Table NOBELBAR® Overview

Brand name	NOBELBAR® 250mg for intravenous injection
Ingredient and content	274 mg of phenobarbital sodium per vial (250 mg as phenobarbital)
Additive	None
pH	9.2 to 10.2 (when 1g is dissolved in 10ml. of water)
Osmotic pressure ratio	2.5 to 2.6 (when 1g is dissolved in 10ml. of water) (ratio to saline)
Indications	Neonatal convulsion/ status epilepticus
Dosage and administration (Neonatal convulsion)	Initial dose: 20 mg/kg of phenobarbital is administered intravenously. The dose should be adjusted according to the patient's condition within the range not exceeding the initial dose, and additional intravenous administration should be performed. Maintenance administration: 2.5 to 5 mg/kg of phenobarbital is intravenously administered once daily.
Usage precautions	Improved consciousness, decreased blood pressure and respiratory depression may occur, and so it is desirable to measure the blood concentration of this drug in order to appropriately adjust the dose.

For phenobarbital sodium preparations approved for intravenous administration overseas, organic solvents such as propylene glycol and alcohol are also used. In addition, it is known that turbidity occurs when diluting 10% Phenobarbital®, an intramuscular injection product in Japan, for intravenous injection, and its safety may be problematic.

In this way, early development of intravenous preparations containing no additives in Japan has been strongly desired by academic societies (Japan Society for Premature and Newborn Medicine, the Japanese Society of Child Neurology, etc.).

The description in the package insert of NOBELBAR® lyophilized formulation that can be intravenously injected is as shown in the table.

Description of review report

Based on this background, investigator-initiated clinical trials were planned and conducted. Please refer to my book, Vol. 3, 6 to 8), for the actual preparation until the execution of the clinical trial and the difficulties and efforts in conducting the clinical trial.

I was sitting in the driver's seat of a used light truck. Mr. O, a person in charge of the company, prepared a new navigation from the clinical trial promotion center in the passenger seat, and one route from Kagawa to the PMDA, and there is a cluster of common practices. The company loaded around 30 cardboard boxes of application materials in a light truck. There were four boxes of cardboard materials that were subjected to a written survey by the clinical trial secretariat and a field survey as a clinical trial facility by PMDA. These materials were created and filed by almost one person after the research expenses were finally cut off.

The company applied for approval with the data from the investigator-initiated clinical trial of only 10 actual subjects. "NOBELBAR®" was finally approved in October 2008 after one year of review by PMDA.

At present, the review report for new drugs can be searched on the website of the Japan Pharmaceutical Information Center (JAPIC)[9]. It is the only document in which the content of the review has been published. This document has been prepared by the reviewers in the Office of New Drug III of the PMDA over a period of one year, and we would like to ask those interested in it to see its efforts.

Presented by MEDICUS SHUPPAN, Publisher Co., Ltd.

Put simply, we have been prepared to state that "Although approval is granted, it cannot be said that it is a safe and effective drug in only 10 clinical studies, and so please thoroughly conduct post-marketing surveillance."

The review report details the reason for the post-marketing surveillance, and we request further information on the following three points.

① Relationship between administration status of this drug (dose, administration rate) and safety and efficacy;
② Relationship between blood concentration and safety and efficacy;
③ Serious skin disorder onset status

① is specified in the protocol to administer the study drug in 5 to 10 minutes, but in many cases, it is 6 minutes. In cases where the study drug was administered in 2 minutes, respiratory depression occurred. In ②, there was a case in which blood concentration increased above the expected value of 45 μg/mL after maintenance administration, and ③ was the result of adverse event that the applicant could not cover in the literature.

And finally,

We would like to ask you to check the "package insert" of the drug in order to reflect the results of the review. If you are also troubled by the "interview form" corresponding to the drug's instructions for use, please read it carefully.

Household appliance can be used without instructions. For drugs, it is equivalent to reading only the "Precautions for use" column on the "Package insert". Wiring and initial settings are also required for home appliances. These are difficult tasks without an instruction manual. This is to view the "package insert" and the "interview form" that corresponds to the instruction manual. It would be appreciated if the drug is not only marketed as a product but also marketed together with "drug information".

The statement "intravenous infusion over 5 to 10 minutes" in the NOBELBAR® package insert, which you may be concerned about, is lastly to state that it is very profound.

In the PMDA reviewers, it is truly not possible to record "intravenous infusion over 5 to 10 minutes" or even further.

As required by the review report, we cannot guarantee that over-the-counter drugs are safe and effective up to administration methods other than those used in clinical trials. Furthermore, as long as there are clinical trial data that "respiratory depression" occurred when administering the drug for a different administration time (2 minutes) than that of the protocol, it is possible to write only the text as per the company's application (= as per the clinical trial protocol) unless there is a description of the intravenous infusion rate and data on the occurrence of adverse drug reactions in the textbook.

So, how was this protocol made?

Presented by MEDICUS SHUPPAN, Publisher Co., Ltd.

When the protocol documents state that "intravenous injection should be given slowly" for face-to-face consultation with PMDA (clinical trial consultation) at the time of the clinical trial plan, the event is ultimately to be given "intravenous injection over 5 to 10 minutes."

This is exactly the way that the safety of the drug is ensured.

As a result of appropriate post-marketing surveillance, we expect users and providers of "NOBELBAR®" to fill in this information.

• Acknowledgments

We would like to express our gratitude for the hard work of planning this investigator-initiated clinical trial, especially to Dr. Shingo Hirano of the Osaka Prefectural General Medical Center for Maternal and Child Health, Dr. Nao Tauchida of the National Center for Child Health and Development, Mr. Hitoshi Shiomura of Nobelpharma Co., Ltd., and Mr. Mitsuo Ozeki.

■ References

1) http://health.med.kagawa-u.ac.jp/jdpt/regulatori/check/index2.html

2) Schmidt, B. et al. Caffeine therapy for apnea of prematurity. N. Engl. J. Med. 354(20), 2006, 2112-21.

3) Schmidt, B. et al. Long-term effects of caffeine therapy for apnea of prematurity. N. Engl. J. Med. 357(19), 2007, 1893-902.

4) http://ja.wikipedia.org/wiki/George Clooney

5) Kensuke Okubo, et al. Survey on the actual use of phenobarbital in neonatal care. For approval of phenobarbital preparation for intravenous injection. Journal of the Japanese Society of Pediatric Clinical Pharmacology and Therapeutics. 18 (1), 2005, 83-5.

6) Kou Kawada. Investigator-initiated clinical trial. Problems after the start: Adjustment and problems. Implementation of "Efficacy and safety research on neonatal convulsions with intravenous phenobarbital." Journal of the Japanese Society of Pediatric Clinical Pharmacology and Therapeutics. 19 (1), 2006, 46-8.

7) Kou Kawada, et al. Current Investigator-Initiated Clinical Trial Practice in the Neonatal Field: an Investigator-Initiated Clinical Trial in Neonates (Intravenous Phenobarbital for Neonatal Convulsions). Journal of the Japanese Society of Pediatric Clinical Pharmacology and Therapeutics. 20 (1), 2007, 30-2.

8) Kou Kawada. Investigator-initiated clinical trial in the field of pediatrics and newborns (intravenous phenobarbital for neonatal seizures). Clinical Pharmacology. 39, 2008, 275-9.

9) http://www.shinsatsuikokusho.jp/dia_us/dat/search/usDatSearch.jsp

Presented by MEDICUS SHUPPAN, Publisher Co., Ltd.



新生児医療の最前線

抗けいれん剤として2008年12月に薬価収載されたノーベルバール®静注用250mg（NOBELBAR® 250mg for Injection，フェノバルビタールナトリウム凍結乾燥製剤）が世の中に出るまでかかわった当事者の話を中心に，本剤について紹介する．

フェノバルビタールナトリウム凍結乾燥製剤「ノーベルバール®」承認に至る治験の概要と添付文書情報

河田 興*

はじめに

筆者は1992年，新生児科医師となるために，大学小児科での研修を開始した．研修2年目の4月よりNICU配属となった筆者は，重症仮死児や超低出生体重児にフェノバルビタール製剤のフェノバール®注をそーっと静注したり，エイッと大腿外側に筋注することを命ぜられた．

立派な指導医に恵まれ，その数は容易に100を超えた．自分の診ていた子どもの何人かは治療の甲斐なく亡くなり，多くの児は元気に成長した．一部の児には，大腿外側に「えくぼ」というにはあまりに大きなクボミを残した．超低出生体重児の大きな筋肉である大腿四頭筋外側への筋注が，果たして彼らにとって一番適切なフェノバルビタール製剤の投与方法・投与場所であったかどうかは疑問である．

フェノバルビタールは水に溶けにくい性質を持つ．フェノバール®注はフェノバルビタールをクロロブタノールなどの有機溶剤に溶かして溶液化している．フェノバール®注の添付文書には「筋注すること」との記載があった．エイッと筋注した薬液の一部は皮下に漏れ，組織障害は子どもの足にクボミを残した．添付文書には「静注しないこと」との記載もあるが，そーっと静注した薬剤はルートの先の血管内で析出しただろう．これは目撃するわけにもいかず，あくまで想像しているに過ぎない．この結晶はどうやって消えていくのであろうか．

子どもが亡くなったのは原疾患が重篤だったためであり，子どもの死亡と静注された薬剤との関係には決して言及されていない．それが約15年前の日本のNICUの風景であった．

今回発売されたフェノバルビタールナトリウム凍結乾燥製剤ノーベルバール®静注用250mg（NOBELBAR® 250mg for Injection）は水に溶解しやすく，かつ溶解すると時間とともに成分が変化する．溶解後は24時間で約6%が分解される比較的不安定なフェノバルビタールナトリウムである．

*国立病院機構香川小児病院総合周産期母子医療センター新生児科医長
（〒765-8501 善通寺市善通寺町2002）

Presented by MEDICUS SHUPPAN, Publisher Co., Ltd.

治験までの道のり

1．子どものくすりの適応外使用問題

当時のボスは大学退官を前に『母乳』と『くすり』をたびたび口にされた．親子の関係を適切に形成するために『いかに母乳哺育と母子相互作用が大切であるか』『いかに子どものくすりがオーファン（孤児）であるか』と．

ボスは大学退官後も薬剤の適応外使用解決に向けて厚生省（現厚生労働省）へ足しげく通い，厚生省班研究事業として，子どものくすりの『オーファン』からの脱出への道筋を模索した．10年近くの月日を経て，現在この活動は種々の適応外使用の問題点を浮かび上がらせた．これら多数の薬剤のうち，ほんの一部はアセトアミノフェンの適正投与量の添付文書記載などの形で，『薬剤適応外使用』解決の実を結び始めている．この適応外使用問題の解決結果の一部は，日本小児臨床薬理学会のホームページでもまとめて紹介されている[1]．

話は2004年5月の岡山に飛ぶ．この年，筆者は岡山で開催された日本小児科学会学術集会で『出生前ステロイド投与が極低出生体重児のCRIB IIに及ぼす影響』について発表した．この数年前には，『極低出生体重児にNasal CPAPを導入してテオフィリン製剤の使用が半減した』とヒストリカルな検討を報告した．この時期，カナダを中心に，世界規模での『カフェインが気管支肺異形成を減らし長期予後も改善する』[2,3]という大規模なランダム化比較試験が終了していた時期である．ちなみにこの臨床研究の結果は，『カフェインの使用によりNasal CPAP使用時期が平均1週間早まる』ことを示している[2]．当時，テオフィリン製剤のアプネカット[2]，アプニション[3]は医薬審第104号通知『適応外使用に係る医療用医薬品の取扱いについて』に則る公知申請中で承認取得時期であり，カフェイン製剤は当時から今なおメーカーの開発（承認取得）の気配はない．まさに製薬企業の活動なしには，オーファンは孤児であり続ける．

2．医師主導治験に向けて

『オーシャンズ11～13』で活躍するジョージ・クルーニー[4]が出世作『ER緊急救命室』のシリーズIで美人MRとフロリダへ出かける話がある．このMRが在籍する架空メーカー（製薬企業）が『ノーベルファーマ』である．同名の当時創業1年の（本邦の）ノーベルファーマ社は，社長以下10数名の本当のベンチャー企業であった．創業社長が『ER』のMRを知っていたかは不明である．ノーベルパール[5]の開発を担当したノーベルファーマ社と具体的に『医師主導治験を実施しましょう』と決定したのがこの2004年5月の会議であった．

この会議では，どんな治験（承認申請を前提に実施する臨床試験）を実施したらよいか議論した．また当時，医師主導治験は制度上可能になったものの，どうやったら薬剤が承認されるかの前例はなく，開発資金は日本医師会治験促

Presented by MEDICUS SHUPPAN, Publisher Co., Ltd.

進センターの研究事業以外にはなかった.

当時筆者は,医師主導治験で2.1億円,メーカー開発費2.5億円の経費がかかり,承認申請認可までに丸4年の歳月を費やすことになるとは思っていなかった.そして,その間,治験の準備ならびに進捗管理を筆者が一手に引き受けることになるとはつゆ知らず,まさに新生児科医師が「治験作業の実地訓練研修」へ出向した状態に陥っていくのであった.

この会議から1ヵ月で医師主導治験のための日本医師会治験促進センターの研究事業応募申請書を書き上げた.「申請用紙の書式が入っているから」と32MBのUSBメモリを手渡されて途方に暮れたのを思い出す.

では,抗けいれん剤のフェノバルビタールは新生児においてどのような目的で使用されていたのであろう.当時大学に導入されていた『Up to Date』で検索したところ,

①新生児けいれん
②人工換気などの鎮静
③重症仮死児の脳保護,けいれん予防
④重症黄疸に対する酵素誘導

などがリストされた.今,振り返れば,このリストの中で「①新生児けいれん」以外で治験を実施するのは至難の業であることは容易にわかる.「②人工換気などの鎮静」は効果の指標の客観性確保が難しく,「③重症仮死児の脳保護,けいれん予防」は重症例では合併症や死亡が頻出してくすりの安全性は確認できない.また「④重症黄疸に対する酵素誘導」は単剤での効果を見るのが難しいことなどは,当時の筆者ではなかなかピンとこないのであった.

日本医師会治験促進センターの研究事業応募申請書に添付した治験概要を眺めると,治験(もしくは薬剤)の安全性に関する配慮や認識が十分でなかったり,主要評価項目が変更になったり,血中濃度測定による薬物動態試験を実施することは記載していなかったりする.一方,当時の新生児におけるフェノバルビタール製剤使用の問題点は明確に指摘されている.つまり,より安全な製剤開発がこの治験の主題であり,当時の新生児でのフェノバルビタール製剤の開発背景であった.

次の項では,当時のフェノバルビタール注射製剤とノーベルバール®の違いを添付文書の記載から明らかにする.

フェノバルビタール製剤の添付文書における記載

1.治験の目的

前出の申請書中の計画する治験に関する概略に示した目的には,「フェノバルビタールは新生児において抗けいれん剤,鎮静剤として最も基本的な薬剤で,国内および欧米において日常的に使用されている.わが国では静注に適した製剤が販売されておらず,筋注・皮下注用製剤を仕方なく使用している.本治験の目的は新生児けいれんに対する静注用フェノバルビタールの教科書的な用法・用量における有効性と安全

Presented by MEDICUS SHUPPAN, Publisher Co., Ltd.

性を評価・確認することである．得られた結果は，既存の成書・文献等における報告と比較し，本邦の新生児けいれんについての承認申請のための評価資料とする」とある．

治験薬は「一般名：フェノバルビタールナトリウム」を使用し，承認を得ようとする効能・効果として「新生児けいれん」としている．なお，「ノーベルバール®」の承認申請の過程で，承認を得ようとする効能・効果には「てんかん重積状態」を併記して，小児・成人でも使用可能な製剤開発が求められたため，新生児には比較的多い用量の250mg製剤が使用された経緯がある．

2．従来のフェノバルビタール製剤の問題点

当時のフェノバルビタール注射製剤は10%フェノバール®（承認取得者：藤永製薬）および10%フェノバルビタール注「マルコ」（同：マルコ製薬）で，効能・効果には「不安緊張状態の鎮静（緊急に必要な場合）」「自律神経発作」「精神運動発作」「てんかんのけいれん発作（強直間代発作：全般けいれん発作・大発作，焦点発作：ジャクソン型発作を含む）」があり，用法・用量として「フェノバルビタールナトリウムとして，通常成人1回50～200mg（1回1/2～2mL）を1日1～2回，皮下または筋肉内注射する．なお，年齢，症状により適宜増減する」とある．その他の記載としては，乳幼児（5ヵ月～1歳6ヵ月）5名にフェノバルビタール10.2mg/kg（平均）を筋注した後の薬物動態データが添付文書上に記載されている10%フェノバール®だけでなく，10%フェノバール®，10%フェノバルビタール注「マルコ」共に添付文書上には，特に「安全性は確立していない」などの否定的な記載はない．筋肉内注射時の注意の項には「なお，乳児，幼児，小児には適用しないことが望ましい」とされており，明らかに乳幼児，小児も投与対象となることを前提に当時のフェノバルビタール注射製剤の添付文書は記載されている．

しかし，次のような問題が存在した．国内では静注用としてフェノバルビタール製剤が市販されておらず，筋注・皮下注用製剤を静注に転用している．添付文書には，「静脈内投与はしないこと」と記載されているにもかかわらず投与されている[5]．これについての添付文書への記載は，10%フェノバール®では「本剤は，水によって主薬を析出するので，静脈内注射及び他の注射剤との混合はしないこと」，10%フェノバルビタール注「マルコ」では「静脈内注射はできない．他の注射剤との混合は避け別々に用いる」となっている．筋注用の製剤10%フェノバール®は難溶の主薬を溶かすために添加物としてクロロブタノール，グリセリンジエチルエーテルなどの有機溶媒が入っているが，同様の有機溶媒であるベンジルアルコールを含有する10%フェノバルビタール注「マルコ」の投与ではベンジルアルコール中毒によるgasping syndromeの合併が考えられ，可能であればこのような添加物の入っていない製剤が特に新

Presented by MEDICUS SHUPPAN, Publisher Co., Ltd.

表　ノーベルバール®概要

販売名	ノーベルバール® 静注用250mg
成分・含量	1バイアル中フェノバルビタールナトリウム274mg（フェノバルビタールとして250mg）
添加物	なし
pH	9.2～10.2（1gを水10mLに溶解時）
浸透圧比	2.5～2.6（1gを水10mLに溶解時）（生理食塩水に対する比）
効能・効果	新生児けいれん・てんかん重積状態
用法・用量（新生児けいれん）	初回投与：フェノバルビタールとして20 mg/kgを静脈内投与する．臨床の状態に応じ初回投与量を超えない範囲で用量を調節し，静脈内に追加投与する．維持投与：フェノバルビタールとして2.5～5mg/kgを1日1回静脈内投与する．
使用上の注意	意識障害，血圧低下，呼吸抑制が現れることがあるので，用量調節を適切に行うために，本剤の血中濃度測定を行うことが望ましい．

生児・低出生体重児に対しては好ましい．海外で静脈内投与の承認を受けているフェノバルビタールナトリウム製剤も，プロピレングリコールやアルコールなど有機溶媒を使用している．また本邦の筋注用製剤10%フェノバール®を静注用に希釈しようとすると混濁が生じることが知られており，その安全性にも問題のある可能性がある．

このように添加物を含まない静注用製剤の本邦における早期開発が学会（日本未熟児新生児学会，日本小児神経学会など）から強く望まれていたのである．

なお，静注可能な凍結乾燥製剤のノーベルバール®の添付文書上の記載は表の通りである．

審査報告書の記載

このような背景を元に医師主導治験は計画・実施された．実際に治験実施に至るまでの準備や，治験実施における困難やその努力については，拙著3編[6-8]を参考にしてほしい．

まさに中古の軽トラックの運転席に私が座り，助手席には企業の担当者のOさん，新品のナビゲーションを治験促進センターから用意してもらって，香川から一路，医薬品医療機器総合機構（PMDA）へ向けて出発した珍道中をまとめてある．企業は軽トラックに申請資料を段ボール30箱あまり積み込んだ．治験事務局としての書面調査と，治験実施施設としての実地調査をPMDAに受けた資料がダンボールで4箱分あった．これらの資料は最終的に研究費が途絶えたあと，ほぼ1名で作成，ファイルを行った．

実際の被験者はたった10例だけの医師主導治験のデータを添えて，企業が承認申請を行った．PMDAによる1年間の審査を経て，ようやく2008年10月に「ノーベルバール®」は承認された．

現在，財団法人日本医薬情報センター（Japan Pharmaceutial Information Center：JAPIC）のホームページで新薬の審査報告書が検索できる[9]．審査の内容が公表されている唯一の資料である．PMDAの新薬審査第3部の審査者た

Presented by MEDICUS SHUPPAN, Publisher Co., Ltd.

ちが1年かけて審査結果をまとめて作成した文書であり，関心のある方はぜひともその力作にも目を通していただきたい．

簡単に言えば，「承認はするけれど，たった10例の臨床試験では十分に安全で有効なくすりとは言えないので，製造販売後調査をしっかりやってください」という内容に仕上がっている．

その審査報告書には詳しく，製造販売後調査を行う理由が述べられており，以下の3点について，さらなる情報の収集を求めている．

①本剤の投与状況（投与量・投与速度）と安全性および有効性との関係
②血中濃度と安全性および有効性との関係
③重篤な皮膚障害の発生状況

①は実施計画書では5～10分で治験薬を投与する規定が，実際は多くの症例で6分であり，2分で投与した症例では呼吸抑制が出現した結果で，②は維持投与して血中濃度が45 μg/mL程度まで予想以上に上昇した症例がある結果で，③は文献的な調査で申請企業がカバーできなかった有害事象内容の結果である．

最後に

審査の結果がまとめられたくすりの「添付文書」はぜひとも確認するようにお願いしたい．くすりの取扱説明書に相当する「インタビューフォーム」も困ったときには，ぜひご一読いただきたい．

家庭電化製品は取扱説明書なしにも使用できる．くすりでは「添付文書」上の「使用上の注意」の欄だけ目を通すことがそれに相当する．家庭電化製品でも配線を施したり，初期設定が必要である．これらは取扱説明書なしには困難な作業である．これはくすりでは「添付文書」やまさに取扱説明書に相当する「インタビューフォーム」を眺めることである．くすりは製品としてだけではなく，「くすりの情報」も一緒に販売されていることを意識していただけるとありがたい．

皆さんが疑問に思われているであろう，ノーベルバール[®]の添付文書の投与方法における「5～10分間かけて静注する」との記載は，非常に奥が深いことについて最後に述べる．

本当に，PMDA審査者にしてみれば，「5～10分間かけて静注する」，それ以上にもそれ以下にも書けないのである．

審査報告書にも求められるように，市販される薬剤が治験で実施した以外の投与方法まで安全で有効であることを保証することはできない．さらに，治験実施計画とは異なる投与時間（2分）で投与した際に「呼吸抑制」を生じたという治験データがある以上，教科書に静注速度と副作用発現のデータ記載がない限り，企業の申請どおり（＝治験の実施計画書どおり）の文章しか記載できないのである．

では，この実施計画書の記載はどのように作られたか．

治験計画時のPMDAとの対面助言（治験相談）のために「ゆっくり静注する」ということ

Presented by MEDICUS SHUPPAN, Publisher Co., Ltd.

を実施計画書の文書に起こした際に、結局「5～10分かけて静注する」となったという経緯がある。

まさに、くすりの安全を保証するということはこういうことである。

適切な製造販売後調査の結果、これらの情報が埋められていくことを『ノーベルバール®』のユーザーとプロバイダーに期待する。

■謝辞

本医師主導治験の計画立案に大変ご尽力いただいた方々、特に大阪府立母子保健総合医療センター・平野慎也先生、国立成育医療センター・土田　尚先生、ノーベルファーマ株式会社・塩村　仁さん、大関三夫さんに感謝いたします。

■参考文献

1) http://health.med.kagawa-u.ac.jp/jdpt/reguratori/check/index2.html
2) Schmidt, B. et al. Caffeine therapy for apnea of prematurity. N. Engl. J. Med. 354 (20), 2006. 2112-21.
3) Schmidt, B. et al. Long-term effects of caffeine therapy for apnea of prematurity. N. Engl. J. Med. 357 (19), 2007. 1893-902.
4) http://ja.wikipedia.org/wiki/ジョージ・クルーニー
5) 大久保賢介ほか. 新生児医療におけるフェノバルビタール使用の実態調査：静注用フェノバルビタール製剤承認に向けて. 日本小児臨床薬理学会雑誌. 18 (1), 2005. 83-5.
6) 河田興. 医師主導型治験　スタート後の問題点. 調整と問題点：「静注用フェノバルビタールの新生児けいれんに対する有効性・安全性に関する研究」実施にあたって. 日本小児臨床薬理学会雑誌. 19 (1), 2006. 46-8.
7) 河田興ほか. 医師主導型治験の現状. 新生児領域における取り組み：新生児における医師主導治験（新生児けいれんに対する静注用フェノバルビタール）. 日本小児臨床薬理学会雑誌. 20 (1), 2007. 50-2.
8) 河田興. 小児・新生児領域における医師主導治験（新生児けいれんに対する静注用フェノバルビタールを実施して）. 臨床薬理. 39. 2008. 275-9.
9) http://www.shinsahoukokusho.jp/dar_us/dar/search/usDarSearch.jsp

Presented by MEDICUS SHUPPAN, Publisher Co., Ltd.

Sun Ex. 1004

ILAE OFFICIAL REPORT

A practical clinical definition of epilepsy

*Robert S. Fisher, †Carlos Acevedo, ‡Alexis Arzimanoglou, §Alicia Bogacz, ¶J. Helen Cross, #Christian E. Elger, **Jerome Engel Jr, ††Lars Forsgren, ‡‡Jacqueline A. French, §§Mike Glynn, ¶¶Dale C. Hesdorffer, ##B.I. Lee, ***Gary W. Mathern, †††Solomon L. Moshé, ‡‡‡Emilio Perucca, §§§Ingrid E. Scheffer, ¶¶¶Torbjörn Tomson, ###Masako Watanabe, and ****Samuel Wiebe

Epilepsia, 55(4):475–482, 2014
doi: 10.1111/epi.12550



Robert S. Fisher
Department of Neurology & Neurological Sciences, Stanford University School of Medicine

Summary

Epilepsy was defined conceptually in 2005 as a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures. This definition is usually practically applied as having two unprovoked seizures >24 h apart. The International League Against Epilepsy (ILAE) accepted recommendations of a task force altering the practical definition for special circumstances that do not meet the two unprovoked seizures criteria. The task force proposed that epilepsy be considered to be a disease of the brain defined by any of the following conditions: (1) At least two unprovoked (or reflex) seizures occurring >24 h apart; (2) one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years; (3) diagnosis of an epilepsy syndrome. Epilepsy is considered to be resolved for individuals who either had an age-dependent epilepsy syndrome but are now past the applicable age or who have remained seizure-free for the last 10 years and off antiseizure medicines for at least the last 5 years. "Resolved" is not necessarily identical to the conventional view of "remission or "cure." Different practical definitions may be formed and used for various specific purposes. This revised definition of epilepsy brings the term in concordance with common use.

KEY WORDS: **Epilepsy, Seizure, Definition, Unprovoked, Recurrence.**

Accepted January 3, 2014

*Department of Neurology & Neurological Sciences, Stanford University School of Medicine, Stanford, California, U.S.A.; †SCH, Past President Chilean League Against Epilepsy, Santiago, Chile; ‡Epilepsy, Sleep and Pediatric Neurophysiology Department, University Hospitals of Lyon (HCL) and Lyon Neuroscience Research Center (CRNL), Lyon, France; §Neurological Institute of Clinical Hospital, Universidad Mayor de la República, Montevideo, Uruguay; ¶UCL-Institute of Child Health, Great Ormond Street Hospital for Children, London & Young Epilepsy, Lingfield, United Kingdom; #Department of Epileptology, University of Bonn Medical Centre, Bonn, Germany; **Neurology, Neurobiology, and Psychiatry and Biobehavioral Sciences, UCLA Seizure Disorder Center, David Geffen School of Medicine at UCLA, Los Angeles, California, U.S.A.; ††Department of Pharmacology and Clinical Neuroscience/Neurology, Umea University, Umea, Sweden; ‡‡Department of Neurology, NYU School of Medicine, New York, New York, U.S.A.; §§CEO, Epilepsy Ireland, Dublin, Ireland; ¶¶GH Sergievsky Center and Department of Epidemiology, Columbia University, New York, New York, U.S.A.; ##Yonsei Epilepsy Research Institute, Yonsei University College of Medicine, Seoul, Korea; ***Departments of Neurosurgery and Psychiatry & BioBehavioral Medicine, Mattel Children's Hospital, David Geffen School of Medicine, University of California, Los Angeles, California, U.S.A.; †††Saul R. Korey Department of Neurology, Dominick P. Purpura Department of Neuroscience and Department of Pediatrics, Laboratory of Developmental Epilepsy, Montefiore/Einstein Epilepsy Management Center, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, New York, U.S.A.; ‡‡‡Department of Internal Medicine and Therapeutics University of Pavia and C. Mondino National Neurological Institute, Pavia, Italy; §§§Departments of Medicine and Paediatrics, Florey Institute, Austin Health and Royal Children's Hospital, The University of Melbourne, Melbourne, Victoria, Australia; ¶¶¶Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden; ###Department of Psychiatry, National Center of Neurology and Psychiatry, Tokyo, Japan; and ****University of Calgary, Calgary, Alberta, Canada

Address correspondence to Robert S. Fisher, Neurology, Stanford University School of Medicine, Room A343, 300 Pasteur Drive, Stanford, CA 94305-5235, U.S.A. E-mail: robert.fisher@stanford.edu

Wiley Periodicals, Inc.
© 2014 International League Against Epilepsy

In 2005, a Task Force of the International League Against Epilepsy (ILAE) formulated conceptual definitions of "seizure" and "epilepsy" (Table 1).[1] Conceptual definitions can be translated for specific purposes into operational (practical) definitions.

The ILAE commissioned a Task Force to formulate an operational definition of epilepsy for purposes of clinical diagnosis. This article summarizes the recommendations of the Task Force, including appended notes and case examples explaining the reasons for these recommendations and occasional dissenting views. In December of 2013, the ILAE Executive Committee adopted the recommendations as a position of the ILAE.

Why alter the definition of epilepsy? Doing so might cause confusion among patients who could be left uncertain as to whether they have or do not have epilepsy. Epidemiologists and other researchers would need to decide whether to use the new or old definition and how this might affect trends and comparisons. Rules and regulations might have to be changed. Arrayed against these potential negatives are positive aspects to reevaluation of the definition. The current definition requires two unprovoked seizures occurring at least 24 h apart.[2] Some epileptologists recognize and feel a need to address circumstances with high risk for future seizures after a first unprovoked seizure. For example, one Delphic study group in Spain[3] voted with high consensus in favor of treatment in five of seven hypothetical scenarios after a first seizure. A decision for treatment does not necessarily equate to a diagnosis of epilepsy, but it can be taken as a marker for belief in a strong enduring predisposition for further seizures. Conversely, a diagnosis of epilepsy does not necessarily require treatment. The current definition does not allow a patient to outgrow epilepsy, yet many older individuals have all but forgotten their two childhood seizures. A definition should conform to how clinicians and patients think, and usefully merge with other individual considerations in helping to make treatment decisions.

Practical Clinical Definition of Epilepsy

Conceptually, epilepsy exists after at least one unprovoked seizure, when there is high risk for another, although the actual required risk is subject to debate. After a single unprovoked seizure, risk for another is 40–52%.[4] With two unprovoked nonfebrile seizures, the chance by 4 years of having another is 73%, with a 95% confidence interval (CI) of 59–87%, subsequently herein portrayed as approximately 60–90%.[5]

The "two unprovoked seizure" definition of epilepsy has served us well, but it is inadequate in some clinical circumstances. A patient might present with a single unprovoked seizure after a remote brain insult, such as a stroke, central nervous system (CNS) infection, or trauma. A patient with such brain insults has a risk of a second unprovoked seizure that is comparable to the risk for further seizures after two unprovoked seizures.[6] When two individuals with a history of at least one unprovoked seizure have the same high risk for having another, an argument can be made that both have epilepsy. Under limits of the current definition, another patient might have photosensitive epilepsy, yet not be considered to have epilepsy because the seizures are provoked by lights. Another might be free of seizures and seizure medications for 50 years, yet still have epilepsy. In order to bring the practical (operational) clinical definition of epilepsy into concordance with how epileptologists think about epilepsy, the ILAE Task Force recommends broadening the definition of epilepsy to include the circumstances enumerated in Table 2. The Task Force also added a time limit to the definition.

Several elements of this definition require clarification.

Table 1. Conceptual definition of seizure and epilepsy – 2005 report

An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain.

Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures, and by the neurobiologic, cognitive, psychological, and social consequences of this condition. The definition of epilepsy requires the occurrence of at least one epileptic seizure.

Disease

Epilepsy has traditionally been referred to as a disorder or a family of disorders, rather than a disease, to emphasize that it is comprised of many different diseases and conditions. The term disorder implies a functional disturbance, not necessarily lasting; whereas, the term disease may (but not always) convey a more lasting derangement of normal function. Many heterogeneous health problems, for example, cancer or diabetes, comprise numerous subdisorders and are still considered to be diseases. The term "disorder" is poorly understood by the public and minimizes the serious nature of epilepsy. The ILAE and the International Bureau for Epilepsy (IBE) have recently agreed that epilepsy is best considered to be a disease.

Two unprovoked seizures

Epilepsy exists in a patient who has had a seizure and whose brain, for whatever reason, demonstrates a pathologic and enduring tendency to have recurrent seizures. This tendency can be imagined as a pathologic lowering of the seizure threshold, when compared to persons without the condition. Table 2, item 1, represents the current commonly employed definition of epilepsy as at least two unprovoked seizures occurring >24 h apart. A seizure that is provoked by a transient factor acting on an otherwise normal brain to temporarily lower the seizure threshold does not count toward a diagnosis of epilepsy. The term "provoked sei-

Epilepsia, 55(4):475–482, 2014
doi: 10.1111/epi.12550

Table 2. Operational (practical) clinical definition of epilepsy

Epilepsy is a disease of the brain defined by any of the following conditions

1. At least two unprovoked (or reflex) seizures occurring >24 h apart
2. One unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years
3. Diagnosis of an epilepsy syndrome

Epilepsy is considered to be *resolved* for individuals who had an age-dependent epilepsy syndrome but are now past the applicable age or those who have remained seizure-free for the last 10 years, with no seizure medicines for the last 5 years.

zure" can be considered as being synonymous with a "reactive seizure" or an "acute symptomatic seizure."[7] Etiology should not be confused with provocative factors, as some etiologies will produce an enduring tendency to have seizures. A brain tumor, for example, might cause a person to have an epileptic seizure, but not as a transient insult.

The condition of recurrent reflex seizures, for instance in response to photic stimuli, represents provoked seizures that are defined as epilepsy. Even though the seizures are provoked,[8] the tendency to respond repeatedly to such stimuli with seizures meets the conceptual definition of epilepsy, in that reflex epilepsies are associated with an enduring abnormal predisposition to have such seizures.

A seizure after a concussion, with fever, or in association with alcohol-withdrawal, each would exemplify a provoked seizure that would not lead to a diagnosis of epilepsy. The term "unprovoked" implies absence of a temporary or reversible factor lowering the threshold and producing a seizure at that point in time. Unprovoked is, however, an imprecise term because we can never be sure that there was no provocative factor. Conversely, identification of a provocative factor does not necessarily contradict the presence of an enduring epileptogenic abnormality. In an individual with an enduring predisposition to have seizures, a borderline provocation might trigger a seizure, whereas in a nonpredisposed individual, it might not. The Definitions Task Force recognizes the imprecise borders of provoked and unprovoked seizures, but defers discussion to another venue.

High recurrence risk

Table 2, item 2 defines another path for diagnosing epilepsy. Its intent is to encompass circumstances for which some practitioners[9] and expert epileptologists[3] manage patients as if epilepsy is present after a single unprovoked seizure, because of a very high risk of recurrence. Such examples may include patients with a single seizure occurring at least a month after a stroke (Hesdorffer et al. 2009)[6] or a child with a single seizure conjoined with a structural or remote symptomatic etiology and an epileptiform electroencephalography (EEG) study.[10] Another example is a patient in whom diagnosis of a specific epilepsy syndrome associated with persistent threshold alteration can be made after the occurrence of a single seizure. A first seizure might present as status epilepticus,[11,12] but this does not in itself imply epilepsy. Recurrence risks are not known for the majority of individual cases. However, if a treating physician is aware that the lesion has generated an enduring predisposition for unprovoked seizures with a risk comparable to those who have had two unprovoked seizures (which we all agree is epilepsy), then that person too should be considered to have epilepsy. Choosing a specific threshold risk number might be excessively precise, but for general comparison, this risk is about 60–90% after two unprovoked seizures.[1] A threshold level of 60% appropriately exceeds the 50% level of recurrence risk found at 5 years after a single seizure in the United Kingdom multicentre study of early epilepsy and single seizures (MESS) study.[13]

It is important to note that a single seizure plus a lesion or a single seizure plus epileptiform EEG spikes does not automatically satisfy criteria for this operational definition of epilepsy, because data may vary among different studies and specific clinical circumstances. In the Dutch Epilepsy Study,[10] children with epileptiform EEG patterns after their first seizure had a 2-year risk for recurrence of 71%, but in the study by Shinnar et al.,[12] children with a first idiopathic seizure and abnormal EEG patterns had recurrence risk of 56% at 3 years. No formula can be applied for additive risks, since data are lacking on how such risks combine; such cases will have to be decided by individualized considerations. Recurrence risk is a function of time, such that the longer the time since the last seizure, the lower the risk.[14]

The revised definition places *no burden* on the treating physician to specify recurrence risk in a particular circumstance. In the absence of clear information about recurrence risk, or even knowledge of such information, the default definition of epilepsy originates at the second unprovoked seizure. On the other hand, if information is available to indicate that risk for a second seizure exceeds that which is usually considered to be epilepsy (about 60%), then epilepsy can be considered to be present.

Epilepsy syndrome

It makes little sense to say that someone has an epilepsy syndrome[15] but not epilepsy. If evidence exists for an epilepsy syndrome, then epilepsy may be presumed to be present, even if the risk of subsequent seizures is low. This is the case with benign epilepsy with centro-temporal spikes (BECTS).

Exceptional syndromic cases may exist in which obvious behavioral seizures may not occur at all, as can be the case with continuous spike and waves during sleep and the Landau-Kleffner Syndrome.[15]

Implications for treatment

Diagnosing epilepsy after a single unprovoked seizure when there is high risk for recurrence may or may not lead to a decision to initiate treatment. The proposed practical definition may provide support to a physician who wishes to treat a patient with high recurrence risk after a single unprovoked seizure. However, a treatment decision is distinct from a diagnosis, and should be individualized depending upon the desires of the patient, the individual risk-benefit ratio and the available options. The physician should weigh the possible avoidance of a second seizure with associated risks against the risk for drug-related side effects and costs for the patients.

To be clear, the diagnosis of epilepsy and a decision to treat are two related but different issues. Many epileptologists treat for a time after an acute symptomatic seizure (for example, with Herpes encephalitis), with no implication of epilepsy. In contrast, patients with mild seizures, with seizures at very long intervals, or those declining therapy might go untreated even when a diagnosis of epilepsy is beyond dispute.

Unprovoked seizures separated in time

The time span between two unprovoked seizures that together qualify as epilepsy is subject to ambiguity. Seizures clustering within 24 h confer approximately the same risk for later seizures as does a single seizure.[17] The Task Force retained the current thinking that unprovoked seizures clustering in a 24 h period be considered to be a single unprovoked seizure for purposes of predicting recurrence risk.

Some authorities[17] consider epilepsy to be present, but in remission, after 5 years of seizure freedom. However, the definition of epilepsy does not specify an outer time limit for occurrence of the second unprovoked seizure to mark the onset of epilepsy. Therefore, epilepsy could be considered present if an unprovoked seizure occurred at age 1 and at age 80, a condition sometimes referred to as oligoepilepsy.[18] The Task Force acknowledges that, in such circumstances, the causes of the seizures occurring at the two time points might be different, and if so then epilepsy would not be present.[11] Otherwise, the Task Force did not agree on a specific interval of time between seizures that would "reset the clock" for counting an event as a second seizure. A rationale for setting such an interval might emerge from future research.

Epilepsy resolved

Is epilepsy, once diagnosed, always present? The traditional definition does not allow for its disappearance. Should a person who has been seizure-free and off medication for decades after absence seizures as a child still be considered to have epilepsy? Likewise, are patients with mesial temporal lobe epilepsy who have been seizure-free off medications for 10 years after resection of their hippocampal sclerosis considered to still have epilepsy? Seizure freedom for long intervals of time can result from one of several different underlying circumstances and treatments. An abnormal tendency to have unprovoked seizures may remain, but the seizures are successfully controlled by therapy. Children can outgrow their epilepsy, as with BECTS. Some persons might have had a definitive treatment, such as brain surgery, rendering them permanently seizure-free.

The Task Force sought a definition that would allow a possible end to the burden of having epilepsy. Medical literature uses the term "remission" to imply an abeyance of a disease, but this term is not well-understood by the public, and remission does not convey absence of the disease. "Cure" implies a risk for future seizures no greater than that of the baseline unaffected population, but after a history of epilepsy such a low risk is never achieved. The Task Force therefore adopted the phrase "resolved."[III] When epilepsy is resolved, it implies that the person no longer has epilepsy, although it does not guarantee that it will not return.

What time intervals and circumstances should characterize resolved epilepsy?[IV] Recurrence risk depends on the type of epilepsy, age, syndrome, etiology, treatment, and many other factors. Juvenile myoclonic epilepsy is known to be subject to an elevated risk of seizures for several decades,[19] but remissions do still occur. Structural brain lesions, such as malformations of cortical development,[20] may elevate risk of seizures long term. Seizures may recur at variable intervals after remission due to removal of an epileptogenic lesion, such as a cavernous malformation.[21] A study[22] of 347 children achieving at least 5-year "complete remission" including at least 5-years free of antiseizure drugs identified late seizure relapses in 6%. One occurred as long as 8 years after the prior seizure. Data were not given for those remaining free of seizures after a 10-year complete remission, but the number would be <6%. After temporal lobe epilepsy surgery,[23] 54.2% of patients relapse within 6 months; whereas, only 1.9% relapse 4 years after surgery. Similar results were seen in another study,[24] with only 0.6% having seizures in the last year of follow-up, provided that they had been seizure-free for 3 years after surgery.

The risk of seizure recurrence after unprovoked seizures diminishes with time, although the risk may never reach levels for normal individuals who have not had a prior seizure. Most relapses are early. After a single unprovoked seizure, 80%[14,17] to 90%[25] of those who had a second did so within 2 years. In one study,[5] after a second unprovoked seizure, subsequent seizures occurred within 4 years, but none in the ensuing 3 years, suggesting that the risk may not be zero but is low. The National General Practice Study of Epilepsy in the United Kingdom[13] identified a 3-year recurrence risk of 44% after a seizure-free period of 6 months, 32% after 12 months,

and 17% after 18 months. No adequate data are available on seizure recurrence risk after being seizure-free and off medication for extended periods of time. Delayed relapses are rare after 5 years.[26] By 10 years off antiseizure medicines, the annual risk for seizures probably is very low.[27,V]

Clinicians will have to individualize a determination of whether epilepsy is resolved. The Task Force chose to define epilepsy as being resolved for individuals who had an age-dependent epilepsy syndrome but are now past the applicable age or those who have remained seizure-free for the last 10 years, with no seizure medicines for the last 5 years. Delineation of circumstances in which epilepsy is definitively cured is beyond the scope of this paper.

Imperfect information

From the clinician's perspective, the new practical definition linking epilepsy to a predefined probability of seizure recurrence brings greater clarity and clinical relevance to the diagnostic process. However, optimal application of this definition often requires specialized diagnostic and interpretative skills—specifically, in assessing recurrence risks, or in diagnosing syndromes—which may not be broadly available in all settings, particularly at the primary care level. Even more important is the inevitable uncertainty in many situations about the potential epileptogenicity of an magnetic resonance imaging (MRI)–demonstrated lesion. For instance, one or more brain cysts in an individual with neurocysticercosis[28] may be incidental findings with no epileptogenic activity in a particular individual. Risk does not equate with causation. When in doubt, practitioners should consider referring a patient to a specialized epilepsy center with experience in diagnosis.

In the absence of a seizure documented by video-EEG recording and typical for a person's recurrent unprovoked seizures, there will be situations where a diagnosis of epilepsy remains uncertain. One approach to these ambiguities would be to define a condition called "probable (or possible) epilepsy."[VI] Such an approach has been adopted with other diseases, such as multiple sclerosis with the McDonald criteria,[29] amyotrophic lateral sclerosis with the El Escorial criteria,[30] migraine,[31] and vascular dementia.[32] The ILAE Task Force recognized the subtle, but important, difference between telling a patient that "you have probable epilepsy" versus "you probably have epilepsy." In the absence of secure information, the latter statement, or another statement simply expressing uncertainty, seemed a more straightforward assertion. Therefore, the Task Force has not defined probable epilepsy as a specific entity, but has left that possibility open for the future.

Consequences of the Practical Definition

Definitions have consequences. From the viewpoint of the patient, epilepsy is associated with stigma and psychological, social, cognitive, and economic repercussions so important as to be built into the conceptual definition of epilepsy.[1] The new practical definition could improve outcomes by sensitizing clinicians about the need to give greater consideration to the risk of recurrence after a single unprovoked seizure, and making the clinicians more comfortable in initiating treatment after some initial unprovoked seizures. This must be individualized, since a diagnosis of epilepsy does not necessarily require prescription of an antiseizure drug, and treatment might be justified in some patients for whom a definitive diagnosis of epilepsy has not been made. A practical definition allowing earlier diagnosis will be especially useful for prevention of unnecessary risks of physical injuries or social consequences resulting from recurrent seizures in patients deemed to be susceptible to a high risk for recurrence. The revised definition also provides an expanded opportunity for disease-modifying interventions that prevent the progression of epilepsy and onset of comorbidities.

How revision of the definition of epilepsy will affect the measured prevalence of epilepsy is unpredictable. Future epidemiologic studies may choose to use the older operational definition for consistency. If the revised definition is used, some patients previously considered to have epilepsy will no longer carry an epilepsy diagnosis because of the provisions for epilepsy being resolved. Other individuals who meet the "single seizure with high risk for another" criteria might be added to the epilepsy group.

The definition of epilepsy will affect diagnosis and treatment in both resource-rich and resource-poor societies. The Task Force has been careful to define epilepsy in a way that can be applied in general with or without expensive technology that may not be universally available.

The correct diagnosis of epilepsy in people who might not have been diagnosed previously may have both negative and positive consequences. For example, economic consequences might include reimbursement by a national health service for medications whose cost otherwise would have to be covered by the affected person. On the other hand, many people with epilepsy have difficulty in obtaining life or medical insurance. Some cannot purchase a first home without a life insurance policy secured at the time of home purchase. Stigma could profoundly affect some people not previously considered to have epilepsy, with serious and misguided consequences such as loss of access to education or marriage bans. Allowing epilepsy to be declared "resolved" may lift the stigma from some who should no longer be considered to have epilepsy. Positive economic and health consequences will accrue when more accurate diagnosis results in appropriate preventative treatment before a second seizure occurs.

People with reflex epilepsies previously have been disenfranchised by the requirement that seizures be unprovoked. The inclusion of reflex epilepsy syndromes in a practical clinical definition of epilepsy now brings these individuals into the epilepsy community.

The revised practical definition described in this report is intended for clinical diagnosis, and might not be suitable for all research studies. Different operational definitions will be used depending on specific purposes, and comparisons could still be made using the traditional "two-unprovoked-seizure" definition of epilepsy whenever appropriate. Investigators must clearly identify the definition used in any study or publication.

A revised definition has implications for legislation and health economics. Regulations affecting individual life activities, such as driving restrictions, relate more to seizure frequency or to risk of seizure recurrence than to a diagnosis of epilepsy, but this is not always the case. In some countries a diagnosis of epilepsy per se limits the period of validity of a driving permit, or the type of permit that can be acquired. Guidelines about participation in certain sports may stipulate restrictions for people with a diagnosis of epilepsy, irrespective of seizure history. Insurance coverage and social benefits might also be affected by the diagnostic label. To the extent that a revised practical definition might affect the number of people diagnosed with epilepsy, there could be cost repercussions for the individual and for the society. Costs to society may not necessarily be higher, however, particularly if the new operational diagnosis codifies the current approach of epileptologists and leads to improved management of individuals who are likely or unlikely to have future seizures.

Conclusion

Epilepsy previously has been defined as at least two unprovoked seizures >24 h apart. The revised practical definition implies that epilepsy also can be considered to be present after one unprovoked seizure in individuals who have other factors that are associated with a high likelihood of a persistently lowered seizure threshold and therefore a high recurrence risk. Such risk should be equivalent to the recurrence risk of a third seizure in those with two unprovoked seizures, approximately at least 60%. The latter risk level occurs with remote structural lesions, such as stroke, CNS infection, certain types of traumatic brain injury, diagnosis of a specific epilepsy syndrome, or in some circumstances with the presence of other risk factors. Those with recurrent reflex seizures, for example, photosensitive seizures, are also considered to have epilepsy. This definition of epilepsy brings the term in concordance with common use by most epileptologists.[VII] Epilepsy is not necessarily life-long, and is considered to be resolved if a person has been seizure-free for the last 10 years, with at least the last 5 year off antiseizure medicines, or when that person has passed the age of an age-dependent epilepsy syndrome. The new definition is more complicated than is the old definition. Studies providing detailed knowledge of seizure recurrence risk are few, so most diagnoses of epilepsy will of necessity still be made by documentation of two unprovoked seizures. As more knowledge of recurrence risks is accrued for specific etiologies, application of the epilepsy definitions will become more precise and more useful.

Case Examples[VIII]

1. *Two seizures.* A 25-year-old woman has two unprovoked seizures, 1 year apart. *Comment*: This person has epilepsy, according to both the old and new definitions.
2. *Stroke and seizure.* A 65-year-old man had a left middle cerebral artery stroke 6 weeks ago and now presented with an unprovoked seizure. *Comment*: With a seizure in this time relation to a stroke (or brain infection or brain trauma) the literature[6] suggests a high (>70%) risk of another unprovoked seizure. Therefore, in the new (but not the old) definition, this man would have epilepsy.
3. *Photic seizures.* A 6-year-old boy has had two seizures 3 days apart while playing a videogame involving flashing lights. There have been no other seizures. EEG shows an abnormal photoparoxysmal response. *Comment*: This boy has epilepsy according to the new definition (but not the old), even though the seizures are provoked by lights, since there is an abnormal enduring predisposition to have seizures with light flashes.
4. *Benign Epilepsy with Centrotemporal Spikes (BECTS).* A 22-year-old man had seizures with face twitching when falling asleep at ages 9, 10, and 14 years; he has had none since. EEG at age 9 years demonstrated centrotemporal spikes. Medications were discontinued at age 16. *Comment*: For this young man, epilepsy is resolved, because of passing the relevant age range of an age-dependent syndrome. The old definition has no provision for considering epilepsy to be resolved.
5. *Single seizure and dysplasia.* A 40-year-old man had a focal seizure characterized by left hand twitching that progressed to a tonic–clonic seizure. This was his only seizure. Magnetic resonance imaging (MRI) shows a probable transmantle dysplasia in the right frontal lobe and EEG shows right frontotemporal interictal spikes. *Comment*: Although many clinicians would reasonably treat this man with antiseizure medications, the recurrence risk for seizures is not precisely known, and therefore epilepsy cannot yet be said to be present according to either definition. Future epidemiologic studies might clarify this situation.
6. *Two seizures long ago.* An 85-year-old man had a focal seizure at age 6 and another at age 8 years. EEG, MRI, blood tests, and family history were all unrevealing. He received antiseizure drugs from age 8 to age 10 years, when they were discontinued. There have been no further seizures. *Comment*: According to the new definition, epilepsy is resolved, since he has been seizure-free for >10 years and off seizure medication for at least the last 5 years. This is not a guarantee against future

seizures, but he has a right to be viewed as someone who does not currently have epilepsy.

7. *Long-interval seizures.* A 70-year-old woman had unprovoked seizures at ages 15 and 70. EEG, MRI, and family history are unremarkable. *Comment:* Both old and new definitions consider this woman to have epilepsy. Despite the diagnosis, many clinicians would not treat because of the low frequency of seizures. Should investigations somehow show that the causes of the two seizures were different, then epilepsy would not be considered to be present.
8. *Questionable information.* A 20-year-old man has had three unobserved episodes over 6 months consisting of sudden fear, difficulty talking, and a need to walk around. He is not aware of any memory loss during the episodes. There are no other symptoms. He has no risk factors for epilepsy and no prior known seizures. Routine EEG and MRI are normal. *Comment:* Declaring this man to have epilepsy is impossible by either the old or new definition. Focal seizures are on the differential diagnosis of his episodes, but both definitions of epilepsy require confidence that the person has had at least one seizure, rather than one of the imitators of seizures. Future discussions may define the boundaries of "possible and probable epilepsy."

Acknowledgments

The Task Force would like to thank a group appointed by the ILAE to review revision of the article specifically in response to the public comments. This group consisted of Lars Forsgren Umeå University Hospital Sweden; Angelina Kakooza, Makerere University College of Health Sciences, Kampala, Uganda; and Akio Ikeda, University of Kyoto.

Disclosure or Conflict of Interest

Robert S. Fisher has received support from, and/or has served as a paid consultant for, the Maslah Saul MD Chair, the Anderson fund for Epilepsy Research, The Susan Horngren Fund, SmartMonitor, and IC-VRs, and has done consulting for Cyberonix, Oracle, and UCB. Alexis Arzimanoglou has received support from, and/or has served as a paid consultant for, Cyberonics, Eisai, GlaxoSmithKline, UCB Pharma, and Viropharma. J. Helen Cross has received support from, and/or has served as a paid consultant for, Eisai, Viropharma, and GlaxoSmithKline. Christian E. Elger has received support from, and/or has served as a paid consultant for, Bial, Eisai, Novartis, Desitin, and UCB. He also received support from the DFG (Deutsche Forschungsgemeinschaft). Jerome Engel, Jr., receives support from the Jonathan Sinay Chair. Lars Forsgren has received support from, and/or has served as a paid consultant for, GSK, UCB, Eisai, and Orion Pharma. Jacqueline A. French has received support from, and/or has served as a paid consultant via the Epilepsy Study Consortium or the HEP project for, Eisai Medical Research, GlaxoSmithKline, Impax, Johnson & Johnson, Mapp Pharmaceuticals, Novartis, Lundbeck, Pfizer, Sepracor, Sunovion, SK Life Science, Supernus Pharmaceuticals, UCB Inc./Schwarz Pharma, Upsher Smith, Vertex, Eisai Medical Research, LCGH, Impax, Mapp Pharmaceuticals, Novartis, UCB, UCB Inc/Schwarz Pharma, Upsher Smith, and Lundbeck. Dale C. Hesdorffer has received support from, and/or has served as a paid consultant for, UCB, Eisai, and UpsherSmith. B.-I. Lee has received support from UCB and GlaxoSmithKline and has done consulting for UCB. Solomon L. Moshé has received support from the Charles Frost Chair In Neurosurgery and Neurology, and has served as a paid consultant for Lundbeck and UCB. Emilio Perucca has received support from, and/or has served as a paid consultant for, Bial, Eisai, GlaxoSmithKline, Lundbeck, Medichem, Pfizer, Sun Pharma, Supernus, UCB Pharma, Viropharma, and Vertex. Ingrid E. Scheffer has received support from, and/or has served as a paid consultant for, UCB, Athena Diagnostics, GlaxoSmithKline, and Janssen-Cilag EMEA. Torbjörn Tomson has received support from, and/or has served as a paid consultant for, GlaxoSmithKline, UCB, Eisai, Sun Pharma, and Bial. S. Wiebe has received support from, and/or has served as a paid consultant for, the Hopewell Professorship in Clinical Neurosciences Research, University of Calgary, and ElectroCore. The remaining authors have no potential conflicts of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Notes

I. Specifying a level of risk for recurrence to quantify the concept of "enduring predisposition" was difficult for the Task Force. All agreed that an individual with two unprovoked seizures had epilepsy. The risk for a third seizure in such an individual is about 3 in 4, but the 95% confidence intervals are about 60–90%. Therefore, the Task Force agreed that an individual having a similar risk after one unprovoked seizure should logically be considered also to have epilepsy. The number "60%" is intended to be an approximate guideline, rather than a sharp cutoff.

II. Some suggested a time limit within which the two spontaneous seizures must occur to diagnose epilepsy. In the absence of consensus and evidence on which to base a specific time, lifetime occurrence was retained as the default.

III. The motivation for this aspect of the definition was twofold. First, many clinicians, patients, and families consider epilepsy to be in the past when seizures no longer occur and no antiseizure medications are employed. Second, the Task Force desired to remove lasting stigma associated with a lifetime diagnosis of epilepsy. Other terms considered included remission, terminal remission, complete remission, inactive epilepsy, epilepsy absent, epilepsy not present, epilepsy no longer present, and cure. Many of these did not convey the concept that epilepsy was gone. Cure implied complete success of some treatment or passage of time, such that risk was that of the baseline population.

IV. Evidence to guide a specific required seizure-free number of years is limited, and existing risk functions show a continuous decline over time, rather than a natural breakpoint. Some argued for 5 years, but as many as 5% annually may have a seizure after a 5-year seizure-free interval. Being seizure-free for the most recent 10 years and off medications for the most recent 5 years predicts future freedom from seizures in a high percentage of cases.

V. Although evidence exists for a (low) relapse rate after 5 years of seizure freedom, no evidence was available at time of writing for relapse rates after being seizure-free for 10 years, which therefore was selected to be a time longer than 5 years, for which relapse rate would be consider likely very low.

VI. Whether to define a condition called "probable epilepsy," "possible epilepsy," or both, generated the most debate in the deliberations, and ultimately the issue was settled by majority view rather than full consensus. Probable epilepsy was considered for two different circumstances: the first in which one seizure had occurred and risks were high but not very high for having another. The second circumstance encompassed limited information in cases that seemed to be epilepsy, but reliable seizure descriptions or other key data were lacking. Allowing a diagnosis of probable epilepsy in the second circumstance could harmfully short-circuit necessary diagnostics to clarify the diagnosis. The Task Force did see value in defining probable epilepsy, but believed that extensive future consideration would be needed in order to make its definition operationally consistent and useful.

VII. An earlier draft of the manuscript was posted for comments on the ILAE website. A total of 315 comments, some very extensive, were received. The majority of opinions were positive, but there also were some very thoughtful and strongly felt disagreements. It was considered

unreasonable to place a burden on a treating physician for knowing the precise risk for a subsequent seizure. The authors agreed with this criticism. Many commenters were for and many others against calling epilepsy a disease, rather than a disorder. This decision was commandeered by the respective IBE and ILAE Executive Committees in favor of the term "disease." The phrase "no longer present" was not embraced by those responding to comments, and it was changed to "resolved." Many commenters wished for epilepsy to be resolved at 5 years of seizure freedom, on or off antiseizure drugs. The Task Force wanted resolved to mean a risk sufficiently low that epilepsy could be put aside, and achieving that requires a more stringent time interval, so we settled on 10 years of seizure freedom, 5 years off medicines. Several commenters wanted to eliminate the slippery concept of provoked versus unprovoked seizures. Such a change would have been quite fundamental, altering our view of acute symptomatic seizures, now comprising 40% of all seizures. We left that discussion for another venue. In general, the authors believed that the "wisdom of the crowd" strengthened and clarified the arguments and, more importantly, moved the definition closer to how working clinicians think of epilepsy.

VIII. These examples were presented on June 24, 2013, to the audience of the ILAE Congress Presidential symposium, with >1,000 epileptologists in attendance. Audience votes on whether epilepsy was present in these cases correlated very strongly with the terms of the revised definition. Although not a scientifically valid survey, the responses indicated that epileptologists thought of epilepsy in ways consistent with the revised definition.

References

1. Fisher RS, van Emde Boas W, Blume W, et al. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia* 2005;46:470–472.
2. Hauser WA, Annegers JF, Kurland LT. Prevalence of epilepsy in Rochester, Minnesota: 1940–1980. *Epilepsia* 1991;32:429–445.
3. Villanueva V, Sanchez-Alvarez JC, Pena P, et al. Treatment initiation in epilepsy: an expert consensus in Spain. *Epilepsy Behav* 2010;19:332–342.
4. Berg AT, Shinnar S. The risk of seizure recurrence following a first unprovoked seizure: a quantitative review. *Neurology* 1991;41:965–972.
5. Hauser WA, Rich SS, Lee JR, et al. Risk of recurrent seizures after two unprovoked seizures. *N Engl J Med* 1998;338:429–434.
6. Hesdorffer DC, Benn EK, Cascino GD, et al. Is a first acute symptomatic seizure epilepsy? Mortality and risk for recurrent seizure. *Epilepsia* 2009;50:1102–1108.
7. Beghi E, Carpio A, Forsgren L, et al. Recommendation for a definition of acute symptomatic seizure. *Epilepsia* 2010;51:671–675.
8. Harding G. The reflex epilepsies with emphasis on photosensitive epilepsy. *Suppl Clin Neurophysiol* 2000;57:433–438.
9. Wilden JA, Cohen-Gadol AA. Evaluation of first nonfebrile seizures. *Am Fam Physician* 2012;86:334–340.
10. Stroink H, Brouwer OF, Arts WF, et al. The first unprovoked, untreated seizure in childhood: a hospital based study of the accuracy of the diagnosis, rate of recurrence, and long term outcome after recurrence. Dutch study of epilepsy in childhood. *J Neurol Neurosurg Psychiatry* 1998;64:595–600.
11. Camfield P, Camfield C. Unprovoked status epilepticus, the prognosis for otherwise normal children with focal epilepsy. *Pediatrics* 2012;130:e501–e506.
12. Shinnar S, Berg AT, Moshe SL, et al. Risk of seizure recurrence following a first unprovoked seizure in childhood: a prospective study. *Pediatrics* 1990;85:1076–1085.
13. Kim LG, Johnson TL, Marson AG, et al. Prediction of risk of seizure recurrence after a single seizure and early epilepsy: further results from the MESS trial. *Lancet Neurol* 2006;5:317–322.
14. Hart YM, Sander JW, Johnson AL, et al. National General Practice Study of Epilepsy: recurrence after a first seizure. *Lancet* 1990;336:1271–1274.
15. Berg AT, Berkovic SF, Brodie MJ, et al. (2010) Revised terminology and concepts for organization of seizures and epilepsies: Report of the ILAE Commission on Classification and Terminology, 2005–2009. *Epilepsia* 2010; 51:676–685.
16. Sinclair DB1, Snyder TJ. Corticosteroids for the treatment of Landau-Kleffner syndrome and continuous spike-wave discharge during sleep. *Pediatr Neurol* 2005;32:300–306.
17. Neligan A, Hauser WA, Sander JW. The epidemiology of the epilepsies. *Handb Clin Neurol* 2012;107:113–133.
18. Rajna P, Selyom A. Oligoepilepsy, a real entity or the benign form of epileptic disorder?. *Ideggyogy Sz* 2011;64:344–349.
19. Geithner J, Schneider F, Wang Z, et al. Predictors for long-term seizure outcome in juvenile myoclonic epilepsy: 25–63 years of follow-up. *Epilepsia* 2012;53:1379–1386.
20. Rowland NC, Englot DJ, Cage TA, et al. A meta-analysis of predictors of seizure freedom in the surgical management of focal cortical dysplasia. *J Neurosurg* 2012;116:1035–1041.
21. Kim W, Stramotas S, Choy W, et al. Prognostic factors for post-operative seizure outcomes after cavernous malformation treatment. *J Clin Neurosci* 2011;18:877–880.
22. Berg AT, Testa FM, Levy SR. Complete remission in nonsyndromic childhood-onset epilepsy. *Ann Neurol* 2011;70:566–573.
23. Goellner E, Bianchin MM, Burneo JG, et al. Timing of early and late seizure recurrence after temporal lobe epilepsy surgery. *Epilepsia* 2013;54:1933–1941.
24. Buckingham SE, Chervoneva I, Sharan A, et al. Latency to first seizure after temporal lobectomy predicts long-term outcome. *Epilepsia* 2010;51:1987–1993.
25. Lindsten H, Stenlund H, Forsgren L. Remission of seizures in a population-based adult cohort with a newly diagnosed unprovoked epileptic seizure. *Epilepsia* 2001;42:1025–1030.
26. Lossius MI, Hessen E, Mowinckel P, et al. Consequences of antiepileptic drug withdrawal: a randomized, double-blind study (Akershus Study). *Epilepsia* 2008;49:455–463.
27. Chadwick D, Taylor J, Johnson T. Outcomes after seizure recurrence in people with well-controlled epilepsy and the factors that influence it. The MRC Antiepileptic Drug Withdrawal Group. *Epilepsia* 1996;37:1043–1050.
28. Monteiro L, Coelho T, Stocker A. Neurocysticercosis--a review of 231 cases. *Infection* 1992;20:61–65.
29. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011;69:292–302.
30. Beghi E, Balzarini C, Boglion G, et al. Reliability of the El Escorial diagnostic criteria for amyotrophic lateral sclerosis. *Neuroepidemiology* 2002;21:265–270.
31. Silberstein S, Loder E, Diamond S, et al. Probable migraine in the United States: results of the American Migraine Prevalence and Prevention (AMPP) study. *Cephalalgia* 2007;27:220–229.
32. Tang WK, Chan SS, Chiu HF, et al. Impact of applying NINDS-AIREN criteria of probable vascular dementia to clinical and radiological characteristics of a stroke cohort with dementia. *Cerebrovasc Dis* 2004;18:98–103.

A Clinical Trial Assessing the Efficacy and Safety of a New Injectable Formula of Sodium Phenobarbital Containing No Additives for the Treatment of Neonatal Seizures

Kou KAWADA[*1], Susumu ITOH[*2], Yasushi ITANI[*3], Hiroyuki KITAJIMA[*3], Hiroyuki YOSHIO[*5], Kazuo ITABASHI[*6], Keiji GOISHI[*7], Yuichi KONDO[*8] and Tomoo NAKAMURA[*9]

[*1] Maternal and Perinatal Center, Facility of Medicine Kagawa University (presently, Kagawa National Children's Hospital), Kagawa, Japan
[*2] Department of Pediatrics, Facility of Medicine, Kagawa University, Kagawa, Japan
[*3] Department of Neonatal Medicine, Kanagawa Children's Medical Center, Kanagawa, Japan
[*4] Department of Neonatal Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan
[*5] Department of Pediatrics, National Hospital Organization Okayama Medical Center, Okayama, Japan
[*6] Department of Pediatrics, Showa University Hospital, Tokyo, Japan
[*7] Department of Pediatrics, The University of Tokyo Hospital, Tokyo, Japan
[*8] Department of Neonatal Care, Maternal and Perinatal Medical Center, Kumamoto Municipal Hospital, Kumamoto, Japan
[*9] Division of Neonatology, Department of Maternal and Perinatal Services, National Center for Child Health and Development, Tokyo, Japan

Aims : A new phenobarbital preparation for injection containing no additives (NOBELBAR®) was developed in Japan for safer use in neonates with seizures. This study was conducted to evaluate the clinical efficacy and safety of NOBELBAR® for the treatment of neonatal seizures.
Methods : This investigator-initiated clinical trial was an open-label, uncontrolled, multicenter study, conducted in compliance with the good clinical practice. Phenobarbital was initially administered intravenously at a loading dose of 20 mg/kg. An additional dose of 20 mg/kg was given if the seizures did not resolve. The primary efficacy was evaluated 30 minutes after the administration of the loading dose. Infants who responded to the loading administration received maintenance therapy with 2.5 to 5 mg/kg intravenous phenobarbital once daily for 6 days.
Results : NOBELBAR® was administered to 10 neonates with seizures. The primary efficacy was evaluated as complete response in all patients. Adverse events were reported in 9 of the 10 patients (90%), most of which were mild. Plasma phenobarbital concentrations ranged from 18.7 to 45.3 μg/mL.
Conclusion : The study demonstrated that NOBELBAR® was effective for the control of neonatal seizures, and plasma drug concentrations were maintained within the therapeutic range. Approved for manufacturing and marketing was obtained based on the results of this study and other relevant data.
(Clinical Trials. number, UMIN-C000000410, JMA-II A00002.)

Key words : phenobarbital, neonatal seizures, good clinical practice, investigator-initiated clinical trial

Introduction

A lyophilized product of phenobarbital sodium entirely free of additives was used in this study.

In Japan, no phenobarbital preparation for intravenous injection was available despite an increasing demand for such product in neonatal care. An intravenous phenobarbital was discontinued in the 1980's, and Japanese neonatologists had no choice but to give intramuscular phenobarbital preparation intravenously or use imported intravenous phenobarbital products. Furthermore, due to the instability of phenobarbital sodium in water, the available phenobarbital injection preparations contain organic sol-

Address for Correspondence : KAWADA K. Kagawa National Children's Hospital, 2693 Zentsuji, Zentsuji, Kagawa 765-8501, Japan
(Manuscript received June 7, 2010 ; revised December 19, 2010 and April 20, 2011 ; accepted April 21, 2011)

vents such as benzyl alcohol[1] and propylene glycol[2-4], which are often hazardous to premature and neonatal infants. Therefore, there is a strong demand for the development of a phenobarbital preparation for intravenous injection that is free of these additives.

The Japan Pediatric Society (JPS) has been appealing to the Ministry of Health, Labour and Welfare (MHLW) for the medical need of appropriate phenobarbital preparations for neonatal seizures. The JPS finally succeeded in negotiating with Norbelpharma Co., Ltd. develop a new additive-free phenobarbital intravenous solution. Nobelpharma Co., Ltd. offered to supply an investigational drug in response to the appeal. This clinical trial, which was an investigator-initiated clinical trial[5-7], was funded by the MHLW and organized by physicians with the aim to obtain regulatory approval of the new phenobarbital preparation.

Methods

Study Design and Patients

This clinical trial was supported by the clinical trial promotion program subsidized by the Japan Medical Association with the Ministry of Health, Labour and Welfare Research Grant. The grant numbers were CCT-A-1601, CCT-B-1605, CCT-C-1661-8. This trial was an open-label, uncontrolled, multicenter study, conducted in compliance with the good clinical practice (GCP). Eight hospitals were enrolled in this trial. The protocol of this study was reviewed and approved by the institutional review board of each study site as well as the relevant regulatory agency. The quality of the trial was controlled by monitoring, data management and an independent assessment committee.

This study was conducted from March 2006 to March 2007, and registered with clinical trials numbers of UMIN-C000000410 and JMA-II A00002.

A diagnosis of neonatal seizure was made by two or more pediatricians using the following criteria[8]: a) at least two episodes of unprovoked movement sequences; b) such movement sequences contained no paroxysms of ankle clonus or premature infant apnea; c) seizures consisted of spontaneous, tonic, myoclonic, focal or multifocal limb movements, or facial and eyelid twitching, sucking, and chewing movements; and d) all these movements being frequent and stereotyped.

To ensure the parents' understanding of the trial in making decisions for enrollment of their child who was acutely experiencing seizures, parents of the candidate infants were explained about the trial beforehand. The criteria for inclusion as candidates were as follows: a) abnormal movements suggestive of neonatal seizures; b) neonatal asphyxia with an Apgar score below 5 at 5 minutes post partum and a base deficit of 10 mmol/L or greater on admission; c) birth trauma; d) infection or anomaly of the central nervous system; and e) hypoglycemia or electrolyte abnormality with abnormal movements suggestive of neonatal seizures.

The candidate infants were further assessed for neonatal seizure. Neonatal seizure was defined as either one of the following: a) acute neonatal seizures in addition to any of the criteria a) to d) for candidate infants (listed above), or b) persistent abnormal movements indicative of neonatal seizures despite correction of hypoglycemia and electrolyte abnormalities in infants fulfilling the criterion e) for candidate infants.

Once the infants met the eligibility criteria for neonatal seizure, they were enrolled in the trial after written informed consent was obtained from the parents.

In the case that the parents were able to receive full explanation of the trial before treatment started, infants whose parents did not receive prior explanation could also be enrolled in the trial after written informed consent was obtained.

Procedures

The treatment consisted of loading administration and maintenance therapy. In the loading administration, the study drug was injected intravenously at a dose equivalent to 20 mg/kg of phenobarbital over 5 to 10 minutes. If neonatal seizures failed to resolve within 30 minutes after initiation of the dose, an additional 20 mg/kg dose was administered over 5 to 10 minutes. Responders (i.e., patients with an excellent or good response) to the loading administration received maintenance therapy with 2.5 to 5 mg/kg phenobarbital intravenously once daily, which was initiated approximately 24 hours after the loading administration or after the last loading dose in the case that any additional dose was given. If recurrence of seizures was observed before initiation of maintenance therapy, an additional dose of 5 to 20 mg/kg intravenous infusion over 5 to 10 minutes was allowed

Table Demographic characteristics of subjects and results of the primary efficacy evaluation

Case No.	Gender	Birth weight (g)	Gestational age	Age in days	Birth place	Cause of seizure*	Seizure type	Investigator's evaluation	IAC's evaluation
1	F	2,274	37w 6d	1	Out-of-hospital	Fetal distress*	Subtle	Excellent	Excellent
2	M	3,650	40w 2d	3	Out-of-hospital	Fetal distress*	Tonic, Clonic	Excellent	Excellent
3	M	2,880	39w 2d	2	Out-of-hospital	Unknown	Clonic	Excellent	Excellent
4	M	2,121	34w 5d	1	Out-of-hospital	Hypoxic ischemic encephalopathy	Myoclonic	Excellent	Excellent
5	M	1,144	33w 1d	4	Out-of-hospital	5p⁻ syndrome	Subtle	Excellent	NA
6	M	3,122	38w 1d	4	In-hospital	Hypocalcemia	Clonic	Excellent	Excellent
7	M	3,178	41w 1d	2	Out-of-hospital	Hypoxic ischemic encephalopathy	Subtle	Excellent	Excellent
8	M	3,530	39w 6d	2	Out-of-hospital	Unknown	Clonic, Subtle	Excellent	NA
9	F	2,982	38w 3d	1	Out-of-hospital	Neonatal cerebral infarction	Tonic, Clonic, Subtle	Good**	Good
10	M	3,796	39w 3d	5	In-hospital	Subarachnoid hemorrhage	Tonic, Subtle	Excellent	Excellent

* : Including suspected causes
** : Changed to excellent after additional dosing
IAC : Independent assessment committee
M : Male
F : Female
NA : not applicable

as long as the patient's plasma drug concentration was confirmed to be below 40 μg/mL.

Outcome Measures

The primary efficacy endpoint was clinical response evaluated 30 minutes after initiation of the dose, and was rated as excellent (seizures resolved), good (seizures reduced in severity or frequency to about half), or poor (seizures reduced in severity or frequency to less than about half). The evaluation was performed by two or more pediatricians. A secondary assessment of the diagnosis of neonatal seizures and clinical efficacy was conducted by an independent assessment committee composed of three members including pediatricians/pediatric neurologists, based on the video of each patient recorded for 30 minutes preceding the initial administration. For each patient, the frequency and type of neonatal seizures reported during the study were assessed at the completion of maintenance therapy. Furthermore, data for adverse events, vital signs, and laboratory tests including hematology and blood biochemistry were collected throughout the follow-up period of one week after the last dosing.

The plasma concentration of phenobarbital was determined 2 hours after initiation of the loading or additional dose at the same time as the evaluation of the primary endpoint, 2 hours after the first maintenance dose, and prior to the final dose of maintenance therapy, or at the time of discontinuation in the case of withdrawal from the study. The relationship between plasma drug concentration and efficacy was analyzed.

Results

The demographic characteristics of the subjects and results of efficacy evaluation are shown in Table.

A total of 10 subjects were enrolled in this clinical trial, with a mean postnatal age of 2.5 days (range : 1

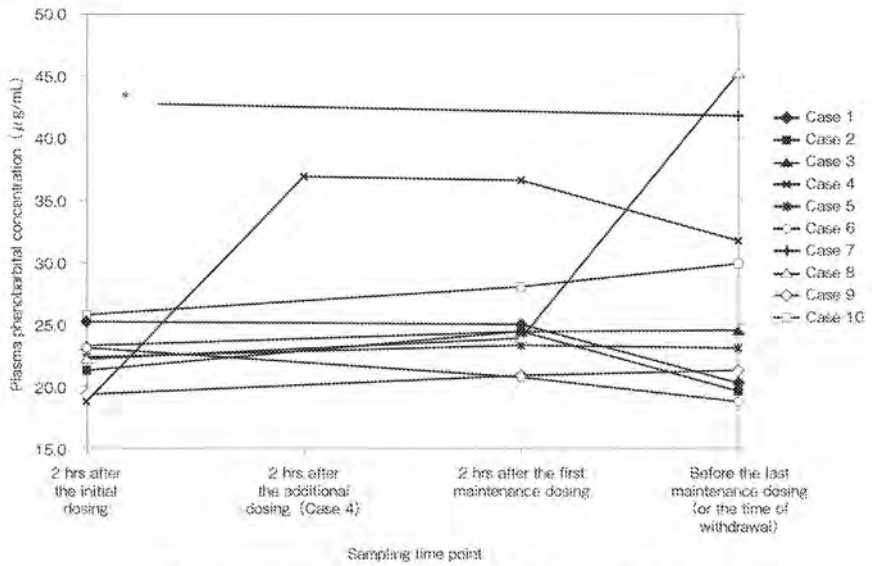


Fig. Plasma concentrations of phenobarbital over time in 10 patients
Plasma phenobarbital concentrations ranged from 20 to 25 μg/mL after the initial loading administration and were approximately 40 μg/mL following additional dosing.
The plasma drug concentrations were maintained at 18 to 32 μg/mL during maintenance therapy in all but one patient.
*2 hrs after the additional dosing (Case 7)

to 5 days), mean birth weight of 2,867 g (range : 1,144 to 3,796 g), and mean gestational age of 38.2 weeks (range : 33.1 to 41.1 weeks). All subjects were confirmed to have neonatal seizures by the independent assessment committee. The results of diagnosis of neonatal seizures provided by the independent assessment committee were consistent with those obtained by the investigators.

Seizures were resolved after administering the 20 mg/kg loading dose in 9 of the 10 patients, and after an additional loading dose (20 mg/kg) in the remaining 1 patient. Therefore, the clinical response evaluated 30 minutes after the loading administration including any additional dosing was rated as excellent in all patients by the investigators on site. Evaluation by the independent assessment committee yielded an excellent response at 30 minutes after the initial administration in 7 patients and good response in 1 patient. The response was not assessable in the remaining 2 patients due to blurred images of the videos. Maintenance therapy was given to all patients, except one (Case 7) who had received pentobarbital, which violated the protocol. One patient (Case 4) received an additional phenobarbital dose of 20 mg/kg for recurrence of seizures before entering the maintenance phase. Eight of 9 patients who started maintenance therapy completed the maintenance therapy. One (Case 9) of nine patients who had received pentobarbital, which violated the protocol. Seizure recurrence was noted in 1 patient during the period of maintenance therapy, and this patient received an additional 20 mg/kg dose.

Plasma phenobarbital concentrations over time for each patient are shown in Figure. The plasma concentrations of the drug were within the range of 20 to 25 μg/mL in all patients with the exception of 2 patients (Cases 4 and 7) who received an additional phenobarbital dose. The plasma phenobarbital concentrations in the 2 patients receiving an additional loading dose were nearly twice as high as those observed after the initial dosing. One patient (Case 8) had a plasma phenobarbital concentration of 45.3 μg/mL before the final maintenance dosing. The plasma drug concentrations were 22.2 μg/mL two hours after the initial dosing and 23.9 μg/mL two hours after the first maintenance dosing. This patient

received an initial loading dose of phenobarbital at 19.8 mg/kg and then maintenance therapy at 5.0 mg/kg/day for 3 days, reducing to 2.5 mg/kg/day thereafter. Excluding this patient, only the patients given an additional loading dose had plasma drug concentrations exceeding 30 μg/mL during the maintenance treatment.

During the study, adverse events were reported in 9 of the 10 patients. Adverse events included respiratory depression in 4 patients (Cases 1, 2, 8 and 9), and bradycardia (Cases 8 and 9), eczema (Cases 1 and 8) and decreased oxygen saturation (Cases 2 and 3) in 2 patients each. Adverse drug reactions were reported in 6 of the 10 patients, including respiratory depression (Cases 1, 2 and 9) in 3 patients, decreased oxygen saturation (Cases 2 and 3) in 2 patients, and bradycardia (Case 9), increased bronchial secretion (Case 7), hypotension (Case 4) and decreased urine volume (Case 3) in 1 patent each.

Most adverse events were mild, except one case each of moderate respiratory depression (Case 2), moderate renal failure (Case 5) and severe patent ductus arteriosus (Case 5). Of these adverse events, only respiratory depression was considered to be related to the study drug. No adverse event was related to high plasma drug concentrations.

The changes in vital signs from baseline were calculated at each assessment point, and there was a decrease in systolic blood pressure 1 hour after the initial loading dose (mean ± SD, −8.8 ± 10.1 mmHg; $p<0.1$). Systolic blood pressure (mean ± SD) before the initial loading dose was 70.1 ± 14.9 mmHg.

No hematological or blood biochemical abnormalities attributable to the administration of the study drug were noted in the present series.

Discussion

This study was performed to evaluate the new injectable phenobarbital preparation containing no additives for the treatment of neonatal seizures.

Since this was an open-label, uncontrolled study, an independent assessment committee was organized to ensure standardized application of the evaluation criteria among study sites. The committee independently assessed the diagnosis of neonatal seizures and the efficacy of the study drug, and the results were compared with those evaluated by each investigator. The primary efficacy evaluation performed by the investigators was found to be consistent with those by the independent assessment committee, corroborating the efficacy of the study drug.

After the loading dose of 20 mg/kg, plasma phenobarbital concentration rapidly reached the therapeutic range of 20 to 25 μg/mL. These levels were consistent with previous reported data[9,10]. The maintenance phenobarbital therapy at 2.5 to 5 mg/kg/day also resulted in stable and sustained therapeutic plasma drug concentrations. One patient (Case 8) had a high plasma drug concentration of 45.3 μg/mL prior to the final dose of maintenance therapy, which was very different from those observed in the remaining patients. It remains unknown why the plasma drug level increased before the final dose of maintenance therapy in this patient, but a low clearance and a protracted elimination half-life of phenobarbital in newborns at the early postnatal stage[11] could explain the elevation, particularly since the maintenance therapy was started at a relatively high dose in this patient. A previous study has shown that maintenance therapy with phenobarbital at 5 mg/kg/day may result in an increased plasma drug level of approximately 40 μg/mL[12].

Adverse drug reactions such as respiratory depression and decreased oxygen saturation were observed in 6 of the 10 patients. Most of these reactions were mild. The adverse reactions observed in this study have already been shown to be associated with the use of phenobarbital. Decreased systolic blood pressure was noted 1 hour after the initial loading dose. The cause of decreased blood pressure was obscure, because it was difficult to distinguish between resolution of convulsion and direct drug action. These findings suggest a need to monitor the neonates' condition carefully for any respiratory depression or decreased blood pressure after intravenous phenobarbital administration.

The adverse event profile observed in the patient with high plasma drug concentration did not differ from those seen in other patients: the elevated plasma drug concentration appeared to have no influence on the adverse event profile within the range of plasma drug concentrations observed in this study (18.7 to 45.3 μg/mL).

The above results thus indicate that the new phenobarbital preparation for injection (NOBELBAR®) is effective for the treatment of neonatal seizures at the dosage examined in the present trial. In addition, there is no particular safety concern except predicta-

ble adverse reactions related to the known pharmacological effects of the drug.

The phenobarbital preparation was approved for manufacturing and marketing based on the results of this study and other relevant data. The product has been marketed for the treatment of neonatal seizures and status epilepticus since December 2008 in Japan under the brand name NOBELBAR® 250 mg for injection. Post-marketing surveillance is currently ongoing to further confirm the safety of this solution.

This is the first investigator-initiated GCP-compliant clinical trial performed in the NICU emergency setting in this country. Although the number of patients was small, the study was conducted using the same standard as industry-sponsored clinical trials. There were two major protocol violations (use of pentobarbital). Apart from that, there were only minor protocol violations. Using the data, the drug was approved in Japan. We hope that other new preparations will be approved with based on investigator-initiated clinical trials in Japan.

Conflict of Interest

There is no conflict of interest in conduct of the present clinical trial and release of the results.

Acknowledgments

The authors would like to express their heartfelt gratitude to Dr. K. Hayashi, Department of Pediatrics, Tokyo Women's Medical University ; Dr. H. Yamamoto, Department of Pediatrics, St. Marianna University School of Medicine ; Dr. H. Nakamura and Dr. N. Tsuchida, National Center for Child Health and Development ; Dr. S. Hirano, Osaka Prefectural General Medical Center for Maternal and Child Health ; Dr. S. Ito, Department of Clinical Pharmacology, Juntendo University School of Medicine ; Dr. M. Takeuchi, the Clinical Statistics Division, Kitasato University Faculty of Pharmacy ; clinical trial staff and clinical trial secretary at the individual study sites ; and the Japan Medical Association Center for Clinical Trials for their cooperation in the conduct of this clinical study, and to Nobelpharma Co. Ltd. for the supply of the investigational product for this study.

References

1) Gershanik J, Boecler B, Ensley H, McCloskey S, George W. The gasping syndrome and benzyl alcohol poisoning. *N Engl J Med*. 1982 ; **307**(22) : 1384-8.
2) Demey H, Daelemans R, De Broe ME, Bossaert L. Propyleneglycol intoxication due to intravenous nitroglycerin. *Lancet*. 1984 ; **1**(8390) : 1360.
3) Glasgow AM, Beeckx RL, Miller MK, MacDonald MG, August GP, Goodman SI. Hyperosmolality in small infants due to propylene glycol. *Pediatrics*. 1983 ; **72**(3) : 353-5.
4) Murch S, Costeloe K. Hyperosmolality related to propylene glycol in an infant. *BMJ*. 1990 ; **301**(6748) : 389.
5) Nakamura H, Ono S. Part III Regulatory guidelines for pediatric drug development : stimulation of pediatric drug research by regulatory authorities 13. Japanese Perseprtive. Mulberg AE, Silber SA, van den Anker JN (Eds). *Pediatric Drug Development*. NJ : Wiley-Blackwell, 2009 ; 153-64.
6) Nakamura H, Ozaki M. Facilitation of Pediatric Research in Japan. Rose K, van den Anker JN (Eds). *Guide to Paediatric Drug Development and Clinical Research*. Basel : Karger, 2010 ; 15-22.
7) http://www.jmacct.med.or.jp/english/whatwedo/investigator/investigator.html
8) Gilman JT, Gal P, Duchowny MS, Weaver RL, Ransom JL. Rapid sequential phenobarbital treatment of neonatal seizures. *Pediatrics*. 1989 ; **83**(5) : 674-8.
9) Lockman LA, Kriel R, Zaske D, Thompson T, Virnig N. Phenobarbital dosage for control of neonatal seizures. *Neurology*. 1979 ; **29**(11) : 1445-9.
10) Gal P, Toback J, Boer HR, Erkaa NV, Wells TJ. Efficacy of phenobarbital monotherapy in treatment of neonatal seizures--relationship to blood levels. *Neurology*. 1982 ; **32**(12) : 1401-4.
11) Heimann G, Gladtke E. Pharmacokinetics of phenobarbital in childhood. *Eur J Clin Pharmacol*. 1977 ; **12**(4) : 305-10.
12) Painter MJ, Pippenger C, MacDonald H, Pitlick W. Phenobarbital and diphenylhydantoin levels in neonates with seizures. *J Pediatr*. 1978 ; **92**(2) : 315-9.

Eur. J. Biochem. 203, 655–661 (1992)
© FEBS 1992

Modifications of hepatic alpha-1-acid glycoprotein and albumin gene expression in rats treated with phenobarbital

Odile BERTAUX[1], Thierry FOURNIER[1], Laurence CHAUVELOT-MOACHON[2], Dominique PORQUET[1], Richard VALENCIA[1] and Geneviève DURAND[1]

[1] Département de Biochimie Générale, UFR des Sciences Pharmaceutiques et Biologiques, Université Paris-Sud, France
[2] Laboratoire Central de Pharmacologie Clinique, CNRS URA 595, Hôpital Cochin, Paris, France

(Received July 18/October 3, 1991) – EJB 91 0944

The serum level of α1-acid glycoprotein (α1-AGP) is significantly increased in various animal species by treatment with cytokines, glucocorticoids and phenobarbital. The mechanisms responsible for the cytokine-induced and glucocorticoid-induced increases are now well documented, but not so in the case of phenobarbital. The main purpose of this study was to assess whether phenobarbital acts on α1-AGP synthesis in the liver at the transcriptional or translational level. Male Dark Agouti rats received 70 mg phenobarbital/kg daily for 7 days. The analysis of total hepatic RNA showed that a single injection of phenobarbital induced an 11-fold increase in phenobarbital-dependent cytochrome *P*450IIB mRNA, whereas seven injections of phenobarbital were required to induce a maximum 5.5-fold increase in α1-AGP mRNA. Concurrently, the transcription rate of the α1-AGP gene rose 3.5-fold. Hepatocytes isolated after the seventh injection of phenobarbital showed a threefold increased capacity to secrete α1-AGP, corresponding to a 3.2-fold increased α1-AGP mRNA content in the liver. In conditions in which its effect on the induction of α1-AGP synthesis was maximum, phenobarbital caused a 30% reduction in liver albumin mRNA and in albumin secretion by isolated hepatocytes, resulting from a 60–70% reduction in the rate of transcription of the albumin gene measured in isolated nuclei. We conclude that the effect of phenobarbital on α1-AGP and albumin gene expression occurs at the transcriptional rather than the translational level.

Alpha1-acid glycoprotein (α1-AGP) or orosomucoid, is one of the major acute-phase reactants in man, mice and rats, and its serum concentration increases several-fold in response to inflammation and infection [1]. Conversely, the serum level of negative acute-phase proteins such as albumin decreases slightly. These changes in serum protein concentrations have been correlated with a sharp increase in α1-AGP mRNA and a slight decrease in albumin mRNA in the liver [2, 3]. Birch and Schreiber [4] have shown that the increase in α1-AGP mRNA is regulated at both the transcriptional and post-transcriptional levels.

The cytokines involved in the hepatic acute-phase reaction can directly stimulate the hepatic synthesis of some acute-phase proteins, particularly α1-AGP, both *in vivo* and *in vitro*; however, contrary to turpentine treatment, they are not able to elicit a full-scale hepatic acute response. The addition of interleukin 1 (IL1) or interleukin 6 (IL6) to primary rat hepatocyte cultures [5, 6], IL1 to cultures of rat hepatoma cells [7, 8], IL1, IL6 or tumor necrosis factor α, to human hepatoma cells [5, 9, 10] and IL1 or IL6 to primary cultures of mouse hepatocytes [11] enhances both the secretion of α1-AGP and the level of the corresponding cellular mRNA. A similar enhancement has been found after treatment of rats with IL6 [12], IL1 [13–16] and tumor necrosis factor α [17]. *In vivo*, IL1 appears to be a more potent inducer of α1-AGP than IL6, in terms of the maximal effect on serum concentrations. As in inflammatory reactions, a decrease in serum albumin and liver albumin mRNA levels also occurs after treatment of rats with IL6 [12] and after addition of IL6 to primary cultures of rat hepatocytes [18]; IL1, IL6 and tumor necrosis factor α have a similar effect on cultured rat hepatoma cells [19].

In the same way, glucocorticoids elicit an increase in α1-AGP mRNA both in primary rat hepatocyte cultures and in the rat hepatoma cell line (Hepatoma Tissue Culture) [20, 21]. A similar increase has been observed in rats [22], associated with an increase in liver albumin mRNA levels [23]. The accumulation of α1-AGP mRNA is modulated at the transcriptional [24] and post-transcriptional levels by deadenylation of mRNA in the nucleus [25] or the cytoplasm [21].

It has been shown that the repeated administration of phenobarbital also increases α1-AGP serum levels in dogs [26] and rats [27–29]. Sensitivity is strain-related in the rat, the Dark Agouti strain showing the highest increase [30]. This high-responder status characteristic of the Dark Agouti strain does not seem to be due to a particular sensitivity to enzyme-

Correspondence to O. Bertaux, Laboratoire de Biochimie, UFR des Sciences Pharmaceutiques et Biologiques, Tour D4, 3ème étage, 5 rue Jean-Baptiste Clément, F-92296 Chatenay Malabry Cédex, France

Abbreviations. α1-AGP, α1-acid glycoprotein; GAPDH, glyceraldehyde phosphate dehydrogenase; IL1, interleukin 1; IL6, interleukin 6.

inducing drugs. Basal levels of cytochrome *P*450 phenobarbital isoenzymes are similar in male Dark Agouti and Sprague Dawley rats [31], and the overall hepatic cytochrome *P*450 content is of the same order of magnitude in Dark Agouti rats and other commonly used rat strains, both at the basal status [32, 33] and after phenobarbital induction [30]. In the light of the high sensitivity of Dark Agouti rats to phenobarbital, Chauvelot-Moachon et al. [30] suggested that this strain should be used to study the effect of cytochrome *P*450 inducers on α1-AGP metabolism.

In order to determine the mechanism of action of phenobarbital on α1-AGP and albumin gene expression, we studied the kinetics of variations of hepatic α1-AGP and albumin mRNA, together with changes in the transcription rates of the corresponding genes. We also studied modifications in the synthesis of these proteins by isolated rat hepatocytes after a 7-day treatment with phenobarbital *in vivo*.

MATERIALS AND METHODS

Treatment of animals

Male Dark Agouti rats (CNRS, Orléans, France), aged 9 – 11 weeks and 210 – 280 g, were housed at 21 – 24 °C under a 14-h – 10-h light-dark cycle with laboratory chow (UAR) and tap water *ad libitum*. Lyophilized sodium phenobarbital (Gardenal for subcutaneous or intramuscular injection, Specia, Paris, France) was dissolved in apyrogenic sterile normal saline and injected subcutaneously in the interscapulum area daily for 1, 3 or 7 days. Each morning, the rats received 75 mg/kg phenobarbital in a volume of 10 ml/kg, while control animals received the solvent alone. After decapitation, 500-mg liver specimens were collected for mRNA analysis according to the following schedule: one injection and sacrifice 12 h later, three injections and sacrifice 12 h later, seven injections and sacrifice 12 h, 1, 2, 3, 5, 7 or 10 days later. Control rats were sacrificed 12 h after the seventh injection of solvent. Some rats received a single subcutaneous injection of 1 ml turpentine to induce a maximal inflammatory response and liver specimens were collected 48 h later. The liver specimens for RNA extraction were immediately frozen in sterile tubes maintained for a few minutes in a dry-ice/ethanol bath and stored at −80 °C until use. Part of the fresh liver (≈ 5 g) was used for the isolation of rat liver nuclei.

The rate of albumin and α1-AGP secretion was evaluated in the culture supernatants of hepatocytes isolated from control and treated rats sacrificed 24 h after the seventh injection of NaCl or phenobarbital and 48 h after the single injection of turpentine.

Isolation of RNA and Northern blot analysis

Total cellular RNA was extracted from frozen pieces of rat liver by the guanidine isothiocyanate/cesium chloride method [34] using a Beckman SW 55 rotor for 16 h at 30000 rpm. Heat-denatured RNA samples were run as previously described [35] in 1.2% agarose gels containing 2.2 M formaldehyde for 16 h at 2 V/cm. RNA was blotted onto Biodyne A nylon membranes (Pall Ultrafine Filtration Corporation) by Northern capillary transfer for at least 48 h. After transfer, the filters were air-dried and baked at 80 °C for 2 h, then prehybridized for at least 3 h at 42 °C in 50% formamide, 5 × NaCl/Cit (750 mM NaCl, 75 mM citrate), 5 × Denhardt's solution (1 g/l Ficoll 400, 1 g/l bovine serum albumin, 1 g/l polyvinyl pyrrolidone), 0.1% SDS, 50 mM sodium phosphate, pH 6.5 and 250 μg/ml denatured herring sperm DNA. Recombinant plasmids containing inserts encoding rat α1-AGP or albumin mRNA were used to probe Northern blots. The RR1 strain of *Escherichia coli* containing the α1-AGP recombinant plasmid was obtained from Taylor [3]; the two strains of *E. coli* containing the 3′ and 5′ ends of the rat albumin gene were cloned in Sala-Trepat's laboratory [36], while the insert encoding rat cytochrome *P*450IIB (Nebert et al. [37]) mRNA was obtained from Gonzalez [38]. Plasmid DNA containing the α1-AGP and albumin inserts, and the purified cytochrome *P*450IIB insert were labelled by nick-translation using [α-^{32}P]dCTP. The specific activity of the plasmid DNA was about 10^8 cpm/μg and 3×10^8 cpm/μg for the insert. Hybridization proceeded for 48 h at 42 °C under the same conditions as for prehybridization. Filters were washed under high stringency using the Milliblot-Blot processor system (Millipore) at room temperature with 1 l 2 × NaCl/Cit (300 mM NaCl, 30 mM citrate) and 0.1% SDS, then at 50 °C with 1 l 0.1 × NaCl/Cit (15 mM NaCl, 1.5 mM citrate) and 0.1% SDS. The wet filters enclosed in Saran film were exposed to hyperfilm MP (Amersham) at −80 °C using Lightning Plus intensifying screens (Dupont Cronex).

Dot-blot analysis

mRNA was quantified by dot-blot analysis using a Biodot apparatus (BioRad). Using serial half-dilutions, 20 μg – 156 ng total cellular RNA was spotted onto Hybond N$^+$ nylon membranes (Amersham) which were processed as recommended by the membrane supplier and under the same conditions as for Northern blots. Autoradiographies were scanned by means of photometric densitometry (Sebia) and results were expressed as variations relative to control rats for 1 μg total cellular RNA.

Isolation of nuclei

4 – 5 g fresh liver was used to isolate nuclei according to the procedure first described by Blobel and Potter [39] and modified as follows: the whole liver was quickly washed with ice-cold phosphate-buffered saline (0.2 g/l KCl, 0.2 g/l KH_2PO_4, 8 g/l NaCl, 2.16 g/l Na_2HPO_4, 7 H_2O), blotted and weighed; a 5-g specimen was then minced and homogenized in 10 ml ice-cold RNase-free 0.3 M sucrose buffer containing 10 mM Tris/HCl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$ and 7.5 mM dithiothreitol, using a 45 ml sterilized Kontes glass homogenizer (Poly Labo) with a motor-driven Teflon pestle with 10 – 15 strokes at 0 °C and 1700 rpm. The homogenate was filtered through four layers of cheese-cloth and then centrifuged at 15000 g for 15 min at 4 °C. The crude nuclear pellet was carefully re-homogenized in 9 ml of the same 0.3 M sucrose buffer using a loose-fitting Dounce homogenizer (pestle A). Two volumes of 2.3 M sucrose, made up in the same buffer, were added to the suspension which was thoroughly mixed by multiple inversions. The suspension was then layered onto 0.8-ml cushions of 2.3 M sucrose made up in the same buffer, without dithiothreitol, in six Beckman SW 55 centrifuge tubes. After centrifugation for 30 min at 4 °C and 33000 rpm, the translucid pellets were pooled in 40% glycerol storage buffer containing 50 mM Tris/HCl, pH 8.3, 5 mM EDTA, 0.1 mM $MgCl_2$ and 10 mM dithiothreitol. The purified nuclei were washed twice with the storage buffer, counted and adjusted to 1.5×10^8 nuclei/ml before storing at −80 °C until use.

Nuclear run-on transcription assay

In vitro transcription assays were carried out essentially as described by Turcotte et al. [40] and Mezger et al. [41] with some modifications. Briefly, nuclei were thawed to 0°C and aliquots containing 3×10^7 nuclei were removed. After eliminating the storage buffer, nuclei were resuspended in 100 µl transcription solution containing 30 mM Tris/HCl, pH 8.0, 150 mM KCl, 2.5 mM $MgCl_2$, 1 mM $MnCl_2$, 0.05 mM EDTA, 2.5 mM dithiothreitol, 0.5 mM each of ATP, GTP and CTP, 100 µCi [α-^{32}P]UTP (800 Ci/mmol, Amersham), 250 U/ml human placental ribonuclease inhibitor (Sigma), 10 mM creatine phosphate, 50 µg/ml creatine phosphokinase, 20% glycerol and 17 U/ml heparin (Choay, 5000 U/ml).

Initiated transcripts were elongated at 26°C for 30 min, then 80 µg yeast tRNA, 10 mM $CaCl_2$ and 25 U RQ1 DNase (Promega Biotec) were added and the incubation was prolonged for 15 min at 26°C. The mixture was then adjusted to 5 mM EDTA and 1% SDS, and incubated at 37°C for 45 min in the presence of 100 ng proteinase K in a final volume of 200 µl. RNA was then extracted twice with 1 vol. phenol/chloroform/isoamyl alcohol (25:24:1) and once with 1 vol. chloroform/isoamyl alcohol (24:1) followed by ethanol precipitation overnight at −20°C. The nucleic acid pellet was redissolved in 250 µl DNase buffer, containing 40 mM Tris/HCl, pH 8.0, 10 mM NaCl, 10 mM $CaCl_2$, 6 mM $MgCl_2$ and 250 U/ml ribonuclease inhibitor, for digestion of the residual DNA with 25 U RQ1 DNase for 1 h at 25°C. The mixture was adjusted to 150 mM NaCl, extracted with phenol as above and RNA was precipitated with ethanol and washed three times. The final RNA pellet was dissolved in 1 ml hybridization buffer.

The recombinant plasmid containing a 1269-bp insert encoding rat glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA (from P. Jeanteur, CNRS URA 1192, Université Montpellier 2 [42]) was linearized by digestion with *Pst*I enzyme (4 U/µg, overnight at 37°C), while *Eco*R1 enzyme was used to linearize the pBR 322 plasmid DNA under the same conditions. The cDNA inserts of α1-AGP and albumin mRNA were amplified by means of the polymerase chain reaction using 20-nucleotide-long primers specific for the 5′ and 3′ regions. Primers were synthesized in the molecular genetics laboratory of B. Granchamps (INSERM U 120, CHU Bichat, Paris).

Hybridization solution contained 50% deionized formamide, 5× NaCl/Cit, 5× Denhardt's solution, 0.1% SDS, 50 mM NaH_2PO_4, pH 6.5, 250 µg/ml yeast tRNA and 100 µg/ml poly(A). Filters (4.0 × 1.7 cm) were prehybridized for 24 h at 42°C in 4 ml hybridization solution, then hybridized for 60 h at 42°C in 1 ml hybridization solution with 10^7 cpm ^{32}P-labelled nuclear RNA transcripts. The filters were washed three times at 20°C with 2× NaCl/Cit, 0.1% SDS, then at 68°C for 1 h with 0.1× NaCl/Cit, 0.1% SDS. Autoradiography of the wet filters was run for 4 days at −80°C. Densitometric scanning of the autoradiographs provided an estimate of the transcription rates relative to that of the GAPDH gene.

Preparation of isolated hepatocytes and quantification of protein secretion

Hepatocytes were isolated according to the procedure of Berry and Friend [43], as modified by Davy et al. [44]. To increase cell viability, the hepatocyte suspensions were incubated in collagen-coated plates at 37°C in a humidified atmosphere of CO_2/air (5:95 mol%), in Hepes buffer, pH 7.4 [45]. The medium was discarded 30 min later, viability checked and Eagle's minimum essential medium supplemented with 1× non-essential amino acids (Gibco), 10 mM lactate, 10 mM Hepes, pH 7.4, was added. Cells were further incubated for 4 h and aliquots of supernatant taken each hour for the determination of protein secretion. At the end of the incubation period, adherent cells were resuspended using collagenase. Viability (80–90%) and cell numbers ($8-12 \times 10^6$ cells) were determined using the trypan-blue exclusion test. Amounts of albumin and α1-AGP secreted by isolated hepatocytes were measured in the culture supernatants using sandwich-type immunoenzymatic methods [46]. Briefly, microplate wells were coated with anti-(α1-AGP) antibody or anti-albumin antibody. After incubation with the supernatants, a second purified antibody labelled with alkaline phosphatase was added. The relevation was obtained with the addition of 4-nitrophenylphosphate as substrate. Results were expressed as µg albumin or α1-AGP synthesized/10^6 hepatocytes.

RESULTS

Analysis and quantification of liver mRNA

The rat liver mRNA of cytochrome *P*450IIB, α1-AGP and albumin was examined specifically by hybridization of total cellular RNA with the corresponding cDNA probe. As seen in Fig. 1a, one injection of phenobarbital was sufficient to induce a maximal increase in cytochrome *P*450 mRNA (slot 3), while seven injections of phenobarbital were required to obtain the maximal increase in α1-AGP mRNA (Fig. 1b, slots 5–7). Cytochrome *P*450IIB mRNA remained at a high level throughout the 7-day administration period (Fig. 1a, slots 4 and 5) and during the 24 h following the last phenobarbital injection (slot 6). Values returned to normal at day 9 (slot 7), were below normal at days 10 and 12 (slots 8 and 9) and were barely detectable at day 14 (slot 10). It is interesting to note that cytochrome *P*450IIB mRNA was at the limit of detection in the livers of turpentine-treated rats (slot 12).

As shown in Fig. 1b, the increase in α1-AGP mRNA in the livers of phenobarbital-treated rats started only 7 days after the first phenobarbital injection (slot 5), reached a maximum 48 h later (slot 7) and decreased gradually to normal levels (slots 8–11). The three upper bands of α1-AGP premessenger mRNA followed the same changes as the mature mRNA. For comparison, slot 12 shows the accumulation of mature and premessenger α1-AGP mRNA in the liver of a turpentine-treated rat.

The seven injections of phenobarbital slightly decreased the amount of albumin mRNA (Fig. 1c). The minimum level was reached 12 h after the third injection of phenobarbital (slot 4) and remained unchanged 3 days after stopping the treatment (slots 5–8). Albumin mRNA returned to normal values 7–10 days later (slots 9–11). In comparison, after a single injection of turpentine, the hepatic albumin mRNA content was much more strongly decreased (slot 12).

Fig. 1d, e and f, shows dot blots of the RNA samples analyzed in Northern blots. Densitometric scanning of the dots revealed the pattern of variations of cytochrome *P*450IIB, α1-AGP and albumin mRNA (Fig. 2). Cytochrome *P*450IIB mRNA increased 11-fold 12 h after the first injection of phenobarbital, while in turpentine-injected rats it decreased 20-fold. α1-AGP mRNA increased 5.5-fold 48 h after the seventh injection of phenobarbital, while a 53-fold increase was

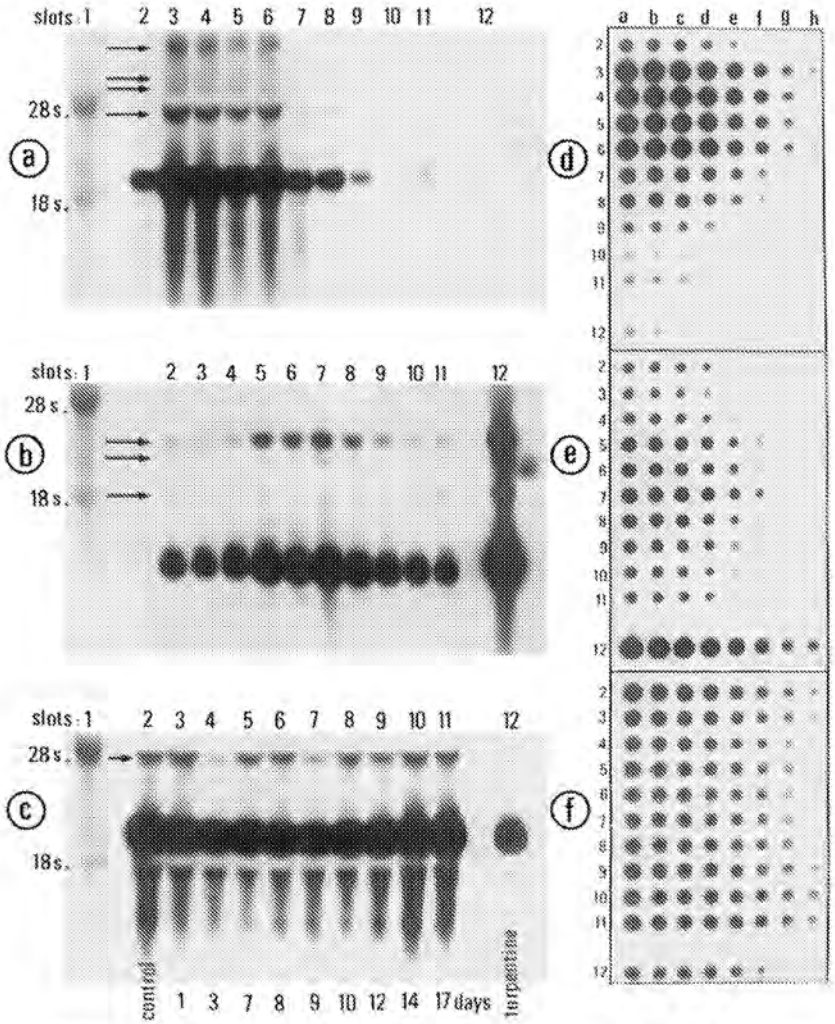


Fig. 1. Northern blot analysis and quantification of cytochrome *P*450IIB, α1-AGP and albumin mRNA in rat liver. Northern blot analysis of cytochrome *P*450IIB (a), α1-AGP (b) and albumin (c) mRNA in liver from control (slot 2), phenobarbital-treated rats (slots 3–11) and a turpentine-treated rat (slot 12). 20 μg total liver RNA was analyzed/slot. Slots 3, 4 and 5 correspond to the liver RNA of rats sacrificed 12 h after the first, third and seventh injection of phenobarbital, respectively. Slots 6 – 11 correspond to the liver RNA of rats sacrificed 1, 2, 3, 5, 7 and 10 days following the 7-day administration period, respectively. As in the Northern blot corresponding to α1-AGP (b), in addition to the major band of mature mRNA (≈ 850 bases), three minor bands of specific premessenger RNA (1850, 2650, 3500 bases, see arrows) are apparent. The molecular mass of these premessenger RNA molecules has been determined by Shiels et al. [25]. Significant amounts of the largest premessenger species (3500 bases) accumulated in rat liver 12 h after the seventh injection of phenobarbital (slot 5), attaining a maximum 2 days later (slot 7) and remaining at a higher level than in normal liver for up to 5 days following the phenobarbital administration period (slot 9). As in the Northern blot of cytochrome *P*450IIB (a), in addition to the major band of mature mRNA, the four upper bands (arrows) probably also correspond to premessenger RNA since they changed parallel to the variations in the mature mRNA. As in the Northern blot of albumin mRNA (c), the major upper band (arrow), which migrated slightly below the 28S ribosomal RNA, also represents albumin premessenger RNA molecules since its hybridization pattern followed the same variations as the mature mRNA. This is particularly clear for the turpentine-treated rats for which this upper band was strongly decreased (slot 12) relative to control rats (slot 2), as was the case for mature mRNA. Therefore, the quantification of mRNA by the dot-blot technique appeared to be accurate since all the signals detected corresponded to either premessenger or mature mRNA. Moreover, quantification of mature mRNA on the Northern blots and of total mRNA on the dot blots with different autoradiographic exposures always gave equivalent relative values. After staining of the Northern blot with methylene blue, rRNA 28 and 18S were used as molecular mass markers (slot 1). Quantitative dot-blot analysis of the mRNA of cytochrome *P*450IIB (d), α1-AGP (e) and albumin (f) from the 11 RNA samples analyzed in parts a – c. Columns a – h correspond to serial half-dilutions from 20 μg (a) – 156 ng (h). Mean integration values of dots giving proportional densities, calculated for 1 μg total cellular RNA, were as follows. Cytochrome *P*450IIB mRNA (d): 573 (2), 6310 (3), 6550 (4), 5254(5), 5754 (6), 1467 (7), 856 (8), 138 (9), 46 (10), 78 (11) and 19 (12). Values for α1-AGP mRNA (e): 150 (2), 105 (3), 194 (4), 616 (5), 501 (6), 1046 (7), 410 (8), 341 (9), 200 (10), 134 (11) and 4016 (12). Albumin mRNA (f): 836 (2), 808 (3), 558 (4), 636 (5), 592 (6), 643 (7), 803 (8), 890 (9), 1011 (10), 1074 (11) and 664 (12).

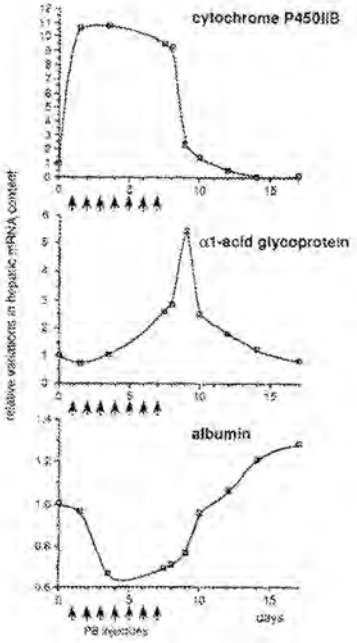


Fig. 2. Time course of variations in hepatic cytochrome *P450IIB*, α1-AGP and albumin mRNA during and after 7 days treatment with phenobarbital. Total RNA was extracted from rat livers over a period of 17 days. Control rats received 7 injections of 0.9% NaCl; control values are reported at day 0. Results are expressed as variations relative to control values. PB, phenobarbital.

observed in turpentine-treated rats. Albumin mRNA showed a maximum decrease of 35% 12 h after the third injection of phenobarbital, while a 65% decrease was observed after the single injection of turpentine.

Analysis of the *in vitro* transcription rates of α1-AGP and albumin genes

To determine the basis of the increase in hepatic α1-AGP mRNA and the associated decrease in albumin mRNA following phenobarbital administration to Dark Agouti rats, the relative changes in the transcription rates of these two genes were evaluated using nuclear run-on assays. As shown in Fig. 3, the transcription rate of the α1-AGP gene did not differ from controls (slot 1) 24 h after the last injection of phenobarbital (day 8, slot 2), but was 3.5-fold increased 24 h later (day 9, slot 3) and returned to normal values within 24 h (day 10, slot 4). The transcription rate of the albumin gene was reduced by 55% at day 8, 70% at day 9 and 60% at day 10 (slots 2, 3 and 4, respectively). For comparison, the transcription rate of the α1-AGP gene in an inflamed rat was increased 7-fold, while that of the albumin gene was reduced by 80% (slot 5). These values were corrected relative to the transcription rate of the GADPH gene used as internal standard [42]. No detectable signal was obtained with pBR322 plasmid DNA used as negative control.

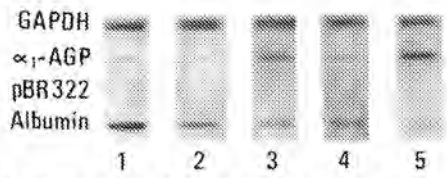


Fig. 3. Slot-blot assays of the radioactive nuclear transcripts from GAPDH, α1-AGP, pBR322 and albumin genes. Comparable amounts of newly transcribed RNA (10^7 cpm/ml) were hybridized against 10 μg/slot linearized GAPDH and pBR 322 plasmid DNA and against 3 μg/slot α1-AGP and albumin cDNA inserts immobilized on Hybond N° nylon membranes (Amersham) using a BioRad slot-blot apparatus. Transcription of GAPDH was used as internal reference [42], while plasmid pBR 322 served as a negative control. Densitometric scanning of the autoradiographies allowed the estimation of the relative transcription rate of the α1-AGP and albumin genes 24 h (slot 2), 48 h (slot 3) and 72 h (slot 4) after the 7-day phenobarbital administration period, relative to control (slot 1) and turpentine-treated rats 24 h after a single injection (slot 5). A 3.5-fold increase in the α1-AGP gene transcription rate was observed 48 h after the last injection of phenobarbital (slot 3), compared to a sevenfold increase in the turpentine-treated rats (slot 5). With regard to the albumin gene transcription rate, a 60–70% decrease occurred throughout (not shown) and after the 7-day phenobarbital treatment period (slots 2–4), compared to an 80% decrease in turpentine-treated rats (slot 5).

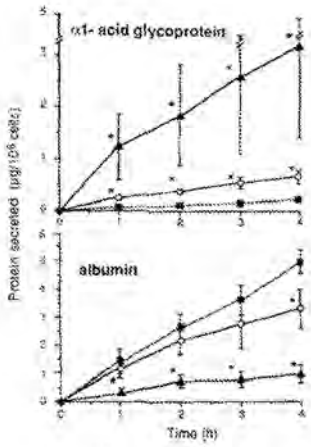


Fig. 4. Secretion of α1-AGP and albumin by hepatocytes isolated from rats 24 h after 7 injections of phenobarbital or 48 h after a single injection of turpentine. Aliquots of supernatant were taken each hour and protein synthesis was measured by enzyme immunoassay. Results are means ± SD of four controls (■——■), five phenobarbital-treated rats (○——○) and three turpentine-treated rats (▲——▲) and are expressed as μg protein secreted/10^6 hepatocytes. (*) $P < 0.05$ (relative to controls).

Synthesis of albumin and α1-AGP by isolated hepatocytes

Secretion of α1-AGP and albumin was determined by means of enzyme immunoassay. During the 4 h of incubation following the isolation of hepatocytes, both proteins accumulated in the culture medium linearly, indicating a constant rate of secretion (Fig. 4). Compared to control rats, the rate of α1-AGP secretion was increased threefold (0.64 versus 0.21 μg · 4 h^{-1} · 10^6 cells $^{-1}$), whereas albumin secretion was de-

creased by 34% (3.30 versus 4.95 μg · 4 h^{-1} · 10^6 cells^{-1}). Protein secretion by hepatocytes isolated from turpentine-treated rats 48 h after the single injection was significantly altered, with a 15-fold increase in α1-AGP (3.15 μg · 4 h^{-1} · 10^6 cells^{-1}) and an 80% decrease in albumin (0.98 μg · 4 h^{-1} · 10^6 cells^{-1}).

DISCUSSION

It is now well established that α1-AGP and albumin gene expression is regulated by a cytokine network involving IL1, IL6 and tumor necrosis factor α [11] and also by glucocorticoids ([22, 23] and [47] for a review). Recent data have suggested that transforming growth factor β is the major cytokine modulating the expression of the albumin gene [48]. As it has been shown that α1-AGP serum concentrations increase after phenobarbital administration [27 – 30], we investigated the effects of repeated administration of phenobarbital on α1-AGP and albumin gene expression in rat liver.

Seven daily injections of phenobarbital induced a maximum 5.5-fold increase in α1-AGP mRNA at day 9, associated with a maximum 35% decrease in albumin mRNA. Nuclei isolated from the liver of animals receiving the same treatment showed parallel alterations in the transcription rates of the corresponding genes, with a 3.5-fold increase and a 60 – 70% decrease for α1-AGP and albumin, respectively. The discrepancy between the relative changes in the transcription rates of these two genes and the hepatic levels of their corresponding mRNA could be due to differences not only in the rate of synthesis of the mRNA and their rate of degradation, but also in the sizes of the mRNA pools. However, nuclear run-on assays showed that the effects of phenobarbital on α1-AGP and albumin synthesis essentially occurred at the transcriptional level. A post-transcriptional mechanism involving changes in the stability of primary transcripts or the rate of cytoplasmic degradation cannot, however, be totally excluded.

In order to examine a possible effect of phenobarbital at the translational level, we measured the secretion rates of α1-AGP and albumin by hepatocytes isolated from phenobarbital-treated rats. Both were modified to the same degree as the corresponding mRNA. Indeed, 24 h after the seventh injection of phenobarbital, the amount of α1-AGP mRNA was increased 3.2-fold, while protein secretion was increased threefold. At the same time, there was a 35% decrease in liver albumin mRNA and protein synthesis by isolated hepatocytes. These results suggest that the effect of phenobarbital on α1-AGP and albumin gene expression occurs at the transcriptional rather than at the translational level.

In turpentine-treated rats, our data showing a 53-fold increase in α1-AGP mRNA and a sevenfold increase in the gene transcription of this gene are in accordance with previous studies [4, 23, 25]. Similarly, the 65% decrease in albumin mRNA and the 80% decrease in the transcription rate of the albumin gene are also in agreement with previously published results [4, 23]. In phenobarbital-treated rats, the maximum inhibition of albumin gene expression was attained at day 3, while the induction of α1-AGP gene expression occurred progressively throughout the 7-day administration period, reaching a maximum at day 9. This discrepancy in the time-course of albumin and α1-AGP gene expression in phenobarbital-treated rats did not occur in turpentine-treated rats, in which the expression of the two genes was modified simultaneously and very rapidly. Therefore, the induction of α1-AGP gene expression consecutive to phenobarbital treatment does not seem to be attributable to an inflammatory response.

A direct effect of phenobarbital in increasing the rate of gene transcription has previously been reported for cytochrome *P*450IIB [49 – 52] while a post-transcriptional effect involving mRNA stabilization has been described for glutathione transferase mRNA [53]. In our study, the 11-fold increase in cytochrome *P*450 mRNA 12 h after the first injection of phenobarbital is in favour of a direct effect. Similar changes have been reported after the addition of phenobarbital to the culture medium of isolated hepatocytes [54]. The enhancement of cytochrome *P*450 mRNA, which remained stable throughout the 7-day administration period, is consistent with previously reported results [54 – 56]. It is important to note that the increase in cytochrome *P*450 mRNA occurred before that in α1-AGP mRNA, suggesting a possible relationship between these two phenomena. Such a relationship is further suggested by the sharp drop in cytochrome *P*450 mRNA levels occurring during the acute-phase reaction [57] and a similar strong decrease after IL1 treatment both *in vivo* [58] and *in vitro* [59].

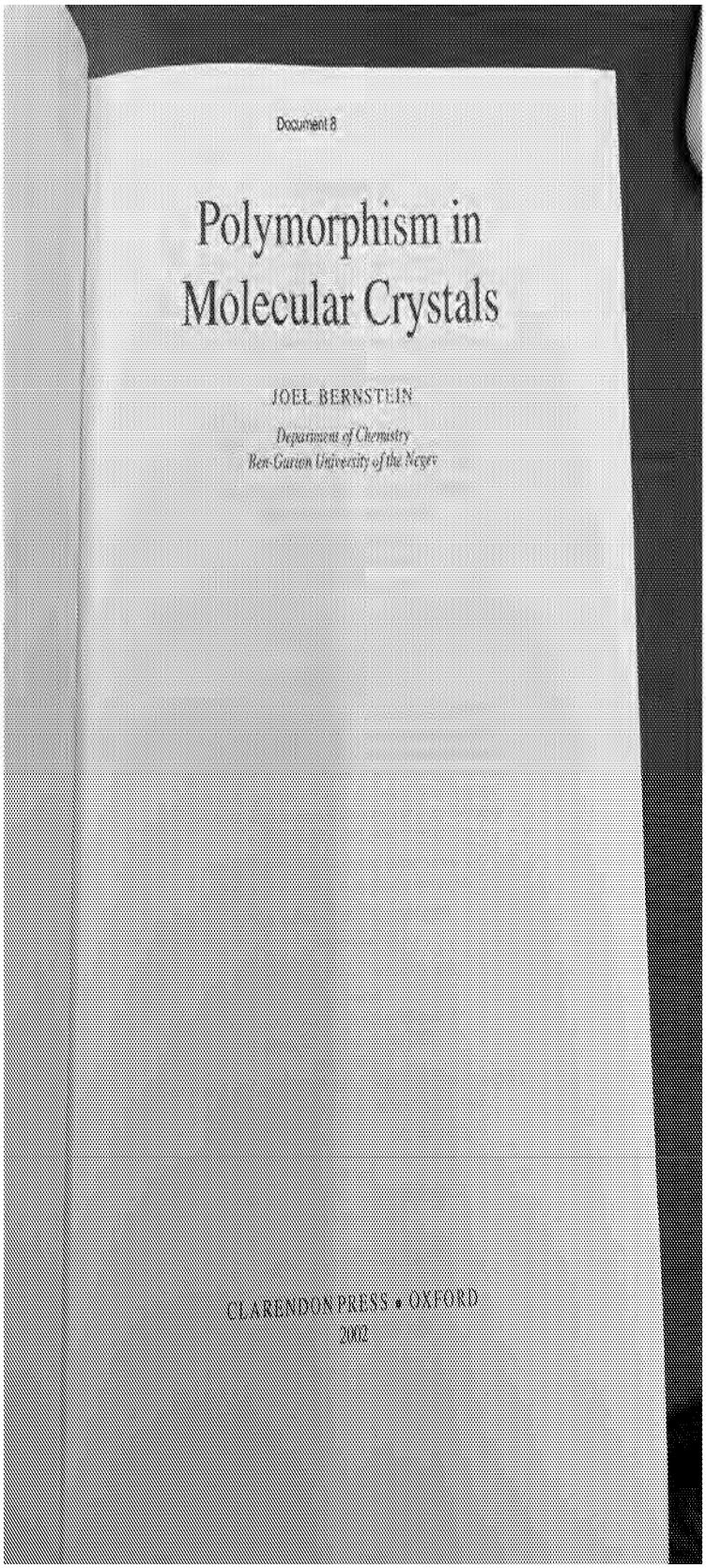
In conclusion, phenobarbital treatment *in vivo* led to an increase in cytochrome *P*450IIB mRNA levels, with a concomitant rise in α1-AGP synthesis and a fall in albumin synthesis, both mediated at the transcriptional level. The significance and possible inter-relationship of these effects remain to be determined.

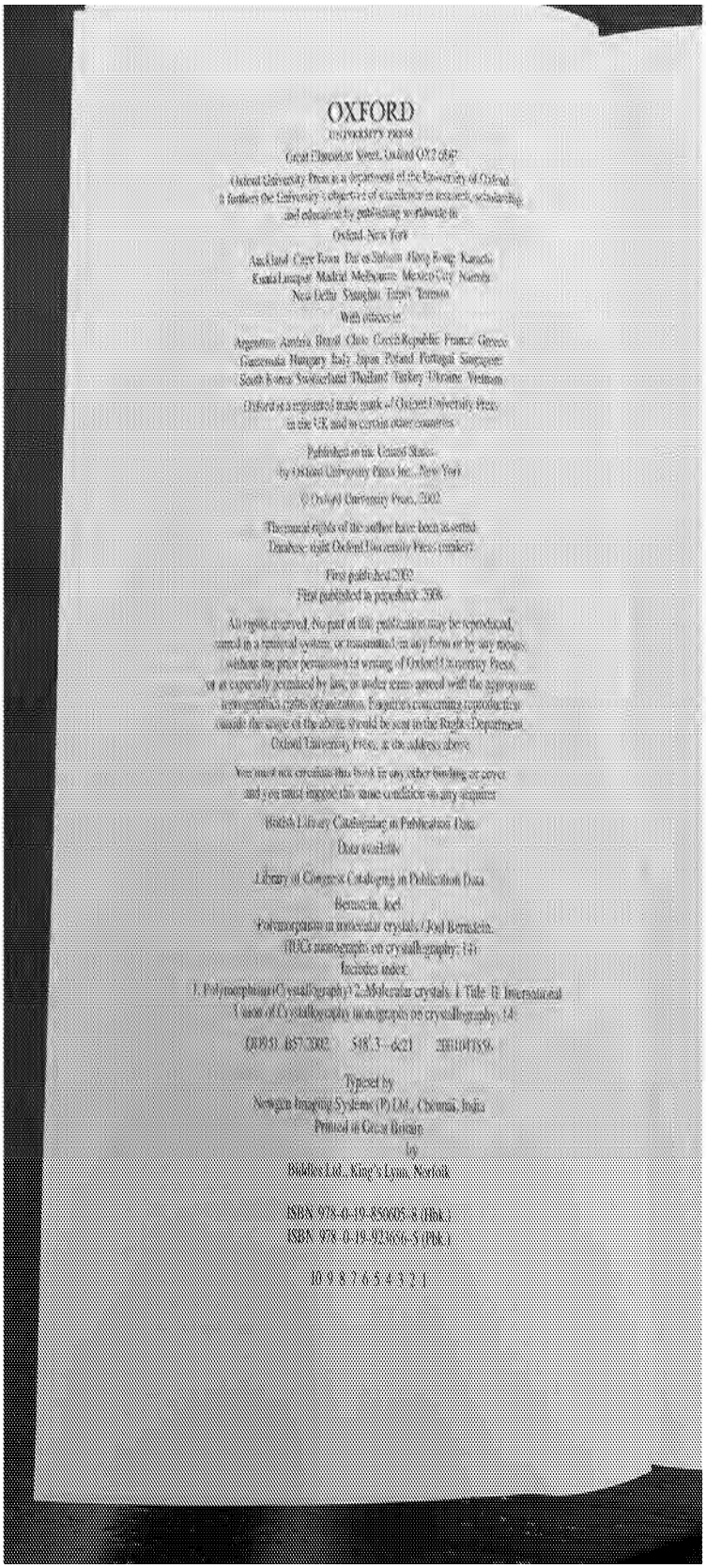
This work was supported by the C.N.R.S. by a *Biennal D.R.E.D. Contrat: xénobiotiques et biotransformation* and by *Contrats de Recherche Externe* INSERM no. 892010: Relationship between hepatocyte functions and inflammatory reaction, and no. 910302: *Induction de l'α$_1$-AGP par le phenobarbital*. The authors wish to thank Prof. F. J. Gonzalez and M. Thomasset for providing the cytochrome *P*450 and GAPDH cDNA probes, respectively. C. Lafarge-Frayssinet and C. Gudal, and C. Lapoumeroulie and Prof. J. Elion for kindly providing laboratory facilities.

REFERENCES

1. Kushner, I. & Mackiewicz, A. (1987) *Disease Markers 5*, 1 – 11.
2. Baumann, H. & Held, W. A. (1981) *J. Biol. Chem. 256*, 10145 – 10155.
3. Ricca, A. G., Hamilton, R. W., McLean, J. W., Conn, A., Kahnyak, J. E. & Taylor, J. M. (1981) *J. Biol. Chem. 256*, 10362 – 10368.
4. Birch, H. E. & Schreiber, G. (1986) *J. Biol. Chem. 261*, 8077 – 8080.
5. Gauldie, J., Richards, C., Harnish, D., Lansdorp, P. & Baumann, H. (1987) *Proc. Natl Acad. Sci. USA 84*, 7251 – 7255.
6. Koj, A., Kurdowska, A., Magielska-Zero, D., Rokita, H., Sipe, J. D., Dayer, J. M., Demiczuk, S. & Gauldie, J. (1987) *Biochem. Int. 14*, 553 – 560.
7. Baumann, H., Onorato, V., Gauldie, J. & Jahreis, G. P. (1987) *J. Biol. Chem. 262*, 9756 – 9768.
8. Geiger, T., Andus, T., Klapproth, J., Northoff, H. & Heinrich, P. C. (1988) *J. Biol. Chem. 263*, 7141 – 7146.
9. Baumann, H., Richards, C. & Gauldie, J. (1987) *J. Immunol. 139*, 4122 – 4128.
10. Darlington, G. J., Wilson, D. R. & Lachman, L. B. (1986) *J. Cell Biol. 103*, 787 – 793.
11. Prowse, K. R. & Baumann, H. (1989) *J. Leukocyte Biol. 45*, 55 – 61.
12. Marinkovic, S., Jahreis, G. P., Wong, G. G. & Baumann, H. (1989) *J. Immunol. 142*, 808 – 812.
13. Poiis, C., Giroud, J. P., Damais, C., Raichvarg, D. & Chauvelot-Moachon, L. (1990) *Drug Metab. Dispos. 18*, 467 – 470.
14. Durand, D., Bertaux, O., Fournier, T., Porquet, D., Valencia, R. & Durand, G. (1990) *FEBS Lett. 263*, 109 – 112.

15. De Jong, F., Birch, H. & Schreiber, G. (1988) *Inflammation 12*, 613–617.
16. Klapproth, J., Castell, J., Geiger, T., Andus, T. & Heinrich, P. C. (1989) *Eur. J. Immunol. 19*, 1485–1490.
17. Delers, F., Mangeney, M., Raffa, D., Vallet-Colom, L., Daveau, M., Tran-Quang, N., Davrinches, C. & Chambaz, J. (1989) *Biochem. Biophys. Res. Commun. 161*, 81–88.
18. Andus, T., Geiger, T., Hirano, T., Kishimoto, T., Tran-Thi, T. A., Decker, K. & Heinrich, P. C. (1988) *Eur. J. Biochem. 173*, 287–293.
19. Andus, T., Geiger, T., Hirano, T., Kishimoto, T. & Heinrich, P. C. (1988) *Eur. J. Immunol. 18*, 739–746.
20. Vannice, J. L., Taylor, J. M. & Ringold, G. M. (1984) *Proc. Natl Acad. Sci. USA 81*, 4241–4245.
21. Carter, K. C., Bryan, S., Gadson, P. & Papaconstantinou, J. (1989) *J. Biol. Chem. 264*, 4112–4119.
22. Reinke, R. & Feigelson, P. (1985) *J. Biol. Chem. 260*, 4397–4403.
23. Kulkarni, A. B., Reinke, R. & Feigelson, P. (1985) *J. Biol. Chem 260*, 15386–15389.
24. Baumann, H. & Maquat, L. E. (1986) *Mol. Cell. Biol. 6*, 2551–2561.
25. Shiels, B. R., Northemann, W., Gehring, M. R. & Fey, G. H. (1987) *J. Biol. Chem. 262*, 12826–12831.
26. Bai, S. A. & Abramson, F. P. (1982) *J. Pharmacol. Exp. Ther. 222*, 589–594.
27. Brinkschulte, M. & Breyer-Pfaff, U. (1982) *Biochem. Pharmacol. 31*, 1749–1754.
28. Monnet, D., Féger, J., Biou, D., Durand, G., Cardon, P., Leroy, Y. & Fournet, B. (1986) *Biochim. Biophys. Acta 881*, 10–14.
29. Lin, T. H., Sugiyama, Y., Sawada, Y., Suzuki, Y., Iga, T. & Hanano, M. (1987) *Drug Metab. Dispos. 15*, 138–140.
30. Chauvelot-Moachon, L., Delers, F., Pons, C., Engler, R., Tallet, P. & Giroud, J. P. (1988) *J. Pharmacol. Exp. Ther. 244*, 1101–1108.
31. Larrey, D., Distelrath, L. M., Dannan, G. A., Wilkinson, G. R. & Guengerich, F. P. (1984) *Biochemistry 23*, 2787–2795.
32. Kahn, G. C., Rubenfield, M., Davies, D. S., Murray, S. & Boobis, A. R. (1985) *Drug Metab. Dispos. 13*, 510–516.
33. Churchill, P. F., Churchill, S. A., Martin, M. V. & Guengerich, F. P. (1987) *Mol. Pharmacol. 31*, 152–158.
34. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular cloning: a laboratory manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA.
35. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry 18*, 5294–5299.
36. Sargent, T. D., Wu, J. R., Sala-Trepat, J. M., Wallace, R. B., Reyes, A. A. & Bonner, J. (1979) *Proc. Natl Acad. Sci. USA 76*, 3256–3260.
37. Nebert, D. W., Adesnik, M., Coon, M. J., Estabrook, R. N., Gonzales, F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Kemper, L. B., Levin, W., Phillips, I. R., Sato, R. & Waterman, M. R. (1989) *DNA 8*, 1–11.
38. Gonzalez, F. J. & Nebert, D. W. (1987) *Annu. Rev. Biochem. 56*, 945–993.
39. Biobel, G. & Potter, Van R. (1966) *Science 154*, 1662–1665.
40. Turcotte, B., Guertin, M., Chevrette, M. & Belanger, L. (1985) *Nucleic Acids Res. 13*, 2387–2398.
41. Mezger, V., Bensaude, O. & Morange, M. (1987) *Develop. Biol. 124*, 544–550.
42. Piechaczyk, M., Blanchard, J. M., Marty, L., Dani, C., Panabieres, F., El Sabouty, S., Fort, P. & Jeanteur, P. (1984) *Nucleic Acids Res. 12*, 6951–6963.
43. Berry, M. N. & Friend, D. S. (1969) *J. Cell Biol. 43*, 506–520.
44. Davy, J., Appel, M., Biou, D., Féger, J. & Agneray, J. (1983) *Biol. Cell. 48*, 203–206.
45. Weigel, P. H. & Oka, J. A. (1982) *J. Biol. Chem. 257*, 1201–1207.
46. Biou, D., Daveau, M., Rigal, O., Hiron, M., Porquet, D. & Lebreton, J. P. (1989) *J. Immunol. Methods 125*, 1–4.
47. Baumann, H. & Gauldie, J. (1990) *Mol. Biol. Med. 7*, 147–159.
48. Morrone, G., Cortese, R. & Sorrentino, V. (1989) *EMBO J. 8*, 3767–3771.
49. Hardwick, J. P., Gonzalez, F. J. & Kasper, C. B. (1983) *J. Biol. Chem. 258*, 8081–8085.
50. Atchison, M. & Adesnik, M. (1983) *J. Biol. Chem. 258*, 11285–11295.
51. Pike, S. F., Shephard, E. A., Rabin, B. R. & Philips, I. R. (1985) *Biochem. Pharmacol. 34*, 2489–2494.
52. Chianale, J., Mulholland, L., Traber, P. G. & Gumucio, J. J. (1988) *Hepatology 8*, 327–331.
53. Francis, V. N. K., Dwarki, V. J. J. & Padmanaban, G. (1986) *Nucleic Acid Res. 14*, 2497–2510.
54. Chianale, J., Dvorak, C., May, M. & Gumucio, J. Y. (1986) *Hepatology 6*, 945–951.
55. Adesnik, M., Bar-Nun, S., Maschin, F., Zunich, M., Lippman, A. & Bard, E. (1981) *J. Biol. Chem. 256*, 10340–10346.
56. Wegiel, E., Dvorak, C., Chianale, J., Traber, P. G., Keren, D. & Gumucio, J. J. (1988) *J. Clin. Invest. 82*, 658–666.
57. Stanley, L., Adams, D., Lindsay, R., Meehan, R., Liao, W. & Wolf, R. (1988) *Eur. J. Biochem. 174*, 31–36.
58. Bertini, R., Bianchi, M., Erroi, A., Villa, P. & Ghezzi, P. (1989) *J. Leukocyte Biol. 46*, 254–262.
59. Sujita, K., Okuno, F., Tanaka, Y., Hirano, Y., Inamoto, Y., Eto, S. & Arai, M. (1990) *Biochem. Biophys. Res. Commun. 168*, 1217–1222.


Document 8
Polymorphism in Molecular Crystals
JOEL BERNSTEIN
Department of Chemistry
Ben-Gurion University of the Negev
CLARENDON PRESS • OXFORD
2002



OXFORD
UNIVERSITY PRESS
Great Clarendon Street, Oxford OX2 6DP
Oxford University Press is a department of the University of Oxford.
It furthers the University's objective of excellence in research, scholarship,
and education by publishing worldwide in
Oxford New York
Auckland Cape Town Dar es Salaam Hong Kong Karachi
Kuala Lumpur Madrid Melbourne Mexico City Nairobi
New Delhi Shanghai Taipei Toronto
With offices in
Argentina Austria Brazil Chile Czech Republic France Greece
Guatemala Hungary Italy Japan Poland Portugal Singapore
South Korea Switzerland Thailand Turkey Ukraine Vietnam
Oxford is a registered trade mark of Oxford University Press
in the UK and in certain other countries
Published in the United States
by Oxford University Press Inc., New York
© Oxford University Press, 2002
The moral rights of the author have been asserted
Database right Oxford University Press (maker)
First published 2002
First published in paperback 2006
All rights reserved. No part of this publication may be reproduced,
stored in a retrieval system, or transmitted, in any form or by any means,
without the prior permission in writing of Oxford University Press,
or as expressly permitted by law, or under terms agreed with the appropriate
reprographics rights organization. Enquiries concerning reproduction
outside the scope of the above should be sent to the Rights Department,
Oxford University Press, at the address above
You must not circulate this book in any other binding or cover
and you must impose this same condition on any acquirer
British Library Cataloguing in Publication Data
Data available
Library of Congress Cataloging in Publication Data
Bernstein, Joel
Polymorphism in molecular crystals / Joel Bernstein.
(IUCr monographs on crystallography; 14)
Includes index.
1. Polymorphism (Crystallography) 2. Molecular crystals. I. Title. II. International
Union of Crystallography. III. Series.
QD931.B57 2002 548'.3—dc21 2001047859
Typeset by
Newgen Imaging Systems (P) Ltd., Chennai, India
Printed in Great Britain
by
Biddles Ltd., King's Lynn, Norfolk
ISBN 978-0-19-850005-6 (Hbk.)
ISBN 978-0-19-921866-5 (Pbk.)
10 9 8 7 6 5 4 3 2 1

Byrn *et al.* 1999; Yu 2001). One increasingly used method to stabilize the amorphous form is to disperse it in a polymer matrix such as polyvinylpyrrolidone, although there is still the possibility that crystalline material will crystallize out (Kumada *et al.* 2000).

Amorphous pharmaceutical materials are typically obtained by employing crystallization procedures far from equilibrium such as (rapid) solidification from the melt, lyophilization (freeze drying) or spray drying, removal of solvent from a solvate, precipitation by changing pH, or by mechanical processing such as granulation, grinding or milling (Guillory 1999). Recently developed supercritical fluid technology is certain to play a role in preparation of amorphous and metastable forms (Shekunov and York 2000). The interest in understanding the processes involved in preparing amorphous materials or those that often result from the inherent tendency of amorphous materials to undergo changes to energetically lower states has led to some comprehensive studies. Many of these involve the same analytical techniques used to characterize crystal modifications. For example, the *formation* of amorphous carbamazepine from the crystalline dihydrate was studied *in situ* by DSC, TGA and variable temperature X-ray diffraction (Li *et al.* 2000). As demonstrated by Ceolin *et al.* (1995) in another study, the amorphous state may also be obtained by quenching the melt. On the other hand, the *degradation* of amorphous quinapril hydrochloride, prepared by two methods, was followed by DSC, TGA, X-ray powder diffraction, polarized optical microscopy, scanning electron microscopy and IR spectroscopy (Gao *et al.* 2000).

Since amorphous material may act as a contaminant, it is often necessary to accurately determine its concentration in a crystalline sample. A variety of techniques of analysis for the quantitative detection of an amorphous phase in a crystalline phase have been developed, and these are undergoing rapid change and improvement (Buckton and Darcy 1999; Stephenson *et al.* 2001). As always, the choice of method will depend on the material and the circumstances, but a few examples are noted here to demonstrate some recent developments. Taylor and Zografi (1998) employed FT-Raman spectroscopy to quantify the degree of crystallinity of indomethacin. They found a linear correlation over a 0–100 per cent range of crystallinity with a limit of detection of 1 per cent crystalline or amorphous content. Diffuse near-infrared reflectance spectroscopy (NIRS) was employed on the same compound together with mixtures of amorphous/crystalline sucrose (a potential excipient) (Seyer *et al.* 2001). This method was found to be slightly more precise than analyses carried out by the more traditional X-ray (Klug and Alexander 1974; Zevin and Kimmel 1995) and DSC techniques.

The evolution in calorimetry technology has also led to the development of protocols for quantitative analysis (Buckton and Darcy 1999). Fiebich and Mato (1985) determined the amorphous content of desferal using both isothermal microcalorimetry and water vapour sorption gravimetry with a level of detection of less than 1 per cent amorphous material. The heat capacity jump associated with the glass transition of amorphous materials MTDSC was used to quantify the amorphous content of a micronized drug substance with a limit of detection of 3 per cent w/w (of amorphous

substance (Gaisford and Leveiller 1999). Significant variations in the chemical stability profiles of an experimental drug could be attributed to differences in amorphous content of around 5 per cent, which was also quantified by MTDSC (Saklatvala *et al.* 1999).

15. Concluding remarks

The development of a pharmaceutical product can be a long, arduous and expensive process. For those that are marketed in solid dosage forms that process deals with many matters of solid state chemistry, among them the detection, characterization and preparation of various modifications. We have attempted here to touch on many of these issues, and as noted at the beginning of the chapter, examples of these various aspects of polymorphism and their manifestations in the pharmaceutical industry may be found throughout this book.

For scientific, regulatory and intellectual property reasons recent years have witnessed increasing efforts to discover and characterize as many different crystal modifications as possible of substances associated with a pharmaceutical product. This effort has been aided and enhanced by the rapid developments in instrumentation and analytical techniques, so that often old compounds have been revisited (or study to the same extent that new ones have been subject to investigation. In light of the new and often more sensitive techniques available, many questions and problems raised 20 or more years ago warrant reinvestigation. One of these involves the large numbers of crystal modifications reported for some substances, for instance, chlorpropamide (thirteen modifications) (Cleverly and Williams 1959; Mosley *et al.* 1966; Stanley Wood and Riley 1972) or cytidine (seven modifications) (Taggetlus and Gerge 1985; Sudo *et al.* 1991). In a recent reexamination of a number of these drugs, Threlfall reported discovering a new polymorph of sulphathiazole, two new polymorphs of sulphapyridine, 120 solvates of sulphathiazole and 30 solvates of sulphapyridine (Threlfall 1999, 2000). Complete structural studies of diverse crystal modifications of a single substance are beginning to appear, for instance the seven solvated forms of 3,5-dinitrosalicylic acid (Kumar *et al.* 1999). In some cases of historically well documented multifarious polymorphism, systematic attempts have recently been made to prepare the various forms for single crystal analysis. For instance, *o*-methylchalcone has been shown to exist in 13 different polymorphic forms (Weygand and Baumgärtel 1929; Eistert *et al.* 1952). Five of the forms that are metastable at room temperature have been prepared; three crystal structures have been carried out, while the other two underwent transformations during the data collection (Janssen and Heinck 1998).

Earlier reports of complex energy–temperature relationships (e.g. dapsone and stanozolol chloride (Kuhnert-Brandstatter and Moser 1979)), or unusual crystal chemistry (e.g. oxyclozanide (Pearson and Varney 1973)) also warrant serious consideration in retrospect. The formulation implications of chemical reactivity in solid-state pharmaceuticals have recently been reviewed by Byrn *et al.* (2001).

Document 2

Deliberation Results Report

September 16, 2008
Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau

[Brand name] Nobelbar for intravenous injection 250 mg
[Generic name] Phenobarbital sodium
[Applicant] Nobel Pharma K.K.
[Application date] September 14, 2007

[Review results]

At the First Committee of Pharmaceutical Preparations held on August 29, 2008, it was judged that approval of this item was no problem, and it was decided to report this to the Pharmaceutical Affairs Section of the Pharmaceutical Affairs and Food and Hygiene Council.

This item does not fall under biological products or specified biological products, and the reexamination period is 10 years for neonatal convulsions and 6 years for status epilepticus, and both the drug substance and drug product are considered to fall under the powerful drug.

Review Report

August 11, 2008
Chief Executive, Pharmaceuticals and Medical Devices Agency

The results of the review by the Pharmaceuticals and Medical Devices Agency concerning the following drug for which the application for approval was submitted are as follows.

Record

[Brand name]	Nobelbar for intravenous injection 250 mg
[Generic name]	Phenobarbital sodium
[Applicant name]	Nobel Pharma K.K.
[Application date]	September 14, 2007
[Formulation/Strength]	lyophilized drug product for intravenous infusion containing 274 mg of phenobarbital sodium (250 mg as phenobarbital) per vial
[Category of application]	Prescription drugs (3) Drugs with new route of administration
[Special notes]	Orphan drugs
[Reviewing Department]	Office of New Drug III

Review results

August 11, 2008

[Brand name] Nobelbar for intravenous injection 250 mg
[Generic name] Phenobarbital sodium
[Applicant name] Nobel Pharma K.K.
[Application date] September 14, 2007
[Review results]

Based on the submitted materials, it is considered that the efficacy and safety of this drug for neonatal convulsion and status epilepticus have been demonstrated. For neonatal convulsions and status epilepticus, further investigation is considered necessary in post-marketing surveillance regarding the relationship between the administration status (dose, administration rate) of the drug and safety and efficacy, the relationship between blood concentration and safety and efficacy, and the occurrence of serious skin disorders.

As a result of the review by the Pharmaceuticals and Medical Devices Agency, it was determined that the following indications and dosage and administration were approved for this item.

[Indications] Neonatal convulsion, status epilepticus
[Dosage and administration] Neonatal convulsion
Initial dose: 20 mg/kg intravenously as phenobarbital. If convulsions cannot be controlled, the dose should be adjusted to the extent not exceeding the initial dose, depending on the patient's condition, and the dose should be additionally administered intravenously.
Maintenance dose: 2.5 - 5 mg/kg of phenobarbital is intravenously administered once daily.
Status epilepticus
15 - 20 mg/kg of phenobarbital is intravenously administered once daily.

Review Report (1)

Prepared on July 4, 2008

I. Application item

[Brand name]	Nobelbar for intravenous injection 250 mg
[Generic name]	Phenobarbital sodium
[Applicant name]	Nobel Pharma K.K.
[Application date]	September 14, 2007
[Formulation/Strength]	lyophilized drug product for intravenous infusion containing 274 mg of phenobarbital sodium (250 mg as phenobarbital) per vial

[Indications at the time of application] Neonatal convulsion, status epilepticus

[Dose and administration at the time of application] Neonatal convulsion

First dose: 20 mg/kg of phenobarbital is intravenously administered once daily. If convulsions cannot be controlled, the same dose should be given as an additional IV infusion.

Maintenance dose: 2.5 to 5 mg/kg/day intravenously as phenobarbital. The maintenance treatment period will last approximately 6 days.

The dose will be increased or decreased as appropriate depending on age and symptoms.

Status epilepticus

15 - 20 mg/kg of phenobarbital is intravenously administered once daily. The dose will be increased or decreased as appropriate depending on age and symptoms.

II. Summary of submitted materials and review

In this application, a summary of the Applicant's response to the materials submitted by the Applicant and inquiries from the Pharmaceuticals and Medical Devices Agency (PMDA) was as follows.

1. Documents regarding the origin or history of discovery and usage status in foreign countries

Phenobarbital (PB), developed by Bayer AG (Germany) in 1912, is a typical barbiturate derivative with anticonvulsant, hypnotic, and sedative effects, and is widely used as a hypnotic, sedative, and seizure treatment for epilepsy in oral, injectable, and suppository formulations, and overseas, as of May 30, 2008, oral formulations of PB (tablets, oral solution, elixirs, sprays, syrups, etc.) are approved in 22 countries worldwide, including the U.S., the U.K., Germany, and France, and suppositories are approved in one country, Switzerland. Oral formulations of phenobarbital sodium (Clozapine) (tablets, oral solution, elixirs, sprays, and syrups) are approved in 3 countries, and injectable formulations are approved in 12 countries for intravenous injection, 13 countries for intramuscular injection, and 1 country for subcutaneous injection.

In Japan, PB has been approved in tablets, powders, elixirs, and subcutaneous/intramuscular preparations since the approval of phenobal® powder in January 1944. Meanwhile, phenobarbital sodium (this drug) was approved for subcutaneous/intramuscular use in 1949, and a suppository was approved in March 1980. Oral drugs and subcutaneous/intramuscular preparations were notified of the results of the re-evaluation in June 1975, and the current indications (sedation of anxiety tone, autonomic seizures, psychomotor seizures, seizure of epilepsy) are described.

However, since there were issues such as the fact that the appropriate intravenous preparation was not marketed in Japan, the efficacy and effect of neonatal convulsions were not established, and the dosage and administration in children were not established except for suppositories, the clinical trial (investigator-initiated clinical trial) was initiated in March 2006 in which the investigator-initiated clinical trial was conducted.

At this time, the Applicant believes that the efficacy and safety of the product for neonatal seizures have been confirmed based on the results of the investigator-initiated clinical trials and existing information, and with regard to status epilepticus, on November 24, 2005, the Japanese Pediatric Society and the Japanese Society of Pediatric Neurology submitted a request to the Minister of Health, Labour and Welfare for an additional indication of intravenous phenobarbital for the treatment of status epilepticus. Based on this, the Applicant conducted a literature review, etc., and determined that the efficacy and safety of the drug for status epilepticus is widely recognized, and that the drug is known to medical and pharmaceutical authorities for status epilepticus, and submitted an application for approval for these two indications.

Regarding the "non-clinical materials" in this application, only the vascular irritation study in rabbits was conducted, and no other documents based on the results of other studies were submitted, but discussion was made based on the published literature, etc.

2. Quality Materials

<Summary of submitted materials>

(1) Drug substance

The drug substance, phenobarbital sodium, is a white crystal or crystalline powder that has general properties of property, solubility, thermal analysis, pH, hygroscopicity, dissociation constant (pKa), and crystalline polymorphs. The drug substance is hygroscopic, has two types of crystalline forms, and is thought to change by hygroscopic absorption at a relative humidity of 68% or higher. However, the drug product is a lyophilized drug product prepared by aliquoting the drug substance into water, and the difference in the crystalline form of the drug substance is judged to have no effect on the manufacture of the drug substance, so physical and chemical examination of the crystalline form generated by hygroscopic absorption have not been performed.

The manufacture of the drug substance consists of the synthesis of [redacted] (Step 1), synthesis of [redacted] (Step 2), synthesis of [redacted] (Step 3), phenobarbital [redacted] (Step 4), phenobarbital [redacted] (Step 5), and synthesis and packaging of phenobarbital sodium (Step 6). Step [redacted] and [redacted] are defined as critical steps, and Step [redacted] phenobarbital is set as the control value as the critical intermediate.

The chemical structure of the drug substance has been demonstrated by infrared absorption (IR), magnetic resonance (^{1}H-NMR), and mass (MS) spectra. As impurities, related substances and residual solvents (ethanol) have been investigated.

The specification and test method of the drug substance are as follows: properties (appearance), confirmatory test (qualitative reaction of IR spectrum, sodium salt), pH, purity test (solution, chloride, sulfate, heavy metal, neutral or basic substance, sulfuric acid color, related substances <liquid chromatography (HPLC)>, residual solvents <gas chromatography (GC)>, loss on drying, endotoxin, and content <HPLC>. Arsenic has not been determined. Based on hygroscopicity of the drug substance, water was set as a new specification during the review process.

As part of the review process, a purity test (related substances <HPLC>), in addition to the specifications listed in the Japanese Pharmacopoeia, was set for the reference substance, phenobarbital standard.

With regard to the stability of the drug substance manufactured at the actual production scale, a long-term storage test (25 ± 2°C/60 ± 5% RH, in a dark location, 60 months), an accelerated test (40 ± 2°C/75 ± 5% RH, dark place, 6 months), and an intermediate test (30 ± 2°C/65 ± 5% RH, dark place, 12 months) were performed in polyethylene bags and the bags were packed in a cardboard box[1]. In these tests, property, solubility, pH, purity test (related substances <thin-layer chromatography (TLC)>), loss on drying, content <titration method> were the test items, and in the acceleration test and intermediate test, confirmatory test (IR spectrum and melting point), purity test (related substances <HPLC>), and microbial limit were the test items. In these stability studies, no clear changes were observed in any of the measurements, and the storage method of the drug substance was set as "tight container, room temperature storage" and the re-test period of the drug substance was set at 5 years.

(2) Drug product

The drug product is a lyophilized injection composed of drug substance only, without additives, with a clear colorless glass vial/rubber stopper/flip cap applied to the container and closure system. An application has been submitted for a formulation containing 274 mg of phenobarbital sodium (250 mg of phenobarbital) per vial. In drug product development, phenobarbital is extremely slightly soluble in water, and the use of a soluble supplement was considered to be unfavorable for neonates. Therefore, it is considered appropriate to use high water solubility of phenobarbital sodium as the drug substance, no additive, and to use the formulation as a lyophilized injection.

The manufacturing process of the drug product consists of Process 1 (redacted), Process 2 (sterile filtration process), Process 3 (redacted), Process 4 (lyophilization process), Process 5 (redacted) and Process 6 (selection and packaging process). The No. [redacted], No. [redacted], and No. [redacted] processes are regarded as critical processes, and control items and control values are set. In the review process, based on the hygroscopicity of the drug product, the [redacted] process is positioned in the critical process, and [redacted] is set as a process control.

The specifications and test methods of the drug product are set as properties (appearance), identification test (IR spectrum, qualitative reaction of sodium salt), pH, purity test (related substances <HPLC>), loss on drying, product homogeneity (mass deviation), endotoxin, foreign insoluble matter, insoluble particulate matter, sterility, content <HPLC>. In addition, based on the hygroscopicity of the formulation, moisture content was newly set as a standard during the review process.

The stability of the formulation was evaluated by long-term storage tests (in paper boxes, 25 ± 2°C/60 ± 5 %RH, in the dark, 36 months), accelerated tests (in paper boxes, 40 ± 2°C/75 ± 5 %RH, in the dark, 6 months), and caustic tests (temperature [in paper boxes, 50 ± 2°C/normal 3 months), and light (unpackaged, total illuminance of at least 1.2 million lux-hr + total near-ultraviolet radiation energy of at least 200 W-h/m²), and relative acceleration studies (paper boxed, upright/inverted, 40 ± 2°C/75 ± 5 %RH/dark, 3 months) were conducted on the formulations manufactured at production scale. In these tests, properties, identification test (qualitative reaction of IR spectrum, sodium salt), solubility, pH, purity test (related substances <HPLC>), water, loss of drying, foreign insoluble matter, insoluble particulate matter, and content were the test items, and endotoxin, product homogeneity (mass deviation) and sterility were the test items for long-term storage test and acceleration test. No clear changes were observed in any of the test items. Based on the above, the drug product is stored at room temperature and the primary packaging is sealed with glass vials, rubber stoppers and caps, and the expiry date of the drug product is set at 3 years.

<Summary of Review>

---

1) The container closure system and container closure system of the drug substance are to be placed in a polyethylene bag (double) and sealed in a fiber drum, which is different from the storage conditions in the stability test

(i) Drug substance

PMDA sought clarification from the Applicant on whether the stability of the drug substance has been appropriately evaluated, although photo stability studies have not been performed for the drug substance.

The Applicant considered that the stability and storage methods of the drug substance were fully evaluated because the drug substance was already listed in the compendial form of the United States Pharmacopeia and other foreign countries, and it was listed up to the Tenth revised Japanese Pharmacopeia in Japan, and explained that photo stability testing was not performed and storage methods (airtight container) were set according to the compendial form above.

PMDA requested the Applicant to explain the appropriateness of the specification value of the content of residual solvent (ethanol) contained in the drug product as [redacted] %, considering the safety of the drug when administered to humans at the dosage and administration.

The Applicant has established that the dosage and administration of the drug should be 20 mg/kg of phenobarbital once daily for neonatal convulsions, with an additional dosage of the same amount if the effect is insufficient, and 15-20 mg/kg of phenobarbital intravenously once daily for status epilepticus. However, considering that the dose may be doubled to 20 mg/kg in clinical practice, the maximum daily dose of ethanol is 184.1 mg/day for adults (assuming a body weight of 60 kg) and 9.2 mg/day for neonates (assuming a body weight of 3.0 kg). While this is higher than the human PDE (permitted daily exposure) of 166.7 mg/day (ICH Guideline-Residual Solvents, *Pharmeuropa, Vol. 9, No. 1 Supplement April*, S58-59, 1997), the blood concentration of 184.1 mg of ethanol administered to an adult is 0.005 % (assuming a circulating blood volume of 4000 mL), which is low compared to the blood ethanol concentration that causes clinical symptoms (0.02 % or higher) (Kono, H. et al., Current Status of Alcohol-Related Problems in Japan, 1993). In addition, the daily intake of ethanol is 376 mg/kg (ICH Guideline-Residual Solvents, *Pharmeuropa Vol. 9, No. 1, Supplement April*, S58-59, 1997), which is based on the acceptable limit for ethanol handling environments established by the ACGIH (American Conference of Governmental Industrial Hygienists). The daily intake of ethanol is 376 mg/kg (ICH Guideline-Residual Solvents, Pharmeuropa Vol. 9, No. 1, Supplement April, S58-59, 1997), and considering that this product is not intended for long-term administration, the effects on humans should be minimal or insignificant. The Applicant also explained that he/she could not find the allowable limit of ethanol intake for children.

Considering the results, etc. of investigator-initiated clinical trials, PMDA believes that there are no particular problems with the safety in neonates at this time, but in post-marketing surveillance, it is necessary to consider the relationship between dosage and administration period and safety of the drug.

Based on the above, PMDA accepted the above and judged that the specifications, test methods, storage and re-test periods, and drug product specifications, test methods, storage and shelf life were appropriate.

3. Non-clinical materials

(i) Summary of pharmacological study results

No new pharmacology studies have been conducted for this application, and publications on the anticonvulsant effect, analgesic effect, hypnotic effect, effects on the central nervous system, respiratory system, cardiovascular system, and digestive system, and effects on juvenile animals of PB or this drug were submitted.

<Summary of submitted materials>

(1)Tests to support the efficacy

1) Anticonvulsant action

① Maximal electroconvulsive seizure method

Mice received PB 0-100 mg/kg SC (s.c.) In addition, when the maximum electroconvulsive action was investigated, the effective dose of this drug (ED_{50}) was 20.7 mg/kg, the toxic dose (TD_{50}: dose that causes 50% of animals to develop toxic symptoms such as ataxia) was 57 mg/kg, and the protective index (PI): TD_{50}/ED_{50}) was 2.7. Phenytoin (s.c.), Trimethadione (s.c.) and phenylacetylurea (oral [p.o.]) ED_{50} was 9.1, 630, and 109 mg/kg, TD_{50} was 92, 660, and 640 mg/kg, respectively, and PI was 10.1, 1.1, and 5.9, respectively (4.3.1.1-4).

When the maximum electroconvulsive effect of oral administration of this drug, ethanol, or methyl parafynol was investigated in rats, ED_{50} was 3.7, 2850, and 50 mg/kg, TD_{50} was 28, 6500, and 108 mg/kg, and PI was 7.6, 2.3, and 2.2, respectively (4.3.1.1-5).

② Electroconvulsive threshold method

PB(s.c.), Trimethadione (s.c.) in rats or phenylacetylurea (p.o.) when administering the study drug, the PI was 1.75 ± 0.19, 1.51 ± 0.17, and 18.7 ± 3.70, respectively. Phenytoin (s.c.) was not effective for electroconvulsive seizures (it was considered effective when there was no convulsion at 1.2 times the threshold current) (4.3.1.1-6).

③ Drug-induced convulsion method

When the inhibitory effect of subcutaneous administration of PB, trimetadione, and phenylacetylurea on metrazole-induced convulsions was investigated in mice, ED_{50} was 24, 480, and 125 mg/kg, respectively, and PI was 2.5, 1.4, and 2.3, respectively. Phenytoin was ineffective for metrazole-induced convulsions (4.3.1.1-4).

In this study, this drug, phenytoin, and trimethadione were orally administered to mice to investigate their inhibitory effects on clonic convulsions, tonic convulsions, and death induced by high doses of metrazole. This drug and trimethadione suppressed clonic convulsions, tonic convulsions, and death (PD_{50} of this drug: 38.2, 20.7, and 26.8 mg/kg, respectively; PD_{50} of trimethadione: 571.4, 498.0, and 459.4 mg/kg, respectively), whereas phenytoin suppressed only tonic convulsions (PD_{50}: 12.0 mg/kg) (4.3.1.1-8).

In a study of the inhibitory effects of aminohexane-induced convulsions following subcutaneous administration of PB, phenytoin, trimetadione, and phenylacetylurea in mice, PB, trimetadione, and phenylacetylurea showed inhibitory effects on convulsions (ED_{50}: 15, 480, and 70 mg/kg, respectively), while phenytoin did not show inhibitory effects on convulsions (4.3.1.1-7).

When PB, phenytoin, and trimetadione were orally administered to mice and investigated for their inhibitory effect on picrotoxin- or strikinine-induced convulsions, PB was shown to be an inhibitory effect on convulsions caused by both picrotoxin and strikinine drugs (ED_{50} 135 and 210 mg/kg, respectively), while phenytoin was ineffective on convulsions caused by both drugs. Trimethadione also showed inhibitory effect on picrotoxin-induced convulsions (ED_{50}: 1300 mg/kg), but no inhibitory effect on striquinine-induced convulsions (4.3.1.1-9).

2) Hypnotic effect

The hypnotic effect of long-term (PB), intermediate (amobarbital) and short (hexobarbital) barbiturates was investigated in mice administered orally and intraperitoneally. The doses (HD_{50}) that lead to sleep in 50% of animals (oral and intraperitoneal) were 89 mg/kg and 75 mg/kg for PB, 129 mg/kg and 61 mg/kg for amobarbital, and 130 mg/kg and 50 mg/kg for hexobarbital, respectively; the LD_{50} for oral administration was 325 mg/kg for PB, 560 mg/kg for amobarbital, and 468 mg/kg for hexobarbital. In addition, when 60% of LD_{50} of each drug was orally administered to examine the onset time and duration of action of hypnotic action, PB, amobarbital, and hexobarbital showed 31, 2 and 1 minutes of onset time, respectively, and the duration of action was 476 ± 14, 89 ± 9, and 91 ± 11 minutes, respectively. In addition, when the effect of prolonged sleep on hexobarbital-induced sleep was examined after oral administration of PB (dosage: HD_{50}: 15, 30, and 60%), the mean sleep duration in the untreated group was 18.1 minutes at all doses, while PB administration prolonged to 30.5, 28.4, and 46.2 minutes, respectively (4.3.1.1-9).

3) Sedating effect

As a result of examination of the inhibitory effect of this drug subcutaneously in mice on hyperkinesia with iminodipropionitrile (IDPN)-induced torsional movement (torsion syndrome), at doses of 20 mg/kg or more, a decrease in activity and 50% decrease in torsional movement were observed, and at 40 mg/kg, a stronger sedative effect was observed, and the effect persisted for more than 3 hours (4.2.1.1-10).

For avoidance of conditions in rats, this drug reduced the avoidance reaction by 60% at a dose of 1/10 of LD_{50} (unknown route of administration) (4.2.1.1-11).

4) Mechanism of action

Anticonvulsants were administered intraperitoneally to mice, and the dose and latency time until the onset of tonic convulsions were investigated after continuous intravenous infusion of the convulsion inducer. As with phenytoin, carbamazepine, and diazepam, PB showed inhibitory action against bicuculline and picrotoxin-induced convulsions, but not against N-methyl-D-aspartate (NMDA)-induced convulsions (4.3.1.1-13).

When the effects of either PB or diazepam on the burst firing of $GABA_A$ receptors were examined by patch clamping of murine fetal cultured neurons, PB did not increase the frequency of opening of GABA-induced chloride channels and prolonged the opening time, whereas diazepam increased the frequency of opening of GABA-induced chloride channels but did not affect the time of opening (4.3.1.1-14).

5) Metabolites

Intravenous administration of p-hydroxyphenobarbital (PHPB), the major metabolite of PB, did not affect clinical symptoms in dogs (100 mg/kg) or mice (500 mg/kg), but 1000 mg/kg IV in mice resulted in sedation, shaking of hands and feet, difficulty in reduction, and disappeared effects several minutes later (4.3.2.4-6).

(2) Secondary pharmacology study

In mice, 150 mg/kg of this drug was administered intraperitoneally 1 hour before the time of the stump, and when the brain creatine phosphate, ATP, glucose, glycogen, and lactate were measured for 10 seconds after the time of the stump, the variation of all parameters was suppressed by the administration of this drug. (4.3.1.2-1)

(3) Safety pharmacology studies

With regard to the effects on general condition, when subcutaneous administration of PB was performed in mice, motor suppression was observed in 2/8 cases at 50 mg/kg and 3/8 cases at 60 mg/kg, ataxia in 6/8 cases at 75 mg/kg and 8/8 cases at 100 mg/kg, and anesthesia was observed in 4/8 cases at 100 mg/kg (4.3.1.3-1). Intramuscular administration of this drug (150 mg/kg or 170 mg/kg) to rabbits resulted in relaxation and bradykinesia of the muscles throughout the body, resulting in lying down and sleeping state. Corneal and light reflexes were absent, ear veins were dilated (followed by contraction), and respiratory rate decreased 30 - 150 minutes after dosing, resulting in a state of deep anesthesia. Body temperature decreased from 1 hour after administration, but there was a tendency for recovery from the event after 8 - 12 hours (4.3.1.3-2).

With regard to the effect on blood pressure, heart rate, and body temperature, when this drug was subcutaneously administered to rats, no changes in blood pressure were observed up to 7 mg/kg, but mild decrease in blood pressure was observed at 10 - 30 mg/kg (4.3.1.3-4). Intraperitoneal administration of this drug at 40 mg/kg did not affect blood pressure or heart rate, but mildly decreased rectal temperature from 40 - 80 minutes after administration (4.3.1.3-5).

Furthermore, although not the results of non-clinical studies, in humans (adults), when a large dose of oral or bolus intravenous administration of hypnotics including barbiturates is administered, mild increase in heart rate due to vagus nerve suppression and decrease in blood pressure due to peripheral vasodilation are observed, and respiratory action is caused by suppression of medullary function resulting in respiratory depression (4.3.1.3-3). Intravenous administration of barbiturates, including PB for anesthesia, causes cough, sneezing, and hiccups, and pharyngeal cramps (4.3.1.3-6). Regarding the effects on the gastrointestinal system, barbiturates including PB act on peripheral or central smooth muscle of the gastrointestinal tract by dose and suppress gastrointestinal motility, but anesthesia did not significantly slow the ability of gastric emptying in humans (4.3.1.3-6).

The following literature has been submitted for effects on juvenile animals:

Fluctuations in hemodynamic parameters were observed in porcine neonates (aged 1 - 3.5 days) following IV administration of PB at 15 or 45 mg/kg, resulting in increased partial pressure of carbon dioxide and decreased cerebral blood flow. The cerebral blood flow by region also showed decreased blood flow in the cerebrum, thalamus, brainstem, and cerebellum, and decreased cerebral oxygen consumption (4.3.1.3-7).

Regarding changes in blood pressure and blood gas pressure, the effect of this drug on changes in cerebral blood flow at the time of blood pressure increase with intravenous administration of phenylephrine and at the time of hypoxic load with 50% N_2 - 50% air gas aspiration was investigated in pig neonates (Days 1 to 2 after birth). Although there was no difference in changes in blood pressure or blood gas pressure between the 20 mg/kg intravenous administration group and the control group (intravenous saline), cerebral blood flow at each site of whole brain and brain was significantly increased in the control group when blood pressure increased and blood pressure increased, compared with the increase in the pemigatinib group when oxygen was applied (4.3.1.3-8).

Based on the above, the Applicant explained that this drug activates the $GABA_A$ receptor in the cranial nerve cells and causes an increase in the opening time of the chloride channel, has anticonvulsant effect in various animal convulsion models, has a hypnotic effect and sedative effect, and that high dose administration causes ataxia, sleep state, increased heart rate, decreased blood pressure, respiratory depression, and suppression of gastrointestinal motility, but these are events that are less likely to occur at higher doses than the clinical dose, and that these symptoms may occur within the range of the normal clinical dose.

<Summary of Review>

PMDA believes that there are no major pharmacological problems based on the fact that the pharmacological action of this drug is widely known, although the data submitted in this application are only from published literature.

(ii) Summary of pharmacokinetic study results

<Summary of submitted materials>

No new nonclinical pharmacokinetic studies have been conducted in this application, and a publication was submitted primarily for reference regarding the pharmacokinetics of intravenous administration and brain penetration of juvenile animals.

(1) Absorption

Following intravenous administration of this drug at 80 mg/kg to female rats, the serum PB concentration disappeared with an elimination half-life ($t_{1/2}$) of 11.1 ± 1.8 hours, and the volume of distribution (Vd) was 0.49 ± 0.085 L/kg (4.3.2.2-1).

Clearance, volume of distribution at steady state (V_{ss}), and $t_{1/2}$ were not dose-dependent and showed no difference when IV doses of 10 or 30 mg/kg of this drug were administered to adult (female) and neonatal (male and female) rabbits; however, in neonatal rabbits, total body clearance was lower than in adult rabbits and elimination was delayed. In addition, following oral or intravenous administration of 10 mg/kg of this drug to adult rabbits, the elimination pattern of PB concentration in serum was similar in both routes of administration, and BA was 119 % with oral administration (4.3.2.2-3).

Following intravenous or oral administration of this drug or PB 10 mg/kg to male and female dogs, plasma PB concentrations were 21 (0 hours post-dose) or 15 (maximum plasma concentration <C_{max}> 6 hours post-dose) μg/mL, respectively, and $t_{1/2}$ was 56 or 52 hours. The oral bioavailability (BA) was 91%, and the pharmacokinetics of PB were similar for both routes of administration (4.3.2.2-2).

After repeated oral administration of 2 mg/kg of this drug (actual dose: 2.06 ± 0.13 mg/kg) three times daily for 6 days to male and female dogs, the serum PB concentration after the final dose reached C_{max} at 3.2 hours after the final dose (13.2 to 24.2 μg/mL), and decreased at 53 hours $t_{1/2}$ (4.3.2.2-4).

(2) Distribution

When 30 or 50 mg/kg of this drug was intravenously administered to male rats, the highest distribution was observed in the liver, and it was also rapidly distributed in the kidney. Distribution to the brain, muscles, and intestines was slow, with brain penetration being the highest at 1 hour post-dosing, and migration was delayed. Re-elevation of PB levels was observed in plasma, liver, and kidney, suggesting involvement of enterohepatic circulation in tissue distribution (4.3.2.3-1).

Following intravenous administration of 20 or 50 mg/kg of this drug (as PB) to female rabbits, the tissue PB concentrations at 5 minutes after administration were in the following order: liver, plasma, heart, kidney, lungs, muscles, and brain. The whole blood PB concentration is similar to the plasma PB concentration, and the penetration of PB into blood cells is expected to be low (4.3.2.3-2).

The blood-brain barrier has been reported to be formed by capillary endothelial cells in the brain (4.3.2.3-5).

In rats, the brain capillary lumen began to form on day 1 after birth, and endothelial cell formation was observed on day 9, and the capillary omentum was completed on day 30 after birth, and the blood-brain barrier in rats was presumed to develop its function at about 1 month after birth (4.3.2.3-6).

Following IV administration of ^{14}C-PB to rabbits (not known as male or female), uptake of PB into the brain parenchyma was higher than in adult animals by 14 days of age, then declined rapidly by 28 days of age, and was at the same level as in adult animals by 28 days of age, suggesting that the rat blood-brain barrier was formed and functioned by approximately 30 days of age (4.3.2.3-7).

When PB was administered intraperitoneally to pregnant rats at 30 mg/kg, PB quickly passed into the fetus, and its changes were similar to those of plasma PB concentration in the mother (4.3.2.3-3).

When ^{3}H-PB was intravenously administered to male rats and showed an inhibitory effect on convulsions due to maximum electroconvulsive action, the brain and plasma PB concentrations were 8.2 and 16.9 μg/mL (median) 3 minutes after the end of administration, respectively, and 61.6% of the plasma PB was unbound PB (4.3.2.3-4).

Protein binding of PB to bovine serum albumin and rabbit plasma (ultrafiltration method) was 44 and 41%. In addition, the binding to liver, heart, kidney, lungs, brain, muscle, and red blood cells ranged from 14 to 32%, and no binding to specific tissues was observed (4.3.2.3-2).

When serum PB concentrations were 15 and 60 μg/mL in neonatal and adult rabbits, mean unbound PB concentrations were 0.72 and 0.68% in neonates and 0.40 and 0.46% in adult animals, respectively, and binding of PB to serum proteins was higher in adult animals than in neonates (4.3.2.2-3).

(3) Metabolism

In addition to the major metabolite p-hydroxyphenobarbital (PHPB), PB-N-glucopyranoside was detected in mouse urine, and 5-(3,4-dihydroxy- 1,5-cyclohexadien-1-yl)-5-ethylbarbituric acid and 5-(1-hydroxyethyl)-5-phenylbarbituric acid were detected in rat and guinea pig urine. In the urine of rats and guinea pigs, 5-(3,4-dihydroxy- 1,5-cyclohexadien-1-yl)-5-ethylbarbituric acid and 5-(1-hydroxyethyl)-5-phenylbarbituric acid were detected (4.3.2.4-1, 4.3.2.4-2, 4.3.2.4-3, 4.3.2.4-4).

Following intraperitoneal administration of ^{14}C-PB at 50 mg/kg to female rats, urinary excretion up to 24 hours after administration was 14 % unchanged drug, 16 % unchanged drug, 9% glucuronide of PHPB, and 4 % unknown acidic water-soluble metabolites. In addition, it is estimated that open ring causes ethylphenylmalonylurea (4.3.2.4-5).

In female dogs following multiple oral doses of PB from 10 - 40 mg/kg and examination of urinary metabolites, the major metabolite was PPHPB, with 67% to 83% of the PPHPB excreted in urine estimated to be glucuronide conjugates (4.3.2.4-6).

The drug metabolizing enzyme was induced by PB administration, and hepatic drug metabolizing enzymes such as CYP molecular species (CYP1A2, CYP2A6, CYP2B, CYP2C (CYP2C9, CYP2C19) and CYP3A) and UDP-glucuronosyltransferace (UGTs) were induced (4.3.2.4-8, 4.3.2.4-9, 4.3.2.4-10, 4.3.2.4-11).

When 4.73 mg/day of this drug was repeatedly administered to male rats (maturity: 3 to 4 months old, age: 32 to 34 months old) for 5 days, the total plasma PB clearance was 83 and 54% higher in adult rats and elderly rats, respectively, than in single dose (20 mg/kg) (4.3.2.4-12).

After a single oral dose of 5.5 or 15 mg/kg/day of PB in male and female dogs, and repeated oral doses of 5.5 or 11 mg/kg/day for 90 days after a 35-day rest period, the total clearance of PB in serum was higher than that after single dose in both dose groups, and the $t_{1/2}$ was shortened (4.3.2.4-13).

Following oral administration of PB to male dogs at 15 to 400 mg/kg, the total clearance of plasma PB was high with increasing dose, and $t_{1/2}$ was shortened (4.3.2.4-14).

These publications explain that repeat administration of this drug may lead to autometabolizing enzyme induction.

For effects on metabolism in neonates, the following documents have been submitted.

The active expression of hepatic drug metabolizing enzymes in rats rapidly developed after birth, peaking at 30 to 60 days of age, and then gradually declined. In neonatal rats 1 and 5 days of age, drug metabolism enzyme activity (Aminopyrine N-demethylation and Pentobarbital hydroxylation) was approximately 2 to 3% and 14% to 20% of patients aged 30 days, with approximately 60% of patients aged 30 days reached at 20 days (4.3.2.4-15, 4.3.2.4-16, 4.3.2.4-17, 4.3.2.4-18).

In rats, the postnatal development pattern of P-450 varies markedly depending on the CYP molecular species (4.3.2.4-19), but many CYP molecular species (CYP2B1, CYP2B2, CYP1A2, CYP3A2) were present at birth and rapidly increased after birth (4.3.2.4-20, 4.3.2.4-21, 4.3.2.4-22, 4.3.2.4-23). In addition, some CYP molecular species (CYP2C11, CYP2C12, and CYP2C7) have been reported to be expressed in the post-natal period (4.3.2.4-24, 4.3.2.4-25).

Induction of hepatic microsomal drug metabolizing enzymes at 3, 6, and 16 days of age and maturation was examined in neonatal and adult rats aged 0, 2, and 12 days after intraperitoneal administration of PB at 80 mg/kg. Although PB administration showed an increase in total P-450 content and induction of drug metabolizing enzymes in all aged rats, the benzpilene hydroxylation activity was approximately 2-fold higher in neonatal rats, but only slightly higher in adult animals, and the benzfetamine N-demethylation activity was 2- to 3-fold higher in adult animals compared with lower in neonates. In addition, unlike in adult animals, aldrin epoxy activity was hardly induced by PB in neonates (Days 3 and 6). Inhibition of benzphetamine N-demethyltransferase in PB-induced neonatal liver microsomes by mature rat PB-induced P-450 antibodies was hardly observed at 3 days of age, but the inhibition rate increased to 30-50 % with increasing age. This suggests that benzphetamine N-demethylation induced by PB in neonatal rats up to several weeks of age is metabolized by a different CYP molecular species than that induced in mature rats (4.3.2.4-26).

(4) Excretion

Following a single dose of ^{14}C-PB at 50 mg/kg in the abdominal cavity in female rats, 79% of the administered radioactivity was excreted in the urine and 9% was excreted in the feces within 8 days after dosing, and the main elimination route was urine. 0.14% of the administered radioactivity was excreted in the breath (4.3.2.5-1).

Following a single 75 mg/kg IV dose of ^{14}C-PB to male rats with bile duct cannulas, 18% of the administered radioactivity was excreted in the bile by 6 hours after dosing (4.3.2.5-2).

Following a single 80 mg/kg intraperitoneal dose of ^{14}C-PB in male rats with bile duct cannulas, 50 to 65% of the administered radioactivity was excreted mainly as conjugates in bile and the remainder was mainly excreted as unchanged drug in urine (4.3.2.5-3).

Based on the above, the Applicant explained that there is no major difference in pharmacokinetics of this drug between intravenous and oral administration, but drug metabolism and blood-brain barrier are undeveloped in neonates, and drug concentration of this drug may increase.

<Summary of Review>

Based on the submitted data, PMDA requested the Applicant to discuss pharmacokinetics in adult and juvenile animals and to discuss the efficacy and safety in neonates and children who are the subjects of administration of this drug.

The Applicant explained that with regard to pharmacokinetics in young animals, the Applicant states that the hepatic drug-metabolizing enzyme system is underdeveloped at birth, and that the expression and production of CYP molecular species reach the levels of mature animals by 30-60 days of age, and that the blood-brain barrier is immature in young animals. This suggests that the uptake of PB into the brain parenchyma may be higher, which may enhance the expression of the pharmacological anticonvulsant effect and increase the incidence of adverse events, and that PB administration induces drug-metabolizing enzymes in young animals as well as in mature animals. However, as it has been reported that induced CYP molecular species differ qualitatively or quantitatively from those in mature animals during development (4.3.2.4-26), repeated administration of this drug to young animals may result in a different metabolite balance in PB than in mature animals. Based on the above, the Applicant explained that "in particular, it is desirable to monitor blood concentration in neonates" in the section of "Precautions for use" in the package insert.

PMDA believes that the pharmacological action of this drug is stronger than that in adults and that many adverse events may occur in neonates. Therefore, it is necessary to pay sufficient attention to the administration of this drug, but it is necessary to make a decision based on the results of clinical studies in detail.

(iii) Summary of Toxicology Results

<Summary of submitted materials>

Only the vascular tolerance study in rabbits was submitted as a new toxicity study conducted in this application. Additionally, publications on single-dose toxicity studies and repeat-dose toxicity studies in neonates were submitted for reference.

(1) Single-dose toxicity study

LD_{50} was 340 mg/kg when this drug was administered intraperitoneally in mice, and hypnosis, depression, etc. were observed as general conditions after administration (4.3.3.1-1). The LD_{50} after oral administration of PB to mice was 325 mg/kg (4.3.3.1-2).

LD_{50} was 190 mg/kg when this drug was administered intraperitoneally to rats, and hypnosis, depression, etc. were observed as general conditions after administration (4.3.3.1-1).

Reductions in body weight, food consumption, and water consumption were observed in rats following rectal or oral administration of PB 150 to 400 mg/kg with LD_{50} ± standard error of 284 ± 38 or 304 ± 36 mg/kg, respectively (4.3.3.1-3).

Following single oral or subcutaneous administration of this drug to neonatal rats (1 day old) and 100 day old rats, the LD_{50} was 121 and 487 mg/kg after single oral administration and 83 and 210 mg/kg after subcutaneous administration, respectively, and these values were lower in neonatal rats than in 100 day old rats, but these were considered to be due to differences in drug metabolism due to maturity. In addition, after repeated oral or subcutaneous administration of 4 mg/kg of this drug to dams from gestation day 15 to delivery, the LD_{50} of this drug after single oral administration or subcutaneous administration of this drug in the neonate (1 day old) was 362 and 396 mg/kg after oral administration and 142 and 132 mg/kg after subcutaneous administration, respectively, and the LD_{50} was similar to that of 100 day old rats. Since the LD_{50} of the neonatal rats did not differ from the untreated group after multiple oral administration of this drug to the maternal animals on or before day 14 of gestation, this drug is thought to be caused by insufficient expression of drug metabolizing enzymes when administered in the second half of gestation to promote induction of drug metabolizing

enzymes and enhance PB processing capacity in the newborn. However, if it is administered before that, liver organogenesis was immature (4.3.3.1-5).

The LD_{50} after a single intravenous dose of this drug in rabbits was 185 mg/kg, and clinical signs after administration included hypnosis and depression (4.3.3.1-1).

Following a single oral dose of 125 to 200 mg/kg of this drug in cats, the LD_{50} was 175 mg/kg, and ataxia and hypnosis were observed as the clinical signs after administration (4.3.3.1-4).

Based on the above, the Applicant explained that there is no significant difference in lethal dose due to the species and route of administration for both this drug and PB, and that the lethal dose of this drug is lower in juvenile animals than in adult animals, and that excessive pharmacological action may cause a stronger toxicity than in adult animals.

(2) Repeat-dose toxicity study

Repeated subcutaneous administration of PB 30 or 60 mg/kg to neonatal rats (3 days of age) from 3 to 21 days after birth resulted in mortality in 8.3% of the 30 mg/kg group and 10.9% of the 60 mg/kg group. Increases in body weight were decreased in the 60 mg/kg group, brain weight (cerebrum and cerebellum), cholesterol decreased in the cerebellum and cerebellum, and DNA, RNA, and protein decreased in the cerebellum, but no difference was observed in the 30 mg/kg group compared to the control group (4.3.3.2-1).

In neonatal rats (4 days of age) that were placed via gastric cannula and forcibly fed, multiple subcutaneous doses of PB 60 mg/kg were administered from 5 to 18 days of age, and there were no deaths, and body weight was not different from the untreated group, but brain weight was significantly increased suppressed compared to the control group, and there was a decrease in the cerebrum and cerebellum RNA, cholesterol, and cerebral DNA, protein (4.3.3.2-2).

Based on the above, the Applicant explained that the brain of the rat newborn is considered to be highly sensitive to PB.

(3) Local tolerance study (4.2.3.6-1)

A solution of 10% of this drug was administered to the auricular veins of male rabbits (5 cases), and 5% phenytoin was administered as a control, and 5% sulfobromophthalein sodium (BSP) was administered in 0.05 mL twice daily for 8 consecutive days as a positive control, and the irritability to blood vessels was examined by using clips. Thrombus formation was observed in macroscopic findings from the first dose in the phenytoin and BSP treatment groups, whereas in the pemigatinib treatment group, thrombus formation was observed from Day 6 of administration, and the incidence and seriousness of the event were highest in BSP, followed by phenytoin and pemigatinib. In addition, the degree of inflammatory changes such as redness and swelling was also strongest in BSP, followed by phenytoin and this drug. Histopathological examination at the injection site showed thrombus, necrosis of the vascular wall, perivascular inflammatory cell infiltration, and fibrosis, and the extent of which, as with macroscopic findings, BSP was strongest, with phenytoin and this drug in that order.

Based on the above, the Applicant explained that the vascular irritation of this drug is considered to be weaker than that of BSP and phenytoin.

(4) Other studies (4.3.3.7-1 - 4.3.3.7-19)

It has been reported that when PB is administered to young animals including perinatally, various metabolic enzyme and hormone secretion abnormalities are continuously observed even after maturation. It is also considered necessary to use the drug in clinical practice with caution when administering long-term and excessive doses to neonates.

While PB has been reported to act as a non-genotoxic carcinogen, there is a threshold for the carcinogenic effect of PB, and at low doses, it has been reported that it has been shown to inhibit carcinogenesis.

<Summary of Review>

PMDA should note that the effect and toxicity of this drug are more potent in juvenile animals than in adult animals, but it is considered necessary to determine the details based on the clinical study results.

4. Clinical materials

(i) Summary of Clinical Pharmacokinetic and Pharmacodynamic Studies

<Summary of submitted materials>

No new clinical pharmacokinetic studies were conducted at the time of this application, and plasma concentration data measured in the investigator-initiated clinical trial (5.3.5.2-1) were submitted. Also, for reference, published literature on the pharmacokinetics of status epilepticus patients or neonatal convulsions in healthy adults and children in Japan and overseas was submitted. In an investigator-initiated clinical trial (5.3.5.2-1), the plasma phenobarbital (PB) concentration was measured using phenobarbital kit "[redacted]" ([redacted]) by fluorescence polarization immunoassay (FPIA), and the lower limit of quantification was 1.1 μg/mL. Pharmacokinetic parameters are presented as mean or mean ± standard deviation, unless otherwise stated.

(1) Plasma concentration data in investigator-initiated clinical trials (5.3.5.2-1)

10 infants with neonatal seizures (gestational weeks: 33.1-41.1 weeks, postnatal age: 1-5 days) were targeted for the study, and 20 mg/kg of this drug was administered intravenously as the initial dose, 20 mg/kg was added if convulsions did not resolve (additional dose I), followed by 5-20 mg/kg was administered additionally (additional dose II) depending on symptoms for patients who were not considered to be inactive (maintenance dose of 2.5-5 mg/kg per day, 6 days) Blood PB concentration at 2 hours after the first dose was 22.4 ± 2.3 μg/mL (range: 18.8 to 25.8 μg/mL) in the 9 subjects who did not receive additional dose I and 42.8 μg/mL in the 1 subject who received additional dose I. 9 maintenance doses were administered, and the first 2-hour maintenance dose blood concentration was 25.3 ± 4.8 μg/mL (range: 20.7 to 36.6 μg/mL), and the last maintenance dose was 27.7 ± 9.5 μg/mL (range: 18.7 to 45.3 μg/mL).

In addition, the following documents were submitted from published literature, etc.

(2) Bioavelability (BA)

Following intramuscular and oral administration of PB, intravenous administration of this drug, and rectal administration in Japanese epilepsy patients (8 to 20 years old), BA was 101, 96, and 80% for intramuscular administration, oral administration, and rectal administration, respectively, and no significant difference was observed in each route of administration (5.4.2.5.3-4).

When the pharmacokinetics of intravenous and intramuscular administration of this drug were compared between healthy non-Japanese adults (19 - 37 years old) and patients with epilepsy (19 - 28 years old), the clearance was slightly higher in epilepsy patients than in healthy adults, but no significant difference was observed. In addition, BA was 101 and 100% when administered

intramuscularly and orally in healthy adults, respectively (5.4.2.4.3-15).

When this drug was intravenously or orally administered to non-Japanese healthy adults (aged 24 - 44 years), the pharmacokinetic parameters of both routes of administration were similar, and the BA was 83.4% when administered orally (5.4.2.4.3-12).

(3) Absorption

In 25 Japanese neonatal patients with seizures, when PB was administered intravenously or intramuscularly at an initial dose of 20-25 mg/kg over 20 minutes within 5 days of birth, followed by a maintenance dose of 1.5 mg/kg intravenously or intramuscularly every 12 hours, the pharmacokinetics of both routes of administration were similar. The plasma PB concentration immediately after the first intravenous dose (mean ± standard deviation at 4 hours and 12 hours post-dose) was 26.4 ± 4.9 μg/mL, and the C_{max} for intramuscular administration was 25.9 ± 3.2 μg/mL. The elimination half-life ($t_{1/2}$) was estimated to be 180 - 200 hours in the first 2 - 3 days and 70 - 90 hours in the first 14 - 17 days of life, and decreased with age (5.4.2.5.1-3).

In 29 Japanese infants with neonatal seizures, this drug was administered intravenously at 5 or 10 mg/kg in 4 infants weighing less than 1 kg and at 10 to 20 mg/kg in 25 infants weighing 1 kg or more. In 21 out of 22 children who weighed 1 kg or more and received 20 mg/kg, the effective blood concentration (15 μg/mL) or higher was achieved in all cases (Patients weighing less than 1 kg were excluded from the analysis due to their high variability) (5.4.2.5.1-1).

Following intravenous administration of PB 10 to 20 mg/kg (6 subjects) or rectal administration of PB 5 to 13 mg/kg (6 subjects) to 12 children with convulsion in Japanese neonates and infants, the maximum observed serum PB concentration (C_{max}) after administration reached 28 ± 14.8 μg/mL (minimum, 15.6; maximum, 60.5 μg/mL) in the intravenous group, and convulsion resolution and seizure prophylaxis were observed. In addition, the C_{max} in the rectal administration group was 10.3 ± 3.4 μg/mL (minimum 4.1, maximum 14.2 μg/mL) (5.4.2.5.3-2).

When PB was administered intravenously to foreign neonates (0-4 weeks of age <14 cases), infants (2-3 months of age <16 cases> or 4-12 months of age <14 cases>), and children (2-5 years of age <7 cases>) at doses of 5-10 mg/kg for neonates and 10 mg/kg for infants and children, $t_{1/2}$ was 118.6 ± 16.1 hours for neonates, 62.9 ± 5.2 hours for infants aged 2-3 months, 63.2 ± 4.2 hours for infants aged 4-12 months, and 68.5 ± 3.2 hours for children. The $t_{1/2}$ in neonates was significantly longer than that in infants and children. Vd at steady state was 0.850 to 0.857 L/kg in neonates and infants 2 - 3 months of age, and 0.570 to 0.666 L/kg in infants and children 4 - 12 months of age, suggesting that the clearance of PB may be delayed due to limited enzymatic treatment in the liver in neonates and limited renal glomerular filtration and tubular excretion (5.4.2.4.3-8).

Adult non-Japanese subjects with epilepsy (6 subjects) received 90 mg of this drug intravenously on the start date, 90 mg/kg of this drug on Days 2-28, and 90 mg/kg to 150 mg/kg of this drug on Days 36-83. Multiple doses of ^{13}C and ^{15}N (this drug) 90 mg/kg IV did not affect Vd, $t_{1/2}$, and CL on Days 29-35 (Week 4) and Days 84-91 (Week 12) (Section 5.4.2.4.3-3).

(4) Distribution

Tissue distribution of PB in neonates and children (15 subjects) was examined, and the ratio of PB concentration in each tissue to plasma was highest in the liver, and was lower in the cerebellum and cerebellum than in other organs. Tissue and plasma PB concentrations correlated, and the correlation coefficients in the cerebellum and cerebellum were 0.82 and 0.81 (5.4.2.5.3-1).

As a result of examining the distribution of PB in tissues of neonate patients (77 patients) and those who died during treatment for neonatal convulsion (17 patients), Vd and $t_{1/2}$ were not affected by gestational age. The PB concentration ratios in each tissue and plasma were 4.3, 6.4, and 2.6 in the liver, heart, and muscle, respectively, and higher than in the brain. In addition, PB concentration ratio of gray matter and white matter in 3 cases measured by separating the gray matter and white matter in the brain was 0.86 to 1.11, respectively, and similar distribution between gray matter and white matter was observed (5.4.2.4.3-13).

PB equivalent to 95% of the serum concentration was detected in the umbilical cord blood during delivery in pregnant women treated with PB, and placental permeability was demonstrated (5.4.2.4.3-10).

The concentration ratio of breast milk to serum PB in lactating women receiving primidone was 0.41, and excretion into breast milk was observed. In addition, the metabolite p-hydroxyphenobarbital (PNPB) concentrations were similar in serum and breast milk (5.2.4.3-11).

Protein binding of PB in neonates and children tended to be lower in the population from 0 - 3 months of age than in the other populations (from 4 months to 2 years of age and from 2 - 15 years of age), with younger children showing a lower trend. In addition, blood albumin concentrations were also low in the population from 0 - 3 months of age, and a positive correlation was observed between PB protein binding and blood albumin concentrations (5.4.2.5.3-3).

The binding of PB to human serum α1 acid glycoprotein and human serum albumin was 5% or less and 27 - 43%, respectively (5.4.2.4.3-1).

Protein binding of PB in plasma of human fetuses, neonates, and healthy adults was examined, and human fetal and neonatal plasma showed a lower binding rate than in adults. Albumin levels were lower in neonates and fetuses compared to adults (5.4.2.4.3-4).

(5) Metabolism

The primary metabolite of this drug in humans is PHPB and its conjugate, and PB is Penobarbital N-glucosylated (PNG), and its metabolism to PPHPB is estimated to mainly involve CYP2C9. However, the metabolic enzymes involved in the metabolism to PNG are unknown (5.4.2.2.1.3-1, 5.4.2.2.1.3-3, 5.4.2.4.3-7). In addition, N-glucosylation activity is lately developed in neonates, but once activated, it has been suggested to be the main pathway of PB metabolism (5.4.2.4.3-2). It has been shown that PB administration induces drug metabolizing enzymes such as the P-450 species CYP1A, CYP2A6, CYP2B, CYP2C, and CYP3A and UGTs, and promotes the metabolism of drugs metabolized by these enzymes (5.4.2.2.2.3-1, 5.4.2.2.2.3-2).

(6) Excretion

The primary route of excretion of PB was urinary excretion, with 78-87% of PB administered within 16 days after oral administration of ^{14}C and ^{15}N-labeled bodies (PB) excreted (5.4.2.4.3-14).

(7) Drug interactions

It has been reported that CYP450 and UGTs, such as CYP1A, CYP2A6, CYP2B, CYP2C, CYP3A, etc. are induced by the enzyme inducing action of PB, and the pharmacological action and physiological activity of drugs, etc. metabolized by these enzymes are altered (5.4.2.2.1.3-4, 5.4.2.2.2.3-2, 5.4.2.4.3-6, 5.4.2.6.3-1). In addition, methylphenidate and valproate inhibit PB metabolism, and St John's Wort promote PB metabolism (5.4.2.2.1.3-5, 5.4.2.4.3-6, 5.4.2.6.3-1, 5.4.2.6.3-2). PB increases glucuronidation of thyroid hormones (T_3, T_4), decreases the concentration of unchanged drug, increases glucuronidation of bilirubin, and promotes biliary excretion of bilirubin, which is presumed to be caused by induction of UGTs by PB (5.4.2.2.1.3-4).

(8) Pharmacodynamic studies

When PB was administered intravenously or intramuscularly in neonates with seizures (38 cases), the initial dose of PB in cases of successful seizure control (12 cases) was 16.2 ± 2.6 mg/kg, and the blood concentration was 19.8 ± 2.7 μg/mL (range: 16.9-25.8 μg/mL), and the PB concentration was significantly higher ($p<0.002$, Student's t-test) than that in the uncontrolled cases (26 cases: initial dose 12.3 ± 3.3 mg/kg, blood concentration 14.3 ± 5.1 μg/mL), but there were a few cases in which the PB concentration was not controlled even when the blood concentration was 16 μg/mL or higher. The ratio of dose to blood concentration was not affected by route of administration, gestational age, or body weight (5.4.2.4.3-9).

In children with neonatal seizures (71 subjects, gestational age: 26-42 weeks, postnatal date: 1-143 days), PB was administered at a dose of 15 mg/kg intravenously as the initial dose, and in the case of seizures that could not be controlled or recurrence, an additional dose of 5-10 mg/kg was administered every 5 to 10 minutes. The plasma concentrations in the successfully controlled cases (60 cases) were 10.1 - 46.4 mg/L, with 36 cases at 20 mg/L or lower, 17 cases at 20 - 30 mg/L, and 7 cases at 30 mg/L or higher (5.4.2.4.3-5).

In children with status epilepticus (12 patients, 7 to 62 months of age), blood concentration was 10 μg/mL within 10 minutes of the start of administration in all cases after IV administration of this drug at a dose of 15 mg/kg as the initial dose, followed by administration of 5 mg/kg as the maintenance dose at 24 and 48 hours after the initial dose, and the blood concentration was maintained at a level of 15 μg/mL or higher within 10 minutes after the start of administration, and 8/12 cases were able to control seizures (5.4.2.4.2.1-3).

<Summary of Review>

(1) Pharmacokinetics and safety of this drug in neonates

Based on the results of the investigator-initiated clinical trial (5.3.5.2-1), PMDA asked the Applicant to explain the relationship between the pharmacokinetics, efficacy, and safety of the drug in neonates.

The Applicant first explained that there was no effect of birth weight, day age, or gestational week on blood PB concentration obtained in the investigator-initiated clinical trial. The Applicant then compared the background factors of one patient (Case No. OKA01), who had a high PB concentration (45.3 μg/mL) immediately before the last dose of maintenance treatment, with the other nine patients, and found that the birth weight was 3530 g, which was higher than the median (3052 g). However, The height and the number of weeks of pregnancy were equivalent to the median values, the blood PB concentrations at 2 hours after the first dose and at 2 hours after the first maintenance dose were 22.2 and 23.9 μg/mL, respectively, and there were no problems in the handling of the specimens. The reason for the high blood PB concentration in this case is not clear, but it was explained that the maintenance dose was started at a relatively high dose (5 mg/kg/day for the first three maintenance doses and 2.5 mg/kg/day for the fourth to sixth maintenance doses), and that he thought it might have been due to the longer $t_{1/2}$ immediately after birth in neonates (5.4.2.5.1-3). The Applicant also explained that the primary efficacy endpoint (efficacy of 30 minutes after the first dose (or after the additional dose (I))) was "complete response" in all cases with regard to the relationship between blood PB concentration and efficacy, and thus, a detailed investigation could not be made. The Applicant further stated that the relationship between blood PB concentration and safety was that in 7 patients, the blood PB concentration of the drug was 30 μg/ml. or less, but in 3 patients, the blood PB concentration exceeded 30 μg/mL (Case No. KNG02 <2 hours after the first dose: 18.8 μg/mL, 2 hours after the second additional dose (II): 36.9 μg/mL, 2 hours after the first maintenance dose: 36.6 μg/mL and before the last maintenance dose: 31.8 μg/mL>, Case No. KNG05 <additional dose (I) after 2 hours: 42.8 μg/mL, at the time of discontinuation: 41.9 μg/mL> and Case No. OKA01 <initial dose 2

hours: 22.2 μg/mL, after the first maintenance dose 2 hours: (2 hours after the first dose: 22.2 μg/mL, 2 hours after the first maintenance dose: 23.9 μg/mL, and before the last maintenance dose: 45.3 μg/mL), the safety profile was not significantly different from the other cases. It is conceivable that the transfer to the brain may be more enhanced than in adults (Bourin M et al. *Dev Pharmacol Ther*, 7: 171-176, 1984, Vajda F et al, *Clin Pharmacol Ther*, 15: 597-603, 1974), and that metabolic enzymes are underdeveloped, and the Applicant explained that since there is a possibility that the clearance of the drug may be low (5.4.2.4.3-2), the package insert states that "it is desirable to monitor blood levels in pediatric patients" and cautions against this.

PMDA considers that the safety profile was not significantly different even in patients with high blood concentration at the dosage and administration in the study in which blood concentration of this drug was measured despite the limited sample size in the investigator-initiated clinical trial (5.3.5.2-1). However, based on the results of the published literature, etc., when this drug is administered to neonates, the blood concentration and intracerebral transfer potential may be high, and if this drug is administered to neonates, the dose should be carefully adjusted while monitoring the patient's condition.

(ii)Summary of efficacy and safety study results

<Summary of submitted materials>

In this application, the results of the investigator-initiated clinical trial (5.3.5.2-1) were submitted as materials for evaluation of efficacy and safety in neonatal convulsions. In addition, publications, guidelines, and completion of clinical studies in Japan and overseas were submitted as reference materials for neonatal convulsions and status epilepticus.

(1) Neonatal convulsion

1) Investigator-initiated clinical trial (5.3.5.2-1 Study Number JNR-NP [March 2006 to March 2007])

An open-label, uncontrolled study was conducted to investigate the efficacy, safety, and pharmacokinetics of this drug in Japanese children with neonatal convulsion (target number of cases: 30).

The dosage and administration in Period I was determined to be 20 mg/kg of this drug administered intravenously over 5 - 10 minutes as the first dose, and if convulsions do not disappear after the first dose, additional administration (I) of this drug at 20 mg/kg was administered intravenously over 5 - 10 minutes as an additional dose 30 minutes after the first dose. In Period II, it was set that as an additional administration (II), 5 - 20 mg/kg of this drug can be administered intravenously over 5 - 10 minutes as needed within a range that does not exceed 40 μg/mL of the blood concentration of this drug to subjects who were determined to have a significant response or efficacy to this drug in Period I. For maintenance dosing[2], 2.5 - 5 mg/kg of this drug was administered intravenously once daily (however, if the blood concentration of this drug exceeded 50 μg/mL, one maintenance dose was avoided), and the maximum duration of administration was set at 6 days (for pharmacokinetics, see "(i) Summary of Clinical Pharmacokinetics and Clinical Pharmacodynamics Study Results"). (For pharmacokinetics, see (i) Summary of clinical pharmacokinetics and pharmacodynamics study results).

All 10 cases of total administration were included in the efficacy and safety analysis set, with 10 cases for the initial administration, 1 case for additional administration (I), 1 case for additional administration (II), 9 cases for maintenance administration, and 8 cases for completion of maintenance administration.

2) The maintenance dose was started after 24 hours from the time of the first dose, additional dose (I), or additional dose (II), whichever was later

The primary efficacy endpoint, which is marked efficiency at 30 minutes after the first dose or additional dose (I), was 100.0% (10/10 subjects).[3]

Adverse events (including laboratory test abnormalities) were observed at 90.0% (9/10 subjects), but there were no deaths. Other serious adverse events were observed in 1 case (1 case of patent ductus arteriosus), but a causal relationship with this drug has been denied. There were no cases of discontinuation due to AEs. Adverse events for which a causal relationship could not be ruled out were observed in 60.0% (6/10 cases), including respiratory depression in 3 cases, oxygen saturation decreased in 2 cases, bradycardia, bronchial secretion increased, blood pressure decreased, body temperature decreased, and urine volume decreased in 1 case each.

Vital signs showed maximal reduction in both systolic and diastolic blood pressure at 1 hour after the first or second dose (I) and significant reduction in systolic blood pressure at 1 and 2 hours after the first or second dose (I) compared to predose. Respiratory rate and heart rate were lower than pre-dose throughout treatment.

Based on the above, the investigator-initiated clinical trial conducted the clinical trial showed efficacy for neonatal convulsion by administering this drug at doses of 2.5 to 5 mg/kg as an additional dose and maintenance dose within a range not exceeding the initial dose of 20 mg/kg and blood concentration of 40 μg/mL, and explained that there were no particular safety issues other than adverse events expected from the pharmacological action of this drug.

2) Text in textbooks, etc. that are international standards

Textbooks that are international standards state that when this drug or PB is used for neonatal convulsions, the first dose of 20 mg can be administered intravenously, with a maximum dose of 40 to 50 mg/kg, the maintenance dose of 2 to 5 mg/kg/day is administered intravenously, intramuscularly, or orally, and the therapeutic range blood concentration is 20 to 40 μg/mL. For the timing of discontinuation of maintenance therapy, although there are no clearly specified criteria, it is stipulated that phenobarbital should be discontinued as soon as possible after neurological examination is normal or if convulsions can be controlled (5.4.2.1.1.1-5, 5.4.2.1.1.1-2, 5.4.2.1.1.1-4). In the textbook (5.4.2.1.2.1-2, 5.4.2.1.2.1-3, and 5.4.2.1.2.1-1) in Japan, 15 - 20 mg/kg was administered intramuscularly or rectally as the initial dose, and if the effect is insufficient, the same dose was administered additionally, 2.5 - 5 mg/kg/day was administered intramuscularly or orally (suppository is acceptable), and the therapeutic range blood concentration was described as 15 - 25 (40) μ g/mL.

[Submitted materials]

(Overseas)

5.4.2.1.1.1-3, 5.4.2.1.1.3-1: Behrman RE, Kliegman RM, Jason HB editors, *Nelson Textbook of Pediatrics (17th edition)*, Saunders, 566-568, 2005-2009, 2004

5.4.2.1.1.1-5: Swaiman KF, Ashwal S, Ferriero DM editors, Pediatric Neurology -*Principles & Practice- (4th edition)*, Mosby Elsevier, 269-277, 2006

5.4.2.1.1.1-1 , 5.4.2.1.1.1-2: McDonald MG, Seshia MM, Mullett MD editors, *Avery's Nephrology - Pathophysiology & Management of the Newborn - (6th edition)*, Lippincott Williams & Wilkins, 1385-1409, Weeping, 1521-1556, 2005

3) Marked efficiency (%) = (number of subjects with significant effect/number of subjects) × 100, "complete effect": disappearance of neonatal convulsion

5.4.2.1.1.1-6: Rennie JM editor, *Roberton's Textbook of Neonatology (4th edition)*, Churchill Livingstone, 1114-1119, 2005

5.4.2.1.1.1-4: Volpe JJ editor, *Neurology of the Newborn (4th edition)*, Saunders, 202-214, 2001 (Domestic)

5.4.2.1.2.1-2; 5.4.2.1.2.1-3: Toshio Kawano, Bedside Neonatal Care, Nanzando, 242-245, 392-394, 2004

5.4.2.1.2.1-1: Yuya Ogawa, Yutaka Tada, Yutaka Nakamura, Hiroshi Nishida, Edited, Neonatal Science, Medica, 973-975, 2000

3) Descriptions in the foreign pharmaceutical formulary, review articles, etc.

The use of pemigatinib or PB for neonatal convulsions is described as the initial dose of 15 to 20 mg/kg (≤1 mg/kg/min) by slow intravenous infusion, the maintenance dose of 2.5 to 5 mg/kg by intravenous infusion once or twice daily, and a therapeutic range of 15 to 40 μg/mL.

[Submitted materials]

5.4.2.1.3.3-3: Sweetman SC editor, *Martindale (35th edition)*, Pharmaceutical Press, 444-446, 2007 5.4.2.1.3.3-2: *British National Formulary for Children (BNFC)*, BMJ Publishing Group and RPS Publishing, 261, 272, 2006

5.4.2.1.3.3-4; 5.4.2.1.3.3-5: *Rote Liste*, Editio Candidate Verlag, 15 001-15 002, 170-172, 2006

5.4.2.1.4.3-1: Takemoto CK, Hodding JH, Kraus DM editors, *Pediatric Dosage Handbook (12th edition)*, Lexi-Comp, 1000-1003, 2005

5.4.2.2.1.1-2: Rennie JM et al, *Curr Opin Neurol*, 16: 177-181, 2003

5.4.2.2.1.1-1: Painter MJ et al, *Curr Treat Options Neurol*, 3: 237-248, 2001 5.4.2.3.1-1: Booth D et al, *The Cochrane Database of Syst Rev*, 3: CD004218, 2004

4) Clinical report, etc.

A comparative controlled study of PB in neonatal convulsion conducted overseas (5.4.2.4.1-1, 5.4.2.4.1-9, 5.4.2.4.1-7), a study (5.4.2.4.1-4) that was referenced in the investigator-initiated study (5.3.5.2-1), and literature on the pharmacokinetics of intravenous administration of PB in neonatal convulsions (5.4.2.5.1-1, 5.4.2.5.1-3) were submitted in Japan.

① Voyager GB et al, *Neurology*, 62: 486-488, 2004 (5.4.2.4.1-1)

An open-label, randomized study was conducted to investigate the efficacy of additional lidocaine or midazolam in neonates who did not respond to PB.

The dosage and administration was randomly assigned to the midazolam group or lidocaine group if children with confirmed seizures in the EEG were administered up to 40 mg/kg of PB and responded within 12 hours after enrollment). The midazolam group was defined as giving a bolus of midazolam 60 μg/kg followed by a continuous infusion at a rate of 150 μg/kg/hr, with no reaction [4] within 12 hours, the infusion rate was increased to 300 μg/kg/hr; the lidocaine group was given a bolus of lidocaine 4 mg/kg, followed by a continuous infusion at a rate of 2 mg/kg/hr, followed by a continuous infusion at a rate of 4 mg/kg/hr, and no reaction [4] within 12 hours. In addition, if the parent/guardian refused to administer the assigned study drug, clonazepam was set to be administered.[4]

---

4) The subject was considered to have responded to the drug if the seizure activity on the electroencephalogram decreased completely or by at least 80% of the seizure activity prior to administration.

A total of 22 patients were treated, all of whom received the first-line drug PB. In addition, 3 subjects in the midazolam group, 5 subjects in the lidocaine group, and 3 subjects in the clonazepam group received the second-line drug.

Response cases, which is the primary efficacy endpoint[5] were 11/22 cases in the PB group, 3/5 cases in the lidocaine group, 0/3 cases in the midazolam group, and 0/3 cases in the clonazepam group. Blood concentration of the additional anticonvulsant was in the therapeutic range.

The safety of adverse events, etc. has not been evaluated in this document.

Based on the above, the efficacy of PB was suggested in approximately half of neonate children with convulsion.

② **Scher MS et al, *Pediatr Neurol*, 28: 277-280, 2003 (5.4.2.4.1-9)**

A randomized study was conducted to investigate the efficacy of intravenous PB and phenytoin in children with neonatal convulsions.

The dosage and administration was set to administer phenytoin if the initial drug was PB, "and to administer phenytoin if the PB was not at least 25 μg/mL (unbound concentration) and phenytoin was at least 3 μg/mL (unbound concentration).

There were 59 subjects who received the total dose (30 subjects in the initial PB group and 29 subjects in the phenytoin group). Seizure activity on the electroencephalogram was only observed in 9 patients, and seizure activity on the electroencephalogram and clinical seizure were observed in 50 patients before drug administration.

With respect to efficacy, the first anti-epileptic drug (PB or phenytoin) led to the disappearance of the seizures on the EEG and the clinical seizure in 24 of the 50 subjects with the seizure activity on the EEG and the clinical seizure. For the 26 subjects with sustained seizure activity or clinical seizures on the EEG, 5 subjects had resolved after the second administration of antiepileptic drugs, 10 subjects had 50% or more suppression, and 11 subjects had 50% or less suppression, and 15 subjects with 50% or more suppression of clinical seizures had sustained seizure activity on the EEG and were dissociated from clinical seizures. In addition, for the 9 subjects with only seizure activity on the EEG, seizure activity on the EEG was observed after administration in all the subjects.

The safety of adverse events, etc. has not been evaluated in this document.

The efficacy rates of PB and phenytoin respectively have not been evaluated.

③ **Painter MJ et al, *N Engl J Med*, 341: 485-489, 1999 (5.4.2.4.1-7)**

A randomized controlled study was conducted to investigate the efficacy and safety of PB and phenytoin in children with neonatal convulsions.

---

5) Patients whose seizure activity on the EEG completely disappeared or decreased by 80% or more in the seizure activity before administration

6) If the 24-hour EEG monitoring shows that the seizure or occupancy due to seizure activity for 2 minutes and 30 seconds is more than half the time, and the unbound concentration of PB and phenytoin is equal to or greater than 25 μg/mL and 3 μg/mL, respectively, it is considered that the patient did not respond to the first drug.

The dosage and administration was set to administer PB or phenytoin intravenously over 5 to 15 minutes as the initial dose, and to measure the unbound plasma concentration 30 minutes after the initial dose, and to administer additional doses of PB 25 μg/mL and phenytoin 3 μg/mL as the index. Thereafter, plasma concentration measurements were performed twice daily, and it was determined that the trough plasma concentration was 22.5 μg/mL for PB and 2.5 μg/mL for phenytoin were indexed and administered once daily. If the patient did not respond to each drug[6], phenytoin was set to be administered if the initial drug was PB, and PB was set to be administered if the initial drug was PB. In addition, if abnormal movements suggestive of seizure were not observed, treatment was discontinued after 7 days, and if clinical seizures persisted for more than 7 days or recurred after discontinuation of treatment, use of other anticonvulsants was set to be considered.

The total number of subjects who received the drug was 59. The first dose was PB in 30 subjects (PB group), and phenytoin in 29 subjects (phenytoin group).

Complete seizure control, an efficacy endpoint[7] was 13/30 subjects in the PB group and 13/29 subjects in the phenytoin group, and there was no significant difference between the groups (p = 1.00, Fisher's exact test).

There were no changes in heart rate, arrhythmia, mean arterial pressure, or respiratory status associated with plasma concentrations of PB or phenytoin with respect to adverse events (causal relationship not provided).

Based on the above, PB was suggested to be as effective as phenytoin for neonatal convulsion.

Regarding this report, the Applicant considers that the same cases as the ones in ② above may be possible based on the site, study design, and the identity of the target cases.

④ Gilman JT et al, *Pediatrics*, 83: 674-678, 1989 (5.4.2.4.1-4)

A clinical study was conducted to investigate the efficacy of PB and the reactivity of additional secondary antiepileptics in children with neonatal convulsions. There is no description of the study design in this document.

The dosing regimen was set to administer a first dose of PB 15 - 20 mg/kg IV over 5 - 15 minutes (first PB dose) with an additional dose of PB 5 - 10 mg/kg q 20 - 30 minutes (additional PB dose) until seizure resolution or serum concentration of 40 μg/mL was achieved. In addition, if seizures persisted, additional repeat doses (high-dose PB) of PB 5-10 mg/kg were established to reach a serum concentration of 100 μg/mL, and a series of PB doses was established to be generally completed within 6 hours. If seizures persisted on PB, the second anticonvulsant (phenytoin or lorazepam) was administered, and if the second anticonvulsant did not respond, the third or more anticonvulsant (phenytoin, lorazepam, paraaldehyde or valproate) was administered.

The total number of subjects treated was 120, with 120 subjects receiving the first dose of PB, 72 subjects receiving the additional PB, and 35 subjects receiving the high dose PB.

Responders, which are efficacy endpoints[8] were the number of subjects who received the first dose of PB, 48/120 subjects; the additional dose of PB, 37/72 subjects; and the high dose of PB, 7/35 subjects.

There were no systemic acute toxicities due to PB with respect to adverse events (causal relationship not provided). Several severely asphyxia subjects had sedation and reduced eating when their serum PB concentration exceeded 50 μg/mL, but this event resolved with the decrease in PB concentration in serum.

Based on the above, adjustment of the serum PB concentration to 40 μg/mL indicated the efficacy and safety of PB for neonatal convulsions, and it was suggested that a second antiepileptic drug should be administered if the effect was insufficient at 40 μg/mL.

---

7) Complete seizure control e.g., complete resolution of seizure activity on EEG

8) Responders: Subjects whose seizures disappeared within 1 hour after the last dose of antiepileptic drugs

⑤ Yukihiro Takebe et al., Journal of the Japanese Society for Neonatal Research, 24: 84-90, 1988 (5.4.2.5.1-3)

An open-label study was conducted to investigate the efficacy and pharmacokinetics of PB in children with neonatal convulsions (for pharmacokinetics, refer to the "(i) Summary of Clinical Pharmacokinetic and Clinical Pharmacodynamic Results" section).

The dose regimen was determined to be PB 20 to 25 mg/kg administered intravenously (IV) or IM over 20 minutes (IM) with maintenance doses of 1.5 mg/kg administered IV or IM every 12 hours. The duration of treatment is not listed in this document.

The total number of subjects administered was 25 subjects, with 17 subjects in the intravenous group and 8 subjects in the intramuscular group.

The efficacy endpoint, effect on convulsion after administration of phenobarbital[9], was marked in 11 cases, effective in 5 cases, and ineffective in 9 cases based on the results of the combined results of the intravenous and intramuscular treatment groups.

Based on the above, the efficacy of PB intravenous administration was suggested.

⑥ Gal P et al, *Neurology*, 32: 1401-1404, 1982 (5.4.2.4.3-5)

An open-label study was conducted to investigate the efficacy and pharmacokinetics of intravenous PB in neonatal seizure patients (for pharmacokinetics, see section (i) Summary of Clinical Pharmacokinetics and Clinical Pharmacodynamics Study Results).

The dose regimen was 15 mg/kg of PB administered intravenously as the initial dose as soon as the patient was diagnosed with convulsive seizures, with an additional dose of 5 - 10 mg/kg every 5 - 10 minutes if seizures were not controlled or recurred, and the maximum dose was set at 40 mg.

The total number of subjects administered was 71 subjects.

Seizure control success cases, an efficacy endpoint, were defined as no recurrence of seizures, and the seizure control success rate was 85% (60/71 cases). The control success rates by plasma PB concentration were 60 % (36/60 subjects), 28 % (17/60 subjects), and 12 % (7/60 subjects) for 20 mg/L or less, 20-30 mg/L, and 30 mg/L or more, respectively.

Regarding safety, it was difficult to distinguish between PB side effects and symptoms and signs of neonatal disease with suppression of activity and muscle tone, but no exacerbation of respiratory function was observed in any of the subjects.

Based on the above, PB for neonatal convulsions showed efficacy that persisted until the plasma concentration reached 40 - 45 mg/L, suggesting that additional drugs should be added if it was ineffective.

5) Results of the investigation on the actual clinical use

① Kensuke Okubo et al., Journal of the Japanese Society of Pediatric Clinical Pharmacology and Therapeutics, 18: 83-85, 2005 (5.4.2.6.1-2)

In 2003, the current status survey on PB use was conducted in 203 physicians (203 sites, 1 site) engaged in neonatal care nationwide, mainly by the Pharmaceutical Affairs Committee of the Japan Society of Prematurity and Neonatal Medicine. A response was obtained from 110 sites, and the total number of hospitalized patients was 25,262, and the number of patients who required the use of anticonvulsants was 2.8% (721/25,262). Of the cases requiring anticonvulsants, 85.7% (618/721 cases) received PB. In addition, 89% (98/110 facilities) of sites raised PB as the first-line drug for neonatal convulsions requiring anticonvulsant drug administration, and 14% (15/110 facilities) as the second-line drug. The sites that were using PB (subcutaneous/intramuscular) intravenously were observed in

9) "Marked": Seizures including microseizures resolved within 12 hours after PB administration, "effective": Seizure frequency decreased to 50% or less including microseizures within 12 hours, and the seizure frequency disappeared within 24 hours, "Failure": Other than "complete response" and "effective"

28% of all sites, and 90% (98 subjects) of physicians responded that PB for intravenous administration was "required". Reasons for the need for intravenous PB preparations include concerns about local side effects associated with intramuscular administration (eg, local necrosis) and pain, and the instability of absorption in the case of suppository administration.

(2) Status epilepticus

1) Text in textbooks, etc. that are international standards

An international standard textbook states that the clinical positioning of PB in the treatment of status epilepticus is benzodiazepines and phenytoin followed by 2nd or 3rd choice drugs, and the initial dose is recommended to be 15 - 20 mg/kg for children and 10 - 20 mg/kg for adults, and the therapeutic range blood concentrations are 10 - 40 μg/mL. In addition, in the literature in Japan, it is stipulated that intramuscular injection or rectal injection of PB preparations will be performed, but it is stated that the onset of effect takes time.

[Submitted materials]

(Overseas)

5.4.2.1.1.2-3: Kasper DL, Fauci AS, Longo DL, Braunwald E, Hauser SL, Jameson JL editors, *Harrison's Principles of Internal Medicine (16th edition)*, McGraw-Hill, 2366-2372, 2005

5.4.2.1.1.2-4: Swaiman KF, Ashwal S, Ferriero DM editors, *Pediatric Neurology: Principles & Practice (4th edition)*, Mosby Elsevier, 1096-1104, 2006

5.4.2.1.1.2-5: Wasterlain CG, Treiman DM editors, *Status Epilepticus: Mechanisms and Management*, MIT Press, 597-605, 2006

5.4.2.1.1.2-6: Wyllie E editor, *The Treatment of Epilepsy: Principles & Practice (4th edition)*, Lippincott Williams & Wilkins, 610-621, 2006

5.4.2.1.1.2-1: Andreoli TE editor, *Cecil Essentials of Medicine (6th edition)*, Saunders, 1057-1059, 2004

5.4.2.1.1.3-1: Behrman RE, Kliegman RM, Jenson HB editors, *Nelson Textbook of Pediatrics (17th edition)*, Saunders, 2005-2009, 2004

5.4.2.1.1.2-2: Engel J, Pedley TA editors, *Epilepsy: A Comprehensive Textbook Vol 2*, Lippincott-Raven, 1317-1323, 1997

(Domestic)

5.4.2.1.2.2-1: Makiko Kagaki, Hiroyuki Sasaki, Kenji Sukai, eds. National Center of Neurology and Psychiatry, Pediatric Diagnosis and Treatment Manual. The Japanese Journal of Diagnosis and Treatment, 279-289, 2003

5.4.2.1.2.2-2: Masaaki Matsushita, total editorial, Clinical Psychiatry Department, Vol. 9, Nakayama Shoten, 118-129, 1998

2) Descriptions in foreign pharmaceutical product associations, etc.

For status epilepticus, initial doses of 15 - 20 mg/kg or 100 - 400 mg for children and 10 - 20 mg/kg or 200 - 600 mg for adults were administered intravenously over 10 - 15 minutes, and the therapeutic range blood concentrations were 15 - 40 μg/mL. It is also stated that maintenance doses should be administered as needed.

[Submitted materials]

5.4.2.1.3.2-2: Sweetman SC editor. *Maindale: The Complete Drug Reference (35th edition)*, Pharmaceutical Press. 422-427, 2007

5.4.2.1.3.3-1: American Society of Health-System Pharmacists, *AHFS Drug Information*, American Hospital Formulary Service. 2177-2178, 2457-2458, 2006

5.4.2.1.3.2-3: *USP-DI 2006 Vol.1: Drug Information for the Health Care Professional (26th edition)*, Thomson Micromedex, 533-535, 2006

5.4.2.1.3.2-1: *British National Formulaly No.51 (BNF)*, BMJ Publishing Group and RPS Publishing, 242-249, 2006

5.4.2.1.3.3-2: *British National Formulaly for Children (BNFC)*, BMJ Publishing Group and RPS Publishing, 261, 272, 2006

5.4.2.1.3.2-4: *ViDAL (82th edition)*, Vidal, 891-893, 2006

5.4.2.1.3.3-4: *Rote Liste*, Editio Cantor Verlag, 2006

5.4.2.1.4.3-1: Takemoto CK, Hodding JH, Kraus DM editors, *Pediatric Dosage Handbook (12th edition)*, Lexi-Comp, 1000-1003, 2005

3) Review articles, guidelines, etc. in Japan and overseas

For status epilepticus in children and adults, it is described that phenobarbital should be used as a second or third-line drug following benzodiazepines and phenytoin, and that the dosage and administration should be 15 - 20 mg/kg administered intravenously at a rate of 100 mg/min or less as the first dose. The Japanese guidelines (5.4.2.2.2.2-2) note that because there are no drugs that can be administered intravenously, and because the use of drugs used for subcutaneous administration or intramuscular administration is unavoidable, PB may crystallize and precipitate, resulting in microvascular embolism, it should not be used except in emergency cases.

[Submitted materials]

5.4.2.2.1.2-4: Kälviäinen R et al, *CNS Drugs*, 19: 759-768, 2005

5.4.2.2.1.2-6: Manno EM, *Mayo Clin Proc*, 78: 508-518, 2003

5.4.2.2.1.2-1: The Status Epilepticus Working Party, *Arch Dis Child*, 83: 415-419, 2000

5.4.2.2.1.2-5: Lowenstein DH et al, *N Engl J Med*, 338: 970-976, 1998

5.4.2.2.1.2-3: Epilepsy Foundation of America, *JAMA*, 270: 854-859, 1993

5.4.2.2.1.2-2: DeLorenzo RJ, *Semin Neurol*, 10: 396-405, 1990

5.4.2.2.2.2-1: Clinical research group on the evidence of drug therapy for status epilepticus in children (Pediatric-004, Principal investigator: Makiko Osawa), Diagnostic and Treatment Guidelines for Status Epilepticus in Children (Draft), March 27, 2005, Version Ver. 8.2

5.4.2.2.2.2-2: Japanese Society of Neurology Treatment Guidelines, Clinical Neurology, 42: 557-597, 2002

5.4.2.3.2-2: Prasad K et al, *The Cochrane Database of Sys Rev*, 4:CD003723, 2005

5.4.2.3.2-1: Appleton R et al, *The Cochrane Database of Sys Rev*, 4: CD001905, 2002

4) Clinical report, etc.

Results of clinical studies of PB in children and adults with status epilepticus conducted overseas, case reports and retrospective studies in patients in Japan and overseas were submitted.

①Investigation of status epilepticus in children (within the scope of the proposed dosage and administration)

i) Kokwaro GO et al, *Br J Clin Pharmacol*, 56: 453-457, 2003 (5.4.2.4.2.1-3)

An open-label study was conducted to investigate the efficacy and pharmacokinetics of pemigatinib in severely ill children with falciparum malaria and status epilepticus who did not respond to treatment with diazepam or paraaldehyde (see (i) Summary of Clinical Pharmacokinetics and Clinical Pharmacodynamics).

The dosage and administration was set to administer diazepam or paraaldehyde as the second-line drug, followed by 15 mg/kg as the initial dose administered intravenously over a period of 20 minutes or more, followed by 5 mg/kg as the maintenance dose administered intravenously 24 and 48 hours after the initial dose.

The total number of subjects treated was 12. The median age was 25 months (range: 7 - 62 months) and the mean weight was 10.7 kg (95% CI: 9.6 - 11.7 kg).

Of the 12 subjects, 66% (8/12 subjects) had seizures that were [10] controlled.

Regarding safety, one death was observed after administration of this drug, and respiratory failure accompanied by an increase in blood pressure occurred 23 hours after administration of this drug, resulting in cardiac arrest 30 minutes after the onset of respiratory failure. In addition, hypoxia was observed in 1 patient after administration of this drug, but hypoxia (oxygen saturation 94%), hypothermia (rectal temperature 35.2°C), and acidosis (base deficiency -16.7) were observed in this patient from before administration of this drug. No significant changes in respiratory depression, blood pressure, heart rate, or middle cerebral artery blood flow were observed after administration of this drug. The definition of adverse events and adverse drug reactions is not provided in this document.

Based on the above, the initial administration of 15 mg/kg of this drug for the status epilepticus in children was able to secure the therapeutic range blood concentration (15-40 μg/mL), suggesting that it is effective for controlling seizures.

ii) Takahiro Shibata et al., Department of Pediatrics, 19: 1329-1334, 1987 (5.4.2.5.2.1-4)

A retrospective study was conducted to investigate the actual state of emergency treatment in children hospitalized for convulsions and status epilepticus.

The total surveillance included 501 cases, of which 56 cases were status epilepticus.

For 56 patients with status epilepticus, the most effective drugs were diazepam in 26 patients, phenytoin in 6 patients, PB in 6 patients, water retention in 4 patients, and thiopental in 1 patient.

The safety of adverse events, etc. has not been evaluated.

Based on the above, the usefulness of PB for status epilepticus was suggested.

②Investigation of status epilepticus in children (within the scope of the proposed dosage and administration)

The following 5 domestic reports and 4 foreign reports were submitted as published literature on high PB dose administration (including parenteral administration) for status epilepticus in children. In addition, a report on high-dose PB therapy for diseases other than status epilepticus was submitted in Japan. There were also reports of septic shock with severe decrease in blood pressure due to barbiturate

10) If sustained seizures resolve within 30 minutes of the start of PB infusion, the seizures are considered suppressed. In addition, if no other anticonvulsants are required 24 hours after PB administration, the seizure is considered to be controlled.

coma (5.4.2.4.2.1-5), but in many reports, there were no reports of blood pressure decreases or dyspnea requiring vasopressors due to the pharmacological action of this drug.

[Submitted materials]

5.4.2.5.2.1-7: Ryo Hayashi, et al, . *Brain and Development*, 38: 359-362, 2006

5.4.2.5.2-1-3: Yuko Sakagami et al., *Clinical Pediatrics*, 57: 2259-2267, 2004

5.4.2.5.2.1-5: Akira Sudo, et al, . *Brain and Development*, 34: 23-29, 2002

5.4.2.5.2.1-1: Sugai K et al, *Epilepsia*, 43: 65-66, 2002

5.4.2.5.2.1-6: Yuichi Tagusa, et al, . *Brain and Development*, 33: 351-356, 2001

5.4.2.5.2.1-8: Hisaka Yamaoka et al., The Japanese Journal of *Clinical Anesthesiology*, 7: 94-98, 1983

5.4.2.4.2.1-4: Lee WK et al, *Pediatr Neurol*, 34: 63-65, 2006

5.4.2.4.2.1-6: Sahin M et al, *Epilepsia*, 42: 1461-1467, 2001

5.4.2.4.2.1-5: Mirski MA et al, *Crit Care Med*, 23: 400-404, 1995

5.4.2.4.2.1-1: Crawford TO et al, *Neurology*, 38: 1035-1040, 1988

③ Study of status epilepticus in adults

i) Treiman DM et al, *N Engl J Med*, 339: 792-798, 1998 (5.4.2.4.2.2-3)

A randomized, double-blind comparative study was conducted to compare the efficacy and safety of 4 anticonvulsants including PB in adult patients with generalized convulsive status epilepticus.

The dosage and administration were as follows: PB 15 mg/kg (PB group, dose rate ≤ 100 mg/min), diazepam 0.15 mg/kg (dose rate ≤ 5 mg/min), followed by phenytoin 18 mg/kg (dose rate ≤ 50 mg/min) (DZP and PHT combination groups), lorazepam 0.1 mg/kg (LZP group, dose rate ≤ 2 mg/min), or phenytoin 18 mg/kg (PHT group, dose rate ≤ 50 mg/min) intravenously.

All 570 subjects were included in the ITT analysis set, and the analysis set for which the diagnosis was confirmed was 518 subjects (124 subjects in the PB group, 131 subjects in the DZP and PHT combination therapy group, 136 subjects in the LZP group, and 127 subjects in the PHT group), and this was the efficacy analysis set. Of the patients with confirmed diagnosis, 384 (91 in the PB group, 95 in the DZP and PHT groups, 97 in the LZP group, and 101 in the PHT group) had clear generalized convulsive status epilepticus (clear status epilepticus), and 134 (33 in the PB group, 36 in the DZP and PHT groups, 39 in the LZP group, and 26 in the PHT group) had subtle generalized convulsive status epilepticus (subtle status epilepticus).[11][12]

The dose, serum concentration after the first dose, and drug administration time for each drug group were as shown in the table below.

Table Dose, Serum Concentration After the First Dose, and Time of Drug Administration for Each Group (Mean ± Standard Deviation)

Treatment group	PB group	DZP and PHT arms		LZP group	PHT group
Drug Administered	PB	DZP	PHT	LZP	PHT
Dose (mg/kg)	14.96 ± 2.53	0.15 ± 0.02	15.08 ± 4.84	0.10 ± 0.01	16.02 ± 3.21
Serum concentration (μg/mL)	31.2 ± 37.2	0.245 ± 0.307	31.8 ± 19.2	0.231 ± 0.299	30.0 ± 13.6
Duration of administration (minutes)	16.6 ± 11.5	42.0 ± 38.1		4.7 ± 7.2	33.0 ± 20.1

11) Defined as recurrent seizures with no complete recovery between attacks

12) Defined as a stage of generalized convulsive state when coma is persistent but only fine convulsive movements are observed

Regarding efficacy, the success rate of initial treatment in patients with overt status of the disease was as shown in the table below, which was significantly higher in the LZP group than in the PHT group ($p = 0.002$, χ^2 test, but no statistically significant difference was observed between the other treatment groups ($p > 0.01$, χ^2 test).

Table Treatment success rate in each group (%)

	PB group	DZP and PHT Combination Administration Arm	LZP group	PHT group
Clear status epilepticus	58.2 (91)	55.8 (95)	64.9 (97)	43.6 (101)

Treatment success, defined as disappearance of all clinical and electroencephalographic seizure activity within 20 minutes of starting drug and no recurrence within 20-60 minutes after starting drug
( ): Number of subjects to be analyzed

With respect to safety, the adverse reactions are as shown in the following table, and there was no difference in the incidence of hypotension, respiratory depression, or cardiac rhythm abnormalities requiring treatment between the treatment groups for either obvious or subtle status.

Table Main Adverse Drug Reactions in the Analysis Set with Confirmed Diagnosis

	PB group	DZP and PHT Combination Administration Arm	LZP group	PHT group
Clear status epilepticus Number of subjects to be analyzed	91	95	97	101
Decreased ventilation (%)	13.2	16.8	10.3	9.9
Hypotension (%)	34.1	31.6	25.8	27.0
Arrhythmia (%)	3.3	2.1	7.2	6.9
Fine status epilepticus Number of subjects to be analyzed	33	36	39	26
Decreased ventilation (%)	15.2	2.9	12.8	7.7
Hypotension (%)	48.5	58.3	59.0	57.7
Arrhythmia	9.1	5.6	7.7	0.0

Based on the above, lorazepam was more effective than phenytoin in patients with overt generalized convulsive status epilepticus and was suggested to be similar to PB or concomitant diazepam and phenytoin.

ii) Shaner DM et al, *Neurology*, 38: 202-207, 1988 (5.4.2.4.2.2-2)

A randomized, open-label study was conducted to evaluate the efficacy and safety of the combination of PB and diazepam and phenytoin in patients with generalized convulsive status epilepticus.

The dosage and administration are as follows: for the PB group, PB 10 mg/kg is administered intravenously at a dose of 100 mg/min, and if the seizure persists after 10 minutes of administration, phenytoin is administered at a dose of 40 mg/min. If the serum phenytoin level is unknown or between 0 and 4 mg/L, the initial dose of 18 mg/kg should be administered, and if the serum phenytoin level is 5 - 9, 10 - 15, or 16 - 20 mg/L, 75, 50, or 25% of the planned initial dose should be administered, respectively. In the diazepam-phenytoin combination group, diazepam was established as 2 mg/min as a stop of convulsion or intravenous administration of up to 20 mg, while phenytoin was administered at the same dose and administration as the PB group. In addition, if seizures persist after 20 mg of diazepam, it was set that the patient would continue to receive diazepam intravenously at 8 mg/h until the end of phenytoin administration.

The total number of subjects treated was 36 (18 subjects in PB group and 18 subjects in diazepam and phenytoin combination group).

Seizures were controlled within 7 hours for all subjects, and while there was a trend for shorter cumulative convulsion time and reaction latency (time from the start of drug administration to the end of the final convulsion) in the PB group than in the combination group with diazepam and phenytoin, there was no significant difference. Seizures were controlled in 11/18 subjects in the PB group with PB monotherapy, and the mean serum concentration in 18 subjects was 17.3 μg/mL, and the mean serum concentration in 11 subjects who were effective as monotherapy was 18.3 μg/mL.

Regarding safety, the incidence of arrhythmia and hypotension was similar in both groups, and 6 subjects each were intubated, and tracheal intubation not caused by respiratory depression was also observed in each group.

Based on the above, it was suggested that PB administration tended to have a higher onset of effect compared to the combination of diazepam and phenytoin, and the safety was similar.

iii) Czapinski P, *J Neurol*, 242: S83, 1995 (5.4.2.4.2.3-1)

A randomized study was conducted to evaluate the efficacy and safety of diazepam in combination with PB or phenytoin in patients with recurrent status epilepticus.

The dose regimen was 10 mg diazepam intravenously followed by 10 - 20 mg/kg PB or 12 to 18 mg/kg phenytoin intravenously over 1 hour (50% of the dose was administered 15 minutes after the start of administration).

The total number of subjects administered was 60.

Status epilepticus was suppressed in 83.3% of patients in the PB group and 86.6% of patients in the phenytoin group (18 minutes and 23 minutes for the mean reaction latency, respectively). The half-life of plasma drug concentration was 101 ± 25 and 84.3 ± 43.2 hours for PB and phenytoin, respectively, and the mean drug concentration in cerebrospinal fluid at 12 hours after administration was 62 and 15% of the plasma concentration for PB and phenytoin, respectively.

With respect to safety, adverse drug reactions occurred in 23.3% of both groups, but none required discontinuation.

Based on the above, it was suggested that PB is almost equivalent efficacy and tolerability to phenytoin.

iv) Clinical reports of status epilepticus in other adults

In addition to the above, a report of a retrospective study on status epilepticus in adults was submitted as a published literature, and PB is administered as a third-line drug.

[Submitted materials]
5.4.2.4.2.2-1: Mayer SA et al, *Arch Neurol*, 59: 205-210, 2002

5) Clinical experience

① Survey on the use of phenobarbital for status epilepticus in children (5.4.2.6.2-3: Kitami Hayashi et al., Brain and Development, 40: 83-85, 2008)

A fact-finding survey on the use of PB was conducted among 329 councilors of the Japanese Society of Pediatric Neurology and the Japanese Epilepsy Society (including 222 pediatricians). As a result, responses from 79 pediatricians among the respondents were tabulated. 53.2% (42/79) of the subjects had used PB formulation, 13 had used intravenous formulation, 20 had used subcutaneous or intramuscular formulation, and 12 had used intravenous formulation. The PB dose ranged from 2 - 30 mg/kg (23 respondents). For the seizure type treated in PB (31 respondents with duplicate responses), 27 patients had generalized tonic-clonic seizures including secondary generalization, 7 had partial seizures, and 2 had other seizure types. The usefulness of PB (30 respondents) was as follows: 15

subjects answered "useful" in 75% or more of cases, 7 answered "useful" in 50% or more and less than 75% of cases, 6 answered "useful" in 25% or more and less than 50% cases, and the remaining 2 answered "useful" or less than 25% cases.

② Experience of use of PB for status epilepticus in adults in Japan

The following 9 reports (including 1 report in children and adults) were submitted as domestic experience with PB in adults with status epilepticus.

[Submitted materials]

5.4.2.5.2.1-2: Akihisa Okumura, et al., *Clinical Pediatrics*, 59: 1938-1942, 2006

5.4.1.2-6: Saito, J. et al, *Medical Journal of Shimane Prefectural Central Hospital*, 29: 3-6, 2004

5.4.1.2-7: Takami Suenaga, et al. Clinical *Psychiatry*, 36: 327-331, 2007

5.4.1.2-11: Yoichi Yanagawa, et al. , *Neurosurgery*, 58: 509-513, 2006

5.4.1.2-5: Fumiko Ide, et al., *Japanese Journal of Toxicology*, 19: 407-408, 2006

5.4.1.2-9: Nobuhiko Instrument et al., Journal of *Japanese Society of Neurology*, 18: 69-72, 2005

5.4.1.2-12: Mariko Yamashita, et al., *Cranial nerve*, 53: 1057-1062, 2001

5.4.1.2-8: Hiroaki Mizushima, et al., Journal *of the Japanese Association for Emergency Medicine*, 7: 197-201, 1996

5.4.1.2-10: Makoto Mukai, et al. *Psychiatry*, 29: 1351-4, 1987

<Summary of Review>

(1) Neonatal convulsion

1) Efficacy of phenobarbital for neonatal convulsion

PMDA asked the Applicant to explain the reason why the efficacy in the investigator-initiated clinical trial (5.3.5.2-1) was higher than that in the submitted clinical reports (5.4.2.4.1-1, 5.4.2.4.1-7, 5.4.2.4.1-4, 5.4.2.4.3-5, 5.4.2.5.1-1, and 5.4.2.5.1-3).

The Applicant explained that the differences in efficacy measures and subject demographics may have influenced the efficacy in the investigator-initiated study (5.3.5.2-1), which was more likely than in the clinical report submitted. First, as clinical seizure and electroencephalographic seizure activity were divergence in clinical seizure activity, and the 25-30% frequency of sustained seizure activity on the EEG was reported after the administration of antiepileptic drugs (5.4.2.4.1-9), an investigator-initiated clinical trial (5.3.5.2-1) with clinical seizure as an efficacy index showed a higher rate than the results in the clinical reports (5.4.2.4.1-1 and 5.4.2.4.1-7) which indicated electroencephalographic seizure activity. Next, in the subjects of the investigator-initiated clinical trial (5.3.5.2-1) and the clinical report (5.4.2.4.1-1, 5.4.2.4.1-7, 5.4.2.5.1-1, and 5.4.2.5.1-3), there was no significant difference in the number of central nervous system diseases as the causative disease, but as shown in the table below, the mean Apgar score in the investigator-initiated clinical trial (5.3.5.2-1) was normal or almost normal at 7 or higher for both the 1 and quintile values. In contrast, the clinical reports (5.4.2.4.1-1, 5.4.2.4.1-7, and 5.4.2.5.1-1) were all in the range of 3 to 5, suggesting that the birth status was worse than that of the affected child in the investigator-initiated clinical trial (5.3.5.2-1), making a detailed study impossible. However, the Applicant explained that the efficacy rate in the investigator-initiated clinical trial (5.3.5.2-1) was considered to be higher than the results in the clinical reports (5.4.2.4.1-1, 5.4.2.4.1-7, 5.4.2.5.1-1, and 5.4.2.5.1-3) due to differences in the severity of the causative disease and the degree of brain function or organic damage related to the causative disease.

Table Efficacy Measures, Apgar Scores, and PB Rates in Investigator-Initiated Clinical Trials and Clinical Reports

Material name	Efficacy measures	Apgar score	PB efficacy rate and results of stratification by causative disease)
Investigator-Initiated Study (5.3.5.2-1)	Clinical attack	1-minute value*: 7.6 ± 2.5 (range 3-10), median 8.5 5 minute value*: 8.3 ± 2.6 (range 3-10), median 9.0 *: Mean ± standard deviation	Overall: 100.0% (10/10 cases) Stratification results by underlying disease: Hypoxic-ischemic encephalopathy 100.0% (2/2 cases) Intracranial hemorrhage 100.0% (1/1 case) Neonatal cerebral infarction 100.0% (1/1 case) Other 100.0% (1/1 case) Unknown: 100.0% (5/5 cases)
Reported by Boylan et al. (5.4.2.4.1-1)	Electroencephalogram findings	All subjects (22 subjects) 1 minute value: mean 3.9 (range 0-9), median 3.5 5 minutes: mean 5.0 (range 0-10), median 5 PB responders (13 subjects) 1 minute value: mean 3.5 (range 0-9), median 4 5 minutes: mean 4.7 (range 0-9), median 5 PB non-responders (11 subjects) 1 minute value: mean 4.3 (range 0 to 9), median 3 5-minute value: mean 5.4 (range 0-10), median 6	Overall: 50.0% (11/22 cases) Stratification results by underlying disease: Hypoxic ischemic encephalopathy 38.5% (5/13 cases) Intracranial hemorrhage 66.7% (2/3 case) Intrauterine growth restriction 100.0% (2/2 cases) End-diastolic blood flow decreased, premature infan 0.0% (0/1) Arteriovenous malformation 0.0% (0/1 cases) Myopathy, Ventilation suppression 100.0% (1/1) Benign neonatal convulsion 100.0% (1/1 case)
Reporting by Painter et al. (5.4.2.4.1-7)	Electroencephalogram findings	Selection criteria: 5th quintile less than 5 (base deficiency greater than 10 mmol/L)	Overall: 43.3% (13/30 cases) Result of stratification by causative disease. Unspecified
Reported by Gilman et al. (5.4.2.4.1-4)	Clinical attack	No description	Overall: 76.7% (92/120 cases) Stratification results by underlying disease: Asphyxia of offspring 72.6%(45/62 cases) Idiopathic 86.7% (26/30 cases) Cerebral hemorrhage 88.9% (16/18 cases) Infectious 55.6% (5/9 cases) Metabolic 0.0% (0/1 cases)
Report by Gal et al (5.4.2.4.3-5)	Clinical attack	No description	Overall: 84.5% 160/74 cases) Stratification results by underlying disease: Asphyxia 80.6% (29/36 cases) Central nervous system hemorrhage 84.6% (11/13 subjects) Infectious 50.0% (1/2 cases) Other 87.5% (7/8 cases) Unknown: 100.0% (12/12 cases)
Report by Shibata et al. (5.4.2.5.1-1)	Clinical attack	1-minute value (mean ± standard deviation): 5.0 ± 3.1	100.0% (22/22 cases) Only patients weighing >1 kg and receiving 20 mg/kg PB were evaluated
Reports by Takebe et al. (5.4.2.5.1-3)	Clinical attack	No description	Overall: 58.8% (10/17 cases) Stratification results by underlying disease: Perinatal anoxia 53.8% (7/13 cases) Pyogenic meningitis 100.0% (1/1 case) Benign familial neonatal convulsion 0.0% (0/1 case) Unknown 100.0% (2/2 subjects)

PMDA requested the Applicant to explain the rationale for thinking that the results of the investigator-initiated clinical trial (5.3.5.2-1) can be extrapolated to children with general neonatal convulsions.

The Applicant explained that the most common causative disorder of neonatal convulsions is hypoxic ischemic encephalopathy, and that other disorders include intracranial hemorrhage, cerebrovascular disorder, central nervous system infection, and metabolic abnormalities such as hypocalcemia, and congenital brain abnormalities, etc., and vary (5.4.2.1.1.1-4, 5.4.2.1.1.2-4, 5.4.2.1.1.1-6). The Applicant explained that although the causative disease was diagnosed as unknown in 5 neonate patients enrolled in the investigator-initiated clinical trial (5.3.5.2-1), it was presumed that the cause was fetal asphyxia in 2 patients and brain malformation in 1 patient, and ischemic hypoxic encephalopathy was approximately half of the incidence of ischemic hypoxic encephalopathy, and intracranial hemorrhage and metabolic disorder were approximately 10%. The patient enrolled in the investigator-initiated clinical trial (5.3.5.2-1) was considered to be a general reflective of the general population.

PMDA considers that the efficacy of the drug for neonatal convulsions is suggested, although a small number of patients were enrolled in the investigator-initiated clinical trial (5.3.5.2-1), and based on information such as other submitted textbooks, pharmaceutical formularies, review articles, and clinical reports, it is acceptable to judge that the drug has shown efficacy for neonatal convulsions. However, PMDA believes that the types and severity of causative diseases causing convulsions vary, and further investigation is necessary in post-marketing surveillance regarding the effects of patient demographics on the efficacy and safety of this drug.

2) Dosage and administration of this drug for neonatal convulsion

PMDA requested the Applicant to explain the appropriateness of setting the initial dose, additional dose, and maintenance dose of the drug for neonatal convulsion.

The Applicant considers that the lower limit of PB therapeutic range blood concentration is 15 μg/mL or more based on clinical reports from Japan and overseas (5.4.2.4.3-5, 5.4.2.5.1-1, and 5.4.2.5.1-3), regarding the upper limit of therapeutic range blood concentration, the efficacy of PB is dose-dependent up to a blood concentration of 40 μg/mL, and explained that the upper limit of the therapeutic range blood concentration is believed to be 40 μg/mL, because there is a report (5.4.2.4.1-4) that decreased food intake and sedation were observed at 50 μg/mL or higher, and the increase in efficacy is reported to be small at blood concentrations above 40 μg/mL (5.4.2.4.3-5). The Applicant also explained that overseas clinical reports (5.4.2.4.3-9) explain the importance of achieving therapeutic range blood concentrations for rapid seizure control, and in order to achieve the lower limit of therapeutic blood concentration (15 μg/mL), it was considered appropriate to set the dosage and administration of 20 mg/kg as the initial intravenous dose and to administer an additional dose of the same amount if the convulsions do not disappear, taking into account the domestic clinical reports (5.4.2.5.1-1, 5.4.2.5.1-3), and explained that the investigator-initiated clinical trial (5.3.5.2-1) was conducted using this dosage and administration. The Applicant also explained that in the investigator-initiated clinical trial (5.3.5.2-1), the blood concentration of PB was 18.8 - 25.8 μg/mL in the initial PB 20 mg/kg administration (9 subjects) and 42.8 and 36.9 μg/mL in the 2 subjects who received the additional PB 20 mg/kg. Therefore, it was considered reasonable to administer the same dose if the initial dose of PB 20 mg/kg cannot be controlled. In addition, the Applicant must administer the maintenance dose to maintain the therapeutic range of blood concentration of PB (15-40 μg/mL) to prevent recurrence of convulsions that disappeared by the first dose. Although the overseas textbook (5.4.2.1.1.1-3, 5.4.2.1.1.3-1) recommends 5 mg/kg/day, and the route of administration includes intramuscular administration, the Applicant explained that there was a report (5.4.2.5.1-3) that a maintenance dose of 3 mg/kg/day maintained plasma PB concentrations between 15.0 and 42.5 μg/mL in Japanese neonatal patients with seizures. Based on the above, in the investigator-initiated clinical trial (5.3.5.2-1), the maintenance dose of PB was set at 2.5 - 5 mg/kg/day, and the blood concentration of PB prior to or at the time of the last maintenance dose (10 subjects) was 18.7 - 45.3 μg/mL. Therefore, the dose of the first dose of PB was considered to be 20 mg/kg, and the same dose was additionally administered when the convulsions did not disappear. The maintenance dose was thus deemed reasonable to be set at 2.5 - 5 mg/kg/day.

PMDA requested the Applicant to explain the appropriateness of approximately 6 days of treatment for neonatal convulsions.

The Applicant explained that in the investigator-initiated clinical trial (5.3.5.2-1), the maintenance treatment period was set at 6 days with reference to the descriptions in the overseas clinical report (5.4.2.4.1-7) and Pediatric Neurology (5.4.2.1.1.1-5). In addition, the Applicant proposes discontinuing PB as soon as possible when convulsion can be controlled in Avery's Nephrology, Pathology & Management of the Newborn (.4.2.1.1.1-2). However, in Pediatric Neurology (5.4.2.1.1.1-5), as the timing of discontinuation of maintenance therapy has not been clearly specified, the Applicant

explained that there is no standardization on how much antiepileptic drug should be administered to treat neonatal seizures, that it is considered necessary to use antiepileptic drugs according to the purpose of clinical use, and that it believes it was appropriate not to set a uniform maintenance dosage period, and for the dosage and administration, while the duration of maintenance administration was set as "ideally approximately 6 days" at the time of application, the Applicant explained that it considered it appropriate to delete this language as well.

PMDA considers that there are no major problems with the initial dose, additional dose, and maintenance dose set for the proposed dosage and administration based on the results of investigator-initiated clinical trials, as the dosage and administration for neonatal convulsions are shown in the literature submitted by the Applicant. Regarding the duration of maintenance administration, the safety of long-term administration of PB has not been clarified, and it is considered appropriate not to administer this drug without warning. However, because the duration of maintenance administration of PB is considered to be different depending on the situation of individual cases, it is considered that there is no need to limit the administration period uniformly. However, in an investigator-initiated clinical trial, the maintenance treatment period is set as 6 days, and it is proposed that the administration should be discontinued as soon as possible after the seizure is controlled (5.4.2.1.1.1-2). Therefore, it is necessary to perform blood concentration monitoring (TDM) when administering this drug continuously, and pay close attention to the safety while measuring the blood PB concentration. The relationship between the dosage of the drug and the administration period of the drug and its efficacy and safety is considered to require further investigation in post-marketing surveillance.

(2) Status epilepticus

1) Validity of the administration of PB for status epilepticus as public knowledge in medical pharmacology

PMDA requested that the Applicant explain the rationale for considering PB to be in the state of status epilepticus and to be in the public knowledge from medical pharmacology.

The applicant believes that the administration of intravenous PB for the treatment of status epilepticus has been approved in Europe and the United States, and explained that based on ① International textbooks (5.4.2.1.1.2-1, 5.4.2.1.1.2-2, 5.4.2.1.1.2-3, 5.4.2.1.1.2-4, 5.4.2.1.1.2-5, 5.4.2.1.1.2-6, 5.4.2.1.1.3-1, and 5.4.2.1.4.3-1) list PB as a treatment for status epilepticus. (5.4.2.1.1.3-1 and 5.4.2.1.4.3-1), PB is listed as a treatment for status epilepticus, and PB has a long history of use as a second- or third-line drug, ② the existence of literature (5.4.2.4.2.2-2, 5.4.2.4.2.2-3) and review articles (5.4.2.2.1.2-1, 5.4.2.2.1.2-2, 5.4.2.2.1.2-3, 5.4.2.2.1.2-4, 5.4.2.2.1.2-5, 5.4.2.2.1.2-6) that can serve as scientific evidence published in internationally reliable journals, and ③ experience of use in Japan (5.4.2.6.2-3, 5.4.2.5.2.1-2, 5.4.1.2-6, 5.4.1.2-7, 5.4.1.2-11, 5.4.1.2-5, 5.4.1.2-9, 5.4.1.2-12, 5.4.1.2-8, 5.4.1.2-10), the efficacy and safety of PB for status epilepticus is considered to be well known in medical and pharmaceutical sciences. In addition, the Applicant explained that the request for this drug was submitted jointly by the Japanese Society of Pediatrics and the Japanese Society of Pediatric Neurology (5.4.2.6.2-4) and was considered medically necessary. Based on the description of the efficacy and safety of PB for status epilepticus in children and adults in both domestic and foreign literature, published literature, and guidelines, etc., PMDA considers that a wide consensus has been reached, and it is considered that there is no particular problem with the efficacy of PB for status epilepticus based on the judgment that it is public knowledge in medical pharmaceuticals.

2) Dosage and administration of PB for status epilepticus

PMDA requested the Applicant to explain the appropriateness of setting the dosage and administration of the drug for status epilepticus.

The Applicant first reported by Kokwaro et al. (5.4.2.4.2.1-3) on dose and dose setting in pediatric status epilepticus that 66% (8/12 subjects) had controlled seizures after the first dose of PB 15 mg/kg intravenously, and the C_{max} of plasma PB in controlled cases was 19.9 μg/mL (median, range: 17.9-27.9; the plasma concentration range was maintained to >15 μg/mL, and the lower concentration range of μg was maintained and the plasma concentration was above, indicating the 15 μg/mL. The Applicant also stated that the upper limit of the therapeutic range blood concentration was not observed, but Booker et al. stated that "the maximum efficacy was achieved by achieving a blood concentration of more than 40 μg/mL, but the possibility of side effects increased in individual patients" (Woodbury DM, Penry JK, Piggenger CE editors, *Antiepileptic drugs, 2nd edition*, Raven Press, 341-350, 1982), and explained that this information is common in the treatment of epilepsy with PB, and that it was considered appropriate to set the upper therapeutic blood concentration limit at 40 μg/mL. In addition, the Applicant also explained that, based on the fact that there is a report that the first dose of 15 - 20 mg/kg of this drug in childhood status epilepticus was next to diazepam (5.4.2.5.2.1-4), and that the intravenous administration of 15 - 20 mg/kg is recommended in the textbooks, formularies, and review articles, etc. in Japan and overseas, it was considered appropriate to set the initial dose to be 15 - 20 mg/kg.

The Applicant then stated that the dosage and administration in adult patients with status epilepticus has been established in overseas textbooks, drug collections (5.4.2.1.3.2-2, 5.4.2.1.3.3-3, 5.4.2.1.3.2-1, 5.4.2.1.3.3-2, 5.4.2.1.3.2-4, 5.4.2.1.4.3-1, 5.4.2.1.1.2-3, 5.4.2.1.1.2-2), etc., the therapeutic range blood concentration is described in the range of 10 to 40 μg/mL, with 15 to 40 μg/mL being most frequently described, and status epilepticus is fatal, and explained that considering the fact that this drug is positioned as a second- or third-line drug, it was judged appropriate to set the therapeutic range blood concentration at 15 - 40 μg/mL, the same as for pediatric patients. The Applicant also explained that there is a report that the efficacy and safety of the initial dose at the administration of PB 15 mg/kg was similar to that of the concomitant administration of diazepam and phenytoin (5.4.2.4.2.2-3), and that it was recommended in the range of 10 - 20 mg/kg in the overseas narrative, formulary, and review articles, etc., and that the majority of the doses were 20 mg/kg. Therefore, it was considered appropriate to set the dose to be 15 - 20 mg/kg.

Based on the above, the Applicant explained that it was considered appropriate to use a dose of 15 - 20 mg/kg for status epilepticus, and that the maintenance dose does not need to be set because it is considered that oral administration of antiepileptic drugs is possible if status epilepticus is controlled.

PMDA stated that the initial dose of 15 - 20 mg/kg should be used for the dosage and administration of this drug in status epilepticus, and the therapeutic range blood concentration should be 15 - 40 μg/mL in the overseas textbooks, drug associations, etc., and that there are no major problems. However, it is considered necessary to further investigate the relationship between dosage of the drug and efficacy and safety in post-marketing surveillance.

(3) Safety

1) Blood concentration and safety of this drug

PMDA requested the Applicant to explain the relationship between PB blood concentration and safety.

In the investigator-initiated clinical trial (5.3.5.2-1), the Applicant explained that the blood PB concentration at 2 hours after the first dose, 2 hours after the first maintenance dose, and before the last dose of maintenance dose (at discontinuation) was 18.7 - 45.3 μg/mL, and that there was no difference in the adverse event profile between the three subjects whose blood concentration exceeded 30 μg/mL and the seven subjects whose blood concentration did not exceed 30 μg/mL. The applicant has also submitted an application for a physician-initiated clinical trial (5.3.5.2-1) and clinical reports (5.4.2.4.1-4, 5.4.2.4.1-7, 5.4.2.4.3-5, Pippenger CE et al, *Clin Perinatol*, 2: 111-115, 1975, DeCarolis MP et al, *Dev Pharmacol Ther*, 14: 84-89, 1990, Shany E, *Eur J Paediatr Neurol*, 8: 323-325, 2004, Wehner J et

al, *Kinderarztl Prax*, 59: S31-34, 1991, Amitai Y et al, *J (Emerg Med*, 8: 449-450, 1990), the relationship between PB blood levels and safety in neonatal convulsions is shown in the table below, and it was explained that side effects such as sedation, decreased feeding (sucking), somnolence, nystagmus, decreased muscle tone, respiratory depression, lethargy, respiratory arrest, bradycardia, decreased body temperature, and decreased peristalsis of the stomach and intestines may occur at 50 μg/mL or higher.

Table PB Blood concentration and side effects report (neonate)

PB blood concentration	Adverse drug reactions that occurred
≤40 μg/mL	Respiratory depression, oxygen desaturation, decreased locomotor activity with decreased blood pressure, etc. (the number of reported cases is low)
≥ 50 μg/mL	Effects on heart rate, decrease in sedation/feeding (feeding), somnolence, nystagmus
≥60 μg/mL	Decreased muscle tone, decreased feeding
≥70 μg/mL	Serious lethargy
≥80 μg/mL	Respiratory irregularity, decreased ventilation
≥90 μg/mL	Temporary cessation of breathing, bradycardia, hypoventilation, lethargy, decreased muscle tone, etc.
High dose administration (majority ≥ 60 μg/mL)	A disorder characterized by a decrease in peristaltic movement in the stomach and intestine.

Next, the Applicant states that in clinical reports (5.4.2.5.2.1-5, 5.4.2.5.2.1-7, 5.4.2.6.2-1, 5.4.2.6.2-5, Pippenger CE et al, *Clin Perinatol*, 2: 111-115, 1975), the relationship between PB blood levels and safety in children and adults with status epilepticus The toxic epidermal necrolysis (TEN) observed in children occurred near the therapeutic range blood concentration. However, since multiple antiepileptic drugs are used concomitantly in patients with status epilepticus, it was thought that the occurrence of adverse events should be noted even when blood concentrations are low, but no adverse reactions of particular concern were observed in children or adults but no adverse reactions of particular concern were observed in children or adults.

Table PB Blood Concentration and Side Effects Report (Pediatrics and Adults)

PB blood concentration	Adverse drug reactions that occurred	
	Children	Adults
≤40 μg/mL	Toxic epidermal necrolysis	Drowsiness, delirium, oversedation, sedation, decreased bone density, depressed heart, hallucinations and delusions
≥ 50 μg/mL	Respiratory depression	γ-GTP transient increase, Reye syndrome
≥60 μg/mL	Decreased muscle tone, reduced feeding (in young children)	
≥70 μg/mL	Strong drowsiness, increased γ-GTP, mild blood pressure decreased	Sleepiness, increased sputum-GTP, mild respiratory depression, mild blood pressure decreased
≥80 μg/mL	Drowsiness, increased γ-GTP	
≥90 μg/mL	Decreased masticatory strength, plastic paresis	Intellectual regression, cerebellar ataxia

PMDA considers that serious adverse events such as respiratory depression, blood pressure reduction, and depressed level of consciousness may occur even at therapeutic range concentrations set for neonatal convulsions and status epilepticus (15-40 μg/mL). However, this drug may be administered under the sufficient observation of a physician, so it is possible to deal with such events, and the benefits of this drug may outweigh the risks by giving sufficient warning. In addition, the possibility that the blood concentration of PB may vary depending on the patient's condition cannot be ruled out, and it is necessary to perform regular TDM especially in neonates, and it is considered that TDM should be performed in other patients as necessary.

The safety of this drug is considered necessary to continue to be investigated in post-marketing surveillance.

2) Administration speed and safety of phenobarbital intravenous administration

PMDA requested the Applicant to explain the relationship between PB administration speed and safety.

The Applicant first stated that, although no report was found that examined the administration rate

and safety of intravenous PB for neonatal convulsions, the textbook (5.4.2.1.1.1-4), which is considered to be the international standard, recommends an administration time of 10 - 15 minutes (PB dose of 20 mg/kg), the Drug Collection (5.4.2.1.3.3-3, 5.4.2.1.3.3-2) recommends 20 mg/kg PB administered intravenously over 20 minutes at a rate of no more than 1 mg/kg/min, and that domestic and foreign clinical reports (5.4.2.4.1-7, 5.4.2.4.1-4, 5.4.2.5.1-1, 5.4.2.5.1-3), the dose range was 5 - 20 minutes (PB dose 15 to 25 mg/kg). In addition, the Applicant explained that the administration rate of PB was set to 5 - 10 minutes (PB 20 mg/kg) in the investigator-initiated clinical trial (5.3.5.2-1), and mild and moderate respiratory depression was observed in 2 cases as an adverse drug reaction at the initial administration, and although blood pressure decreased during the maintenance administration period, it was non-serious, so it was reasonable to set the administration rate of PB for neonatal convulsions to 5 - 10 minutes (20 mg/kg).

Next, the Applicant explained that no report on the administration rate and safety of PB intravenous administration for status epilepticus was found, and the setting of administration rate in textbooks, formulary, review articles, and clinical reports is as shown in the table below, and the administration rate of PB intravenous administration for status epilepticus is set at 100 mg/min or less in both children and adults, and thus, it is reasonable to set the administration rate of PB intravenous administration for status epilepticus to be at 10 minutes or longer and 100 mg/min or less.

Table Setting of Infusion Rate in the Textbook, Formulary, Review Article, and Clinical Report

Infusion rate	Initial Dose	Children	Adults
30 mg/min or less	20 mg/kg	Treat Epilepsy (textbook, 5.4.2.1.1.2-6)	
≤ 50 mg/min	20 mg/kg	Ped Neurol (textbook, 5.4.2.1.1.2-4) DeLorenzo 1990 (Review article, 5.4.2.2.1.2-2)	
≤ 60 mg/min	100-400 mg or 20 mg/kg	AHFS (Pharmaceutical Formulary, 5.4.2.1.3.3-1)	AHFS (Pharmaceutical Formulary, 5.4.2.1.3.3-1)
≤ 50-75 mg/min	20 mg/kg	Lowenstein 1998 (Review article, 5.4.2.2.1.2-5)	Harrison (textbook, 5.4.2.1.1.2-3) Lowenstein 1998 (Review article, 5.4.2.2.1.2-5)
≤ 100 mg/min	20 mg/kg		Epilepsy Comp Text (5.4.2.1.1.2-2)
≤ 100 mg/min	10 mg/kg		Martindale (Pharmaceutical Formulary, 5.4.2.1.3.2-2, 5.4.2.1.3.2-3) BNF/BNFC (Pharmaceutical Formulary, 5.4.2.1.3.2-1, 5.4.2.1.3.3-2)
	15 mg/kg		Treiman 1998 (Clinical Report, 5.4.2.4.2.2-3)
	15-20 mg/kg	Kalviainen 2005 (Review article, 5.4.2.2.1.2-4)	
	20 mg/kg	Treat Epilepsy (target, older children)	Treat Epilepsy (textbook, 5.4.2.1.1.2-6) DeLorenzo 1990 (Review article, 5.4.2.2.1.2-2)
100 mg/min	10 mg/kg		Shaner 1988 (Clinical Report, 5.4.2.4.2.2-2)
	15-20 mg/kg	Nunuma 1987 (Clinical report, 5.4.2.5.2.1-4)	
	20 mg/kg	Cecil (textbook, 5.4.2.1.1.2-1) Epilepsy Foundation of America 1993 (Review, 5.4.2.2.1.2-3) Japanese Society of Neurology Treatment Guidelines 2002 (5.4.2.2.2.2-2)	
10 minutes or more	20 mg/kg	Appleton 2000 (Review, 5.4.2.2.1.2-1)	
10-15 minutes	15-20 mg/kg	USP DI (Pharmaceutical Formulary, 5.4.2.1.3.2-3)	
10-30 minutes	15-20 mg/kg	Nelson Ped (textbook, 5.4.2.1.1.3-1)	
20 minutes	15 mg/kg	Kokwaro 2003 (Clinical Report, 5.4.2.4.2.1-3)	
1 hour	10-20 mg/kg	Czapinski 1995 (Clinical Report, 5.4.2.4.2.3-1)	
≤ 1 mg/kg/min	20 mg/kg	BNF/BNFC (age 12-18 years, 5.4.2.1.3.2-1, 5.4.2.1.3.3-2)	
Low speed	10-20 mg/kg		USP DI (Pharmaceutical Formulary, 5.4.2.1.3.2-3)
	200 mg		Rote Liste (Pharmaceutical Formulary, 5.4.2.1.3.3-4, 5)

*: Administration rate up to 10 mg/kg is 100 mg/min. If convulsions persist, the administration rate should be decreased to 50 mg/kg and the administration should be additionally administered.
**: 200 mg, may be 2 - 3 doses per day, maximum daily dose 800 mg

PMDA believes the relationship between the dosing rate of PB and safety in neonatal convulsions has not been clarified, and the dosing rate (5-10 min (20 mg/kg)) set in the investigator-initiated

clinical trial (5.3.5.2-1) was also studied in a small number of cases, and although it cannot be said that sufficient studies have been conducted, no serious events have been observed in these cases, the drug is administered only under the observation of a physician, and overseas clinical reports (5.4.2.4.3-9) indicate that it is important to reach the therapeutic range concentration promptly. in light of the fact that PB is administered at a dose rate of 5 - 10 minutes for neonatal convulsions, PMDA sees no significant issues regarding setting the PB dose rate at 5 - 10 minutes.

While the relationship between PB administration rate and safety is unclear for status epilepticus, PMDA considers that the slow administration rate for status epilepticus in children and adults should be not exceed 10 mg/min based on the fact that it has been indicated that status epilepticus should be administered over 10 minutes or longer based on textbooks, review papers, etc. in Japan and overseas, and that the Japanese guidelines (5.4.2.2.2.2-2) indicate that it should be administered over 100 mg/min.

The relationship between the administration rate and safety is considered necessary in post-marketing surveillance.

3) Skin disorder caused by this drug

PMDA asked the Applicant to explain the possibility of skin disorders occurring as a result of administration of this drug.

The Applicant stated that the incidence of skin rash caused by antiepileptic drugs in adults is high for phenytoin (5.9%), lamotrigine (4.8%), and carbamazepine (3.7%), and low for levetiracetam (0.6%), gabapentin (0.3%), and valproate (0.7%), and it is reported that the frequency of occurrence in PB was in between (approximately 1.0%[13] (Arif H et al, *Neurology*, 68: 1701-1709, 2007), and that the incidence of serious skin disorders (Stevens-Johnson syndrome (SJS), and the risk of developing serious skin disorders (Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN)) in 10,000 newly medicated patients with antiepileptic drugs was reported to be 8.1 for PB, 8.3 for phenytoin, 2.5 for lamotrigine, 1.4 for carbamazepine, and 0.4 for valproate, which is as high as that of phenytoin as reported (Mockenhaupt M et al, *Neurology*, 64: 1134-1138, 2005). In addition, the Applicant reported that the onset time of SJS and TEN was within 63 days for PB, and that sufficient observation is required for at least 6 weeks after the start of administration (Mockenhaupt M et al, *Neurology*, 64: 1134-1138, 2005). The frequency of occurrence by route of administration was found to be difficult in both domestic and overseas literature survey results (5.4.2.6.2-5, 5.4.2.6.2-1), and the frequency of administration was not clearly explained, but the frequency of these events was not clearly explained.

PMDA should pay sufficient attention to skin disorders caused by administration of this drug, but the risks are considered to be similar to those of other antiepileptic drugs, and at present, it is considered that there is no problem to pay attention to the skin disorder by performing the same intramuscular injection preparation of PB. It is also considered necessary to consider the occurrence status of serious skin disorders (SJS and TEN) in post-marketing surveillance.

4) Safety of intravenous administration of phenobarbital in extremely low birth weight infants

PMDA requested the Applicant to explain the safety of PB intravenous administration in extremely low birth weight infants.

13) Calculated by visual inspection from the graph presented in the report

In the investigator-initiated clinical trial (5.3.5.2-1), very low birth weight was not incorporated, and one extremely low birth weight infant (registration number KNG02, birth weight 1144 g) was incorporated, and serious adverse events (patent ductus arteriosus) were observed. However, the causal relationship was ruled out, so the other adverse events were renal failure, hyponatremia, body temperature increased, CRP increased, and impetigo, but the causal relationship was ruled out. Ruth et al. (5.4.2.4.1-8) reported that when PB was administered intravenously (initial dose: 30 mg/kg, maintenance dose: 5 mg/kg, serum concentration before maintenance dose: 43 μg/mL) to 47 preterm infants (weight: 1160 ± 210 g (mean ± standard deviation, hereinafter the same)), no problematic adverse effects were observed except for bilirubin Yamamoto et al. (5.4.2.5.1-4) reported that when PB was administered to prevent intracranial hemorrhage in 18 extremely heavy-birth-weight infants (weight: 1075.5 ± 298.9 g) (20 mg once, mean serum concentration at 24 hours: 25.3 ± 4.2 μg/mL), no adverse effects were observed, and although limited information is available, the safety of intravenous administration of this drug is not expected to be significantly different between very low birth weight infants and other neonates within the range of the dosage and administration for which the Applicant has applied. PMDA considers that the safety of this drug in extremely low birth weight infants has not been sufficiently studied. However, when this drug is administered in such cases, it is necessary to administer it particularly carefully, and it is considered that it should be administered while carefully monitoring the patient's condition. The efficacy and safety in these children should be further investigated in post-marketing surveillance.

(4) Positioning of this drug for treatment of neonatal convulsion and status epilepticus

PMDA asked the Applicant to explain the positioning of this drug in the treatment of neonatal convulsions.

The Applicant is aware that other drugs such as phenytoin, lidocaine, and benzodiazepines exist for the treatment of neonatal convulsions, but regarding this drug, as shown in ① the relationship between the dose and blood concentration and the therapeutic blood concentration have been clarified (5.4.2.5.1-3, 5.4.2.5.1-1, 5.4.2.5.3-2, 5.4.2.4.3-8, 5.4.2.4.3-3, 5.4.2.4.3-13), ② the half-life of blood concentration is longer than that of other drugs, and the effect lasts longer (5.4.2.1.1.1-5), and ③ since there are investigator-initiated clinical trials (5.3.5.2-1) and research reports (5.4.2.5.1-3, 5.4.2.5.1-1, 5.4.2.5.3-2, 5.4.2.4.3-8, 5.4.2.4.3-3, 5.4.2.4.1-4, 5.4.2.4.1-1, 5.4.2.4.1-9, 5.4.2.4.1-7), and as the safety profile is considered to be clear, as stated in domestic and foreign textbooks (5.4.2.1.1.1-5, 5.4.2.1.1.1-1, 5.4.2.1.1.1-2, 5.4.2.1.1.1-6, 5.4.2.1.1.1-4:, 5.4.2.1.2.1-2, 5.4.2.1.2.1-3) and review studies (5.4.2.2.1.1-2, 5.4.2.2.1.1-1), the Applicant explained that it believes it would be a first-line treatment for neonatal convulsions.

PMDA asked the Applicant to explain the positioning of this drug in the treatment of status epilepticus.

The Applicant explained that the first-line drugs for status epilepticus are diazepam in Japan and benzodiazepines such as lorazepam overseas, and they are fast-acting and have a strong anticonvulsant effect, but respiratory depression may occur due to short duration and rapid intravenous administration, and respiratory depression is considered to be a safety problem. In addition, as a second-line drug, phenytoin is used in Japan, and PB or phenytoin is used overseas. There have been reports that the efficacy of PB was comparable to that of lorazepam and higher than that of phenytoin (5.4.2.4.2.2-3), and that the onset of efficacy was faster with PB than with the combination of diazepam and phenytoin (5.4.2.4.2.2.-2). Additionally, the incidence of respiratory depression and decreased level of consciousness is lower with phenytoin than with PB (5.4.2.1.1-6, 5.4.2.2.1.2-1). However, the Applicant explained that due to its high irritant effect on blood vessels, the administration rate of intravenous administration must be slowed, which makes it less effective as an initial treatment, and that it is thought to be useful in combination with benzodiazepines. Based on the above, the Applicant

explained that this drug is considered to be positioned as a second or third-line drug for treatment of status epilepticus depending on the patient's status.

PMDA understands the above, and the drug is considered to provide valuable options for treatment of neonatal convulsion and status epilepticus in Japan.

III. Results of the compliance investigation related to materials to be attached to the application for approval by PMDA/MHLW and PMDA's judgment

1. PMDA judgment on the results of the compliance documentary inspection

As the documents to be attached to the approval application form were investigated in writing based on the provisions of the Pharmaceutical Affairs Law, no particular problems were observed as a result of the examination, it was judged that there was no problem to perform the review based on the submitted documents.

2. PMDA's judgment on the results of GCP on-site inspection

GCP on-site inspection was performed on materials to be attached to the approval application form (5.3.5.2-1: JNR-NP) based on the provisions of the Pharmaceutical Affairs Law. As a result, at some of the investigational sites, the opinions of the Clinical Trial Review Committee had not been heard on the monitoring reports and audit reports received by the head of the site, and while deviations from the study protocol (incorrect conversion of doses to weight) were found, none were serious were found, and PMDA judged that there were no obstacles in conducting the review based on the submitted materials for approval.

IV. Overall Assessment

Based on the data submitted, it is concluded that the efficacy and safety of the drug have been demonstrated when used for neonatal convulsions and status epilepticus. We believe that the effect of the patient demographics, the administration period of this drug and the administration speed of this drug during maintenance administration, etc., on the efficacy and safety, etc. of this drug should be continuously investigated in post-marketing surveillance.

This drug may be approved if it can be judged that there is no particular problem based on the consideration in the expert consultation.

PMDA's thinking was supported in the expert discussion. Based on the examination in the expert consultation, Pharmaceuticals and Medical Devices Agency (PMDA) additionally examined the following points and handled them as necessary. The expert committee member of this expert consultation has requested that the items for this application do not fall under items 1 and 2 (1) of "Handling of conflicts of interest issues for expert committee members of the Pharmaceuticals and Medical Devices Agency" dated May 8, 2007.

(1) Post-marketing surveillance

PMDA will conduct a post-marketing surveillance of the use of the drug in neonatal seizures and status epilepticus to investigate the relationship between the administration of the drug (dose and administration rate) and safety and efficacy, the relationship between blood concentration and safety and efficacy, the relationship between EEG and efficacy of the drug, and the occurrence of serious skin disorders due to the administration of the drug. In addition, PMDA asked the applicant to examine the relationship between birth weight and safety and efficacy in neonatal seizures, and the relationship between drugs used in the treatment of epilepsy and previous treatments for status epilepticus and safety and efficacy in status epilepticus.

The Applicant agreed, and explained that the post-marketing surveillance for neonatal seizures will be conducted on all patients who have used the drug at the registered facilities, and that the post-marketing surveillance for status epilepticus will be conducted on a total of 400 patients (200 adults and 200 children), and the Applicant explained that the survey items, survey forms, etc. would be prepared in consideration of the above contents.

PMDA considers that the drug use-results survey described above should be performed promptly, and the results obtained should be appropriately provided to clinical sites.

As for other points, the dosing rate of this drug was set to take 5 - 10 minutes for the first dose in the investigator-initiated clinical trial. However, in reality, the drug was administered over 2 - 8 minutes (only one case of 2-minute administration was administered in less than 5 minutes), and adverse events such as respiratory depression or decreased oxygen saturation were observed in cases where the administration time was 2 - 6 minutes. Therefore, in the section of precautions for use related to dosage and administration, it is recommended that "the drug be administered slowly over 5 - 10 minutes in neonates. However, depending on the patient's condition, consideration should be given to administering the drug more slowly" and it was deemed appropriate to alert the public.

Based on the review above, PMDA considers it acceptable to approve this drug after adjusting the indications and dosage and administration as follows. Since the re-examination period for this drug is a rare disease drug for neonatal convulsion, it is appropriate to set it for 10 years and a 6-year period for status epilepticus, considering that it is a newly developed drug product, as it is a new route of administration. In addition, the drug substance and drug product are considered to be a powerful drug and not biological products or specified biological products.

[Indications] Neonatal convulsion, status epilepticus

[Dosage and administration] Neonatal convulsion

Initial dose: 20 mg/kg intravenously as phenobarbital. If convulsions cannot be controlled, the dose should be adjusted to the extent not exceeding the initial dose, depending on the patient's condition, and the dose should be additionally administered intravenously.

Maintenance dose: 2.5 - 5 mg/kg of phenobarbital is intravenously administered once daily.

Status epilepticus

15 - 20 mg/kg of phenobarbital is intravenously administered once daily.

Document 2 (Japanese original)

審議結果報告書

平成 20 年 9 月 16 日
医薬食品局審査管理課

[販　売　名] ノーベルバール静注用 250mg
[一　般　名] フェノバルビタールナトリウム
[申　請　者] ノーベルファーマ株式会社
[申請年月日] 平成 19 年 9 月 14 日

[審 議 結 果]
平成 20 年 8 月 29 日に開催された医薬品第一部会において、本品目を承認して差し支えないとされ、薬事・食品衛生審議会薬事分科会に報告することとされた。
なお、本品目は生物由来製品及び特定生物由来製品に該当せず、再審査期間は、新生児けいれんについては 10 年、てんかん重積状態については 6 年とし、原体及び製剤ともに劇薬に該当するとされた。

審査報告書

平成 20 年 8 月 11 日
独立行政法人医薬品医療機器総合機構

承認申請のあった下記の医薬品にかかる医薬品医療機器総合機構での審査結果は，以下のとおりである。

記

[販 売 名]	ノーベルバール静注用 250 mg
[一 般 名]	フェノバルビタールナトリウム
[申 請 者 名]	ノーベルファーマ株式会社
[申請年月日]	平成 19 年 9 月 14 日
[剤型・含量]	1 バイアル中にフェノバルビタールナトリウム 274 mg（フェノバルビタールとして 250 mg）を含有する静注用凍結乾燥製剤
[申 請 区 分]	医療用医薬品（3）新投与経路医薬品
[特 記 事 項]	希少疾病用医薬品
[審査担当部]	新薬審査第三部

審査結果

平成 20 年 8 月 11 日

［販　売　名］　ノーベルバール静注用 250 mg
［一　般　名］　フェノバルビタールナトリウム
［申 請 者 名］　ノーベルファーマ株式会社
［申請年月日］　平成 19 年 9 月 14 日
［審 査 結 果］

提出された資料から、本剤の新生児けいれん、てんかん重積状態に対する有効性及び安全性は示されていると判断する。なお，新生児けいれん及びてんかん重積状態において、本剤の投与状況（投与量、投与速度）と安全性及び有効性との関係、血中濃度と安全性及び有効性との関係、重篤な皮膚障害の発現状況等については、製造販売後調査においてさらに検討が必要と考える。

以上、医薬品医療機器総合機構における審査の結果、本品目については、以下の効能・効果、用法・用量で承認して差し支えないと判断した。

［効能・効果］　新生児けいれん、てんかん重積状態
［用法・用量］　新生児けいれん
初回投与：フェノバルビタールとして、20 mg/kg を静脈内投与する。けいれんがコントロールできない場合は、患者の状態に応じ、初回投与量を超えない範囲で用量を調節し、静脈内に追加投与する。
維持投与：フェノバルビタールとして、2.5～5 mg/kg を 1 日 1 回静脈内投与する。
てんかん重積状態
フェノバルビタールとして、15～20 mg/kg を 1 日 1 回静脈内投与する。

審査報告（1）

平成20年7月4日作成

Ⅰ．申請品目

［販　売　名］	ノーベルバール静注用250 mg
［一　般　名］	フェノバルビタールナトリウム
［申請者名］	ノーベルファーマ株式会社
［申請年月日］	平成19年9月14日
［剤型・含量］	1バイアル中にフェノバルビタールナトリウム274 mg（フェノバルビタールとして250 mg）を含有する静注用凍結乾燥製剤
［申請時効能・効果］	新生児けいれん、てんかん重積状態
［申請時用法・用量］	新生児けいれん 初回投与：フェノバルビタールとして、20 mg/kgを1日1回静脈内投与する。けいれんがコントロールできない場合は、同量を追加静脈内投与する。 維持投与：フェノバルビタールとして、2.5～5 mg/kg/日を静脈内投与する。維持投与期間は6日間をめどとする。 なお、年齢、症状により適宜増減する。 てんかん重積状態 フェノバルビタールとして、15～20 mg/kgを1日1回静脈内投与する。 なお、年齢、症状により適宜増減する。

Ⅱ．提出された資料の概略及び審査の概略

本申請において、申請者が提出した資料及び医薬品医療機器総合機構（機構）からの照会事項に対する申請者の回答の概略は、下記のようなものであった。

1．起原又は発見の経緯及び外国における使用状況等に関する資料

フェノバルビタール（PB）は、1912年バイエル社（ドイツ）により開発された抗けいれん作用と催眠・鎮静作用を有する代表的なバルビツール酸誘導体で、催眠・鎮静薬又はてんかんのけいれん発作治療薬として経口剤、注射剤、坐剤等が広く使用されており、海外では、2008年5月30日現在、PBの経口剤（錠剤、内用液、エリキシル剤、散剤、シロップ剤等）は米国、英国、ドイツ及びフランス等世界22ヶ国、坐剤はスイス1ヶ国で承認されており、フェノバルビタールナトリウム（本薬）の経口剤（錠剤、内用液、エリキシル剤、散剤、シロップ剤）は3ヶ国、注射剤は、静注用製剤12ヶ国、筋注用製剤13ヶ国、皮下注用製剤1ヶ国で承認されている。

本邦においては、PBは1944年1月にフェノバール®末が承認されて以来、錠剤、散剤、エリキシル剤、皮下・筋注用製剤が承認されている。一方、フェノバルビタールナトリウム（本薬）は、1949年に皮下・筋注用製剤が承認され、1980年3月に坐剤が承認されている。経口剤及び皮下・筋注用製剤については、1975年6月に再評価結果通知が発出され、現在の効能・効果（不安緊張状態の鎮静、自律神経発作、精神運動発作、てんかんのけいれん発作）となっている。

しかしながら、本邦においては適切な静注用製剤が販売されていないこと、新生児けいれんの

効能・効果が設定されていないこと，小児における用法・用量は坐剤を除き設定されていないこと等の課題が残されていたため，2006年3月から自ら治験を実施するものによる治験（医師主導治験）が開始された。

今般，申請者は，新生児けいれんについては，医師主導治験の結果及び既存の情報を整理し有効性及び安全性が確認されたと考えたこと，てんかん重積状態については，2005年11月24日，日本小児科学会及び日本小児神経学会から，静注用フェノバルビタール製剤のてんかん重積状態に対する適応追加の要望書が厚生労働大臣に提出されたことを踏まえ，申請者は文献調査等を実施し，てんかん重積状態に対する本剤の有効性及び安全性は広く認められており，てんかん重積状態については医学薬学上公知と判断し，これら2つの効能・効果について承認申請を行った。

なお，本申請における「非臨床に関する資料」については，ウサギを用いた血管刺激性試験のみが実施され，その他の新たな試験成績に基づく資料は提出されていないが，公表文献等に基づく考察が行われている。

2．品質に関する資料

＜提出された資料の概略＞

（1）原薬

原薬であるフェノバルビタールナトリウムは，白色の結晶又は結晶性の粉末であり，一般特性として性状，溶解性，熱分析，pH，吸湿性，解離定数（pKa），結晶多形が検討されている。原薬は吸湿性を有し，2種類の結晶形が存在し，相対湿度68 %以上において吸湿により結晶形が変化すると考えられているが，製剤は原薬を水に溶解した後，バイアルに分注し凍結乾燥した製剤であり，原薬の結晶形の相違が製剤の製造に影響しないと判断されたことから，吸湿により生成する結晶形の物理的及び化学的検討は実施されていない。

原薬の製造は[redacted]及び[redacted]を出発物質として，[redacted]の合成（Step 1），[redacted]の合成（Step 2），[redacted]の合成（Step 3），フェノバルビタール[redacted]（Step 4），フェノバルビタールの[redacted]（Step 5），フェノバルビタールナトリウムの合成及び包装（Step 6）からなる。Step[redacted]及び[redacted]が重要工程と規定され，Step[redacted]のフェノバルビタールが重要中間体として管理値が設定されている。

原薬の化学構造は，赤外吸収（IR）スペクトル，核磁気共鳴（^{1}H-NMR）スペクトル，質量（MS）スペクトルにより確認されている。不純物として，類縁物質及び残留溶媒（エタノール）について検討されている。

原薬の規格及び試験方法として，性状（外観），確認試験（IRスペクトル，ナトリウム塩の定性反応），pH，純度試験（溶状，塩化物，硫酸塩，重金属，中性又は塩基性物質，硫酸呈色物，類縁物質＜液体クロマトグラフィー（HPLC）＞，残留溶媒＜ガスクロマトグラフィー（GC）＞，乾燥減量，エンドトキシン，含量＜HPLC＞）が設定され，ヒ素については，検討されたが規格は設定されていない。なお，原薬の吸湿性を踏まえ，審査の過程において水分が新たに規格として設定された。

原薬の標準物質であるフェノバルビタール標準品について，審査の過程において，日本薬局方収載規格に加え，純度試験（類縁物質＜HPLC＞）が設定された。

原薬の安定性については，実生産スケールで製造された原薬について，ポリエチレン袋に詰め，

その袋を厚紙の箱[1]に入れた状態で長期保存試験（25±2℃/60±5 %RH、暗所、60ヶ月）、加速試験（40±2℃/75±5 %RH、暗所、6ヶ月）及び中間的試験（30±2℃/65±5 %RH、暗所、12ヶ月）が実施された。これらの試験では、性状、溶状、pH、純度試験（類縁物質＜薄層クロマトグラフィー（TLC）＞）、乾燥減量、含量＜滴定法＞が試験項目であり、加速試験及び中間的試験においては確認試験（IR スペクトル及び融点）、純度試験（類縁物質＜HPLC＞）及び微生物限度も試験項目とされた。これらの安定性試験において、いずれの測定項目においても明確な変化は認められず、原薬の貯蔵方法は「気密容器、室温保存」、原薬のリテスト期間は5年と設定された。

（2）製剤

製剤は、添加剤を含まず、原薬のみからなる凍結乾燥注射剤であり、無色透明ガラスバイアル/ゴム栓/フリップキャップを容器及び施栓系とする。1バイアルあたりフェノバルビタールナトリウムとして274 mg（フェノバルビタールとして250 mg）を含有する製剤が申請されている。製剤の開発においては、フェノバルビタールは水に極めて溶けにくく、溶解補助剤の使用は新生児にとって好ましくないと考えられたことから、水溶性の高いフェノバルビタールナトリウムを原薬とし、添加剤は使用せず、剤型を凍結乾燥注射剤にすることが適切と判断されている。

製剤の製造工程は、第一工程（[redacted]工程）、第二工程（無菌ろ過工程）、第三工程（[redacted]工程）、第四工程（凍結乾燥工程）、第五工程（[redacted]工程）及び第六工程（選別・包装工程）からなる。第[redacted]、第[redacted]及び第[redacted]工程が重要工程とされ、管理項目及び管理値が設定されている。なお、審査の過程において、製剤の吸湿性を踏まえて、第[redacted]工程が重要工程に位置付けられ、[redacted]が工程管理として設定されている。

製剤の規格及び試験方法としては、性状（外観）、確認試験（IR スペクトル、ナトリウム塩の定性反応）、pH、純度試験（類縁物質＜HPLC＞）、乾燥減量、製剤均一性（質量偏差）、エンドトキシン、不溶性異物、不溶性微粒子、無菌、含量＜HPLC＞が設定されている。また、製剤の吸湿性を踏まえ、審査の過程において水分が新たに規格として設定されている。

製剤の安定性については、パイロット生産スケールで製造された製剤について、長期保存試験（紙箱入り、25±2℃/60±5 %RH/暗所、36ヶ月）、加速試験（紙箱入り、40±2℃/75±5 %RH/暗所、6ヶ月）及び苛酷試験（温度［紙箱入り、50±2℃/なりゆき湿度/暗所、3ヶ月］、光［無包装、総照度120万 lux·hr以上+総近紫外放射エネルギー200W·h/m^{2}以上］）が実施され、また実生産スケールで製造された製剤について相対加速試験（紙箱入り、正立/倒立、40±2℃/75±5 %RH/暗所、3ヶ月）が実施された。これらの試験では、性状、確認試験（IR スペクトル、ナトリウム塩の定性反応）、溶状、pH、純度試験（類縁物質＜HPLC＞）、水分、乾燥減量、不溶性異物、不溶性微粒子、含量が試験項目とされ、長期保存試験及び加速試験においてはエンドトキシン、製剤均一性（質量偏差）及び無菌も試験項目とされた。その結果いずれの試験項目においても明確な変化は認められなかった。以上から製剤の貯法は室温保存、一次包装はガラス製バイアルとゴム栓及びキャップによる密封包装とされ、製剤の有効期限は3年と設定されている。

＜審査の概略＞

---

1) 原薬の容器及び施栓系は、ポリエチレン袋（二重）に入れ、ファイバードラムで遮光保存することとなっており、安定性試験の保存条件と異なっている。

（1）原薬

機構は，原薬について光安定性試験が実施されていないが、原薬の安定性について適切な評価がなされているか、申請者に説明を求めた。

申請者は，本原薬は米国薬局方などの海外公定書には既に収載されており，本邦においても第十改正日本薬局方まで収載されていたことから、その安定性及び貯蔵方法について十分に評価されていると考え，光安定性試験を実施せず，上記の公定書に従った貯法（気密容器）を設定したと説明した。

機構は、製剤に含まれる残留溶媒（エタノール）の含量の規格値が[redacted]%と設定されていることについて，本剤の用法・用量でヒトに投与した際の安全性を考慮し、その妥当性について申請者に説明を求めた。

申請者は、本剤の用法・用量は、新生児けいれんにおいてフェノバルビタールとして 20 mg/kg を 1 日 1 回投与，効果不十分な場合は同量を追加投与、てんかん重積状態においてはフェノバルビタールとして，15～20 mg/kg を 1 日 1 回静脈内投与すると設定されているが、臨床現場では 20 mg/kg の倍量まで投与される可能性があることを考慮すると、エタノールの 1 日最大投与量は成人（体重 60 kg と仮定）で 184.1 mg/日，新生児（体重 3.0 kg と仮定）で 9.2 mg/日となり、成人ではヒトの PDE（permitted daily exposure）である 166.7 mg/日（ICH Guideline-Residual Solvents, *Pharmeuropa, Vol.9, No.1 Supplement April*, S58-59, 1997）を上回るが、成人に 184.1 mg のエタノールを投与した場合の血中濃度は 0.005 %（循環血液量 4000 mL と仮定）であり、臨床症状が認められる血中エタノール濃度（0.02 %以上）と比較して低値であること（河野裕明ら，*わが国のアルコール関連問題の現状*, 1993）、また、ACGIH（米国産業衛生専門家会議）により設定されたエタノール取扱い作業環境での許容限界に基づくエタノールの 1 日摂取量は，376 mg/kg（ICH Guideline-Residual Solvents, *Pharmeuropa Vol.9, No.1 Supplement April*, S58-59, 1997）であること、本剤は長期間投与するものでないことを考慮すると、ヒトに及ぼす影響はほとんどないか、あっても軽微であると考えることを説明した。なお申請者は，小児についてのエタノール摂取量についての許容限界についての記載は見出せなかったことをあわせて説明した。

機構は、医師主導治験の成績等を考慮すると、現段階で新生児における安全性に特段の問題はないと考えるが、製造販売後調査において、投与量及び投与期間と本剤の安全性との関連性については、検討が必要と考える。

その上で機構は、以上について了承し、原薬の規格、試験方法、貯法及びリテスト期間並びに製剤の規格、試験方法、貯法及び有効期間について妥当であると判断した。

3．非臨床に関する資料

（i）薬理試験成績の概要

本申請に際して新たな薬理試験は実施されておらず、PB 又は本薬の抗けいれん作用、鎮痛作用、催眠作用、中枢神経系、呼吸器系、循環器系及び消化器系に及ぼす影響及び幼若動物への影響に関する公表文献が提出された。

＜提出された資料の概略＞

（1）効力を裏付ける試験

1）抗けいれん作用

①最大電撃けいれん法

マウスに PB 0～100 mg/kg を皮下投与（s.c.）し、最大電撃けいれん抑制作用について検討したところ、本薬の 50 %有効用量（ED_{50}）は 20.7 mg/kg、50 %中毒量（TD_{50}; 50 %の動物に運動失調等の中毒症状を発現させる用量）は 57 mg/kg、防御指数（PI（protective index）: TD_{50}/ED_{50}）は 2.7 であった。また、フェニトイン（s.c.）、トリメタジオン（s.c.）及びフェニルアセチル尿素（経口投与（p.o.））の ED_{50} はそれぞれ 9.1、630 及び 109 mg/kg、TD_{50} はそれぞれ 92、660 及び 640 mg/kg、PI はそれぞれ 10.1、1.1 及び 5.9 であった（4.3.1.1-4）。

ラットに本薬、エタノール又は methyl parafynol を経口投与し、最大電撃けいれん抑制作用について検討したところ、ED_{50} はそれぞれ 3.7、2850 及び 50 mg/kg、TD_{50} はそれぞれ 28、6500 及び 108 mg/kg、PI はそれぞれ 7.6、2.3 及び 2.2 であった（4.3.1.1-5）。

②電撃けいれん閾値法

ラットに PB（s.c.）、トリメタジオン（s.c.）又はフェニルアセチル尿素（p.o.）を投与し，電撃けいれん抑制作用について検討したところ，PI はそれぞれ 1.75 ± 0.19、1.51 ± 0.17、18.7 ± 3.70 であった。なお、フェニトイン（s.c.）は電撃けいれんに対して無効（閾値の 1.2 倍の電流でもけいれんを起こさない場合を有効と判断）であった（4.3.1.1-6）。

③薬物誘発けいれん法

マウスに PB、トリメタジオン及びフェニルアセチル尿素を皮下投与し、メトラゾール誘発けいれんに対する抑制作用について検討したところ、ED_{50} はそれぞれ 24、480 及び 125 mg/kg であり、PI はそれぞれ 2.5、1.4 及び 2.3 であった。なお、フェニトインはメトラゾール誘発けいれんに対して無効であった（4.3.1.1-4）。

マウスに本薬，フェニトイン及びトリメタジオンを経口投与し、高用量のメトラゾールにより誘発される間代性けいれん、強直性けいれん及び死亡に対する抑制作用について検討したところ、本薬及びトリメタジオンは間代性けいれん，強直性けいれん及び死亡を抑制した（本薬の 50 %防御量（PD_{50}）はそれぞれ、38.2、20.7 及び 26.8 mg/kg、トリメタジオンの PD_{50} はそれぞれ、571.4、498.0 及び 459.4 mg/kg）が、フェニトインは強直性けいれんのみを抑制した（PD_{50}; 12.0 mg/kg）（4.3.1.1-8）。

マウスに PB、フェニトイン，トリメタジオン及びフェニルアセチル尿素を皮下投与し，アミノヘキサン誘発けいれん抑制作用について検討したところ、PB、トリメタジオン及びフェニルアセチル尿素はけいれん抑制作用を示した（それぞれの ED_{50}; 15、480 及び 70 mg/kg）が、フェニトインはけいれん抑制作用を示さなかった（4.3.1.1-7）。

マウスに PB、フェニトイン及びトリメタジオンを経口投与し、ピクロトキシン又はストリキニーネ誘発けいれん抑制作用について検討したところ、PB はピクロトキシン及びストリキニーネのいずれの薬物によるけいれんに対しても抑制作用を示した（ED_{50} はそれぞれ 135 及び 210 mg/kg）のに対し，フェニトインはいずれの薬物によるけいれんに対しても無効であった。また、トリメタジオンはピクロトキシン誘発けいれんに対して抑制作用を示したが（ED_{50}; 1300 mg/kg）、ストリキニーネ誘発けいれんに対する抑制作用は認められなかった（4.3.1.1-9）。

2）催眠作用

マウスに、長時間型（PB）、中間型（アモバルビタール）及び短時間型（ヘキソバルビタール）のバルビツール酸系薬物を経口及び腹腔内投与し、催眠作用について検討した。50 %の動物が睡眠に至る投与量（HD_{50}）は、経口及び腹腔内投与でそれぞれ、PB で 89 mg/kg 及び 75 mg/kg、アモバルビタールで 129 mg/kg 及び 61 mg/kg、ヘキソバルビタールで 130 mg/kg 及び 50 mg/kg であり、経口投与時の LD_{50} は PB で 325 mg/kg、アモバルビタールで 560 mg/kg、ヘキソバルビタールで 468 mg/kg であった。また、各薬物の LD_{50} の 60 %を経口投与し、催眠作用発現時間と作用持続時間を検討したところ、PB、アモバルビタール及びヘキソバルビタールで、作用発現時間はそれぞれ 31、2 及び 1 分、作用持続時間は 476 ± 14、89 ± 9 及び 91 ± 11 分であった。さらに、PB（投与量: HD_{50} の 15、30 及び 60 %）を経口投与し、ヘキソバルビタール誘発睡眠に対する睡眠延長作用について検討したところ、無処置群における平均睡眠時間はいずれの投与量でも 18.1 分であったのに対し、PB の投与によりそれぞれ 30.5、28.4 及び 46.2 分まで延長した（4.3.1.1-9）。

3）鎮静作用

マウスに本薬を皮下投与し、iminodipropionitrile（IDPN）により誘発される回転運動を伴う運動亢進（回転症候群）に対する抑制効果を検討したところ、20 mg/kg 以上の用量で活動性の低下、回転運動の 50 %減少がみられ、40 mg/kg ではより強い鎮静作用が認められ、その作用は 3 時間以上持続した（4.2.1.1-10）。

ラット条件回避行動に対して、本薬は LD_{50} の 1/10 の投与量（投与経路不明）で回避反応を 60 %減少させた（4.2.1.1-11）。

4）作用機序

マウスに抗けいれん薬を腹腔内投与し、その後けいれん誘発物質を静脈内持続注入し、強直性けいれんの発現までの投与量と潜伏時間について検討した。PB はフェニトイン、カルバマゼピン、ジアゼパムと同様に、ピクロリン及びピクロトキシン誘発けいれんに対して抑制作用を示したが、N-methyl-D-aspartate（NMDA）誘発けいれんに対しては抑制作用を示さなかった（4.3.1.1-13）。

マウス胎児培養神経細胞を用いてパッチクランプ法により、PB 又はジアゼパムによる $GABA_A$ 受容体のバースト発火に対する作用について検討したところ、PB は GABA によるクロライドチャネルの開口頻度を増加させず、開口時間を延長したのに対して、ジアゼパムは GABA によるクロライドチャネルの開口頻度を増加させたが、開口時間に影響を及ぼさなかった（4.3.1.1-14）。

5）代謝物

PB の主要代謝物である p-hydroxyphenobarbital（PHPB）をイヌ（100 mg/kg）又はマウス（500 mg/kg）に静脈内投与したとき、一般症状に影響は認められなかったが、マウスに 1000 mg/kg を静脈内投与すると、鎮静、手足の振るえ、整復困難が認められ、数分後に作用は消失した（4.3.2.4-6）。

（2）副次的薬理試験

マウスに本薬 150 mg/kg を断頭の 1 時間前に腹腔内投与し、断頭後 10 秒間の脳内クレアチンリン酸、ATP、グルコース、グリコーゲン、乳酸を測定したところ、本薬投与によりすべてのパラ

メータの変動が抑制された。(4.3.1.2-1)

（3）安全性薬理試験

全身状態に対する影響について、マウスに PB を皮下投与すると，50 mg/kg で 2/8 例、60 mg/kg で 3/8 例に運動抑制、75 mg/kg で 6/8 例、100 mg/kg で 8/8 例に歩行失調を示し、100 mg/kg では 4/8 例に麻酔状態が認められた（4.3.1.3-1)。ウサギに本薬（150 mg/kg 又は 170 mg/kg）を筋肉内投与すると、全身の筋肉が弛緩し運動緩慢となり、横臥・睡眠状態となった。投与 30～150 分後に角膜反射、対光反射の消失、耳静脈の拡張（後に収縮）、呼吸数の減少が認められ、深麻酔の状態となった。投与 1 時間後より体温低下が認められたが、8～12 時間後には回復傾向が認められた（4.3.1.3-2)。

血圧・心拍数・体温に対する作用について、ラットに本薬を皮下投与したところ、7 mg/kg までは血圧の変動は認められなかったが，10～30 mg/kg では軽度の血圧低下が認められた（4.3.1.3-4)。また、ラットに本薬 40 mg/kg を腹腔内投与したところ，血圧及び心拍数に対して影響を及ぼさなかったが，投与 40 分から 80 分にわたり直腸温を軽度低下させた（4.3.1.3-5)。

また，非臨床試験成績ではないが，ヒト（成人）において，バルビツール酸系薬剤を含む催眠剤の大量経口投与又は急速静脈内投与をした場合は、逆走神経抑制による心拍数の軽度増加及び末梢血管拡張による血圧低下が認められ、呼吸器系に対する作用については、延髄機能抑制により呼吸抑制を生じる（4.3.1.3-3)。麻酔を目的とした PB を含むバルビツール酸系薬剤の静脈内投与では咳、くしゃみ、しゃっくりが認められ、咽頭けいれんが認められる（4.3.1.3-6)。また、消化器系に対する作用について、PB を含むバルビツール酸系薬剤は用量により末梢性又は中枢性に消化管平滑筋に作用し、消化管運動を抑制するが、ヒトにおいて麻酔量で胃排出能を著しく遅らせることはなかった（4.3.1.3-6)。

幼若動物に対する作用について、以下の文献が提出されている。

循環動態パラメータの変動について、ブタ新生児（生後 1～3.5 日齢）に PB を 15 又は 45 mg/kg で静脈内投与したとき、二酸化炭素分圧の上昇、脳血流量の低下が認められた。脳の部位別血流量でも、大脳、視床、脳幹、小脳で血流の減少が認められ、また脳内酸素消費量の低下が認められた（4.3.1.3-7)。

血圧、血液ガス圧の変動について、ブタ新生児（生後 1～2 日）に、フェニレフリン静脈内投与による血圧上昇時及び 50 % N_2-50 % air ガス吸引による低酸素負荷時における脳血流の変化に対する本薬の影響を検討した。本薬 20 mg/kg 静脈内投与群と対照群（生理食塩水静脈内投与）の間に血圧変動、血液ガス圧の変動に差は認められなかったが，全脳及び脳内各部位における脳血流が、対照群で血圧上昇時及び血圧上昇かつ低酸素負荷時に有意に増加したのに対し，本薬投与群ではこれらの増加が抑制された（4.3.1.3-8)。

以上より申請者は、本薬は脳神経細胞の $GABA_A$ 受容体を活性化させ、クロライドチャネルの開口時間の延長を引き起こすことにより、様々な動物けいれんモデルに対し抗けいれん作用を有し、催眠作用及び鎮静作用を有すること、また大量投与により歩行失調，睡眠状態，心拍数の増加，血圧低下，呼吸抑制、消化管運動の抑制を引き起こすが、これらは臨床用量よりも高用量で

認められる事象であり、通常の臨床用量の範囲において、これらの症状が発現する可能性は低いと考えることを説明した。

＜審査の概略＞

機構は、本申請で提出された資料は公表文献のみであるが、本薬の薬理作用については広く知られていることを踏まえると、薬理学的に大きな問題はないと考える。

（ii）薬物動態試験成績の概要

＜提出された資料の概要＞

本申請に際して新たな非臨床薬物動態試験は実施されておらず、参考資料として、主として静脈内投与時の薬物動態及び幼若動物の脳移行性に関する公表文献が提出された。

（1）吸収

雌性ラットに本薬 80 mg/kg を静脈内投与したとき、血清中 PB 濃度は 11.1 ± 1.8 時間の消失半減期（$t_{1/2}$）で消失し、分布容積（Vd）は 0.49 ± 0.085 L/kg であった（4.3.2.2-1）。

成熟ウサギ（雌性）及び新生児ウサギ（雌雄）に本薬 10 又は 30 mg/kg を静脈内投与したとき、クリアランス、定常状態の分布容積（V_{ss}）及び $t_{1/2}$ は用量に依存せず、差異は認められなかったが、新生児ウサギでは、全身クリアランスが成熟ウサギより低値で、消失も遅延した。また、成熟ウサギに本薬 10 mg/kg を経口又は静脈内投与したとき、血清中 PB 濃度の消失パターンは両投与経路で類似しており、経口投与時の BA は 119 %であった（4.3.2.2-3）。

雌雄イヌに本薬又は PB 10 mg/kg を静脈内又は経口投与したとき、血漿中 PB 濃度はそれぞれ 2)（投与 0 時間値）又は 15（最高血漿中濃度<C_{max}>: 投与 6 時間後）μg/mL であり、$t_{1/2}$ は 56 又は 52 時間であった。経口投与時の生物学的利用率（BA）は 91 %であり、両投与経路での PB の薬物動態は類似していた（4.3.2.2-2）。

雌雄イヌに本薬 2 mg/kg（実際の投与量は 2.06 ± 0.13 mg/kg）を 1 日 3 回、6 日間反復経口投与したとき、最終投与後の血清中 PB 濃度は、投与 3.2 時間後に C_{max}（13.2～24.2 μg/mL）に達した後、53 時間の $t_{1/2}$ で減少した（4.3.2.2-4）。

（2）分布

雄性ラットに本薬 30 又は 50 mg/kg を静脈内投与したとき、最も高い分布は肝臓に認められ、その他に腎臓等にも速やかに分布した。脳、筋肉及び腸管への分布は遅く、特に脳への移行は投与 1 時間後に最高値を示し、移行の遅れが認められた。血漿、肝臓及び腎臓では PB 濃度の再上昇が認められ、組織内分布への腸肝循環の関与が示唆された（4.3.2.3-1）。

雄性ウサギに本薬（PB として）20 又は 50 mg/kg を静脈内投与したとき、投与 5 分後における組織内 PB 濃度は、肝臓、血漿、心臓、腎臓、肺、筋肉、脳の順であった。全血中 PB 濃度は血漿中 PB 濃度と同程度であり、PB の血球への移行性は低いと推察されている（4.3.2.3-2）。

血液脳関門は、脳内の毛細血管内皮細胞により形成されると報告されている（4.3.2.3-5）。

ラットにおいて、生後 1 日において脳毛細血管内腔は形成され始め、9 日目で内皮細胞の形成が認められ、毛細血管網は生後約 30 日で完成し、ラットの血液脳関門は生後約 1 ヶ月でその機能

を発現すると推察されている（4.3.2.3-6）。

ウサギ（雌雄不明）に、^{14}C-PB を静脈内投与したとき、PB の全脳内濃度に対する脳実質内への取り込み率は、生後 14 日齢まで成熟動物と比較して高値を示し、その後 28 日齢までに急速に低下し、28 日齢では成熟動物と同レベルを示したことから、ラットの血液脳関門は生後約 30 日までに形成され機能を発現すると考えられている（4.3.2.3-7）。

妊娠ラットに PB を 30 mg/kg で腹腔内投与したとき、PB は速やかに胎児に移行し、その推移は母体の血漿中 PB 濃度推移と類似していた（4.3.2.3-3）。

雄性ラットに ^{3}H-PB を静脈内投与し、最大電撃けいれんによるけいれん抑制作用を示した際の脳内及び血漿中 PB 濃度は、投与終了 3 分後においてそれぞれ 8.2 及び 16.9 μg/mL（中央値）であり、血漿中 PB の 61.6 %が非結合型 PB であった（4.3.2.3-4）。

ウシ血清アルブミン及びウサギ血漿に対する PB のタンパク結合率（限外ろ過法）は 44 及び 41 %であった。また、肝臓、心臓、腎臓、肺、脳、筋肉、赤血球との結合率は 14～32 %であり、特定の組織への結合は認められなかった（4.3.2.3-2）。

新生児ウサギ及び成熟ウサギにおいて、血清中 PB 濃度が 15 及び 60 μg/mL のとき、平均非結合型 PB 濃度は、新生児で 0.72 及び 0.68 %、成熟動物で 0.40 及び 0.46 %であり、PB の血清タンパク質との結合は、新生児よりも成熟動物において高かった（4.3.2.2-3）。

（3）代謝

マウス、ラット及びモルモットでの代謝を検討した結果、主要代謝物 p-hydroxyphenobarbital（PHPB）の他に、マウスの尿中では PB-N-glucopyranoside が検出され、ラット及びモルモットの尿中では、5-(3,4-dihydroxy-1,5-cyclohexadien-1-yl)-5-ethylbarbituric acid 及び 5-(1-hydroxyethyl)-5-phenylbarbituric acid が検出された（4.3.2.4-1、4.3.2.4-2、4.3.2.4-3、4.3.2.4-4）。

雄性ラットに ^{14}C-PB を 50 mg/kg で腹腔内投与したとき、投与 24 時間後までの尿中には未変化体 14 %、PHPB 16 %、PHPB のグルクロン酸抱合体 9 %、未知の酸性水溶性代謝物 4 %が排泄された。また、開環によって ethylphenylmalonylurea が生じることが推定されている（4.3.2.4-5）。

雄性イヌに PB を 10～40 mg/kg で反復経口投与し、尿中代謝物について検討したとき、主代謝物は PHPB であり、尿中に排泄された PHPB の 67～83 %がグルクロン酸抱合体と推定されている（4.3.2.4-6）。

PB 投与により、薬物代謝酵素が誘導され、CYP 分子種（CYP1A2、CYP2A6、CYP2B、CYP2C（CYP2C9、CYP2C19）及び CYP3A）並びに UDP-glucuronosyltransferace（UGTs）等の肝薬物代謝酵素が誘導された（4.3.2.4-8、4.3.2.4-9、4.3.2.4-10、4.3.2.4-11）。

雄性ラット（成熟：3～4 ヶ月齢、老齢：32～34 ヶ月）に本薬 4.73 mg/日を 5 日間反復腹腔内投与したとき、血漿中 PB の総クリアランスは単回投与時（20 mg/kg）と比較して成熟ラット及び老齢ラットでそれぞれ 83 及び 54 %高値であった（4.3.2.4-12）。

雌雄イヌに PB を 5.5 又は 15 mg/kg/日で単回経口投与し、35 日間の休薬後にそれぞれ 5.5 又は 11 mg/kg/日で 90 日間反復経口投与したとき、いずれの投与群においても反復投与時では単回投与時より血清中 PB の総クリアランスが高値を示し、$t_{1/2}$ は短縮した（4.3.2.4-13）。

雄性イヌに PB を 15～400 mg/kg で経口投与したとき、投与量の増加に伴って血漿中 PB の総クリアランスは高値を示し、$t_{1/2}$ は短縮した（4.3.2.4-14）。

以上の公表文献から、申請者は、本薬の反復投与により、自己代謝酵素誘導が起こることが示唆されていることを説明した。

なお、新生児における代謝への影響については、以下の資料が提出されている。

ラットにおける肝薬物代謝酵素の活性発現は、生後急速に発達して 30～60 日齢でほぼ最高に達し、その後徐々に低下した。生後 1 及び 5 日齢の新生児ラットにおける薬物代謝酵素活性（Aminopyrine N-demethylation 及び Pentobarbital hydroxylation）は 30 日齢の約 2～3 %及び約 14～20 %であり、20 日齢では 30 日齢の約 60 %に達した（4.3.2.4-15、4.3.2.4-16、4.3.2.4-17、4.3.2.4-18）。

ラットにおいて、P-450 の生後発達パターンは CYP 分子種により著しく異なるが（4.3.2.4-19）、CYP 分子種の多く（CYP2B1、CYP2B2、CYP1A2、CYP3A2）は、出生時に発現しており、出生後、急速に増加した（4.3.2.4-20、4.3.2.4-21、4.3.2.4-22、4.3.2.4-23）。また、一部の CYP 分子種（CYP2C11、CYP2C12、CYP2C7）は生後に発現することが報告されている（4.3.2.4-24、4.3.2.4-25）。

生後 0、2 及び 12 日齢の新生児ラット及び成熟ラットに PB を 80 mg/kg で腹腔内投与し、生後 3、6 及び 16 日齢及び成熟期における肝ミクロソーム薬物代謝酵素の誘導について検討された。全ての日齢のラットにおいて、PB 投与により P-450 総含量の増加及び薬物代謝酵素の誘導が示されたが、ペンツピレン水酸化活性は、新生児ラットでは約 2 倍上昇したものの成熟動物での上昇はわずかであり、ペンツフェタミン N-脱メチル化活性は、成熟動物では 2～3 倍の上昇が認められたのに対して新生児ではその程度は低かった。また、アルドリンエポキシ化活性は、成熟動物と異なり、新生児（生後 3 及び 6 日）では、PB によりほとんど誘導されなかった。なお、PB 誘導新生児肝ミクロソームにおけるペンツフェタミン N-脱メチル化代謝酵素に対する成熟ラット PB 誘導 P-450 抗体による抑制は、3 日齢ではほとんど認められなかったが、その後の日齢増加により抑制率が 30～50 %まで増加したことから、生後数週までの新生児ラットにおける PB で誘導されたペンツフェタミン N-脱メチル化は、成熟ラットで誘導された CYP 分子種とは異なる CYP 分子種により代謝されていることが示唆された（4.3.2.4-26）。

（4）排泄

雌性ラットに ^{14}C-PB を腹腔内に 50 mg/kg 単回投与したとき、投与後 8 日間に投与放射能の 79 %が尿中に、9 %が糞中にそれぞれ排泄され、主排泄経路は尿中であった。呼気中には投与放射能の 0.14 %が排泄された（4.3.2.5-1）。

胆管カニューレを装着した雄性ラットに ^{14}C-PB を静脈内に 75 mg/kg 単回投与したとき、投与 6 時間後までに投与放射能の 18 %が胆汁中に排泄された（4.3.2.5-2）。

胆管カニューレを装着した雄性ラットに ^{14}C-PB を腹腔内に 80 mg/kg 単回投与したとき、投与放射能の 50～65 %が主に抱合体として胆汁中に排泄され、残りは主に未変化体として尿中に排泄された（4.3.2.5-3）。

以上より申請者は、本薬の薬物動態は静脈内投与と経口投与で大きな相違はないが、新生児では薬物代謝能及び血液脳関門が未発達であり、本剤の薬物濃度が上昇する可能性があることを説明した。

＜審査の概略＞

機構は、提出された資料を踏まえ、成熟動物と幼若動物での薬物動態について考察し、本薬の投与対象である新生児及び小児における有効性及び安全性について考察するよう、申請者に求めた。

申請者は、幼若動物における薬物動態については、肝薬物代謝酵素系は、出生時には未発達であり、生後 30～60 日で CYP 分子種の発現及び生成量が成熟動物のレベルに達すること、幼若動物では血液脳関門は未成熟であることから、脳実質への PB の取り込みは高くなり、薬理作用である抗けいれん作用の発現が強まるとともに有害事象発現率が上昇する可能性が示唆されたこと、また幼若動物においては、成熟動物と同様に、PB 投与による薬物代謝酵素の誘導が認められるが、誘導される CYP 分子種が発達の過程で成熟動物と質的又は量的に異なることが報告されていること（4.3.2.4-26）から、幼若動物に本薬を反復投与した場合、PB の代謝物バランスが成熟動物と異なる可能性があることを説明した。以上を踏まえ、申請者は、添付文書の「使用上の注意」の項において、「特に新生児では血中濃度をモニタリングすることが望ましい」旨を記載したことを説明した。

機構は、新生児においては、成人と比較して本薬の薬理作用は強く発現し、有害事象は多く発現する可能性があると考えられることから、本薬の投与に際しては十分な注意喚起が必要と考えるが、詳細については臨床試験成績も踏まえて判断する必要があると考える。

（iii）毒性試験成績の概要

＜提出された資料の概略＞

本申請に際し新たに実施された毒性試験として、ウサギを用いた血管刺激性試験のみが提出された。また、参考資料として単回投与毒性試験、新生児を用いた反復毒性試験に関する公表文献が提出された。

（1）単回投与毒性試験

マウスに本薬を腹腔内投与したときの LD_{50} は 340 mg/kg であり、投与後の一般状態として、催眠、振うつ等が認められた（4.3.3.1-1）。また、マウスに PB を経口投与したときの LD_{50} は 325 mg/kg であった（4.3.3.1-2）。

ラットに本薬を腹腔内投与したときの LD_{50} は 190 mg/kg であり、投与後の一般状態として、催眠、振うつ等が認められた（4.3.3.1-1）。

ラットに PB 150～400 mg/kg を直腸内又は経口投与したとき、体重、摂餌量及び摂水量の減少が認められ、LD_{50} ± 標準誤差はそれぞれ 284 ± 38 又は 304 ± 36 mg/kg であった（4.3.3.1-3）。

新生児ラット（1 日齢）及び 100 日齢ラットに本薬を単回経口投与又は皮下投与したとき、LD_{50} は単回経口投与時でそれぞれ 121 及び 487 mg/kg、皮下投与時でそれぞれ 83 及び 210 mg/kg であり、新生児ラットでは 100 日齢ラットと比較して低値であったが、成熟度による薬物代謝能の差に起因するものと考えられている。また、母動物に妊娠 15 日から分娩まで、本薬 4 mg/kg を反復経口又は皮下投与したとき、その新生児（1 日齢）において本薬を単回経口又は皮下投与した時の LD_{50} は、経口投与時でそれぞれ 362 及び 396 mg/kg、皮下投与時でそれぞれ 142 又は 132 mg/kg であり、100 日齢ラットにおける LD_{50} と同程度であった。なお、母動物に妊娠 14 日以前に本薬を

反復経口投与した場合、新生児ラットの LD_{50} は無処置群と変わらなかったことから、本薬は胎生期後半に投与した場合、薬物代謝酵素の誘導を促進し、新生児の PB 処理能を亢進させるが、それ以前に投与した場合、肝臓の器官形成が未熟であることから、薬物代謝酵素の発現が不十分だったことが原因と考えられている（4.3.3.1-5）。

ウサギに本薬を単回静脈内投与したときの LD_{50} は、185 mg/kg であり、投与後の一般状態として、催眠、振うつ等が認められた（4.3.3.1-1）。

ネコに本薬 125～200 mg/kg を単回経口投与したとき、LD_{50} は 175 mg/kg であり、投与後の一般状態として、運動失調、催眠が認められた（4.3.3.1-4）。

以上より申請者は、本薬、PB とも動物種及び投与経路による致死量に大きな違いはないこと、幼若動物では成熟動物よりも本薬の致死量が低く、過度の薬理作用により成熟動物よりも強い毒性を示す可能性があることを説明した。

（2）反復投与毒性試験

新生児ラット（3 日齢）に PB 30 又は 60 mg/kg を生後 3～21 日まで反復皮下投与したとき、30 mg/kg 群で 8.3 %、60 mg/kg 群で 10.9 %に死亡が認められた。また、60 mg/kg 群では体重増加の抑制、脳重量（大脳及び小脳）の減少、大脳及び小脳のコレステロールの減少並びに大脳の DNA、RNA 及びタンパク質の減少が認められたが、30 mg/kg 群では対照群と差は認められなかった（4.3.3.2-1）。

胃カニューレを留置し強制哺乳した新生児ラット（4 日齢）に、PB 60 mg/kg を生後 5～18 日まで反復皮下投与したとき、死亡例はなく、体重は無処置群と差は認められなかったが、脳重量は対照群に比べて有意な増加抑制が認められ、大脳及び小脳の RNA、コレステロールの減少及び大脳の DNA、タンパク質の減少が認められた（4.3.3.2-2）。

以上より申請者は、ラット新生児の脳は PB に高い感受性を有するものと考えられることを説明した。

（3）局所刺激性試験（4.2.3.6-1）

雄性ウサギ（5 例）の耳介静脈に本薬 10 %水溶液、比較対照として 5 %フェニトイン、陽性対照として 5 %スルホブロモフタレインナトリウム（BSP）を 0.05 mL、1 日 2 回、8 日間連続投与し、クリップを用いて 3 分間貯留させ血管に対する刺激性が検討された。フェニトイン投与群及び BSP 投与群では、初回投与後から肉眼所見において血栓形成が観察されたのに対し、本薬投与群では投与 6 日目から観察され、その発生頻度及び重篤度は BSP が最も強く、次いでフェニトイン、本薬の順であった。また、充血及び腫脹等の炎症性変化の程度も、BSP が最も強く、次いでフェニトイン、本薬の順であった。投与部位における病理組織学的検査では、血栓、血管壁の壊死、血管周囲の炎症性細胞浸潤及び線維化が観察され、その程度は肉眼所見と同様に BSP が最も強く、フェニトイン、本薬の順であった。

以上より申請者は、本薬の血管刺激性は BSP 及びフェニトインと比べ弱いものと考えられることを説明した。

（4）その他の試験（4.3.3.7-1～4.3.3.7-19）

周産期を含む若齢動物に対して PB を投与すると種々の代謝酵素、ホルモン分泌等の異常が成熟後にも継続して認められることが報告されている。臨床使用に際しても、新生児への長期、過剰量投与には慎重に対応する必要があると考えられている。

PB は非遺伝毒性発がん物質としての作用を有することが報告されているが、一方で PB の発がん誘発作用には閾値が存在し、低用量では発がん抑制を示すことが報告されている。

＜審査の概略＞

機構は、本薬の作用や毒性は、成熟動物よりも、幼若動物でより強く認められることについて留意する必要があるが、詳細については臨床試験成績も踏まえて判断する必要があると考える。

4．臨床に関する資料

（i）臨床薬物動態及び臨床薬力学試験成績の概要

＜提出された資料の概略＞

本申請に際して、新たな臨床薬物動態試験は実施されておらず、医師主導治験（5.3.5.2-1）で測定された血漿中濃度データが提出された。また、参考資料として、国内外の健康成人及び小児のてんかん重積状態患者又は新生児けいれんでの薬物動態に関する公表文献が提出された。なお、医師主導治験（5.3.5.2-1）で、血漿中フェノバルビタール（PB）濃度は、フェノバルビタールキット（[redacted]）を用い、蛍光偏光免疫測定法（Fluorescence Polarization Immunoassay: FPIA）により測定され、定量下限は 1.1 μg/mL であった。なお、薬物動態パラメータは特に記載のない限り、平均値又は平均値 ± 標準偏差で示されている。

（1）医師主導治験における血漿中濃度データ（5.3.5.2-1）

新生児けいれん患児 10 例（在胎週数: 33.1～41.1 週、生後: 1～5 日）を対象として、初回投与量として本薬 20 mg/kg を静脈内投与、けいれんが消失しない場合に 20 mg/kg を追加投与（追加投与 I）、その後症状に応じて 5～20 mg/kg を追加投与（追加投与 II）し、無効であると判断されない患者について、維持投与量として 2.5～5 mg/kg を 1 日 1 回、6 日間静脈内投与した。初回投与 2 時間後の血中 PB 濃度は、追加投与 I を実施しなかった 9 例では 22.4 ± 2.3 μg/mL（範囲: 18.8～25.8 μg/mL）、追加投与 I を実施した 1 例では 42.8 μg/mL であった。維持投与は 9 例で実施され、1 回目の維持投与 2 時間後の血中濃度は 25.3 ± 4.8 μg/mL（範囲: 20.7～36.6 μg/mL）であり、維持投与最終投与前又は中止時における血中濃度は 27.7 ± 9.5 μg/mL（範囲: 18.7～45.3 μg/mL）であった。

また、公表文献等から、以下のような資料が提出された。

（2）バイオアベラビリティ（BA）

日本人てんかん患者（8～20 歳）に PB を筋肉内投与及び経口投与、本薬を静脈内投与及び直腸内投与したとき、筋肉内投与、経口投与及び直腸内投与時の BA はそれぞれ 101、96 及び 80 %であり、各投与経路で有意な差は認められなかった（5.4.2.5.3-4）。

外国人健康成人（19～37 歳）と外国人てんかん患者（19～28 歳）に本薬を静脈内投与及び筋肉内投与した際の薬物動態を比較したとき、クリアランスは健康成人と比較して、てんかん患者で

やや大きかったが、有意差は認められなかった。また、健康成人での筋肉内投与及び経口投与時のBAはそれぞれ101及び100％であった（5.4.2.4.3-15）。

外国人健康成人（24～44歳）に本薬を静脈内投与又は経口投与したとき、両投与経路における薬物動態パラメータは類似しており、経口投与時のBAは83.4％であった（5.4.2.4.3-12）。

（3）吸収

日本人新生児けいれん患児25例を対象として、生後5日以内にPBを初回投与量20～25 mg/kgを20分かけて静脈内又は筋肉内投与し、その後維持用量として1.5 mg/kgを12時間おきに静脈内又は筋肉内投与したとき、両投与経路における薬物動態は類似しており、静脈内投与時の初回投与直後（投与4時間後及び12時間後の平均値 ± 標準偏差）の血漿中PB濃度は26.4 ± 4.9 μg/mL、筋肉内投与時のC_{max}は25.9 ± 3.2 μg/mLであった。消失半減期（$t_{1/2}$）は、出生直後から2～3日間で180～200時間、生後14～17日で70～90時間と推定され、加齢と共に短縮した（5.4.2.5.1-3）。

日本人新生児けいれん患児29例を対象として、体重1 kg未満の患児（4例）には本薬5又は10 mg/kg、1 kg以上の患児（25例）には本薬10～20 mg/kgを静脈内投与したとき、20 mg/kgを投与した体重1 kg以上の患児では、22例中21例において有効血中濃度（15 μg/mL）以上に達し、全例が有効例であった（体重1 kg未満の患児はバラツキが大きく解析対象外とされている）（5.4.2.5.1-1）。

日本人新生児及び乳児のけいれん患児12例にPB 10～20 mg/kgを静脈内投与（6例）又は5～13 mg/kgを直腸内投与（6例）したとき、静脈内投与群において、投与後の最高血清中PB濃度（C_{max}）は28 ± 14.8 μg/mL（最小15.6、最大60.5 μg/mL）に達し、けいれん消失及びけいれん予防が認められた。また、直腸内投与群でのC_{max}は10.3 ± 3.4 μg/mL（最小4.1、最大14.2 μg/mL）であった（5.4.2.5.3-2）。

外国人新生児（生後0～4週<14例>）、乳児（生後2～3ヶ月<16例>又は4～12ヶ月<14例>）及び小児（2～5歳<7例>）にPBを新生児には5～10 mg/kg、乳児及び小児には10 mg/kgを静脈内投与したとき、$t_{1/2}$は、新生児で118.6 ± 16.1時間、生後2～3ヶ月の乳児で62.9 ± 5.2時間、生後4～12ヶ月の乳児では63.2 ± 4.2時間、小児では68.5 ± 3.2時間であり、新生児における$t_{1/2}$は乳児及び小児に比べて有意に長かった。定常状態のVdは、新生児及び生後2～3ヶ月の乳児で0.850～0.857 L/kg、生後4～12ヶ月の乳児及び小児で0.570～0.666 L/kgであり、差が認められたことから、新生児では肝臓での酵素的処理に限界があり、同時に腎臓での糸球体濾過、尿細管からの排泄が少ないために、PBの排泄が遅延するものと推察されている（5.4.2.4.3-8）。

外国人成人てんかん患者（6例）に本薬90 mgを投与開始日は静脈内投与、投与2～28日目は本薬90 mg/kg、投与36～83日目は本薬90～150 mg/kgを反復経口投与した。投与29～35日目（4週目）及び投与84～91日目（12週目）は^{13}C及び^{15}N標識体（本薬）90 mg/kgを静脈内投与したとき、反復投与により Vd，$t_{1/2}$及びClに対する影響は認められなかった（5.4.2.4.3-3）。

（4）分布

新生児及び小児（15例）におけるPBの組織内分布を検討した結果，各組織と血漿中のPB濃度比は肝臓で最も高く，大脳及び小脳では他の臓器と比較して低値を示した。また，組織中PB濃度と血漿中PB濃度は相関し、大脳及び小脳における相関係数は0.82及び0.81であった

(5.4.2.5.3-1)。

新生児けいれん患児（77 例）及び新生児けいれんの治療中に死亡した患児（17 例）における PB の組織への分布を検討した結果、Vd 及び $t_{1/2}$ は在胎齢の影響を受けなかった。各組織と血漿中の PB 濃度比は、肝臓、心臓、筋肉でそれぞれ 4.3、6.4、2.6 であり、脳よりも高い濃度であった。また、脳の灰白質と白質とを分けて測定した 3 例における灰白質と白質の PB 濃度比は、それぞれ 0.86～1.11 で、灰白質と白質の間で同程度の分布が認められた（5.4.2.4.3-13）。

PB を投与した妊婦の分娩時の臍帯血中には、血清中濃度の 95 %に相当する PB が検出され、胎盤透過性が示された（5.4.2.4.3-10）。

プリミドンを投与された授乳婦における母乳と血清中 PB の濃度比は 0.41 であり、乳汁中への移行が認められた。また、代謝物である p-hydroxyphenobarbital（PHPB）濃度は、血清と母乳中でほぼ同じであった（5.2.4.3-11）。

新生児及び小児における PB のタンパク結合率は、生後 0～3 ヶ月の集団においてそれ以外の集団（生後 4 ヶ月～2 歳及び 2～15 歳）よりも低く、年少児ほど低い傾向が認められた。また、血中アルブミン濃度についても生後 0～3 ヶ月の集団において、低値を示し、PB のタンパク結合率と血中アルブミン濃度に、正の相関関係が認められた（5.4.2.5.3-3）。

PB のヒト血清 α_1 酸性糖タンパク及びヒト血清アルブミンへの結合率は、それぞれ 5 %以下及び 27～43 %であった（5.4.2.4.3-1）。

ヒト胎児、新生児及び健康成人の血漿における PB のタンパク結合率を検討した結果、ヒト胎児及び新生児血漿は、成人と比較して低い結合率であった。アルブミン濃度は、新生児、胎児で成人に比べて低かった（5.4.2.4.3-4）。

（5）代謝

本薬のヒトにおける主要代謝物は、PHPB 及びその抱合体、PB が N-グルコシル化を受けた phenobarbital N-glucopyranoside（PNG）であり、PHPB への代謝については主に CYP2C9 が関与していると推察されているが、PNG への代謝に関与する代謝酵素については、明らかになっていない（5.4.2.2.1.3-1、5.4.2.2.1.3-3、5.4.2.4.3-7）。また、新生児では、N-グルコシル化活性は遅れて発達するが、一旦活性化されると、PB の代謝の主要経路になることが示唆されている（5.4.2.4.3-2）。PB 投与により、P-450 分子種である CYP1A、CYP2A6、CYP2B、CYP2C 及び CYP3A と UGTs 等の薬物代謝酵素が誘導され、これらの酵素により代謝される薬物の代謝が促進されることが明らかにされている（5.4.2.2.2.3-1、5.4.2.2.2.3-2）。

（6）排泄

PB の主排泄経路は尿中排泄であり、^{14}C 及び ^{15}N 標識体（PB）を経口投与後 16 日間に投与した PB の 78～87 %が排泄された（5.4.2.4.3-14）。

（7）薬物相互作用

PB の酵素誘導作用により、CYP1A、CYP2A6、CYP2B、CYP2C、CYP3A 等の CYP450 と UGTs 等が誘導され、これらの酵素によって代謝を受ける薬物等の薬理作用及び生理活性を変動させることが報告されている（5.4.2.2.1.3-4、5.4.2.2.2.3-2、5.4.2.4.3-6、5.4.2.6.3-1）。また、メチルフェニ

デート及びバルプロ酸は PB の代謝を抑制し、セイヨウオトギリソウは PB の代謝を促進する（5.4.2.2.1.3-5、5.4.2.4.3-6、5.4.2.6.3-1、5.4.2.6.3-2）。PB は甲状腺ホルモン（T_3、T_4）のグルクロン酸抱合を増加し、未変化体濃度を減少させ、ビリルビンのグルクロン酸抱合を増強しビリルビンの胆汁排泄を促進させるが、これらは PB による UGTs の誘導によるものと推察されている（5.4.2.2.1.3-4）。

（8）薬力学試験

新生児けいれん患児（38 例）を対象として、PB を静脈内又は筋肉内投与したとき、けいれんのコントロール成功例（12 例）における PB の初回投与量は 16.2 ± 2.6 mg/kg、血中濃度は 19.8 ± 2.7 μg/mL（範囲: 16.9～25.8 μg/mL）であり、コントロール不成功例（26 例: 初回投与量 12.3 ± 3.3 mg/kg、血中濃度は 14.3 ± 5.1 μg/mL）よりも有意に高値であった（$p<0.002$、Student の t 検定）が、血中 PB 濃度が 16 μg/mL 以上でもコントロールができなかった患児が数例存在した。なお、投与量と血中濃度の比は、投与経路、在胎週数又は体重により影響を受けなかった（5.4.2.4.3-9）。

新生児けいれん患児（71 例、在胎週数: 26～42 週、出生後日数: 1～143 日）を対象として、PB を初回投与量として 15 mg/kg 静脈内投与し、けいれんがコントロールできなかった場合又は再発した場合に 5～10 分ごとに 5～10 mg/kg を追加投与した。コントロール成功例（60 例）における血漿中濃度は 10.1～46.4 mg/L であり、20 mg/L 以下が 36 例、20～30 mg/L が 17 例、30 mg/L 以上が 7 例であった（5.4.2.4.3-5）。

てんかん重積状態の患児（12 例、生後 7～62 ヶ月）を対象として、本薬を初回投与量として 15 mg/kg 静脈内投与し、その後維持量として 5 mg/kg を初回投与 24 及び 48 時間後に投与したとき、すべての症例で血中濃度は投与開始後 10 分以内に 10 μg/mL に達し、その後 15 μg/mL 以上が維持され、8/12 例でけいれん発作のコントロールが可能であった（5.4.2.4.2.1-3）。

＜審査の概略＞

（1）新生児における本剤の薬物動態と安全性について

機構は、医師主導治験（5.3.5.2-1）の成績に基づき、新生児における本剤の薬物動態と有効性及び安全性の関連性について説明するよう申請者に求めた。

申請者は、まず、医師主導治験で得られた血中 PB 濃度に関して、出生体重、日齢及び在胎週数による影響は認められなかったことを説明した。その上で申請者は、1 例（症例番号 OKA01）において維持投与最終投与直前の血中 PB 濃度が高値（45.3 μg/mL）を示したが、当該症例の背景因子を他の 9 例と比較したところ、出生体重は 3530 g と中央値（3052 g）よりも高値であったが、身長及び在胎週数は中央値に相当する値であったこと、初回投与 2 時間後及び 1 回目の維持投与 2 時間後における血中 PB 濃度はそれぞれ 22.2 及び 23.9 μg/mL であったこと、検体の取り扱いにも問題はなかったことを述べ、当該症例で血中 PB 濃度が高値を示した理由については明らかではないが、比較的高用量で維持投与を開始（維持投与 1～3 回は 5 mg/kg/日、4～6 回は 2.5 mg/kg/日）しており、新生児の生後直後の $t_{1/2}$ が長くなること（5.4.2.5.1-3）等に起因した可能性があると考えることを説明した。また申請者は、血中 PB 濃度と有効性の関連性について、有効性の主要評価項目（初回投与後（又は追加投与（1）後）30 分の有効性）は全例で「著効」であり、詳細な検討はできなかったことを説明した。さらに申請者は、血中 PB 濃度と安全性の関係につい

て、7例において本剤の血中PB濃度は30 μg/mL以下であったが、30 μg/mLを超えた3例（症例番号KNG02<初回投与2時間後: 18.8 μg/mL、追加投与（II）2時間後: 36.9 μg/mL、1回目の維持投与2時間後: 36.6 μg/mL及び維持投与最終投与前: 31.8 μg/mL>、症例番号KNG05<追加投与（I）2時間後: 42.8 μg/mL、投与中止時: 41.9 μg/mL>及び症例番号OKA01<初回投与2時間後: 22.2 μg/mL、1回目の維持投与2時間後: 23.9 μg/mL及び維持投与最終投与前: 45.3 μg/mL>）においても、安全性プロファイルはそれ以外の症例と大きく異ならなかったことを説明し、新生児では、成人と比較して脳血液関門が未熟であり、脳への移行が成人よりも高まる可能性が考えられること（Bourin M et al, *Dev Pharmacol Ther*, 7: 171-176, 1984、Vajda F et al, *Clin Pharmacol Ther*, 15: 597-603, 1974）、代謝酵素が未発達であり本剤のクリアランスが低くなる可能性があること（5.4.2.4.3-2）から、添付文書において「小児では血中濃度をモニタリングすることが望ましい」旨を記載し、注意喚起していること説明した。

機構は、医師主導治験（5.3.5.2-1）において、限られた症例数ではあるものの、本剤の血中濃度が測定され、当該試験での用法・用量において、高い血中濃度が認められた症例においても、安全性プロファイルは大きく異なることはなかったが、公表文献等の結果を踏まえると、本剤を新生児に投与した際には、血中濃度及び脳内移行性が高くなる可能性があり、本剤を新生児に投与する場合には、患者の状態を観察しながら慎重に用量等を調節し、投与すべきであると考える。

（ii）有効性及び安全性試験成績の概要

＜提出された資料の概略＞

本申請では、新生児けいれんに対する有効性及び安全性に関する評価資料として医師主導治験（5.3.5.2-1）の成績が提出された。また、新生児けいれん及びてんかん重積状態に対する参考資料として国内外の公表文献、ガイドライン、成書等が提出された。

（1）新生児けいれん

1）医師主導治験（5.3.5.2-1: 試験番号 JNR-NP＜2006年3月～2007年3月＞）

日本人新生児けいれん患児（目標症例数30例）を対象に、本剤の有効性、安全性及び薬物動態を検討するため、非盲検非対照試験が実施された。

用法・用量は、第I期では、初回投与として本剤20 mg/kgを5～10分かけて静脈内投与し、初回投与でけいれんが消失しない場合には、初回投与30分後に追加投与（I）として本剤20 mg/kgを5～10分かけて静脈内に追加投与すると設定された。第II期では、第I期で本剤が著効又は有効と判定された被験者に対して、追加投与（II）として、本剤の血中濃度が40 μg/mLを超えない範囲で必要に応じ本剤5～20 mg/kgを5～10分かけて静脈内投与することが可能と設定された。また、維持投与[2)]については、本剤2.5～5 mg/kgを1日1回静脈内投与（ただし、本剤の血中濃度が50 μg/mLを超えた場合は1回維持投与を回避）、投与期間は最長6日間と設定された（薬物動態に関しては、「（i）臨床薬物動態及び臨床薬力学試験成績の概要」の項参照）。

総投与症例10例全例が有効性及び安全性解析対象であり、初回投与例10例、追加投与（I）実施例1例、追加投与（II）実施例1例、維持投与例9例、維持投与完了例8例であった。

2) 維持投与は初回投与終了、追加投与（I）、追加投与（II）のいずれか遅い時点から24時間以降を目安に開始した。

有効性の主要評価項目である初回投与又は追加投与（I）の投与 30 分後における著効率[3]は 100.0 %（10/10 例）であった。

有害事象（臨床検査値異常を含む）は 90.0 %（9/10 例）で認められたが、死亡例は認められなかった。その他の重篤な有害事象は、1 例（動脈管開存症 1 例）に認められたが、本剤との因果関係は否定されている。有害事象による投与中止例は認められていない。因果関係の否定できない有害事象は 60.0 %（6/10 例）で認められ、呼吸抑制 3 例、酸素飽和度低下 2 例、徐脈、気管支分泌増加、血圧低下、体温低下及び尿量減少各 1 例であった。

バイタルサインは、収縮期血圧及び拡張期血圧ともに初回投与又は追加投与（I）の投与 1 時間後で最大の低下を示し、収縮期血圧は初回投与又は追加投与（I）の投与 1 及び 2 時間後で初回投与前と比較して有意な低下が認められた。呼吸数及び心拍数は本剤投与中を通じて投与前よりも低値を示した。

以上より自ら治験を実施した者は、本剤を初回投与 20 mg/kg、血中濃度 40 μg/mL を超えない範囲で追加投与及び維持投与として本剤 2.5～5 mg/kg を投与することにより新生児けいれんに対し有効性を示し、本剤の薬理作用から予測される有害事象以外に特に安全性上問題となることはなかったことを説明した。

2）国際的に標準とされている教科書等における記載

国際的に標準とされている教科書においては，本薬又は PB を新生児けいれんに対して用いる場合には、初回投与量として 20 mg を静脈内投与し、最大投与量として 40～50 mg/kg まで投与可能であること、維持投与量として、2～5 mg/kg/日を静脈内、筋肉内又は経口投与すること、治療域血中濃度は 20～40 μg/mL であること等が記載されている。また、維持療法の中止時期については、明確に規定された基準はないが、神経学的検査が正常となったとき又はけいれんのコントロールが可能となった場合は、可能な限り速やかにフェノバルビタールの投与を中止すること等が記載されている（5.4.2.1.1.1-5、5.4.2.1.1.1-2、5.4.2.1.1.1-4），本邦における教科書（5.4.2.1.2.1-2、5.4.2.1.2.1-3 及び 5.4.2.1.2.1-1）では，初回投与量として 15～20 mg/kg を筋肉内又は直腸内投与し、効果不十分であれば同量を追加投与すること、維持投与量として 2.5～5 mg/kg/日を筋肉内又は経口投与（坐剤でも可）すること、治療域血中濃度は 15～25（40）μg/mL と記載されている。

【提出された資料】

（海外）

5.4.2.1.1.1-3、5.4.2.1.1.3-1: Behrman RE, Kliegman RM, Jenson HB editors, *Nelson Textbook of Pediatrics (17th edition)*, Saunders, 566-568, 2005-2009, 2004

5.4.2.1.1.1-5: Swaiman KF, Ashwal S, Ferriero DM editors, Pediatric Neurology *-Principles & Practice-(4th edition)*, Mosby Elsevier, 269-277, 2006

5.4.2.1.1.1-1、5.4.2.1.1.1-2: McDonald MG, Seshia MM, Mullett MD editors, *Avery's Neonatology -Pathophysiology & Management of the Newborn- (6th edition)*, Lippincott Williams & Wilkins, 1385-1409, 1521-1556, 2005

3) 著効率（%）＝（著効の例数/対象例数）×100，「著効」：新生児けいれん消失

5.4.2.1.1.1-6: Rennie JM editor, *Roberton's Textbook of Neonatology (4th edition)*, Churchill Livingstone, 1114-1119, 2005

5.4.2.1.1.1-4: Volpe JJ editor, *Neurology of the Newborn (4th edition)*, Saunders, 202-214, 2001

（国内）

5.4.2.1.2.1-2、5.4.2.1.2.1-3: 河野寿夫編、*ベッドサイドの新生児の診かた*、南山堂, 242-245, 392-394, 2004

5.4.2.1.2.1-1: 小川雄之亮、多田裕、中村肇、仁志田博司編、*新生児学*、メディカ出版, 973-975, 2000

3）海外医薬品集、総説等における記載

新生児けいれんに対する本薬又はPBの使用について、初回投与量として15～20 mg/kgを緩徐に（1 mg/kg/分以下）静脈内投与し、維持投与量は2.5～5 mg/kgを1日1～2回静脈内投与、治療域血中濃度は15～40 μg/mLと記載されている。

【提出された資料】

5.4.2.1.3.3-3: Sweetman SC editor, *Martindale (35th edition)*, Pharmaceutical Press, 444-446, 2007

5.4.2.1.3.3-2: *British National Formulary for Children (BNFC)*, BMJ Publishing Group and RPS Publishing, 261, 272, 2006

5.4.2.1.3.3-4、5.4.2.1.3.3-5: *Rote Liste*, Editio Cantor Verlag, 15 001-15 002, 170-172, 2006

5.4.2.1.4.3-1: Takemoto CK, Hodding JH, Kraus DM editors, *Pediatric Dosage Handbook (12th edition)*, Lexi-Comp, 1000-1003, 2005

5.4.2.2.1.1-2: Rennie JM et al, *Curr Opin Neurol*, 16: 177-181, 2003

5.4.2.2.1.1-1: Painter MJ et al, *Curr Treat Options Neurol*, 3: 237-248, 2001

5.4.2.3.1-1: Booth D et al, *The Cochrane Database of Syst Rev*, 3: CD004218, 2004

4）臨床報告等

海外で実施された新生児けいれんを対象としたPBの比較対照試験（5.4.2.4.1-1、5.4.2.4.1-9、5.4.2.4.1-7）、医師主導治験（5.3.5.2-1）において参考とされた1試験（5.4.2.4.1-4）及び国内で新生児けいれんを対象としたPBの静脈内投与時の薬物動態に関する文献（5.4.2.5.1-1、5.4.2.5.1-3）が提出された。

① Boylan GB et al, *Neurology*, 62: 486-488, 2004（5.4.2.4.1-1）

PBに反応しなかった新生児けいれん患児を対象に、リドカイン又はミダゾラム追加投与時の有効性を検討するために、非盲検無作為化試験が実施された。

用法・用量は、脳波で発作が確認された患児に、PBを40 mg/kgまで投与し、登録後12時間以内に反応[4]が認められなかった場合に、ミダゾラム群又はリドカイン群に無作為に割り付けられた。ミダゾラム群は、ミダゾラム60 μg/kgをボーラス投与した後、150 μg/kg/hrの速度で持続注入し、12時間以内に反応[4]が認められなかった場合に注入速度を300 μg/kg/hrに増量すると設定さ

4) 脳波上の発作活動が完全消失又は投与前発作活動の80 %以上減少した場合、投与後反応したと判断された。

れ、リドカイン群は、リドカイン 4 mg/kg をボーラス投与した後、2 mg/kg/hr の速度で持続注入を行い、12 時間以内に反応[5]が認められなかった場合は、注入速度を 4 mg/kg/hr に増量すると設定された。また、割り付けられた試験薬の投与を保護者が拒否した場合は、クロナゼパムを投与することと設定された。

総投与症例は 22 例であり、全例で第一選択薬である PB が投与された。また、第二選択薬の投与例は、ミダゾラム投与群 3 例、リドカイン投与群 5 例及びクロナゼパム投与群 3 例であった。

有効性の主要評価項目である反応例[6]数は、PB 投与群で 11/22 例、リドカイン投与群で 3/5 例、ミダゾラム投与群 0/3 例、クロナゼパム投与群 0/3 例であった。なお、追加投与された抗けいれん薬の血中濃度は治療域であった。

有害事象等の安全性については、本資料では評価されていない。

以上より、約半数の新生児けいれん患児において、PB の有効性が示唆された。

② Scher MS et al, *Pediatr Neurol*, 28: 277-280, 2003（5.4.2.4.1-9）

新生児けいれん患児を対象に、PB 及びフェニトイン静脈内投与の有効性を検討するために無作為化試験が実施された。

用法・用量は、PB は 25 μg/mL（非結合型濃度）以上、フェニトインは 3 μg/mL（非結合型濃度）以上となるよう投与し、各薬剤に反応[6]しなかった場合、初回投与薬剤が PB の場合はフェニトインを、フェニトインの場合は PB を追加投与すると設定された。

総投与症例は 59 例（初回 PB 投与群 30 例、フェニトイン群 29 例）であった。薬剤投与前に脳波上の発作活動のみが認められたのは 9 例、脳波上の発作活動及び臨床上の発作が認められたのは 50 例であった。

有効性について、脳波上の発作活動及び臨床上の発作が認められた 50 例中 24 例で最初の抗てんかん薬（PB 又はフェニトイン）により脳波上及び臨床上の発作が消失した。脳波上の発作活動又は臨床上の発作が持続した 26 例については、2 番目の抗てんかん薬投与後に臨床上の発作は、5 例が消失、10 例が 50 %以上の抑制、11 例が 50 %未満の抑制であり、臨床上の発作が 50 %以上の抑制が認められた 15 例では、脳波上の発作活動は持続し臨床上の発作と乖離した。また、脳波上の発作活動のみが認められた 9 例については、全例で投与後にも脳波上の発作活動が認められた。

有害事象等の安全性については、本資料では評価されていない。

なお、PB 及びフェニトインの各々の有効率については評価されていない。

③ Painter MJ et al, *N Engl J Med*, 341: 485-489, 1999（5.4.2.4.1-7）

新生児けいれん患児を対象に、PB 及びフェニトインの有効性及び安全性を検討するために無作為化比較試験が実施された。

用法・用量は、初回投与として PB 又はフェニトインを 5～15 分かけて静脈内投与し、初回投

---

5) 脳波上の発作活動が完全消失又は投与前発作活動の 80 %以上減少した症例

6) 24 時間脳波モニタリングにおいて、2 分 30 秒の発作又は発作活動による占有が 5 分間の半分以上となった場合、並びに PB 及びフェニトインの非結合型濃度がそれぞれ 25μg/mL 及び 3 μg/mL と等しいかそれ以上になった場合、最初の薬剤に反応しなかったとみなす。

与30分後の血漿中非結合型濃度を測定し，PBは25 μg/mL、フェニトインは3 μg/mLを指標として追加投与すると設定された。以後は1日2回の血漿中濃度測定を行い、トラフ血漿中濃度としてPBでは22.5 μg/mLを、フェニトインでは2.5 μg/mLを指標に用量調節し、1日1回投与すると設定された。各薬剤に反応[6]しなかった場合、初回投与薬剤がPBの場合はフェニトインを，フェニトインの場合はPBを投与すると設定された。また、発作を示唆する異常運動が認められなかった場合、7日後に治療を中止し、臨床発作が7日を越えて持続又は治療中止後に再発した場合、他の抗けいれん薬の使用を検討すると設定された。

総投与症例は59例であった。初回投与がPBであったのは30例（PB群）、フェニトインであったのは29例（フェニトイン群）であった。

有効性の評価項目である完全な発作コントロール[7]例数は、PB群で13/30例，フェニトイン群で13/29例であり、両群間に有意差は認められなかった（p = 1.00、Fisherの正確確率検定）。

有害事象（因果関係についての記載はない）に関して、PB又はフェニトインの血漿中濃度に関連した心拍数、不整脈、平均動脈圧及び呼吸状態の変化は認められなかった。

以上より，PBは新生児けいれんに対して、フェニトインと同程度の有効性が示唆された。

なお，本報告について申請者は，実施施設、試験デザイン，対象例数の同一性から、上記②での症例と同一症例の可能性がある旨を考察している。

④ Gilman JT et al, *Pediatrics*, 83: 674-678, 1989（5.4.2.4.1-4）

新生児けいれん患児を対象に、PBの有効性及び2次的な抗てんかん薬の追加投与の反応性を検討する臨床試験が実施された。本資料においては試験デザインの記載はない。

用法・用量は、初回投与としてPB 15～20 mg/kgを5～15分かけて静脈内投与（PB初回投与）し、発作が持続する場合には、発作の消失又は血清中濃度40 μg/mLに達するまで、PB 5～10 mg/kgを20～30分間隔で追加投与（PB追加投与）すると設定された。また、さらに発作が持続する場合には、PB 5～10 mg/kgを血清中濃度100 μg/mLに達するまで繰り返し追加投与（PB高用量投与）することと設定され、一連のPB投与は通常6時間以内に完了すると設定された。PB投与により発作が持続した場合には、2剤目の抗けいれん薬（フェニトイン又はロラゼパム）を投与し、2剤目の抗けいれん薬で反応が見られなかった場合には、3剤目以上の抗けいれん薬（フェニトイン、ロラゼパム、パラアルデヒド又はバルプロ酸）投与すると設定された。

総投与症例数は120例であり、PB初回投与例が120例、PB追加投与例が72例、PB高用量投与例が35例であった。

有効性の評価項目である反応例[8]数は、PB初回投与48/120例、PB追加投与37/72例、PB高用量投与7/35例であった。

有害事象（因果関係についての記載はない）に関して、PBによる全身的急性毒性は認められなかった。数例の重度仮死被験者において、血清中PB濃度が50 μg/mLを超えたときに鎮静及び摂食減少が認められたが、血清中PB濃度の減少に伴い本事象は消失した。

以上より、血清中PB濃度を40 μg/mLを目標に調節することで、新生児けいれんに対するPBの有効性及び安全性が示され、40 μg/mLで効果不十分な場合には，2剤目の抗てんかん薬を投与

---

7) 完全な発作コントロール例：臨床上の発作活動が完全に消失した被験者
8) 反応例：抗てんかん薬の最終投与後1時間以内に発作が消失した被験者

すべきであることが示唆された。

⑤ 武部幸佩ら，*日本新生児学会雑誌*, 24: 84-90, 1988（5.4.2.5.1-3）

新生児けいれん患児を対象に、PB の有効性及び薬物動態を検討するために非盲検試験が実施された（薬物動態については、「（ｉ）臨床薬物動態及び臨床薬力学試験成績の概要」の項参照）。

用法・用量は、PB 20～25 mg/kg を 20 分かけて静脈内投与（静脈内投与群）又は筋肉内投与（筋肉内投与群）し、維持投与として 12 時間ごとに 1.5 mg/kg を静脈内投与又は筋肉内投与すると設定された。投与期間については、本資料で記載されていない。

総投与症例数は 25 例であり、静脈内投与群 17 例、筋肉内投与群 8 例であった。

有効性の評価項目であるフェノバルビタール投与後のけいれんに対する効果[9]は、静脈内投与群及び筋肉内投与群の併合結果では、著効は 11 例、有効は 5 例、無効は 9 例であった。

以上より、PB 静脈内投与時の有効性が示唆された。

⑥ Gal P et al, *Neurology*, 32: 1401-1404, 1982（5.4.2.4.3-5）

新生児けいれん患児を対象に、PB の静脈内投与による有効性及び薬物動態を検討するために、非盲検試験が実施された（薬物動態については、「（ｉ）臨床薬物動態及び臨床薬力学試験成績の概要」の項参照）。

用法・用量は、けいれん発作と診断され次第、初回投与量として PB 15 mg/kg を静脈内投与し、発作がコントロールされない場合又は再発した場合に 5～10 分ごとに 5～10 mg/kg を追加投与、最高用量は 40 mg と設定された。

総投与症例数は 71 例であった。

有効性の評価項目である発作コントロール成功例は発作の再発がないことと定義され、発作コントロール成功率は 85 %（60/71 例）であった。血漿中 PB 濃度別でのコントロール成功率は、20 mg/L 以下、20～30 mg/L 及び 30 mg/L 以上でそれぞれ、60 %（36/60 例）、28 %（17/60 例）、及び 12 %（7/60 例）であった。

安全性については、PB の副作用と活動性及び筋緊張の抑制を伴う新生児疾患の症候を区別することは困難であったが、いずれの被験者においても、呼吸機能の悪化は認められなかった。

以上より、新生児けいれんに対して PB は血漿中濃度が 40～45 mg/L となるまで持続した有効性が示され、無効な場合に他剤を追加していく必要があることが示唆された。

5）臨床使用実態調査結果

① 大久保賢介ら，*日本小児臨床薬理学会雑誌*, 18: 83-85, 2005（5.4.2.6.1-2）

2003 年に、日本未熟児新生児学会薬事委員会を中心に、全国の新生児医療に携わる医師 203 名（203 施設、1 施設 1 名）に対して PB の使用状況に関する実態調査が実施された。110 施設より回答が得られ、総入院患者数は 25,262 例であり、抗けいれん薬の使用を要した症例は 2.8 %（721/25,262 例）であった。抗けいれん薬を要した症例のうち PB が投与されたのは 85.7 %（618/721 例）であった。また、抗けいれん薬投与が必要な新生児けいれんに対する第 1 選択薬として PB

9) 「著効」：PB 投与 12 時間以内に後続発作も含めて発作が消失、「有効」：12 時間以内に後続発作も含めて発作回数が 50 %以下に減少し、24 時間以内に消失したもの、「無効」：「著効」及び「有効」以外

をあげた施設は 89 % (98/110 施設)、第 2 選択薬としたのは 14 % (15/110 施設) であった。PB (皮下・筋注用製剤) を静脈内投与に転用している施設は、全体の 28 %に認められ、静注用 PB 製剤が「必要である」と回答した医師は、90 % (98 名) であった。静注用 PB 製剤を必要とする理由としては、筋肉内投与に伴う局所の副作用 (局所壊死等) や痛みが心配であること、坐薬投与における吸収の不安定さ等が述べられている。

(2) てんかん重積状態

1) 国際的に標準とされている教科書等における記載

国際的に標準とされている教科書で、てんかん重積状態の治療における PB の臨床的位置付けはベンゾジアゼピン系薬剤、フェニトインに続く第 2 又は第 3 選択薬であり、初回投与量として、小児で 15～20 mg/kg、成人で 10～20 mg/kg の静脈内投与が推奨され、治療域血中濃度は 10～40 μg/mL である旨などが記載されている。また、国内における成書においては、PB 製剤の筋肉内注射や直腸内投与を行なうことが記載されているが、効果発現に時間を要することが記載されている。

【提出された資料】

(海外)

5.4.2.1.1.2-3: Kasper DL, Fauci AS, Longo DL, Braunwald E, Hauser SL, Jameson JL editors, *Harrison's Principles of Internal Medicine (16th edition)*, McGraw-Hill, 2366-2372, 2005

5.4.2.1.1.2-4: Swaiman KF, Ashwal S, Ferriero DM editors, *Pediatric Neurology: Principles & Practice (4th edition)*, Mosby Elsevier, 1096-1104, 2006

5.4.2.1.1.2-5: Wasterlain CG, Treiman DM editors, *Status Epilepticus: Mechanisms and Management*, MIT Press, 597-605, 2006

5.4.2.1.1.2-6: Wyllie E editor, *The Treatment of Epilepsy: Principles & Practice (4th edition)*, Lippincott Williams & Wilkins, 610-621, 2006

5.4.2.1.1.2-1: Andreoli TE editor, *Cecil Essentials of Medicine (6th edition)*, Saunders, 1057-1059, 2004

5.4.2.1.1.3-1: Behrman RE, Kliegman RM, Jenson HB editors, *Nelson Textbook of Pediatrics (17th edition)*, Saunders, 2005-2009, 2004

5.4.2.1.1.2-2: Engel J, Pedley TA editors, *Epilepsy: A Comprehensive Textbook, Vol 2*, Lippincott-Raven, 1317-1323, 1997

(国内)

5.4.2.1.2.2-1: 加我牧子, 佐々木征行, 須貝研司編, *国立精神・神経センター 小児神経科 診断・治療マニュアル*, 診断と治療社, 279-289, 2003

5.4.2.1.2.2-2: 松下正明総編集, *臨床精神医学講座 第9 巻*, 中山書店, 118-129, 1998

2) 海外医薬品集等における記載

てんかん重積状態に対し、初回投与量として小児では 15～20 mg/kg 又は 100～400 mg、成人では 10～20 mg/kg 又は 200～600 mg を 10～15 分かけて静脈内投与し、治療域血中濃度は 15～40 μg/mL であること等が記載されている。また、必要に応じて維持投与を行うことが記載されてい

る。

【提出された資料】

5.4.2.1.3.2-2: Sweetman SC editor. *Matindale: The Complete Drug Reference (35th edition)*, Pharmaceutical Press, 422-427, 2007

5.4.2.1.3.3-1: American Society of Health-System Pharmacists, *AHFS Drug Information*, American Hospital Formulary Service, 2177-2178, 2457-2458, 2006

5.4.2.1.3.2-3: *USP-DI 2006 Vol.1: Drug Information for the Health Care Professional (26th edition)*, Thomson Micromedex, 533-535, 2006

5.4.2.1.3.2-1: *British National Formulaly No.51 (BNF)*, BMJ Publishing Group and RPS Publishing, 242-249, 2006

5.4.2.1.3.3-2: *British National Formulaly for Children (BNFC)*, BMJ Publishing Group and RPS Publishing, 261, 272, 2006

5.4.2.1.3.2-4: *ViDAL (82th edition)*, Vidal, 891-893, 2006

5.4.2.1.3.3-4: *Rote Liste*, Editio Cantor Verlag, 2006

5.4.2.1.4.3-1: Takemoto CK, Hodding JH, Kraus DM editors. *Pediatric Dosage Handbook (12th edition)*, Lexi-Comp, 1000-1003, 2005

3）国内外の総説、ガイドライン等

小児及び成人のてんかん重積状態に対し、フェノバルビタールはベンゾジアゼピン系薬剤、フェニトインに続く第2又は第3選択薬として使用すること、その際の用法・用量は初回投与として15～20 mg/kgを100 mg/分以下の速度で静脈内投与すること等が記載されている。なお、国内ガイドライン（5.4.2.2.2.2-2）では、静脈内投与ができる薬剤がなく、皮下投与又は筋肉内投与に使用する薬剤を用いざるを得ないことから、PBが結晶化して析出し、微細血管の塞栓症が起こる可能性があるため、緊急の場合以外は使用しないことが注意喚起されている。

【提出された資料】

5.4.2.2.1.2-4: Kälviäinen R et al, *CNS Drugs*, 19: 759-768, 2005

5.4.2.2.1.2-6: Mamo EM, *Mayo Clin Proc*, 78: 508-518, 2003

5.4.2.2.1.2-1: The Status Epilepticus Working Party, *Arch Dis Child*, 83: 415-419, 2000

5.4.2.2.1.2-5: Lowenstein DH et al, *N Engl J Med*, 338: 970-976, 1998

5.4.2.2.1.2-3: Epilepsy Foundation of America, *JAMA*, 270: 854-859, 1993

5.4.2.2.1.2-2: DeLorenzo RJ, *Semin Neurol*, 10: 396-405, 1990

5.4.2.2.2.2-1: 小児のけいれん重積に対する薬物療法のエビデンスに関する臨床研究研究班（H14-小児-004、主任研究者：大澤真木子）、小児のけいれん重積状態の診断・治療ガイドライン（案）, 2005.3.27版, Ver.8.2

5.4.2.2.2.2-2: 日本神経学会治療ガイドライン, *臨床神経学*, 42: 557-597, 2002

5.4.2.3.2-2: Prasad K et al, *The Cochrane Database of Sys Rev*, 4:CD003723, 2005

5.4.2.3.2-1: Appleton R et al, *The Cochrane Database of Sys Rev*, 4: CD001905, 2002

4）臨床報告等

海外で実施された小児及び成人てんかん重積状態の患者を対象とした PB の臨床試験成績、国内外の患者を対象とした症例報告及び後向き研究等が報出された。

① 小児のてんかん重積状態に対する検討（申請用法・用量範囲内）

i）**Kokwaro GO et al, *Br J Clin Pharmacol*, 56: 453-457, 2003（5.4.2.4.2.1-3）**

ジアゼパム又はパラアルデヒド投与で効果が認められない重症の熱帯熱マラリア及びてんかん重積状態の患児を対象に、本薬の有効性及び薬物動態を検討するために非盲検試験が実施された（薬物動態については、「（i）臨床薬物動態及び臨床薬力学試験の概略」参照）。

用法・用量は、ジアゼパム又はパラアルデヒド投与に続き、第 2 選択薬として本薬を、初回用量として 15 mg/kg を 20 分以上かけて点滴静脈内投与し、初回投与 24 及び 48 時間後に維持用量として 5 mg/kg を静脈内投与すると設定された。

総投与症例数は 12 例であった。年齢（中央値）は生後 25 ヶ月（範囲: 7～62 ヶ月）、平均体重は 10.7 kg（95 %信頼区間: 9.6～11.7 kg）であった。

12 例の被験者のうち、66 %（8/12 例）で発作がコントロール[19]された。

安全性について、本薬投与後に死亡 1 例が認められ、当該症例は本薬投与 23 時間後に血圧上昇を伴う呼吸不全が出現し、呼吸不全出現 30 分後に心停止に至った。また、本薬投与後に低酸素症が 1 例認められたが、当該症例では本薬投与前より低酸素症（酸素飽和度 94 %）、低体温（直腸内体温 35.2℃）及びアシドーシス（塩基欠乏-16.7）が認められていた。なお、本薬投与後に、呼吸抑制、血圧、心拍数、中大脳動脈血流における著しい変化は認められなかった。本資料では有害事象及び副作用に関する定義は記載されていない。

以上より、小児のけいれん重積状態に対して、本薬 15 mg/kg の初回投与により、治療域血中濃度（15～40 μg/mL）の確保が可能であり、発作のコントロールに有効であることが示唆された。

ii）**杉田隆博ら, *小児内科*, 19: 1329-1334, 1987（5.4.2.5.2.1-4）**

けいれん及びけいれん重積症で入院した患児を対象に、救急治療の実態調査を目的として、後向き研究が実施された。

総調査対象例は 501 例であり、このうちけいれん重積症は 56 例であった。

けいれん重積症 56 例に対し、最も有効であった薬剤は、ジアゼパムが 26 例、フェニトインが 6 例、PB が 6 例、抱水クロラールが 4 例、チオペンタールが 1 例であった。

有害事象等の安全性については、評価されていない。

以上より、けいれん重積症に対する PB の有用性が示唆された。

② 小児のてんかん重積状態に対する検討（申請用法・用量範囲以外）

小児のてんかん重積状態に対して PB 高用量投与（非経静脈投与を含む）に関する公表文献として、下記の国内 5 報及び海外 4 報が報出された。また、国内において、てんかん重積状態以外

---

16) PB の点滴静脈内投与開始から 30 分以内に持続性けいれん発作が消失したとき、発作が抑制されたとみなす。また、PB の投与 24 時間後に、前の抗けいれん薬を必要としなかったとき、発作はコントロールされたとみなす。

の疾患を対象とした PB 大量療法に関する報告 1 例が提出された。バルビツレート昏睡による重症の血圧低下を伴う敗血症ショックが認められた（5.4.2.4.2.1-5）との報告もあったが、多くの報告で、本薬の薬理作用による昇圧剤を必要とする血圧低下や呼吸困難は認められていないと報告されている。

【提出された資料】

5.4.2.5.2.1-7: 林泰佑ら, *脳と発達*, 38: 359-362, 2006

5.4.2.5.2.1-3: 阪上由子ら, *小児科臨床*, 57: 2259-2267, 2004

5.4.2.5.2.1-5: 須藤章ら, *脳と発達*, 34: 23-29, 2002

5.4.2.5.2.1-1: Sugai K et al, *Epilepsia*, 43: 65-66, 2002

5.4.2.5.2.1-6: 田草雄一ら, *脳と発達*, 33: 351-356, 2001

5.4.2.5.2.1-8: 山岡久泰ら, *臨床脳波*, 7: 94-98, 1983

5.4.2.4.2.1-4: Lee WK et al, *Pediatr Neurol*, 34: 63-65, 2006

5.4.2.4.2.1-6: Sahin M et al, *Epilepsia*, 42: 1461-1467, 2001

5.4.2.4.2.1-5: Mirski MA et al, *Crit Care Med*, 23: 400-404, 1995

5.4.2.4.2.1-1: Crawford TO et al, *Neurology*, 38: 1035-1040, 1988

③ 成人のてんかん重積状態に対する検討について

i）Treiman DM et al, *N Engl J Med*, 339: 792-798, 1998（5.4.2.4.2.2-3）

成人の全般けいれん性てんかん重積状態の患者を対象に、PB を含む抗けいれん薬 4 剤の有効性及び安全性を比較するために、無作為化二重盲検比較試験が実施された。

用法・用量は、PB 15 mg/kg（PB 群、投与速度 100 mg/分以下）、ジアゼパム 0.15 mg/kg（投与速度 5 mg/分以下）投与後にフェニトイン 18 mg/kg（投与速度 50 mg/分以下）（DZP 及び PHT 併用群）、ロラゼパム 0.1 mg/kg（LZP 群、投与速度 2 mg/分以下）、又はフェニトイン 18 mg/kg（PHT 群、投与速度 50 mg/分以下）を静脈内投与すると設定された。

総登録症例数 570 例全例が ITT 解析対象集団であり、診断が確定した解析対象は 518 例（PB 群 124 例、DZP 及び PHT 併用群 131 例、LZP 群 136 例、PHT 群 127 例）であり、有効性解析対象集団であった。診断が確定した解析対象のうち、明らかな全般けいれん性てんかん重積状態（明らかな重積状態）[11] は 384 例（PB 群 91 例、DZP 及び PHT 併用群 95 例、LZP 群 97 例、PHT 群 101 例）、微細な全般けいれん性てんかん重積状態（微細な重積状態）[12] は 134 例（PB 群 33 例、DZP 及び PHT 併用群 36 例、LZP 群 39 例、PHT 群 26 例）であった。

各薬剤群の投与量、初回投与後の血清中濃度及び薬剤投与時間は下表のとおりであった。

表　各群の投与量、初回投与後の血清中濃度及び薬剤投与時間（平均値 ± 標準偏差）

投与群	PB 群	DZP 及び PHT 群		LZP 群	PHT 群
投与薬剤	PB	DZP	PHT	LZP	PHT
投与量（mg/kg）	14.96 ± 2.53	0.15 ± 0.02	15.08 ± 4.84	0.10 ± 0.01	16.02 ± 3.21
血清中濃度（μg/mL）	31.2 ± 37.2	0.245 ± 0.307	31.8 ± 19.2	0.231 ± 0.299	30.0 ± 13.6
投与時間（分）	16.6 ± 11.5	42.0 ± 38.1		4.7 ± 7.2	33.0 ± 20.1

11）発作と発作間に完全な回復のない反復性のけいれんと定義

12）昏睡状態が持続しているが、微細なけいれん運動のみ認められる場合の全般けいれん状態の増悪と定義

有効性について、明らかな重積状態患者における初回治療成功率は、下表のとおりであり、PHT群よりもLZP群で有意（$p = 0.002$、χ^2検定）に高値であったが、それ以外の治療群間において統計的な有意差は認められなかった（$p>0.01$、χ^2検定）。

表　各群の治療成功率（%）

	PB群	DZP及びPHT併用群	LZP群	PHT群
明らかな重積状態	58.2 (91)	55.8 (95)	64.9 (97)	43.6 (101)

治療成功：薬剤投与開始20分以内における全ての臨床時及び脳波的発作活動の消失し、かつ投与開始後20～60分にわたって再発がない状態と定義
（　）：解析対象例数

安全性について、副作用は下表のとおりであり，明らかな重積状態又は微細な重積状態のいずれにおいても、治療を要した低血圧、呼吸抑制及び心調律異常の発現率は各治療群間で差は認められなかった。

表　診断が確定した解析対象集団における主な副作用

	PB群	DZP及びPHT併用群	LZP群	PHT群
明らかな重積状態				
解析対象例数	91	95	97	101
換気低下（%）	13.2	16.8	10.3	9.9
低血圧（%）	34.1	31.6	25.8	27.0
不整脈（%）	3.3	2.1	7.2	6.9
微細な重積状態				
解析対象例数	33	36	39	26
換気低下（%）	15.2	2.9	12.8	7.7
低血圧（%）	48.5	58.3	59.0	57.7
不整脈	9.1	5.6	7.7	0.0

以上より、明らかな全般けいれん性てんかん重積状態患者に対し、ロラゼパムはフェニトインと比較して効果的であり、PB又はジアゼパム及びフェニトインの併用投与とは同程度であることが示唆された。

ii）Shaner DM et al, *Neurology*, 38: 202-207, 1988（5.4.2.4.2.2-2）

全般けいれん性てんかん重積状態の患者を対象に、PB及びジアゼパムとフェニトインの併用投与時の有効性及び安全性を検討するために、無作為化非盲検試験が実施された。

用法・用量は、PB群はPB 10 mg/kgを100 mg/分で静脈内投与し、投与開始10分後に発作が持続する場合、フェニトインを40 mg/分で投与し、血清中フェニトイン濃度が不明又は0～4 mg/Lの場合初回投与量18 mg/kgを、血清中フェニトイン濃度が5～9、10～15又は16～20 mg/Lの場合、それぞれ予定初回投与量の75、50又は25 %を投与すると設定された。ジアゼパム・フェニトイン併用群では、ジアゼパムを2 mg/分でけいれんの停止又は20 mgまで静脈内投与し、同時にフェニトインをPB群と同様の用法・用量で投与すると設定された。また、ジアゼパム20 mg投与後も発作が持続する場合、フェニトインの投与終了時まで、継続してジアゼパムを8 mg/時で静脈内投与すると設定された。

総投与症例数は36例（PB群18例、ジアゼパムとフェニトインの併用投与群18例）であった。

全被験者で、発作は7時間以内にコントロールされ、累積けいれん時間及び反応潜時（薬剤投与開始から最終のけいれん終了までの時間）はPB群でジアゼパム・フェニトイン併用群よりも

短い傾向が認められたが、有意差は認められなかった。PB 群では 11/18 例が PB 単剤投与で発作がコントロールされ、18 例の平均血清中濃度は 17.3 μg/mL、単剤投与有効例 11 例での平均血清中濃度は 18.3 μg/mL であった。

安全性について、不整脈及び低血圧の発現率は両群で同程度であり、各群でそれぞれ 6 例に気管挿管が行なわれ、呼吸抑制に起因しない気管挿管も認められた。

以上より、PB 投与は、ジアゼパムとフェニトインの併用投与と比較して、効果発現が速い傾向にあり、安全性についても同程度であることが示唆された。

ⅲ）Czapinski P, *J Neurol*, 242: S83, 1995（5.4.2.4.2.3-1）

てんかん重積状態を繰り返す患者を対象に、ジアゼパムと PB 又はフェニトインを併用投与した際の有効性及び安全性を検討するために、無作為化試験が実施された。

用法・用量は、ジアゼパム 10 mg を静脈内投与し、その後 PB 10～20 mg/kg 又はフェニトイン 12～18 mg/kg を 1 時間かけて静脈内投与する（投与量の 50 %を投与開始 15 分で投与する）と設定された。

総投与症例数は 60 例であった。

てんかん重積状態は、PB 群で 83.3 %、フェニトイン群で 86.6 %の症例で抑制された（平均反応潜時は、それぞれ 18 分及び 23 分）。血漿中薬物濃度の半減期は PB 及びフェニトインでそれぞれ 101 ± 25 及び 84.3 ± 43.2 時間であり、投与 12 時間後の脳脊髄液中の平均薬物濃度は PB 及びフェニトインにおいて、それぞれ血漿中濃度の 62 及び 15 %であった。

安全性について、副作用は両群で 23.3 %に発現したが、いずれも投与中止を必要とするものではなかった。

以上より、PB はフェニトインとほぼ同等の有効性及び忍容性を有することが示唆された。

ⅳ）その他の成人のてんかん重積状態に対する臨床報告

上記以外にも公表文献として、成人のてんかん重積状態を対象とした後方視的研究 1 報が提出され、PB は第 3 選択薬として投与されている。

【提出された資料】

5.4.2.4.2.2-1: Mayer SA et al, *Arch Neurol*, 59: 205-210, 2002

5）臨床使用経験

① 小児のてんかん重積状態に対するフェノバルビタールの使用実態に関する調査（5.4.2.6.2-3: 林北見ら, *脳と発達*, 40: 83-85, 2008）

日本小児神経学会評議員及び日本てんかん学会評議員 329 名（うち小児科医 222 名）に対して、PB の使用状況に関する実態調査が実施された。その結果、回答者のうち小児科医 79 名からの回答が集計された。PB 製剤の使用経験者は 53.2 %（42/79 名）であり、静注用製剤を静脈内投与した経験ありが 13 名、皮下・筋注用製剤を筋肉内投与した経験ありが 20 名及び皮下・筋注用製剤を静脈内投与した経験ありが 12 名であった。PB の用量は、2～30 mg/kg の範囲であった（回答者 23 名）。PB での治療対象となった発作型（回答者 31 名、重複回答あり）は、27 名が二次性全般

化を含む全身性強直間代発作、7名が部分発作、2名がその他の発作型であった。PBの有用性（回答者30名）は、15名が75％以上の症例で「有用」、7名が50％以上75％未満の症例で「有用」、6名が25％以上50％未満の症例で「有用」、残り2名が25％未満の症例で「有用」又は「有用」を認めなかったと回答した。

② 本邦における成人のてんかん重積状態に対するPBの使用経験

成人のてんかん重積状態におけるPBの国内の使用経験として、下記の9報（小児及び成人を対象とした報告1報が含まれる）が提出された。

【提出された資料】

5.4.2.5.2.1-2: 奥村彰久ら, *小児科臨床*, 59: 1938-1942, 2006
5.4.1.2-6: 斎藤潤ら, *島根県中病医誌*, 29: 3-6, 2004
5.4.1.2-7: 末永貴美ら, *臨床精神医学*, 36: 327-331, 2007
5.4.1.2-11: 柳川洋一ら, *脳神経*, 58: 509-513, 2006
5.4.1.2-5: 井出文子ら, *中毒研究*, 19: 407-408, 2006
5.4.1.2-9: 道具伸治ら, *日本神経救急学会雑誌*, 18: 69-72, 2005
5.4.1.2-12: 山下真理子ら, *脳神経*, 53: 1057-1062, 2001
5.4.1.2-8: 水島靖明ら, *日救急医会誌*, 7: 197-201, 1996
5.4.1.2-10: 向井誠ら, *精神医学*, 29: 1351-4, 1987

＜審査の概略＞

（1）新生児けいれん

1）フェノバルビタールの新生児けいれんに対する有効性について

機構は、医師主導治験（5.3.5.2-1）における有効性が、提出された臨床報告（5.4.2.4.1-1、5.4.2.4.1-7、5.4.2.4.1-4、5.4.2.4.3-5、5.4.2.5.1-1及び5.4.2.5.1-3）での結果よりも高くなった原因について説明するよう申請者に求めた。

申請者は、医師主導治験（5.3.5.2-1）での有効性が、提出した臨床報告での結果よりも高くなった原因として、有効性評価指標及び被験者背景の相違が影響したと考えられることを説明した。まず、有効性評価指標については、臨床的な発作と脳波上の発作活動には乖離があり、抗てんかん薬投与後に臨床的な発作が消失したにも関わらず脳波上の発作活動が持続することが25～30％の頻度で認められたことが報告されているため（5.4.2.4.1-9）、臨床的な発作を有効性評価指標とした医師主導治験（5.3.5.2-1）では、脳波上の発作活動を指標とした臨床報告（5.4.2.4.1-1及び5.4.2.4.1-7）での結果よりも有効率が高くなったと考えられたことを説明した。次に、医師主導治験（5.3.5.2-1）及び臨床報告（5.4.2.4.1-1、5.4.2.4.1-7、5.4.2.5.1-1及び5.4.2.5.1-3）の被験者では、原因疾患として中枢神経系疾患が多く大きな差は認められなかったが、下表のように医師主導治験（5.3.5.2-1）におけるアプガースコアの平均値は1及び5分値ともに7以上と正常又はほぼ正常であったのに対し、臨床報告（5.4.2.4.1-1、5.4.2.4.1-7及び5.4.2.5.1-1）ではいずれも3～5の範囲で、医師主導治験（5.3.5.2-1）における患児よりも出生状態が悪かったと考えられ、詳細な検討は不可能であるが、原因疾患の重症度及び原因疾患に関連する脳機能又は器質障害の

程度の差により、医師主導治験（5.3.5.2-1）における有効率が臨床報告（5.4.2.4.1-1、5.4.2.4.1-7、5.4.2.5.1-1 及び 5.4.2.5.1-3）での結果よりも高くなったと考えられることを説明した。

表　医師主導治験及び臨床報告の有効性評価指標、アプガースコア及び PB の有効率

資料名	有効性評価指標	アプガースコア	PB の有効率及び原因疾患による層別結果）
医師主導治験（5.3.5.2-1）	臨床発作	1 分値*: 7.6±2.5（範囲 3～10）、中央値 8.5 5 分値*: 8.3±2.6（範囲 3～10）、中央値 9.0 *：平均値 ± 標準偏差	全体: 100.0 %（10/10 例） 原因疾患による層別結果: 低酸素性虚血性脳症 100.0 %（2/2 例） 頭蓋内出血 100.0 %（1/1 例） 先天性脳異常 100.0 %（1/1 例） その他 100.0 %（1/1 例） 不明 100.0 %（5/5 例）
Boylan らの報告（5.4.2.4.1-1）	経皮報告所見	全被験者（22 例） 1 分値: 平均値 3.9（範囲 0～ 9）、中央値 3.5 5 分値: 平均値 5.0（範囲 0～10）、中央値 5 PB 反応例（11 例） 1 分値: 平均値 3.5（範囲 0～ 9）、中央値 4 5 分値: 平均値 4.7（範囲 0～9）、中央値 5 PB 非反応例（11 例） 1 分値：平均値 4.3（範囲 0～ 9）、中央値 3 5 分値：平均値 5.4（範囲 0～10）、中央値 6	全体: 50.0 %（11/22 例） 原因疾患による層別結果: 低酸素性虚血性脳症 38.5 %（5/13 例） 頭蓋内出血 66.7 %（2/3） 子宮内胎児死亡疑い 100.0 %（2/2 例） 奇形症候群疑い、未熟児 0.0 %（0/1 例） 脳静脈奇形 0.0 %（0/1 例） ミオパチー 感染関連 100.0 %（1/1 例） 良性新生児けいれん 100.0 %（1/1 例）
Painter らの報告（5.4.2.4.1-7）	経皮報告所見	薬剤基準: 5 分値 5 点満（血清次之 10mmol/L 以上）	全体: 43.3 %（13/30 例） 原因疾患による層別結果: 記載なし。
Gilman らの報告（5.4.2.4.1-4）	臨床発作	記載なし	全体: 76.7 %（92/120 例） 原因疾患による層別結果: 出生児仮死 72.6 %（45/62 例） 肝疾患 86.7 %（26/30 例） 脳出血 88.9 %（16/18 例） 感染症 55.6 %（5/9 例） 代謝性 0.0 %（0/1 例）
Gal らの報告（5.4.2.4.3-5）	臨床発作	記載なし	全体: 84.5 %（60/71 例） 原因疾患による層別結果: 仮死 80.6 %（29/36 例） 中枢神経系出血 84.6 %（11/13 例） 感染症 50.0 %（1/2 例） その他 87.5 %（7/8 例） 不明 100.0 %（12/12 例）
柴田らの報告（5.4.2.5.1-1）	臨床発作	1 分値（平均値 ± 標準偏差）: 5.0 ± 3.1	100.0 %（22/22 例） 体重 3kg 以上かつ PB 20 mg/kg 投与例のみが評価された
武部らの報告（5.4.2.5.1-3）	臨床発作	記載なし	全体: 58.8 %（10/17 例） 原因疾患による層別結果: 周産期脳障害脳症 53.8 %（7/13 例） 化膿性髄膜炎 100.0 %（1/1 例） 良性家族性新生児けいれん 0.0 %（0/1 例） 不明 100.0 %（2/2 例）

機構は、医師主導治験（5.3.5.2-1）での成績を一般的な新生児けいれん患児に外挿できると考える根拠について説明するよう申請者に求めた。

申請者は、新生児けいれんの原因疾患は、低酸素性虚血性脳症が原因として最も多く、その他には頭蓋内出血、脳血管障害、中枢神経系感染症、低カルシウム血症等の代謝異常及び先天性脳異常等があり多彩であること（5.4.2.1.1.1-4、5.4.2.1.1.2-4、5.4.2.1.1.1-6）を説明した。そして申請者は、医師主導治験（5.3.5.2-1）に組み入れられた新生児けいれん患児における原因疾患は 5 例で不明と診断されたが、2 例で胎児仮死、1 例で脳奇形と推察され、虚血性低酸素性脳症が約半数、頭蓋内出血及び代謝障害が約 10 %の頻度であり、医師主導治験（5.3.5.2-1）に組み入れられた患児は、一般集団を概ね反映していると考えられることを説明した。

機構は、医師主導治験（5.3.5.2-1）に組み入れられた症例は少数であるが、新生児けいれんに対

する本剤の有効性は示唆されており，他の提出された教科書、医薬品集、総説及び臨床報告等の情報も踏まえると、本剤の新生児けいれんに対する有効性は示されたと判断して差し支えないと考える。しかしながら機構は、けいれんを引き起こす原因疾患の種類やその重症度は様々であると考えられ、患者背景が本剤の有効性及び安全性に及ぼす影響については、製造販売後調査においてさらに検討が必要と考える。

2）新生児けいれんに対する本剤の用法・用量について

機構は、新生児けいれんに対する本剤の初回投与量、追加投与量及び維持投与量の設定の妥当性について説明するよう申請者に求めた。

申請者は、PBの治療域血中濃度の下限は、国内外臨床報告（5.4.2.4.3-5、5.4.2.5.1-1及び5.4.2.5.1-3）を踏まえ、15 μg/mL以上であると考えられたこと、治療域血中濃度の上限については、PBの有効性は血中濃度40 μg/mLまでは用量依存的であり、50 μg/mL以上では摂食減少及び鎮静が認められるとの報告（5.4.2.4.1-4）があること，40 μg/mLを超える血中濃度では有効率の増加は少ないと報告されていること（5.4.2.4.3-5）から、治療域血中濃度の上限は40 μg/mLと考えられることを説明した。また申請者は、海外臨床報告（5.4.2.4.3-9）では、速やかな発作抑制のために、治療域血中濃度を達成することの重要性が説明されており、治療域血中濃度の下限（15 μg/mL）を達成するには、国内臨床報告（5.4.2.5.1-1、5.4.2.5.1-3）も踏まえると、静脈内投与による初回投与量を20 mg/kg、けいれんが消失しない場合には、同量を追加投与することを用法・用量として設定することが妥当と考え、医師主導治験（5.3.5.2-1）は当該用法・用量で実施したことを説明した。また申請者は、医師主導治験（5.3.5.2-1）において、PBの血中濃度は、初回PB 20 mg/kg投与（9例）では18.8～25.8 μg/mL、PB 20 mg/kgを追加投与した2例では42.8及び36.9 μg/mLであったことから、PB 20 mg/kgの初回投与により、けいれんがコントロールできない場合は、同量を追加投与することが妥当であると考えられたことを説明した。さらに申請者は、維持投与量について、初回投与により消失したけいれんの再発防止を目的にPBの治療域血中濃度（15～40 μg/mL）を維持できる用量を投与することが必要であり，海外の教科書（5.4.2.1.1.1-3、5.4.2.1.1.3-1）では5 mg/kg/日が推奨されていること、投与経路として筋肉内投与を含むものであるが、日本人の新生児けいれん患児で3 mg/kg/日の維持投与量により、血漿中PB濃度を15.0～42.5 μg/mLに維持したとの報告（5.4.2.5.1-3）があることを説明した。以上を踏まえ申請者は、医師主導治験（5.3.5.2-1）では、PBの維持投与量を2.5～5 mg/kg/日と設定した結果，維持投与最終投与前又は中止時のPB血中濃度（10例）は18.7～45.3 μg/mLであったことから、申請用量として、PBの初回投与の用量を20 mg/kg、けいれんが消失しないときには同量を追加投与し、維持投与量を2.5～5 mg/kg/日と設定することが妥当であると考えたことを説明した。

機構は、新生児けいれんに対する本剤の投与期間について、6日間をめどとすることの妥当性を説明するよう申請者に求めた。

申請者は、医師主導治験（5.3.5.2-1）では、海外臨床報告（5.4.2.4.1-7）及びPediatric Neurology（5.4.2.1.1.1-5）での記載を参考に維持投与期間を6日間と設定したことを説明した。また申請者は、Avery's Neonatology, Pathophysiology & Management of the Newborn（5.4.2.1.1.1-2）では、けいれんのコントロールが可能となった場合、可能な限り速やかにPBの投与を中止することが提案されているが、Pediatric Neurology（5.4.2.1.1.1-5）では維持療法の中止時期は明確に規定されてい

ないことから，新生児けいれんに対し，抗てんかん薬をどの程度投与するかは標準化されておらず、臨床使用目的に応じた使用が必要と考えられ、維持投与期間を一律に設定しないことが妥当であると考えたことを説明し、用法・用量として維持投与の期間を申請時には「6日間をめどに」と設定しているが、この記載についても削除することが適切と考える旨を説明した。

機構は、新生児けいれんに対する用法・用量について、申請者から提出された文献より示されており、医師主導治験の結果を踏まえると、申請された用法・用量で設定された初回投与量、追加投与量及び維持投与量については特に大きな問題はないと考える。また、維持投与の期間については、PBの長期投与による安全性は明確になっておらず、本剤を漫然と投与しないことが適切と考えられるが、個々の症例の状況により PB を維持投与する期間は異なると考えられることから、一律に投与期間を限定する必要はないと考える。しかしながら、医師主導治験では維持投与期間が6日間と設定されており、さらなる長期間の投与は実施されていないこと、けいれん発作がコントロール可能となった時点で、可能な限り速やかに投与を中止することが提案されている（5.4.2.1.1.1-2）ことから、本剤を継続して投与する際には血中濃度モニタリング（Therapeutic Drug Monitoring: TDM）を実施し、血中PB濃度を測定しながら、安全性に十分に注意を払うことが必要であると考える。なお，本剤の投与量並びに投与期間と有効性及び安全性との関係については、製造販売後調査でさらに検討が必要であると考える。

（2）てんかん重積状態

1）PBのてんかん重積状態に対する投与が医学薬学上公知とすることの妥当性について

機構は、PBのてんかん重積状態に対する投与が、医学薬学上公知であると考えた根拠を説明するよう申請者に求めた。

申請者はてんかん重積状態に対するPB静注用製剤の投与は，欧米においては承認されており、① 国際的な教科書（5.4.2.1.1.2-1、5.4.2.1.1.2-2、5.4.2.1.1.2-3、5.4.2.1.1.2-4、5.4.2.1.1.2-5、5.4.2.1.1.2-6、5.4.2.1.1.3-1 及び 5.4.2.1.4.3-1）では、てんかん重積状態の治療薬として記載され、PBは第2又は第3選択薬として長年の使用実績があること、② 国際的に信頼できる学術雑誌に掲載された科学的根拠となり得る文献（5.4.2.4.2.2-2、5.4.2.4.2.2-3）及び総説（5.4.2.2.1.2-1、5.4.2.2.1.2-2、5.4.2.2.1.2-3、5.4.2.2.1.2-4、5.4.2.2.1.2-5、5.4.2.2.1.2-6）が存在すること、③ 国内における使用経験があること（5.4.2.6.2-3、5.4.2.5.2.1-2、5.4.1.2-6、5.4.1.2-7、5.4.1.2-11、5.4.1.2-5、5.4.1.2-9、5.4.1.2-12、5.4.1.2-8、5.4.1.2-10）から、PBのてんかん重積状態に対する有効性及び安全性については医学薬学上公知と考えられることを説明した。また申請者は、本剤は日本小児科学会及び日本小児神経学会から共同で要望書（5.4.2.6.2-4）が提出されており、医学的にも必要と考えられていることを説明した。

機構は、小児及び成人のてんかん重積状態に対する PB の有効性及び安全性については、国内外の成書、公表文献及びガイドライン等での記載から、広くコンセンサスが得られていると考えられ、PBのてんかん重積状態に対する有効性は、医学薬学上公知と判断することについては特に大きな問題はないと考える。

2）てんかん重積状態に対するPBの用法・用量について

機構は、てんかん重積状態に対する本剤の用法・用量の設定の妥当性について説明するよう申請者に求めた。

申請者は、まず小児てんかん重積状態における用法・用量の設定について、Kokwaro らの報告（5.4.2.4.2.1-3）から、PB 15 mg/kg の初回静脈内投与を行ったとき、66 %（8/12 例）で発作がコントロールされ、コントロールされた症例での血漿中 PB 濃度の C_{max} は 19.9 μg/mL（中央値、範囲: 17.9～27.9）であり、15 μg/mL を超える血漿中 PB 濃度が維持されたことから、治療域血中濃度の下限を 15 μg/mL とすることは適切と考えられたことを説明した。また申請者は、治療域血中濃度の上限については、具体的なデータは認められなかったが、Booker らは「40 μg/mL を超える血中濃度を達成することで有効性は最大化するが、個々の患者では副作用発現の可能性が増加する」と述べており（Woodbury DM, Penry JK, Piggenger CE editors. *Antiepileptic drugs, 2nd edition*, Raven Press, 341-350, 1982）、当該記載は、PB 投与によるてんかん治療では一般的な情報であり、治療域血中濃度上限を 40 μg/mL とすることは適切と考えられたことを説明した。また申請者は、小児てんかん重積状態における初回投与量として、日本人で本薬 15～20 mg/kg を投与したとき、ジアゼパムに次いで有効であるとの報告（5.4.2.5.2.1-4）があること、国内外の教科書、医薬品集、総説等に、15～20 mg/kg の静脈内投与を推奨していることを踏まえ、初回投与量を 15～20 mg/kg とすることが妥当と判断したことを説明した。

次に申請者は、成人てんかん重積状態における用法・用量の設定について、海外教科書、医薬品集（5.4.2.1.3.2-2、5.4.2.1.3.3-3、5.4.2.1.3.2-1、5.4.2.1.3.3-2、5.4.2.1.3.2-4、5.4.2.1.4.3-1、5.4.2.1.1.2-3、5.4.2.1.1.2-2）等で治療域血中濃度は、10～40 μg/mL の範囲で記載されており、15～40 μg/mL との記載が最も多いこと、てんかん重積状態は救死的であり、本薬は第 2 又は第 3 選択薬の位置付けであることを踏まえると、小児と同様に治療域血中濃度を 15～40 μg/mL とすることは妥当と判断したことを説明した。また、初回投与量については、PB 15 mg/kg 投与時の有効性及び安全性がジアゼパム・フェニトインを併用投与時と同程度であったとの報告（5.4.2.4.2.2-3）があること、海外成書、医薬品集及び総説等で 10～20 mg/kg の範囲で推奨されており、多くは 20 mg/kg であったことから、投与量は 15～20 mg/kg と設定することが適切と判断したことを説明した。

以上から申請者は、てんかん重積状態において 15～20 mg/kg を用量とすることは妥当と判断したこと、維持投与については、てんかん重積状態がコントロールできた場合、抗てんかん薬の経口投与が可能となると考えられることから、設定する必要はないと考えたことを説明した。

機構は、てんかん重積状態における本剤の用法・用量について、15～20 mg/kg を初回投与量とし、治療域血中濃度を 15～40 μg/mL と設定することは、海外教科書、医薬品集等でも述べられており、特に大きな問題はないと考えられる。しかしながら、本剤の投与量と有効性及び安全性の関係については、製造販売後調査でさらに検討する必要があると考える。

（3）安全性について

1）本薬の血中濃度と安全性について

機構は、PB の血中濃度と安全性の関係について説明するよう申請者に求めた。

申請者は、医師主導治験（5.3.5.2-1）において、初回投与 2 時間後、1 回目の維持投与 2 時間後及び維持投与最終投与前（中止時）の PB 血中濃度は 18.7～45.3 μg/mL であり、血中濃度が 30 μg/mL を超えた 3 例及び 30 μg/mL を超えなかった 7 例では、有害事象プロファイルには差異はなく、いずれも非重篤であったことを説明した。また申請者は、医師主導治験（5.3.5.2-1）及び臨床報告（5.4.2.4.1-4、5.4.2.4.1-7、5.4.2.4.3-5、Pippenger CE et al, *Clin Perinatol*, 2: 111-115, 1975、DeCarolis MP

et al, *Dev Pharmacol Ther*, 14: 84-89, 1990、Shany E, *Eur J Paediatr Neurol*, 8: 323-325, 2004、Wehner J et al, *Kinderarzti Prax*, 59: S31-34, 1991、Amitai Y et al, *J Emerg Med*, 8: 449-450, 1990）において、新生児けいれんにおけるPB血中濃度と安全性の関係は下表のとおりであり、50 μg/mL以上では鎮静・摂食（哺乳）減少、傾眠、眼振、筋緊張の低下、呼吸抑制、嗜眠、呼吸停止、徐脈、体温低下、胃・腸の蠕動運動の低下等の副作用の発現が起こる可能性があることを説明した。

表　PB血中濃度と副作用に関する報告（新生児）

PB血中濃度	発現した副作用
40 μg/mL 以下	呼吸抑制、痙攣閾値低下、意識の低下を伴う自発運動低下等（発生例数が低い）
50 μg/mL 以上	心拍数への影響、鎮静・摂食（哺乳）減少、傾眠、眼振
60 μg/mL 以上	筋緊張の低下、哺乳減少
70 μg/mL 以上	重篤な嗜眠
80 μg/mL 以上	呼吸下垂傾向、痙攣閾の低下
90 μg/mL 以上	一時的な呼吸の停止、徐脈、低換気、嗜眠、筋緊張の低下等
高用量投与 （血中濃度が60 μg/mL 以上）	胃・腸の蠕動運動の低下

次に申請者は、臨床報告（5.4.2.5.2.1-5、5.4.2.5.2.1-7、5.4.2.6.2-1、5.4.2.6.2-5、Pippenger CE et al, *Clin Perinatol*, 2: 111-115, 1975）において、小児及び成人のてんかん重積状態におけるPB血中濃度と安全性の関係は下表のとおりであり、小児に認められた中毒性表皮壊死症（toxic epidermal necrolysis: TEN）は、治療域血中濃度近くで発現しているものであったが、血中濃度との関係が明らかであった報告のみを抽出したものであり、小児において特に注意すべき事象であるかどうか不明であること、てんかん重積状態の場合は、複数の抗てんかん薬が併用されることから、血中濃度が低い場合でも有害事象の発現に注意すべきと考えられたが、小児又は成人で特に懸念すべき副作用は認められなかったことを説明した。

表　PB血中濃度と副作用に関する報告（小児及び成人）

PB血中濃度	発現した副作用	
	小児	成人
40 μg/mL 以下	中毒性表皮壊死融解症	嘔気、せん妄、過鎮静、傾静、胃痛後減少、支持ヨウの状態、幻覚妄想状態
50 μg/mL 以上	呼吸抑制	γ-GTP一過性上昇、Reye症候群
60 μg/mL 以上	筋緊張の低下、哺乳減少（幼児）	—
70 μg/mL 以上	強い嗜眠、γ-GTP上昇、修度血圧低下	嘔気、γ-GTP上昇、軽度呼吸抑制、軽度血圧低下
80 μg/mL 以上	嘔気、γ-GTP上昇	—
90 μg/mL 以上	嚥下力低下、胃生下垂性潰瘍	知的障害、不眠失調

機構は、新生児けいれん及びてんかん重積状態に対し設定された治療域濃度（15～40 μg/mL）においても、呼吸抑制、血圧低下及び意識水準の低下等の重篤な有害事象が発現する可能性はあるが、本剤は、医師による十分な観察下で投与されると考えられることから、これらの事象が発現した場合においても対処は可能と考えられ、十分な注意喚起を行うことで本剤のベネフィットはリスクを上回るもの考える。また、患者の状態によりPBの血中濃度が変動する可能性は否定できず、特に新生児においては定期的なTDMの実施が必要であり、それ以外の患者においても必要に応じてTDMを実施すべきと考える。

なお、本剤の安全性については、製造販売後調査において、引き続き検討が必要と考える。

2）フェノバルビタール静脈内投与における投与速度と安全性について

機構は、PBの投与速度と安全性の関係について説明するよう申請者に求めた。

申請者は、まず、新生児けいれんについて、PBの静脈内投与における投与速度と安全性を検討

した報告は認められなかったが、国際的に標準とされている教科書（5.4.2.1.1.1-4）では投与時間として10～15分（PB投与量20 mg/kg）を推奨していること、医薬品集（5.4.2.1.3.3-3, 5.4.2.1.3.3-2）ではPB 20 mg/kgを1 mg/kg/分以下の速度で20分かけて静注することが推奨されていること、国内外の臨床報告（5.4.2.4.1-7、5.4.2.4.1-4、5.4.2.5.1-1、5.4.2.5.1-3）では5～20分（PB投与量15～25 mg/kg）と設定されていたことを説明した。その上で申請者は、医師主導治験（5.3.5.2-1）における投与速度は5～10分（PB 20 mg/kg）と設定されており、初回投与時の副作用として軽度及び中等度の呼吸抑制が2例に認められ、維持投与期には血圧低下が認められたものの、いずれも非重篤であったことから、新生児けいれんに対するPBの投与速度は5～10分（20 mg/kg）と設定することが妥当と考えることを説明した。

次に申請者は、てんかん重積状態について、PBの静脈内投与における投与速度と安全性を検討した報告は認められず、教科書、医薬品集、総説及び臨床報告での投与速度の設定は下表のとおりであり、小児及び成人ともに投与速度は100 mg/分以下と設定されていることから、てんかん重積状態に対するPBの静脈内投与の投与速度は10分以上かつ100 mg/分以下と設定することが妥当であると考えることを説明した。

表　教科書、医薬品集、総説及び臨床報告での投与速度の設定

投与速度	初回投与量	小児	成人
30mg/分以下	20 mg/kg	Treat Epilepsy（教科書、5.4.2.1.1.2-6）	
50mg/分以下	20 mg/kg	Ped Neurol（教科書、5.4.2.1.1.2-4） DeLorenzo 1990（総説、5.4.2.2.1.2-2）	
60mg/分以下	100～400 mg（又は20 mg/kg）	AHFS（医薬品集、5.4.2.1.3.3-1）	AHFS（医薬品集、5.4.2.1.3.3-1）
50～75mg/分以下	20 mg/kg	Lowenstein 1998（総説、5.4.2.2.1.2-5）	Harrison（教科書、5.4.2.1.1.2-4） Lowenstein 1998（総説、5.4.2.2.1.2-5）
100mg/分未満	20 mg/kg		Epilepsy Comp Text（教科書、5.4.2.1.1.2-2）
100mg/分以下	10 mg/kg		Martindale（医薬品集、5.4.2.1.3.2-2、5.4.2.1.3.3-3） BNF/BNFC（医薬品集、5.4.2.1.3.2-1、5.4.2.1.3.3-2）
	15 mg/kg		Treiman 1998（臨床報告、5.4.2.4.2.2-3）
	15～20 mg/kg	Kalviainen 2005（総説、5.4.2.2.1.2-4）	
	20 mg/kg	Treat Epilepsy（教科書、対象：年長児）	Treat Epilepsy（教科書、5.4.2.1.1.2-6） DeLorenzo 1990（総説、5.4.2.2.1.2-2）
100mg/分	10 mg/kg*		Shamer 1988（臨床報告 5.4.2.4.2.2-2）
	15～20 mg/kg	杉田 1987（臨床報告、5.4.2.5.2.1-4）	
	20 mg/kg	Cecil（教科書　5.4.2.1.1.2-1） Epilepsy Foundation of America 1993（総説、5.4.2.2.1.2-3） 日本神経学会治療ガイドライン2002（5.4.2.2.2.2-2）	
10分以下	20 mg/kg	Appleton 2000（総説、5.4.2.2.1.2-1）	
10～15分	15～20 mg/kg	USP DI（医薬品集、5.4.2.1.3.2-3）	
10～30分	15～20 mg/kg	Nelson Ped（教科書、5.4.2.1.1.3-1）	
20分	15 mg/kg	Kokwaro 2003（臨床報告、5.4.2.4.2.1-3）	
1時間	10～20 mg/kg	Czapinski 1995（臨床報告、5.4.2.4.2.3-1）	
1 mg/kg/分以下	20 mg/kg	BNF/BNFC（12～18歳、5.4.2.1.3.2-1、5.4.2.1.3.3-2）	
既述	10～20 mg/kg		USP DI（医薬品集、5.4.2.1.3.2-3）
	200 mg**		Rote Liste（医薬品集、5.4.2.1.3.3-4,5）

* 10 mg/kgまで投与速度100 mg/分、けいれんの持続する場合は、投与速度は50 mg/kgに減速し、追加投与する。
** 200 mg投与、場合によって1日2～3回投与可、1日最大量800 mg

機構は、新生児けいれんにおいて、PBの投与速度と安全性の関係は明確になっておらず、医師主導治験（5.3.5.2-1）で設定された投与速度（5～10分（20 mg/kg））についても、少数例の検討であり、十分な検討が行われたとは言えないものの、これらの症例において、特に重篤な事象は認

められておらず、本剤は医師の観察下でのみ投与されること、海外の臨床報告（5.4.2.4.3-9）では速やかに治療域濃度に到達させることが重要であるとされていることなどを踏まえると、新生児けいれんに対するPBの投与速度を5～10分と設定することについて、特に大きな問題はないと考える。

また機構は、てんかん重積について、PBの投与速度と安全性の関係は明確になっていないものの、国内外の教科書、総説等から、てんかん重積状態においては、10分以上かけて投与すると示されていること、国内のガイドライン（5.4.2.2.2.2-2）では100 mg/分で投与すると記載されていることなどを踏まえると、小児及び成人におけるてんかん重積に対する投与速度を、本剤を10分以上かけて緩徐に投与すること（100 mg/分を超えないこと）と設定することは妥当と考える。

なお、投与速度と安全性との関係については、製造販売後調査において、さらに検討が必要であると考える。

3）本薬による皮膚障害について

機構は、本薬の投与により皮膚障害が発現する可能性について、申請者に説明を求めた。

申請者は、成人における抗てんかん薬による皮疹の発現頻度は、フェニトイン（5.9 %）、ラモトリギン（4.8 %）及びカルバマゼピン（3.7 %）では高く、レベチラセタム（0.6 %）、ガバペンチン（0.3 %）、バルプロ酸（0.7 %）では低い発現頻度であり、PBにおける発現頻度はその中間（約1.0 %[13]）であったと報告されていること（Arif H et al, *Neurology*, 68: 1701-1709, 2007）、また重篤な皮膚障害（スティーブンス・ジョンソン症候群（Stevens-Johnson syndrome: SJS）、中毒性表皮壊死融解症（TEN））については、抗てんかん薬の新規投薬患者10,000人に対する発現危険率は、PB 8.1人、フェニトイン8.3人、ラモトリギン2.5人、カルバマゼピン1.4人、バルプロ酸0.4人とフェニトインと同程度に高い頻度であったと報告（Mockenhaupt M et al, *Neurology*, 64: 1134-1138, 2005）されていることから、PBによるSJS及びTENの発現には注意を要するが、他の抗てんかん薬より皮膚障害の発現頻度が著しく高いものではないと考えられることを説明した。また申請者は、SJS及びTENの発現時期は、PBでは63日以内に認められ、少なくとも投与開始6週間は十分な観察が必要との報告（Mockenhaupt M et al, *Neurology*, 64: 1134-1138, 2005）があること、投与経路別の発現頻度については、国内外の文献調査結果（5.4.2.6.2-5、5.4.2.6.2-1）では、使用頻度が高い経口投与でこれらの事象が多く認められているが、発現頻度が明らかではなく、投与経路による発現頻度の差を考察することは困難であったことを併せて説明した。

機構は、本剤投与による皮膚障害について、十分な注意が必要であるが、そのリスクは他の抗てんかん薬と同様と考えられ、現時点においては、注意喚起についても、PBの筋肉内注射用製剤と同様に行うことで問題ないと考える。なお、重篤な皮膚障害（SJS及びTEN）の発現状況については、製造販売後調査においても検討が必要であると考える。

4）超低出生体重児に対するフェノバルビタール静脈内投与の安全性について

機構は、超低出生体重児に対するPB静脈内投与の安全性について説明するよう申請者に求めた。

13）報告中に掲示されているグラフより目視にて算出

申請者は、医師主導治験（5.3.5.2-1）においては、超低出生体重児は組み込まれておらず、極低出生体重児1例（登録番号KNG02、出生体重1144 g）が組み込まれ、重篤な有害事象（動脈管開存症）が認められたが、先天性のものであると考えられたことから因果関係は否定されていること、その他の有害事象として腎不全、低Na血症、体温上昇、CRP上昇、膿瘍疹が認められたが、因果関係は否定されていることを説明した。また申請者は、Ruthら（5.4.2.4.1-8）は、早産児47例（体重1160 ± 210 g（平均 ± 標準偏差、以下同様））に対してPBを静脈内投与（初回投与量: 30 mg/kg、維持投与量: 5 mg/kg、維持投与前の血清中濃度: 43 μg/mL）した時、ビリルビン低下以外には問題となる副作用は認められなかったと報告していること、田本ら（5.4.2.5.1-4）は、極出生体重児18例（体重1075.5 ± 298.9 g）の頭蓋内出血の予防のためにPBを投与（20 mg 1回投与、24時間後平均血中濃度: 25.3 ± 4.2 μg/mL）した時、副作用は認められなかったと報告していることを述べ、限られた情報ではあるものの、本剤静脈内投与の安全性は、申請用法・用量の範囲において、超低出生体重児と他の新生児との間で、大きな差異はないと考えられることを説明した。

機構は、超低出生体重児における本剤の安全性は十分に検討されていないと考えるが、このような症例に対して本剤を投与する場合には、特に慎重に投与することが必要であり、患者の状態を十分に観察しながら投与すべきと考える。なお、これらの患児に対する有効性及び安全性については、製造販売後調査においてさらに検討する必要があると考える。

（4）新生児けいれん及びてんかん重積状態の治療における本剤の位置付けについて

機構は、新生児けいれんの治療における本剤の位置付けについて、申請者に説明を求めた。

申請者は、新生児けいれんの治療薬として、本剤以外にフェニトイン、リドカイン、ベンゾジアゼピン系薬剤が存在するが、本剤については、① 投与量と血中濃度の関係及び治療域血中濃度が明らかにされていること（5.4.2.5.1-3、5.4.2.5.1-1、5.4.2.5.3-2、5.4.2.4.3-8、5.4.2.4.3-3、5.4.2.4.3-13）、② 他剤と比較して血中濃度の半減期が長く、効果が長時間持続すること（5.4.2.1.1.1-5）、③ 医師主導治験（5.3.5.2-1）や研究報告（5.4.2.5.1-3、5.4.2.5.1-1、5.4.2.5.3-2、5.4.2.4.3-8、5.4.2.4.3-3、5.4.2.4.1-4、5.4.2.4.1-1、5.4.2.4.1-9、5.4.2.4.1-7）があり、安全性プロファイルが明らかになっていると考えられることから、国内外の教科書（5.4.2.1.1.1-5、5.4.2.1.1.1-1、5.4.2.1.1.1-2、5.4.2.1.1.1-6、5.4.2.1.1.1-4、5.4.2.1.2.1-2、5.4.2.1.2.1-3）及び総説（5.4.2.2.1.1-2、5.4.2.2.1.1-1）で示されているように、新生児けいれんに対する治療の第1選択薬になるものと考えていることを説明した。

機構は、てんかん重積状態の治療における本剤の位置付けについて説明するよう申請者に求めた。

申請者は、てんかん重積状態の第1選択薬は国内ではジアゼパム、海外ではロラゼパムなどのベンゾジアゼピン系薬剤であり、速効性がありけいれん抑制作用も強力であるが、持続時間が短いこと及び急速静脈内投与により呼吸抑制が生じることがあり、安全性に問題があると考えられていることを説明した。また第2選択薬として、国内ではフェニトイン、海外ではPB又はフェニトインが使用されており、PBの有効性は、ロラゼパムと同程度で、フェニトインよりも高かったとの報告（5.4.2.4.2.2-3）や、ジアゼパム及びフェニトイン併用投与と比較してPBでは効果発現が早かったとの報告（5.4.2.4.2.2.-2）があること、また安全性については、フェニトインはPBと比較して呼吸抑制及び意識水準の低下の発現は少ない（5.4.2.1.1-6、5.4.2.2.1.2-1）ものの、血管に対する刺激性が高いため静脈内投与での投与速度を遅くする必要があることから初期治療とし

ての有効性が低く、ベンゾジアゼピン系薬剤との併用が有用であると考えられていることを説明した。以上を踏まえ申請者は、てんかん重積状態の治療において、本剤は患者の治療状況に応じて第２又は第３選択薬の位置付けとなると考えられることを説明した。

機構は、以上について了承し、本剤は本邦における新生児けいれん及びてんかん重積状態の治療において、貴重な選択肢を与えるものと考える。

Ⅲ．機構による承認申請書に添付すべき資料に係る適合性調査結果及び機構の判断

１．適合性書面調査結果に対する機構の判断

薬事法の規定に基づき承認申請書に添付すべき資料に対して書面による調査を実施し、その結果、特に問題は認められなかったことから、提出された資料に基づき審査を行うことについては支障のないものと判断した。

２．GCP 実地調査結果に対する機構の判断

薬事法の規定に基づき承認申請書に添付すべき資料（5.3.5.2-1: JNR-NP）に対して GCP 実地調査を実施した。その結果、一部の治験実施医療機関において実施医療機関の長が受け取ったモニタリング報告書及び監査報告書について治験審査委員会の意見を聴いていなかったこと、治験実施計画書からの逸脱（投与量の体重換算の誤り）が認められたが、特に重大な問題は認められず、提出された承認申請資料に基づき審査を行うことについては支障のないものと機構は判断した。

Ⅳ．総合評価

提出された資料から、新生児けいれん及びてんかん重積状態に対し、本剤を使用した際の有効性及び安全性は示されていると判断する。なお、患者背景、維持投与における本剤の投与期間及び本剤の投与速度などが本剤の有効性及び安全性等に及ぼす影響については、製造販売後調査において引き続き検討する必要があると考える。

専門協議での検討を踏まえて、特に問題がないと判断できる場合には、本剤を承認して差し支えないと考える。

平成20年8月1日作成

専門協議では，機構の考え方は支持された。専門協議における検討を踏まえ、医薬品医療機器総合機構（機構）で以下の点について追加で検討し，必要な対応を行った。なお、本専門協議の専門委員からは、本申請品目について、平成19年5月8日付「医薬品医療機器総合機構専門委員の利益相反問題への当面の対応について」1及び2（1）各項に該当しない旨の申し出がなされている。

（1）製造販売後調査について

機構は，製造販売後に新生児けいれん及びてんかん重積状態を対象とした使用成績調査を実施し、本剤の投与状況（投与量、投与速度）と安全性及び有効性との関係、血中濃度と安全性及び有効性との関係、脳波と本剤の有効性との関係、本剤投与による重篤な皮膚障害の発現状況等について調査するとともに、新生児けいれんにおいては、出生体重と安全性及び有効性との関係、てんかん重積状態においては，てんかん治療に用いられていた薬剤，てんかん重積状態における前治療薬と安全性及び有効性との関係について検討するよう申請者に求めた。

申請者は了承し、上記内容について新生児けいれんについては登録された施設における本剤を使用した全症例を対象に製造販売後調査を実施すること，てんかん重積状態については、成人200例及び小児200例の計400例を対象に製造販売後調査を実施することを説明し、調査項目、調査票等について上記内容を検討できるよう配慮し作成することを説明した。

機構は、上記の使用成績調査を速やかに実施し、得られた結果について、適切に臨床現場に情報提供する必要があると考える。

なお、その他の点として、本剤の投与速度については、医師主導治験では初回投与時に5～10分かけて投与することと設定されていたが、実際には2～8分かけて投与され（5分未満で投与された症例は2分投与の1例のみ）、投与時間が2～6分の症例で呼吸抑制又は酸素飽和度低下の有害事象が認められていることから、用法・用量に関連する使用上の注意の項で，「新生児では5～10分かけて緩徐に投与すること。ただし，患者の状態に応じ，より緩徐に投与することも考慮すること」と記載し、注意喚起することが適切と判断した。

以上の審査を踏まえ、機構は、効能・効果及び用法・用量を以下のように整備した上で、本剤を承認して差し支えないと判断する。本剤の再審査期間は、新生児けいれんについては希少疾病用医薬品であることから10年、てんかん重積状態については新投与経路医薬品であり、新規に開発された製剤であることを考慮して6年を設定することが適切と判断する。また、原体及び製剤は劇薬に該当し、生物由来製品及び特定生物由来製品のいずれにも該当しないと判断する。

［効能・効果］　新生児けいれん、てんかん重積状態

［用法・用量］　新生児けいれん

初回投与：フェノバルビタールとして、20 mg/kg を静脈内投与する。けいれんがコントロールできない場合は、患者の状態に応じ、初回投与量を超えない範囲で用量を調節し、静脈内に追加投与する。

維持投与：フェノバルビタールとして、2.5～5 mg/kg を 1 日 1 回静脈内投与する。

てんかん重積状態

フェノバルビタールとして、15～20 mg/kg を 1 日 1 回静脈内投与する。

Polymorphism in Pharmaceutical Solids

edited by
Harry G. Brittain
Discovery Laboratories, Inc.
Milford, New Jersey



MARCEL DEKKER, INC. NEW YORK • BASEL

ISBN: 0-8247-0237-9

This book is printed on acid-free paper.

Headquarters
Marcel Dekker, Inc.
270 Madison Avenue, New York, NY 10016
tel: 212-696-9000; fax: 212-685-4540

Eastern Hemisphere Distribution
Marcel Dekker AG
Hutgasse 4, Postfach 812, CH-4001 Basel, Switzerland
tel: 44-61-261-8482; fax: 44-61-261-8896

World Wide Web
http://www.dekker.com

The publisher offers discounts on this book when ordered in bulk quantities. For more information, write to Special Sales/Professional Marketing at the headquarters address above.

Copyright © 1999 by Marcel Dekker, Inc. All Rights Reserved.

Neither this book nor any part may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, microfilming, and recording, or by any information storage and retrieval system, without permission in writing from the publisher.

Current printing (last digit):
10 9 8 7 6 5 4

PRINTED IN THE UNITED STATES OF AMERICA

Pref

Since
mole
prop
meth
the
extr
phe
othe
ver
pou
sta
co
cry
co
he
ph
de

Table 5 Continued

Compound	Method used	Reference
Digoxin	Milling in a Glen Creston Model M270 ball mill for 8 hours	[110]
	Comminution of 1 g at 196°C for 15 minutes in a freezer mill	[111]
Amobarbital	Ball-milling with methylcellulose, microcrystalline cellulose, or dextran 2000	[112,113]
Acetaminophen	Ball milling for 24 hours with α- and β-cyclodextrin	[114]
6-Methylenandrosta-1, 4-diene-3,17-dione	Co-grinding with β-cyclodextrin for 2 hours	[115]

C. Spray-Drying

In the pharmaceutical industry, spray-drying is used to dry heat-sensitive pharmaceuticals, to change the physical form of materials for use in tablet and capsule manufacture, and to encapsulate solid and liquid particles. This methodology is also used extensively in the processing of foods [116]. In the spray-drying process, a liquid feed stream is first atomized for maximal air spray contact. The particles are then dried in the airstream in seconds owing to the high surface area in contact with the drying gas. Spray-drying can produce spherical particles that have good flow properties, and the process can be optimized to produce particles of a range of sizes required by the particular application. The process can be run using either aqueous or nonaqueous solutions. Examples of pharmaceuticals obtained in the form of amorphous powders by spray-drying are found in Table 6.

D. Lyophilization

Lyophilization (also known as freeze-drying) is a technique that is widely employed for the preparation of dry powders to be reconstituted at the time of administration. It is a particularly useful technique in the

Table 6 Amorphous Pharmaceuticals Obtained by Spray-Drying

Compound	Method used	Reference
YM022	Spray-drying a methanol solution	[117]
α-Lactose monohydrate	Spray-drying in a Buchi 190	[118]
	Spray-drying a solution or suspension	[119]
4″-O-(4-methoxy-phenyl) acetyltylosin	Spray drying a dichloromethane solution	[120]
Salbutamol sulfate	Spray-drying of an aqueous solution in Buchi 90 spray dryer	[121]
Lactose	Spray-drying an aqueous solution	[118,122]
Furosemide	Spray-drying from a 4:1 chloroform: methanol solution at 50 and 150°C inlet temperature	[123,124]
Digoxin	Spray-drying an aqueous solution containing hydroxypropyl methylcellulose	[125]
Cefazolin sodium	Spray-drying from a 25% aqueous solution with an inlet temperature of 150°C and an outlet temperature of 100°C	[126]
9,3″-Diacetyl-midecamycin	Spray-drying of aqueous solution in the presence and absence of ethylcellulose	[127]

case of compounds that are susceptible to decomposition in the presence of moisture but that are more stable as dry solids. The physical form, chemical stability, and dissolution characteristics of lyophilized products can be influenced by the conditions of the freeze-drying cycle. In most pharmaceutical applications, lyophilization is performed on aqueous solutions containing bulking agents, and these often are chosen so as to form a coherent cake after completion of the freeze-drying process. However, lyophilization also can be employed to convert crystalline materials into their amorphous counterparts. The lyophilization process usually consists of the three stages of freezing, primary drying,

and secondary drying. For the preparation of amorphous materials, rapid freezing is employed so as to avoid the crystallization process. Both aqueous solutions and solutions containing organic solvents have been lyophilized. The primary drying phase involves sublimation of frozen water or vaporization of another solvent. This step is carried out by reducing the pressure in the chamber and supplying heat to the product. The secondary drying phase consists of the desorption of moisture (or residual solvent) from the solid.

Recently, excipients of various types have been employed in frozen solutions so as to inhibit crystallization. Cyclodextrins appear to be particularly useful for this purpose, although it is generally necessary to employ rapid freezing to liquid nitrogen temperatures to ensure that the freeze-dried product is noncrystalline. When α-cyclodextrin, which has a larger cavity than does β-cyclodextrin, is frozen at a relatively slow rate, it will cocrystallize with compounds such as benzoic acid, salicylic acid, *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, and methyl *p*-hydroxybenzoate [128]. However, rapid freezing of a methyl *p*-hydroxybenzoate solution containing α-cyclodextrin at a benzoate/cyclodextrin ratio of 0.33 yields an amorphous solid after freeze-drying [29].

β-Cyclodextrin and its derivatives have been shown to form amorphous lyophilized products with a number of compounds, principally nonsteroidal antiinflammatory agents. Examples from the literature of excipients and pharmaceuticals prepared as amorphous materials by lyophilization are given in Table 7.

E. Removal of Solvent from a Solvate or Hydrate

Solids can sometimes be rendered amorphous by the simple expedient of allowing solvent molecules of crystallization to evaporate at modest temperatures. If the solvent merely occupies channels in the crystal structure, the structure often remains intact, but when the solvent is strongly bonded to molecules of the host, the structure frequently will collapse when the solvent is removed and one obtains an amorphous powder. A few examples of amorphous solids obtained in this manner are found in Table 8.

Phenobarbital Induction of α_1-Acid Glycoprotein in Primary Rat Hepatocyte Cultures

THIERRY FOURNIER,[1] NAJET MEJDOUBI,[1] DAGUI MONNET,[2] GENEVIÈVE DURAND[1] AND DOMINIQUE PORQUET[1]*

[1]*General Biochemistry Laboratory, Pharmacy Faculty, University of South Paris, 92290 Châtenay-Malabry, France; and* [2]*Biochemistry Laboratory, Pharmacy Faculty, University of Abidjan, BP V 34 Abidjan, Ivory Coast*

The serum level of rat α_1-acid glycoprotein is significantly increased by treatment with phenobarbital, and *in vivo* studies have shown that phenobarbital seems to act mainly at the transcriptional level. To show the direct mediating effect of phenobarbital on α_1-acid glycoprotein gene expression, we investigated the ability of primary cultured rat hepatocytes to respond to *in vitro* phenobarbital administration. Phenobarbital increased both α_1-acid glycoprotein secretion and corresponding mRNA levels in primary rat hepatocytes cultured on matrigel. Used in combination with interleukin-1, interleukin-6 and dexamethasone, phenobarbital had an additive or synergistic effect on α_1-acid glycoprotein synthesis. These results show that (a) phenobarbital acts directly on hepatocytes by increasing α_1-acid glycoprotein gene expression and (b) this effect is mediated by a specific mechanism independent of pathways involved in α_1-acid glycoprotein induction by interleukin-1, interleukin-6 and glucocorticoids. (HEPATOLOGY 1994;20:1584-1588.)

Hepatic production of AGP is strongly increased after systemic tissue injury (1, 2). The induction of this response is mediated mainly by soluble cytokines interacting with specific receptors on hepatocytes (3-5). A major role is played by IL-1 and tumor necrosis factor-α, and IL-6 acts synergistically in the induction of AGP (6-11). However, maximal expression of AGP requires the presence of glucocorticoids (12-14) but this does not rule out additional effects of other cytokines, including leukemia inhibitory factor (15, 16), IL-11 (17) and oncostatin M (18). Recently we showed that phenobarbital, a strong inducer of liver drug-metabolizing enzymes, increases AGP gene expression *in vivo*. This effect seems to occur mainly at the transcriptional level (19). In the last few years, major progress has been made in the search for a suitable experimental tissue culture system to show a direct effect of mediators on liver cells. This direct effect on hepatocytes *in vitro* was considered the defining criterion to ascertain the role of a mediator in the control of gene expression (4). Moreover, such *in vitro* studies permit assessment of the relative effectiveness of several mediators and their interactions in determining the level of expression of a given gene (4).

The aim of this study was to examine the direct effect of phenobarbital *in vitro* on AGP production and gene expression. Because *in vivo* induction of AGP synthesis seems to require several administrations of phenobarbital (19), we chose primary cultures of rat hepatocytes on matrigel, which persist for several days, as the experimental system. The results presented here show a direct stimulating action of phenobarbital on AGP gene expression. Additive and synergistic effects of IL-1, IL-6 and dexamethasone in phenobarbital-mediated activation were also observed.

MATERIALS AND METHODS

Preparation of Hepatocytes and Primary Cultures. Hepatocytes were prepared from male 9- to 11-wk-old Dark Agouti rats (19). All animals were anesthetized before liver perfusion and received humane care. Matrigel (20) is an extract of the extracellular matrix produced by EHS transplantable mouse sarcoma (21). The EHS gel was applied as a thin layer (approximately 1 mg/60 mm plate) and allowed to solidify at 37° C for 1 hr. Cells (1.5×10^6) were plated in 3 ml of William's E culture medium, 2 mmol/L L-glutamine, 10 mmol/L sodium pyruvate, 315 mmol/L insulin, 100 U/ml penicillin, 100 μg/ml streptomycin and 250 μg/ml fungizone. After a 3-hr adhesion period, cultures were maintained at 37° C in an atmosphere of 5% CO_2 in air, and the culture medium was changed daily. Chemicals were added to the culture medium just after adhesion and daily for 4 days.

Chemicals. Lyophilized sodium phenobarbital (Gardenal for subcutaneous or intramuscular injection; Specia, Paris, France), recombinant human IL-1β (kindly provided by Rhône Poulenc, Alfortville, France), recombinant human IL-6 (Genzyme, Cambridge MA) and dexamethasone (Soludecadron; Merck Sharp & Dohme Chibret, Paris, France) were all dissolved or diluted in William's E medium and added to primary cultures.

Quantification of Protein Secretion. Amounts of AGP secreted by hepatocytes in primary culture were measured in

Received March 29, 1994; accepted June 16, 1994.

Other abbreviations used in the text: AGP, α_1-acid glycoprotein; EHS, Engelbreth-Holm-Swarm; GST, glutathione-S-transferase; IL-1, interleukin-1; IL-6, interleukin-6; SSC, standard sabre citrate.

This work was supported by INSERM external research contract 91 0302 and by Fondation pour la Recherche Médicale.

Address reprint requests to: Dr. D. Porquet, Laboratoire de Biochimie Générale, Tour D4, 1er étage, UFR des Sciences Pharmaceutiques et Biologiques, 5 rue JB Clément, 92296 Châtenay-Malabry Cédex, France.

Copyright © 1994 by the American Association for the Study of Liver Diseases.

0270-9139/94 $3.00 + 0 31/1/59575

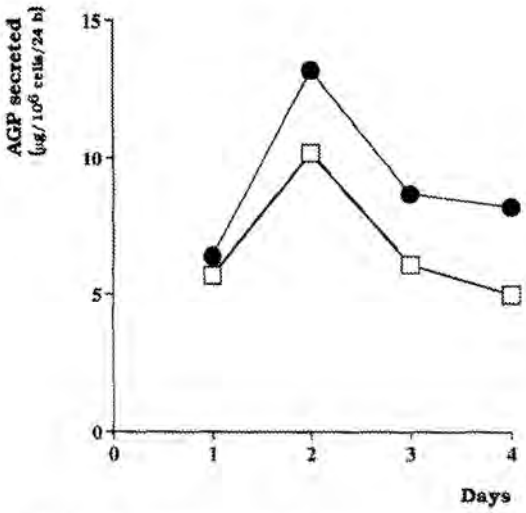


FIG. 1. Effect of phenobarbital on AGP secretion by hepatocytes in primary culture. Phenobarbital (1 mmol/L) was added daily after an adhesion period of 4 hr on matrigel. Aliquots of supernatant were taken each day, and AGP synthesis was measured by means of EIA. Values represent the mean ± SD of triplicate samples in one experiment representative of five. □, control; ●, phenobarbital treatment. $p < 0.05$.

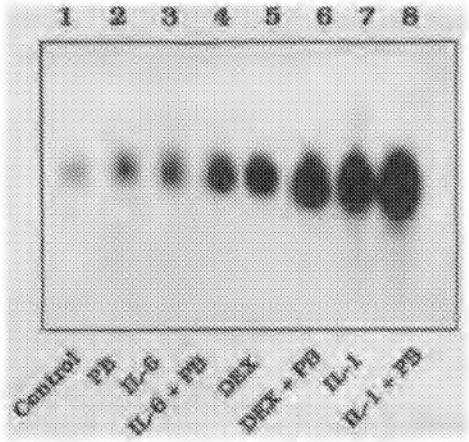


FIG. 2. Effects of phenobarbital, IL-6, dexamethasone and IL-1 alone and in combination on AGP mRNA levels. Four hours after isolation, hepatocytes were treated daily (three plates/treatment) with 1 mmol/L PB, 100 U/ml IL-1, 100 U/ml IL-6 or 10^{-6} mol/L dexamethasone or combinations of IL-6 + phenobarbital, dexamethasone + phenobarbital or IL-1 + phenobarbital. Total RNA was extracted 84 hr later, and AGPm RNA was analyzed by means of Northern blotting.

the supernatants by means of sandwich-type immunoenzymatic methods (22). Purified AGP and antiserum were prepared in our laboratory. Results are expressed as micrograms of AGP secreted per 10^6 hepatocytes per 24 hr or as variations relative to control values on day 4.

Analysis and Quantification of Liver mRNA. Total cellular RNA was extracted from a pellet of hepatocytes with 200 µl RNAzol B (Bioprobe Systems, Paris, France)/10^6 cells. Heat-denatured RNA samples were subjected to electrophoresis on 1.2% agarose gels as described previously (19, 23) and blotted onto Hybond N$^+$ membranes (Amersham, Les Ulis, France) by means of Northern capillary transfer for 3 hr with 0.05 mol/L NaOH. After transfer, the filters were rinsed in 3× SSC and prehybridized (19). A recombinant plasmid containing an insert encoding AGP mRNA was obtained from Taylor (24) and labeled by means of random priming with [α^{32}P]dATP and [α^{32}P]dCTP. The specific activity of the plasmid DNA was about 5×10^8 cpm/µg, and hybridization proceeded for 20 hr at 42° C. Filters were washed at room temperature with 2× SSC, 0.1% SDS and then at 65° C with 0.1× SSC, 0.1% SDS. Autoradiographs were scanned by means of photometric densitometry (Sebia France, Paris, France), and results are expressed as variations relative to control values. Hybridization of membranes with a control probe (recombinant plasmid containing a 1,269-bp insert encoding rat GAPDH mRNA, a gift of P. Jeanteur, CNRS URA 1192, Université de Montpellier, Montpellier France) was used to standardize variations in the layering of total RNA.

RESULTS

Primary Cultures and Concentration of Inducers. Preliminary experiments were performed to define primary culture conditions in which the addition of phenobarbital, IL-1, IL-6 and dexamethasone to cultures induced AGP synthesis (data not shown). Hepatocytes were maintained for 4 days on matrigel. In these conditions, albumin secretion was relatively stable at 100 µg/24 hr in 3 ml of culture medium. In the range of concentrations tested (0.1 mmol/L to 8 mmol/L), 1 mmol/L phenobarbital appeared to induce the strongest increase in AGP secretion with no cytotoxic effect. Recombinant human IL-1β (100 U/ml) was chosen because it was nearly as effective as concentrations of 500 and 1,000 U/ml, whereas 100 U/ml recombinant human IL-6 and 1 µmol/L dexamethasone yielded the maximal effect in our conditions of culture.

Synthesis of AGP by Hepatocytes. Figure 1 shows the phenobarbital-induced effect on AGP secretion during 4-day primary hepatocyte culture. The rate of AGP secretion was maximal on day 4, with a 1.6-fold increase relative to control cultures (8.2 vs. 5 µg/10^6 cells/24 hr). The regulation of AGP is characterized by additional and synergistic actions by various combinations of IL-1, IL-6 and dexamethasone. Although it has previously been shown in rat primary hepatocyte cultures that the presence of dexamethasone is necessary for induction of AGP by IL-1 and IL-6, dexamethasone was not necessary for induction by cytokines in matrigel cultures; phenobarbital, IL-1, IL-6 and dexamethasone were thus tested alone and in combination (Table 1). The potential for AGP induction by the test compounds can be classified in the following order: IL-6 < PB < IL-1 < dexamethasone (lanes 2 to 5). Additive or synergistic effects between IL-1, IL-6 and dexameth-

TABLE 1. Responses of rat primary hepatocytes to phenobarbital, IL-1, IL-6 and dexamethasone

Lane	Treatment	Group A	Group B
1	Control	5.3	1
2	PB	8.3*	1.6
3	IL-1	32.5	6.1
4	IL-6	6.4	1.2
5	DEX	43.0	8.1
6	PB + IL-1	39.4*	7.4
7	PB + IL-6	9.1*	1.7
8	PB + DEX	47.9*	9
9	IL-1 + DEX	68.4	12.9
10	IL-6 + DEX	45.4	8.6
11	IL-1 + IL-6	34.0	6.4
12	PB + DEX + IL-1	87.5	16.5
13	PB + DEX + IL-6	54.1	10.2
14	IL-1 + IL-6 + DEX	91.3	17.2
15	PB + IL-1 + IL-6	42.0	7.9
16	PB + DEX + IL-1 + IL-6	108.7	20.5

Group A: primary rat hepatocyte cultures on matrigel were treated for 96 hr in William's E culture medium and stimulated daily with the indicated inducers and fresh medium (1 mmol/L phenobarbital, 1 mmol/L dexamethasone, 100 U/ml IL-1, 100 U/ml IL-6). Supernatants were collected after 72 to 96 hr of incubation. Group B: AGP contents were determined by means of EIA. The values represent the mean of three to six separate cultures. Results were expressed as μg/24 hr/10^6 cells (A) or as variations relative to control values (B). Student's paired *t* test was used to compare phenobarbital and control, phenobarbital + dexamethasone vs. dexamethasone, phenobarbital + IL-1 vs. IL-1 and phenobarbital + IL-6 vs. IL-6 (*$p < 0.05$).

asone (lanes 9 to 11) were observed, as reported in mouse primary hepatocytes (11) and HepG2 cells (13). Phenobarbital acted in an additive fashion with dexamethasone (compare lanes 2 and 5 with lane 8), IL-6 (compare lanes 2 and 4 with lane 7) and IL-1 (compare lanes 2 and 3 with lane 6).

These results are in keeping with a direct effect of phenobarbital on AGP induction, independent from those mediated by cytokines and dexamethasone. However, some hepatocyte cell lines produce IL-6, which can act in an autocrine manner (25, 26), suggesting that phenobarbital may act by stimulating the expression of IL-6 in our cultures. However, Gauldie et al. (27) did not detect IL-6 mRNA on Northern-blot analysis or with the polymerase chain reaction in primary rat hepatocytes in the presence of dexamethasone, which inhibits IL-6 expression.

In the same experiments, we tested combinations of three of the four inducers (lanes 12 through 16). Synergistic effects were observed in every case. In combinations including dexamethasone (i.e., when endogenous IL-6 production was blocked), phenobarbital still increased AGP secretion (compare lanes 12 and 9 with lane 2; lanes 13 and 10 with lane 2; and lanes 16 and 14 with lane 2). These results strongly suggest that endogenous IL-6, if produced, did not play a role in the phenobarbital effect on AGP induction.

AGP mRNA Levels. Rat hepatocyte AGP mRNA levels were examined specifically by means of hybridization of 15 μg of total cellular RNA with the corresponding DNA probe. As shown in Figure 2, phenobarbital, IL-1, IL-6 and dexamethasone alone and combinations of phenobarbital with each of the other inducers *(slots 2 to 8)* induced increases in AGP mRNA at day 3.5 compared with the control *(slot 1)*. As shown in Table 2, dexamethasone induced a 7.1-fold increase in the amount of AGP mRNA. The 9.4-fold and 1.6-fold increases obtained with IL-1 and IL-6, respectively, at day 3.5 corresponded to the protein augmentation observed 12 hr later and were in agreement with the lag time between the appearance of the mRNA and the protein. Treatment with phenobarbital alone resulted in a 2.6-fold increase in the amount of AGP mRNA. When combinations of phenobarbital and one of the three factors were used, AGP levels increased more strongly than with the factor used alone (phenobarbital + IL-1, 12.6-fold; PB + IL-6, 3.7-fold; PB + dexamethasone, 9.1-fold). Under the same conditions used for protein synthesis, combination of three inducers evidenced synergistic effects; when dexamethasone was added, phenobarbital still enhanced AGP synthesis, showing the nonintervention of endogenous IL-6 in the phenobarbital-mediated effects on AGP expression.

DISCUSSION

A major impediment to cellular and molecular studies of phenobarbital-elicited responses is the absence of a suitable culture system (28). Recently, a significant increase in the responsiveness of primary rat hepatocytes to phenobarbital was observed with cells cultured in William's E medium on plates coated with matrigel (29). Using this model, we found that phenobarbital induced AGP gene expression, as measured on the basis of specific mRNA accumulation and protein secretion. These findings are in keeping with our previous work showing the inducing effect of phenobarbital treatment of rats on AGP mRNA levels and gene transcription (19). Moreover, they suggest that phenobarbital acts directly on liver cells, as do other regulators of AGP gene expression (dexamethasone, IL-1 and IL-6).

The modulation of AGP gene expression by phenobarbital *in vitro* was obtained with the same concentrations used in similar models to induce drug- and steroid-metabolizing enzymes, mainly cytochrome P-450 II B (30-32) and GST (33). However, whereas transcriptional activation of the cytochrome P-450 IIB gene was detected only a few hours after a single administration of PB, induction of GST required several days of regular phenobarbital treatment. These kinetic differences could be explained as follows: in the case of cytochrome P-450 IIB, phenobarbital seems to act essentially at the transcriptional level (34-37), whereas in GST induction posttranscriptional mechanisms predominate (38). With regard to AGP modulation, direct transcriptional activation by PB seems to predominate (19), and the lag time before increased gene expression could be the consequence of a more complex pathway involving several intermediate steps.

Of interest are the *in vitro* effects of dexamethasone, IL-6, IL-1 and combinations of these inducers. The induction of AGP by phenobarbital was stronger than that by IL-6 but weaker than that by IL-1 and dexamethasone. Moreover, when IL-1, IL-6, dexamethasone and phenobarbital were used in combination, the increased AGP secretion and mRNA levels displayed at least additive effects, particularly with the combinations of two inducers, and synergistic effects with the combination of three or four inducers. It is now known that inducible effects of IL-1, IL-6 and dexamethasone on the AGP gene occur respectively through specific glucocorticoid-responsive element and cytokine-responsive element *cis*-acting sequences and that a combination of the two generates an additive and in some instances synergistic interaction (39, 40). However, the precise nature of this interaction has not yet been elucidated, and it could thus occur at the transcriptional or posttranscriptional level (41). In our study, protein secretion changed parallel to the mRNA concentration with all the combinations tested. These results strongly suggest that the interactions between IL-1, IL-6, dexamethasone and phenobarbital occur at a pretranslational level and that phenobarbital may act on the AGP gene in a manner similar to that with IL-1, IL-6 and dexamethasone (i.e. at the transcriptional level through a specific *cis*-acting element). Although a PB receptor protein has not yet been identified, in the 5′ flanking DNA sequences of rat cytochrome P-450 II B1 and cytochrome P-450 II B2 genes, a 17-bp sequence corresponding to a barbiturate-responsive element has been found to interact with a phenobarbital-regulated activator protein (42). The question remains of whether PB acts in the same way on AGP regulation gene expression, either directly as a transacting factor or by way of a complex sequence of activation.

In conclusion, the results presented here show that (a) phenobarbital acts directly on hepatocytes by inducing an increase in AGP gene expression; (b) this effect is additive with that of IL-1, IL-6 and glucocorticoids, the principal mediators of AGP gene expression, suggesting a specific pathway of AGP regulation by phenobarbital involving a specific *cis*-acting sequence in the promoter region of the AGP gene; and (c) endogenous secretion of IL-6 did not play a role in the PB effect on AGP induction in our culture conditions. The culture system presented here should facilitate transfection studies with deletion derivatives of the AGP promoter to identify the putative specific *cis*-acting sequence involved in phenobarbital-mediated effects.

TABLE 2. Effects of phenobarbital, IL-1, IL-6 and dexamethasone on the expression of AGP mRNA in rat primary hepatocytes

Treatment	Variation relative to control values
Control	1
PB	2.6
IL-1	9.4
IL-6	1.6
DEX	7.1
PB + IL-1	12.6
PB + IL-6	3.7
PB + DEX	9.1
IL-1 + DEX	13.5
IL-6 + DEX	9.8
IL-1 + IL-6	10.2
PB + DEX + IL-1	19
PB + DEX + IL-6	12.4
IL-1 + IL-6 + DEX	17.4
PB + IL-1 + IL-6	13
PB + DEX + IL-1 + IL-6	21

Four hours after isolation, hepatocytes were treated daily (three plates/treatment) with 1 mmol/L phenobarbital, 100 U/ml IL-1, 100 U/ml IL-6 or 10^{-6} mol/L dexamethasone. Total RNA was extracted 84 hr later, and AGP mRNA was analyzed by means of Northern blotting. Autoradiographs were scanned using films that were not overexposed, and results were expressed after standardization of variations in the layering of total RNA by means of a GAPDH control probe, as variations relative to control values for 15 μg total cellular RNA.

Acknowledgments: We thank Mrs. H. Kleinman for providing the EHS transplantable mouse sarcoma and B. Clement (INSERM U 49, Rennes, France) for helpful discussion.

REFERENCES

1. Kushner I. The phenomenon of the acute-phase response. Ann N Y Acad Sci 1982;389:39-48.
2. Kushner I, Mackiewicz A. Acute-phase proteins as disease markers. Dis Markers 1987;5:1-11.
3. Baumann H. Hepatic acute phase reaction in vivo and in vitro. In Vitro 1989;25:115-126.
4. Baumann H, Prowse KR, Marinkovic S, Won KA. Jahreis GP. Stimulation of hepatic acute phase response by cytokines and glucocorticoids. Ann N Y Acad Sci 1989;557:280-295.
5. Baumann H, Gauldie J. Regulation of hepatic acute-phase plasma protein genes by hepatocyte stimulating factors and other mediators of inflammation. Mol Biol Clin 1990;7:147-159.
6. Gauldie J, Richards C, Harnish D, Lansdorp P, Baumann H. Interferon β2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. Proc Natl Acad Sci U S A 1987;84:7251-7255.
7. Gauldie J, Richards C, Northemann W, Fey G, Baumann H. IFN beta2/IL-6 is the monocyte-derived HSF that regulates receptor-

specific acute phase gene regulation in hepatocytes. Ann N Y Acad Sci 1989;557:46-59.
8. Heinrich P, Castell JV, Andus T. Interleukin-6 and the acute phase response. Biochem J 1990;265:621-636.
9. Gauldie J, Richards CD, Harnish D, Baumann H. In: Monokines and other non-lymphocytic cytokines. Pavanda M, Oppenheim JJ, Kluger MJ, Dinarello CA, eds. New York: Alan R. Liss, Inc., 1988:15-21.
10. Andus T, Geiger T, Hirano T, Kishimoto T, Heinrich PC. Action of recombinant human interleukin 6, interleukin 1β and tumor necrosis factor alpha on the mRNA induction of acute-phase proteins. Eur J Immunol 1988;18:739-746.
11. Prowse KR, Baumann H. Interleukin-1 and interleukin-6 stimulate acute-phase protein production in primary mouse hepatocytes. J Leukoc Biol 1989;45:55-61.
12. Vannice JL, Taylor JM, Ringold MR. Glucocorticoid-mediated induction of α1-acid glycoprotein: evidence for hormone-regulated RNA processing. Proc Natl Acad Sci U S A 1984;81:4241-4245.
13. Baumann H, Richards C, Gauldie J. Interaction among hepatocyte-stimulating factors, interleukin-1 and glucocorticoids for regulation of acute-phase plasma proteins in human hepatoma (HepG2) cells. J Immunol 1987;139:4122-4128.
14. Kulkarni AB, Reinke R, Feigelson P. Acute phase mediators and glucocorticoids elevate α1-acid glycoprotein gene transcription. J Biol Chem 1985;260:15386-15389.
15. Baumann H, Won KA, Jahreis GP. Human hepatocyte-stimulating factor III and interleukin-6 are structurally and immunologically distinct but regulate the production of the same acute phase plasma proteins. J Biol Chem 1989;264:8046-8051.
16. Baumann H, Wong GG. Hepatocyte-stimulating factor III shares structural and function identity with leukemia inhibitory factor. J Immunol 1989;143:1163-1167.
17. Baumann H, Schendel P. Interleukin-11 regulates the hepatic expression of the same plasma protein genes as interleukin-6. J Biol Chem 1991;266:20424-20427.
18. Richards CD, Brown TJ, Shoyab M, Baumann H, Gauldie J. Recombinant oncostatin M stimulates the production of acute phase proteins in HepG2 cells and rat primary hepatocytes in vitro. J Immunol 1992;148:1731-1736.
19. Bertaux O, Fournier T, Chauvelot-Moachon L, Porquet D, Valencia R, Durand G. Modifications of hepatic α1-acid glycoprotein and albumin gene expression in rats treated with phenobarbital. Eur J Biochem 1992;203:655-661.
20. Bissel DM, Arenson DM, Maher JJ, Roll FJ. Support of cultured hepatocytes by a laminin-rich gel: evidence for a functionally significant subendothelial matrix in normal rat liver. J Clin Invest 1987;79:801-812.
21. Kleinman HK, McGarvey M, Hassel JR, Star VL, Cannon FB, Laurie GW, Martin GR. Basement membrane complexes with biological activity. Biochemistry 1986;25:312-318.
22. Biou D, Daveau M, Rigal O, Hiron M, Porquet D, Lebreton JP. An enzyme-linked immunoassay for the measurement of rat α_1-acid glycoprotein synthesized by cultured hepatocytes. J Immunol Methods 1989;125:1-4.
23. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ. Isolation of biologically active RNA from sources enriched in ribonuclease. Biochemistry 1979;18:5294-5299.
24. Ricca AG, Hamilton RW, McLean JW, Conn A, Kalinyak JE, Taylor JM. Rat alpha 1 acid glycoprotein mRNA: cloning of double-stranded cDNA and kinetics of induction of mRNA levels following acute inflammation. J Biol Chem 1981;256:10362-10368.
25. Northemann WM, Hattori G, Baffet TA, Braciak RG, Fletcher LJ, Abraham J, Gauldie M, et al. Production of interleukin-6 by hepatoma cells. Mol Biol Med 1990;7:273-276.
26. Lotz M, Zuraw BK, Carson DA, Jirik FR. Hepatocytes produce IL-6. Ann N Y Acad Sci 1989;557:509-512.
27. Gauldie J, Northemann W, Fey H. IL-6 functions as an exocrine hormone in inflammation. J Immunol 1990;144:3804-3808.
28. Waxman DJ, Azaroff L. Phenobarbital induction of cytochrome P-450 gene expression. Biochem J 1992;581:577-592.
29. Schuetz EG, Li D, Omiecinski CJ, Muller-Eberhard U, Kleinman HK, Elswick B, Guzelian PS. Regulation of gene expression in adult rat hepatocytes cultured on a basement membrane matrix. J Cell Physiol 1988;134:309-323.
30. Turner NA, Pitot HC. Dependence of the induction of cytochrome P-450 by phenobarbital in primary cultures of adult rat hepatocytes on the composition of the culture medium. Biochem Pharmacol 1989;38:2247-2251.
31. Guzelian PS, Li D, Schuetz EG. Induction of cytochromes P-450b/e by phenobarbital in primary culture of adult rat hepatocytes: test of differentiated liver gene expression. Drug Metab Rev 1989;20:793-809.
32. Hars RG, Mitchel AM, Wolf R, Elcombe CR. Induction of cytochrome P-450 in cultured rat hepatocytes. The heterogeneous localization of specific isoenzymes using immunocytochemistry. Biochem J 1989;262:151-158.
33. Sasaki H, Miyaura S, Horie K, Isono H. Induction of glutathione S-transferase by phenobarbital in rat hepatocyte culture. J Pharmacobio-Dyn 1989;12:775-780.
34. Hardwick JP, Gonzalez FJ, Kasper CB. The effect of phenobarbital on the transcriptional activity of liver. J Biol Chem 1983; 258:8081-8085.
35. Atchison M, Adesnik M. A cytochrome P-450 multigene family: characterization of a gene activated by phenobarbital administration. J Biol Chem 1983;258:11285-11295.
36. Pike SF, Shephard EA, Rabin BR, Philipps I.R. Induction of cytochrome P-450 by phenobarbital is mediated at the level of transcription. Biochem Pharmacol 1985;34:2489-2494.
37. Chianale J, Mulholland L, Traber PG, Gumucio JJ. α_1-Acid glycoprotein concentrations and protein binding of propranolol in Sprague-Dawley and Dark Agouti rat strains treated by phenobarbital. HEPATOLOGY 1988;8:327-331.
38. Francis VNK, Dwarki VJ, Padmanaban G. A comparative study of the regulation of cytochrome P-450 and glutathione transferase gene expression in rat liver. Nucleic Acids Res 1986;14:2497-2510.
39. Won KA, Baumann H. The cytokine response element of the rat α1-acid glycoprotein gene is a complex of several interacting regulatory sequences. Mol Cell Biol 1990;10:3965-3978.
40. Klein ES, Reinke R, Feigelson P, Ringold GM. Glucocorticoid-regulated expression from the 5′-flanking region of the rat alpha-1-acid glycoprotein gene. J Biol Chem 1987;262:520-523.
41. Baumann H, Jahreis GP, Morella KK. Interaction of cytokine- and glucocorticoid-response elements of acute-phase plasma protein genes. J Biol Chem 1990;265:22275-22281.
42. He JS, Fulco AJ. A barbiturate-regulated protein binding to a common sequence in the cytochrome P450 genes of rodents and bacteria. J Biol Chem 1991;266:7864-7869.

FREEZE DRYING AS A METHOD OF PROCESSING SOME PHARMACEUTICAL PRODUCTS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

JOSEPH HERSCHEL KERN, B.S., M.S.

The Ohio State University

1954

Approved by:

Earl P. Lineal

.....

Adviser

ACKNOWLEDGEMENTS

Mere words will not justly express the author's sincere and humble appreciation to the many, who have so willingly and sincerely given their time, advice, suggestions and much needed guidance.

To Dr. Earl P. Guth, Professor, College of Pharmacy, my major adviser, I can only say, "Thank you," your continuous encouragement and guidance during this investigation cannot be measured. I would also like to express my deep appreciation to this great teacher and loyal friend for those intangibles that may not appear in this paper but which he has instilled in me.

To Dr. Bernard V. Christensen, Dean, College of Pharmacy, I am indebted for the teaching appointments which I have held for the past five years. His every readiness to offer suggestions, encouragement and extend assistance has been invaluable.

To Dr. John W. Nelson, Professor, College of Pharmacy, I wish to offer my most sincere appreciation for his many helpful criticisms and suggestions pertaining to the pharmacological phase of this investigation.

To Dr. Frank W. Bope, Associate Professor, College of Pharmacy and Dr. Loyd E. Harris, Professor, College of Pharmacy, I extend my thanks for their valuable advice and

A 49099

suggestions.

To the other members of the faculty of the College of Pharmacy who have extended assistance, I am very grateful.

To my fellow graduate students and friends who have in many ways been a source of encouragement and assistance, I extend my sincere thanks.

I wish also to extend my thanks to the American Foundation for Pharmaceutical Education for granting to me a Teaching Fellowship.

To my wife, Shermania R. Kern, without whose inspiration, encouragement and sacrifice during the past five years this could not have been accomplished, I dedicate this paper.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS i

PART I

INTRODUCTION

CHAPTER I INTRODUCTION 2

CHAPTER II HISTORICAL 5

CHAPTER III FUNDAMENTAL PRINCIPLES OF FREEZE DRYING 19

CHAPTER IV THE FREEZE DRYER AND ITS USE 24

PART II

EXPERIMENTAL

CHAPTER V INFUSIONS OF DIGITALIS 28

Source 28

Moisture Determination 28

Total Ash and Acid Insoluble Ash 29

Preparation of the Infusions . . . 30

Determination of the Amount of Water Soluble Extractive in Infusions Prepared Under Different Conditions 34

Chemical Assay 35

Nitrogen Determination 38

Sugar Determination 44
Toxicity Studies 49
Absorption Studies 52
Determination and Comparison of Potency of Infusion A by U.S.P. XIV Method 57

CHAPTER VI CALCIUM GLUCONATE 59
Lyophilization 59
Solubility Tests 59
Rate of Solubility 61
Moisture Determination and Analysis 61

CHAPTER VII CAFFEINE 63
Lyophilization 63
Solubility Tests 63
Rate of Solubility 64
Moisture Determination 65

CHAPTER VIII BENZOCAINE 66
Lyophilization 66
Solubility Tests 66
Stability of Lyophilized Benzocaine Solutions 71

CHAPTER IX	SULFANILAMIDE.	73
	Lyophilization	73
	Preparation of the Buffers	73
	Solubility Tests	74
	Rate of Solubility	79
	Stability of Lyophilized Sulfanilamide Solutions.	80
CHAPTER X	PHENOBARBITAL.	81
	Lyophilization	81
	Solubility Tests	81
CHAPTER XI	OTHER MEDICINAL CHEMICALS.	83
	Theophylline.	83
	Calcium Lactate	84
	Arsenic Trioxide.	84
	Aspirin.	85
	Caffeine and Citric Acid	85

PART III

CONCLUSION

CHAPTER XII	DISCUSSION.	87
	Infusions of Digitalis.	87
	Calcium Gluconate.	99
	Caffeine.	102
	Benzocaine.	105

Sulfanilamide. 106
Phenobarbital. 107
Other Medicinal Chemicals. 108

CHAPTER XIII SUMMARY AND CONCLUSIONS. 110

BIBLIOGRAPHY. 114

AUTOBIOGRAPHY 120

FREEZE DRYING AS A METHOD OF PROCESSING SOME PHARMACEUTICAL PRODUCTS

PART I

INTRODUCTION

CHAPTER I

INTRODUCTION

Freeze drying, drying by sublimation and lyophilization are all terms used to denote low temperature evaporation of water under vacuum following freezing. The common laboratory expression "freeze drying" well describes the process. Harris (1) indicates that he prefers the term drying by sublimation which has been in use for over fifty years. Lyophilization seems to be of commercial coinage.

Regardless of the term used the method of freeze drying has assumed a role of major importance among the developments of the pharmaceutical industry in recent years. This process made possible the supply of blood plasma and blood serum to the Armed Forces stationed everywhere in the world during the last global conflict.

The stabilization of penicillin is a major contribution of the freeze drying process and the food industry has made very advantageous use of it. Desiccation from the frozen state is now serving as an essential research tool.

Tremendous strides have been taken during the last ten years in the manner of carrying out the process and in

the equipment used. The extent to which these developments in the process have recently made it of usefulness in research may be measured by the major increase in reference to desiccation from the frozen state in the scientific literature (2).

In the past the distribution of biologicals and other perishable forms of medication has not only presented a problem to industry but has involved waste. First, due to deterioration between the time of manufacture and the time of use, and secondly, many such products are totally lost because of having passed their date of expiration before use. The value of a means for reducing this two-fold waste to a minimum is too obvious to require stress (3). Freeze drying has in many respects aided in overcoming some of these heretofor unsolved problems.

Even with the rapid development of freeze drying as indicated by the number of scientific articles, applications of this process of drying is in its infancy. Flosdorf (4) reports that of the references which deal with freeze drying and its applications, fewer than ten occurred before 1930 and over 350 since that date. No patents on the process were issued before 1934. The potential and possibility for its application seem unlimited. Up to now the greatest use for this process has been in connection with biologicals and with certain foodstuffs. Little has been reported in the literature of its application to

galenicals and medicinal chemicals.

Before drying by sublimation the product is frozen; the ice is then sublimed rapidly under high vacuum. With many materials the over all volume of desiccated material corresponds closely to that of the original frozen product. Blood plasma is typical of such products, showing little over all reduction in volume after drying from the frozen state. The advantages of drying materials while in the frozen state are several. Two of the major advantages are; first, the properties of labile substances are unchanged during drying, with consequent prolonged preservation of desired characteristics during the storage period which follows, and second, the solubility of products dried in this way is remarkably fast and complete. As a result the life of commercial labile products can be greatly extended.

CHAPTER II

HISTORICAL

Leslie (5) reported in 1811 an experiment consisting of a chamber containing sulfuric acid in a broad flat pan and vessel containing water. On exhaustion of the chamber by an air pump the water froze through loss of latent heat of vaporization; the ice gradually wasted away by sublimation of water vapor to the sulfuric acid.

The evaporation of water under vacuum at low temperature following rapid freezing is a process so old that Wollaston (6) was apologetic in exhibiting it before the Royal Society of London in 1813.

Neither Leslie nor Wollaston indicated the use of sublimation for drying. It would be difficult if not impossible to trace definite connection between their experiments and developments of the present day application of drying by sublimation.

The first clearly recorded use of sublimation for preserving biological substances after drying by sublimation is that of Shackell (7) in 1909.

Strumae, McGraw and Reichel (8) credit d'Arsonval and Bordas and Vansteenberghe with the principles of drying by sublimation. The subsequent history of development of freeze drying during the present century has been

recorded (3,8), including reference to the work of Shackell (7), Harris and Shackell (9), Hammer (10), Rogers (11), Swift (12), Sawyer (13), and Elser (14).

Desiccation from the frozen state became available by 1935 on a laboratory research scale, for production of human serum and other substances for parenteral use, guinea pig complement and other laboratory reagents and for industrial production. This represented a culmination of the cited work and contributions of many in overcoming the difficulties involved in self regulation of temperature during desiccation in individual containers. Subsequent workers described successful use of chemical desiccants (15) and of direct evacuation (16) of water vapor in procedures for preservation by desiccation in vacuo. Other apparatuses for drying by sublimation have since been described by Folsom (17), Hill and Pfeiffer (18), Strumia, McGraw, and Reichel (8), Taylor and Beard (19), Hays and Koch (20), Dykstra and Dubuy (21), Campbell and Pressman (22), Pomes and Irving (23), Pratt and Yamaguchi (24), and Holzman (25).

Falk, Frankel and McKee (26) in 1919 successfully dried meat, fruit and vegetables at low temperature under vacuum. They reported an analysis of the fresh and dried meat. No mention was made of freezing.

In 1920, Ewe (27) studied the effects of cold and freezing upon magnesia magma and other pharmaceutical

preparations. Ewe found that when magnesia magma is frozen and thawed again, the stable milk like appearance is altered and a clear colorless liquid, with a thick white sediment results. He explained this as being due to the conversion of the normal water content of the magnesium hydroxide gel into ice crystals with the results that the gel condition is disturbed and the molecules of magnesium hydroxide unite to form a fine crystalline powder. This form is practically irreversible. This is cited as an example of incidents that occur that apparently have no bearing on freeze drying, however many of the relevant basic factors of freeze drying are present.

Buchanan and Zook (28) found it difficult to dehydrate apples and pears. However, the product had good appearance after dehydration with a loss of 2.5 to 5.5 per cent of water. They also found a loss in total sugars. The loss of sucrose was corresponding in an increase invert sugar. Invert sugar formation was due to the acid hydrolysis and enzyme action at the favorable temperature conditions. Small amounts of esters lost indicated there was little breaking of the cell structure. They made no mention of freezing before dehydration. Today freezing and drying of this type of product is commonly accepted without unfavorable reactions during the process.

Breedis and Furth (29) reported the feasibility of preserving neoplastic cells in the frozen state. They found that neoplastic cells could be preserved and the virulence of those tested had not altered after one year at -70°C.

The survival of plant cells immersed in liquid air was studied by Luyet and Thoennes (30). They found that to save cells from disintegration three factors must be observed; first, dehydrate, second, cool rapidly, and third, warm rapidly. The more complete the dehydration the more resistant the cells to injury. They suggested that the resistance of gels to crystallization was the same fundamental process as in this case.

In 1941, Hailton, Cunningham and Kirk (31) reported on the preparation of sterile proteins in the lyophiled state and discussed its application.

Francis and Salk (32) reported a simplified procedure involving freeze drying for the concentration and purification of influenza virus.

The nature of type C Botulinus toxin fractions was studied by Coburn (33) and he used freeze drying for concentration. Coburn indicated that the reduction of fraction B to powder dryness by lyophil process results in only partial loss of toxicity as indicated by the inoculation route.

Casals (34) reported in 1943 the use of non-virulent frozen and dried antigens for complement fixation tests with central nervous system virus infections.

A study of crystalline beef liver catalase by Dounce and Howland (35) showed that when crystalline beef liver catalase had been dried by the lyophil process and the properties of the dried preparation compared to those of the undried, the dried material was not crystallizable. It possessed only one-third the activity per dry weight of undried and in contrast to undried material its hematin iron could be reduced by sodium hydrosulfite. They reported that the process of drying or subsequent solution of dried material produces some change in the molecular structure such as mild denaturation. Svihla and Osterman (36) gave another example of the far reaching applications of freeze drying. They reported the growth of orchid seeds after dehydration from the frozen state. In 1945 Nymon, Gunsalus and Gortner (37) published a paper dealing with the application of the lyophil process to the maintenance of cultures for microbiological assay. In the same year Seegers (38) reported methods and apparatus for the drying of proteins from the frozen state.

Another application of freeze drying has to do with frozen dried preparations for the electron microscope. Wyckoff (39) reported that most native proteins

are strongly hydrated whether they are in molecular suspension or form part of an organized biological tissue. When this water is lost during air drying for electron microscopy, it is likely that the elementary particles of many proteins will shrink and distort. Dehydration cannot now be avoided and therefore techniques are needed which will desiccate with a minimum of alteration. Quick freezing and desiccation from the frozen state does this. He describes how to prepare material for electron microscopy. Frozen dried preparations containing appreciable quantities of salt have not yet given useful micrographs, because in its extreme dispersion this salt tends to smear over the fine details that are present.

French, Holt and Powell (40) indicate that freezing and drying through disintegration of chloroplasts or precipitation of ammonium sulfate results in preparations that still retain considerable activity. Ability to produce oxygen is a property not only of intact chloroplasts but also of material which has been exposed to above mentioned conditions.

Bradish (41) studies of freeze drying indicated that many biological fluids and other solutions which deteriorate at high concentrations and elevated temperatures can be dried in the frozen state.

There are empirical observations reported in the literature that cells can be dried without the movement of salt only if they are quickly frozen to produce a glass ice and then if the subsequent dehydration is accomplished at temperature below -55°C. Ordinary lyophilization temperatures are higher than this and cannot be expected to immobilize inorganic ions (42).

A method of drying partial protein hydrolysates and other hygroscopic materials for nutritional studies was devised by Westfall, Miller and Westfall (43). They indicated that drying a protein hydrolysate by lyophil is a procedure often desired as a means of preparing diets for studies of nutritive value of the hydrolysate. The major disadvantages of the lyophil method was a tendency for the hydrolysate to fuse during lyophilization and the hygroscopic nature of the dry material. They overcame this by concentrating the solution in vacuo and adding dextrin to the concentrated solution. Of common dietary carbohydrates only dextrin or starch were found suitable. Sucrose or glucose does not aid in subsequent lyophilization. Dextrin is added to give 30-50 per cent protein in the dry product. It was also found that the same procedure was useful in drying hygroscopic materials, such as liver extract.

The concentration and preservation of urinary

substances by lyophilization was studied by Steinitz and his co-workers (44). They found that drying depended on the size of the sample, the concentration of the sample and the temperature and relative humidity. The advantages of using this process in work with urine they indicated were three-fold. First, the apparatus is simple and of moderate cost, second, the process requires little attention once the initial steps are taken, and third, the concentration of urine is at below zero degrees temperature and unfavorable to chemical alteration.

In 1947, Plank (45), a German worker published a paper which included a discussion covering the characteristics and development of freeze drying, types of vacuum pumps, choices of vacuum and the freeze drying of foodstuffs. From a discussion of the energy requirements and plant problems Plank concluded that while freeze drying is undoubtedly economical for very valuable temperature sensitive materials it is probably too expensive for the food industry.

Strickler and Shaffer (46) reported on the use of the Campbell-Pressman lyophilizing apparatus (22) for urinary extractives. They used this apparatus as means of removing organic solvents from mixtures and for the removal of water from mixtures remaining after enzymatic hydrolysis of small amounts of steroid glucuronides.

Richins (47) used the freezing and drying technique for study of vasomotor activity. After administration of the drug he exposed the intestine by incision and froze a section in a Dewar flask. Then he removed a part of the duodenum and made sections.

Strumia (48) found that the hemolysis of blood by freezing or heating is overcome by freeze drying, following specific procedures and regulated temperature.

Barratt and Tatum (49) showed that lyophilization of microorganisms is essential for their preservation without change of characteristics. They report it to be effective for bacteria, for yeast and fungi.

Freeze drying with radiant energy was studied by Zanezow and Marshall (50). They indicated that frozen material retained on a supporting medium transparent to infrared radiation was dried with radiant energy emitted from electrically heated wires. Drying time curves were obtained for various conditions of cake thickness, initial moisture content and energy concentration.

Chambers and Nelson (51) have shown that freeze drying is applicable to Belladonna leaf, as a method of preservation. They showed that lyophilization reduces the moisture content comparable to oven drying. They also indicated that lyophilization preserves certain constituents better than oven drying at 50°C.

Adams (52), in 1952, reported a new laboratory method for drying wheat gluten. Wet gluten was frozen in liquid nitrogen or dry ice, ground and dried on trays and freeze dried. All drying methods gave gluten superior baking tests to that dried in air by the usual method.

Valenzuela and Feliciano (53) reported what precautions should be exercised during the first stage since they found this to be the critical stage of the process. They indicated that one must be sure that the solution is completely frozen before drying and that the temperature of the drying chamber is not raised to a point that will cause puffing or liquefaction and consequent loss of the product. Several advantages of freeze drying over the ordinary solution method are given. They also indicated that freeze dried products defy oxidation, and that freeze drying halts aging, preserves potency and solves prolonged storage problems. Various other applications of freeze drying in medicine, pharmacy, veterinary science, food industry and other industrial applications are enumerated.

Cosgrove and Guth (54) studied the effects of freeze drying on the glycosidal content of the leaves of Digitalis purpurea L. and Digitalis lutea L. The results indicated that freeze drying is applicable as a means of drying leaves of these two species of digitalis. They reported that the moisture content of the lyophilized

material compared favorably with that found for the corresponding oven dried samples. In regards to the toxicity tests, it was found that no significant differences were present with samples made from either the lyophilized or oven dried material. The chemical assays indicated notable differences in the majority of samples compared.

Cosgrove and Guth (55) further reported a method for preparing lyophilized water soluble extracts of Digitalis purpurea L. and Digitalis lutea L. The extracts which are instantaneously soluble in water, lend themselves readily to the extemporaneous preparation of infusions. Preliminary tests on the extracts stored approximately sixty days indicated that apparently the powders are stable and have potencies comparable with tinctures prepared from corresponding samples when equal concentrations are compared.

In a still later paper, Cosgrove and Guth (56) reported on the sugar and chlorophyll content of alcoholic extracts of oven dried and lyophilized leaves of Digitalis purpurea L. and Digitalis lutea L. They showed that freeze drying preserves more total chlorophyll and Chlorophyll "a" than oven drying and that freeze drying does not have anymore effect than oven drying on the amount of carbohydrate material extracted with alcohol.

Wurster et al, (57) recently reported that steroids can be prepared in the form of submicron particles by rapidly freezing solutions of them and removing the solvent by sublimation from the frozen mass. Data given showed that the average particle size obtained is influenced by the steroid chosen, the concentration of the steroid in the initial solution, the solvent used, the temperature employed in the process, and the presence of certain additives.

It is interesting to note that throughout this brief but encompassing historical survey of the literature regarding freeze drying and its applications that little reference is made to specific medicinals. That is, medicinals in the sense of chemical entities. Several references were made to galenicals and there is often mentioned the application of freeze drying to medicinals and pharmaceuticals. It is not meant to imply that blood plasma and the materials belonging to this group are not medicinals, but there has been no reference in the literature to compounds or medicinal chemicals like calcium gluconate, benzocaine, phenobarbital, caffeine or sulfanilamide.

Valenzuela and Feliciano (53) did mention a lyophilized sodium salt of para amino salicylic acid which was instantly soluble upon addition of the diluent.

They also indicated that freeze drying of this parenteral has to be undertaken at a lower temperature than a lyophilized vitamin B-complex product mentioned, because of the low melting point of the sodium salt of para amino salicylic acid, m.p. 78°C. A temperature not higher than 40°C. was employed in drying the frozen sodium salt of para amino salicylic acid because its thermoplasticity combined with its low surface tension and its low melting point made it easily susceptible to bubbling and subsequent liquefaction. Other applications of freeze drying in the field of medicine and pharmacy are the drying of antibiotics, like amorphous penicillin, streptomycin, hormones, amino acids, bile, enzymes and other biologicals.

While a considerable amount of work has been done on the application of freeze drying to biologicals and several other types of products very little has been published regarding its application to specific medicinal chemicals and like pharmaceuticals.

This study is concerned with the application of freeze drying to Infusions of Digitalis, Calcium Gluconate, Benzocaine, Sulfanilamide, Caffeine, Phenobarbital and several other medicinal chemicals. The purpose of the research reported in this study is three-fold:

1. To study and determine the effects of freeze drying on Infusions of Digitalis with emphasis upon

possible changes in some of its chemical or physical properties and its pharmacology.

2. To study and determine the effects of freeze drying on Calcium Gluconate, Benzocaine, Sulfanilamide, Caffeine, Phenobarbital, and several other medicinal chemicals with emphasis upon possible changes in some of their chemical and physical properties, and,

3. To report any additional information regarding methods of preparation, stability and other implications as to its practical and possible use in the field of pharmaceutical medicinal chemicals.

CHAPTER III

FUNDAMENTAL PRINCIPLES OF FREEZE DRYING

"Freeze drying" is the process of drying a substance or mixture of substances while in the frozen state under high vacuum without passing through the liquid state. Sublimation is the chemical process by which vapor is formed directly from a solid without passing through the liquid state. Freeze drying is therefore, a reverse process of sublimation. In freeze drying, the residue rather than the vapor is the product desired, which is the case in the manufacture of pharmaceutical products like antibiotics, vitamins, blood plasma, and several others. Freeze dried products are termed "lyophilized" (solvent-loving) meaning dehydrated or desiccated from the frozen state at low temperature under vacuo (53).

Whether a substance or mixture of substances will normally sublime or melt and then vaporize depends on the location of the triple point, which is the point of intersection of the sublimation, vaporization and melting point curves and all three phases are in equilibrium. It is evident that vaporization of a solid without prior melting can be accomplished only at pressures or partial pressures below that of the triple point. If the triple point of a substance is above atmospheric pressure, the

substance will normally sublime like "dry ice". But if the triple point is below atmospheric pressure like iodine and most substances which are normally solid, sublimation can only be accomplished by reducing the pressure or the partial pressure below atmospheric pressure by the use of high vacuum in order to reduce the total pressure or by the presence of an inert gas in order to reduce the partial pressure of the substance.

It follows, therefore, that simple sublimation or freeze drying will ordinarily require the use of vacuum in order to reduce the total pressure. An ordinary vaporization or distillation process differs with sublimation in that the solid first melts and then vaporizes.

Greaves (58) points out that control of the supply of latent heat of sublimation is the most important factor in freeze drying. How soon heat can be applied will depend upon the highest safe temperature at which the material can be dried and the rate of heat transfer through the frozen material. If the heat input is too high, there is a tendency to thaw at the point of contact of the frozen material with the heated surface. In other words, the temperature of the product during drying should not be allowed to rise above its liquefying point. Regulated temperatures of hot water circulated through jackets and hollow shelves supply the latent heat of vaporization during freeze drying.

It has long been known that the freezing point of water, or other solvent, is lowered by the addition of a solute, and it has also been pointed out that the depression of the freezing point is directly proportional to the concentration of the solution. At the freezing point, solid and liquid are in equilibrium, and both have the same vapor pressure. If the solid has a greater pressure than the liquid, it will distill over into the liquid phase; if the liquid has the greater pressure, it will change into the solid. When solid and solvent are in equilibrium, and a solute is dissolved in the liquid, the solid solvent will disappear if the temperature remains constant, but this may be stopped and a state of equilibrium established by lowering the temperature. The vapor pressure-temperature curve of a solid is steeper than its corresponding liquid because the heat of sublimation of the solid is greater than the heat of vaporization of the liquid. At the intersection of the two curves, solid and liquid are in equilibrium and the corresponding temperature is the freezing point. The higher the concentration of the solution, the lower is the vapor pressure curve, and the lower is the freezing point of the solution, that is, the temperature at which the vapor curve of the solution intersects the vapor curve of the ice.

For carrying out the freeze drying process, the product to be dried must be frozen. Pre-freezing may be

accomplished by means of external cooling or by vacuum evaporation to induce "self-freezing". Medicinal products in small vials are frozen directly within vacuum chambers by lowering the temperature to -40°C. The shape and position in which the products are frozen in the container are highly important. The layer of frozen material should be kept as thin as possible and the evaporating area as large as possible. In general, a volume of product not in excess of 50 per cent of the size of the bottle should be used. There are four general shapes or positions in which products are frozen; 1) flat freezing, in which the bottles or vials are placed on their sides for freezing, 2) slant freezing in which the bottle is raised slightly at the neck or mouth end to prevent the product from running out of the container before freezing is completed, 3) plug freezing in which the bottles are allowed to stand upright with the material frozen on the bottom, and 4) shell freezing used in cases where any one of the three preceding methods would result in too thick a layer or too small an evaporating surface. This is necessary in cases where a big volume is being frozen per container like blood plasma.

When the freezing process of the solution in their final containers is completed, the materials are ready for the drying process which consists of two stages: first,

ice is evaporated from the frozen mass; and second, moisture is removed from the final dry solid leaving less than 0.5 per cent moisture in the finished product. During the first stage of drying, care should be exercised in applying heat to the drying chambers. Various products require different drying temperatures. Vitamin preparations dry well at temperatures between -5°C. to -10°C., plasma between -20°C. to -25°C., and antibiotics between -25°C. to -32°C. Heat must not be carried to the walls of the container which holds the product faster than the heat can be conducted through the frozen mass to a free surface where it is utilized to induce evaporation. Otherwise, melting adjacent to the container wall will occur, and the product may liquefy, followed by puffing and consequent loss of the contents of the container. After observing the behaviour of different products, heat is gradually raised to 37°C. from 5 to 24 hours, and finally to 48°C., from 10 to 12 hours, in the case of certain vitamins.

This is already the second and final stage of drying where the rate of supplying heat is not so critical because the actual weight of water being evaporated is small. Heat is then reduced to 37°C. for another three hours to allow temperature of the product to cool to room temperature. The vacuum is then released and the material placed in suitable containers or the containers removed are sealed.

CHAPTER IV

THE FREEZE DRYER AND ITS USE

A Stokes Freeze Dryer, Model 2004-L, attached with a Stokes Microvac Model F Pump was used in this study. This is a self-contained and complete unit for freeze drying. The two lower shelves are refrigerated with freon and the two upper shelves are heated with circulating hot water.

In order to carry out the desiccation process, the materials are placed in pyrex glass trays of proper size and shape and frozen in the freezing area of the freeze dryer. Depending on the quantity of material and its depth in the trays, from two to three hours may be required for completing the freezing operation. The temperature should be taken to -20°C. or -30°C. Most of this work was done at temperatures from -35°C. to -40°C. After freezing the materials, the trays are removed and placed on the upper shelves within the same chamber, the door quickly closed and the vacuum pump turned on to exhaust the chamber as quickly as possible to below 2000 microns. This will arrest the tendency to thaw and from then on the pressure will continue downward to around 500 microns and lower. When this pressure is reached, the circulating pump is turned on to carry water through the shelves and rapidly

dry the material. The water vapor during drying is then condensed on the cold plates.

As the processing is carried on, the vacuum may fall off considerably, but as long as the material does not thaw, one need not be concerned about the fall of vacuum. The vacuum may stay at about this point until nearly the end of the drying cycle, when it will again fall finally to the order of 200 microns. This is a partial index of the completion of desiccation.

Both the temperature of the material being desiccated and the pressure in the system are indexes of proper operation during the processing of the material. During the desiccation, the temperature of the material is controlled by the temperature of the water circulated through the cabinet shelves. The temperature of this should be adjusted so that in the early stages of drying the temperature of the material does not rise above the point at which the material will thaw or soften. In general, the temperature of the shelves should be raised as quickly as possible in order to accelerate the process of desiccation, but it should not be raised to the point where the material reaches a temperature of fusion. Near the very end of the process of desiccation, the temperature will rise and it should be maintained at as high a level as possible, without destroying the dried material, in order to insure

completeness of desiccation. Upon completion of the process, the large valve in the vapor line is closed and dry air admitted to the vacuum chamber. After the vacuum has been released, the material is removed from the chamber and stored in suitable containers (59,60).

PART II

EXPERIMENTAL

CHAPTER V

INFUSIONS OF DIGITALIS

Source

Digitalis purpurea L., U.S.P. obtained from a commercial source was used in the preparation of all the infusions studied. The portion of the gross weight of the U.S.P. Digitalis powder which passed a number twenty sieve using a Ro-tap was used throughout this work.

Moisture Determination

The moisture content was determined by the U.S.P. XIV method for drugs containing no volatile principles at 100°C. Ten gram samples of the number twenty Digitalis, U.S.P. powder were placed in the Bradender Semi Automatic Moisture Tester and dried at 65°C. After five hours of drying, the samples were checked for percentage of moisture lost. The drying and checking were continued at one hour intervals until two consecutive readings differed by less than 0.25 per cent.

After twenty-one hours of drying a constant weight representing a loss of 6.4 per cent moisture was recorded.

This exceeds the limits specified for Digitalis, U.S.P. The U.S.P. XIV, states that not more than 6 per cent moisture may be present.

Total Ash and Acid Insoluble Ash

The ash determinations were made according to the U.S.P. XIV method. The results are indicated in Table I and Table II, (page 29).

TABLE I

PERCENTAGE OF TOTAL ASH

Sample	Digitalis, U.S.P.	Water Soluble Extract
	8.30 8.29 9.00	7.40 6.91 6.66
Average	8.53	6.99

TABLE II

PERCENTAGE OF ACID INSOLUBLE ASH

Sample	Digitalis, U.S.P.	Water Soluble Extract
	0.72 0.91 0.68	0.40 0.44 0.61
Average	0.77	0.48

The powdered digitalis samples are within the U.S.P. XIV requirements for Acid Insoluble Ash. The U.S.P. requires that Digitalis yield not more than 5 per cent acid insoluble ash.

Preparation of the Infusions

Infusion A.

Nine hundred milliliters of boiling distilled water were poured upon 15 Gm. of digitalis powder contained in a one-quart, wide-mouth thermos bottle. The thermos was stoppered and allowed to stand for one hour. At the end of one hour the contents of the thermos were filtered and volume made to 1000 ml. The clear amber infusion was placed in previously chilled 13 1/2 x 8 1/2 inch, interior dimension pyrex glass trays. The depth of the solution placed in the pyrex glass trays was controlled at approximately one-fourth inch.

The pyrex glass trays containing the infusion were covered with aluminum foil and placed in the freezing chamber of a Stokes Freeze Dryer at a temperature of -30°C. A temperature of -35°C. was recorded fifteen hours after placing the material in the freezing chamber. The frozen infusion was allowed to remain in the freezing chamber one hour after recording the -35°C. temperature.

The frozen infusion was then transferred to the

drying chamber. After the drying chamber was exhausted which required from 10 to 15 minutes, the vacuum line was closed. In approximately 15 minutes when the pressure registered 300 microns the circulating water pump and heater were turned on. A temperature of 25°C. was maintained throughout for the circulating water by thermostatic control. One hour after the temperature of the drying chamber and the material reached equilibrium at 28°C. and 175 microns pressure the vacuum was broken. The material was then removed and placed in amber colored wide-mouth bottles. The bottles were stored in a desiccator until the material was used.

From the time the material was placed in the drying chamber until the vacuum was broken required nine hours. The lyophilized powder was buff in color and very fine and fluffy. The small amount adhering to the sides of the pyrex glass trays became tacky and darkened upon exposure to air after a very short time.

Infusion B.

Infusion B was prepared like Infusion A with the following modifications. The temperature of the water poured on the digitalis powder contained in a one-quart thermos was 50°C. The time in the freeze chamber was fifteen hours and the final temperature was -36°C. Time required in the drying chamber was twelve hours and the

final temperature was 27°C. at 175 microns pressure. The lyophilized powder was a lighter buff color than observed in Infusion A.

Infusion C.

Infusion C was prepared in the same manner and under the same conditions as Infusion B with the following exceptions. The temperature of the water poured on the digitalis contained in the thermos was 31°C., (room temperature). The conditions of freezing and drying are identical to Infusion B, as both Infusion B and Infusion C were prepared and processed at the same time.

Infusion D.

Infusion D was prepared in the same manner and under the same conditions as Infusion A with the following modifications. Fifty grams of digitalis powder were used instead of 15 Gm. The time in the freeze chamber was twenty-one hours with a final temperature of -44°C. Time required in the drying chamber was twelve hours at a final temperature of 24°C. for the chamber and 27°C. for the material at a pressure of 175 microns.

The color of the infusion although clear was darker than in Infusion A and had a red hue. The lyophilized product was coarse; the surface, which was

clear, had a shiny gum-like appearance.

Infusion E.

Infusion E was prepared in the same manner and under the same conditions as Infusion D with the following modifications. One hundred grams of digitalis powder was used in preparing Infusion E whereas only 50 Gm. was used in preparing Infusion D.

The conditions of freezing and drying are identical as both Infusion D and Infusion E were prepared and processed at the same time. The color of the product of Infusion E was darker than any of the other products from the other infusions. The final product was reddish brown in color. The appearance was as reported in Infusion D.

Infusions A-1, B-1, C-1, D-1, and E-1.

Infusions A-1, B-1, C-1, D-1, and E-1 were prepared like Infusions A, B, C, D, and E respectively, with the exception that they were not lyophilized.

Determination of the Amount of Water Soluble Extractive in Infusions Prepared Under Different Conditions

Two, 300 ml. portions of Infusion A-1 were placed in tared beakers and concentrated nearly to dryness on a steam plate. The beakers containing the partially dried water soluble extract were then transferred to an oven, heated at 120°C. for twelve hours, cooled in a desiccator and weighed.

Two, 200 ml. portions of Infusion B-1 were placed in beakers and concentrated to approximately 50 ml. on a steam plate. The concentrations were transferred to tared evaporating dishes and dried in an oven at 60°C. for twelve hours, cooled in a desiccator and weighed.

The amount of water soluble extractive from Infusions C-1, D-1 and E-1 was determined in the same manner as for Infusion B-1. The results of the determination of the water soluble extractive in infusions prepared under different conditions are indicated in Table III, (page 35).

TABLE III

AMOUNT OF WATER SOLUBLE EXTRACTIVE IN INFUSIONS PREPARED UNDER DIFFERENT CONDITIONS

Grams per 1000 ml.

Infusion	A-1	B-1	C-1	D-1	E-1
	5.180	5.140	5.065	16.610	31.675

Chemical Assay

Total Glycosides.

Total glycosides were determined by using a modified Knudson-Dresbach (61) method. The assay is based on a comparison using a standardized commercial U.S.P. Digitalis Tincture as the standard. Fifty milliliters of the U.S.P. Digitalis Tincture were concentrated on a water bath to 10 ml. and this made up to original volume with distilled water, to eliminate the ethanol.

Twenty milliliters of this aqueous solution were diluted to 100 ml. with distilled water. This was used as the standard solution and represented 2 Gm. of digitalis powder.

The amount of lyophilized water soluble extractive

of each infusion used was calculated on the basis of digitalis represented by the amount of tincture used in the standard. The standard was prepared so that 2 Gm. of powdered digitalis was represented in each 100 ml. of solution. Based upon the determination of water soluble extractive in each infusion (Table III, page 35), Table IV, (page 36) indicates the amount of the water soluble extractive used to prepare the test solutions for each infusion.

TABLE IV

GRAMS OF WATER SOLUBLE EXTRACTIVE
USED TO PREPARE 100 ML.
OF TEST SOLUTION

Infusion	A	B	C	D	E
	0.6906	0.6853	0.6753	0.6644	0.6335

A 5 ml. sample of the solution to be tested was measured into a 25 ml. flask. This was diluted with 15 ml. of water and 5 ml. of 10 per cent W/V aqueous solution of neutral lead acetate. To precipitate the excess lead 2.5 ml. of a 10 per cent W/V aqueous solution of sodium diphosphate was added. The mixture was shaken and made to

volume with distilled water, mixed thoroughly and filtered through a dry filter. The addition of the sodium diphosphate solution to the lead acetate mixture before filtration is a modification of the Knudson-Dresbach (61) method. The filtrate was repassed through the same filter until the filtrate was clear.

To develop the color reaction, 12.5 ml. of an alkaline picric acid solution was added to a 12.5 ml. aliquot of the clear filtrate and the mixture allowed to stand for 20 minutes. The alkaline picric acid solution was prepared by mixing 95 parts of a 1 per cent W/V aqueous solution of picric acid with 5 parts of a 10 per cent W/V aqueous solution of sodium hydroxide.

The transmission of light was then compared at 525 mu. in a 25 ml. absorption tube by using a Fisher Electrophotometer. The results expressed in percentage of transmission for five different infusions of digitalis are compared to results of a U.S.P. Digitalis Tincture taken as the standard. The results appear in Table V, (page 38).

TABLE V

TOTAL GLYCOSIDE CONTENT OF FIVE DIFFERENT INFUSIONS OF DIGITALIS COMPARED TO A U.S.P. DIGITALIS TINCTURE AS STANDARD

Transmission in Percentage

Sample	Standard	Infusion A	B	C	D	E
	45.50	43.50	46.00	50.50	45.50	49.00
	45.25	42.50	45.50	49.50	46.00	48.50
	48.50	42.00	46.50	50.50	45.50	49.50
			47.00	50.00	46.50	49.00
Average	46.42	42.67	46.25	50.12	45.88	49.00

Nitrogen Determination

The percentage of nitrogen in Digitalis Tincture, U.S.P., Infusion A and Infusion C was determined by Folin-Farmer (62) Microkjeldahl Method Modified. A 1 ml. sample of the solution to be tested was measured into a 100 ml. Kjeldahl flask. To this was added the digestive mixture and 0.65 Gm. of potassium sulfate. The digestive mixture was prepared by adding 2 Gm. of cupric sulfate to 100 ml. of concentrated sulfuric acid. The mixture was digested by heat until a clear solution was obtained.

Using a microkjeldahl apparatus, the digested sample was transferred to the distillation flask. The washings from the kjeldahl flask were added and then 5 ml. of a 50 per cent W/V aqueous solution of sodium hydroxide were added. Distillation was carried on for five minutes, starting with the first bubble observed in the receiving flask. At the end of five minutes the receiving flask was lowered and any distillate was collected for one additional minute. Twenty milliliters of a 0.01N hydrochloric acid were used as the receiving liquid. The receiving liquid and rinsings of the receiving flask were transferred to a 50 ml. flask. To this solution, 8 ml. of Nessler's Reagent was added and the contents made to volume with distilled water. A 25 ml. aliquot of the colored solution was transferred to a 25 ml. absorption tube. After twenty minutes transmission was compared at 425 mu by using a Fisher Electrophotometer.

Nessler's Reagent was prepared by transferring 37.5 Gm. of potassium iodide and 27.5 Gm. of iodine to a 250 ml. flask, then adding 250 ml. of distilled water and 35 to 40 Gm. of mercury. The flask was shaken continuously and vigorously for 7 to 15 minutes or until nearly all the iodine had disappeared. When the red iodine solution began to pale the flask was cooled in running water and

shaken until the reddish color changed to green. The aqueous solution was separated from the excess mercury by decantation. The mercury was washed with distilled water and the washings added to the decanted solution. The clear solution was made to 500 ml. volume with distilled water. One hundred-fifty milliliters of the solution were mixed with 700 ml. of 10 per cent W/V aqueous solution of sodium hydroxide in a 1000 ml. flask and made up to volume with distilled water.

A standard curve (Table VI, page 41) was constructed for the determination of nitrogen using an ammonium chloride solution as the standard. The stock solution of ammonium chloride was prepared by dissolving 3.818 Gm. of ammonium chloride in sufficient distilled water to make 1000 ml. The standard solution was prepared by taking 10 ml. of the stock solution and diluting it to 1000 ml. Each milliliter of the standard solution represented 0.01 mgm. of nitrogen.

To five, 50 ml. flasks containing 20 ml. of 0.01N hydrochloric acid was added 1 ml., 3 ml., 6 ml., 9 ml., and 15 ml. of ammonium chloride standard solution respectively. A sixth, 50 ml. flask containing 20 ml. of 0.01N hydrochloric acid served as a blank.

To each mixture was added 8 ml. of Nessler's Reagent and distilled water to make it to volume. A 25 ml. aliquot of this solution was measured into a 25 ml. absorption tube

and the transmission was compared at 425 mu after 20 minutes using a Fisher Electrophotometer. The results in percentage of transmission for construction of the standard curve are indicated in Table VI, (page 41).

TABLE VI

AMMONIUM CHLORIDE STANDARD

Milliliters of Ammonium Chloride Standard Solution	Milligrams of Nitrogen in Standard Solution	Milligrams of Nitrogen per 25 ml. Aliquot	Percentage of Transmission*
0	0.00	0.000	100.00
1	0.01	0.005	91.00
3	0.03	0.015	70.88
6	0.06	0.030	51.25
9	0.09	0.045	37.00
15	0.15	0.075	21.88

* Average of three readings.

One milliliter samples of Digitalis Tincture, U.S.P. were used in the nitrogen determination. The results calculated on the basis of a 20 per cent concentration appear in Table VII, (page 42).

TABLE VII

PERCENTAGE OF TOTAL NITROGEN IN DIGITALIS TINCTURE, U.S.P. CALCULATED AS MILLIGRAMS OF NITROGEN PER 20 PER CENT CONCENTRATION BASED ON CRUDE DRUG

Sample	Percentage Transmission*	Milligrams of Nitrogen per 25 ml. aliquot from Standard Cruve	Milligrams of Nitrogen in 100 ml. of a 20 per cent concentration	Percentage of Nitrogen Based on Crude Drug
1	11.50	0.103	4.12	0.206
2	11.17	0.104	4.16	0.208
3	10.00	0.108	4.32	0.216
Average	10.89	0.105	4.20	0.210

* Average of three readings.

One milliliter samples of Infusion A and Infusion C were analyzed for their nitrogen content. The test solutions were prepared based on the amount of water soluble extractive equivalent to 2 Gm. of powdered digitalis, Table IV,(page 36). The results calculated as percentage of total nitrogen per 100 ml. of solution are indicated in Table VIII, (page 43) and Table IX,(page 44).

TABLE VIII

PERCENTAGE OF TOTAL NITROGEN IN INFUSION A

CALCULATED AS MILLIGRAMS OF NITROGEN PER 100 ML.

BASED ON CRUDE DRUG

Sample	Percentage Transmission**	Milligrams of Nitrogen per 25 ml. aliquot from Standard Curve	Milligrams of Nitrogen per 100 ml. Sample	Percentage of Nitrogen Based on Crude Drug
1	39.00	0.0440	8.80	0.440
2	46.50	0.0360	7.20	0.360
3*	79.50	0.0103	8.24	0.412
4*	76.50	0.0122	9.76	0.488
5	51.00	0.0310	6.20	0.310
6	46.00	0.0360	7.26	0.363
7	50.50	0.0320	6.40	0.320
8	51.00	0.0315	6.30	0.315
9	50.50	0.0320	6.40	0.320
Average			7.395	0.3697

* Dilution factor different.
** Average of three readings.

TABLE IX

PERCENTAGE OF TOTAL NITROGEN IN INFUSION C

CALCULATED AS MILLIGRAMS OF NITROGEN PER 100 ML.

BASED ON CRUDE DRUG

Sample	Percentage Transmission*	Milligrams of Nitrogen per 25 ml. aliquot from Standard Curve	Milligrams of Nitrogen per 100 ml. Sample	Percentage of Nitrogen Based on Crude Drug
1	54.33	0.0290	5.80	0.290
2	56.92	0.0260	5.20	0.260
3	56.33	0.0270	5.40	0.270
4	55.17	0.0279	5.58	0.279
5	54.92	0.0280	5.60	0.280
Average	55.534	0.02758	5.516	0.2758

* Average of three readings.

Sugar Determination

The sugar content of Digitalis Tincture, U.S.P., Infusion D-1, Infusion C, Infusion C-1 and Infusion A was determined by Folin-Wu (63) Method. The solutions to be tested were prepared in the same manner and on the same basis as indicated under the determination of glycosides, (see pages 35-36).

A 5 ml. portion of the solution to be tested was measured into a 100 ml. flask and diluted to 60 ml. with

distilled water. To this was added 5 ml. of a 10 per cent W/V aqueous solution of neutral lead acetate, to eliminate tannins, phenols and other interferring substances. The mixture was thoroughly mixed by shaking and 2.5 ml. of a 10 per cent W/V aqueous solution of sodium diphosphate were added to precipitate excess lead. The mixture was then thoroughly shaken, diluted to the mark and filtered through a dry filter paper, discarding the first 10 ml. A 2 ml. aliquot of the clear filtrate was transferred to a Folin-Wu tube and 2 ml. of an alkaline copper solution added.

The alkaline copper solution was prepared by dissolving 40 Gm. of anhydrous sodium carbonate in 400 ml. of distilled water in a 1000 ml. flask. To this was added 7.5 Gm. of tartaric acid. When the tartaric acid had dissolved 4.5 Gm. of crystallized copper sulfate was added and made to volume with distilled water.

The Folin-Wu tube containing 2 ml. of the clear filtrate and 2 ml. of alkaline copper solution was placed in boiling water for 8 minutes. It was then cooled for three minutes, 2 ml. of phosphomolybdic acid solution added and diluted to the mark with distilled water. A 25 ml. aliquot was transferred to an absorption tube and the percentage of transmission compared at 425 mu.

The phosphomolybdic acid solution was prepared by

placing 35 Gm. of molybdic acid and 5 Gm. of sodium tungstate in a beaker, add 200 ml. of 10 per cent W/V aqueous sodium hydroxide solution and 200 ml. of distilled water. This mixture was boiled for 30 minutes to remove ammonia. The mixture was cooled, diluted to about 350 ml. and 125 ml. of 85 per cent phosphoric acid added. The mixture was transferred to a 500 ml. flask and made to volume with distilled water.

A standard sugar solution was prepared for the construction of a standard curve, Table X,(page 47). A 1 per cent W/V aqueous sugar stock solution was prepared with benzoic acid as preservative. Standard solution number one was prepared by diluting a 1 ml. aliquot of the stock solution to 100 ml. with distilled water. Standard solution number two was prepared by using a 2 ml. aliquot of the stock solution. One milliliter of standard solution number one represented 0.1 mgm. of sugar and 1 ml. of standard solution number two represented 0.2 mgm. of sugar.

The points of the standard curve were determined by taking 0.5 ml., 1.0 ml., and 1.5 ml. of standard solution number one and 1.0 ml., and 2.0 ml. of standard solution number two. Each measured amount of the standard solutions were placed in a Folin-Wu tube and treated as described, (see pages 44-45). The results are indicated in Table X, (page 47).

TABLE X

STANDARD SUGAR CURVE

Sample	Milliliters of Standard Solution	Standard Solution	Milligrams of Sugar	Percentage of Transmission*
1	0.0	Blank	0.00	100.00
2	0.5	No. 1	0.05	84.06
3	1.0	No. 1	0.10	66.31
4	1.5	No. 1	0.15	52.66
5	1.0	No. 2	0.20	42.84
6	2.0	No. 2	0.40	17.44

* Average of six readings.

The results of the determination of sugar in Digitalis Tincture, U.S.P., Infusion A, Infusion C, Infusion C-1 and Infusion D-1 appear in Table XI, (page 48).

TABLE XI

PERCENTAGE OF TOTAL SUGARS IN DIFFERENT DIGITALIS PREPARATIONS CALCULATED AS MILLIGRAMS OF SUGAR PER 100 ML.

Sample	Percentage Transmission*	Milligrams of Sugar Calculated From Standard Curve	Percentage of Total Sugars
Digitalis Tincture, U.S.P.**	72.500	0.07800	7.8000
	73.120	0.07500	7.5000
	71.880	0.08000	8.0000
	72.120	0.07900	7.9000
Average	72.405	0.07800	7.8000
Infusion A	58.000	0.12900	6.4500
	58.060	0.12800	6.4000
	58.250	0.12600	6.3000
Average	58.103	0.12760	6.3830
Infusion C	54.000	0.14500	7.2500
	53.250	0.14800	7.4000
	54.320	0.14300	7.1500
	53.320	0.14700	7.3500
Average	53.722	0.14575	7.2875
Infusion C-1	67.920	0.09000	4.5000
	67.540	0.09300	4.6500
	67.800	0.09100	4.5500
Average	67.753	0.09130	4.5670
Infusion D-1	69.500	0.08800	4.4000
	69.750	0.08500	4.2500
	70.120	0.08400	4.2000
	68.120	0.09000	4.5000
Average	69.372	0.08675	4.3375

* Average of four readings.

** Five milliliters, Digitalis Tincture, U.S.P. used as sample. Percentage of total sugars calculated on 100 ml. of 20 per cent concentration.

Toxicity Studies

A comparison of the toxicity of Infusion A with Digitalis Tincture, U.S.P. was made. A 20 per cent solution prepared by taking 20 ml. of tincture which had been evaporated to remove the ethanol was diluted to 100 ml. with physiological salt solution. The test solution was prepared by dissolving in physiological salt solution an amount of extractive from Infusion A equivalent to 2 Gm. of the powdered digitalis.

The toxicity was compared by using the frog heart chamber method (64). This method is based on the production of systolic stoppage of the heart. The digitalis solutions are applied to and held in contact with the heart by means of a heart chamber. When frogs of uniform size and standardized conditions are employed fairly constant systolic stoppage times can be recorded.

The time required to produce systolic stoppage is used as a basis for the potency of the solutions tested. The results obtained using Digitalis Tincture, U.S.P. and Infusion A are indicated in Table XII, (page 50).

Although this method measures the toxicity of digitalis it may be used for comparing digitalis preparations. A typical digitalis response is indicated in Figure 1, (page 51). Figure 2, (page 51) shows the heart response to Infusion A.

TABLE XII

A COMPARITIVE TOXICITY STUDY OF DIGITALIS

MEASURED ON THE FROG HEART

Heart Stoppage Time in Minutes

Sample	Digitalis Tincture, U.S.P.	Infusion A
	21	17
	21	17
	22	19
	22	19
	22	21
	23	26
	23	26
	24	27
	24	28
	26	29
	26	35
	26	39
	29	40
	29	44
	29	44
	30	44
	31	46
	33	47
	33	48
	41	55
	44	56
	44	63
	45	66
	49	
Average Mean	29.9	37.2
Variance	73.3	220.2
Standard Deviation	± 8.6	± 14.8
Standard Error	± 1.7	± 3.1

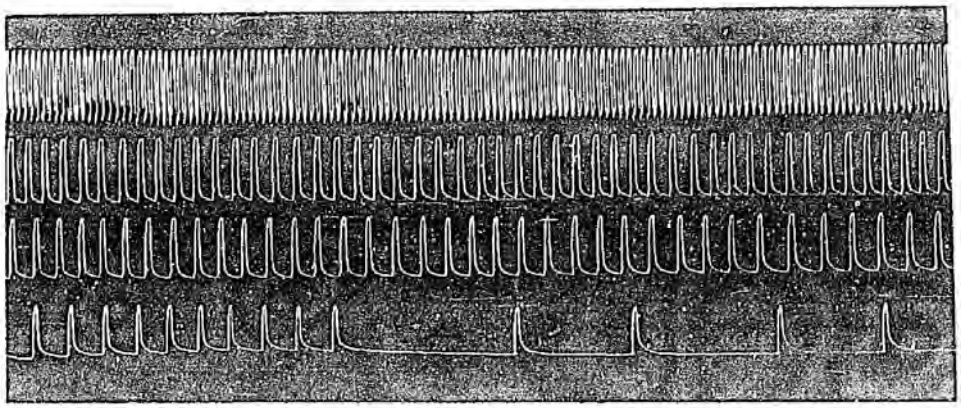


FIGURE 1

Frog heart response to Digitalis Tincture, U.S.P.

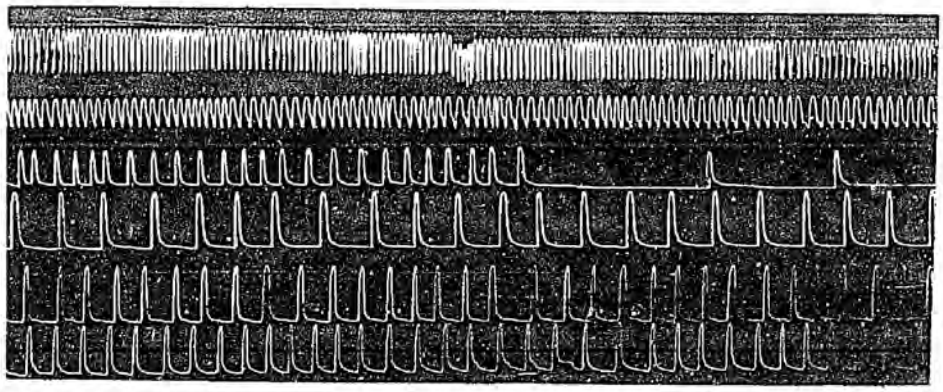


FIGURE 2

Frog heart response to Infusion A.

Absorption Studies

As a preliminary study various doses of lyophilized digitalis infusions were administrated orally to rats in order to determine a lethal dose. White male albino rats weighing between 250 and 365 Gm. were given the digitalis infusions orally by attaching a 10 ml. syringe to a rubber catheter. To facilitate administration the catheter was inserted into the stomach of the rat by passing it through a glass tube approximately 2 inches long. The glass tube was made fast by inserting it into a cork held in a burette clamp and fastened to a ring stand at about a 135° angle. After the syringe was filled by drawing the liquid through the catheter, the plunger was depressed until the syringe held the correct dose. To prevent the teeth of the animal from coming into contact with the rubber catheter it was inserted into the glass tube so as not to protrude beyond the lower end. Holding the animal in such a position so that the glass tube was forced into its mouth the catheter was cautiously slipped into the stomach and the total contents of the syringe slowly forced into the stomach. The results of this preliminary study are recorded in Table XIII, (page 54).

Since it was impossible to determine a lethal dose for lyophilized digitalis infusions when administrated

orally, a continuous administration of a uniform dose was tried. The uniform dose was calculated on the basis of the intravenous fatal dose (65), to allow for the difference in route of administration and the possible destruction of some of the drug in the stomach.

A diluted Digitalis Tincture, U.S.P. was prepared so as to represent 200 mgm. of digitalis powder per milliliter. The lyophilized Infusion D was prepared to represent 117 mgm. of digitalis powder per milliliter. The dose administrated was based on 2 ml. of test solution per 300 Gm. rat. One dose was administrated orally every 24 hours to each rat for 12 consecutive days and the rats were observed for 20 days following discontinuance of administration. The results of the oral administration of Digitalis Tincture, U.S.P. and Infusion D appear in Table XIV, (page 55) and Table XV, (page 56).

TABLE XIII

PRELIMINARY ABSORPTION STUDIES AFTER ORAL ADMINISTRATION OF DIGITALIS INFUSIONS TO RATS

Rat	Fasting Time in Hours	Weight of Rat Grams	Dose ml.	Infusion	Milligram Equivalent of Water Soluble Extract	Gram Equivalent Digitalis Powder	Deaths after 14 Days
1	--	--	2	E	13	0.040	--
2	10	322	2	D	54	0.163	--
3	10	364	2	D	54	0.163	--
4	11	342	2	E	138	0.435	--
5	11	280	2	E	138	0.435	--
6	11	340	2	E	933	2.943	--
7	12	322	2	E	933	2.943	--
8	12	348	2	E	933	2.943	--
9	12	326	2	E	933	2.943	--

TABLE XIV

CONTINUOUS ORAL ADMINISTRATION OF A UNIFORM DOSE OF INFUSION D TO RATS

Rat	Weight of Rat Grams	Dose 2 ml./300 Gm.	Milligram Equivalent Digitalis Powder	Deaths
1	230	1.53	179	--
2	290	1.93	226	--
3	270	1.80	211	--
4	274	1.82	213	--
5	240	1.60	187	--
6	257	1.71	200	--
7	270	1.81	212	--
8	245	1.63	191	--
9	340	2.27	266	--
10	320	2.13	249	--
11	270	1.80	211	--
12	358	2.39	280	--

TABLE XV

CONTINUOUS ORAL ADMINISTRATION OF A UNIFORM DOSE OF DIGITALIS TINCTURE, U.S.P. TO RATS

Rat	Weight of Rat Grams	Dose 2 ml./300 Gm.	Milligram Equivalent Digitalis Powder	Deaths
1	310	2.07	414	--
2	300	2.00	400	--
3	310	2.07	414	7th Day
4	310	2.07	414	--
5	280	1.87	374	--
6	230	1.53	306	--
7	225	1.50	300	--
8	365	2.43	486	--
9	290	1.93	386	--
10	310	2.07	414	--
11	280	1.87	374	--
12	250	1.67	334	--

Determination and Comparison of Potency of Infusion A by U.S.P. XIV Method

The potency of digitalis infusion A was determined by the U.S.P. XIV Method, with one modification, sodium pentobarbital, 40 mgm. per kilogram of body weight was used for the anesthesia instead of ether. The results of the assay are recorded in Table XVI, (page 57).

TABLE XVI

NUMBER OF INJECTIONS

Pigeon	Reference Standard	Infusion A
1	14	15
2	17	18
3	13	15
4	19	14
5	14	14
6	17	16
Average	15.67	15.33

The potency of infusion A was calculated to be 0.82 U.S.P. Digitalis unit. This is below the limits set forth in the U.S.P. XIV, which states that the potency of Digitalis is satisfactory if the results of the assay is not less than 0.85 U.S.P. Digitalis Unit and not more than 1.20 U.S.P. Digitalis Unit.

The standard error of the assay was calculated according to the U.S.P. XIV method and found to be 0.061 U.S.P. Digitalis Unit. This is within the limits set forth in the U.S.P. XIV, which states that the standard error of the assay should fall within the limit of 0.08 U.S.P. Digitalis Unit.

CHAPTER VI

CALCIUM GLUCONATE

Lyophilization

Approximately 20 Gm. of calcium gluconate were placed in 1000 ml. of distilled water and heated at about 60°C. for 30 minutes. The solution was allowed to cool to room temperature and filtered. The filtrate was placed in pyrex glass trays, covered with aluminum foil and lyophilized as described under digitalis, (see page 30). The only modification being the temperature of the circulating water which was maintained at 35°C.

Solubility Tests

The lyophilized calcium gluconate as with all other materials in this study presented a bulky fluffy appearance and texture. Preliminary experiments indicated that the lyophilized material was rapidly soluble and more soluble in water than the official salt. (1 Gm. in 30 ml.) Several methods of determining the solubility of lyophilized calcium gluconate were attempted only one of which was considered satisfactory.

A series of test tubes each containing 6 ml. of distilled water were set up. To each tube was added

weighed amounts of lyophilized calcium gluconate. Each tube was shaken and the results observed. See Table XVII, (page 60).

TABLE XVII

SOLUBILITY OF LYOPHILIZED CALCIUM GLUCONATE

Grams of Lyophilized Calcium Gluconate in 6 ml. of Distilled Water	Solubility	Rate of Solubility	Remarks
0.1	Very soluble	Very rapid	
0.2*	Very soluble	Very rapid	
0.3	Very soluble	Rapid	
0.4	Very soluble	Fairly rapid	
0.5	Soluble	Fairly rapid	
0.6	Soluble	Fairly rapid	
0.7	Soluble	Fairly rapid	
0.8	Soluble	Fairly rapid	
0.9	Soluble	Fairly rapid	
1.0	Soluble	Fairly rapid	
1.5	Soluble	Fairly rapid	
3.0	Soluble	Slowly	Crystallized out
6.0	Difficulty Soluble	Very slowly	Crystallized out

* Normal solubility. (1 Gm. in 30 ml.)

One gram of lyophilized calcium gluconate was placed in 1 ml. of distilled water. The powder crystallized out in 1 hour and gave the appearance of a completely dry powder. The amount of distilled water was increased to 30 ml. by adding to the crystallized out salt, 9 ml. of

distilled water one milliliter at a time and then 20 ml., 10 ml. at a time. It was not until a total of 30 ml. of distilled water, including the original 1 ml. were added did the calcium gluconate go into solution.

Rate of Solubility

One-half gram of non-lyophilized calcium gluconate and lyophilized calcium gluconate were each placed in 15 ml. of distilled water. Time was recorded starting with the placing of the salt in the distilled water until by observation it was dissolved. Observation was based on a clear solution with no evidence of granules or crystals being present.

Lyophilized calcium gluconate was observed to go into solution in 10 seconds. For the same amount of non-lyophilized calcium gluconate, solution was nearly complete, although there was some cloudiness plus a few granules or crystals, in 150 seconds.

Moisture Determination and Analysis

The determination of moisture in lyophilized calcium gluconate by using an oven for drying proved unsatisfactory due to possible deterioration, indicated by color change. The determination was carried out by placing the lyophilized and non-lyophilized calcium gluconate in tared

aluminum foil boats and inserting them into a Abderhaldem Drying Pistol, using phorphous pentoxide as the desiccant. The pistol was connected to a vacuum pump for 1 hour, then sealed by closing the stop-cock and the vacuum turned off. The material was held under this condition for 12 hours. The results of the determination of moisture and the analysis are indicated in Table XVIII, (page 62).

TABLE XVIII

PERCENTAGE OF MOISTURE IN NON-LYOPHILIZED AND LYOPHILIZED CALCIUM GLUCONATE

Sample	Non-lyophilized Calcium Gluconate	Lyophilized Calcium Gluconate
	0.0139	0.89
	0.0310	1.21
	0.0297	1.28
Average	0.0272	1.13

CHAPTER VII

CAFFEINE

Lyophilization

Approximately 20 Gm. of caffeine were placed in 1000 ml. of distilled water and heated at about 50°C. for 30 minutes. The solution was allowed to cool to room temperature and filtered. The filtrate was placed in pyrex glass trays, covered with aluminum foil and lyophilized as described under digitalis, (see page 30).

Solubility Tests

Lyophilized caffeine presented a bulky fluffy appearance and texture. Preliminary experiments indicated that the lyophilized material was rapidly soluble and more soluble in water than the official salt. (1 Gm. in 50 ml.). Several methods of determining the solubility of lyophilized caffeine were attempted only one of which was considered satisfactory.

A series of test tubes each containing 10 ml. of distilled water were set up. To each tube was added weighed amounts of lyophilized caffeine. Each tube was shaken and the results observed. The results are indicated in Table XIX, (page 64).

TABLE XIX

SOLUBILITY OF LYOPHILIZED CAFFEINE

Grams of Lyophilized Caffeine In 10 ml. of Distilled Water	Solubility	Rate of Solubility	Remarks
0.05	Very soluble	Very rapid	
0.10	Very soluble	Very rapid	
0.20*	Soluble	Rapid	
0.30	Soluble	Fairly rapid	
0.40	Soluble	Slowly	Soluble in 30 minutes.
0.50	Insoluble	Insoluble	Very little solubility long needle crystals in 5 hours.

*Normal solubility. (1 Gm. in 50 ml.)

Rate of Solubility

Definite weights of the non-lyophilized caffeine and lyophilized caffeine were placed in 10 ml. of distilled water. Time was recorded starting with the placing of the salt in the water until by observation it was dissolved. Solubility was based upon observance of a clear solution with no granules or crystals present.

Results showed that 0.2 Gm of lyophilized caffeine went into solution in 10 ml. of distilled water in 10

seconds. For an equal amount of non-lyophilized caffeine in 10 ml. of distilled water complete solution was observed in 220 seconds.

Moisture Determination

Samples of non-lyophilized caffeine and lyophilized caffeine were dried in an 80°C. oven for 4 hours and the percentage of moisture loss calculated.

The results for the determination of moisture in non-lyophilized and lyophilized caffeine are indicated in Table XX (page 65).

TABLE XX

PERCENTAGE OF MOISTURE IN
NON-LYOPHILIZED CAFFEINE AND LYOPHILIZED CAFFEINE

Sample	Non-lyophilized Caffeine	Lyophilized Caffeine
	0.1485 0.3037	0.0607 0.0442
Average	0.2261	0.0525

CHAPTER VIII

BENZOCAINE

Lyophilization

Approximately 1 Gm. of benzocaine was placed in 1000 ml. of distilled water and heated at about 50°C. for 30 minutes. The solution was allowed to cool to room temperature and filtered. The filtrate was placed in pyrex glass trays, covered with aluminum foil and lyophilized as described under digitalis (see page 30). The temperature of the circulating water was maintained at 35°C.

Solubility Tests

Lyophilized benzocaine presented a bulky, fluffy appearance and texture. Preliminary tests indicated that the lyophilized material was practically insoluble in water (1 Gm. in 2500 ml.). Because of the non-wetability of the lyophilized benzocaine an aqueous mixture was centrifuged at 5000r.p.m. for 10 minutes and at 10,000 r.p.m. for 30 minutes on a Servall Super-speed Angle Centrifuge with little of the salt going to the bottom of the centrifuge tube.

Using 5 ml. of a 10 per cent V/V aqueous solution

of alcohol as solvent 0.1 Gm. of lyophilized benzocaine was found to be insoluble. The amount of solvent was increased by 5 ml. increments until a total of 40 ml. without solubilizing the material. The amount of solute and solvent used were calculated on the basis of the alcoholic solubility of benzocaine. (1 Gm. in 5 ml.).

A series of test tubes each containing 1 ml. of alcohol were arranged to test the solubility of lyophilized benzocaine in alcohol. To each tube was added weighed amounts of lyophilized benzocaine. Each tube was shaken and the results observed. The results are indicated in Table XXI (page 68).

TABLE XXI

SOLUBILITY OF LYOPHILIZED BENZOCAINE IN ALCOHOL

Grams of Lyophilized Benzocaine In 1 ml. of Alcohol	Solubility	Rate of Solubility	Remarks
0.10	Soluble	Fairly rapid	
0.20*	Soluble	Fairly rapid	
0.30	Soluble	Very slowly	
0.12	Soluble	Fairly rapid	On the addition of 0.1 ml. of water crystallized out immediately.

* Normal solubility (1 Gm. in 5 ml.).

The determination of the solubility of lyophilized benzocaine was made by using a modified colorimetric method (66). A standard curve was first constructed by placing 10 mgm. of benzocaine, U.S.P. in a 100 ml. flask containing 25 ml. of distilled water. This mixture was allowed to stand for several hours to insure solubility. To the benzocaine solution was added 0.5 ml. of a 10 per cent V/V hydrochloric acid solution, 1 ml. of a 2 per cent W/V aqueous solution of sodium nitrite with gentle shaking and 1 ml. of concentrated

ammonium hydroxide. After the addition of each reagent the mixture was subjected to gentle shaking, except after the addition of the concentrated ammonium hydroxide, the mixture was shaken once or twice until turbid, and made to volume with distilled water. Each milliliter of this standard solution represented 0.1mgm. of benzocaine.

Five, different amounts of the standard solution were accurately measured into a 25 ml. flask and made to volume with distilled water. The 25 ml. colored solution was transferred to a 25 ml. absorption tube and the percentage of transmission compared at 425 mu. Blanks were prepared for each sample using the proper amounts of reagents and distilled water. Three samples were prepared for each different weight. Readings were taken 10 minutes after the dilution of the standard solution and within 30 minutes after preparation of the standard solution. The results for construction of the standard curve for benzocaine appear in Table XXII (page 70).

A series of test tubes each containing 25 ml. of distilled water were arranged for determining the solubility of lyophilized benzocaine in water. To each tube was added weighed amounts of lyophilized benzocaine. Each tube was stoppered, shaken fifty times and allowed to stand at room temperature for 3 hours. The mixtures were then filtered through a sintered glass filter of medium

porosity. To a 10 ml. aliquot of the clear filtrate in a 100 ml. flask was added 0.5 ml. of a 10 per cent hydrochloric acid solution, 1 ml. of a 2 per cent sodium nitrite solution and 1 ml. of concentrated ammonium hydroxide. The turbid solution was then made to volume with distilled water. A 2 ml. aliquot of the colored solutions was measured into 25 ml. flasks and made to volume with distilled water. The solutions were then transferred to a 25 ml. absorption tube and percentage transmission compared at 425 mμ. The results of the determination of the solubility of lyophilized benzocaine are indicated in Table XXIII (page 71).

TABLE XXII

STANDARD CURVE FOR BENZOCAINE

Transmission in Percentage

Standard Solution*	0.1 ml.	1.0 ml.	1.25 ml.	2.0 ml.	2.5 ml.
	92.00	54.00	49.00	33.50	27.50
	91.50	54.00	49.00	33.50	26.00
	92.50	54.00	49.50	33.00	26.00
Average	92.00	54.00	49.17	33.33	26.50

* 1 ml. of standard solution represents 0.1 mgm. of benzocaine.

TABLE XXIII

COLORIMETRIC DETERMINATION OF THE SOLUBILITY OF LYOPHILIZED BENZOCAINE

Transmission in Percentage

Milligrams Lyophilized Benzocaine in 25 ml. of Distilled Water	Percentage Transmission*	Milligrams of Benzocaine from Standard Curve	Calculated Milligrams of Benzocaine in Original 25 ml. Solution
10.00**	65.50	0.0760	9.500
20.20	43.17	0.1500	18.750
30.70	27.17	0.2325	29.063
40.00	26.17	0.2390	29.875
51.10	24.17	0.2540	31.750

* Average of three readings.
** Normal solubility. (1 Gm. in 2500 ml.)

Stability of Lyophilized Benzocaine Solutions

The remaining portions of the original solutions used in the colorimetric determination of the solubility of lyophilized benzocaine were stored in stoppered vials at room temperature for 18 days. The solutions were then filtered and treated as previously described (see pages 69 and 70). The results of the determination of the stability of lyophilized benzocaine solutions after 18 days are recorded in Table XXIV (page 72).

TABLE XXIV

STABILITY OF LYOPHILIZED BENZOCAINE

SOLUTIONS AFTER 18 DAYS

Transmission in Percentage

Milligrams Lyophilized Benzocaine in 25 ml. of Distilled Water After 18 Days	Percentage Transmission*	Milligrams of Benzocaine from Standard Curve	Calculated Milligrams of Benzocaine in Original 25 ml. Solution
10.00**	66.33	0.0740	9.250
20.20	43.33	0.1500	18.750
30.70	37.33	0.1750	21.875
40.00	30.67	0.2100	26.250
51.10	36.00	0.1810	22.625

* Average of three readings.
** Normal solubility. (1 Gm. in 2500 ml.)

CHAPTER IX

SULFANILAMIDE

Lyophilization

Approximately 5 Gm. of sulfanilamide were placed in 1000 ml. of a series of buffer solutions and heated at about 50°C. for 30 minutes. The solutions were allowed to cool to room temperature and filtered. The filtrate was placed in pyrex glass trays, covered with aluminum foil and lyophilized. (See page 30). The circulating water of the drying chamber was maintained at 35°C.

Preparation of the Buffers

Five different buffer solutions were prepared for use as solvent for sulfanilamide. The pH range of the buffers was from a pH 4.5 to a pH 8.2. Table XXV (page 74) shows the composition of the buffer solutions prepared.

TABLE XXV

BUFFERS FOR THE pH RANGE 4.5 TO 8.2

2M Sodium Acetate ml.	3.5M Acetic Acid ml.	0.0667M Acid Sodium Phosphate ml.	0.0667M Dibasic Sodim Phosphate ml.	pH
10	16.85			4.5
10	1.85			5.3
		90	10	6.3
		40	60	7.2
		5	95	8.2

Solubility Tests

Lyophilized sulfanilamide was bulky and had a fluffy appearance and texture. Preliminary experiments indicated that the lyophilized sulfanilamide was more soluble at a pH 4.5 than at pH 8.2. There were also indications that the lyophilized material was more soluble at the acid pH than the official salt (1 Gm. in 125 ml.). Several methods of determining the solubility of lyophilized sulfanilamide at different pH values were attempted only two were considered satisfactory.

Two series of test tubes each containing 20 ml. of buffer solution were set up. To each tube was added weighed amounts of lyophilized sulfanilamide. Each tube was shaken and the results recorded. See Table XXVI,

(page 75) and Table XXVII (page 76).

TABLE XXVI

SOLUBILITY OF LYOPHILIZED SULFANILAMIDE AT pH 4.5

Grams of Lyophilized Sulfanilamide in 20 ml. of Distilled Water	Solubility	Rate of Solubility	Remarks
0.0803	Soluble	Slowly	
0.1609*	Soluble	Slowly	
0.2413	Soluble	Very slowly	
0.3224	Very slightly soluble	--	Very little noticable solubility

*Normal solubility (1 Gm. in 125 ml.).

TABLE XXVII

SOLUBILITY OF LYOPHILIZED SULFANILAMIDE AT pH 8.2

Grams of Lyophilized Sulfanilamide In 20 ml. of Distilled Water	Solubility	Rate of Solubility	Remarks
0.0813	Soluble	Slowly	
0.1612*	Soluble	Slowly	
0.2407	Slightly soluble	Very slowly	Incompletely soluble
0.3206	Very slightly soluble	--	Very little solubility

*Normal solubility (1 Gm. in 125 ml.)

Two series of test tubes each containing 10 ml. of alcohol were set up. To each tube was added weighed amounts of lyophilized sulfanilamide. Each tube was shaken and the results recorded. See Table XXVIII (page 77).

TABLE XXVIII

SOLUBILITY OF LYOPHILIZED SULFANILAMIDE

AT pH 4.5 IN ALCOHOL

Grams of Lyophilized Sulfanilamide In 10 ml. of Alcohol	Solubility	Rate of Solubility	Remarks
0.1376	Soluble	Rapid	
0.2810*	Soluble	Fairly rapid	
0.3780	Soluble	Slowly	Soluble with difficulty.
0.4746	Slightly soluble	Very slowly	Incompletely soluble

*Normal solubility (1 Gm. in 37 ml. alcohol).

Although, there probably was some solubility of lyophilized sulfanilamide at pH 8.2 the degree of solubility was not apparent.

Weighed amounts of lyophilized sulfanilamide were added to 25 ml. of water in test tube, each tube was stoppered, shaken fifty times and allowed to stand at room temperature for 6 hours. The mixtures were filtered through a sintered glass filter of medium porosity. A 10 ml. aliquot of the clear filtrate was assayed for sulfanilamide by the U.S.P. XIV method for sulfanilamide. The results of the assay are indicated in Table XXIX

(page 78)and Table XXX (page 79)

TABLE XXIX

SOLUBILITY OF LYOPHILIZED SULFANILAMIDE AT pH 4.5

DETERMINED BY U.S.P. XIV METHOD

Milligrams Lyophilized Sulfanilamide in 25 ml. of Distilled Water	Milliliters of Sodium Nitrate Required*	Milligrams of Sulfanilamide per 10 ml. Aliquot	Calculated Milligrams in Original Sample
100.20	2.275	39.1755	97.9387
201.50**	4.595	79.1259	197.8147
301.80	5.850	100.7370	252.5425
401.60	6.650	114.5130	286.2825
503.00	6.600	113.6520	284.1300

*Average of three determinations
**Normal solubility (1 Gm. in 125 ml.).

TABLE XXX

SOLUBILITY OF LYOPHILIZED SULFANILAMIDE AT pH 8.2

DETERMINED BY U.S.P. XIV METHOD

Milligrams Lyophilized Sulfanilamide in 25 ml. of Distilled Water	Milliliters of Sodium Nitrite Required*	Milligrams of Sulfanilamide per 10 ml. Aliquot	Calculated Milligrams in Original Sample
101.30	2.05	35.301	88.2525
204.70**	4.00	68.880	172.2000
302.00	5.80	99.876	249.6900
400.30	6.70	115.374	288.4350
502.20	6.10	105.042	262.6050

*Average of three determinations
**Normal solubility (1 Gm. in 125 ml.).

Rate of Solubility

Definite weights of non-lyophilized sulfanilamide and lyophilized sulfanilamide at pH 4.5 were placed in 25 ml. of buffer and 25 ml. of distilled water respectively. Time of solution was recorded starting with the placing of the material in the solvent until it was observed to have dissolved.

Lyophilized sulfanilamide was observed to go into solution in 150 seconds. For an equal amount of non-lyophilized sulfanilamide in a pH 4.5 buffer solution, solution was complete in 335 seconds.

Stability of Lyophilized Sulfanilamide Solutions.

After standing for 8 days at room temperature, the remaining portions of the original lyophilized sulfanilamide solutions buffered at pH 4.5 were assayed to determine stability. The solutions were filtered and treated as previously described, using the U.S.P., XIV method for the assay (see page 77). The results of the determination are indicated in Table XXXI (page 80).

TABLE XXXI

STABILITY OF LYOPHILIZED SULFANILAMIDE SOLUTIONS AT pH 4.5 DETERMINED BY U.S.P., XIV METHOD

Milligrams of Lyophilized Sulfanilamide in 25 ml. of Distilled Water	Aliquot of Original Solutions	Milliliters of Sodium Nitrite Required*	Milligrams of Sulfanilamide per Aliquot	Calculated Milligrams in Original Sample
100.20	10 ml.	2.20	37.8840	94.710
201.50**	3 ml.	1.35	23.2470	193.725
301.80	1 ml.	0.57	9.8154	245.385
401.60	10 ml.	5.70	98.1540	245.385
503.00	10 ml.	5.80	99.8760	249.690

*Average of three determinations
**Normal solubility (1 Gm. in 125 ml.).

CHAPTER X

PHENOBARBITAL

Lyophilization

Approximately 1 Gm. of phenobarbital was placed in 1000 ml. of a series of buffer solutions and heated at about 80°C. for 30 minutes. The solutions were allowed to cool to room temperature and filtered. The filtrate was placed in pyrex glass trays, covered with aluminum foil and lyophilized (see page 30). The temperature of the circulating water in the drying chamber was maintained at 35°C. The buffers used were prepared as described under sulfanilamide (see page 73).

Solubility Tests

Lyophilized phenobarbital as with all other materials in this study presented a bulky fluffy appearance and texture. Preliminary experiments indicated that the lyophilized material was practically insoluble in distilled water at all pH values.

Several experiments consisting of 10 per cent and 20 per cent alcohol as the solvent indicated that lyophilized phenobarbital was practically insoluble in these solvents. When 0.025 Gm. of lyophilized phenobarbital

buffered at pH 4.5 was placed in 50 ml. of a 10 per cent alcohol solution complete solubility was not apparent. When 0.1 Gm. samples of lyophilized phenobarbital buffered at pH 4.5 and pH 8.2 were placed in 2 ml. of alcohol no solubility was evident.

A lyophilized phenobarbital was prepared as previously described using isotonic dextrose solution as the solvent. After lyophilization the resultant residue was hard and gave the appearance of crystallized sugar.

CHAPTER XI

OTHER MEDICINAL CHEMICALS

Theophylline

Approximately 4 Gm. of theophylline were placed in 1000 ml. of distilled water and heated at 80°C. for 30 minutes. The solution was allowed to cool to room temperature and filtered. The filtrate was placed in pyrex glass trays, covered with aluminum foil and lyophilized (see page 30). The temperature of the circulating water of the drying chamber was maintained at 35°C.

A series of test tubes each containing 25 ml. of distilled water were arranged to determine the solubility. To each tube was added weighed amounts of lyophilized theophylline. Each tube was shaken and the results observed. See Table XXXII (page 84).

TABLE XXXII

SOLUBILITY OF LYOPHILIZED THEOPHYLLINE

Grams of Lyophilized Theophylline In 25 ml. of Distilled Water	Solubility	Rate of Solubility	Remarks
0.1015	Very soluble	Slowly	
0.2064*	Soluble	Slowly	
0.3010	Soluble	Slowly	
0.4013	Soluble	Very slowly	Soluble with difficulty

*Normal solubility (1 Gm. in 120 ml. water).

Calcium Lactate

Calcium lactate was lyophilized as previously described (see page 30). As with all other lyophilized material in this study it presented a bulky fluffy appearance and texture. Preliminary experiments indicated the lyophilized material was rapidly soluble and more soluble in distilled water than the official salt (1 Gm. in 20 ml.).

Arsenic Trioxide

An amount of arsenic trioxide was lyophilized as previously described. (See page 30). The lyophilized material presented a bulky fluffy appearance and texture.

Preliminary experiments did not indicate an increase in solubility of the lyophilized material compared to the official compound.

Aspirin

When prepared and lyophilized as previously described aspirin sublimed in the drying chamber.

Caffeine and Citric Acid

When lyophilized as previously described solutions of caffeine with different per cent concentrations of citric acid were tested. Preliminary tests show that the solubility of citrated caffeine can be equalled with 15 per cent citric acid, when lyophilized.

PART III

CONCLUSION

CHAPTER XII

DISCUSSION

Infusions of Digitalis

Preliminary studies concerning the lyophilized water soluble extracts of Digitalis purpurea L. were first reported by Cosgrove and Guth (55). They indicated that the dry extracts were stable and had potencies comparable with tinctures prepared from the same sample of drug. It was also reported by these workers that the extracts were instantaneously soluble in water and suggested that these lyophilized extracts be used to prepare infusions extemporaneously.

One objective of this research is to report the effects of freeze drying on the glycosidal, nitrogen and sugar content, the toxicity, and the potency of lyophilized infusions of digitalis.

Five different infusions were prepared. Each infusion was prepared in duplicate, one sample dried by lyophilization and the other on a steam plate. The infusions varied in that the temperature of the extracting water varied and the ratio of the drug to the amount of water varied (see pages 30 to 33).

The infusions prepared with water at different temperatures showed a difference in color and this color difference was apparent in the lyophilized powder. When the ratio of the drug to water was reduced a still greater color difference in the infusions resulted and this difference in color was carried over to the lyophilized powders.

The lyophilized products had greater bulk, were of finer texture and more fluffy than the dried non-lyophilized water soluble extracts. These latter extracts had the appearance of porous brittle caramel. When exposed to air all extracts were hygroscopic and darkened considerably. The extracts became sticky and the rate of solution in water was decreased. The rate of solution in water of the lyophilized water extracts was rapid. However when placed in a solvent consisting of 1 part water and 4 parts alcohol a dark brown dispersion resulted with a gummy insoluble mass adhering to the sides and bottom of the container. The increase in the intensity of color observed when the amount of powdered drug used to prepare the infusions was increased corresponds to the total increase in the amount of extractive. (Table III, page 35).

The lyophilized products of the infusions prepared with small amounts of powdered digitalis were light brown or buff in color and produced a faintly amber solution

when dissolved in water. The lyophilized products of the more concentrated infusions produced darker infusions when dissolved in water. With all the variation in the lyophilized extractives there appeared to be little or no difference in the total amount of extractive in proportion to the amount of powdered drug used. This is shown by the determination of the amount of water soluble extractive in the different infusions. (Table III, page 35). Irrespective of the proportion of drug to water or to the temperature of the water, approximately 33 per cent of extractive was obtained. This shows that the water soluble materials are easily and quickly extracted from digitalis.

Total Ash and Acid Insoluble Ash

Total ash determinations were made on digitalis powder and on the lyophilized water soluble extracts. Results (Table I, page 29) indicate digitalis leaf contains 8.53 per cent of total ash and that the water soluble extract had 6.99 per cent ash. The 6.99 per cent of total ash found in the water soluble extract represents 27 per cent of the total ash found in the powdered digitalis.

The determination of acid insoluble ash (Table II, page 29) indicates 0.77 per cent in digitalis powder and 0.48 per cent in the water soluble extract. The water soluble extract therefore contains approximately 20 per cent of the acid insoluble ash found in the digitalis leaf.

Since acid insoluble ash is considered to be silica it is difficult to understand why the water soluble extract contains such material. However, it may be possible that the ash including the acid insoluble ash represents certain elements or balance of elements that contribute to the over all pharmacological action of digitalis infusion.

Glycosidal Content

A modified Knudson-Dresbach (61) method was used to determine the total glycosidal content by comparing the five different lyophilized digitalis infusions with a Digitalis Tincture, U.S.P. The tincture used as a standard and the test solutions of the lyophilized infusions were prepared to represent the activity of 2 Gm. of powdered digitalis (Table IV, page 36).

From the results (Table V, Page 38) it is concluded that the differences in glycosidal content are insignificant, even though Infusions A, B and D did show less transmittancy of light compared to the standard than Infusions C and E. The apparent difference, if significant would indicate Infusions A, B and D to be of slightly greater potency than the standard. Infusions C and E were less potent since an increase in transmittancy of light occurred.

The difference between the minimum and maximum average percentage transmittancy for the infusions compared to the average percentage transmittancy for the standard is

±3.75 per cent. This is too small a difference to indicate a significance between the infusions and the tincture. When the averages of percentage transmittancy for all infusions are totalled, the mean is calculated to be 46.78 per cent. This is a variation of +0.36 per cent from the average of percentage transmittancy of the standard and is too slight a variation to be of significance. Therefore it is concluded that the potency of the five lyophilized infusions are comparable in potency to Digitalis Tincture, U.S.P. when determined by a modified Knudson-Dresbach method.

It has been shown that the Knudson-Dresbach method as modified by Bell and Krantz for the chemical evaluation of digitalis preparations does not appear to satisfy all the requirements for an official chemical assay (67). Other investigators have shown that values obtained by this method are generally higher than values obtained by biological methods (68,69).

When the aglycones are assayed colorimetrically the color intensities are twice those produced by equal weights of their respective glycosides. It seems that the degree of complexity of the glycoside mixture, that is the primary and secondary glycosides and aglycones have an effect on the colorimetric assay. The presence of materials which are pharmacologically inactive, but give the Baljet

Reaction will favor a higher colorimetric assay (70,71).

Due to the absence of a satisfactory chemical assay for digitalis many facets of this problem have not yet been investigated. Correlations between chemical methods and biological assays are not substantiated. Many workers claim that there is little if any correlation between the chemical and biological methods. If a significance in the results of this analysis is assumed then it becomes apparent that Infusions A, B and D are not comparable to the standard. However when compared with the studies on the frog heart and the official pigeon assay the chemical and biological methods do have a correlation. If this assumptions is made for Infusions A, B and D then just the opposite must be assumed for Infusions C and E. The answer to the problem may lie in the suggestions made by Cosgrove and Guth (60), that it may be possible to determine the proportions of primary and secondary glycosides as well as aglycones by means of chromatography. It would then be possible to develop a satisfactory assay. It is further suggested that digitalis studies along this line be made using electrophoresis.

Nitrogen

Total nitrogen in Digitalis Tincture, U.S.P., Infusion A and Infusion C was determined colorimetrically using the Folin-Farmer (62) Microkjeldahl Method. The determinations

were made in order to determine whether any reaction had taken place during preparation of the infusions which would increase the nitrogenous material extracted.

The results (Tables VII, VIII, and IX, pages 42, 43 and 44) indicate Infusion A and Infusion C contained more total nitrogen than Digitalis Tincture, U.S.P. The percentage of total nitrogen in Infusions A and C is higher than the total nitrogen in the tincture. This difference is not surprising since it is known that water is a better solvent for proteins then is alcohol or hydroalcohol. However the difference in total nitrogen in the infusions, there being more in Infusion A than in Infusion C, is probably quite significant. The reason for this difference is not known but if the increase in nitrogen in hot water extractions represents protein this could be an important factor if the extracts were to be injected into the blood system.

It can be assumed that increases in temperature of the extracting water increases the amount of water soluble nitrogenous products. Furthermore, there seems to be no correlation between the amount of nitrogenous materials extracted and the glycosidal content. This is also demonstrated in this study but unexplained.

Sugar

Digitalis Tincture, U.S.P. and Infusions A,C,C-1 and D-1 were assayed for sugar content. The assay was made using a modified Folin-Wu Method (63). The results indicate that more total sugars are present in the tincture than in the water extracts. This appears contrary to fact, since sugars in general are more soluble in water than in alcohol. However it should be pointed out that the tincture was not prepared from the same digitalis as were the infusions and therefore, the difference in sugar content could be explained on that basis. This does not explain however the apparent significant difference in the percentage of total sugars in the water extracts.

Results (Table XI, page 48) indicate that those water extracts subjected to freeze drying contain more total sugars than those dried on a steam plate. This may be explained by assuming that pentoses are changed to furfural, a volatile substance, whereas any hexoses present would not be so effected. It is also feasible that due to the manner of drying, the sugars present could conceivably form some non-reducing complex that would prevent disclosure of their presence. Buchanan and Zook (28) reported a similar incident. They found that after dehydration apples and pears showed a loss in total sugars. They assumed that the loss in total sugars was due to decomposition of levulose. Other than the above postulations this condition

cannot be explained.

Toxicity Studies on the Frog Heart

Studies were made on the frog heart to determine the toxicity of Infusion A as compared to the toxicity of Digitalis Tincture, U.S.P.

The results (Table XII, page 50) show a mean of 29.9 for the tincture with a standard deviation of ±8.6 and a standard error of ±1.7 as compared to a mean of 37.2 for the infusion with a standard deviation of ±14.8 and a standard error of ±3.1. This seems to be ample evidence of a significant difference in the potency of the lyophilized extract as compared to the potency of the tincture. As previously pointed out this method measures the toxicity of digitalis. It then becomes apparent after a study of figure 1 (page 51) and figure 2 (page 51) that toxicity is not the only facet of this phase of the problem.

Figure 1 (page 51) indicates the typical response of the frog heart to digitalis tincture by this method and clearly indicates the normal heart beat with strong amplitude followed by a slowing of the beat and some decrease in amplitude. Finally, there is the typical digitalis heart block and escape with further decrease in amplitude, continuing until complete systolic stoppage.

In contrast, figure 2 (page 51) represents the

response of a frog heart to a test solution of lyophilized water soluble extract of digitalis, comparable in strength to the tincture. Here also is a normal heart beat with fairly strong amplitude. Following administration of the test solution of digitalis infusion is shown a slowing of the heart with some decrease in amplitude (same as the tincture). This is followed by heart block and increased amplitude until finally a very strong beat, quite uniform and with an increase in amplitude. If the results produced by the test solution had been a single incident there probably would be no cause to mention it. However, of the total 23 frogs used this condition was demonstrated in more than 45 per cent.

Based upon the results of the toxicity study on the frog heart it appears that there may be a cardiotonic activity present in the lyophilized extract not present or apparent in the tincture. This cardiotonic activity is not measurable by this method. It is suggested that further studies regarding this activity be carried out. In conclusion, it seems that the results of the toxicity studies indicate that the lyophilized extract is less toxic than the tincture but possesses a cardiotonic activity not apparent in the tincture.

Absorption Studies

In order to determine the lethal dose of the lyophilized extract of digitalis various concentrations were administrated orally to rats. The results (Table XIII, page 54) indicate that of the nine rats receiving a dose of lyophilized extract equivalent to between 0.04 Gm. to 2.94 Gm. of digitalis powder not a single animal showed any outward signs of toxicity and not one fatality was recorded. From this one would assume that the rats were either highly resistant to digitalis when administrated orally or that the drug was destroyed in the stomach, rapidly eliminated or not absorbed.

Using a continuous uniform dose of lyophilized water extract and Digitalis Tincture, U.S.P. oral administration to rats was made every 24 hours. Digitalis Tincture, U.S.P. and Infusion D were each tested on a separate group of 12 rats. No deaths were recorded in the group of rats that received the lyophilized water extract. The amount of lyophilized extract given in a 12 day period ranged from a low equivalent of 2.15 Gm. of digitalis powder to a high of 3.36 Gm. of digitalis powder. The reported fatal dose for rats intravenously is from 3.3 Gm. to 3.6 Gm. per kilogram of body weight (65). The total amount administrated orally exceeded this amount from 7 to 11 times.

In the group of rats receiving the Digitalis Tincture, U.S.P. one fatality was recorded on the seventh day. In this case it was highly suspected for good reason that during administration the lungs were damaged. The amount of tincture administrated in a 12 day period ranged from a low equivalent of 3.67 Gm. of digitalis powder to a high of 5.83 Gm. of digitalis powder. This exceeds by 12 to 20 times the average fatal intravenous dose.

From the above results it must be concluded that the determination of toxicity by the oral administration of lyophilized extract of digitalis and digitalis tincture to rats is not practical. Further study is indicated and it is suggested that, along with this study, the rate of absorption after oral administration be investigated. It is quite possible, assuming this study is an indication, that a different laboratory animal other than the rat will have to be used. It is further suggested that the studies be made pertaining to the intravenous administration of lyophilized infusions of digitalis.

Digitalis Potency

Infusion A was assayed for its potency by using the U.S.P. XIV method for assaying digitalis. On a comparative basis it is interesting to note that there is a correlation between this assay and the results of the toxicity study on the frog heart. Both methods indicating

the lyophilized infusion to be less toxic. An observation that seems significant, although not measurable by either method and not recordable by the official method is the fact that a change in the character of the heart beat was noticed after the second or third injection of the lyophilized extract. The change in the character of the heart beat was not apparent in the pigeons receiving the reference standard until after the ninth or tenth injection. Furthermore, it was observed in the group of pigeons receiving the lyophilized extract that this change in the heart beat was continuous until the heart stopped beating. With reference to what has been recorded in the toxicity studies on the frog heart this serves to further substantiate the presence of a cardiotonic principle in the lyophilized infusion not apparent in the tincture.

It can be assumed, based upon both biological studies that the lyophilized infusion is pharmacologically potent, less toxic than the tincture and has a cardiotonic activity not present in the tincture.

Calcium Gluconate

The solubility of lyophilized calcium gluconate was studied. Results (Table XVII, page 60) show that the amount of lyophilized calcium gluconate that would go into solution in distilled water was increased by approximately 15 times over the normal solubility (1 Gm. in 30 ml.).

This seems to be in agreement with reports in the literature which indicate that the solubility of biologicals are increased by freeze drying. As previously pointed out the investigation of medicinal chemicals subjected to freeze drying has been almost nil. Assuming that freeze drying does increase the solubility of lyophilized calcium gluconate by 15 times it seems possible that larger doses of calcium gluconate might be administrated with a decrease in solvent. It then becomes apparent that at present a dose of calcium gluconate within the range of 1 Gm. to 5 Gm. would require 30 ml. to 150 ml. of water. Using lyophilized calcium gluconate it now becomes possible to give the same dose in 1 ml. to 5 ml. of water.

It was interesting to note that when lyophilized calcium gluconate crystallized out of an aqueous solution it seemed to assume its original solubility properties. This was well demonstrated when 1 Gm. of lyophilized calcium gluconate was dissolved in 1 ml. of distilled water. It crystallized out in 30 minutes and upon the additon of more distilled water would not go into solution until its normal solubility had been reached. This may be an indication that lyophilized products might revert to their normal state once in solution.

Further studies of lyophilized calcium gluconate seem to be in order, especially pharmacologically. It is

apparent that an investigation of lyophilized calcium gluconate on animals must be undertaken to determine whether there is any change in the physiological activity of the drug due to freeze drying.

The rate of solubility of lyophilized calcium gluconate was determined. In comparison with the non-lyophilized the lyophilized calcium gluconate is instantaneously soluble in distilled water. When equal weights of the lyophilized and non-lyophilized calcium gluconate were placed in an equal amount of distilled water the lyophilized went into solution 15 times faster.

The possible application of freeze drying to calcium gluconate in order to give a large dose in less volume of solvent with less time for solution is conclusive. A great deal of further study and investigation is needed and it is suggested that emphasis at first be placed on means and methods for determining more accurately, solubility, rate of solubility, stability and the pharmacology of lyophilized products.

The percentage of moisture in non-lyophilized and lyophilized calcium gluconate was determined by using a Abderholdem Drying Pistol. Results (Table XVIII, page 62) indicate that more moisture was present in the lyophilized than in the non-lyophilized calcium gluconate. The excess moisture in the lyophilized calcium gluconate cannot be

accounted for.

It is well to point out that the effect of freeze drying undoubtly makes for smaller particle size, thus exposing a greater surface area. Upon this assumption it might be concluded that this partially explains the increase in solubility and the increased rate of solubility.

Caffeine

The effect of freeze drying on caffeine was studied with particular attention to solubility and rate of solubility. Observations (Table XIX, page 64) show that the solubility in water of caffeine was increased by approximately 50 per cent over the normal solubility (1 Gm. in 50 ml.) when lyophilized.

Results show that when equal amounts of non-lyophilized and lyophilized caffeine are placed in the same amount of distilled water the rate of solubility of the lyophilized caffeine was 22 times greater. However this does not parallel the finding for lyophilized calcium gluconate in respect to total solubility versus the rate of solubility. The solubility of lyophilized calcium gluconate was 15 times greater than the non-lyophilized while the rate of solubility was increased 15 times. It would seem that an increase in rate of solubility would coincide with an increase in solubility. However as

indicated by the experiments with lyophilized caffeine this is not necessarily true.

It is evident that the process of freeze drying increases the amount of caffeine that will go into solution in distilled water and that the rate at which it will go into solution is greater. It is possible that this is due to the finer subdivision of particles, thus exposing a greater surface area to the solvent. In freeze drying of medicinal chemicals a solution is made of the substance under investigation. When this solution is subjected to rapid freezing at a low temperature the solvent, in this case distilled water, freezes rapidly. It is then possible that we might visualize a field of particles, the solute, of colloidal size or smaller dispersed throughout the frozen solvent, ice. When this frozen solution is then subjected to drying with heat under vacuum the ice sublimes, leaving the solute in submicron particles as the dried residue. We can further assume that the spaces where there had once been solvent are probably now filled with air. This phenomenon of increase solubility is rarely observed because of the lack of conditions required to decrease the particle size of matter. The possible use of the electronic microscope may be a valuable tool in determining what takes place regarding particles size and possible change in crystalline structure during the process of freeze drying

of medicinal chemicals.

The total moisture content of both lyophilized and non-lyophilized caffeine was determined. Results indicate that non-lyophilized caffeine lost more moisture on drying than lyophilized caffeine. This is as may be expected, for as pointed out previously lyophilization deprives a substance of almost 99 per cent of its total moisture. However these results are contrary to the results of the moisture content found in calcium gluconate. The hygroscopic nature of the calcium gluconate may account partially for this condition.

It was observed during this study that care must be taken when handling lyophilized substances. Proper storage is essential. This was demonstrated by the fact that samples of lyophilized materials exposed to air seem to become less soluble and exhibited a decrease in the rate of solubility over similar material not exposed to air. There seems to be a tendency for lyophilized materials to revert to their original state if not properly stored. Lyophilized materials stored in dry, tightly stoppered bottles, and handled in a manner to prevent over exposure to air seemed to retain properties characteristic of lyophilized materials.

Benzocaine

A property of lyophilized benzocaine observed in preliminary studies indicated that the wetability of benzocaine was reduced. A phenomenon recorded but not explained showed that centrifuging at 10,000 r.p.m. for 30 minutes failed to cause the lyophilized benzocaine to settle at the bottom of the centrifuge tube.

The solubility of lyophilized benzocaine in alcohol was studied. Results indicate that the lyophilized material was insoluble in 10 per cent alcohol. However its solubility in alcohol was determined to be 50 per cent greater than the normal solubility (1 Gm. in 5 ml.).

Determined colorimetrically the amount of benzocaine after lyophilization that would go into solution in distilled water was found to increase approximately 3 times over the normal solubility (1 Gm. in 2500 ml.).

Results (Table XXIII, page 71) indicate that as the amount of lyophilized benzocaine placed in water is increased, the amount going into solution increases. This was evident for each sample tested. Noteworthy too, is the fact that as the weight of the sample is increased the per cent of the total weight going into solution decreased. It is difficult at this time to explain this phenomenon. However, it might be assumed that the commonly accepted

theory of a compound being made up of like molecules, each molecule having the same properties requires further study. It is also recommended that pharmacological studies be made using lyophilized benzocaine.

A test for the stability of lyophilized benzocaine in solution after 18 days was made. Results (Table XXIV, page 72) show that although not totally stable after 18 days more benzocaine remains in solution than would normally be soluble (1 Gm. in 2500 ml.).

Sulfanilamide

Preliminary studies of the solubility of lyophilized sulfanilamide buffered at pH 4.5 and pH 8.2 were made. Results (Tables XXVI and XXVII, pages 75 and 76) show that lyophilized sulfanilamide at pH 4.5 is more soluble than lyophilized sulfanilamide at pH 8.2. This is as may be expected for the literature indicates sulfanilamide solutions to be either neutral or slightly acid. The amount of sulfanilamide at pH 4.5 that would go into solution when placed in distilled water increased by 50 per cent over the solubility of non-lyophilized sulfanilamide (1 Gm. in 125 ml.).

The solubility of lyophilized sulfanilamide at pH 4.5 in alcohol was determined to be 36 per cent greater than the normal solubility (1 Gm. in 37 ml.).

The solubility of lyophilized sulfanilamide buffered at pH 4.5 and pH 8.2 was determined according to the U.S.P. XIV method for determining sulfanilamide. Results (Tables XXIX and XXX, pages 78 and 79) show again that lyophilized sulfanilamide at pH 4.5 is more soluble than that buffered at pH 8.2. The amount of lyophilized sulfanilamide buffered at pH 4.5 that went into solution in distilled water was determined to be approximately 43 per cent greater than the normal solubility (1 Gm. in 125 ml.).

The rate of solubility of lyophilized sulfanilamide at pH 4.5 was determined and found to be a little more than twice as fast when compared to the non-lyophilized compound.

The stability of lyophilized sulfanilamide at pH 4.5 in solution after 8 days was determined. Results (Table XXXI, page 80) indicate sulfanilamide solutions are not totally stable after 8 days. However it should be pointed out that an amount in excess of what normally goes into solution was found present in the 8 day old solutions.

Phenobarbital

Freeze drying seems to reduce the wetability of phenobarbital. Several preliminary solubility studies were made. Results indicated that other methods will have to

be used to determine the solubility and rate of solubility of lyophilized phenobarbital.

An isotonic dextrose solution was used as solvent on the assumption that by using a protective colloid solubility might have been enhanced. Results indicated that dextrose crystallizes when subjected to freeze drying. It is suggested that further studies be carried out on the use of protective colloids in conjunction with freeze drying.

Other Medicinal Chemicals

Theophylline

Lyophilized theophylline was studied for possible increase in its solubility. Results (Table XXXII, page 84) indicate that the amount of lyophilized theophylline that would go into solution in distilled water was increased 44 per cent over the normal solubility (1 Gm. in 120 ml.).

Calcium Lactate

Lyophilized calcium lactate appears to have characteristics similar to lyophilized calcium gluconate.

Arsenic Trioxide

Lyophilized arsenic trioxide may have a greater solubility than the non-lyophilized but to what degree was not determined. A possible means of increasing solubility

of compounds of this type that is recommended for further study is the re-lyophilization of an already lyophilized substance.

Aspirin

When dried at 35°C. under vacuum aspirin sublimes. It is possible that regulation of the temperature below 35°C. at 100 to 200 microns pressure might prove of value with substance of this nature.

Caffeine and Citric Acid

It has already been indicated that freeze drying increases the solubility of caffeine. It is now possible to assume that citrated caffeine may have exceeded its usefulness. However studies indicate that freeze drying permits decreasing the amount of citric acid normally required to solubilize the alkaloid. As low as 15 per cent concentration of citric acid gave results in respect to solubility comparable to citrated caffeine. It was not determined whether the increase in solubility was due to the citric acid or freeze drying. It is possible that a combination of citric acid and freeze drying increases the solubility of the alkaloid beyond that reported for caffeine (See pages 63 to 65).

CHAPTER XIII

SUMMARY AND CONCLUSIONS

PART I

1. A study was made of the effect of freeze drying on infusions of digitalis.

2. The lyophilized digitalis water extracts had greater bulk, were of finer texture and more fluffy than the dried non-lyophilized water extracts.

3. The lyophilized water extracts were more soluble and went into solution more rapidly than the non-lyophilized extracts.

4. Regardless of the proportion of drug to water or to temperature of water, approximately 33 per cent of extractive was obtained.

5. Water soluble materials are easily and quickly extracted from digitalis.

6. The lyophilized water extract contained 20 per cent of the total acid insoluble ash found in powdered digitalis.

7. The glycosidal content of digitalis tincture and digitalis infusions is approximately equal when assayed chemically.

8. There is no apparent correlation between the

glycosidal content determined chemically and the cardiac activity measured biologically.

9. The total nitrogen content of the lyophilized infusions is higher than the total nitrogen content of a standard tincture of equal concentration.

10. Increases in temperature of the extracting water increases the amount of water soluble nitrogenous products, extracted when preparing infusions of digitalis.

11. Lyophilization of water extracts prevents destruction of sugars.

12. Lyophilized infusion of digitalis is less toxic than a standard tincture of equal concentration when determined on the frog heart.

13. It is apparent that a cardiotonic activity is present in the lyophilized infusion of digitalis that is not present in a standard tincture when test solutions of each are applied to the frog heart.

14. White male albino rats are either resistant to large amounts of digitalis when it is administrated orally or the drug is destroyed in the stomach, rapidly eliminated or not absorbed.

15. Biological studies using frogs and pigeons show the lyophilized infusion is pharmacologically potent, less toxic than the tincture and has a cardiotonic activity not present in the tincture.

PART II

16. A study was made on the effect of freeze drying on several different medicinal chemicals.

17. Freeze drying increases the solubility and the rate of solubility of calcium gluconate and caffeine in distilled water.

18. Lyophilized products apparently revert to their original and normal state once in solution or after crystallizing out.

19. The moisture content of lyophilized calcium gluconate is higher than the moisture content of non-lyophilized calcium gluconate.

20. Non-lyophilized caffeine has a higher moisture content than lyophilized caffeine.

21. The solubility and the rate of solubility of benzocaine in water and in alcohol is increased by freeze drying.

22. Solutions of lyophilized benzocaine are not totally stable after 18 days but the amount of benzocaine remaining in solution after this period is greater than the amount normally soluble in distilled water.

23. Lyophilized sulfanilamide buffered at a pH 4.5 is more soluble and has a more rapid rate of solubility in distilled water than lyophilized sulfanilamide buffered at a pH 8.2.

24. Solutions of lyophilized sulfanilamide buffered at a pH 4.5 are not totally stable after 8 days but a greater amount remains in solution after this time than is normally soluble.

25. Freeze drying increases the non-wetability of sulfanilamide, phenobarbital and benzocaine.

26. Freeze drying of an aqueous solution of theophylline increases its solubility.

27. Aspirin sublimes when dried from the frozen state at 35°C. and 200 microns pressure.

28. The effect of freeze drying on an aqueous solution of arsenic trioxide was not apparent.

29. Freeze drying of a 15 per cent citric acid and caffeine mixture increased solubility comparable to citrated caffeine.

30. Lyophilized products require special handling and controlled storage conditions.

31. The application of freeze drying as to its practical and possible use in the field of pharmaceutical medicinal chemicals is feasible.

BIBLIOGRAPHY

1. Harris, R.J.C., Freezing and Drying. New York, Hafner Publishing Co. Inc., 1952.

2. Flosdorf, E.W., Hull, L.W. and Mudd, S., "Drying By Sublimation". J. of Immunol., 50, 21 (1945).

3. Flosdorf, E.W. and Mudd, S., "Procedure and Apparatus for Preservation in 'Lyophile' Form of Serum and Other Biological Substances". J. of Immunol., 29, 389 (1935).

4. Flosdorf, E.W., Freeze-Drying (Drying by Sublimation). 330 West Forth-second Street, New York 18, New York, Reinhold Publishing Corporation, 1949.

5. Leslie, J., "Methode Nouvelle de Produice el d'entretenir la Congelation". Ann. de chem. et de physique 78, 177 (1811).

6. Wollaston, W.H., "On a Method of Freezing At a Distance". Philos. Trans. Royal Soc. London. 103, 71 (1813).

7. Shackell, L.F., "An Improved Method of Desiccation With Some Applications to Biological Problems". Am. J. Physiol., 24, 325 (1909).

8. Strumia, M.M., McGraw, J.J., and Reichel, J., "The Preparation and Preservation of Human Plasma. IV. Drying of Plasma from the Frozen State by Low Temperature Condensation". Am. J. Clin. Path., 11, 480 (1941).

9. Harris, D.L. and Shackell, L.F., "The Effect of Vacuum Desiccation Upon the Virus of Rabies With Remarks Upon a New Method". Amer. J. Publ. Health, 1, 52 (1911).

10. Hammer, B.W., "A Note on the Vacuum Desiccation of Bacteria". Jour. Med. Res., 24, 527 (1911).

11. Rogers, L.A., "The Preparation of Dried Cultures". Jour. Infect. Dis., 14, 100 (1914).

12. Swift, H. F., "Preservation of Stock Cultures of Bacteria by Freezing and Drying". J. Exper. Med., 33, 69 (1921).

13. Sawyer, W.A., Lloyd, W.D.M. and Kitchen, S.F., "The Preservation of Yellow Fever Virus". J. Exper. Med., 50, 1 (1929).

14. Elser, W.J., Thomas, R. A., and Steffen, G.I., "The Desiccation of Sera and Other Biological Products (Including Micro Organisms) in the Frozen State With the Preservation of the Original Qualities of Products So Treated". J. Immunol., 28, 433 (1935).

15. Flosdorf, E.W. and Mudd, S., "An Improved Procedure and Apparatus for Preservation of Sera, Micro-organisms and Other Stubstances--the Cryochem-Process". J. Immunol., 34, 469 (1938).

16. Flosdorf, E.W. and Stokes, F. J., and Mudd, S., "The Desivac Process for Drying from the Frozen State". J. Amer. Med. Assoc., 115, 1095 (1940).

17. Folsom, T.R., Blood Substitutes and Blood Transfusion. C.C. Thomas, Springfield, Ill., pp. 49-63, (1942).

18. Hill, J. M., and Pfeiffer, D.C., "A New and Economical Desiccating Process Particularly Suitable for the Preparation of Concentrated Plasma or Serum for Intravenous Use; the Adtevac Process". Ann. Int. Med., 14, 201 (1940).

19. Taylor, A. R. and Beard, J. W., "An Inexpensive Apparatus for Drying from the Frozen State". Science, 92, 611 (1940).

20. Hays, E.E. and Koch, F.C., "An Apparatus for Vacuum Drying in the Frozen State". Science, 95, 633 (1942).

21. Dykstra, T.P. and DuBuy, H.G., "Preserving Plant Viruses in Vitro by Means of a Simplified Lyophil Apparatus". Science, 96, 189 (1942).

22. Campbell, D.H. and Pressman, D., "A Simplified Lyophil Apparatus". Science, 99, (1944).

23. Pomes, A.F., and Irving, G.W. Jr., "Lyophilization Apparatus". Science, 101, 22 (1945).

24. Pratt, H.K. and Yamaguchi, M., "Modified Laboratory Lyophil Apparatus". Science, 112, 560 (1950).

25. Holzman, G., "A Laboratory Lyophil Apparatus". Science, 111, 550 (1950).

26. Falk, K.G., Frankel, E.M. and McKee, R.H., "Low Temperature Vacuum Food Dehydration". J. Ind. Eng. Chem., 11, 1036 (1919).

27. Ewe, G.E., "The Effects of Cold and Freezing Upon Magnesia Magma and Other Pharmaceutical Preparation". Am. Drug., 68, 11 (1920).

28. Buchanan, J.H. and Zook, P.A., "What Happens to Fruit in Vacuum Dehydration". Chem. Met. Eng., 29, 1097 (1923).

29. Breedis, C. and Furth, J., "The Feasibility of Preserving Neoplastic Cells in the Frozen State". Science, 88, 531 (1938).

30. Luyet, B.J. and Thoennes, G., "The Survival of Plant Cells Immersed in Liquid Air". Science, 88, 284 (1938).

31. Hailton, I.R., Cunningham, B. and Kirk, P.L., "The Preparation of Sterile Proteins in the 'Lyophiled' State". Science, 94, 469 (1941).

32. Francis, T. Jr. and Salk, J.E., "A Simplified Procedure for the Concentration and Purification of Influenza Virus". Science, 96, 499 (1942).

33. Coburn, D.R., "Concerning the Nature of Type C Botulinus Toxin Fractions". Science, 95, 389 (1942).

34. Casals, J., "Non-Virulent Frozen and Fried Antigens for Complement Fixation Tests with Central Nervous System Virus Infections". Science, 97, 337 (1943).

35. Dounce, A.L. and Howland, J.W., "A Study of Crystalline Beef Liver Catalase Dried in the Frozen State". Science, 97, 21 (1943).

36. Svihla, R.D. and Osterman, E., "Growth of Orchid Seeds After Dehydration from the Frozen State". Science, 98, 23 (1943).

37. Nymon, M.C., Gunsalus, I.C. and Gortner, W.A., "An Application of the Lyophil Process to the Maintenance of Cultures for Microbiological Assay". Science, 102, 125 (1945).

38. Seegers, W.H., "Arrangement for Drying Proteins from the Frozen State". Science, 101, 284 (1945).

39. Wyckoff, R.W.G., "Frozen-Dried Preparations for the Electron Microscope". Science, 104, (1946).

40. French, C.S., Holt, A.S. and Powell, R.D., "The Evaluation of Oxygen from Illuminated Suspensions of Frozen Dried and Homogenized Chloroplasts". Science, 103, 505, (1946).

41. Bradish, C.J., "Freeze Drying". Chem. Products, 10, 60, (1947).

42. Pease, D.C., "The Disappearance of Salt from Glass Ice During Low-Temperature Dehydration and its Implication in Electron Microscopy". Science, 106, 543 (1947).

43. Westfall, R.J., Miller, C. and Westfall, I.S., "A Method of Drying Partial Protein Hydrolysates and Other Hygroscopic Materials for Nutritional Studies". Science, 105, 530, (1947).

44. Friedgood, H.B., Haagen-Smit, A.J., Garst, J.B. and Steinitz, L., "The Concentration and Preservation of Urinary Substances by Lyophilization". Science, 105, 99, (1947).

45. Plank, R., "Freeze Drying". Angen. Chem., B19, 36 (1947) through Chemical Abstracts 41, 5347 (1947).

46. Strickler, H.S. and Shaffer, C.B., "On the Use of the Campbell-Pressman Lyophilizing Appartus for Urinary Extractives". Science, 107, 71 (1948).

47. Richins, C.A., "Use of the Freezing-Drying Technique for Study of Vasomotor Activity". Science, 107, 25 (1948).

48. Strumia, M.M., "Freezing of Whole Blood". Science, 110, 398 (1949).

49. Barratt, R.W. and Tatum, E., "A Simplified Method of Lyophilizing Microorganisms". Science, 112, 122 (1950).

50. Zanezow, W.H. and Marshall, W.R. Jr., "Freeze Drying with Radiant Energy". Chem. Eng. Progress, 48, 21 (1950).

51. Chambers, H. and Nelson, J.W., "An Investigation of High-Vacuum Freeze-Drying as a Means of Drug Preservation". J.A. Ph. A., 39, 323 (1950).

52. Adams, G.A., "New Laboratory Methods for Drying Wheat Gluten". Cereal Che., 29, 312 (1952).

53. Valenzuela, A.C. and Feliciano, L.P., "Pharmaceutical Applications of Freeze-Drying". J. Philippine Pharm. Assoc., 34, 233 (1952).

54. Cosgrove, F.P. and Guth, E.P., "The Effect of Freeze-Drying on the Glycosidal Content of Digitalis Purpurea and Digitalis Lutea L." J.A.Ph. A., 43, 90 (1954).

55. Cosgrove, F.P. and Guth, E.P., "Preliminary Studies Concerning the Lyophilized Water-Soluble Extracts of Digitalis Purpurea L. and Digitalis Lutea L." J.A.Ph.A., 43, 266 (1954).

56. Cosgrove, F.P. and Guth, E.P., "Carbohydrate and Chlorophyll Content of Leaves of Digitalis Purpurea L. and Digitalis Lutea L. After Freeze-Drying and Oven-Drying". J.A.Ph.A., 43, 268 (1954).

57. Lemberger, A.P., Higuchi, T., Busse, L.W., Swintosky, J.V. and Wurster, D.E., "Preparation of Some Steroids in Microcrystalline Form by Rapid Freeze-Sublimation Technique". J.A.Ph.A., 43, 338 (1954).

58. Greaves, R.I.N., "The Preservation of Proteins by Drying With Special Reference to the Production of Dried Human Serum and Plasma for Transfusion". Med. Res. Council, Special Report Series, 258, (1946).

59. Brochure, Instruction for Installation and Operation. F. J. Stokes Machine Company, Philadelphia, Pa.

60. Cosgrove, F.P. and Guth, E.P., "Freeze-Drying As a Pharmaceutical Process for Drying Leaves of Digitalis purpurea L. and Digitalis lutea L." A Thesis presented to the Graduate School, The Ohio State University, 1953.

61. Bell, F.K. and Krantz, J.G. Jr., "A Chemical Evaluation of Digitalis". J. Pharmacol. Exptl. Therapeutics. 83, 213 (1945).

62. Folin, O., and Farmer, C.J., "A New Method for the Determination of Total Nitrogen In Urine". J. Biol. Chem., 11, 493 (1913).

63. Folin, O. and Wu, H., "A System of Blood Analysis - Supplement I - Improved Method for Determination of Sugar". J. Biol. Chem., 41, 367 (1920).

64. Hiner, D.L., "Demonstration of Cardiac Drugs". Am. J. Pharm., 3, 79 (1939).

65. Farris and Griffith, The Rat in Laboratory Investigation. 2nd Ed., Lippincott Company, Philadelphia, Pa.

66. Riegel, B.R. and Williams, J.F., "A New Color Reaction for Procaine and Some Other Local Anesthetics, and Its Application to the Determination of Procaine". J.Am. Chem. Soc., 48, 2871 (1926).

67. Goldstein, S.W., "A Study of the Modified Knudson-Dresbach Colorimetric Evaluation of Digitalis: Extraction Studies on Digitalis". J. A. Ph. A., 36, 296 (1947).

68. Bell, F.K. and Krantz, J.C. Jr., "Digitalis II--A Chemical Evaluation of Tablets of Digitoxin". J. Pharmacol., 87, 148 (1946).

69. Vos, B.J. and Welsh, L.H., "A Note on the Bell-Krantz Modification of Knudson-Dresbach Chemical Assay of Digitalis". J. Pharmacol., 85, 346 (1945).

70. Bell, F.K. and Krantz, J.C. Jr., "Digitalis VIII--The Baljet Reaction, Digitoxin and Digitoxigenin". J.A.Ph.A., 38, 107 (1949).

71. Eastland, C.J., Lawday, D.P. and Sellwood, E.B., "Digitalis Glycosides". J. Pharm. and Pharmacol., 4, 811 (1952).

AUTOBIOGRAPHY

I, Joseph Herschel Kern, was born in Chillicothe, Ohio, January 31, 1920. My elementary education was completed at St. Peter's Parochial School, Chillicothe, Ohio, June, 1935. In June, 1939, I was graduated from the Catholic Central High School, Chillicothe, Ohio, and in September, of that year enrolled in the College of Arts and Sciences, The Ohio State University, transferring to the College of Pharmacy, October, 1941.

I was honorably discharged from the United States Army, January 17, 1946, enrolled in the College of Pharmacy, The Ohio State University, March, 1946 and received the degree Bachelor of Science in Pharmacy, September, 1949. I qualified as a registered pharmacist by examination in the State of Ohio, October, 1949.

I acted in the capacity of assistant and part-time instructor while working toward the degree Master of Science which I received in June, 1951. I received an appointment as Instructor in the College of Pharmacy for the academic years 1951-1952 and 1952-1953. For the academic year 1953-1954, I was granted a Teaching Fellowship by the American Foundation for Pharmaceutical Education and appointed Instructor in the College of Pharmacy. I held this position while completing the requirements for the degree Doctor of Philosophy.

※※ Revised in November 2020 (Version 15)
※ Revised in June 2019

Anticonvulsant

NOBELBAR® 250 mg for Injection

(Phenobarbital sodium freeze-dried preparation)

Powerful drug, psychotropic
Addictive drug Note 1)
Prescription medication Note 2)

Japan Standard Commodity Classification No.
871139

Approval No.	22000AMX02274000
NHI Price listing	December 2008
Start of sales	December 2008
Re-examination results	December 2017 (Status epilepticus)
International birth date	October 2008

Storage method: Room temperature
Expiration date: Use by the expiration date printed on the outer box and vial

Note 1) Warning – This drug may be habit-forming.
Note 2) Warning – This drug should be used as prescribed by a physician, etc.

[Contraindications (Do not use to treat the following patients)]
(1) Patients with a history of hypersensitivity to the ingredients in this drug or to barbiturate compounds
(2) Patients with acute intermittent porphyria [there may be an increase in porphyrin synthesis, worsening the symptoms]
(3) Patients using voriconazole, tadalafil (when indicated for pulmonary hypertension), asunaprevir, daclatasvir, macitentan, elbasvir, grazoprevir, ticagrelor, artemether / lumefantrine, daclatasvir / asunaprevir / beclabuvir, darunavir / cobicistat, doravirine, rilpivirine, rilpivirine / tenofovir disoproxil / emtricitabine, rilpivirine / tenofovir alafenamide / emtricitabine, bictegravir / emtricitabine / tenofovir alafenamide, elvitegravir / cobicistat / emtricitabine / tenofovir alafenamide, elvitegravir / cobicistat / emtricitabine / tenofovir disoproxil, sofosbuvir / velpatasvir, or dolutegravir / rilpivirine

※※

[See the section on [Interactions]]

[Composition and Characteristics]

Brand name	Nobelbar 250 mg for injection
Ingredients and content	One vial contains 274 mg of phenobarbital sodium (250 mg as phenobarbital)
Additives	None
Formulation, color	White aggregate or powdered freeze-dried injection agent
pH	9.2 ~ 10.2 (when dissolving 1 g in 10 mL of water)
Ratio of osmotic pressure	2.5 ~ 2.6 (when dissolving 1 g in 10 mL of water) (the ratio in relation to physiological saline)

[Indications and Efficacy]
Neonatal seizures
Status epilepticus

<<Precautions in use regarding the indications and efficacy.>>
This drug is a long-acting drug with slow onset of activity, so the first line of treatment for patients with status epilepticus should be a fast-acting drug, and this drug should be used as a second-line treatment or thereinafter. (See the section on [Pharmacology])

[Dosage and Administration]
Neonatal seizures
Initial dose: Perform intravenous administration of 20 mg/kg as phenobarbital. If it is not possible to control the convulsions, the dose may be adjusted for additional intravenous administration according to the patient's condition within a range that does not exceed the initial dose used.
Maintenance treatment: Perform intravenous administration of 2.5 ~ 5 mg/kg as phenobarbital once a day.
Status epilepticus
Perform intravenous administration of 15 ~ 20 mg/kg as phenobarbital once a day.

<<Precautions in use regarding the dosage and administration>>
<Neonatal seizures and status epilepticus>
There may be onset of consciousness disorder, decreased blood pressure, or respiratory depression, so it is preferable to measure the blood concentration of this drug in order to enable appropriate adjustment of the dose. Also, if there is onset of respiratory depression, the appropriate measures should be taken immediately, such as artificial respiration. (See the sections on [Pediatric use] and [Overdose])
<Neonatal seizures>
Dosing should be performed slowly to neonatal patients over a period of 5 ~ 10 minutes. However, consideration may also be given to even slower dosing according to the patient's condition. Also, when performing additional dosing, the patient's condition should be observed and additional dosing should be performed after ensuring an adequate interval after the initial dose. (See the section on [Clinical results])
<Status epilepticus>
For pediatric patients and adults, dosing should be performed slowly over a period of at least 10 minutes. However, a dosing rate of 100 mg/min should not be exceeded.

[Precautions in Use]
1. Careful dosing (Perform careful dosing to the following patients)
(1) Low birth-weight infants [There is little experience of using this drug to treat these cases. (See the section on [Pediatric use])
(2) Elderly patients (See the section on [Geriatric use])
(3) Infirm patients with decreased respiratory function [There may be onset of respiratory depression.]
(4) Patients with post-traumatic syndrome after head injury or advanced arteriosclerosis [There may be intensification of the activity of this drug.]
(5) Patients with cardiac disorder [There may be onset of decreased blood pressure or decreased heart rate.]
(6) Patients with hepatic disorder or renal disorder [There may be worsening of these symptoms or increased blood concentration.]
(7) Patients with drug hypersensitivity
(8) Alcoholic patients [There may be intensification of the central nervous system depressant action.]
(9) Patients with a tendency for or a history of drug dependence [There may be psychological dependence or physical dependence.]
(10) Patients with serious neuropathy [There may be dependence.]
(11) Patients with hypothyroidism [There may be onset of abnormal thyroid function.]
2. Important basic precautions
(1) There may be onset of **status epilepticus** as a result of **a sudden decrease of the dose during use or sudden discontinuation of the treatment**, so when discontinuing the treatment, this should be performed carefully, such as by gradually reducing the dose. Particular caution is required for elderly or infirm patients.
(2) It is preferable to perform periodic **liver/kidney function tests and blood tests** during the use of this drug.
(3) Sleepiness or decreased attentiveness, concentration, or reflex actions, etc., may occur, so patients using this drug should be warned to **avoid operating machinery that may present a risk, such as driving an automobile**.

3. Drug interactions

This drug has activity to induce drug metabolizing enzyme CYP3A or the like. [1]

※※ (1) Contraindications for coadministration (do not use this drug together with the following drugs)

Drug name, etc.	Clinical symptoms, intervention method	Mechanism, risk factors
Voriconazole (VFEND) Tadalafil (when indicated for pulmonary hypertension: Adcirca) Asunaprevir (Sunvepra) Daclatasvir (Daklinza) Macitentan (Opsumit) Elbasvir (Zepatier) Grazoprevir (Grazytra) Ticagrelor (Brilinta) Artemether/lumefantrine (Riamet combination tablet) Daclatasvir/asunaprevir/beclabuvir (Ximency combination tablet) Darunavir/cobicistat (Prezcobix combination tablet) Dutasteride (Pfeltia)	There may be promotion of the metabolism of these drugs, resulting in decreased blood concentration.	As a result of the action of this drug to induce liver drug metabolizing enzymes (CYP3A).
Rilpivirine (Edurant) Rilpivirine/tenofovir disoproxil/emtricitabine (Complera combination tablet)	There may be promotion of the metabolism of rilpivirine, resulting in decreased blood concentration.	
Rilpivirine/tenofovir alafenamide/emtricitabine (Odefsey combination tablet)	There may be decreased blood concentration of rilpivirine and tenofovir alafenamide.	As a result of the action of this drug to induce liver drug metabolizing enzymes (CYP3A) and to induce P-glycoprotein.
Bictegravir/emtricitabine/tenofovir alafenamide (Biktarvy combination tablet)	There may be decreased blood concentration of bictegravir and tenofovir alafenamide.	
Elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (Genvoya combination tablet)	There may be decreased blood concentration of elvitegravir, cobicistat, and tenofovir alafenamide.	
Elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil (Stribild combination tablet)	There may be decreased blood concentration of elvitegravir and cobicistat.	
Sofosbuvir / velpatasvir (Epclusa combination tablet)	There may be decreased blood concentration of sofosbuvir and velpatasvir.	
Dolutegravir/rilpivirine (Juluca combination tablet)	There may be decreased blood concentration of dolutegravir and rilpivirine.	As a result of the action of this drug to induce liver drug metabolizing enzymes (CYP3A) and to induce UGT1A1.

※ (2) Careful coadministration (care is required when using this drug together with the following drugs)

Drug name, etc.	Clinical symptoms, intervention method	Mechanism, risk factors
Central nervous system depressants Phenothiazine derivatives Barbiturates Tranquilizers Hypnotics, etc. Antihistamine drugs Diphenhydramine, etc. Alcohol	There may be mutual intensification of the activity, so care is required, such as reducing the dose.	As a result of additive central nervous system depressant action.
MAO inhibitors		Unknown mechanism
Tricyclic antidepressants Imipramine, etc. Tetracyclic antidepressants Maprotiline, etc.	(1) There may be mutual intensification of the activity, so care is required, such as reducing the dose. (2) There may be a decrease in the blood concentration of these antidepressants. [Note 1]	(1) As a result of additive central nervous system depressant action. (2) As a result of the action of this drug to induce liver drug metabolizing enzymes.
Methylphenidate	There may be an increase in the blood concentration of this drug, so care is required, such as reducing the dose of this drug.	It is believed that methylphenidate will inhibit liver metabolism.
Valproic acid Sulpenitol	(1) There may be an increase in the blood concentration of this drug, intensifying the action. (2) There may be a decrease in the blood concentration of these drugs. [Note 1]	(1) These drugs can inhibit liver metabolism. (2) As a result of the action of this drug to induce liver drug metabolizing enzymes.
Clobazam	(1) There may be an increase in the blood concentration of this drug. (2) There may be a decrease in the blood concentration of clobazam. [Note 2]	(1) Mechanism unknown. (2) As a result of the action of this drug to induce liver drug metabolizing enzymes.
Irinotecan [1]	There may be a decrease in the blood concentration of the irinotecan active metabolite, resulting in weakening of the action, so it is preferable to avoid concomitant dosing.	As a result of the action of this drug to induce liver drug metabolizing enzymes.

Drug name, etc.	Clinical symptoms, intervention method	Mechanism, risk factors

Drug name, etc.	Clinical symptoms, intervention method	Mechanism, risk factors
Drugs having CYP3A as the primary substrate Aprepitant [**] Ignatinoxi Imatinib Carbamazepine Cyclosporin Zonisamide Tacrolimus Felodipine Verapamil Montelukast [*], etc Adrenocortical hormones Dexamethasone, etc Follicular hormones, corpus luteum hormones Norgestrel, ethinyl estradiol, etc. PDE5 inhibitors Tadalafil (when indicated for erectile dysfunction or dysuria associated with benign prostatic hypertrophy: Cialis, Zalasta), sildenafil, vardenafil	There may be a decrease in the blood concentration of these drugs, resulting in weakening of the action, so care is required in selecting the dose. [**][**]	As a result of the action of this drug to induce liver drug metabolizing enzymes
Aminophylline hydrate Chlorpromazine Theophylline Fluvoxetine [**] Enoxacin		
Lansoprazole Dexlansoprazole Clarithromycin	There may be a decrease in the blood concentration of these drugs. [**][**]	This drug may promote the gluconeogenic acid conjugation of these drugs
Rifampicin		Mechanism unknown
Ritonavir		As a result of the action of this drug to induce liver drug metabolizing enzymes and P-glycoprotein
Sofosbuvir Ledipasvir/sofosbuvir Daclatasvir/asunaprevir/beclabuvir Tenofovir alafenamide		As a result of the action of this drug to induce P-glycoprotein
Doxycycline	There may be shortening of the blood concentration half-life of doxycycline.	As a result of the action of this drug to induce liver drug metabolizing enzymes
Coumarin-based anticoagulants Warfarin	The action of the coumarin anticoagulant may be weakened, so the blood clotting time should be measured more frequently than usual, and the dose of the coumarin anticoagulant should be adjusted.	
Albendazol	The blood concentration of the albendazole active metabolite may be decreased, resulting in weakening of the drug efficacy.	Mechanism unknown
Diuretics Thiazide-based hypotensive diuretics, etc	There may be intensification of orthostatic hypotension, so care is required, such as reducing the dose.	The mechanism is unknown, but at high doses, this drug may cause a drop in blood pressure.

Drug name, etc.	Clinical symptoms, intervention method	Mechanism, risk factors
Acetazolamide	Rickets or osteomalacia may easily occur.	It is believed that this is caused by the promotion of vitamin D inactivation of this drug, or by renal tubular disorder or metabolic acidosis, etc., caused by acetazolamide.
Acetaminophen [**]	Patients using this drug continuously for a long period of time are susceptible to hepatopathy resulting from the metabolites of acetaminophen.	It is believed that this is caused by the action of this drug to induce liver drug metabolizing enzymes, or the promotion of the metabolism of acetaminophen to the hepatotoxic N-acetyl-p-benzoquinone imine.
Food products containing St. John's Wort	The metabolism of this drug may be promoted, reducing the blood concentration, so the patient should be warned to avoid ingesting food products containing St. John's Wort when using this drug.	It is believed that this is caused by the action of St. John's Wort to induce liver drug metabolizing enzymes.

Note 1) When decreasing the dose of this drug or when discontinuing the use of this drug, attention should be paid to any increase in the blood concentration of these drugs.

4. Adverse drug reactions

Amongst the 10 cases for which the safety was evaluated in a domestic clinical study on neonatal seizures, adverse drug reactions (including clinical laboratory test value abnormalities) occurred in 6 cases (60%), including 3 instances of respiratory depression in 3 cases (30%), 2 instances of decreased oxygen saturation in 2 cases (20%), 1 instance of blood pressure decreased in 1 case (10%), 1 instance of bradycardia in 1 case (10%), 1 instance of body temperature decreased in 1 case (10%), 1 instance of increased bronchial secretion in 1 case (10%), and 1 instance of urinary output diminished in 1 case (10%). (At the time of approval)

[*] [**] Amongst the 329 cases for which the safety was evaluated in a treatment outcome survey that was conducted on neonatal seizures, there were 22 instances of adverse drug reactions (including clinical laboratory test value abnormalities) that occurred in 17 cases (5.2%), and the primary adverse drug reactions were blood pressure decreased (4 instances in 4 cases, 1.2%) and respiratory depression (2 instances in 2 cases, 0.6%). (At the completion of the re-examination)

Amongst the 834 cases for which the safety was evaluated in a treatment outcome survey that was conducted on status epilepticus, there were 176 instances of adverse drug reactions (including clinical laboratory test value abnormalities) that occurred in 107 cases (12.8%), and the primary adverse drug reactions were hepatic dysfunction (33 instances in 30 cases, 3.6%), sleepiness (14 instances in 13 cases, 1.6%), blood pressure decreased (12 instances in 12 cases, 1.4%), and respiratory depression (7 instances in 6 cases, 0.7%). (At the completion of the re-examination)

(1) Serious adverse drug reactions [Note 4)]

1) **Toxic Epidermal Necrolysis (TEN)** [*], **Stevens-Johnson syndrome** [*], **erythroderma (exfoliative dermatitis** [*] [**]**) (unknown frequency).** Adequate monitoring should be performed, and if abnormalities such as fever, erythema, blistering/erosion, pruritus, pharyngeal pain, eye hyperemia, or stomatitis are observed, the treatment should be discontinued and the appropriate actions, such as the administration of an adrenocortical hormone, should be taken. [*]

2) **Hypersensitivity syndrome (0.1%).** There may be onset of initial symptoms such as rash or fever, followed by delayed, serious hypersensitivity symptoms such as lymphadenopathy, organ disorder such as hepatic dysfunction, increased white blood cell count, eosinophilia, or the appearance of atypical lymphocytes, so adequate monitoring should be performed, and if these types of symptoms are observed, the treatment should be discontinued and the appropriate actions should be taken. Hypersensitivity syndrome may often be accompanied by viral reactivation such as human herpes virus 6 (HHV-6), and there may be recurrence or protraction of symptoms such as rash, fever, and hepatic dysfunction, so care is required.

3) **Dependence (unknown frequency)**: Continuous use of this drug may result in drug dependence, so adequate monitoring should be performed, and dosing should be performed carefully, paying attention to the dosage and treatment period. Also, a sudden decrease in the dose or sudden discontinuation of the treatment during continuous use may result in the onset of withdrawal symptoms such as anxiety, insomnia, seizures, nausea, hallucinations, delusions, excitability, confusion, or a depressed state, so when discontinuing the use of this drug, the treatment should be discontinued carefully, such as by gradually decreasing the dose.

4) **Granulocytopenia (0.4%), thrombocytopenia (unknown frequency)**: Adequate monitoring should be performed, and if any abnormalities are observed, the treatment should be discontinued and the appropriate actions should be taken.

5) **Hepatic dysfunction (3.6%)**: There may be onset of hepatic dysfunction associated with increased AST (GOT), ALT (GPT), or γ-GTP, so adequate monitoring should be performed, and if any abnormalities are observed, the treatment should be discontinued and the appropriate actions should be taken.

6) **Respiratory depression** [16] **(0.7%)**: Adequate monitoring should be performed, and if any abnormalities are observed, the appropriate actions should be taken, such as discontinuing the treatment.

(2) Other adverse drug reactions [Note 4]

	1 – less than 3%	Less than 1%	Unknown frequency
Hypersensitivity [Note 5]		Toxicodermatosis, Exanthi	Scarlet fever-like rash, measles-like rash
Blood [Note 6]			Platelets decreased, megaloblastic anemia
Liver [Note 7]	Hepatic dysfunction such as elevated AST (GOT), ALT (GPT), or γ-GTP		Jaundice
Kidneys [Note 8]		Renal disorder such as proteinuria	
Neuropsychiatric system	Sleepiness	Vertigo, headache, mental function deteriorated	Amnesia, delirium, stupor, dullness, dysbulia, paresthesia, ataxia, excitement, hyperkinesia
Circulatory system	Blood pressure decreased	Bradycardia	
Digestive system		Anorexia	
Bone, teeth			Rickets [Note 9], osteomalacia, Kaschin-Beck disease, hypoplasia [Note 9], hypocalcemia
Endocrine system			Abnormal thyroid function test values (uptake, T3 value, etc.)
Other		Oxygen saturation decreased, increased bronchial secretion, urinary output diminished, fever	Decreased serum folate values, hemoglobinuria [Note 10], body temperature decreased

Note 4) The frequency of occurrence is noted based on the results of treatment outcome survey that was conducted on status epilepticus. Adverse drug reactions that did not occur in this survey are noted as having unknown frequency.

Note 5) The treatment should be discontinued.

Note 6) The appropriate actions should be taken, such as discontinuing the treatment.

Note 7) Adequate monitoring should be performed, and if any abnormalities are observed, the appropriate actions should be taken, such as discontinuing the treatment.

Note 8) This may occur as a result of continuous use.

Note 9) This may occur as a result of continuous use, so adequate monitoring should be performed, and if any abnormalities (such as elevated serum alkaline phosphatase levels, decreased serum calcium, or increased serum inorganic phosphorus) are observed, the appropriate actions should be taken, such as decreasing the dose or administering vitamin D.

5. Geriatric use

Dosing should be performed carefully, such as starting the treatment from a low dose. When discontinuing the use of the drug, the treatment should be discontinued carefully, such as by gradually decreasing the dose. [Elderly patients are susceptible to the onset of respiratory depression, excitement, depression, or confusion (See the section on [Important basic precautions]).]

6. Use in pregnant women, parturient women, or women who are nursing, etc.

(1) This drug should only be used to treat women who are pregnant or who may become pregnant if it is judged that the benefits of the treatment (preventing the frequent occurrence of seizures in the mother and protecting the fetus from hypoxia) outweigh the risks. [There is a report of an epidemiological survey that showed a large number of cases that gave birth to infants showing deformities (cleft lip, cleft palate, heart malformation [17], [18], or coarctation of the aorta, etc.) amongst patients treated with this drug, either in monotherapy or combination therapy, during pregnancy.]

(2) The use of this drug during pregnancy may induce a bleeding tendency or respiratory depression, etc., in the newborn infant.

(3) When used continuously prior to delivery, there may be onset of withdrawal symptoms (hyperkinesis, tremors, hyperreflexia, hypertonicity, etc.) in the newborn infant after birth.

(4) There has been a report of the occurrence of decreased folic acid as a result of the use of this drug during pregnancy. [19]

(5) It is preferable to avoid using this drug to treat women who are nursing, but if the use of this drug is unavoidable, the patient should avoid nursing. [There may be transfer to human breast milk, which could induce somnolence or decreased feeding in the newborn infant or suckling infant. [20], [21]]

7. Pediatric use

It has been reported that the primary metabolic system for this drug will be completed 10 ~ 20 days after birth, so it is preferable to perform blood concentration monitoring particularly for low birth-weight infants and newborn infants. [22]

8. Overdose [23]

(1) Symptoms:

Central nervous system and cardiovascular system depression. Sleepiness, nystagmus, or ataxia may occur at a blood concentration of 40 ~ 45 μg/mL or more, and the patient may fall into a comatose state during severe toxicosis. Respiration will be depressed at an early stage, with weakening of the pulse and cold sweats in the skin, resulting in a drop in the body temperature. There is also the risk of pulmonary complications or renal disorder.

(2) Intervention:

Respiratory care. The excretion of the drug should be promoted through the administration of diuretics or through urine alkalization by administering sodium hydrogen carbonate. In severe cases, consideration should be given to hemodialysis or blood perfusion.

9. Precautions in application

(1) This drug should only be administered intravenously.

(2) Preparation method

1) Normally, one vial should be dissolved in 5 mL of water for injection or physiological saline for administration (concentration after dissolution: 50 mg/mL as phenobarbital). The solution should be used promptly (within 6 hours) after dissolution.

2) It has been confirmed that changes in composition may occur with the following injection agents, so these drugs should not be mixed. Dopamine hydrochloride, L-potassium aspartate, menatetrenone (vitamin K), vecuronium bromide, amikacin sulfate, gentamycin sulfate, erythromycin for injection (erythromycin lactobionate)

10. Other precautions

(1) There have been reports of the retardation of brain growth during repeated dosing of large doses to neonatal rats (60 mg/kg subcutaneous dosing) in comparison to the control group. [24], [25]

(2) There have been reports of a significant increase in the occurrence of hepatic tumors when performing long-term dosing of large doses to rats and mice (rats: 25 mg/kg, mice: 75 mg/kg) in comparison to the control group. [26], [27]

(3) There may be the occurrence of serum immunoglobulin (IgA, IgG, etc.) abnormalities. [28]

(4) There was a report of the induction of cross-hypersensitivity (cutaneous hypersensitivity including hypersensitivity syndrome) between this drug and other anticonvulsants (phenytoin, carbamazepine).

[Pharmacokinetics]

Blood drug concentration

This drug was administered intravenously for neonatal seizures at an initial dose of 20 mg/kg, with administration of the same dose as an additional dosage if the seizures could not be controlled. A maintenance dose of 2.5 ~ 5 mg/kg was intravenously administered once a day. As a result, the initial dose was 17.5 ~ 20.8 mg/kg, the additional dose was 20.1 mg/kg, and the maintenance dose was 2.2 ~ 5 mg/kg. The blood concentration (9 cases) at 2 hours after the initial dose was 22.38 ± 2.34 μg/mL (18.8 ~ 25.8 μg/mL) as the mean ± standard deviation (minimum value ~ maximum value), and the blood concentration was 42.8 μg/mL in the one case that received an additional dose (30 minutes after the initial dose). Also, the blood concentration (10 cases) prior to the final administration of the maintenance dose was 27.65 ± 9.46 μg/mL (18.7 ~ 45.3 μg/mL).[26), 30)]

[Clinical results]

Neonatal seizures

A total of 10 patients having neonatal seizures (age: mean age in days 2.5 days old (range: 1 ~ 5 days old), body weight at birth: mean 2,867.7 g (range: 1,144 ~ 3,796 g), fetus week number: mean 38.23 weeks (range: 33.1 ~ 41.1 weeks)) were used in a non-controlled, open-label, multi-center study having the existence of seizures based on the clinical findings at 30 minutes after the initial dose of this drug (with the administration of an additional dose at 30 minutes after the initial dose if the seizures failed to disappear) as the primary endpoint. As a result, the drug was judged to be "markedly effective" in all of the cases. One case received the administration of an additional dose, and the drug was judged to be "effective" at 30 minutes after the initial dose in this case, but there was persistence of intermittent seizures, so an additional dose was administered, and the treatment was judged to be "markedly effective", with disappearance of the seizures following the additional dose. Also, in this study, a 6-day maintenance treatment period was established, and the recurrence of seizures was observed in 2 of the 9 cases that received the maintenance dose treatment. Further, the recurrence of seizures was observed in 4 cases, including one case that discontinued the study, during the follow-up observation period after the completion of the treatment with this drug.

Status epilepticus

No clinical studies have been conducted in Japan.

[Pharmacology]

This drug acts on the central nervous system, and will primarily inhibit the reticular activating system, displaying sedative-hypnotic action. The onset of action is slow, and this drug is classified as a long-acting drug. At the sedative-hypnotic dose or lower, it will inhibit seizures induced by electric stimulation or a drug.[31 ~ 34)]

[Physicochemical information related to the active ingredient]

Generic name: Phenobarbital sodium

Chemical name: 5-Ethyl-5-phenyl-2,4,6 (1*H*,3*H*,5*H*)-pyrimidinetrione, monosodium salt

Molecular formula: $C_{12}H_{11}N_2NaO_3$

Molecular weight: 254.22

Structural formula:



Characteristics: This product is a white crystal or crystalline powder. The product is extremely readily soluble in water, somewhat readily soluble in ethanol (95), and mostly insoluble in diethyl ether and chloroform. This product is hygroscopic and will gradually decompose when left to sit within a humid atmosphere. The pH of an aqueous solution (when dissolving 1.0 g in 10 mL of water) is 9.2 ~ 10.2.

[Packaging]

Nobelbar 250 mg for injection: 6 vials

[Pivotal literature]

1) Iwahashi K, et al. Jpn J Clin Psychopharmacol. 1998; 1 (7): 721-727
2) Crews KR, et al. Clin Cancer Res. 2002; 8: 2200-2206
3) Capewell S, et al. Lancet. 1988; ii: 480-482
4) Holland S, et al. Clin Pharmacol Ther. 1988; 43: 231
5) Greb WH, et al. Acta Psychiatr Scand. 1980; 80 (suppl 280): 95-98
6) Gray GF, et al. Human Experimental Toxicol. 1992; 11: 265-270
7) Matsumoto R, et al. Rinsho derma (Tokyo). 1970; 12 (11): 973-983
8) Tomita H, et al. J Jpn Pediatr Soc. 1987; 91 (6): 1465-1471
9) Inoue M, et al. J Yamada Red Cross Hospital. 1988; 10 (10): 100-103
10) Osaki M, et al. Acta dermatologica (Kyoto). 1979; 74 (1,2): 51-55
11) Sexton DL, et al. J Am Med Assoc. 1941; 116 (8): 700-701
12) Kano Y. The Allergy in Practice. 2001; 21 (5): 355-359
13) Tashiro T, et al. Psychiat Neurol Jap 1986, 88 (6): 441-442
14) Holmes LB, et al. Arch Neurol. 2001; 61 (5): 673-678
15) Arpino C, et al. Epilepsia. 2000; 41 (11): 1436-1443
16) Thakker JC, et al. Indian Pediatr. 1991; 28 (1): 73-75
17) Anderson RC, J Pediatr. 1976; 89 (2): 318-319
18) Annegers JF, et al. Birth Defects Orig Artic Ser. 1975; 11 (5): 137-160
19) Delgado-Escueta AV, et al. Neurology. 1982; 32 (Suppl 5): 139-160
20) Suzuki K, et al. Perinatal Medicine. 1979; 9 (8): 1259-1264
21) Isobe K, et al. Jpn J Dev Pharmacol Ther. 1992, 5 (1): 68-70
22) Bhargava VO, et al. Dev Pharmacol Ther. 1988; 11: 8-15
23) Yamashita M. Jpn J of Pediatrics. 1987; Special Issue 40: 2923-2936
24) Schain RJ, et al. Exper Neurol. 1979; 47: 509-515
25) Diaz J, et al. Biol Neonate. 1977; 32: 77-82
26) Thorpe E, et al. Fund Chemet Toxicol. 1973; 11: 433-442
27) Rossi L, et al. Int J Cancer. 1977; 19: 179-185
28) Hisano Y, et al. Jpn J of Pediatrics. 1978; 31 (9): 1681-1684
29) Ito S, et al. Clinical study on neonatal seizures: Physician-led clinical trial. Internal material.
30) Kawada K, et al. Jpn J Clin Pharmacol Ther. 2011; 42 (4): 209-210
31) Brunton LL, et al. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th ed., NewYork, McGraw-Hill, 2006. 510-513
32) Kumagaya H Ed. Survey of Clinical Pharmacology, Vol. 4. Tokyo, Nakayama Shoten 1964. 102-104
33) Sato H. J Practical Pharmacy. 1984; 35 (1): 81-86
34) 17th Edition of the Japanese Pharmacopoeia Handbook. Tokyo, Hirokawa Shoten. 2016. C-4403-4408

[Contact for literature requests and product information inquiries]

Please contact the following regarding any internal materials listed in the pivotal literature.

Nobelpharma Co., Ltd., Customer Center
1-17-24 Shinkawa, Chuo-ku, Tokyo 104-0033
Toll-free: 0120-003-140

Manufacturer **NOBELPHARMA CO., LTD.**
1-17-24 Shinkawa, Chuo-ku, Tokyo 104-0033

NBR2011-12

※※2020年11月改訂(第12版)
※2019年5月改訂

抗けいれん剤

劇薬、向精神薬
習慣性医薬品※1)
処方箋医薬品※2)

ノーベルバール®静注用250mg

NOBELBAR® 250mg for Injection
(フェノバルビタールナトリウム凍結乾燥製剤)

日本標準商品分類番号
8 7 3 1 2 9

承認番号	22000AMX02374000
薬価収載	1968年12月
発売開始	2008年12月
再審査結果	2017年12月 (てんかん重積状態)
国際誕生	1988年10月

貯　法：室温保存
使用期限：外箱及びバイアルに表示の使用期限内に使用すること

注1) 注意-習慣性あり
注2) 注意-医師等の処方箋による使用に限ること

【禁忌（次の患者には投与しないこと）】

(1)本剤の成分又はバルビツール酸系化合物に対して過敏症の既往歴のある患者
(2)急性間欠性ポルフィリン症の患者〔ポルフィリン合成が増加し、症状が悪化するおそれがある。〕
※※(3)ボリコナゾール、タダラフィル（肺高血圧症を適応とする場合）、アスナプレビル、ダクラタスビル、マシテンタン、エルバスビル、グラゾプレビル、チカグレロル、アルチメテル・ルメファントリン、ダクラタスビル・アスナプレビル・ベクラブビル、ダルナビル・コビシスタット、トラビリン、リルピビリン、リルピビリン・テノホビル ジソプロキシル・エムトリシタビン、リルピビリン・テノホビル アラフェナミド・エムトリシタビン、ビクテグラビル・エムトリシタビン・テノホビル アラフェナミド、エルビテグラビル・コビシスタット・エムトリシタビン・テノホビル アラフェナミド、エルビテグラビル・コビシスタット・エムトリシタビン・テノホビル ジソプロキシル、ソホスブビル・ベルパタスビル、ドルテグラビル・リルピビリンを投与中の患者
〔「相互作用」の項参照〕

【組成・性状】

販売名	ノーベルバール静注用250mg
成分・含量	1バイアル中フェノバルビタールナトリウム 274mg (フェノバルビタールとして250mg)
添加物	なし
剤形・色調	白色の塊又は粉末・凍結乾燥注射剤
pH	9.2～10.2（1.0gを水10mLに溶解時）
浸透圧比	2.5～2.6（1.0gを水10mLに溶解時） (生理食塩液に対する比)

【効能・効果】

新生児けいれん
てんかん重積状態

〈効能・効果に関連する使用上の注意〉
本剤は、作用発現が遅く、長時間作用型に属することから、てんかん重積状態の患者では、速効性の薬剤を第一選択とし、本剤は第二選択以降に使用することが望ましい。〔「薬効薬理」の項参照〕

【用法・用量】

新生児けいれん
初回投与：フェノバルビタールとして、20mg/kgを静脈内投与する。けいれんがコントロールできない場合は、患者の状態に応じ、初回投与量を超えない範囲で用量を調節し、静脈内に追加投与する。
維持投与：フェノバルビタールとして、2.5～5mg/kgを1日1回静脈内投与する。

てんかん重積状態
フェノバルビタールとして、15～20mg/kgを1日1回静脈内投与する。

〈用法・用量に関連する使用上の注意〉
〈新生児けいれん及びてんかん重積状態〉
意識障害、血圧低下、呼吸抑制があらわれることがあるので、用量調節を適切に行うために、本剤の血中濃度測定を行うことが望ましい。また、呼吸抑制があらわれた場合には、直ちに人工呼吸など適切な処置を行うこと。〔「小児等への投与」、「過量投与」の項参照〕
〈新生児けいれん〉
新生児では、5～10分かけて緩徐に投与すること。ただし、患者の状態に応じ、より緩徐に投与することも考慮すること。また、追加投与を行う際には、患者の状態を観察し、初回投与から十分な間隔をあけた上で、実施すること。〔臨床成績〕の項参照〕
〈てんかん重積状態〉
小児及び成人では、10分以上かけて緩徐に投与すること。ただし、100mg/分の投与速度を超えないこと。

【使用上の注意】

1、慎重投与（次の患者には慎重に投与すること）
(1)低出生体重児〔これらの症例に対する投与経験が少ない。〔「小児等への投与」の項参照〕。〕
(2)高齢者〔「高齢者への投与」の項参照〕
(3)虚弱者、呼吸機能の低下している患者〔呼吸抑制を起こすことがある。〕
(4)頭部外傷後遺症又は進行した動脈硬化症の患者〔本剤の作用が強くあらわれることがある。〕
(5)心障害のある患者〔血圧低下や心拍数減少を起こすおそれがある。〕
(6)肝障害、腎障害のある患者〔これらの症状の悪化、また血中濃度上昇のおそれがある。〕
(7)薬物過敏症の患者
(8)アルコール中毒のある患者〔中枢抑制作用が増強される。〕
(9)薬物依存の傾向又は既往歴のある患者〔精神依存及び身体依存を示すことがある。〕
(10)重篤な神経症の患者〔依存を示すおそれがある。〕
(11)甲状腺機能低下症の患者〔甲状腺機能の異常をきたすおそれがある。〕

2、重要な基本的注意
(1)適用中における投与量の急激な減少ないし投与の中止により、てんかん重積状態があらわれることがあるので、投与を中止する場合には、徐々に減量するなど慎重に行うこと。なお、高齢者、虚弱者の場合は特に注意すること。
(2)適用中は定期的に肝・腎機能、血液検査を行うことが望ましい。
(3)眠気、注意力・集中力・反射運動能力等の低下が起こることがあるので、本剤投与中の患者には自動車の運転等危険を伴う機械の操作に従事させないよう注意すること。

3．相互作用

本剤は薬物代謝酵素CYP3A等の誘導作用を有する[*1]。

※※(1)併用禁忌（併用しないこと）

薬剤名等	臨床症状・措置方法	機序・危険因子
ボリコナゾール （ブイフェンド） タダラフィル （肺高血圧症を適応とする場合：アドシルカ） アスナプレビル （スンベプラ） ダクラタスビル （ダクルインザ） マシテンタン （オプスミット） エルバスビル （エレルサ） グラゾプレビル （グラジナ） チカグレロル （ブリリンタ） アルテメテル・ルメファントリン （リアメット配合錠） ダクラタスビル・アスナプレビル・ベクラブビル （ジメンシー配合錠） ダルナビル・コビシスタット （プレジコビックス配合錠） ドロピリン （ピフェルトロ）	これらの薬剤の代謝が促進され、血中濃度が低下するおそれがある。	本剤の肝薬物代謝酵素（CYP3A）誘導作用による。
リルピビリン （エジュラント） リルピビリン・テノホビル ジソプロキシル・エムトリシタビン （コムプレラ配合錠）	リルピビリンの代謝が促進され、血中濃度が低下するおそれがある。	
リルピビリン・テノホビル アラフェナミド・エムトリシタビン （オデフシィ配合錠）	リルピビリン及びテノホビル アラフェナミドの血中濃度が低下するおそれがある。	本剤の肝薬物代謝酵素（CYP3A）誘導作用及びP糖蛋白誘導作用による。
ビクテグラビル・エムトリシタビン・テノホビル アラフェナミド （ビクタルビ配合錠）	ビクテグラビル及びテノホビル アラフェナミドの血中濃度が低下するおそれがある。	
エルビテグラビル・コビシスタット・エムトリシタビン・テノホビル アラフェナミド （ゲンボイヤ配合錠）	エルビテグラビル、コビシスタット及びテノホビル アラフェナミドの血中濃度が低下するおそれがある。	
エルビテグラビル・コビシスタット・エムトリシタビン・テノホビル ジソプロキシル （スタリビルド配合錠）	エルビテグラビル及びコビシスタットの血中濃度が低下するおそれがある。	
ソホスブビル・ベルパタスビル （エプクルーサ配合錠）	ソホスブビル及びベルパタスビルの血中濃度が低下するおそれがある。	
ドルテグラビル・リルピビリン （ジャルカ配合錠）	ドルテグラビル及びリルピビリンの血中濃度が低下するおそれがある。	本剤の肝薬物代謝酵素（CYP3A）誘導作用及びUGT1A1誘導作用による。

※(2)併用注意（併用に注意すること）

薬剤名等	臨床症状・措置方法	機序・危険因子
中枢神経抑制剤 フェノチアジン誘導体 バルビツール酸誘導体 トランキライザー トピラマート等 抗ヒスタミン剤 ジフェンヒドラミン等 アルコール	相互に作用が増強されることがあるので、減量するなど注意すること。	相加的中枢神経抑制作用による。
MAO阻害剤		機序不明
三環系抗うつ剤 イミプラミン等 四環系抗うつ剤 マプロチリン等	(1)相互に作用が増強されることがあるので、減量するなど注意すること。 (2)これらの抗うつ剤の血中濃度が低下することがある[*2]。	(1)相加的中枢神経抑制作用による。 (2)本剤の肝薬物代謝酵素誘導作用による。
メチルフェニデート	本剤の血中濃度が上昇することがあるので、本剤を減量するなど注意すること。	メチルフェニデートが肝代謝を抑制すると考えられている。
バルプロ酸 スチリペントール	(1)本剤の血中濃度が上昇し、作用が増強されることがある。 (2)これらの薬剤の血中濃度が低下することがある[*2]。	(1)これらの薬剤が肝代謝を抑制する。 (2)本剤の肝薬物代謝酵素誘導作用による。
クロバザム	(1)本剤の血中濃度が上昇することがある。 (2)クロバザムの血中濃度が低下することがある[*2]。	(1)機序不明 (2)本剤の肝薬物代謝酵素誘導作用による。
イリノテカン[*1]	イリノテカンの活性代謝物の血中濃度が低下し、作用が減弱することがあるので、併用を避けることが望ましい。	本剤の肝薬物代謝酵素誘導作用による。

薬剤名等	臨床症状・措置方法	機序・危険因子
主にCYP3Aの基質となる薬剤 アゼルニジピン[11] イダラビモド イマチニブ カルバマゼピン シクロスポリン ゾニサミド タクロリムス フェロジピン ベラパミル モンテルカスト[11]等 副腎皮質ホルモン剤 デキサメタゾン等 卵胞ホルモン剤・黄体ホルモン剤 ノルゲストレル・エチニルエストラジオール等 PDE5阻害剤 タダラフィル（勃起不全、前立腺肥大症に伴う排尿障害等を適応とする場合：シアリス、ザルティア、シルデナフィル、バルデナフィル	これらの薬剤の血中濃度が低下し、作用が減弱することがあるので、用量に注意すること[11]。	本剤の肝薬物代謝酵素誘導作用による。
アミノフィリン水和物 クロラムフェニコール デオキシリン パロキセチン[11] フレカイニド		
ラセトリモン デフェラシロクス ナナダリフロジン	これらの薬剤の血中濃度が低下することがある[11]。	本剤がこれらの薬剤のグルクロン抱合を促進する。
ルフィナミド		機序不明
アピキサバン		本剤の肝薬物代謝酵素誘導作用及びP糖蛋白誘導作用による。
ソホスブビル レジパスビル・ソホスブビル グレカプレビル・ピブレンタスビル テノホビル アラフェナミド		本剤の P糖蛋白誘導作用による。
ドキシサイクリン	ドキシサイクリンの血中濃度半減期が短縮することがある。	本剤の肝薬物代謝酵素誘導作用による。
クマリン系抗凝血剤 リルファリン	クマリン系抗凝血剤の作用が減弱することがあるので、通常より頻回に血液凝固時間の測定を行い、クマリン系抗凝血剤の量を調整すること。	
アルベンダゾール	アルベンダゾールの活性代謝物の血中濃度が低下し、効果が減弱することがある。	機序不明

薬剤名等	臨床症状・措置方法	機序・危険因子
利尿剤 チアジド系降圧利尿剤等	起立性低血圧が増強されることがあるので、減量するなど注意すること。	機序は不明であるが、高用量の本剤は血圧を低下させることがある。
アセタゾラミド	くる病、骨軟化症があらわれやすい。	本剤によるビタミンDの不活性化促進、又はアセタゾラミドによる骨吸収抑制、代謝性アシドーシス等が考えられている。
アセトアミノフェン[11]	本剤の長期連用者は、アセトアミノフェンの代謝物による肝障害を生じやすくなる。	本剤の肝薬物代謝酵素誘導作用により、アセトアミノフェンから肝毒性を持つ*N*-アセチル-*p*-ベンゾキノンイミンへの代謝が促進されると考えられている。
セイヨウオトギリソウ（St. John's Wort、セント・ジョーンズ・ワート）含有食品	本剤の代謝が促進され血中濃度が低下するおそれがあるので、本剤投与時はセイヨウオトギリソウ含有食品を摂取しないよう注意すること。	セイヨウオトギリソウの肝薬物代謝酵素誘導作用によると考えられている。

注3）本剤を減量又は中止する場合には、これらの薬剤の血中濃度の上昇に注意すること。

4．副作用

新生児けいれんを対象として実施された国内臨床試験において安全性を評価した10例中、副作用（臨床検査値異常を含む）発現症例は6例（60%）で、呼吸抑制3例3件（30%）、徐脈傾向低下2例2件（20%）、血圧低下1例1件（10%）、体重減少1例1件（10%）、体温低下1例1件（10%）、気管支分泌増加1例1件（10%）、尿量減少1例1件（10%）であった。（本認可時）

参※新生児けいれん寛解量として実施された使用成績調査において安全性を評価した320例中、副作用（臨床検査値異常を含む）発現症例は17例22件（5.2%）で、主な副作用は、血圧低下4例4件（1.2%）、呼吸抑制2例2件（0.6%）であった。（再審査終了時）

てんかん重積状態を対象として実施された使用成績調査において安全性を評価した334例中、副作用（臨床検査値異常を含む）発現症例は107例176件（32.8%）で、主な副作用は、肝機能障害30例33件（9.0%）、傾眠13例14件（3.9%）、血圧低下12例13件（3.6%）、呼吸抑制6例7件（1.8%）であった。（再審査終了時）

(1)重大な副作用[11]

1）**中毒性表皮壊死融解症（Toxic Epidermal Necrolysis：TEN）**[11]、**皮膚粘膜眼症候群（Stevens-Johnson症候群）**[11]、**紅皮症（剥脱性皮膚炎**[11]**）（頻度不明）**：観察を十分に行い、発熱、紅斑、水疱・びらん、そう痒感、眼脂、眼充血、口内炎等の異常が認められた場合には、投与を中止し、副腎皮質ホルモン剤の投与等の適切な処置を行うこと[11]。

2）**過敏症症候群（0.1%）**：初期症状として発疹、発熱がみられ、さらにリンパ節腫脹、肝機能障害等の臓器障害、白血球増加、好酸球増多、異型リンパ球出現等を伴う遅発性の重篤な過敏症状があらわれることがあるので、観察を十分に行い、このような症状があらわれた場合には、投与を中止し、適切な処置を行うこと。なお、ヒトヘルペスウイルス6（HHV-6）等のウイルスの再活性化を伴うことが多く、発疹、発熱、肝機能障害等の症状が再燃あるいは遷延化することがあるので注意すること[11]。

5) **依存性（頻度不明）**：連用により薬物依存を生じることがあるので、観察を十分に行い、用量及び使用期間に注意し慎重に投与すること。また、連用中における投与量の急激な減少ないし投与の中止により、不安、不眠、けいれん、悪心、幻覚、妄想、興奮、錯乱又は抑うつ状態等の離脱症状があらわれることがあるので、投与を中止する場合には、徐々に減量するなど慎重に行うこと。

6) **顆粒球減少（0.4%）、血小板減少（頻度不明）**：観察を十分に行い、異常が認められた場合には、投与を中止し、適切な処置を行うこと。

5) **肝機能障害（3.6%）**：AST（GOT）、ALT（GPT）、γ-GTPの上昇等を伴う肝機能障害があらわれることがあるので、観察を十分に行い、異常が認められた場合には、投与を中止するなど適切な処置を行うこと。

6) **呼吸抑制[注7]（0.7%）**：観察を十分に行い、異常が認められた場合には、投与を中止するなど適切な処置を行うこと。

(2)その他の副作用[注4]

	1～5%未満	1%未満	頻度不明
過敏症[注5]		中毒疹様発疹	紅斑、蕁麻疹、麻疹様発疹
血液[注6]			血小板減少、巨赤芽球性貧血
肝臓[注6]	AST(GOT)・ALT(GPT)・γ-GTP上昇等の肝機能障害		黄疸
腎臓[注6]		蛋白尿等の腎障害	
精神神経系	眠気	眩暈、頭痛、精神機能低下	アステリキシス(asterixis)、せん妄、昏迷、錯乱、構音障害、知覚異常、運動失調、興奮、多動
循環器	血圧低下	徐脈	
消化器		食欲不振	
骨・歯			くる病[注9]、骨軟化症[注9]、歯牙の形成不全[注9]、低カルシウム血症
内分泌			甲状腺機能検査値（血清T4値等）の異常
その他		炭素酸塩濃度低下、気管支分泌増加、尿量減少、発熱	血清無機塩の低下、ヘマトポルフィリン尿[注9]、体温低下

注4）てんかん重積状態を対象として実施された使用成績調査の結果に基づき、発現頻度を記載した。なお、本調査では発現しなかった副作用については頻度不明として記載した。
注5）投与を中止すること。
注6）投与を中止するなど適切な処置を行うこと。
注7）観察を十分に行い、異常が認められた場合には、投与を中止するなど適切な処置を行うこと。
注8）連用によりあらわれることがある。
注9）連用によりあらわれることがあるので、観察を十分に行い、異常（血清アルカリフォスファターゼ値の上昇、血清カルシウム・無機リンの低下等）があらわれた場合には、減量又はビタミンDの投与等適切な処置を行うこと。

5．高齢者への投与

少量から投与を開始するなど慎重に投与すること。なお、投与を中止する場合には、徐々に減量するなど慎重に行うこと。[高齢者では、呼吸抑制、興奮、抑うつ、錯乱等があらわれやすい（「重要な基本的注意」の項参照）。]

6．妊婦、産婦、授乳婦等への投与

(1)妊婦又は妊娠している可能性のある婦人には、治療上の有益性（母体のてんかん発作頻発を防ぎ、胎児を低酸素状態から守る）が危険性を上回ると判断される場合にのみ投与すること。[妊娠中に本剤を単独、又は併用投与された患者の中に、奇形を有する児（口唇裂、口蓋裂、心奇形[注10]、大動脈縮窄等）を出産した例が多いとの疫学的調査報告がある。]

(2)妊娠中の投与により、新生児に出血傾向、呼吸抑制等を起こすことがある。

(3)分娩前に連用した場合、出産後新生児に離脱症状（多動、振戦、反射亢進、痙攣等）があらわれることがある。

(4)妊娠中の投与により、葉酸低下が生じるとの報告がある[注8]。

(5)授乳中の婦人への投与は避けることが望ましいが、やむを得ず投与する場合には、授乳を避けさせること。[ヒト母乳中へ移行し、新生児、乳児に傾眠、哺乳量低下を起こすことがある[注8、注9]。]

7．小児等への投与

本剤の主要代謝系は生後10日～20日に完成するとの報告があることから、特に低出生体重児及び新生児では血中濃度モニタリングを実施することが望ましい[注8]。

8．過量投与[注8]

(1)**症状**：

中枢神経系及び心血管系抑制。血中濃度40～45μg/mL以上で眠気、眼振、運動失調が起こり、重症の中毒では昏睡状態となる。呼吸は早期より抑制され、脈拍は弱く、皮膚には冷汗があり、体温は下降する。脳の合併症や腎障害の危険性もある。

(2)**処置**：

呼吸管理。炭酸水素ナトリウム投与による尿アルカリ化、利尿剤投与により薬物の排泄を促進させる。重症の場合は、血液透析や血液灌流を考慮すること。

9．適用上の注意

(1)本剤は静脈内にのみ投与すること。

(2)調製方法

1)投与に際しては、通常、1バイアルを5mLの注射用水又は生理食塩液に溶解する（溶解後の濃度：フェノバルビタールとして50mg/mL）。なお、溶解後は、速やか（6時間以内）に使用すること。

2)以下に示す注射薬との配合変化を起こすことが確認されているので、混合しないこと。ドパミン塩酸塩、L-アスパラギン酸カリウム、メサデトレノン（ビタミンB1）、パクロニウム臭化物、アミカシン硫酸塩、ゲンタマイシン硫酸塩、注射用エリスロマイシン（エリスロマイシンラクトビオン酸塩）。

10．その他の注意

(1)ラット新生児への大量反復投与（60mg/kg皮下投与）で、脳の成長が対照群に比較して抑制されたとの報告がある[注8、注9]。

(2)ラット及びマウスに長期間大量投与（ラット：25mg/kg、マウス：75mg/kg）したところ、対照群に比較して肝腫瘍の発生が有意に増加したとの報告がある[注8、注9]。

(3)血清免疫グロブリン（IgA、IgG等）の異常があらわれることがある[注9]。

(4)本剤と他の抗てんかん薬（フェニトイン、カルバマゼピン）との間に交差過敏症（過敏症症候群を含む皮膚過敏症）を起こしたとの報告がある。

【薬物動態】

薬物血中濃度

本剤を新生児けいれんに対し、初回投与量として20mg/kgを、けいれんがコントロールできない場合は追加投与として同量を、維持投与量として1日1回2.5～5mg/kgを、静脈内投与した。その結果、初回投与量は17.5～20.8mg/kg、追加投与量は20.1mg/kg、維持投与量は2.2～5mg/kgであった。初回投与後2時間における血中濃度（9例）は、22.38±2.31μg/mL（18.8～25.8μg/mL）；平均±標準偏差（最小値～最大値）であり、追加投与（初回投与30分後）を実施した1例では42.8μg/mLであった。また、維持投与最終投与前（中止時）の血中濃度（10例）は、27.65±9.46μg/mL（18.7～45.3 μg/mL）であった[1]。

【臨床成績】

新生児けいれん

新生児けいれんを有する患児10症例（年齢：平均日齢25日（範囲：日齢1～5日）、出生体重：平均2867g（範囲：1144～3796g）、在胎週数：平均37.25週（範囲：33.1～41.1週））を対象に、本剤の初回投与（けいれんが消失しなかった場合は、初回投与30分後に追加投与を実施）30分後での臨床所見に基づくけいれんの有無を主要評価項目として非対照、非盲検の多施設共同試験を行った結果、全例で「著効」と判定された。なお、追加投与が実施された症例は、1例であり、本症例については、初回投与後30分では、「有効」と判定されたものの、断続的にけいれんが持続したため、追加投与が行われ、追加投与後にけいれんが消失し「著効」と判定された。また、本試験では、6日間の維持投与期間を設定して実施したが、維持投与が実施された9例のうち、2例にけいれんの再発が認められた。また、本剤投与終了後の後観察期では、中止例1例を含む4例にけいれんの再発が認められた[2][3]。

てんかん重積状態

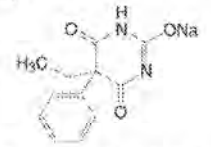
国内では臨床試験を実施していない。

【薬効薬理】

本剤は、中枢神経系に作用し、主に網様体賦活系を抑制して、鎮静催眠作用をあらわす。作用の発現は遅く、長時間作用型に分類される。鎮静催眠量以下で電気刺激あるいは薬物によるけいれんを抑制する[4][5]。

【有効成分に関する理化学的知見】

一般名：フェノバルビタールナトリウム
(Phenobarbital Sodium)
化学名：5-Ethyl-5-phenyl-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione, monosodium salt
分子式：$C_{12}H_{11}N_2NaO_3$
分子量：254.22
構造式：



性　状：白色の結晶又は結晶性の粉末である。水に極めて溶けやすく、エタノール(95)にやや溶けやすく、ジエチルエーテル及びクロロホルムにほとんど溶けない。吸湿性で、湿った空気中に放置すると、徐々に分解する。水溶液（1.0gを水10mLに溶解時）のpH9.2～10.2である。

【包　装】

ノーベルバール静注用250mg：5バイアル

【主要文献】

1) 岩橋和彦, 他. 臨床精神薬理. 1998 ; 1(7) : 721-727
2) Crews KR, et al. Clin Cancer Res. 2002 ; 8 : 2202-2209
3) Capewell S, et al. Lancet. 1988 ; ii : 480-482
4) Holland S, et al. Clin Pharmacol Ther. 1998 ; 63 : 231
5) Greb WH, et al. Acta Psychiatr Scand. 1989 ; 80(suppl 350) : 95-98
6) Bray GP, et al. Human Experimental Toxicol. 1992 ; 11 : 265-270
7) 松本敬一, 他. 皮膚科の臨床. 1970 ; 12(11) : 973-982
8) 富樫　斉, 他. 日本小児科学会雑誌. 1987 ; 91(6) : 1463-1471
9) 井上正和, 他. 国立療育センター病院雑誌. 1988 ; 10(10) : 100-103
10) 尾崎元昭, 他. 皮膚科紀要. 1979 ; 74(1.2) : 51-55
11) Sexton DL, et al. J Am Med Assoc. 1941 ; 116(8) : 700-701
12) 神野美子, アレルギーの臨床. 2001 ; 21(5) : 355-359
13) 田代邦雄, 他. 精神神経学雑誌. 1986 ; 88(6) : 441-442
14) Holmes LB, et al. Arch Neurol. 2001 ; 61(5) : 673-678
15) Arpino C, et al. Epilepsia. 2000 ; 41(11) : 1436-1443
16) Thakker JC, et al. Indian Pediatr. 1991 ; 28(1) : 73-75
17) Anderson RC. J Pediatr. 1976 ; 89(2) : 318-319
18) Annegers JF, et al. Birth. Defects Orig. Artic Ser. 1975 ; 11(5) : 157-160
19) Delgado-Escueta AV, et al. Neurology. 1982 ; 42(Suppl 5) : 149-160
20) 鈴木喜八郎, 他. 周産期医学. 1979 ; 9(8) : 1259-1264
21) 織部健一, 他. 発達薬理誌. 1992 ; 5(1) : 65-70
22) Bhargava VO, et al. Dev Pharmacol Ther. 1988 ; 11 : 8-13
23) 山下　衛. 小児科臨床. 1987 ; 40増 : 2923-2936
24) Schain RJ, et al. Exper Neurol. 1975 ; 47 : 509-515
25) Diaz J, et al. Biol Neonate. 1977 ; 32 : 77-82
26) Thorpe E, et al. Food Cosmet Toxicol. 1973 ; 11 : 423-442
27) Rossi L, et al. Int J Cancer. 1977 ; 19 : 179-185
28) 久野保夫, 他. 小児科臨床. 1978 ; 31(9) : 1681-1684
29) 伊藤進, 他. 新生児けいれんに対する臨床試験：医師主導治験. 社内資料
30) Kawada K, et al. Jpn J Clin Pharmacol Ther. 2011 ; 42(4) : 205-210
31) Brunton LL, et al. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th ed. New York, McGraw-Hill. 2006. 510-511
32) 薬谷 寿 監修. 臨床薬理学大系. 第4巻. 東京, 中山書房. 1963. 102-104
33) 佐藤公道. 薬局. 1984 ; 35(1) : 81-86
34) 第十七改正日本薬局方解説書. 東京, 廣川書店. 2016. C-4403-4408

【文献請求先・製品情報お問い合わせ先】

下記文献に記載の社内資料につきましては下記にご請求下さい。

ノーベルファーマ株式会社　カスタマーセンター
〒104-0033　東京都中央区新川1-17-24
フリーダイヤル：0120-003-140

製造販売元　ノーベルファーマ株式会社
東京都中央区新川1－17－24

NSR2011-12

Electronic Patent Application Fee Transmittal					
Application Number:	17025881				
Filing Date:	18-Sep-2020				
Title of Invention:	Lyophilized Compositions of Phenobarbital Sodium Salt				
First Named Inventor/Applicant Name:	Bala Tripura Sundari Chodavarapu				
Filer:	Jay Philip Lessler				
Attorney Docket Number:	104078.0001US				
Filed as Large Entity					
Filing Fees for					
Description		**Fee Code**	**Quantity**	**Amount**	**Sub-Total in USD($)**
Basic Filing:					
DOCUMENT FEE FOR THIRD-PARTY SUBMISSIONS		1818	1	180	180
Pages:					
Claims:					
Miscellaneous-Filing:					
Petition:					
Patent-Appeals-and-Interference:					
Post-Allowance-and-Post-Issuance:					

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	180

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
17/025,881	09/18/2020	Bala Tripura Sundari Chodavarapu	104078.0001US	8113

149344 7590 09/02/2021

Umberg Zipser LLP
1920 Main Street Suite 750
Irvine, CA 92614

EXAMINER	
ZHANG, YANZHI	

ART UNIT	PAPER NUMBER
1617	

NOTIFICATION DATE	DELIVERY MODE
09/02/2021	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patents@umbergzipser.com
rdean@umbergzipser.com

PTOL-90A (Rev. 04/07)

UNITED STATES DEPARTMENT OF COMMERCE
U.S. Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450

APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR/ PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
17/025,881	09/18/2020	Chodavarapu et al.	104078.0001US

	EXAMINER	
Umberg Zipser LLP 1920 Main Street Suite 750 Irvine, CA 92614	MARIANNE C SEIDEL	
	ART UNIT	PAPER
	1600	20210901

DATE MAILED: _____

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner for Patents

The third-party submission under 37 CFR 1.290 filed on 8/31/2021 for the instant application has been determined to be compliant with 35 U.S.C. 122(e) and 37 CFR 1.290 and is being entered in the application. Please allow a few days for the submission to be visible in the Patent Application Information Retrieval (PAIR) system.

/MARIANNE C SEIDEL/
Quality Assurance Specialist, Art Unit 1600

PTO-90C (Rev.04-03)

To: rdean@umbergzipser.com,patents@umbergzipser.com,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 149344

Sep 03, 2021 05:10:50 AM

Dear PAIR Customer:

Umberg Zipser LLP
1920 Main Street Suite 750
Irvine, CA 92614
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 149344 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:
The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
17025881	M327	09/02/2021	104078.0001US

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

To: rdean@umbergzipser.com,patents@umbergzipser.com,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 149344

Sep 23, 2021 05:04:49 AM

Dear PAIR Customer:

Umberg Zipser LLP
1920 Main Street Suite 750
Irvine, CA 92614
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 149344 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:
The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
17025881	CTFR	09/23/2021	104078.0001US
	892	09/23/2021	104078.0001US

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
17/025,881	09/18/2020	Bala Tripura Sundari Chodavarapu	104078.0001US	8113

149344 7590 09/23/2021

Umberg Zipser LLP
1920 Main Street Suite 750
Irvine, CA 92614

EXAMINER
ZHANG, YANZHI

ART UNIT	PAPER NUMBER
1617	

NOTIFICATION DATE	DELIVERY MODE
09/23/2021	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patents@umbergzipser.com
rdean@umbergzipser.com

PTOL-90A (Rev. 04/07)

Office Action Summary	Application No. 17/025,881	Applicant(s) Chodavarapu et al.	
	Examiner YANZHI ZHANG	Art Unit 1617	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 08/24/21.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.

2a)☑ This action is **FINAL.** 2b) ☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 1-2,4-20 and 24-27 is/are pending in the application.

5a) Of the above claim(s) ____ is/are withdrawn from consideration.

6) ☐ Claim(s) ____ is/are allowed.

7) ☑ Claim(s) 1-2,4-20 and 24-27 is/are rejected.

8) ☐ Claim(s) ____ is/are objected to.

9) ☐ Claim(s) ____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on ____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. ____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date ____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date ____.

4) ☐ Other: ____.

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Claim Status

This action is a response to papers filed on August 24, 2021. Claims 1, 2, 4, 6-11, and 13-16 are amended. Claims 3, 21-23 are cancelled. New claims 24-27 are added. Accordingly, claims 1, 2, 4-20, 24-27 are pending in the application and under consideration on the merit.

Withdrawn Claim Objection

The objection to claim 22 is hereby withdrawn in view amendments dated 08/24/21.

Applicant's amendments dated 08/24/21 necessitate the new grounds of rejection set forth below.

Claim Rejections - 35 U.S.C. 112(b)

The following is a quota

The following is a quotation of 35 U.S.C. 112(b):
(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1, 2, 4-16, and 24-27 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and

distinctly claim the subject matter which the inventor or a joint inventor (or for applications subject to pre-AIA 35 U.S.C. 112, the applicant), regards as the invention.

Claims 1 and 2 recites "... solution with a pH at or between 9.3 to 9.6 ..." in line 6 and 5, respectively. As written, it is unclear what pH the claimed solution. The conjunction word "at" should be followed a number not a range. It's proper to say "a pH at 9.3 or 9.6" or "a pH between 9.3 and 9.6". Thus, the metes and bounds of the claimed scope are not defined.

Since claims 1 and 2 are independent claims, the dependents are included in the rejection.

It is suggested to correct the pH range in claims 1 and 2 or amend claims 1 and 2 by reciting the pH is at 9.3 or 9.6

Claim Rejections - 35 U.S.C. 112(d)

The following is a quotation of 35 U.S.C. 112(d):

> (d) REFERENCE IN DEPENDENT FORMS.—Subject to subsection (e), a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

The following is a quotation of pre-AIA 35 U.S.C. 112, fourth paragraph:

> Subject to the following paragraph [i.e., the fifth paragraph of pre-AIA 35 U.S.C. 112], a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

Claim 7 is rejected under 35 U.S.C. 112(d) or pre-AIA 35 U.S.C. 112, 4th paragraph, as being of improper dependent form for failing to further limit the subject matter of the claim upon which it depends, or for failing to include all the limitations of the claim upon which it depends.

As written, the solution in claim 1 has a pH at 9.3 or 9.6 or alternatively between 9.3 and 9.6 (independent claim). However, the pH range between 9.2 and 10.2 is broader than the range in claim 1.

Applicant may cancel the claim(s), amend the claim(s) to place the claim(s) in proper dependent form, rewrite the claim(s) in independent form, or present a sufficient showing that the dependent claim(s) complies with the statutory requirements.

Claim Rejections - 35 U.S.C. 103

The text of those sections of Title 35 of the U.S. Code not included in this action can be found in a prior Office action.

Claims 1-2, 8-13, 15, and 21-27 are rejected under 35 U.S.C. 103 as obvious over Parker et al (US 20170143719 A1, publication date: May 25, 2017, "Parker", of record) in view of FDA document of Lyophilization of Parenteral (7/93) (non-paten literature, published 11/11/2014, "FDA_7/93").

Claims 1, 2, 6-16, and 24-27 embrace a pharmaceutical composition comprising a storage-stable powder form of lyophilized amorphous Phenobarbital Sodium that forms no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution, wherein the storage- stable powder form of lyophilized amorphous Phenobarbital Sodium is produced by a process describe in clam 1.

In addition, claims 1 (product) and 2 (method claim) use the open-ended transitional phrase "comprising". Thus, they allow for the presence of additional unrecited steps or components.

Clam 1 is a product-by-process claim. According to MPEP 2113I: "[E]ven though product-by-process claims are limited by and defined by the process, determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process."

Parker is directed to stable formulation of **phenobarbital sodium** injection (title, read on the limitation of the active ingredient instant claim 1 and 2). Parker teaches that stable phenobarbital sodium **solutions for injection** include phenobarbital sodium in an amount of 15 to 200 mg/mL; a C1-C4 alcohol in an amount of 105 to 160 mg/mL; and propylene glycol in an amount of between 620 and 830 mg/mL and have a **pH** of between 9 and 12 (abstract, encompassing the limitations of pH range in the instant claims 1-2 and 7). Parker also teaches that the solutions for injection are extremely **stable** and do not generate any significant amounts of impurities, including phenyl ethyl acetyl urea (PEAU) and phenyl butyric acid (PBA, during extended storage ([0017]), i.e. impurity-free formulations ([0016]), implying the limitation of no more than 0.2%, 0.1% or 0.05% PEAU in the instant claims 1-2, 8-9, 12, and 23).

While teaching adjusting pH conditions, Parker does not expressly teach lyophilization. These deficiencies are cured by FDA_7/93.

FDA_7/93 is directed to Lyophilization of Parenteral (title). FDA_7/93 teaches that the lyophilization process consists of three separate, unique, and interdependent processes; freezing, primary drying (sublimation), and secondary drying (1st para of page 1/22, read on the process of removing water in the instant claim 1). FDA_7/93 also teaches the following steps:

- Dissolving the drug and excipients in a suitable solvent, generally water for injection (WFI).
- Sterilizing the bulk solution by passing it through a 0.22 micron bacteria-retentive filter.
- Filling into individual sterile containers and partially stoppering the containers under aseptic conditions.
- Transporting the partially stoppered containers to the lyophilizer and loading into the chamber under aseptic conditions.
- Freezing the solution by placing the partially stoppered containers on cooled shelves in a freeze-drying chamber or pre-freezing in another chamber.
- Applying a vacuum to the chamber and heating the shelves in order to evaporate the water from the frozen state.
- Complete stoppering of the vials.

FDA_7/93 indicates that products are manufactured in the lyophilized form due to their instability when in solution (2nd para. under the heading PRODUCT TYPE/FORMULATION on page 2/22) and the **free water** in a product is that water that is absorbed on the surfaces of the product and must be removed to limit further biological and chemical reactions (see 4th and 6th para. on page 16/22). FDA_7/93 further teaches that an obvious concern with the lyophilized product is the **amount of moisture** present in vials. The manufacturer's data for the establishment of moisture specifications for both product release and stability should be reviewed. Stability testing should also include provisions for the assay of aged samples and subsequent **reconstitution** of these aged samples for the maximum amount of time specified in the labeling (see 1st and 4th para. under the heading Stability Testing on page 13/22). Additionally, FDA_7/93 teaches that the product is frozen at a **temperature** well below the eutectic point. It is desirable after freezing and during primary drying to hold the drying temperature (in the product) at least 4-50 below the eutectic point. Obviously, the manufacturer

should know the eutectic point and have the necessary instrumentation to assure the uniformity of product temperatures (see LYOPHILIZATION CYCLE AND CONTROLS on pages 5-6/22). Some of the variables would include freezing rate and temperature ramping rate (see CYCLE VALIDATION on page 8/22). Furthermore, FDA_7/93 teaches that particular sections that might introduce contamination (e.g. filling) and provided solutions to address these sections including filling of 3000 vials and stoppering on line or loading in the lyophilizer, and exposure to a portion of the **nitrogen flush** and then stoppering (see 3rd para. on page 4/22).

It would have been obvious for one of ordinary skill in the art, as of the effective filing date of the claimed invention, to choose lyophilization as taught by FDA_7/93 as the particular means for an aqueous drug solution because the particular option identified by FDA_7/93 are predictable solutions to the problem of drying an unstable drug like phenobarbital sodium, for example, enhancing the product stability in a dry state (page 1/22). The person of ordinary skill in the art would have a reasonable expectation to be of success in choosing the option. See MPEP 2141, part (III)(A) and (D). Additionally, one of ordinary skill in the art would take advantages of lyophilization taught by FDA_7/93 as set forth above.

Regarding the limitation of "a storage-stable powder form of" lyophilized amorphous in claims 1 and 2, it is reasonable to believe that, when lyophilized as taught by Snyder, the pharmaceutical composition of Phenobarbital Sodium has the same physical and chemical properties as claimed, absent evidence to the contrary.

Regarding the preamble of "no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution" in claim 2, "no more than 0.1% phenylethylacetylurea" in claim 8, "no more than 0.05% phenylethylacetylurea" in claim 9, no detectable amount of 2-ethyl-2-phenylmalonamide, etc. in claim 10, and stable up to at least 6 months at accelerated stability

conditions in claim 11; these are considered properties of the lyophilized Phenobarbital sodium. The lyophilized Phenobarbital sodium as set forth in the rejection above would meet these limitations. In the absence of the USPTO to have at its disposal the tools or facilities deemed necessary to make physical determinations of this sort, Applicants have the burden to show that this, in fact, is not the case.

Regarding the temperatures of freezing and drying and the vacuum pressure in claims 24-27, FDA_7/93 teaches the temperature needs to be well below the eutectic point, at least 4-50 below the eutectic point and the rate and manner of freezing has been shown to have an effect on the physical form (polymorph) of the drug substance. Thus, it would have been obvious to control rate and manner of freezing in order to obtain the freeze-dry product desired.

Claims 4-7, and 14 are rejected under 35 U.S.C. 103 as obvious over Parker et al (US 20170143719 A1, publication date: May 25, 2017, "Parker") in view of FDA document of Lyophilization of Parenteral (7/93) (non-paten literature, published 11/11/2014, "FDA_7/93") as applied to claims 1-2, 8-13, 15, and 21-27 in further view of Roskos et al ("Roskos", US 6077545 A, date issued: June 20, 2000, of record).

The teaching of Parker, and Snyder have been discussed as applied to claims 1-2, 8-13, 15, and 21-22. Parker, and Snyder do not expressly teach the water is water for injection (WFI), nor nitrogen purge. These deficiencies are cured by Roskos.

Roskos is directed to an improved diluent for the preparation of cisplatin suspensions from a lyophilized powder (abstract). Roskos teaches that lyophilized CDDP (a drug for cancer) is intended for reconstitution into a solution and the standard vehicle for such solution reconstitution is **Water for Injection** (WFI) (col. 3, lines 4-6, read on the limitation of the instant claim 4). Roskos teaches that the solution of example 1 is sparged with nitrogen for 30

minutes (gas flushing), when the required amount of epinephrine is added, and the mixture is stirred under a nitrogen blanket until the epinephrine dissolved, **pH is adjusted** with HCl and/or NaOH (Table of col. 5) if necessary, and additional WFI was added to achieve the final desired volume. The solution was **sparged with nitrogen** for an additional 30 minutes, and then sterile **filtered** through a 0.22 micron filter. Filtered solution was filled into 5 ml vials, the vial headspace was flushed with nitrogen, and the vials are stoppered (interpreted as sealed) (col. 5, lines 45-58, read on the limitations of the instant claims 4-5, 7, and 14).

It would have been obvious for one of ordinary skill in the art, as of the effective filing date of the claimed invention, to choose nitrogen sparge and filtering prior to adding the drug as taught by Roskos as the particular means to ensure the drug is stable and meet the Food and drug administration requirements. The person of ordinary skill in the art would have a reasonable expectation to be of success in choosing the steps because Roskos provides the exact solution of processing a stable lyophilized drug product. See MPEP 2141, part (III)(A) and (G). In addition, selection of any order of performing process steps is prima facie obvious in the absence of unexpected results (see MPEP 2144).

Claims 16-20 are rejected under 35 U.S.C. 103 as obvious over Kelly et al ("Kelly", non-patent literature, Critical Care Trauma Centre, pp. 1-3, last update: October 11, 2018, of record) and Sun et al ("Sun", US 20100035904 A1, publication-date: February 11, 2010, of record).

Claims 16-20 embrace a method of treating an individual in need of Phenobarbital Sodium, the method comprising: adding saline or dextrose to a composition comprising lyophilized amorphous Phenobarbital Sodium immediately prior to administration to form a

Phenobarbital Sodium solution; and administering the Phenobarbital Sodium solution to the individual.

Kelly is directed to **phenobarbital sodium** for **intravenous** (IV) direct **injection** or infusion (title, and the section of Administration on page 1, read on the limitation of the drug in the instant claim 16 and the administering routes in the instant claim 20). Kelly discloses that the drug, anticonvulsant, sedative, is diluted to 10mL with **sodium chloride** 0.9%, **dextrose** 5% or Ringer's Lactate and is used to **treat seizures** (i.e. epilepsy) (the section of Classification on page 1, read on the limitations of treating, saline in the instant claims 16-18). Kelly also discloses the loading dose is 10 - 15 mg/kg IV or 1000 mg in 250 mL for infusion (the section of Dose and Administration on page 1, read on the limitation of the instant claim 19).

While teaching phenobarbital sodium for intravenous Kelly does not expressly teach phenobarbital sodium is a lyophilized amorphous product as claimed. This deficiency is cured by Sun.

Sun is directed to **phenobarbital** salts; **methods of making**; and methods of use thereof (title). Sun teaches non-crystalline or **amorphous forms** of phenobarbital salts including **phenobarbital sodium** ([0011], the same drug as the instant claim 1-2). Sun also discloses non-crystalline or amorphous forms of phenobarbital salts including phenobarbital sodium ([0011], the same physical form in the instant claim 16). Sun further teaches that the concentration of phenobarbital in selected buffers or **pH conditions** will be determined using a **stability indicating assay** that distinguishes the active agent from its degradation products, and also detects any degradation that may appear in the salt solution ([0060]). Sun recognizes that phenobarbital sodium is a known salt freely soluble in water in contrast to the free acid form, but

phenobarbital sodium is hygroscopic and degrades when exposed to humid environments ([0004]).

It would have been obvious for one of ordinary skill in the art, as of the effective filing date of the claimed invention, to choose phenobarbital sodium with proper pH as taught by Sun as the particular drug to be incorporated into the method of Kelly because all of the particular options identified by Sun are predictable solutions to the drug formulations,. The person of ordinary skill in the art would have a reasonable expectation to be of success in choosing any of those options. See MPEP 2143, part (I)(B) or (E).

Regarding treating a newborn in claim 18, it would have been obvious to a pedestrian to follow the guidelines set forth for infants.

Response to Arguments

Applicant's Declaration under 37 CFR 1.132 filed 05/13/2021 by Dr. Dasaradhi Lakakraju and arguments pertaining to the obviousness of the instant claims have been previously submitted and fully considered. But, it is not persuasive for the reasons of record (see non-final rejection mailed 06/01/21).

Applicant argues that the pH of Parker is for a liquid solution, not a lyophilized powder composition of Phenobarbital Sodium.

In response, it is believed that the pH of Parker applies to the Phenobarbital Sodium solution as well lyophilized product claimed.

Applicant argues that Parker discusses using further components of the liquid solution, such as C1- C4 alcohol, propylene glycol, etc. which affect the degradation processes.

In response, it should be noted that the transition phrase "comprising" in claim 1 allows the presence of unrecited ingredients. There is no evidence of record showing the presence of alcohol adversely affects the claimed pharmaceutical composition.

For the reason above at lease, the instant claims are obviousness over the prior art.

Relevant Art

Bardat et al (non-patent literature, titled Moisture measurement: a new method for monitoring freeze-drying cycles; J Parenter Sci Technol, Nov-Dec 1993; vol. 47(6); pp. 293-9. e) is provided, but not cited, to show the state of art at the time when the invention was filed.

Bardat indicates that quality of the final product largely depends on the freeze-drying process. In turn this largely depends on an adequate control of the amount of residual moisture after freeze-drying (abstract).

CONCLUSION

No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37

CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

CONTACT INFORMATION

Any inquiry concerning this communication or earlier communications from the examiner should be directed to YANZHI ZHANG whose telephone number is (571)272-3117. The examiner can normally be reached on Monday-Friday 8am-5pm.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Johann Richter can be reached on 5712720646. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/YANZHI ZHANG/

Primary Examiner, Art Unit 1617

Notice of References Cited	Application/Control No. 17/025,881	Applicant(s)/Patent Under Reexamination Chodavarapu et al.	
	Examiner YANZHI ZHANG	Art Unit 1617	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
	A					
	B					
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Bardat et al; title: Moisture Measurement: A New Method for Monitoring Freeze-drying Cycles; TECHNOLOGY I APPLICA TIONS; vol. 47. No. 6 / November-December 1993; pp.293- 299. (Year: 1993)
	V	FDA documnet: GUIDE TO INSPECTIONS OF LYOPHILIZATION OF PARENTERALS, title: Lyophilization of Parenteral; published 11/11/2014. (Year: 2014)
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Downloaded from on September 7, 2021

Moisture Measurement: A New Method for Monitoring Freeze-drying Cycles

A. BARDAT[Δ], J. BIGUET, E. CHATENET, and F. COURTEILLE

Centre National de Transfusion Sanguine, Les Ulis, France

ABSTRACT: Quality of the final product largely depends on the freeze-drying process. In turn this largely depends on an adequate control of the amount of residual moisture after freeze-drying. Measuring this amount in the chamber of the freeze-dryer to determine the end point of sublimation and the end point of secondary drying provides a reliable control with regard to the methods traditionally used (for example rapid increase in product temperature). The purpose of this study is to evaluate the benefits and disadvantages of the different methods recommended for the monitoring of a freeze-drying cycle. Two systems for the measurement of the moisture in the freeze dryer are evaluated here: the Pirani vacuum gauge, and the moisture sensor. The moisture sensor appears to be the most sensitive and reliable way of determining both the end of sublimation and the end of secondary drying of the full load batch when placed on a freeze-dryer. The immediate benefit for the industry is to allow to scale-up without the risks of under or over estimating the freeze-drying cycle.

Introduction

A listing of the different means proposed for the determination of the ends of primary and secondary drying is presented in this study. Among the techniques used, those which adequately monitor the variations of the partial pressure of water vapor seem to be more promising. M. Roy et al., (1) have suggested using a moisture sensor whereas S. Nail (2) has proposed a Pirani gauge for the determination of the end point of the primary drying.

The aim of this study is therefore to compare the respective sensitivity of these methods as a function of: the number of overfilled vials, the level of pressure inside the chamber, and the number of vials.

A new test has been designed. It is presented herewith. Data show it offers the advantage of discriminating between the two methods on the basis of their respective ability to determine the end point of the secondary drying in addition to the end point of the primary drying.

Materials and Equipment

Materials

The materials used for this work were:

- Water for injection (Laboratoires Bruneau, Boulogne Billancourt, France),
- Mannitol (Roquette, Lestrem, France),
- Protein solution (C.N.T.S., Les Ulis, France): 50 g/l of immunoglobulins, 50 g/l of sucrose, 30 g/l of glycine.

Equipment

- A laboratory freeze-dryer—Edwards Lyoflex 0.4 freeze-dryer (0.4 m^2 shelf surface) with 3 shelves. The pressure is monitored by a capacitance manometer Datametrics Barocel 600 AB, and maintained at the required value by a closed-loop controller. The temperature sensors in contact with the product are Pt 100 resistance temperature detectors (the "probes"). The cycles are automatically monitored by a computerized system consisting of an IBM AT and a GOULD controller. The freeze-dryer is equipped with a trap and an arm to allow sampling during the cycle.
- An industrial freeze-dryer—USIFROID SMH 1000 freeze-dryer (10 m^2 shelf surface), with pressure monitored by a Pirani vacuum gauge, and temperatures by Pt 100 probes.
- Sensors—Humidity sensor: Endress+Hauser Hygrolog WMY 770 Z (Boissy St-Léger, France); Pirani vacuum gauge: Balzers TPR 100 (Meudon, France).
- Analyzers—Differential scanning calorimetry: DSC 7 Perkin Elmer (Saint Quentin en Yvelines, France) for the determination of the glass transition (Tg) of the products at different drying stages; Karl Fischer equipment: Mettler DL 18 (Mettler Instrument S.A., Viroflay, France) to determine the residual moisture content of the products.

Various Methods for Monitoring a Freeze-Drying Cycle

The end point of the different stages of the freeze-drying cycle can be determined using several methods.

Received October 14, 1992. Accepted for publication May 26, 1993.
[Δ] Author to whom correspondence should be addressed: Centre National de Transfusion Saguine, 3 Avenue des Tropiques, B.P. 100, 91943 Les ULIS CEDEX, France.

Downloaded from on September 7, 2021

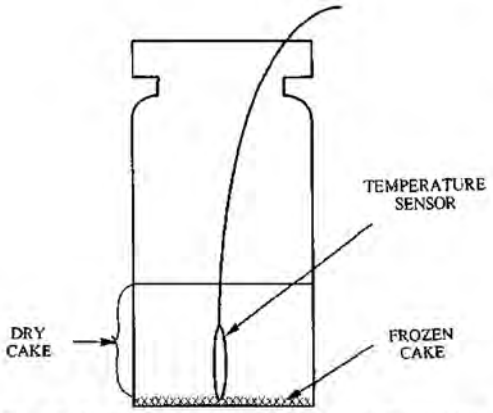


Figure 1—Diagram showing sublimation in a vial containing a temperature probe.

Product Temperature Response

This is the oldest and the most common method still used today. As the product approaches the end of primary drying, the major part of the ice has been removed by sublimation and only a thin layer remains on the bottom of the vials (Fig. 1). This ice layer is removed by sublimation and the sensor loses thermal contact with the ice. One can then observe a rapid increase in the product temperature curve. This means the end of sublimation (Fig. 2): all ice has been removed.

Secondary drying can then begin, although this is traditionally done once the temperature of the product equals the shelf temperature. Many users prefer waiting several hours before increasing the shelf temperature for secondary drying. This practice minimizes the risk of collapse or eutectic melt in those vials that dry more slowly than the monitored vials. Vials containing a temperature probe generally display more rapid primary drying (1) than the vials not monitored. Also, the vials not monitored do not exhibit perfectly uniform drying behavior, even in a freeze dryer with perfectly uniform

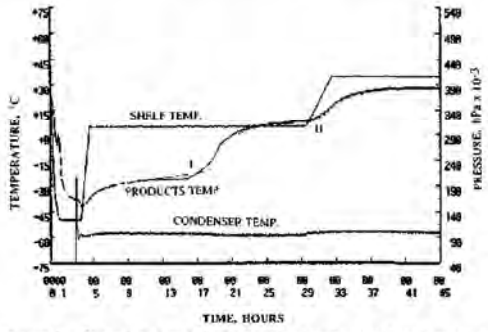


Figure 2—Curve for a lyophilization cycle showing the sharp temperature increase: (I) at 16 hrs reflects the end point of the primary drying. (II) shows the product temperature response to the rise in temperature of the shelf for the secondary drying.

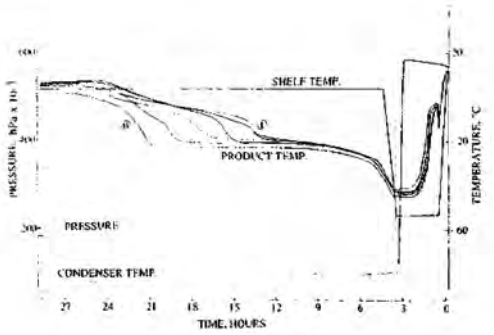


Figure 3—Curve showing the sharp temperature increase in 6 vials at various places on the shelf. Each 125 ml vial contains 20 ml of 3% mannitol solution (p/vol).

shelf temperature (3). In this research, we observed faster sublimation in those vials located at the edge of the vial array. Figure 3 gives an example showing a time interval of 8 hours between the temperature increase in vial 1 and vial 6. Due to the large variation in drying behavior, one cannot have confidence that all vials are dry based upon observations from a small sub-set of vials line (i.e., the vials containing temperature sensors).

In addition, the end point of secondary drying cannot be detected with this method because of the lack of a precise thermal event. The benefits and disadvantages are described in Table I.

Pressure Rise Test

Basically the test consists of recording the rise in pressure in the drying chamber after it has been isolated from the condenser. If either ice or a large amount of residual moisture remains, the partial pressure of water rises because the water vapor cannot be condensed. In this case the cycle must be continued until the pressure stabilizes. This test which, in the past was recommended to evaluate the end of primary drying is now no longer used by most operators due to the risks of early melting of the product.

This test is, however, very useful in detecting the end of secondary drying, in order to optimize the cycles. This prevents product to remain in the desorption stage too long. This test is far from convenient: the benefits and disadvantages are described in Table II.

Sample Extractor

This device installed on the freeze-drying chamber comprises an arm with tongs which enables vials to be

TABLE I
Benefits and Disadvantages of the Method of the Temperature Response

Benefits	Disadvantages
Automatic	Modifies the behavior of the sample
Easy to operate	Not representative of the whole load
	Needs volumes in order to completely immerse the probe
	Does not indicate the end of desorption

Downloaded from on September 7, 2021

TABLE II
Benefits and Disadvantages of the Pressure Rise Test

Benefits	Disadvantages
Automatic	The condenser must be separated from the freeze-drying chamber. Computerized system compulsory
Provides evidence of end of secondary drying	Expensive
Representative of the full load batch	Risks of early melting of the product if used for the determination of the end of the sublimation
Noninvasive	

sampled during the drying process without breaking the vacuum. The vial is sealed in the drying chamber, then removed by the air lock. One benefit of the system is that it enables product characteristics to be monitored during drying: e.g., residual moisture, glass transition temperature, activity. The disadvantages and other benefits of this system are shown in Table III.

Residual Gas Analysis with a Mass Spectrometer

This methodology, as proposed by Jennings (5), allows the monitoring of changes in partial pressure of water or other gases. However, the size of the equipment, in addition to the strain in cleaning it and its cost (which is at least 35 times the price of a Pirani gauge or 10 times the price of a moisture sensor) makes it hardly suitable for common use in this application. In our experience, the mass spectrometer finds much better uses in other applications as described by Jennings (5), such as the intermittent determination of the presence of non-water vapors during validation tests.

Pirani Vacuum Gauge

This instrument is a thermal conductivity gauge. The measurements made with this sensor depend on the varying gas composition (4). S. Nail (2) suggested using drift in the Pirani gauge response as an indication of the end of primary drying. Here, the pressure measured by a capacitance manometer (insensitive to gas composition) must be held constant.

When sublimation begins, the partial pressure of water increases: the pressure displayed by the gauge increases whereas the pressure given by the capacitance sensor remains constant. At the end of primary drying, the composition of vapor in the drying chamber stabilizes and the response of the Pirani vacuum gauge becomes constant: according to the author, sublimation can then be considered to be completed. This technique is very attractive because it is easy to carry out, cost-effective and suitable for every kind of freeze-dryer.

However two questions remain:

- What is the sensitivity of this procedure?
- Can this procedure be used to study the desorption, or secondary drying stage?

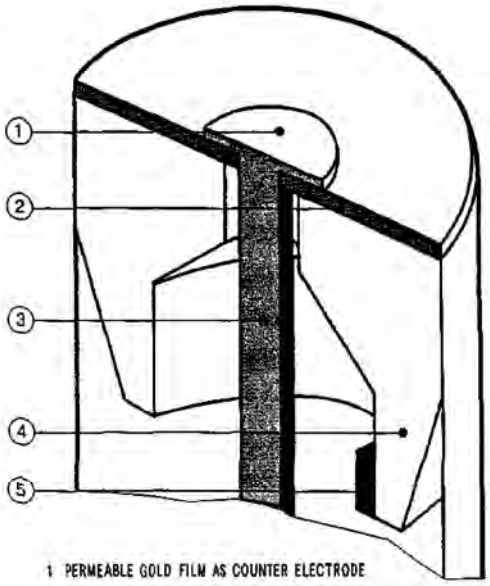
TABLE III
Benefits and Disadvantages of the Sample Extractor

Benefits	Disadvantages
Noninvasive	Automation not possible
Enables analysis of the product according to several criteria	Not suitable for a vapor sterilizable production freeze-dryer
Enables determination of the end of sublimation	Only few vials can be sampled
Enables determination of the end of desorption	

Moisture Sensor

The use of this kind of sensor on a freeze-dryer was first reported by M. Roy et al. (1). This instrument (sensor DY 70) has an aluminum cylinder which acts as the basic electrode. The dielectric is made up of an aluminum oxide film (Al_2O_3).

The counter-electrode has a gold film which is permeable to water vapor (Fig. 4). The water molecules adsorb on the oxide film and alter the capacitance of the circuit. The amount of adsorption, and hence the capacitance, is determined by the partial pressure of water vapor and the temperature of the oxide. The change in capacity is converted by the electronics into a PFM signal (pulse frequency modulation) via the Hygrolog WMY 770 Z.



1 PERMEABLE GOLD FILM AS COUNTER ELECTRODE
2 METAL OXIDE AL203 AS DIELECTRIC
3 CONTACT PIN FOR COUNTER ELECTRODE
4 HIGHEST GRADE ALUMINIUM WITH TIP AS GROUND ELECTRODE OF THE CAPACITOR
5 PT 100 THERMOELEMENT

Figure 4—Diagram showing the moisture sensor with aluminum oxide film (in Endress+Hauser literature).

Downloaded from on September 7, 2021

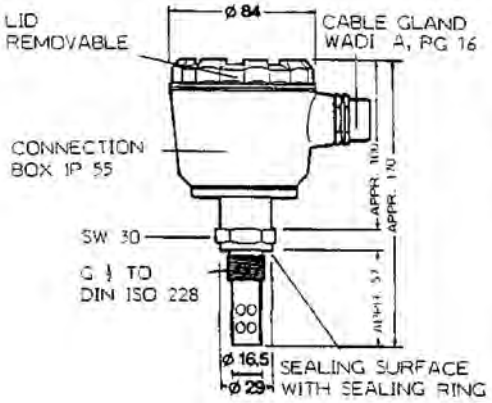
According to the authors (1), the sensor is highly sensitive so that it is possible to detect as few as 0.3% of vials with incomplete sublimation. This instrument, however, did not seem to be suitable for the analysis of secondary drying. The authors indicated desorption of water from the aluminum as the metal increases in temperature (an occurrence which happens during the secondary drying). The device can be fitted on any freeze-dryer equipped with a flange. The advantage is its compactness (Fig. 5).

Since both systems (Pirani gauge and humidity sensor) appear potentially promising, our work was thus undertaken to assess their respective sensitivities and limits.

Results and Discussion

Detection Threshold

The study was carried out in the laboratory freeze-dryer on a batch of 200 vials (8 ml) containing 1 ml of water for injection. Some batches were modified as required with a variable number of vials containing 2 ml instead of 1 ml. The data in Figure 6 demonstrate that the moisture sensor can detect 0.5% of vials not yet finished with primary drying. The Pirani vacuum gauge for the pressure control at 0.3 hPa (1 hectopascal = 0.75 Torr) is much less sensitive: the change in the slope of the curve is observed with only 1% of overfilled vials.



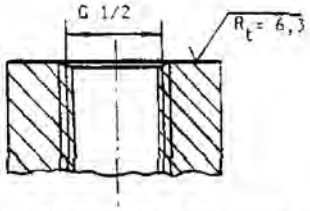


Figure 5—Diagram of the DY 70 hygrometer from Endress+Hauser

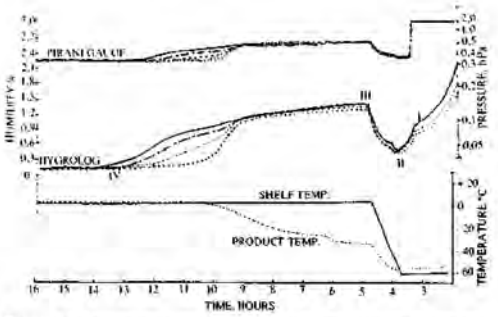


Figure 6—Comparative sensitivity of the Pirani vacuum gauge and the moisture sensor: (I) end of freezing; (II) vacuum; (III) initial sublimation step; (IV) end of sublimation. Pressure is maintained at 300 hPa × 10^{-3} during the cycle. Product temperature measures in a normal fill vial: ×××× 0% overfilled vials; ···· 0.5% overfilled vials; ---- 1% overfilled vials; ·-·-· 3% overfilled vials; — 5% overfilled vials.

The product temperature is the temperature in a normal fill vial and the sharp increase observed at time T. 6.10 hours reflects the beginning of lack of contact between the Pt100 sensor and the ice. After the sublimation of water, the temperature probe gradually loses any contact with ice (Fig. 1), under such a situation it is impossible to determine the end of the primary drying with enough accuracy. The same problem occurs in vials with low volume of filling. Temperature probes are too large to allow a good contact with the cake.

Effects of Pressure on Sensitivity

The response of the Pirani gauge is not linear in pressure, and at higher pressure the gauge seems to lose sensitivity. Thus we carried out studies at a lower pressure to see if the sensitivity to detect vials "not yet dry" would be improved. The test was done on the laboratory freeze-dryer loaded with 5% of overfilled vials containing 2 ml of water instead of 1 ml.

Figure 7 shows that, whatever the chamber pressure, the moisture sensor clearly indicates the end point of primary drying despite the low partial pressure of water. However, if the "end point" of the primary drying is more accurately defined at the lower pressure than at the higher one, the end point indicated by the moisture sensor is always later than this indicates by the Pirani gauge. This could be interpreted as a lack of sensitivity of the Pirani gauge or alternatively as a problem of desorption of the moisture sensor. This issue is further discussed below.

Both tests demonstrate that the Pirani vacuum gauge or the moisture sensor have the potential to detect the end point of primary drying irrespective of the pressure; the moisture sensor however is more sensitive especially at higher pressures (above 0.2 hPa). In order to improve the sensitivity of the Pirani gauge it would have been necessary, albeit impracticable, to linearize electronically the pressure signal and to plot it on linear coordinates.

Downloaded from on September 7, 2021

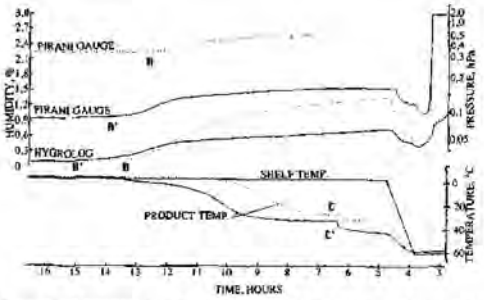


Figure 7—Comparative sensitivity of the Pirani vacuum gauge and the Endress+Hauser hygrometer as a function of pressure: ····· test at 300 hPa × 10^{-3}; — test at 80 hPa × 10^{-3}; A,A' end of sublimation according to the Pirani sensor; B,B' end of sublimation according to the moisture sensor respectively at 300 and 80 hPa × 10^{-3}; C,C' end of sublimation according to the temperature probe in a vial filled with a 1 ml sample at 300 and 80 hPa × 10^{-3} respectively.

Effects of the Size of the Load on Sensitivity

The "load" or number of vials subliming in the freeze-dryer chamber is another parameter likely to influence the ability of the sensors to detect end point.

The test was run in the lyoflex on variable loads of 8 ml vials containing 1 ml of water for injection. Figure 8 shows the effects of this on the sublimation period. Drying times increase as a function of the batch sizes. As no other parameter was changed, excepted the size of the load this event could be the result of radiation heat transfer differences.

The Pirani vacuum gauge and the moisture sensor show that there has to be an increased time interval of 5 hours for the primary drying cycle between the minimal batch of 5 vials and the full load batch. As the load increases, the differences between the "product temperature end point" and "vapor composition end points" become much larger, suggesting a possible scale-up problem if one uses only product temperature measurement for end point determination. Therefore, monitoring the moisture during the freeze-drying cycle can be an important parameter to ensure quality of the product during scale-up tests.

Relationship Between the Moisture in the Chamber and Moisture in the Product During Secondary Drying

In Figures 6, 7 and 8, one can see that the moisture probe responses reach a low constant value, suggesting the end of secondary drying, one or two hours later than constant response of the Pirani gauge suggests dryness.

The question arises as to whether this difference in response reflects residual moisture in the product and therefore, a greater sensitivity of the moisture probe, or simply reflects slow desorption of moisture from the moisture probe (1).

The fact that both sensors (Pirani and moisture) record a simultaneous slight rise (recorded as a "bump" in Fig. 8) at the beginning of the primary drying (e.g., before any increase in product ice temperature) indicates their fine sensitivity.

This transitory phenomenon (likely to be due to the desorption of the walls of the freeze-dryer) suggests there is no problem with the desorption of the aluminum layer of the moisture sensor. To test the sensitivity of these sensors, samples were taken from the chamber at the end of sublimation, when the Pirani vacuum gauge reached a step, whereas the moisture sensor still indicated a decrease of the partial pressure in water in the chamber (Fig. 9).

The product used as a substrate was a protein base of 50 g/l mixed with various excipients (sugar and amino acids) distributed in 5 ml doses in 30 ml vials. The samples were analyzed on DSC and the residual moisture content was determined for each sample. The results in Table IV and Figure 9 show that after the third sample time, the values of the Pirani gauge response are constant, but the moisture probe response continues to show a slight decrease in partial pressure of water as time increases. The moisture probe response correlates well with both the residual moisture in the product and the glass transition temperature of the product (Table IV). Thus, the moisture sensor response near the end of the process is not an artifact due to slow desorption of moisture from the sensor. The moisture probe is respond-

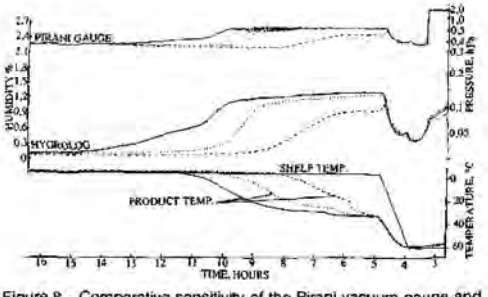


Figure 8—Comparative sensitivity of the Pirani vacuum gauge and the Endress+Hauser hygrometer as a function of load = — 5 vials; ····· freeze-dryer loaded at ½; — full freeze-dryer. In each case plotted temperature reflects the temperature of one representative vial.

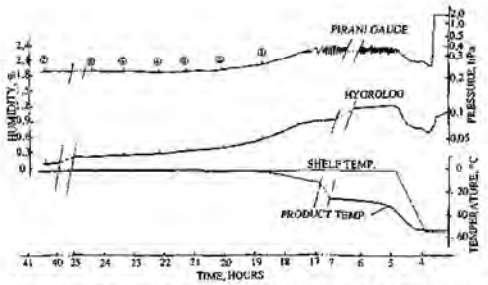


Figure 9—Relationship between the moisture of the chamber and that of the product. Sampling at the end of drying. Marks 1,2,3,4,5,6,7 indicate the hours at which sampling took place.

Downloaded from on September 7, 2021

TABLE IV
Relationship Between Moisture in the Chamber and Moisture in the Product

Samples Obtained as Described in Fig. 9	% Residual Moisture in the Product[a]	Tg[b]	% Relative Humidity in the Chamber (Hygrometer)	Pressure hPa (Pirani)
1	4.4	31°C	0.54	0.26
2	3.7	32°C	0.36	0.25
3	3.0	33°C	0.30	0.24
4	2.8	33°C	0.27	0.23
5	2.8	35°C	0.21	0.23
6	2.6	40°C	0.18	0.23
7	1.9	54°C	0.10	0.23

[a] Means of 3 samples (the same used for the determination of the Tg) determined by Karl Fischer. Samples were weighted in a dry box (moisture < 3 ppm).

[b] Tg determined by DSC: samples were placed in sealed pans and scanned at a rate of 20°C/min for the temperature range 0 to 100°C. Samples were prepared in a dry box (moisture < 10 ppm). Tg reported are the onsets of the transition obtained on three samples with an estimated experimental error of ±0.5°C.

ing to variable moisture in the product during secondary drying.

The Pirani vacuum gauge is then a convenient means to determine the end of primary drying particularly at low pressure. However, the moisture sensor offers the advantage of a greater sensitivity. Its sensitivity enables using it for the determination of the end of the secondary drying. The newly proposed additional option to monitor the humidity inside the chamber during the cycle allows to avoid undesirable over- or under-drying of delicate products. This can be done after the correlation curves have been established between the moisture of the chamber and that of the product.

Tests with an Industrial Freeze-Dryer

In order to test the sensitivity of the moisture sensor on an industrial plant with large capacity, we used a home-made set-up (Fig. 10) on the flange (DN 10) of the equipment which was mounted above the chamber. A system was specially designed for the positioning of: the Pirani vacuum gauge, the capacitance gauge, and the moisture sensor.

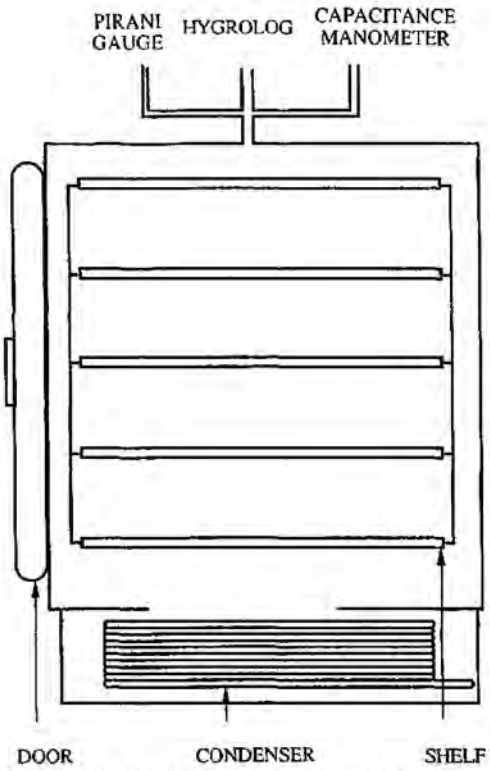


Figure 10—Mounting diagram of the sensors on the industrial freeze-dryer.

When used under actual industrial conditions, the moisture sensor led us to increase the duration of the primary dessication by 4 hours, as compared to the duration of the cycle of the pilot plant. In the absence of the electronic moisture sensor, we would have thus inadvertantly started to increase the temperature before the end of the sublimation of all vials of the batch. With a hygrometer it is therefore possible to avoid the errors

TABLE V
Summary of the Benefits and Disadvantages of the Various Methods Given

	Suggested System				
Tested Options	Product Temperature Response	Pressure Rise Test	Sample Extractor	Pirani Gauge	Moisture Sensor
Indication of the end of sublimation	Not representative of the whole load	Risk of melting of the product	Good but not possible to automate	Good	Good
Indication of the end of desorption	Not possible	Good	Good but not possible to automate	Not possible	Good
Suitable for any freeze-dryer	Yes	No	No	Yes	Yes
Ability to demonstrate heterogeneity of the load	Poor	Good	Poor	Good	Very Good

Downloaded from on September 7, 2021

stemming from the scale-up. The benefits and the disadvantages of the different systems can be summed up in Table V.

Conclusion

The extremely high sensitivity of the moisture sensor to quantify water vapor during the freeze-drying cycles is obvious. This sensor enables monitoring of the end of the primary drying as described in M. Roy (1). In addition, our study shows that the moisture sensor is also able to pinpoint the end of the secondary drying. This kind of sensor used on freeze-dryers avoids the potential risks stemming from:

- Cycle adaptation from one unit to others with various sizes and designs
- Heterogeneous sublimation kinetics from one vial to the other
- Size differences of the load for freeze-drying.

Adding such a sensor enables to optimize the cycles, to improve the quality of the product and to increase productivity. Our results have thus led us to further develop an automated computerized close loop feedback system.

Acknowledgements

We would like to thank Dr. M. Pikal for his helpful suggestions on the manuscript. We are particularly grateful to Dr. G. Rolland for his help in the preparation of this manuscript.

References

1. M.-L. Roy and M.-J. Pikal, "Process control in freeze-drying: determination of the point of sublimation drying by an electronic moisture sensor," *J. Parenter. Sci. Technol,* **43**, 60–66, (1989).
2. S. L. Nail, "Methodology for in process determination of residual water in freeze drying products," International symposium on biological product freeze-drying and formulation, Bethesda, USA 1990: *Develop. Biol. Standard,* **74**, 137–151 (Karger, Basel, 1991).
3. D. Greiff, "Factors affecting the statistical parameters and patterns of distribution of residual arrays of samples following lyophilization," *J. Parenter. Sci. Technol.*, **44**, 118–129, (1990).
4. J. Grant Armstrong, "Use of the capacitance manometer gauge in vacuum freeze-drying," *J. Parenteral Drug Association,* **34**, 6, 473–483, (1980).
5. T. A. Jennings, "Residual gas analysis and vacuum freeze-drying," *J Parenteral Drug Association,* **34**, 1, 62–69, (1980).

Downloaded from on September 7, 2021

PDA Journal
of Pharmaceutical Science and Technology



An Authorized User of the electronic PDA Journal of Pharmaceutical Science and Technology (the PDA Journal) is a PDA Member in good standing. Authorized Users are permitted to do the following:

·Search and view the content of the PDA Journal
·Download a single article for the individual use of an Authorized User
·Assemble and distribute links that point to the PDA Journal
·Print individual articles from the PDA Journal for the individual use of an Authorized User
·Make a reasonable number of photocopies of a printed article for the individual use of an Authorized User or for the use by or distribution to other Authorized Users

Authorized Users are not permitted to do the following:

·Except as mentioned above, allow anyone other than an Authorized User to use or access the PDA Journal
· Display or otherwise make any information from the PDA Journal available to anyone other than an Authorized User
·Post articles from the PDA Journal on Web sites, either available on the Internet or an Intranet, or in any form of online publications
·Transmit electronically, via e-mail or any other file transfer protocols, any portion of the PDA Journal
·Create a searchable archive of any portion of the PDA Journal
·Use robots or intelligent agents to access, search and/or systematically download any portion of the PDA Journal
·Sell, re-sell, rent, lease, license, sublicense, assign or otherwise transfer the use of the PDA Journal or its content
·Use or copy the PDA Journal for document delivery, fee-for-service use, or bulk reproduction or distribution of materials in any form, or any substantially similar commercial purpose
·Alter, modify, repackage or adapt any portion of the PDA Journal
·Make any edits or derivative works with respect to any portion of the PDA Journal including any text or graphics
·Delete or remove in any form or format, including on a printed article or photocopy, any copyright information or notice contained in the PDA Journal

Lyophilization of Parenteral (7/93)

GUIDE TO INSPECTIONS OF LYOPHILIZATION OF PARENTERALS

Note: This document is reference material for investigators and other FDA personnel. The document does not bind FDA, and does no confer any rights, privileges, benefits, or immunities for or on any person(s).

INTRODUCTION

Lyophilization or freeze drying is a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor without passing through a liquid phase. The process consists of three separate, unique, and interdependent processes; freezing, primary drying (sublimation), and secondary drying (desorption).

The advantages of lyophilization include:

Ease of processing a liquid, which simplifies aseptic handling

Enhanced stability of a dry powder

Removal of water without excessive heating of the product

Enhanced product stability in a dry state

Rapid and easy dissolution of reconstituted product

Disadvantages of lyophilization include:

Increased handling and processing time

Need for sterile diluent upon reconstitution

Cost and complexity of equipment

The lyophilization process generally includes the following steps:

- Dissolving the drug and excipients in a suitable solvent, generally water for injection (WFI).
- Sterilizing the bulk solution by passing it through a 0.22 micron bacteria-retentive filter.
- Filling into individual sterile containers and partially stoppering the containers under aseptic conditions.

- Transporting the partially stoppered containers to the lyophilizer and loading into the chamber under aseptic conditions.
- Freezing the solution by placing the partially stoppered containers on cooled shelves in a freeze-drying chamber or pre-freezing in another chamber.
- Applying a vacuum to the chamber and heating the shelves in order to evaporate the water from the frozen state.
- Complete stoppering of the vials usually by hydraulic or screw rod stoppering mechanisms installed in the lyophilizers.

There are many new parenteral products, including anti-infectives, biotechnology derived products, and in-vitro diagnostics which are manufactured as lyophilized products. Additionally, inspections have disclosed potency, sterility and stability problems associated with the manufacture and control of lyophilized products. In order to provide guidance and information to investigators, some industry procedures and deficiencies associated with lyophilized products are identified in this Inspection Guide.

It is recognized that there is complex technology associated with the manufacture and control of a lyophilized pharmaceutical dosage form. Some of the important aspects of these operations include: the formulation of solutions; filling of vials and validation of the filling operation; sterilization and engineering aspects of the lyophilizer; scale-up and validation of the lyophilization cycle; and testing of the end product. This discussion will address some of the problems associated with the manufacture and control of a lyophilized dosage form.

PRODUCT TYPE/FORMULATION

Products are manufactured in the lyophilized form due to their instability when in solution. Many of the antibiotics, such as some of the semi-synthetic penicillins, cephalosporins, and also some of the salts of erythromycin, doxycycline and chloramphenicol are made by the lyophilization process. Because they are antibiotics, low bioburden of these formulations would be expected at the time of batching. However, some of the other dosage forms that are lyophilized, such as hydrocortisone sodium succinate, methylprednisolone sodium succinate and many of the biotechnology derived products, have no antibacterial effect when in solution.

For these types of products, bioburden should be minimal and the bioburden should be determined prior to sterilization of these bulk solutions prior to filling. Obviously, the batching or compounding of these bulk solutions should be controlled in order to prevent any potential increase in microbiological levels that may occur up to the time that the bulk solutions are filtered (sterilized). The concern with any microbiological level is the possible increase in endotoxins that may develop. Good practice for the compounding of lyophilized products would also include batching in a controlled environment and in sealed tanks, particularly if the solution is to be held for any length of time prior to sterilization.

In some cases, manufacturers have performed bioburden testing on bulk solutions after prefiltration and prior to final filtration. While the testing of such solutions may be meaningful in determining the bioburden for sterilization, it does not provide any information regarding the potential formation or presence of endotoxins. While the testing of 0.1 ml samples by LAL methods of bulk solution for endotoxins is of value, testing of at least 100 ml size samples prior to prefiltration, particularly for the presence of gram negative organisms, would be of greater value in evaluating the process. For example, the presence of Pseudomonas sp. in the bioburden of a bulk solution has been identified as an objectionable condition.

FILLING

The filling of vials that are to be lyophilized has some problems that are somewhat unique. The stopper is placed on top of the vial and is ultimately seated in the lyophilizer. As a result the contents of the vial are subject to contamination until they are actually sealed.

Validation of filling operations should include media fills and the sampling of critical surfaces and air during active filling (dynamic conditions).

Because of the active involvement of people in filling and aseptic manipulations, an environmental program should also include an evaluation of microbiological levels on people working in aseptic processing areas. One method of evaluation of the training of operators working in aseptic processing facilities includes the surface monitoring of gloves and/or gowns on a daily basis. Manufacturers are actively sampling the surfaces of personnel working in aseptic processing areas. A reference which provides for this type of monitoring is the USP XXII discussion of the Interpretation of Sterility Test Results. It states under the heading of "Interpretation of Quality Control Tests" that review consideration should be paid to environmental control data, including...microbial monitoring, records of operators, gowns, gloves, and garbing practices. In those situations in which manufacturers have failed to perform some type of personnel monitoring, or monitoring has shown unacceptable levels of contamination, regulatory situations have resulted.

Typically, vials to be lyophilized are partially stoppered by machine. However, some filling lines have been noted which utilize an operator to place each stopper on top of the vial by hand. At this time, it would seem that it would be difficult for a manufacturer to justify a hand-stoppering operation, even if sterile forceps are employed, in any type of operation other than filling a clinical batch or very small number of units. Significant regulatory situations have resulted when some manufacturers have hand-stoppered vials. Again, the concern is the immediate avenue of contamination offered by the operator. It is well recognized that people are the major source of contamination in an aseptic processing filling operation. The longer a person works in an aseptic operation, the more microorganisms will be shed and the greater the probability of contamination.

Once filled and partially stoppered, vials are transported and loaded into the lyophilizer. The transfer and handling, such as loading of the lyophilizer, should take place under primary barriers, such as the laminar flow hoods under which the vials were filled. Validation of this handling should also include the use media fills.

Regarding the filling of sterile media, there are some manufacturers who carry out a partial lyophilization cycle and freeze the media. While this could seem to greater mimic the process, the freezing of media could reduce microbial levels of some contaminants. Since the purpose of the media fill is to evaluate and justify the aseptic capabilities of the process, the people and the system, the possible reduction of microbiological levels after aseptic manipulation by freezing would not be warranted. The purpose of a media fill is not to determine the lethality of freezing and its effect on any microbial contaminants that might be present.

In an effort to identify the particular sections of filling and aseptic manipulation that might introduce contamination, several manufacturers have resorted to expanded media fills. That is, they have filled approximately 9000 vials during a media fill and segmented the fill into three stages. One stage has included filling of 3000 vials and stoppering on line; another stage included filling 3000 vials, transportation to the lyophilizer and then stoppering; a third stage included the filling of 3000 vials, loading in the lyophilizer, and exposure to a portion of the nitrogen flush and then stoppering. Since lyophilizer sterilization and sterilization of the nitrogen system used to backfill require separate validation, media fills should primarily validate the filling, transportation and loading aseptic operations.

The question of the number of units needed for media fills when the capacity of the process is less than 3000 units is frequently asked, particularly for clinical products. Again, the purpose of the media fill is to assure that product can be aseptically processed without contamination under operating conditions. It would seem, therefore, that the maximum number of units of media filled be equivalent to the maximum batch size if it is less than 3000 units.

After filling, dosage units are transported to the lyophilizer by metal trays. Usually, the bottom of the trays are removed after the dosage units are loaded into the lyophilizer. Thus, the dosage units lie directly on the lyophilizer shelf. There have been some situations in which manufacturers have loaded the dosage units on metal trays which were not removed. Unfortunately, at one manufacturer, the trays warped which caused a moisture problem in some dosage units in a batch.

In the transport of vials to the lyophilizer, since they are not sealed, there is concern for the potential for contamination. During inspections and in the review of new facilities, the failure to provide laminar flow coverage or a primary barrier for the transport and loading areas of a lyophilizer has been regarded as an objectionable condition. One manufacturer as a means of

correction developed a laminar flow cart to transport the vials from the filling line to the lyophilizer. Other manufacturers building new facilities have located the filling line close to the lyophilizer and have provided a primary barrier extending from the filling line to the lyophilizer.

In order to correct this type of problem, another manufacturer installed a vertical laminar flow hood between the filling line and lyophilizer. Initially, high velocities with inadequate return caused a contamination problem in a media fill. It was speculated that new air currents resulted in rebound contamination off the floor. Fortunately, media fills and smoke studies provided enough meaningful information that the problem could be corrected prior to the manufacture of product. Typically, the lyophilization process includes the stoppering of vials in the chamber.

Another major concern with the filling operation is assurance of fill volumes. Obviously, a low fill would represent a subpotency in the vial. Unlike a powder or liquid fill, a low fill would not be readily apparent after lyophilization particularly for a biopharmaceutical drug product where the active ingredient may be only a milligram. Because of the clinical significance, sub-potency in a vial potentially can be a very serious situation.

For example, in the inspection of a lyophilization filling operation, it was noted that the firm was having a filling problem. The gate on the filling line was not coordinated with the filling syringes, and splashing and partial filling was occurring. It was also observed that some of the partially filled vials were loaded into the lyophilizer. This resulted in rejection of the batch.

On occasion, it has been seen that production operators monitoring fill volumes record these fill volumes only after adjustments are made. Therefore, good practice and a good quality assurance program would include the frequent monitoring of the volume of fill, such as every 15 minutes. Good practice would also include provisions for the isolation of particular sections of filling operations when low or high fills are encountered.

There are some atypical filling operations which have not been discussed. For example, there have also been some situations in which lyophilization is performed on trays of solution rather than in vials. Based on the current technology available, it would seem that for a sterile product, it would be difficult to justify this procedure.

The dual chamber vial also presents additional requirements for aseptic manipulations. Media fills should include the filling of media in both chambers. Also, the diluent in these vials should contain a preservative. (Without a preservative, the filling of diluent would be analogous to the filling of media. In such cases, a 0% level of contamination would be expected.)

LYOPHILIZATION CYCLE AND CONTROLS

After sterilization of the lyophilizer and aseptic loading, the initial step is freezing the solution. In some cycles, the shelves are at the temperature needed for freezing, while for other cycles, the product is loaded and then the shelves are taken to the freezing temperature necessary for

product freeze. In those cycles in which the shelves are precooled prior to loading, there is concern for any ice formation on shelves prior to loading. Ice on shelves prior to loading can cause partial or complete stoppering of vials prior to lyophilization of the product. A recent field complaint of a product in solution and not lyophilized was attributed to preliminary stoppering of a few vials prior to exposure to the lyophilization cycle. Unfortunately, the firm's 100% vial inspection failed to identify the defective vial.

Typically, the product is frozen at a temperature well below the eutectic point.

The scale-up and change of lyophilization cycles, including the freezing procedures, have presented some problems. Studies have shown the rate and manner of freezing may affect the quality of the lyophilized product. For example, slow freezing leads to the formation of larger ice crystals. This results in relatively large voids, which aid in the escape of water vapor during sublimation. On the other hand, slow freezing can increase concentration shifts of components. Also, the rate and manner of freezing has been shown to have an affect on the physical form (polymorph) of the drug substance.

It is desirable after freezing and during primary drying to hold the drying temperature (in the product) at least 4-5o below the eutectic point. Obviously, the manufacturer should know the eutectic point and have the necessary instrumentation to assure the uniformity of product temperatures. The lyophilizer should also have the necessary instrumentation to control and record the key process parameters. These include: shelf temperature, product temperature, condenser temperature, chamber pressure and condenser pressure. The manufacturing directions should provide for time, temperature and pressure limits necessary for a lyophilization cycle for a product. The monitoring of product temperature is particularly important for those cycles for which there are atypical operating procedures, such as power failures or equipment breakdown.

Electromechanical control of a lyophilization cycle has utilized cam-type recorder-controllers. However, newer units provide for microcomputer control of the freeze drying process. A very basic requirement for a computer controlled process is a flow chart or logic. Typically, operator involvement in a computer controlled lyophilization cycle primarily occurs at the beginning. It consists of loading the chamber, inserting temperature probes in product vials, and entering cycle parameters such as shelf temperature for freezing, product freeze temperature, freezing soak time, primary drying shelf temperature and cabinet pressure, product temperature for establishment of fill vacuum, secondary drying shelf temperature, and secondary drying time.

In some cases, manufacturers have had to continuously make adjustments in cycles as they were being run. In these situations, the lyophilization process was found to be non-validated.

Validation of the software program of a lyophilizer follows the same criteria as that for other processes. Basic concerns include software development, modifications and security. The Guide to Inspection of Computerized Systems in Drug Processing contains a discussion on potential problem areas relating to computer systems. A Guide to the Inspection of Software Development Activities is a reference that provides a more detailed review of software requirements.

Leakage into a lyophilizer may originate from various sources. As in any vacuum chamber, leakage can occur from the atmosphere into the vessel itself. Other sources are media employed within the system to perform the lyophilizing task. These would be the thermal fluid circulated through the shelves for product heating and cooling, the refrigerant employed inside the vapor condenser cooling surface and oil vapors that may migrate back from the vacuum pumping system.

Any one, or a combination of all, can contribute to the leakage of gases and vapors into the system. It is necessary to monitor the leak rate periodically to maintain the integrity of the system. It is also necessary, should the leak rate exceed specified limits, to determine the actual leak site for purposes of repair.

Thus, it would be beneficial to perform a leak test at some time after sterilization, possibly at the beginning of the cycle or prior to stoppering. The time and frequency for performing the leak test will vary and will depend on the data developed during the cycle validation. The pressure rise found acceptable at validation should be used to determine the acceptable pressure rise during production. A limit and what action is to be taken if excessive leakage is found should be addressed in some type of operating document.

In order to minimize oil vapor migration, some lyophilizers are designed with a tortuous path between the vacuum pump and chamber. For example, one fabricator installed an oil trap in the line between the vacuum pump and chamber in a lyophilizer with an internal condenser. Leakage can also be identified by sampling surfaces in the chamber after lyophilization for contaminants. One could conclude that if contamination is found on a chamber surface after lyophilization, then dosage units in the chamber could also be contaminated. It is a good practice as part of the validation of cleaning of the lyophilization chamber to sample the surfaces both before and after cleaning.

Because of the lengthy cycle runs and strain on machinery, it is not unusual to see equipment malfunction or fail during a lyophilization cycle. There should be provisions in place for the corrective action to be taken when these atypical situations occur. In addition to documentation of the malfunction, there should be an evaluation of the possible effects on the product (e.g., partial or complete meltback. Refer to subsequent discussion). Merely testing samples after the

lyophilization cycle is concluded may be insufficient to justify the release of the remaining units. For example, the leakage of chamber shelf fluid into the chamber or a break in sterility would be cause for rejection of the batch.

The review of Preventive Maintenance Logs, as well as Quality Assurance Alert Notices, Discrepancy Reports, and Investigation Reports will help to identify problem batches when there are equipment malfunctions or power failures. It is recommended that these records be reviewed early in the inspection.

CYCLE VALIDATION

Many manufacturers file (in applications) their normal lyophilization cycles and validate the lyophilization process based on these cycles. Unfortunately, such data would be of little value to substantiate shorter or abnormal cycles. In some cases, manufacturers are unaware of the eutectic point. It would be difficult for a manufacturer to evaluate partial or abnormal cycles without knowing the eutectic point and the cycle parameters needed to facilitate primary drying.

Scale-up for the lyophilized product requires a knowledge of the many variables that may have an effect on the product. Some of the variables would include freezing rate and temperature ramping rate. As with the scale-up of other drug products, there should be a development report that discusses the process and logic for the cycle. Probably more so than any other product, scale-up of the lyophilization cycle is very difficult.

There are some manufacturers that market multiple strengths, vial sizes and have different batch sizes. It is conceivable and probable that each will have its own cycle parameters. A manufacturer that has one cycle for multiple strengths of the same product probably has done a poor job of developing the cycle and probably has not adequately validated their process. Investigators should review the reports and data that support the filed lyophilization cycle.

LYOPHILIZER STERILIZATION/DESIGN

The sterilization of the lyophilizer is one of the more frequently encountered problems noted during inspections. Some of the older lyophilizers cannot tolerate steam under pressure, and sterilization is marginal at best. These lyophilizers can only have their inside surfaces wiped with a chemical agent that may be a sterilant but usually has been found to be a sanitizing agent. Unfortunately, piping such as that for the administration of inert gas (usually nitrogen) and sterile air for backfill or vacuum break is often inaccessible to such surface "sterilization" or treatment. It would seem very difficult for a manufacturer to be able to demonstrate satisfactory validation of sterilization of a lyophilizer by chemical "treatment".

Another method of sterilization that has been practiced is the use of gaseous ethylene oxide. As with any ethylene oxide treatment, humidification is necessary. Providing a method for introducing the sterile moisture with uniformity has been found to be difficult.

A manufacturer has been observed employing Water For Injection as a final wash or rinse of the lyophilizer. While the chamber was wet, it was then ethylene oxide gas sterilized. As discussed above, this may be satisfactory for the chamber but inadequate for associated plumbing.

Another problem associated with ethylene oxide is the residue. One manufacturer had a common ethylene oxide/nitrogen supply line to a number of lyophilizers connected in parallel to the system. Thus, there could be some ethylene oxide in the nitrogen supply line during the backfilling step. Obviously, this type of system is objectionable.

A generally recognized acceptable method of sterilizing the lyophilizer is through the use of moist steam under pressure. Sterilization procedures should parallel that of an autoclave, and a typical system should include two independent temperature sensing systems. One would be used to control and record temperatures of the cycle as with sterilizers, and the other would be in the cold spot of the chamber. As with autoclaves, lyophilizers should have drains with atmospheric breaks to prevent back siphonage.

As discussed, there should also be provisions for sterilizing the inert gas or air and the supply lines. Some manufacturers have chosen to locate the sterilizing filters in a port of the chamber. The port is steam sterilized when the chamber is sterilized, and then the sterilizing filter, previously sterilized, is aseptically connected to the chamber. Some manufacturers have chosen to sterilize the filter and downstream piping to the chamber in place. Typical sterilization-in-place of filters may require steaming of both to obtain sufficient temperatures. In this type of system, there should be provisions for removing and/or draining condensate. The failure to sterilize nitrogen and air filters and the piping downstream leading into the chamber has been identified as a problem on a number of inspections.

Since these filters are used to sterilize inert gas and/or air, there should be some assurance of their integrity. Some inspections have disclosed a lack of integrity testing of the inert gas and/or air filter. The question is frequently asked how often should the vent filter be tested for integrity? As with many decisions made by manufacturers, there is a level of risk associated with the operation, process or system, which only the manufacturer can decide. If the sterilizing filter is found to pass the integrity test after several uses or batches, then one could claim its integrity for the previous batches. However, if it is only tested after several batches have been processed and if found to fail the integrity test, then one could question the sterility of all of the previous batches processed. In an effort to minimize this risk, some manufacturers have resorted to redundant filtration.

For most cycles, stoppering occurs within the lyophilizer. Typically, the lyophilizer has some type of rod or rods (ram) which enter the immediate chamber at the time of stoppering. Once the rod enters the chamber, there is the potential for contamination of the chamber. However, since the vials are stoppered, there is no avenue for contamination of the vials in the chamber

which are now stoppered. Generally, lyophilizers should be sterilized after each cycle because of the potential for contamination of the shelf support rods. Additionally, the physical act of removing vials and cleaning the chamber can increase levels of contamination.

In some of the larger units, the shelves are collapsed after sterilization to facilitate loading. Obviously, the portions of the ram entering the chamber to collapse the shelves enters from a non-sterile area. Attempts to minimize contamination have included wiping the ram with a sanitizing agent prior to loading. Control aspects have included testing the ram for microbiological contamination, testing it for residues of hydraulic fluid, and testing the fluid for its bacteriostatic effectiveness. One lyophilizer fabricator has proposed developing a flexible "skirt" to cover the ram.

In addition to microbiological concerns with hydraulic fluid, there is also the concern with product contamination.

During steam sterilization of the chamber, there should be space between shelves that permit passage of free flowing steam. Some manufacturers have placed "spacers" between shelves to prevent their total collapse. Others have resorted to a two phase sterilization of the chamber. The initial phase provides for sterilization of the shelves when they are separated. The second phase provides for sterilization of the chamber and piston with the shelves collapsed.

Typically, biological indicators are used in lyophilizers to validate the steam sterilization cycle. One manufacturer of a Biopharmaceutical product was found to have a positive biological indicator after sterilization at 1210C for 45 minutes. During the chamber sterilization, trays used to transport vials from the filling line to the chamber were also sterilized. The trays were sterilized in an inverted position on shelves in the chamber. It is believed that the positive biological indicator is the result of poor steam penetration under these trays.

The sterilization of condensers is also a major issue that warrants discussion. Most of the newer units provide for the capability of sterilization of the condenser along with the chamber, even if the condenser is external to the chamber. This provides a greater assurance of sterility, particularly in those situations in which there is some equipment malfunction and the vacuum in the chamber is deeper than in the condenser.

Malfunctions that can occur, which would indicate that sterilization of the condenser is warranted, include vacuum pump breakdown, refrigeration system failures and the potential for contamination by the large valve between the condenser and chamber. This is particularly true for those units that have separate vacuum pumps for both the condenser and chamber. When there are problems with the systems in the lyophilizer, contamination could migrate from the condenser back to the chamber. It is recognized that the condenser is not able to be sterilized in many of the older units, and this represents a major problem, particularly in those cycles in which there is some equipment and/or operator failure.

As referenced above, leakage during a lyophilization cycle can occur, and the door seal or gasket presents an avenue of entry for contaminants. For example, in an inspection, it was noted that during steam sterilization of a lyophilizer, steam was leaking from the unit. If steam could leak from a unit during sterilization, air could possibly enter the chamber during lyophilization.

Some of the newer lyophilizers have double doors - one for loading and the other for unloading. The typical single door lyophilizer opens in the clean area only, and contamination between loads would be minimal. This clean area, previously discussed, represents a critical processing area for a product made by aseptic processing. In most units, only the piston raising/lowering shelves is the source of contamination. For a double door system unloading the lyophilizer in a non-sterile environment, other problems may occur. The non-sterile environment presents a direct avenue of contamination of the chamber when unloading, and door controls similar to double door sterilizers should be in place.

Obviously, the lyophilizer chamber is to be sterilized between batches because of the direct means of contamination. A problem which may be significant is that of leakage through the door seal. For the single door unit, leakage prior to stoppering around the door seal is not a major problem from a sterility concern, because single door units only open into sterile areas. However, leakage from a door gasket or seal from a non-sterile area would present a significant microbiological problem. In order to minimize the potential for contamination, it is recommended that the lyophilizers be unloaded in a clean room area to minimize contamination. For example, in an inspection of a new manufacturing facility, it was noted that the unloading area for double door units was a clean room, with the condenser located below the chamber on a lower level.

After steam sterilization, there is often some condensate remaining on the floor of the chamber. Some manufacturers remove this condensate through the drain line while the chamber is still pressurized after sterilization. Unfortunately, some manufacturers have allowed the chamber to come to and remain at atmospheric pressure with the drain line open. Thus, non-sterile air could contaminate the chamber through the drain line. Some manufacturers have attempted to dry the chamber by blowing sterile nitrogen gas through the chamber at a pressure above atmospheric pressure.

In an inspection of a biopharmaceutical drug product, a Pseudomonas problem probably attributed to condensate after sterilization was noted. On a routine surface sample taken from a chamber shelf after sterilization and processing, a high count of Pseudomonas sp. was obtained. After sterilization and cooling when the chamber door was opened, condensate routinely spilled onto the floor from the door. A surface sample taken from the floor below the door also revealed Pseudomonas sp. contamination. Since the company believed the condensate remained in the chamber after sterilization, they repiped the chamber drain and added a line to a water seal vacuum pump.

FINISHED PRODUCT TESTING FOR

LYOPHILIZED PRODUCTS

There are several aspects of finished product testing which are of concern to the lyophilized dosage form. These include dose uniformity testing, moisture and stability testing, and sterility testing.

(a) Dose Uniformity

The USP includes two types of dose uniformity testing: content uniformity and weight variation. It states that weight variation may be applied to solids, with or without added substances, that have been prepared from true solutions and freeze-dried in final containers. However, when other excipients or other additives are present, weight variation may be applied, provided there is correlation with the sample weight and potency results. For example, in the determination of potency, it is sometimes common to reconstitute and assay the entire contents of a vial without knowing the weight of the sample. Performing the assay in this manner will provide information on the label claim of a product, but without knowing the sample weight will provide no information about dose uniformity. One should correlate the potency result obtained form the assay with the weight of the sample tested.

(b) Stability Testing

An obvious concern with the lyophilized product is the amount of moisture present in vials. The manufacturer's data for the establishment of moisture specifications for both product release and stability should be reviewed. As with other dosage forms, the expiration date and moisture limit should be established based on worst case data. That is, a manufacturer should have data that demonstrates adequate stability at the moisture specification.

As with immediate release potency testing, stability testing should be performed on vials with a known weight of sample. For example, testing a vial (sample) which had a higher fill weight (volume) than the average fill volume of the batch would provide a higher potency results and not represent the potency of the batch. Also, the expiration date and stability should be based on those batches with the higher moisture content. Such data should also be considered in the establishment of a moisture specification.

For products showing a loss of potency due to aging, there are generally two potency specifications. There is a higher limit for the dosage form at the time of release. This limit is generally higher than the official USP or filed specification which is official throughout the entire expiration date period of the dosage form. The USP points out that compendial standards apply at any time in the life of the article.

Stability testing should also include provisions for the assay of aged samples and subsequent reconstitution of these aged samples for the maximum amount of time specified in the labeling. On some occasions, manufacturers have established expiration dates without performing label claim reconstitution potency assays at the various test intervals and particularly the expiration date test interval. Additionally, this stability testing of reconstituted solutions should include the most concentrated and the least concentrated reconstituted solutions. The most concentrated reconstituted solution will usually exhibit degradation at a faster rate than less concentrated solutions.

(c) Sterility Testing

With respect to sterility testing of lyophilized products, there is concern with the solution used to reconstitute the lyophilized product. Although products may be labeled for reconstitution with Bacteriostatic Water For Injection, Sterile Water For Injection (WFI) should be used to reconstitute products. Because of the potential toxicities associated with Bacteriostatic Water For Injection, many hospitals only utilize WFI. Bacteriostatic Water For Injection may kill some of the vegetative cells if present as contaminants, and thus mask the true level of contamination in the dosage form.

As with other sterile products, sterility test results which show contamination on the initial test should be identified and reviewed.

FINISHED PRODUCT INSPECTION - MELTBACK

The USP points out that it is good pharmaceutical practice to perform 100% inspection of parenteral products. This includes sterile lyophilized powders. Critical aspects would include the presence of correct volume of cake and the cake appearance. With regard to cake appearance, one of the major concerns is meltback.

Meltback is a form of cake collapse and is caused by the change from the solid to liquid state. That is, there is incomplete sublimation (change from the solid to vapor state) in the vial. Associated with this problem is a change in the physical form of the drug substance and/or a pocket of moisture. These may result in greater instability and increased product degradation.

Another problem may be poor solubility. Increased time for reconstitution at the user stage may result in partial loss of potency if the drug is not completely dissolved, since it is common to use in-line filters during administration to the patient.

Manufacturers should be aware of the stability of lyophilized products which exhibit partial or complete meltback. Literature shows that for some products, such as the cephalosporins, that the crystalline form is more stable than the amorphous form of lyophilized product. The amorphous form may exist in the "meltback" portion of the cake where there is incomplete sublimation.

GLOSSARY

ATMOSPHERE, THE EARTH

The envelope of gases surrounding the earth, exerting under gravity a pressure at the earth's surface, which includes by volume 78% nitrogen, 21% oxygen, small quantities of hydrogen, carbon dioxide, noble gases, water vapor, pollutants and dust.

ATMOSPHERIC PRESSURE

The pressure exerted at the earth's surface by the atmosphere. For reference purposes a standard atmosphere is defined as 760 torr or millimeters of mercury, or 760,000 microns.

BACKSTREAMING

A process that occurs at low chamber pressures where hydrocarbon vapors from the vacuum system can enter the product chamber.

BLANK-OFF PRESSURE

This is the ultimate pressure the pump or system can attain.

BLOWER (see Mechanical Booster Pump)

This pump is positioned between the mechanical pump and the chamber. It operates by means of two lobes turning at a high rate of speed. It is used to reduce the chamber pressure to less than 20 microns.

BREAKING VACUUM

Admitting air or a selected gas to an evacuated chamber, while isolated from a vacuum pump, to raise the pressure towards, or up to, atmospheric.

CIRCULATION PUMP

A pump for conveying the heat transfer fluid.

CONDENSER (Cold trap)

In terms of the lyophilization process, this is the vessel that collects the moisture on plates and holds it in the frozen state. Protects the vacuum pump from water vapor contaminating the vacuum pump oil.

CONDENSER/RECEIVER

In terms of refrigeration, this unit condenses (changes) the hot refrigerant gas into a liquid and stores it under pressure to be reused by the system.

COOLING

The lowering of the temperature in any part of the temperature scale.

CONAX CONNECTION

A device to pass thermocouple wires through and maintain a vacuum tight vessel.

CONTAMINATION

ln the vacuum system, the introduction of water vapor into the oil in the vacuum pump, which then causes the pump to lose its ability to attain its ultimate pressure.

DEFROSTING

The removal of ice from a condenser by melting or mechanical means.

DEGREE OF CRYSTALLIZATION

The ratio of the energy released during the freezing of a solution to that of an equal volume of water.

DEGREE OF SUPERCOOLING

The number of degrees below the equilibrium freezing temperature where ice first starts to form.

DESICCANT

A drying agent.

DRY

Free from liquid, and/or moisture.

DRYING

The removal of moisture and other liquids by evaporation.

EQUILIBRIUM FREEZING TEMPERATURES

The temperature where ice will form in the absence of supercooling.

EUTECTIC TEMPERATURE

A point of a phase diagram where all phases are present and the temperature and composition of the liquid phase cannot be altered without one of the phases disappearing.

EXPANSION TANK

This tank is located in the circulation system and is used as a holding and expansion tank for the transfer liquid.

FILTER OR FILTER/DRIER

There are two systems that have their systems filtered or filter/dried. They are the circulation and refrigeration systems. In the newer dryers this filter or filter/dryer is the same, and can be replaced with a new core.

FREE WATER

The free water in a product is that water that is absorbed on the surfaces of the product and must be removed to limit further biological and chemical reactions.

FREEZING

This is the absence of heat. A controlled change of the product temperature as a function of time, during the freezing process, so as to ensure a completely frozen form.

GAS BALLAST

Used in the vacuum system on the vacuum pump to decontaminate small amounts of moisture in the vacuum pump oil.

GAS BLEED (Vacuum control)

To control the pressure in the chamber during the cycle to help the drying process. In freeze-drying the purpose is to improve heat-transfer to the product.

HEAT EXCHANGER

This exchanger is located in the circulation and refrigeration systems and transfers the heat from the circulation system to the refrigeration system.

HEAT TRANSFER FLUID

A liquid of suitable vapor pressure and viscosity range for transferring heat to or from a component, for example, a shelf or condenser in a freeze-dryer. The choice of such a fluid may depend on safety considerations. Diathermic fluid.

HOT GAS BYPASS

This is a refrigeration system. To control the suction pressure of the BIG FOUR (20-30 Hp) compressors during the refrigeration operation.

HOT GAS DEFROST

This is a refrigeration system. To defrost the condenser plates after the lyophilization cycle is complete.

ICE

The solid, crystalline form of water.

INERT GAS

Any gas of a group including helium, radon and nitrogen, formerly considered chemically inactive.

INTERSTAGE

In a two stage compressor system, this is the cross over piping on top of the compressor that connects the low side to the high side. One could also think of it as low side, intermediate, and high side.

INTERSTAGE PRESSURE REGULATING VALVE

This valve controls the interstage pressure from exceeding 80 - 90 PSI. This valve opens to suction as the interstage pressure rises above 80 - 90 PSI.

LEXSOL

A heat transfer fluid (high grade kerosene).

LIQUID SUB-COOLER HEAT EXCHANGER (see Sub-cooled Liquid)

The liquid refrigerant leaving the condenser/receiver at cooling water temperature is sub-cooled to a temperature of +150F (-100C) to -150F (-250C).

LYOPHILIZATION

A process in which the product is first frozen and then, while still in the frozen state, the major portion of the water and solvent system is reduced by sublimation and desorption so as to limit biological and chemical reactions at the designated storage temperature,

MAIN VACUUM VALVE (see Vapor Valve)

This valve is between the chamber and external condenser to isolate the two vessels after the process is finished. This is the valve that protects the finished product.

MATRIX

A matrix, in terms of the lyophilization process, is a system of ice crystals and solids that is distributed throughout the product.

MECHANICAL BOOSTER PUMP (see Blower)

A roots pump with a high displacement for its size but a low compression ratio. When backed by an oil-seal rotary pump the combination is an economical alternative to a two-stage oil-sealed rotary pump, with the advantage of obtaining a high vacuum.

MECHANICAL VACUUM PUMP

The mechanical pumping system that lowers the pressure in the chamber to below atmospheric pressure so that sublimation can occur.

MELTING TEMPERATURE (Melt-back)

That temperature where mobile water first becomes evident in a frozen system.

MICRON (see Torr)

A unit of pressure used in the lyophilization process. One micron = one Mtorr or 25,400 microns = 1" Hg., or 760,000 microns = one atmosphere.

NONCONDENSABLES

A mixture of gases such as nitrogen, hydrogen, chlorine, and hydrocarbons. They may be drawn into the system through leaks when part of the system is under a vacuum. Their presence reduces the operating efficiency of the system by increasing the condensing pressure.

NUCLEATION

The formation of ice crystals on foreign surfaces or as a result of the growth of water clusters.

OIL-MIST FILTER

In vacuum terminology a filter attached to the discharge (exhaust) of an oil-sealed rotary pump to eliminate most of the "smoke" of suspended fine droplets of oil which would be discharged into the environment.

OIL SEALED ROTARY PUMP

A standard type of mechanical vacuum pump used in freeze-drying with a high compression ratio but having a relatively low displacement (speed) for its size. A two-stage pump is effectively two such pumps in series and can obtain an ultimate vacuum.

OIL SEPARATOR

Separates the oil from the compressor discharge gas and returns the oil through the oil float trap and piping to the compressor crankcase.

REAL LEAK

A real leak is a source of atmospheric gases resulting from a penetration through the chamber.

RECONSTITUTE

The dissolving of the dried product into a solvent or diluent.

RELIEF VALVE

Used for safety purposes to prevent damage in case excessive pressure is encountered.

ROTARY VANE PUMP

A mechanical pumping system with sliding vanes as the mechanical seal. Can be single or two stages.

SHELF COMPRESSOR (Controlling Compressor)

Used for controlling the shelf temperature, either cooling or from overheating.

SELF LIQUID HEAT EXCHANGER

The transfer of heat from the shelf fluid to the refrigeration system through tubes in the exchanger causing compressor suction gas to warm.

SHELVES

In terms of the lyophilization process, they are a form of heat exchanger, within the chamber, that have a serpentine liquid flow through them, entering one side and flowing to the other side. They are located in the circulation system.

SINGLE STAGE COMPRESSOR

This is a normal type compressor used in refrigeration. In the lyophilization process it is used to control the shelf temperature, both for cooling and keeping the shelf temperature from overheating using a temperature controller.

SILICONE OIL

A heat transfer fluid.

STERILIZATION

The use of steam and pressure to kill any bacteria that may be able to contaminate that environment or vessel.

SUBLIMATION

The conversion of a material from a solid phase directly to a vapor phase, without passing through the liquid phase. This is referred to as the primary drying stage.

SUB-COOLED LIQUID (See Liquid Sub-cooler Heat Exchanger)

The liquid refrigerant is cooled through an exchanger so that it increases the refrigerating effect as well as reduces the volume of gas flashed from the liquid refrigerant in passage through the expansion valve.

SUCTION LINE ACCUMULATOR

To provide adequate refrigerant liquid slug protection (droplets of liquid refrigerant) from returning to the compressor, and causing damage to the compressor.

TCE

Trichloroethylene - A heat transfer fluid.

TEMPERATURE

The degree of hotness or coldness of a body.

THERMOCOUPLE

A metal-to-metal contact between two dissimilar metals that produces a small voltage across the free ends of the wire.

THERMOSTATIC EXPANSION VALVE

An automatic variable device controlling the flow of liquid refrigerant.

TORR (See Micron)

A unit of measure equivalent to the amount of pressure in 1000 microns.

TWO STAGE COMPRESSOR (see Interstage)

This is a specially built compressor. Its function is to be able to attain low temperatures by being able to operate at low pressures. It is two compressors built into one. A low stage connected internally and a high stage connected externally with piping, called interstage.

UNLOADING VALVE

This valve connects the interstage with suction to equalize both pressures during pump-down.

VACUUM

Strictly speaking, a space in which the total pressure is less than atmospheric.

VACUUM CONTROL (Gas Bleed)

To assist in the rate of sublimation, by controlling the pressure in the lyophilizer.

VACUUM PUMP

A mechanical way of reducing the pressure in a vessel below atmospheric pressure to where sublimation can occur. There are three types of pumps, rotary vane, rotary piston and mechanical booster.

VAPOR BAFFLE

A target shaped object placed in the condenser to direct vapor flow and to promote an even distribution of condensate.

VACUUM VALVES

The vacuum valves used are of a ball or disk type that can seal without leaking. The ball types are used for services to the chamber and condenser. They are also used for drains and isolation applications. The disk types are used in the vacuum line system and are connected to the vacuum pump, chamber and condenser.

VAPOR VALVE (See Main Vacuum Valve)

The vacuum valve between the chamber and external condenser. When this valve is closed the chamber is isolated from the external condenser. Also known as the main vapor valve.

VIAL

A small glass bottle with a flat bottom, short neck and flat flange designed for stoppering.

VIRTUAL LEAK

In the vacuum system a virtual leak is the passage of gas into the chamber from a source that is located internally in the chamber.

Return to: Page Top | Inspection Start (/inspections-compliance-enforcement-and-criminal-investigations/inspection-guides/inspection-guides)

Google amount of water in lyophilized powder

All Shopping Images News Videos More Tools

About 6,260,000 results (0.63 seconds)

Ad · https://www.argonautms.com/go_ambient (888) 834-8892

Lyophilization Experts - Microbead, Vial & Bulk Powders

Located in Carlsbad, CA. cGMP Compliance. Call to Find Out More. Feasibility Studies. Multiple Excipients, Types, Fill, Finish, Analytical QC & QA.

FAQ's · Analytical Testing · Why A Lyophilization CMO? · Get a Quote

Ad · https://www.pharm-int.com/aseptic-filling/lyophilization (410) 884-0001

Lyophilization Know How™ - Pharmaceutical Know-How™

Pi's integrated software allows real time analysis + optimization - saving costly product. Lyophilization - High Potency - Stabilization. 2 - 50ml vials achieving your drug outcomes. cGMP Certified. Flexible Services. US Based CDMO. Minimal Human Contact.

Dosage Forms · Aseptic Fill · Leadership · Contact Us

After the drying process, the final residual water content in the product is very low with around **1% to 4%**. Nevertheless, higher amounts of water in the lyophilized drug formulation can reduce the stability of some drugs.

https://www.mt.com › ... › Titration

Water Content of Lyophilized vaccines - Titration - Mettler Toledo

About featured snippets · Feedback

https://www.pharmtech.com › view › residual-moisture-...

Residual Moisture Testing Methods for Lyophilized Drug ..

Apr 22, 2018 — Because water can be present in a lyophilized product in a number of ways, measurement of the residual moisture and the method used to ...

https://www.researchgate.net › post › How_does_the_mov...

How does the movement of water / moisture in lyophilized ...

The water from the atmosphere then hydrates the powder homogeneously and establishes an equilibrium with the entire product. You can check the amount of ...

2 answers · 0 votes: I have got some experience preparing hydrated protein powders. The pro...

https://www.americanpharmaceuticalreview.com › 8855 ...

Considering Residual Moisture Measurement in Lyophilized ...

Dec 23, 2019 — The transfer of stopper moisture to the product is critical for products with a low fill volume as the transfer of water from the stopper ...

https://pubmed.ncbi.nlm.nih.gov › ...

Determining the optimum residual moisture in lyophilized ...

by CC Hsu · 1992 · Cited by 135 — The amount of water adsorbed on each protein can be determined and approximated as a monolayer by the Brunauer-Emmett-Teller method. The ...

https://www.fda.gov › inspection-guides › lyophilizatio...

Lyophilization of Parenteral (7/93) | FDA - US Food and Drug ...

Nov 11, 2014 — Lyophilization or freeze drying is a process in which water is removed from a product after it is ... Enhanced stability of a dry powder.

Google amount of water in lyophilized powder

by A Duralliu · 2020 · Cited by 5 — Case Study 1: Freeze Drying of a High Concentration IgG ... mL volume screw capped vials (41.5 × 18 mm) (e.g. Schott VC005, Adelphi Tubes, ...

https://www.pharmaceuticalonline.com › doc › moistur...

Moisture Determination of Dry Product - Pharmaceutical Online

... stability and shelf life of lyophilized pharmaceutical product, sterile powders, ... Studies have demonstrated that the amount of headspace water vapor ...

https://www.drugdiscoverytrends.com › lyophilization-t...

Lyophilization: The Basics - Drug Discovery and Development

Mar 7, 2017 — Lyophilization, defined as a freeze-drying process that removes water ... Enhancing the stability of a dry powder as well as the product ...

https://core.ac.uk › download › pdf

Determination of moisture content in lyophilized mannitol ...

by CR Muzzio · Cited by 17 — total amount of water in lyophilized mannitol through intact glass vials with a reflectance ... particle size and powder packing, several authors (Shenk ...

10 pages

Related searches

usp <921 method 1c

how does freeze drying work

lyophilization

Fairfax County, Virginia - From your IP address - Update location

Help Send feedback Privacy Terms

Doc Code:IDS.3P
Document Description: Third-Party Submission Under 37 CFR 1.290

PTO/SB/429(08-12)
Approved for use through 07/31/2015. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	17025881

U.S. PATENTS

Cite No	Patent Number	Kind Code[1]	Issue Date (YYYY-MM-DD)	First Named Inventor

U.S. PATENT APPLICATION PUBLICATIONS

Cite No	Publication Number	Kind Code[1]	Publication Date (YYYY-MM-DD)	First Named Inventor

FOREIGN PATENTS AND PUBLISHED FOREIGN PATENT APPLICATIONS

Cite No	Foreign Document Number[3]	Country Code[2]	Kind Code[1]	Publication Date (YYYY-MM-DD)	Applicant, Patentee or First Named Inventor	T[5]
						☐

NON-PATENT PUBLICATIONS (e.g., journal article, Office action)

Cite No	Author (if any), title of the publication, page(s) being submitted, publication date, publisher (where available), place of publication (where available).	T[5]	E[6]

EFS Web 2.1.17

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Y.Z/

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	17025881

1 /Y.Z/	K. Kawada, Phenobarbital sodium lyophilized formulation: Overview of the clinical trial leading to approval of NOBELBAR and package insert information, Neonatal Care, Vol. 22, No. 7, pp. 657-663, 2009	☒	☐
2 /Y.Z/	R.S. Fisher et al., A practical clinical definition of epilepsy, Epilepsia, Vol. 55, No. 4, pp. 475-482, 2014	☐	☐
3 /Y.Z/	K. Kawada et al., A clinical Trial Assessing the Efficacy and Safety of a New Injectable Formula of Sodium Phenobarbital Containing No Additives for the Treatment of Neonatal Seizures, Jpn. J. Clin. Phamacol. Ther., Vol. 42, No. 6, pp. 205-10, July 2011	☐	☐

EFS Web 2.1.17

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Y.Z/

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	17025881

4 /Y.Z/	O. Bertaux et al., Modification of hepatic alpha-1-glycoprotein and albumin gene expression in rats treated with phenobarbital, Eur. J. Biochem., Vol. 203, pp. 655-61, 1992	☐	☐
5 /Y.Z/	J. Bernstein, Polymorphism in Molecular Crystals, Oxford University Press, Oxford, GB, 2002, ISBN 978-0-19-923656-5	☐	☐
6 /Y.Z/	Japan Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Deliberation Results Report for Nobelbar for intravenous injection 250 mg, 43 pages, September 16, 2008	☒	☐

EFS Web 2.1.17

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Y.Z/

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	17025881

7 /Y.Z/	H. G. Brittain, Polymorphisms in Pharmaceutical Solids, Marcel Dekker, Inc., New York, NY, 1999, ISBN 0-8247-0237-9	☐	☐
/Y.Z/ 8	T. Fournier et al., Phenobarbital Induction of α1-Acid Glycoprotein in Primary Rat Hepatocyte Cultures, Hepatology, Vol. 20, No. 6, pp. 1584-88, 1994	☐	☐
/Y.Z/ 9	J.H. Kern, Freeze Drying as a Method of Processing Some Pharmaceutical Products, Doctor of Philosophy Thesis, The Ohio State University, 1954	☐	☒

EFS Web 2.1.17

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Y.Z/

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	17025881

/Y.Z/ 10	Nobelpharma Co., Ltd., NOBELBAR 250 mg for Injection, version 12, November 2020	☒	☐

STATEMENTS

The party making the submission is not an individual who has a duty to disclose information with respect to the above-identified application under 37 CFR 1.56.

This submission complies with the requirements of 35 U.S.C. 122(e) and 37 CFR 1.290.

☒ The fee set forth in 37 CFR 1.290(f) has been submitted herewith.

☐ The fee set forth in 37 CFR 1.290(f) is not required because this submission lists three or fewer total items and, to the knowledge of the person signing the statement after making reasonable inquiry, this submission is the first and the only submission under 35 U.S.C 122(e) filed in the above-identified application by the party making the submission or by a party in privity with the party.

☐ This resubmission is being made responsive to a notification of non-compliance issued for an earlier filed third-party submission. The corrections in this resubmission are limited to addressing the non-compliance. As such, the party making this resubmission: (1) requests that the Office apply the previously-paid fee set forth in 37 CFR 1.290(f), or (2) states that no fee is required to accompany this resubmission as the undersigned is again making the fee exemption statement set forth in 37 CFR 1.290(g).

Signature	/Jay P Lessler/		
Name/Print	Jay P Lessler	Registration Number (if applicable)	41151

Examiner Signature	/YANZHI ZHANG/	Date Considered	09/07/2021

*EXAMINER: Signature indicates all documents listed above have been considered,except for citations through which a line is drawn. Draw line through citation if not considered. Include a copy of this form with next communication to applicant. 1. If known, enter kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16. See MPEP 901.04(a). 2. Enter the country or patent office that issued the document, by two-letter code under WIPO standard ST.3. See MPEP 1851. 3. For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. 4. If known, enter the kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 . See MPEP 901.04(a). 5. Check mark indicates translation attached. 6. Check mark indicates evidence of publication attached.

EFS Web 2.1.17

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Y.Z/

Doc Code:IDS.3P
Document Description: Third-Party Submission Under 37 CFR 1.290

PTO/SB/429(08-12)
Approved for use through 07/31/2015. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	17025881

U.S. PATENTS

Cite No	Patent Number	Kind Code[1]	Issue Date (YYYY-MM-DD)	First Named Inventor
1 /Y.Z/	8436190	B2	2013-05-07	Jason Brittain

U.S. PATENT APPLICATION PUBLICATIONS

Cite No	Publication Number	Kind Code[1]	Publication Date (YYYY-MM-DD)	First Named Inventor
1 /Y.Z/	20170143719	A1	2017-05-25	Michael G. Parker

FOREIGN PATENTS AND PUBLISHED FOREIGN PATENT APPLICATIONS

Cite No	Foreign Document Number[3]	Country Code[2]	Kind Code[1]	Publication Date (YYYY-MM-DD)	Applicant, Patentee or First Named Inventor	T[5]
						☐

NON-PATENT PUBLICATIONS (e.g., journal article, Office action)

Cite No	Author (if any), title of the publication, page(s) being submitted, publication date, publisher (where available), place of publication (where available).	T[5]	E[6]

EFS Web 2.1.17

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Y.Z/

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	17025881

/Y.Z/ 1	Access to Ohio State Dissertations and Theses, https://gradsch.osu.edu/access-distribution (accessed on July 14, 2021)	☐	☐
/Y.Z/ 2	Lyophilization of Parenteral (7/93) (LoP) (https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-guides/lyophilization-parenteral-793) (dated November 11, 2014) (accessed on July 19, 2021)	☐	☐
/Y.Z/ 3	D.S. Przic, et al., Lyophilization - The Process and Industrial Scale, Chem. Ind. Vol. 58, No. 12, pp. 552-562, 2004	☐	☐

EFS Web 2.1.17

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Y.Z/

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	17025881

/Y.Z/ 4	OhioLINK: Frequently Asked Questions, https://etd.ohiolink.edu/apexprod/-f?p=1501:2:310637464866699 (accessed on July 14, 2021)	☐	☐
/Y.Z/ 5	Entry from OhioLINK database for Kern, https://etd.ohiolink.edu/apexprod/-rws_olink/r/1501/10?clear=10&p10_accession_num=osu1486476025025146 (accessed on July 14, 2021)	☐	☐
/Y.Z/ 6	I.B. Butler, et al., Removal of dissolved oxygen from water: a comparison of four common techniques, Talanta, Vol. 41, No. 2, pp. 211-215, 1994	☐	☐

EFS Web 2.1.17

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Y.Z/

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	17025881

/Y.Z/ 7	R. Sandhyarani, Development and Evaluation of Lyophilized Product of Apo-Acetozolamide, IOSR Journal of Pharmacy; Vol. 6, Issue 9 Version. 1, pp. 48-63, Sept. 2016	☐	☐
/Y.Z/ 8	Encyclopedia of Pharmaceutical Technology (2007), Vol 1, Third Edition; James Swarbrick; Freeze, Freeze Drying, Scale-Up Considerations (Pg. 1807-1874)	☐	☐

STATEMENTS

The party making the submission is not an individual who has a duty to disclose information with respect to the above-identified application under 37 CFR 1.56.

This submission complies with the requirements of 35 U.S.C. 122(e) and 37 CFR 1.290.

☒ The fee set forth in 37 CFR 1.290(f) has been submitted herewith.

☐ The fee set forth in 37 CFR 1.290(f) is not required because this submission lists three or fewer total items and, to the knowledge of the person signing the statement after making reasonable inquiry, this submission is the first and the only submission under 35 U.S.C 122(e) filed in the above-identified application by the party making the submission or by a party in privity with the party.

☐ This resubmission is being made responsive to a notification of non-compliance issued for an earlier filed third-party submission. The corrections in this resubmission are limited to addressing the non-compliance. As such, the party making this resubmission: (1) requests that the Office apply the previously-paid fee set forth in 37 CFR 1.290(f), or (2) states that no fee is required to accompany this resubmission as the undersigned is again making the fee exemption statement set forth in 37 CFR 1.290(g).

EFS Web 2.1.17

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Y.Z/

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	17025881

Signature	/Jay P, Lessler/		
Name/Print	Jay P. Lessler	Registration Number (if applicable)	41151

Examiner Signature	/YANZHI ZHANG/	Date Considered	09/07/2021

*EXAMINER: Signature indicates all documents listed above have been considered,except for citations through which a line is drawn. Draw line through citation if not considered. Include a copy of this form with next communication to applicant. 1. If known, enter kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16. See MPEP 901.04(a). 2. Enter the country or patent office that issued the document, by two-letter code under WIPO standard ST.3. See MPEP 1851. 3. For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. 4. If known, enter the kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 . See MPEP 901.04(a). 5. Check mark indicates translation attached. 6. Check mark indicates evidence of publication attached.

EFS Web 2.1.17

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Y.Z/

Electronic Acknowledgement Receipt	
EFS ID:	44587340
Application Number:	17025881
International Application Number:	
Confirmation Number:	8113
Title of Invention:	Lyophilized Compositions of Phenobarbital Sodium Salt
First Named Inventor/Applicant Name:	Bala Tripura Sundari Chodavarapu
Customer Number:	149344
Filer:	Martin Fessenmaier/Danielle Moroz
Filer Authorized By:	Martin Fessenmaier
Attorney Docket Number:	104078.0001US
Receipt Date:	22-DEC-2021
Filing Date:	18-SEP-2020
Time Stamp:	12:40:37
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$1000
RAM confirmation Number	E2021BLC41094462
Deposit Account	601823
Authorized User	Danielle Moroz
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.21 (Miscellaneous fees and charges) 37 CFR 1.20 (Post Issuance fees)	

37 CFR 1.19 (Document supply fees)

37 CFR 1.17 (Patent application and reexamination processing fees)

37 CFR 1.16 (National application filing, search, and examination fees)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Continued Examination (RCE)	RCE-02.pdf	1364401 759af26416d229a87f4607677222f701528c4af9	no	3
Warnings:					
Information:					
2		Response-04_FOA_9-23-21_REV001.pdf	117212 de3e673aaeaa5ca0c3097a68acc30810d8cab1e	yes	7
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Amendment Submitted/Entered with Filing of CPA/RCE		1	1	
	Claims		2	4	
	Applicant Arguments/Remarks Made in an Amendment		5	7	
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	37595 876d6ae3d24861f29c4aa0c9db9aaf99f4b6b8e	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1519208		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Doc code: RCEX
Doc description: Request for Continued Examination (RCE)

PTO/SB/30EFS (02-18)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL
(Submitted Only via EFS-Web)

Application Number	17/025,881	Filing Date	2020-09-18	Docket Number (if applicable)	104078.0001US	Art Unit	1617
First Named Inventor	Bala Tripura Sundari Chodavarapu			Examiner Name	Yanzhi ZHANG		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV

SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

- ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
- ☐ Other _____

☒ Enclosed

- ☒ Amendment/Reply
- ☐ Information Disclosure Statement (IDS)
- ☐ Affidavit(s)/ Declaration(s)
- ☐ Other _____

MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____
(Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.
☒ The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to Deposit Account No 601823

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

☒ Patent Practitioner Signature

Applicant Signature

EFS - Web 2.1.16

Doc code: RCEX
Doc description: Request for Continued Examination (RCE)

PTO/SB/30EFS (02-18)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Signature of Registered U.S. Patent Practitioner			
Signature	/Martin Fessenmaier Ph.D./	Date (YYYY-MM-DD)	2021-12-22
Name	Martin Fessenmaier Ph.D.	Registration Number	46697

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

EFS - Web 2.1.16

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Appl No.:	17/025,881
First Inventor:	Bala Tripura Sundari Chodavarapu
Filing Date:	2020-09-18
Art Unit:	1617
Examiner:	Yanzhi ZHANG
Attorney Docket No.:	104078.0001US
Title	Lyophilized Compositions of Phenobarbital Sodium Salt

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO FINAL OFFICE ACTION

Sir:

Concurrent with filing an RCE, this paper is in response to the Office Action dated 23 September 2021. Please enter the amendments shown herein.

- Claim amendments begin on page 2.
- Remarks begin on page 5.

AMENDMENTS TO THE CLAIMS

This listing of claims represents the current version of claims before the Office:

1-27.(canceled)

28. (new) A method of producing a storage-stable form of lyophilized amorphous phenobarbital sodium composition, comprising:

adding phenobarbital sodium to water to form a phenobarbital sodium solution having a pH of between 9.2 and 10.2, wherein the phenobarbital sodium solution does not contain an organic solvent, and wherein the phenobarbital sodium solution optionally further comprises sodium chloride;

lyophilizing the phenobarbital sodium solution in a container under a protocol comprising a freezing step, a primary drying step, and a secondary drying step to thereby produce the lyophilized amorphous phenobarbital sodium composition;

wherein the freezing step comprises freezing the lyophilized amorphous phenobarbital sodium to a temperature of about -50 °C without application of vacuum;

wherein the primary drying step comprises application of vacuum at a pressure of between about 50mTorr and 75 mTorr at a temperature of between about -50 °C and about 0 °C for at duration of at least 1,000 minutes;

wherein the secondary drying step comprises application of vacuum at a pressure of between about 50 mTorr and 75 mTorr at a temperature of between about 25 °C and about 50 °C for at duration of at least 200 minutes;

wherein the lyophilized amorphous phenobarbital sodium has an initial moisture content of equal or less than 1.5%; and

wherein the lyophilized amorphous phenobarbital sodium forms, upon storage over at least 3 months, no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution.

29. (new) The method of claim 28, wherein the sodium chloride is present in the phenobarbital sodium solution in an amount that, upon reconstitution of the lyophilized amorphous phenobarbital sodium in the aqueous solution, yields a sodium chloride concentration of 0.9% in the reconstituted solution.

30. (new) The method of claim 28, wherein the phenobarbital sodium solution in the container is present in an amount of about 65 mg, or about 100 mg, or about 130 mg, or about 200 mg.

31. (new) The method of claim 28, wherein the water is sparged with nitrogen.

32. (new) The method of claim 28, wherein the phenobarbital sodium solution does not contain a preservative.

33. (new) The method of claim 28, wherein the primary drying step is performed using at increasing temperatures that increase from about -50 °C to about 0 °C when the amount of phenobarbital sodium in the container is between 65 mg and 130 mg.

34. (new) The method of claim 28, wherein the primary drying step is performed using at constant temperature of about -42 °C when the amount of phenobarbital sodium in the container is 200 mg.

35. (new) The method of claim 28, wherein the secondary drying step is performed using at decreasing temperatures that decrease from about 50 °C to about 25 °C when the amount of phenobarbital sodium in the container is between 65 mg and 130 mg.

36. (new) The method of claim 28, wherein the secondary drying step is performed using at constant temperature of about 35 °C when the amount of phenobarbital sodium in the container is 200 mg.

37. (new) The method of claim 28, wherein the lyophilized amorphous phenobarbital sodium has an initial moisture content of equal or less than 1.2%.

38. (new) The method of claim 28, wherein the lyophilized amorphous phenobarbital sodium has an initial moisture content of equal or less than 1.0%.

39. (new) The method of claim 28, wherein the lyophilized amorphous phenobarbital sodium forms, upon storage over at least 3 months, no more than 0.1% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution.

40. (new) The method of claim 28, wherein the lyophilized amorphous phenobarbital sodium forms, upon storage over at least 3 months, no detectable quantities of 2-ethyl-2-phenylmalonamide (2EPMM) or alpha-phenylbutyrylguanidine (PBG) when reconstituted in an aqueous solution.

41. (new) The method of claim 28, wherein the storage is at 40 °C and 75% relative humidity.

42. (new) The method of claim 28, wherein the lyophilized amorphous phenobarbital sodium composition contains at least 98% lyophilized amorphous phenobarbital sodium.

43. (new) A pharmaceutical composition comprising a storage-stable form of lyophilized amorphous phenobarbital sodium, comprising:

sterile lyophilized amorphous phenobarbital sodium composition in a container, wherein the amount of the lyophilized amorphous phenobarbital sodium in the container is between 50 mg and 250 mg;

wherein the sterile lyophilized amorphous phenobarbital sodium composition does not contain an organic solvent, and optionally further comprises sodium chloride;

wherein the lyophilized amorphous phenobarbital sodium composition has an initial moisture content of equal or less than 1.5%; and

wherein the lyophilized amorphous phenobarbital sodium forms, upon storage over at least 3 months, no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution.

44. (new) The pharmaceutical composition of claim 43, wherein the sodium chloride is present in the phenobarbital sodium solution in an amount that, upon reconstitution of the lyophilized amorphous phenobarbital sodium in the aqueous solution, yields a sodium chloride concentration of 0.9% in the reconstituted solution.

45. (new) The pharmaceutical composition of claim 43, wherein the lyophilized amorphous phenobarbital sodium forms, upon storage over at least 3 months, no detectable quantities of 2-ethyl-2-phenylmalonamide (2EPMM) or alpha-phenylbutyrylguanidine (PBG) when reconstituted in an aqueous solution.

46. (new) The pharmaceutical composition of claim 43, wherein the composition is free of preservatives.

47. (new) The pharmaceutical composition of claim 43, wherein the lyophilized amorphous phenobarbital sodium composition contains at least 98% lyophilized amorphous phenobarbital sodium.

REMARKS/ARGUMENTS

The Office action dated 23 September 2021 has been received and carefully considered. By this amendment, new claims 28-47 are added and previous claims 1-27 are canceled. The new claims were added to enhance clarity and are based on previous independent claims 1 and 2. After entry of this Amendment, claims 28-47 will be pending. With these amendments, no new matter is added. In view of these amendments and the following remarks, Applicants respectfully request reconsideration.

35 U.S.C. §112(b)

Claims 1 and 2 were rejected for lack of clarity with regard to recitation of ranges. The applicant agrees and the new claims no longer contain the objected phrases. The rejection should therefore be overcome.

35 U.S.C. §112(d)

Claim 7 was rejected for no further limiting the scope of the claim from which it depended. lack of clarity with regard to recitation of ranges. The applicant once more agrees and the new claims no longer contain the objected phrase. The rejection should therefore be overcome.

35 U.S.C. §103

The Office rejected **claims 1-2, 8-13, 15, and 21-27** under 35 U.S.C. 103 as obvious over Parker et al (US 20170143719 A1, publication date: May 25, 2017, "Parker", of record) in view of FDA document of Lyophilization of Parenteral (7 /93) (non-patent literature, published 11/11/2014 "FDA_7/93"). As all previously pending claims were canceled, the present rejections are moot.

Nevertheless, with regard to new claims 28-47 and Parker it is noted that the solution to the known instability of phenobarbital sodium in Parker was to avoid the presence of water and to substitute water with organic solvents, and particularly a C1-C4 alcohol and a glycol. In contrast, the present claims expressly require that the phenobarbital sodium compositions must be free of organic solvents, and that in the preparation of the solution for lyophilization, the phenobarbital sodium is dissolved in an aqueous solution that does not contain organic solvents. Clearly, Parker fundamentally requires organic solvents, whereas the present claims expressly exclude such

solvents. As such Parker not only fails to teach or suggest the limitations of the present claims, but also directly teaches against the claimed subject matter. Consequently, the PHOSITA would not have a reasonable expectation of success to modify Parker by removing organic solvents. FDA_7/93 fails to remedy these defects, and the combination of Parker and FDA_7/93 does not render the presently pending claims obvious.

In addition, and equally significant, it should be appreciated that while the claims expressly require a method of lyophilization of an aqueous solution containing phenobarbital sodium or a pharmaceutical composition containing lyophilized amorphous phenobarbital sodium, Parker is concerned with liquid formulations having extended storage stability. While possibly not apparent at first glance, it must be recognized that the solvent system of Parker is entirely unsuitable to lead to a lyophilized phenobarbital sodium form as alcohol/glycol mixtures will not sublimate and desorb, particularly under conditions that would preserve chemical integrity of phenobarbital sodium. Should the Office believe that pharmaceutical active ingredients, and especially phenobarbital sodium, can be lyophilized from an alcohol/glycol mixtures into an amorphous product, the applicant respectfully requests documentary evidence to support such belief.

For at least these reasons, the presently pending claims are new and non-obvious over Parker and FDA_7/93, alone or in combination.

***

The Office also rejected **claims 4, 7, and 14** under 35 U.S.C. 103 as obvious over Parker et al (US 20170143719 A1, publication date: May 25, 2017, "Parker", of record) in view of FDA document of Lyophilization of Parenteral (7 /93) (non-patent literature, published 11/11/2014 "FDA_7/93") as applied to claims 1-2, 8-13, 15, and 21-27 in further view of Roskos et al. ("Roskos", US 6077545 A, date issued: June 20, 2000, of record). As all previously pending claims were canceled, the present rejections are moot.

Nevertheless, with regard to new claims 28-47 and the combination of Parker and FDA_7/93, the same arguments and deficiencies as pointed out above apply and are not reiterated here, and Roskos et al. fail to remedy these defects.

***

The Office further rejected **claims 16-20** under 35 U.S.C. 103 as obvious over Kelly et al ("Kelly", non-patent literature, Critical Care Trauma Centre, pp. 1-3, last update: October 11, 2018, of record) and Sun et al ("Sun", US 20100035904 Al, publication-date: February 11, 2010, of record). As all previously pending claims were canceled, and as the currently pending claims are not directed to a method of treatment of seizures, the present rejections are moot.

Request For Allowance

Claims 28-47 are pending in this application. Applicant requests allowance of all pending claims.

Respectfully submitted,
Umberg Zipser LLP

Date: December 22, 2021

By: /Martin Fessenmaier Ph.D./
Martin Fessenmaier Ph.D.
Reg. No. 46697

Umberg Zipser LLP
1920 Main Street, Suite 750
Irvine, CA 92614
Telephone (949) 679-0052
Fax (949) 679-0461

Electronic Patent Application Fee Transmittal				
Application Number:	17025881			
Filing Date:	18-Sep-2020			
Title of Invention:	Lyophilized Compositions of Phenobarbital Sodium Salt			
First Named Inventor/Applicant Name:	Bala Tripura Sundari Chodavarapu			
Filer:	Martin Fessenmaier/Danielle Moroz			
Attorney Docket Number:	104078.0001US			
Filed as Small Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	**Fee Code**	**Quantity**	**Amount**	**Sub-Total in USD($)**
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
RCE- 2ND AND SUBSEQUENT REQUEST	2820	1	1000	1000
		Total in USD ($)		**1000**

To: rdean@umbergzipser.com,patents@umbergzipser.com,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 149344

Feb 08, 2022 04:56:48 AM

Dear PAIR Customer:

Umberg Zipser LLP
1920 Main Street Suite 750
Irvine, CA 92614
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 149344 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:
The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
17025881	CTRS	02/08/2022	104078.0001US

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
17/025,881	09/18/2020	Bala Tripura Sundari Chodavarapu	104078.0001US	8113

149344 7590 02/08/2022

Umberg Zipser LLP
1920 Main Street Suite 750
Irvine, CA 92614

EXAMINER
AZPURU, CARLOS A

ART UNIT	PAPER NUMBER
1617	

NOTIFICATION DATE	DELIVERY MODE
02/08/2022	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patents@umbergzipser.com
rdean@umbergzipser.com

PTOL-90A (Rev. 04/07)

Office Action Summary	Application No. 17/025,881	Applicant(s) Chodavarapu et al.	
	Examiner CARLOS A AZPURU	Art Unit 1617	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 2 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 12/22/2021.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.

2a)☐ This action is **FINAL.** 2b) ☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 28-47 is/are pending in the application.

5a) Of the above claim(s) ____ is/are withdrawn from consideration.

6) ☐ Claim(s) ____ is/are allowed.

7) ☐ Claim(s) ____ is/are rejected.

8) ☐ Claim(s) ____ is/are objected to.

9) ☑ Claim(s) 28-47 are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on ____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. ____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date ____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date ____.

4) ☐ Other: ____.

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Election/Restriction

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 28-42, drawn to a method of producing, classified in A1K 9/19.

II. Claims 43-47, drawn to pharmaceutical composition, classified in A61K 31/515.

The inventions are independent or distinct, each from the other because:

Inventions Group I and Group II are related as process of making and product made. The inventions are distinct if either or both of the following can be shown: (1) that the process as claimed can be used to make another and materially different product or (2) that the product as claimed can be made by another and materially different process (MPEP § 806.05(f)). In the instant case , the product as claimed can be made by the process of WO 2017/085687 (Parker et al).

Restriction for examination purposes as indicated is proper because all the inventions listed in this action are independent or distinct for the reasons given above and there would be a serious search and/or examination burden if restriction were not required because one or more of the following reasons apply:

Groups I and II are independent and distinct as set out above, and the search and/or examination of both Groups would entail an undue burden on this office.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of an invention to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected invention**.

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103 or pre-AIA 35 U.S.C. 103(a) of the other invention.

A telephone call was not made to Martin Fessenmaier, Ph.D. on 02/03/2022 to request an oral election to the above restriction requirement.

Inventorship

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be corrected in compliance with 37 CFR 1.48(a) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. A request to correct inventorship under 37 CFR 1.48(a) must be accompanied by an application data sheet in accordance with 37 CFR 1.76 that identifies each inventor by his or her legal name and by the processing fee required under 37 CFR 1.17(i).

Rejoinder

The examiner has required restriction between product or apparatus claims and process claims. Where applicant elects claims directed to the product/apparatus, and all product/apparatus claims are subsequently found allowable, withdrawn process claims that include all the limitations of the allowable product/apparatus claims should be considered for rejoinder. All claims directed to a nonelected process invention must include all the limitations of an allowable product/apparatus claim for that process invention to be rejoined.

In the event of rejoinder, the requirement for restriction between the product/apparatus claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103 and 112. Until all claims to the elected product/apparatus are found allowable, an otherwise proper restriction requirement between product/apparatus claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowable product/apparatus claim will not be rejoined. See MPEP § 821.04. Additionally, in order for rejoinder to occur, applicant is advised that the process claims should be amended during prosecution to require the limitations of the product/apparatus claims. **Failure to do so may result in no rejoinder.** Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

Correspondence

Any inquiry concerning this communication or earlier communications from the examiner should be directed to CARLOS A AZPURU whose telephone number is (571)272-0588. The examiner can normally be reached 9 am- 3 pm, 4 pm-8pm.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Johann R Richter can be reached on 571-272-0646. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: https://patentcenter.uspto.gov. Visit https://www.uspto.gov/patents/apply/patent-center for more information about Patent Center and https://www.uspto.gov/patents/docx for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/CARLOS A AZPURU/
Primary Examiner, Art Unit 1617

caz

Electronic Acknowledgement Receipt	
EFS ID:	45189868
Application Number:	17025881
International Application Number:	
Confirmation Number:	8113
Title of Invention:	Lyophilized Compositions of Phenobarbital Sodium Salt
First Named Inventor/Applicant Name:	Bala Tripura Sundari Chodavarapu
Customer Number:	149344
Filer:	Martin Fessenmaier/Danielle Moroz
Filer Authorized By:	Martin Fessenmaier
Attorney Docket Number:	104078.0001US
Receipt Date:	09-MAR-2022
Filing Date:	18-SEP-2020
Time Stamp:	19:22:57
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Response-05_RRNFOA_2-8-22_REV002.pdf	127430 916ad9196fcd9360e72d7a86919facae1b0 98fed	yes	7

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Response to Election / Restriction Filed	1	1
	Claims	2	5
	Applicant Arguments/Remarks Made in an Amendment	6	7
Warnings:			
Information:			
	Total Files Size (in bytes):	127430	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Appl No.:	17/025,881
First Inventor:	Bala Tripura Sundari Chodavarapu
Filing Date:	2020-09-18
Art Unit:	1617
Examiner:	Yanzhi ZHANG
Attorney Docket No.:	104078.0001US
Title	Lyophilized Compositions of Phenobarbital Sodium Salt

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO RESTRICTION REQUIREMENT

Sir:

This paper is in response to the Office Action dated 2/8/2022. Please enter the amendments shown herein.

- Claim amendments begin on page 2.
- Remarks begin on page 6.

AMENDMENTS TO THE CLAIMS

This listing of claims represents the current version of claims before the Office:

1-27.(canceled)

28. (currently amended) A method of producing a storage-stable form of lyophilized amorphous phenobarbital sodium composition, comprising:

adding phenobarbital sodium to water to form a phenobarbital sodium solution having a pH of between 9.2 and 10.2, wherein the phenobarbital sodium solution does not contain an organic solvent, and wherein the phenobarbital sodium solution optionally further comprises sodium chloride;

lyophilizing the phenobarbital sodium solution in a container under a protocol comprising a freezing step, a primary drying step, and a secondary drying step to thereby produce the lyophilized amorphous phenobarbital sodium composition;

wherein the freezing step comprises freezing the lyophilized amorphous phenobarbital sodium to a temperature of about -50 °C without application of vacuum;

wherein the primary drying step comprises application of vacuum at a pressure of between about 50mTorr and 75 mTorr at a temperature of between about -50 °C and about 0 °C for at duration of at least 1,000 minutes;

wherein the secondary drying step comprises application of vacuum at a pressure of between about 50 mTorr and 75 mTorr at a temperature of between about 25 °C and about 50 °C for at duration of at least 200 minutes;

wherein the lyophilized amorphous phenobarbital sodium has an initial moisture content of equal or less than 1.5%; and

wherein the lyophilized amorphous phenobarbital sodium forms, upon storage over at least <u>24 months at room temperature</u> ~~3 months~~, no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution.

29. (previously presented) The method of claim 28, wherein the sodium chloride is present in the phenobarbital sodium solution in an amount that, upon reconstitution of the lyophilized amorphous phenobarbital sodium in the aqueous solution, yields a sodium chloride concentration of 0.9% in the reconstituted solution.

30. (previously presented) The method of claim 28, wherein the phenobarbital sodium solution in the container is present in an amount of about 65 mg, or about 100 mg, or about 130 mg, or about 200 mg.

31. (previously presented) The method of claim 28, wherein the water is sparged with nitrogen.

32. (previously presented) The method of claim 28, wherein the phenobarbital sodium solution does not contain a preservative.

33. (previously presented) The method of claim 28, wherein the primary drying step is performed using at increasing temperatures that increase from about -50 °C to about 0 °C when the amount of phenobarbital sodium in the container is between 65 mg and 130 mg.

34. (previously presented) The method of claim 28, wherein the primary drying step is performed using at constant temperature of about -42 °C when the amount of phenobarbital sodium in the container is 200 mg.

35. (previously presented) The method of claim 28, wherein the secondary drying step is performed using at decreasing temperatures that decrease from about 50 °C to about 25 °C when the amount of phenobarbital sodium in the container is between 65 mg and 130 mg.

36. (previously presented) The method of claim 28, wherein the secondary drying step is performed using at constant temperature of about 35 °C when the amount of phenobarbital sodium in the container is 200 mg.

37. (previously presented) The method of claim 28, wherein the lyophilized amorphous phenobarbital sodium has an initial moisture content of equal or less than 1.2%.

38. (previously presented) The method of claim 28, wherein the lyophilized amorphous phenobarbital sodium has an initial moisture content of equal or less than 1.0%.

39. (currently amended) The method of claim 28, wherein the lyophilized amorphous phenobarbital sodium forms, upon storage over at least 24 months at room temperature ~~3 months~~, no more than 0.1% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution.

40. (currently amended) The method of claim 28, wherein the lyophilized amorphous phenobarbital sodium forms, upon storage over at least 24 months at room temperature ~~3~~

~~months~~, no detectable quantities of 2-ethyl-2-phenylmalonamide (2EPMM) or alpha-phenylbutyrylguanidine (PBG) when reconstituted in an aqueous solution.

41. (previously presented) The method of claim 28, wherein the storage is at 40 °C and 75% relative humidity.

42. (previously presented) The method of claim 28, wherein the lyophilized amorphous phenobarbital sodium composition contains at least 98% lyophilized amorphous phenobarbital sodium.

43. (withdrawn) A pharmaceutical composition comprising a storage-stable form of lyophilized amorphous phenobarbital sodium, comprising:

sterile lyophilized amorphous phenobarbital sodium composition in a container, wherein the amount of the lyophilized amorphous phenobarbital sodium in the container is between 50 mg and 250 mg;

wherein the sterile lyophilized amorphous phenobarbital sodium composition does not contain an organic solvent, and optionally further comprises sodium chloride;

wherein the lyophilized amorphous phenobarbital sodium composition has an initial moisture content of equal or less than 1.5%; and

wherein the lyophilized amorphous phenobarbital sodium forms, upon storage over at least 3 months, no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution.

44. (withdrawn) The pharmaceutical composition of claim 43, wherein the sodium chloride is present in the phenobarbital sodium solution in an amount that, upon reconstitution of the lyophilized amorphous phenobarbital sodium in the aqueous solution, yields a sodium chloride concentration of 0.9% in the reconstituted solution.

45. (withdrawn) The pharmaceutical composition of claim 43, wherein the lyophilized amorphous phenobarbital sodium forms, upon storage over at least 3 months, no detectable quantities of 2-ethyl-2-phenylmalonamide (2EPMM) or alpha-phenylbutyrylguanidine (PBG) when reconstituted in an aqueous solution.

46. (withdrawn) The pharmaceutical composition of claim 43, wherein the composition is free of preservatives.

47. (withdrawn) The pharmaceutical composition of claim 43, wherein the lyophilized amorphous phenobarbital sodium composition contains at least 98% lyophilized amorphous phenobarbital sodium.

REMARKS/ARGUMENTS

Restriction/Election

The Office required restriction/election to a single invention as the application would contain the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under 35 U.S.C.121:

I. **Claims 28-42**, drawn to a method of producing, classified in A1K 9/19.

II. **Claims 43-47**, drawn to pharmaceutical composition, classified in A61K 31/515.

The applicant respectfully disagrees. Nevertheless, the applicant elects without traverse **Group I**, reading on **claims 28-42**.

Amendment

Claims 28, 39, and 40 were amended to even more clearly point to the unexpected benefits of the claimed method. Support can be found, *inter alia*, at [0071], [0091], and [0101] in the specification as originally filed.

Request For Allowance

Claims 28-47 are pending in this application, with claims 43-47 withdrawn. Applicant requests allowance of all pending claims.

Respectfully submitted,
Umberg Zipser LLP

Date: March 9, 2022

By: /Martin Fessenmaier Ph.D./
Martin Fessenmaier Ph.D.
Reg. No. 46697

Umberg Zipser LLP
1920 Main Street, Suite 750
Irvine, CA 92614
Telephone (949) 679-0052
Fax (949) 679-0461

Examiner-Initiated Interview Summary	Application No. 17/025,881	Applicant(s) Chodavarapu et al.		
	Examiner CARLOS A AZPURU	Art Unit 1617	AIA (First Inventor to File) Status Yes	Page 1 of 2

All Participants (applicant, applicants representative, PTO personnel)	Title	Type
CARLOS A AZPURU	Primary Examiner	Telephonic
Martin Fessenmaier, Ph.D.	Attorney of Record	

Date of Interview: 15 March 2022

Issues Discussed:

35 U.S.C. 112

Dr Zfessenmaier was called because the new limitation of "at least 24 months at room temperature" is based on a statement of equivalence with extreme conditions for 6 months, but has no support from any data.
The pending rejections under 35 USC 112(b) and 35 USC 112(d) are withdrawn in view of applicant's response filed amendment of 12/22/2021.

35 U.S.C. 103

The rejections under 35 USC 103 based on Park (US 20170143719) are withdrawn in view of Parker's inclusion of organic solvents.

Proposed Amendment(s)

It was agreed that the original claim limitation of 3 months would be restored by Examiner's amendment. The "at least' wording would also be removed since it would entail an open ended time period starting at 3 months.

/CARLOS A AZPURU/ Primary Examiner, Art Unit 1617	

Applicant is reminded that a complete written statement as to the substance of the interview must be made of record in the application file. It is the applicants responsibility to provide the written statement, unless the interview was initiated by the Examiner and the Examiner has indicated that a written summary will be provided. See MPEP 713.04
Please further see:
MPEP 713.04
Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b)
37 CFR § 1.2 Business to be transacted in writing

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete

and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

To: rdean@umbergzipser.com,patents@umbergzipser.com,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 149344

Mar 30, 2022 05:33:32 AM

Dear PAIR Customer:

Umberg Zipser LLP
1920 Main Street Suite 750
Irvine, CA 92614
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 149344 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:
The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
17025881	NOA	03/30/2022	104078.0001US
	EXIN	03/30/2022	104078.0001US
	892	03/30/2022	104078.0001US

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

Transaction History Date 2022-03-30
Date information retrieved from USPTO Patent Application Information Retrieval (PAIR) system records at www.uspto.gov

Notice of Allowability	**Application No.** 17/025,881	**Applicant(s)** Chodavarapu et al.	
	Examiner CARLOS A AZPURU	**Art Unit** 1617	**AIA (FITF) Status** Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to the amendment of 03/09/2022.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are 28-42 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some* c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☑ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☑ Interview Summary (PTO-413), Paper No./Mail Date. 0312022.
5. ☑ Examiner's Amendment/Comment
6. ☑ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/CARLOS A AZPURU/
Primary Examiner, Art Unit 1617

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Withdrawn Rejections

The rejections under 35 USC 112(b), 35 USC 112(d) are withdrawn in view of the amendments of 12/22/2021. The rejections 35 USC 103 based on Parker (US 2017/0143719) are withdrawn in view of the fact that Parker uses organic solvents which are excluded by the instant claims.

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in an interview with Martin Fessenmaier, Ph.D. on 03/15/2021.

The application has been amended as follows:

In claim 28, lines 20-21, delete "at least 24 months at room temperature", and replace it with:

3 months

In claim 39, line 2, delete "at least 24 months at room temperature" , and replace it with:

3 months

In claim 40, line 2, delete "at least 24 months at room temperature", and replace it with:

3 months

Claims 43-47 are canceled.

Reasons for Allowance

The following is an examiner's statement of reasons for allowance: The prior art neither teaches, nor fairly suggests the instantly claimed method of making a lyophilized sodium phenobarbital. The closest prior art is that of Parker (US 2017/0143719), which includes organic solvents which are excluded by the instantly claimed method. The instantly claimed method is therefore allowable over the prior art of record.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Correspondence

Any inquiry concerning this communication or earlier communications from the examiner should be directed to CARLOS A AZPURU whose telephone number is (571)272-0588. The examiner can normally be reached 9 am- 3 pm, 4 pm-8pm.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Johann R Richter can be reached on 571-272-0646. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: https://patentcenter.uspto.gov. Visit https://www.uspto.gov/patents/apply/patent-center for more information about Patent Center and https://www.uspto.gov/patents/docx for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/CARLOS A AZPURU/
Primary Examiner, Art Unit 1617

caz