

A History of Vitamin A and Retinoids

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This is a historical account of the recognition of human vitamin A deficiency from ancient Egypt to the present century, as well as a history of the discovery of vitamin A and the functions of vitamin A and the retinoids.

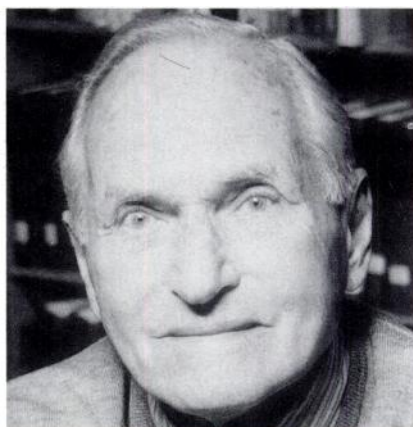
VITAMIN A DEFICIENCY

Vitamin A has probably been known for at least 3500 years as a factor that can cure a deficiency disease. Ancient Egyptians appear to have recognized night blindness, which we now know to be caused by vitamin A deficiency, and found a cure for it (1). However, this view has been disputed by modern scholars (2): the papyrus Ebers (ca. 1500 B.C.) (1) mentions an unspecified eye disease, *sharew*, but not night blindness; the word "night" does not appear. The recommended cure was "roasted ox liver, pressed, applied [to the eye], really effective."

Vitamin A deficiency can affect two parts of the eye: the epithelial covering (cornea and conjunctiva) and the retina (rhodopsin and iodopsin). Normally, the vitamin is delivered to the cornea via the tears and by diffusion through eye tissue. Vitamin A deficiency leads to destruction of the cornea, a major cause of blindness in children. It can be treated topically with vitamin A or retinoic acid. On the other hand, vitamin A is delivered to the retina via the bloodstream; hence night blindness, caused by loss of rhodopsin from the retina, would not yield to topical treatment. Therefore, topically applied juice of ox liver, though rich in vitamin A, would not be effective as a cure for night blindness. Egyptian medical practice was partly empirical, partly magical. The ox supplied many medicaments with the idea of "transfer" of the strength of the animal to the afflicted part. Thus, juice of ox liver applied to the night-blind eyes would be a typical example of Egyptian medical ritual. An amusing sidelight onto this "magic" treatment of night blindness was thrown by Hussaini et al. (3): these authors observed several treatment sessions in rural Java in our own days (1978). The juice of lamb liver was applied topically to the eyes of night-blind children. The procedure was exactly as described by the ancient Egyptians, "except for one small addition: rather than discarding the remaining organ, the [practitioners] fed it to the affected child...This was never considered part of the therapy itself." Similarly, in 1928 Aykroyd (4) wrote: "I have been told of a custom of steaming the eyes over cooking liver, which is then eaten," as a remedy for night blindness in Newfoundland. Perhaps the ancient Egyptian ritual treatment also ended with the patient eating the liver.

Another ancient Egyptian papyrus, Kahun 1 (ca. 1825 B.C.) (2), a gynecological treatise, mentions "instructions for a woman [with] sickness [so that] she cannot see: then you shall cause her to eat raw liver of an ass" (2). The sickness may have been night blindness, and the cure more rational. The case for the ancient Egyptians thus is not proved, but it is strong; the connection between liver, the richest source of vitamin A in food, with blindness is highly suggestive.

The first indisputable recognition of night blindness was made by the ancient Greeks, who called it *nyktalopia*



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(*nyx*, *nyktos*, night; *alaos*, blind; *ops*, *opteos*, eye). Hippocrates (460–327 B.C.) described "nyktalopia" in the second book of "Prognostics" (5). This description has given rise to some controversy: the collection of writings that has come down to us states "those who *do see* at night, whom we call *nyktalopes*, experi-

ence the disease when young, as children or youths"; this clearly means patients who *can see* at night (*italics added*). It was interpreted as "day blindness" by 19th century scientists (6). However, a handwritten text by Hippocrates in the 14th century, discovered by the Greek philologist A. Korais (1748–1833), shows a gap in the text between "do" and "see," and in the margin is written the word "not," resulting in "those who *do not see* at night" (7). This interpretation seems by far the most sensible, because later Greeks, like Galen (129–199 A.D.), a follower of Hippocrates, very clearly described *nyktalopia* as *night blindness* (7). Oribasius, a follower of Galen, born ca. 325 A.D., defines night blindness as: "vision is good during the day and declines at sundown; one cannot distinguish anything any longer at night" (8). Also convincing for this interpretation is the cure for *nyktalopia*, mentioned in the collection from the school of Hippocrates (ca. 300 B.C.), entitled *Concerning Vision* (5). There,

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nyctalopia is reported to afflict children with infectious diseases like otitis, sore throat, or fever. To cure nyctalopia, the work recommends "raw beef liver, as large as possible, soaked in honey [presumably to make it more palatable], to be taken once or twice by mouth." Galen recommends: "continuous eating of...liver of goats." This is exactly what modern medical practice would prescribe were it not possible to obtain fish-liver extract or vitamin A pills. Today, it is well known that vitamin A deficiency occurs principally in malnourished children. The vitamin deficiency predisposes them to infections because of an impaired immune system. One marvels at the acuity of observation and the knowledge of effective therapy by the Hippocratic school of medicine.

Assyrian medical texts (ca. 700 B.C.) describe night blindness, thought to be caused by rays of the moon and cured by application of ass's liver to the eyes. Chinese medicine recognized night blindness, and Sun-szu-mo (7th century A.D.) in his *1000 Golden Remedies* describes a cure by administration of pig's liver (9). Arabic medicine, often derived from translation of ancient Greek medical texts, returned to topical application of liver extract (Hunain Ibn Ishaq, 9th century A.D.). A Hebrew treatise from Muslim Spain (Abraham Ibn Ezra, 12th century A.D.) again recommends topical treatment with goat liver, but followed by eating of the liver (8).

In the later Middle Ages, the Dutch physician Jacob van Maerlandt (1235–1299) wrote the following poem (10):

Who does not at night see right

Eats the liver of goat.

He will then see better at night.

During the time of the colonial expansion of European nations, two physicians of the Netherlands drew attention to night blindness and its cure (10). Jacobus Bontius (1592–1631), the first medical doctor working in the Dutch East Indies, describes in *De Medicina Indorum* a condition of disturbed vision leading to blindness that can be cured by a specific medicament used by the natives of Java: liver of shark. The other, Willem Piso (1611–1678), working in Brazil, mentions the occurrence of night blindness, especially among the poor, and its cure by shark liver. In support he cites the Hippocratic collection "*Concerning Vision*," mentioned above, in connection with the liver therapy. In the 18th century, a German physician, C. A. von Bergen (1754), described epidemics of night blindness in rural Russia, postulated a nutritional cause, and mentioned liver as a cure, known from its use in China (11).

An early experiment in human nutrition was carried out by a young ship's doctor, Eduard Schwarz (1831–1862). When Austria still had a navy (based in Trieste), the naval frigate Novara, a sailing ship, was sent around the world on a scientific exploration (1857–1859). The ship, with 352 men on board including Schwarz, counted also a geologist, two botanists, two zoologists, a geographer/anthropologist, and even an orchestra of seven men (jolly tunes were played every morning from 9 to 11

to keep up morale). Schwarz accurately described the 75 cases of night blindness (then termed "hemeralopia") (12), of which 60 occurred during the long haul from Cape Horn to Gibraltar, often in conjunction with scurvy. Curiously, most of the members of the musical band were afflicted; perhaps they received less nourishment than the regular sailors. Every evening at dusk, they lost their vision and had to be led about like the blind. Schwarz states (12) that before his departure from Vienna, several physicians asked him to test the administration of boiled ox liver against night blindness, and thus to confirm or refute the effectiveness of this old folk medicine. He reports that its action as a cure was "a true miracle" (12). Pork liver was equally effective. The cure was permanent, and he diagnosed night blindness correctly and emphatically as a nutritional disease. It is interesting that he kept a number of the afflicted sailors below deck in darkness for 3–5 days and observed that they regained normal vision, but became night-blind again when brought into daylight. We can conclude, from contemporary knowledge, that during dark adaptation, small amounts of vitamin A reserves remaining in the body reached the retina to re-form the depleted visual pigment, only to be bleached again during light exposure.

Curiously, Schwarz was attacked viciously upon publication of his report; his view of night blindness as a nutritional disease was called a "frivolity" and its description as such a case of "self-aggrandizement" (13). No scientific reason for this critique was given. Three years after his return from the exceedingly strenuous 3-year expedition, Eduard Schwarz died of tuberculosis at age 31.

Epithelial defects caused by vitamin A deficiency were first reported by the English physician George Budd (1808–1882) (14). He based his conclusions on the experiments of F. Magendie (1817), in which dogs were fed a diet composed entirely of sugar and water. After only 3 weeks, the dogs became sick and emaciated, and developed ulceration of the cornea. These ulcers increased in size and depth until the corneas were perforated and the aqueous humor and lens escaped (keratomalacia). Magendie ascribed this event to the lack of nitrogen in the diet, but Budd wrote (14): "...this is far too exclusive. It does not recognize the importance of other principles which are wanting...but which, although less in amount yet...enter constantly into the composition of the body and are therefore required for its support." He then described similar corneal ulcerations developed by East Indians on their sea voyage to England on diets we can now recognize as very low in vitamin A.

The earliest association between night blindness and a corneal defect was made by V. von Hubbenet, who in 1860 observed that night blindness was associated with "silver scales at the cornea" caused by a faulty diet and treatable with beef liver (8). Similar observations were made by Bitot in 1862 who described the famous "Bitot's spots," foamy white spots on the corneas of night-blind children (8), now regarded as a strong indicator of vitamin A deficiency. The explorer of Africa, David Livingstone, in 1857 also noted the

corneal lesions in African natives who subsisted on a diet of coffee, manioc, and meal, and wrote: "The eye became affected as in the case of animals fed pure gluten or starch" (15). Guggenheim (8) mentioned the prevalence of xerophthalmia (dry eye) associated with night blindness and ending in keratomalacia in infants in Russia in the late 19th century, during the great Lenten fast of 7 weeks, when no animal food was allowed. This condition, recognized as a nutritional disease, could be easily cured by eating liver (curiously not forbidden during Lent).

An important contribution was that of M. Mori (16), who in 1904 described widespread xerophthalmia in Japanese children, including large numbers showing keratomalacia (softening of the cornea, ending in corneal ulceration and generalized liquefactive necrosis, characteristic as we now know of severe vitamin A deficiency). Mori reported that their diet consisted of rice, barley, cereals, "and other vegetables," and found that liver and especially cod-liver oil were curative, thus connecting the disease to a nutritional cause. In a similar vein, S. Ishihara, in 1913 writes (17): "Xerosis of the conjunctiva combined with night blindness...seems to occur mostly in childhood and to be associated with overall malnutrition....It often deprives juvenile patients of their eyesight by supervention of keratomalacia. Fortunately, we have in cod-liver oil an excellent, almost specific medication....Indeed, in most cases, the effect is so rapid that by evening the children with night blindness are already dancing around briskly, to the joy of their mothers."

Coming close to the actual discovery of vitamin A, as described in the next section, were the observations of C. E. Bloch (18), who studied children in Denmark afflicted with night blindness and keratomalacia during World War I. As a result of the war, the children had subsisted on a diet of fat-free milk, oatmeal, and barley soup. In an important early experiment, Bloch divided a group of 32 institutionalized children (1–4 years old) into two groups; one received whole milk, the other only vegetable fat (margarine). The latter showed eight cases of corneal xerosis, the former remained healthy. All the xerosis cases were cured rapidly with cod-liver oil. Bloch correctly surmised that whole milk, creamery butter, eggs, and cod-liver oil contained lipid substances that protected against corneal and conjunctival xerosis.

THE DISCOVERY OF VITAMIN A

The effects of vitamin A deficiency on growth were first described by G. Lunin (19) in 1881. He found that mice could not survive on a diet of pure casein, fat, sucrose, minerals, and water, but lived and grew normally with whole dried milk. The outcome of similar experiments led F. G. Hopkins (20) to postulate "minimal qualitative factors" in the diet necessary for growth and survival.

An interesting and exciting account of the beginnings of experimental nutrition that ultimately led to the discovery of vitamin A is given by E. V. McCollum (21). When McCollum began his research (1907), chemical analysis of foods and animal feed was well advanced. He

fed cows rations of hay with wheat or oats or yellow maize, of identical composition as far as protein, carbohydrate, fat and minerals were concerned. The wheat-fed cows did not thrive, became blind and gave birth to dead calves prematurely. The oat-fed cows fared somewhat better, but the yellow maize-fed cows were in excellent condition, produced vigorous calves, and had no miscarriages. That was a big puzzle at the time; McCollum spent 4 years trying to determine the cause for these remarkable results: did the wheat contain a toxic substance? What was lacking in wheat that was present in yellow maize? At about that time, McCollum conceived the important idea that such nutrition experiments could be done much faster and better with small animals, such as mice or rats: they eat much less, they can be given purified diets, reproduce rapidly, and many more can be housed at one time. His idea met with much opposition, mainly because he worked in the Wisconsin College of Agriculture, and was told by the dean "to experiment with economically valuable animals—the rat was a pest to farmers!" (21).

Surreptitiously, McCollum started a rat colony in a basement of the Agricultural Hall. He found that whereas young rats on a diet of pure protein, pure milk sugar, minerals, and lard (or olive oil) failed to grow, addition to their diet of butter fat or an ether extract of egg yolk restored their health. He soon determined that an ether extract of alfalfa leaves or, even better, of liver or kidney, when added to the rat diet greatly improved its growth-promoting quality. Clearly, he had found a fat-soluble factor that promoted growth in rats. He saponified butter fat, extracted the unsaponifiable mixture into ether, and added the extract to olive oil: now the olive oil, with pure protein, sugar, and minerals, supported growth. This and subsequent experiments proved that there was a fat-soluble growth factor (other than fat itself) in butter fat that could be transferred from one fat to another. McCollum ultimately showed that this factor was essential for growth and survival: this was the discovery of vitamin A, then named "fat-soluble factor A," as opposed to other accessory dietary factors, called "water-soluble B" (22).

Osborne and Mendel (23), at exactly the same time, performed similar experiments. They found that young rats fed a diet consisting of purified protein, sugar, starch and lard would not grow, though mature rats on the same diet remained healthy. When the diet was supplemented with evaporated whole-milk powder, growth was excellent. It was concluded that milk contained something other than protein that was necessary for the growth of young animals. When protein-free milk powder was given over prolonged periods, growth also declined, but resumed with whole-milk powder. Detailed analysis of the difference between whole-milk powder and protein-free milk powder showed a lack of milk fat in the latter. Addition of butter to a casein, starch, and protein-free milk powder diet again resulted in normal growth. "It would seem, therefore, as if a substance exerting a marked influence upon growth were present in butter..." (23). This

paper, published just 5 months after McCollum and Davis's study (22), came to the same conclusion. Later, Osborne and Mendel (24) found that cod-liver oil could substitute for butter; in its absence the rats developed xerophthalmia. Thus, the early work of Mori (16), in which children were cured of keratomalacia by cod-liver oil, was reproduced precisely in experimental animals. McCollum's "fat-soluble factor A" (later, factor A; then vitamin A) could maintain growth and prevent keratomalacia. In attempts to isolate and identify the factor, Osborne and Mendel (24) obtained an active yellow oil from butter fat, egg yolk fat, and cod-liver oil, but not from lard or olive oil. It was noted by H. Steenbock (25) that active extracts such as those made from butter, egg yolk, or certain plants (carrots) were yellow, whereas extracts from lard, inactive in growth promotion, were white. He realized, however, that active fat-soluble factors existed in white extracts from liver or kidney. H. Steenbock and P. W. Boutwell (26) thereupon proposed the hypothesis, not fully confirmed for another 10 years, that fat-soluble factor A was *associated* with a yellow pigment (now known to be β -carotene) and proposed that it was converted to an active colorless form (vitamin A or retinol). In the meantime, L. S. Palmer and H. L. Kempster (27) threw the subject into confusion. They fed chickens a diet free of yellow pigments: white maize, skim milk, and bone meal. When the birds stopped growing and began to fail, they supplemented the diet with a small amount of pork liver (containing no yellow pigment) and found that, though the birds had received no yellow pigment, they grew normally and laid eggs. The resulting egg yolks were colorless, but when hatched, the eggs produced normal chicks.

It was not until 1930 that T. Moore (28) resolved the conflict by proving that the yellow pigment extracted from plant sources, butter fat, or egg yolk and purified as carotene, was converted to the active factor in the animal body. He fed pure, crystalline carotene (yellow) to young rats and observed the accumulation of retinol (colorless) in their livers. He correctly concluded that yellow carotene is the provitamin or precursor of colorless vitamin A (retinol).

DISCOVERY OF THE FUNCTIONS OF VITAMIN A

In the 1930s, the isolation and chemical structure of β -carotene and retinol were determined by Karrer et al. (29). The enzymatic conversion of β -carotene to retinol was later shown to take place in the intestinal mucosa of animals and humans. The requirements and recommended daily allowances of β -carotene and retinol for animals and humans were determined. Isler et al. (30) succeeded in the total synthesis of retinol in 1947.

Early research on a function of retinol was based on physiological and biochemical responses of experimental animals to a diet lacking in β -carotene or retinol. When

weanling rats are fed a vitamin A-deficient diet, their liver stores of retinyl esters become exhausted after about 4 weeks and their growth begins to decline after 5 weeks. They then become night-blind and their corneas begin to show lesions. After about 6 weeks they lose weight precipitously, their corneas ulcerate, and they die. This simple feeding experiment highlights three of the most important physiological functions of vitamin A: vision, normal differentiation of epithelia (such as the cornea), and growth.

The visual function of vitamin A was elucidated in great detail and with exceptional brilliance (culminating in the Nobel Prize) by G. Wald (31). Night blindness (defective vision at low illumination) is one of the earliest lesions to appear in vitamin A-deficient animals and humans. It was therefore fitting that the function of vitamin A in vision was the first to be defined with a precise mechanism at the molecular level. Moore (32) comments: "It may be an inspiring thought...that Man's knowledge of the existence of the stars and the vast universe which appears in the heavens each night, comes in the first place from the stimulation by the light rays of delicately poised molecules of vitamin A."

As long ago as 1877, W. Kühne (33) discovered that retinas from dark-adapted frogs, purple in color, changed to yellow when exposed to light. In a little-known work, M. Parinaud (34), only a few years later (1881), related "visual purple" (now known as rhodopsin) to night blindness. He surmised correctly that rhodopsin "serves as an intermediate in visual excitation." Rhodopsin, he suggested, is secreted by the pigment epithelium of the retina. In night blindness, sensitivity to light is altered because of a modification of rhodopsin. "Too strong an illumination develops night blindness...because it destroys rhodopsin." He clearly distinguished between rod vision in the periphery of the retina (after Kühne, 33), based on rhodopsin and thus susceptible to night blindness, and cone vision in the macula of the retina, serving color vision, independent of rhodopsin and thus not involved in night blindness.

Ishihara in 1913 (14) was the first to connect keratomalacia to night blindness and "visual purple" (rhodopsin). He proposed that a "fatty substance" in blood needed for the synthesis of rhodopsin and "keratohyalin" (the surface layer of the cornea), when lacking in malnourished children leads to night blindness and keratomalacia.

The connection between night blindness and vitamin A was made in 1925 by L. S. Fridericia and E. Holm (35), who recognized that vitamin A-deficient rats, when light-adapted, formed visual purple at a slower rate than did normal rats, when put into the dark. Holm (36) observed the presence of vitamin A in the retina. However, it was Wald who, starting in 1935 and continuing into subsequent years, found that the visual purple of the retina, called rhodopsin, consists of a protein, opsin, combined with "retinene" (later shown by R. A. Morton to be retinaldehyde). G. Wald and co-workers (31) described the

visual cycle thus: the 11-*cis*-isomer of retinaldehyde, while attached to opsin in rhodopsin, is isomerized to the all-*trans* form by light, the event that triggers the nerve impulse to the brain as perception of light. The all-*trans*-retinaldehyde is released from the opsin and reduced to all-*trans*-retinol. In the dark, this is isomerized to 11-*cis*-retinol and oxidized to 11-*cis*-retinaldehyde, which recombines with opsin to re-form rhodopsin (dark adaptation), completing the cycle.

Elucidation of the function of vitamin A in cell differentiation lagged behind the recognition of its role in vision. The observations by Wolbach and Howe (37) defined the epithelial lesions resulting from vitamin A deficiency, particularly the keratinization of epithelial cells, especially of the cornea, but also of the respiratory, intestinal, and genitourinary tracts. It seemed that vitamin A influenced the differentiation of epithelial cells, from the normal, simple, and pseudostratified phenotype to squamous, metaplastic lesions that start focally and ultimately spread throughout the epithelium.

Apart from the function of maintaining normal epithelial differentiation, the pathology of vitamin A deficiency revealed a function of vitamin A in bone growth, shown by the thickened bones in deficient growing animals; in male reproduction, by cessation of spermatogenesis and atrophy of the testes; in the female by a defective uterus, placental necrosis, and ultimately resorption of the fetus in vitamin A deficiency. Indeed, as long ago as 1933, the teratogenic effect of marginal (as opposed to complete) vitamin A deficiency, resulting in ocular and genitourinary defects in pig embryos, underlined the necessity of vitamin A to support normal fetal development.

In the 1950s and 1960s, the metabolism of vitamin A was delineated by J. A. Olson (38) and many others, followed by recognition of the importance of its transport proteins: by D. S. Goodman (39) in regard to the plasma retinol-binding protein, and by F. Chytil and D. E. Ong (40) with respect to the intracellular-binding proteins. K. R. Norum and R. Blomhoff (41) and others established the basic facts of absorption and liver storage mechanisms for retinol.

In the 1970s and 1980s, the discovery by U. Saffiotti (42) of an anticarcinogenic action of vitamin A led to an outburst of research effort, though its anticancer action in the human is still somewhat controversial. In animal experiments, retinol and particularly its metabolite, retinoic acid, could be demonstrated indisputably to have anticarcinogenic properties. As applied to human cancer patients, however, a search for similar activity was disappointing. A number of isomeric forms and synthetic chemical derivatives of retinoic acid were found to be active against some forms of cancer: for instance, acute promyelocytic leukemia has been successfully treated with all-*trans*-retinoic acid; certain skin and head-and-neck cancers have yielded to 13-*cis*-retinoic acid; and retinoic acid and some of its synthetic chemical deriva-

tives have exhibited spectacular cures in a number of skin diseases.

A new era of vitamin A research dawned in 1987, when P. Chambon in Strasbourg (France) (43) and R. M. Evans in San Diego (U.S.) (44), and their respective co-workers, simultaneously discovered the retinoic acid receptors in cell nuclei. These receptor proteins can bind retinoic acid, and when thus liganded, can activate specific genes to stimulate the cells to produce specific proteins (enzymes) or to inhibit the expression of other genes. In this way, one can arrive at an explanation at the molecular level of the many metabolic functions of vitamin A with regard to embryonic development, differentiation, and growth. As Sporn and Roberts (45) had already stated in 1983: "Ultimately, it would appear that the problem of the molecular mechanism of action of retinoids in control of differentiation and carcinogenesis is converging on one of the central problems of all biology, the control of gene expression." This type of action—gene activation—establishes vitamin A (in the form of its metabolite, retinoic acid) as a hormone, similar to the steroid hormones and the thyroid hormone." As predicted in 1970: "There is no a priori reason why a hormone should have to be made by the animal itself. It is quite conceivable that a hormone can be taken in the diet [as vitamin A], stored in the liver, and secreted into the bloodstream when needed. The liver then acts as the endocrine organ" (46).

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Erratum

Neuronal nitric oxide synthase, a modular enzyme formed by convergent evolution: structure studies of a cysteine thiolate-liganded heme protein that hydroxylates L-arginine to produce NO[•] as a cellular signal. B. S. S. Masters, K. McMillan, E. A. Sheta, J. S. Nishimura, L. J. Roman, and P. Martasek [*FASEB J.* (1996) **10**, 552–558]

The editors regret that several words were inadvertently dropped from the bottom of the second column on page 552. The correct sentence appears below with the omitted line in boldface.

It has been suggested that NO[•] produced in central nervous system neurons upon activation of glutamate receptors (6), as well as a non-NMDA glutamate receptors [α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate (7, 8), can regulate cerebral blood flow.