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N-Nitrosamines in Foods 357

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Phillip Issenberg, referee. B. S., Ph.D., Massachusetts Institute of Technology. Professor and Associate Director, Eppley Institute for Research in Cancer, Omaha, Nebraska.

N-nitrosamines are an extremely hazardous class of compounds. The possibility of the occurrence of these compounds in our food and in other environmental situations has caused concern over the past decade. This review examines the literature which provides an understanding of the chemistry of *N*-nitrosamine formation. Particular attention is paid to the more recent reports concerning methodology for determining *N*-nitrosamines and to the reports since 1970 on the occurrence of *N*-nitrosamines in foods. The literature concerning factors which influence *N*-nitrosamine formation in foods is evaluated and discussed.

High Altitude Food Preparation and Processing 403

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The preparation and the general quality characteristics of our foods are affected by atmoshperic pressure. Taste is perceived as a function of solubility and not volatility as in the case of odor. Therefore, no one would expect a change in taste sensitivity with changes in altitude. However, basic taste sensitivity decreases with increasing elevation. Photomicrographs have indicated that certain starches in dilute suspensions increased in particle size. Water binding capacities and viscosities of these starches were significantly affected by atmospheric pressure. Amylograph and mixograph curve characteristics vary considerably when these instruments are operated under varying atmospheric pressures. Many commercial bakeries, flour mills, and cereal chemistry laboratories are located in the high altitude region (above 2,500 ft) and have to consider altitude when examining or reporting amylograph or mixograph data. Breads have larger volumes and cookies are larger in diameter when baked at higher elevations. The internal temperature of baked products decreases with increasing elevation. Cake formulations adjusted for sea level will produce cakes which rise too high in the oven and flow over the top of the pan when baked at higher elevations. Formula adjustments in leavening and moisture content, therefore, are required. Vegetables and meats require a longer cooking in the high altitude region than they would at sea level. The high altitude region comprises about one third of the U.S. Although the area is generally sparsely populated, about 15 million people make their homes there.

Bread Staling 443

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Retardation of bread staling has historically been an important economic consideration that has resulted in numerous scientific investigations. This review attempts to summarize the history of bread staling beliefs and practices. A major portion deals with factors that have been reported to influence the rate of bread staling. Other areas discussed include staling theories, methods employed to measure staling changes, and nutritional considerations associated with the staling phenomenon.

Meat Flavor 487

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The flavor of meat is due to a complex mixture of compounds produced by heating a heterogenous system containing nonodorous precursors and consists of volatile compounds with odor properties, nonvolatile compounds with taste and tactile properties, and potentiators and synergists. The chemistry of these compounds, their precursors, and the influence of enzymes found in muscle on the overall flavor of cooked meat are discussed. A short discussion on the flavor of ripened, dry sausages is also included.

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MEAT FLAVOR

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INTRODUCTION

The flavor of meat is attributed to a complex mixture of compounds produced by heating a heterogeneous system containing nonodorous precursors, and it is comprised of:

1. Volatile compounds with odor properties.
2. Nonvolatile compounds with taste and tactile properties.
3. Potentiators and synergists.

The chemistry of the compounds responsible for the flavor of cooked meat, and how these flavor compounds are produced during the process of cooking, have been the subject of considerable research in the last two decades. Two obvious facts about cooked meat flavor are

1. The nature of the flavor depends upon the way in which meat is cooked.
2. Each meat has its own characteristic flavor.

Since all types of meat consist of broadly similar components – such as proteins and other amino compounds, lipids, carbohydrates, and mineral salts – it is apparent that there must be a significant difference either in the proportion in which these compounds are present or in the way in which they interact. The differences in flavor due to cooking are probably a direct function of temperature and degree of moisture in the meat.[1] Because it is necessary to heat meat to produce the desired flavor, it follows that meat must contain substances which interact or degrade during cooking to produce compounds responsible for the characteristic flavor of meat. These substances are termed flavor precursors. A general discussion on the importance of flavor precursors and enzymes in the development of various food flavors was given in an earlier review.[2] This review deals with the nature of volatile and nonvolatile compounds, flavor precursors, and enzymes that may, directly or indirectly, influence the flavor of cooked meat and ripened sausage.

NATURE OF MEAT FLAVOR

Volatile Compounds

The list of volatile compounds isolated from cooked meat is quite extensive and their contribution to the 'typical' flavor of meat uncertain.

Tables 1 and 2 list most of the volatile compounds identified so far from beef and pork products. Most of these volatiles are produced during cooking from nonvolatile precursors. Unlike fruit, dairy, and fermented food flavors, enzymes have little significance in the development of cooked meat aroma from precursors.

The nature of volatile compounds responsible for the characteristic aroma of meat has not been resolved. However, significant advances have been made in the last 15 years. Hornstein et al.[6,7] in 1960 reported the isolation of two major fractions of volatile compounds from a lean beef slurry:

1. Highly volatile fraction containing ammonia, traces of methylamine, hydrogen sulfide, methyl mercaptan, formaldehyde, acetaldehyde, acetone, and other unidentified highly volatile compounds. A rather disagreeable aroma was noticed in this fraction.
2. Relatively less volatile fraction having a pleasant meaty aroma. Lactic acid and its ammonium salt were the only compounds identified in this fraction.

Of the large number of volatile compounds identified in meat products, Herz and Chang[30] believe that lactones, furan ring compounds that do not contain sulfur, and aliphatic sulfur compounds make a direct contribution to the meat flavor profile. They, however, concede that none of the representatives of the above classes of compounds isolated from cooked meat by their group[3,23,30] has a characteristic meaty aroma. On the other hand, these researchers have reported the presence of four volatile compounds in cooked beef that have a meaty aroma.[3,23] These compounds include: 5-thiomethylfurfural, thiophencarboxy-2-aldehyde, 2,5-dimethyl-1,3,4-trithiolane, and 2,4,5-trimethyl-3-oxazoline.

Watanabe and Sato[26] reported that in shallow fried beef, the most likely compounds responsible for the typical roasted flavor are alkyl-substituted pyrazines and pyridines. This deduction was based on their observation that the removal of basic compounds from the total flavor extract in diethyl ether with hydrochloric acid solution resulted in a considerable loss of the roasted flavor. In a subsequent study with shallow fried beef, Watanabe and Sato[25] isolated a volatile fraction with a typical heated beef flavor and observed that no single compound isolated in this fraction had a typical heated beef flavor. It was suggested that the heated beef flavor might be a complex sensation resulting from a mixture of several different compounds: methional, 2-acetylfuran, 2-furfuryl methyl ketone, 1-methyl-2-acetylpyrrole, benzothiazole, and others.

It appears likely that the question of the nature of compounds responsible for the characteristic cooked meat flavor would remain unresolved for some time to come. It may, however, be pointed out that most of the odorous compounds present in cooked meat are likely to play a significant role in determining its flavor character. It is also common knowledge that odorous compounds, when presented in a mixture, often give an odor sensation somewhat different from the one predicted on the basis of odor characterstics of the individual components in the mixture. It may, therefore, be futile to look for the "characteristic" compounds responsible for the typical meat flavor. Most likely, the subtle quantitative balance of the various odorous components in cooked meat determines the aroma character of meat. For example, the aromas of cooked beef and cooked pork liver are easily distinguishable, although a cursory look at the volatile compounds identified in them (Tables 1 and 2) shows a striking similarity.

Unlike the cooked meat aroma produced from their precursors during heat treatment, the characteristic aroma of dry or fermented sausages is related, in part, to the hydrolytic and oxidative changes occurring in the lipid fraction during ripening.[31,32] The bacterial,[33-35] muscle, and adipose tissue[36] lipases liberate fatty acids from the sausage lipids. The unsaturated fatty acids thus liberated undergo nonenzymatic oxidation and produce lipid peroxides and carbonyl compounds.[31,37] The lipid peroxides are probably further metabolized by the sausage microflora to carbonyl compounds and fatty acids.[38,39] Demeyer et al.[40] have recently investigated the specificity of lipolysis during dry sausage ripening and report that unsaturated fatty acids are selectively liberated by the lipases from the sausage lipids. In this study, linoleic acid was liberated into the free fatty acid fraction at a much faster rate than all other acids. The rate of lipolysis decreased in the order: linoleic > oleic > stearic > palmitic acid. Since pork fat is known to have most of the linoleic and oleic acids (50 to 60%) at position 3 of triglyceride molecule,[41] it has been suggested

TABLE 1

Volatile Compounds Isolated from Heat-treated Beef

Compound	Meat sources and treatment*	References
Acids		
Formic	9	15
Acetic	9,13	15,24
Propionic	9	15
Butanoic	2,6,13	10,16,24
Hexanoic	7	3
2-Methylpropionic	1,9	4,5,15
Aldehydes (aliphatic)		
Formaldehyde	3,6	6,7,10
Acetaldehyde	1,3,4,6,7	3,4,5,6,7,8,10,11
Propanal	1,3,6,7	3,4,5,6,7,10,11
Pentanal	7,8,12,13	3,12,13,14,24
Hexanal	3,7,8,12,13	3,6,7,12,13,14,24
Heptanal	7,8,12,13	3,12,13,14,24
Octanal	7,8,12,13	3,12,13,14,24
Nonanal	3,7,8,12,13	3,6,7,12,13,14,24
Decanal	12,13	24
Undecanal	8,12,13	12,13,14,24
Dodecanal	12,13	24
Tridecanal	13	24
Hexadecanal	7	3
Methional	8,14	12,13,14,25
2-Methylpropanal	1,6	4,5,10
2-Methylbutanal	12,13	24
3-Methylbutanal	1,4,7,8,12,13	3,4,5,12,13,14,24
2-Hexenal	13	24
2-Heptenal	12,13	24
2-Octenal	7,12,13	3,24
2-Nonenal	12,13	24
2-Decenal	12,13	24
2-Undecenal	12,13	24
2-Dodecenal	13	24
2-Tridecenal	13	24
6-Methylhepten-1-al	7	3
2,4-Octadienal	12,13	24
2,4-Nonadienal	13	24
2,4-Decadienal	7,12,13	11,24
2,4-Undecadienal	13	24
2,4-Dodecadienal	12,13	24
Esters		
Methyl formate	2	6
Ethyl acetate	7	3
Acetol acetate	14	25
Ethers		
Pentyl ether	7	3
Pyrroles		
Acetylpyrrole	13	24
1-Methyl-2-acetylpyrrole	14	25

TABLE 1 (continued)

Volatile Compounds Isolated from Heat-treated Beef

Compound	Meat sources and treatment*	References
Alcohols		
Methanol	1	4,5
Ethanol	1,7,8,9	3,4,5,12,13,14,15
Propanol	7,13	3,24
2-Propanol	8	12,13,14
2-Methylpropanol	7	3
Butanol	6,7,8,12,13	3,10,12,13,14,24
3-Methylbutanol	8	12,13,14
Pentanol	7,8,12,13	3,12,13,14,24
Hexanol	8,12	12,13,14,24
Heptanol	8,12,13	12,13,14,24
2-Heptanol	14	25
3-Heptanol	12,13	24
4-Heptanol	13	24
Octanol	7,8,12,13	3,12,13,14,24
3-Octanol	13,14	24,25
4-Octanol	13,14	24,25
Nonanol	14	25
Decanol	14	25
Undecanol	14	25
2-Butoxyethanol	12	24
2-Hexenol	7	3
1-Pentene-3-ol	7	3
1-Octene-3-ol	7	3
2-Octenol	12	24
2,3-Butanediol	12,13	24
Ketones		
Acetone	2,3,4	6,7,8,16
Propanone	1,3,9	4,5,6,7,15
2-Butanone	1,6,8	4,5,10,12,13,14
Acetoin	7,8	3,12,13,14
Diacetyl	5,7,9	3,9,15
2-Heptanone	12,13	24
3-Heptanone	13	24
2-Octanone	12,13	24
3-Octanone	12,13	24
4-Octanone	7	3
2-Nonanone	12,13	24
3-Nonanone	7	3
2-Decanone	12,13	24
2-Undecanone	12,13	24
2-Dodecanone	13	24
3-Dodecanone	7	3
2-Tridecanone	12,13	24
2-Pentadecanone	13	24
3-Hydroxy-2-butanone	12,13	24
2,3-Butanedione	12	24
2,3-Pentanedione	12	24
2,3-Octanedione	12	24
2-Furfuryl methyl ketone	14	25

TABLE 1 (continued)

Volatile Compounds Isolated from Heat-treated Beef

Compound	Meat sources and treatment*	References
Hydrocarbons		
Butane	5	9
Pentane	5	9
Hexane	5,7	3,9
Heptane	5	9
Octane	5	9
Nonane	12,13	24
Decane	12,13	24
Undecane	12,13	24
Dodecane	7,12,13	3,24
Tridecane	12,13	24
Tetradecane	12,13	24
Pentadecane	7,12,13	3,24
Hexadecane	7,12,13	3,24
Heptadecane	12,13	24
Octadecane	7,13	3,24
6(?)-Methyltetradecane	12	24
1-Pentadecene	7	3
1-Heptadecene	13	24
1-Undecene	7	3
Benzene compounds		
Benzene	7,8	3,12,13,14
Trimethylbenzene	12,13	24
Diethylbenzene	12	24
1,4-Dimethylbenzene	12,13	24
1,2-Dimethylbenzene	12	24
Propylbenzene	7	3
Butylbenzene	12	24
Benzaldehyde	4,7,8,12,13	3,12,13,14,24,29
3-Methylbenzaldehyde	7	3
Phenylacetaldehyde	8,13	12,13,14,24
Paradichlorobenzene	7	3
Toluene	7,12,13	3,24
Lactones		
δ-Valerolactone	13	24
γ-Valerolactone	7	3
γ-Butyrolactone	12,13	24
γ-Hexalactone	13	24
δ-Heptalactone	13	24
γ-Heptalactone	13	24
δ-Octalactone	13	24
γ-Octalactone	13	24
γ-Nonalactone	12,13	24
δ-Nonalactone	13	24
γ-Decalactone	13	24
Furans		
2-Acetylfuran	14	25
2-Pentylfuran	7,12,13	3,24
2-Hexylfuran	13	24
2-Heptylfuran	12,13	24
2-Octylfuran	13	24
2-Methyltetrahydrofuran-3-one	7	3
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	4	28
4-Hydroxy-5-methyl-3(2H)-furanone	4	28

TABLE 1 (continued)

Volatile Compounds Isolated from Heat-treated Beef

Compound	Meat sources and treatment*	References
Sulfur compounds		
Hydrogen sulfide	1,2,3,7,9	4,5,6,7,11,15,16
Methyl mercaptan	1,2,4,7,10	4,5,8,11,15,16
Ethyl mercaptan	1,2,4,7	4,5,8,11,16
Propyl mercaptan	2	16
Butyl mercaptan	2,7	3,16
Isobutyl mercaptan	10	17
Dimethyl sulfide	1,4,7,9,10,12	3,4,5,8,15,16,17,24
Dimethyl disulfide	8,10	12,13,14,17
Ethyl methyl disulfide	10	17
Methyl vinyl disulfide	10	17
Diethyl disulfide	10	17
Methyl propyl sulfide	7	3
Methyl allyl sulfide	7	3
Diallyl sulfide	7	3
Thiophene	10	17
2-Methyl thiophene	10	17
2-Ethyl thiophene	10	17
2-*n*-Butyl thiophene	10	17
2-*n*-Amyl thiophene	10	17
n-Octyl thiophene	10	17
n-Tetradecyl thiophene	10	17
2-Thiophene carboxaldehyde	10	17
5-Methyl-2-thiophene carboxaldehyde	10	17
2,5-Dimethyl-3-thiophene carboxaldehyde	10	17
2-Thiophene acrolein	10	17
2-Acetyl thiophene	10	17
3-Acetyl thiophene	10	17
5-Methyl-2-acetylthiophene	10	17
1-(2-Thienyl)-1-propanone	10	17
1-(2-Methyl-5-thienyl)-1-propanone	10	17
2-Thiophene methanol	10	17
Tetrahydrothiophen-3-one	10	17
2-Methyl tetrahydrothiophen-3-one	10	17
Thiazole	10	17
2-Methylthiazole	10	17
4-Methylthiazole	10	17
2,4-Dimethylthiazole	10	17
5-Ethyl-4-methylthiazole	10	17
4-Ethyl-2-methylthiazole	10	17
2,4,5-Trimethylthiazole	10	17
2,4-Dimethyl-5-vinylthiazole	10	17
2-Acetylthiazole	10	17
Benzothiazole	10,14	17,25
3,5-Dimethyl-1,2,4-trithiolane	4,7,10	17,23,29
5,6-Dihydro-2,4,6-trimethyl-1,3,5-dithiazine	10	17
Trithioacetaldehyde	10	17
Trithioacetone	10	17
Methylthioacetate	10	17
1,1-bis-Methylthioethane	10	17
Dimethyl sulfone	12,13	24
2-Acetyl-2-thiazoline	4	27
1-Methylthio-ethanethiol	4	29
Thialdine	4	29

TABLE 1 (continued)

Volatile Compounds Isolated from Heat-treated Beef

Compound	Meat sources and treatment*	References
Nitrogen compounds		
Ammonia	3,9	6,7,15
Methylamine	3	6
2,4,5-Trimethyloxazoline	7	23
Pyrazine	11	18
Methylpyrazine	11,14	18,26
2,5-Dimethylpyrazine	11,14	18,26
2,6-Dimethylpyrazine	11,14	18,26
2,3-Dimethylpyrazine	11,14	18,26
Ethylpyrazine	11	18
2,5-Dimethyl-3-ethylpyrazine	14	26
2-Ethyl-6-methylpyrazine	11	18
2-Ethyl-5-methylpyrazine	11,14	18,26
Trimethylpyrazine	11,14	18,26
2,6-Diethyl-3-methylpyrazine	14	26
Tetramethylpyrazine	11,14	18,26
5-Ethyl-2,3-dimethylpyrazine	11	18
2-Ethyl-3,5-dimethylpyrazine	11	18
2-Ethyl-3,6-dimethylpyrazine	11	18
2,6-Diethylpyrazine	11	18
Methylpropylpyrazine	11	18
2-Methyl-3,5-diethylpyrazine	11	18
2-Methyl-3,6-diethylpyrazine	11	18
5-Methyl-2,3-diethylpyrazine	11	18
3,6-Diethyl-2,5-dimethylpyrazine	11	18
Triethylpyrazine	11	18
6,7-Dihydro-5(H)-cyclopentapyrazine	11	18
5-Methyl-6,7-dihydro-5(H)-cyclopentapyrazine	11	18
2(3),5-Dimethyl-6,7-dihydro-5(H)-cyclopentapyrazine	11	18
5,6,7,8-Tetrahydroquinoxaline	11	18
2-Methyl-5,6,7,8-tetrahydroquinoxaline	11	18
Vinylpyrazine	11	18
2-Methyl-6-vinylpyrazine	11	18
Isopropenylpyrazine	11	18
Acetylpyrazine	11	18
2-Methyl-5-acetylpyrazine	11	18
2-Ethyl-5-acetylpyrazine	11	18
1-Pyrazinyl-2-propanone	11	18

*Volatile compounds were isolated from the following sources:

1. Ox meat during boiling for meat extract.
2. Canned beef in broth.
3. Lyophilized lean muscle extract.
4. Boiled beef in broth.
5. Enzyme inactivated beef in cans.
6. Ground beef heated in water or fat.
7. Boiled beef.
8. Enzyme inactivated beef.
9. Boiled beef in slurry.
10. Pressure cooked beef (163° and 182°C) in slurry.
11. Pressure cooked beef (162.7°C) in slurry.
12. Roast beef heated to 163°C.
13. Roast beef drippings.
14. Shallow fried beef.

TABLE 2

Volatile Compounds from Processed Pork and Ham

Compound	Meat sources and treatment*	References
Acids		
Formic	2	20
Acetic	1,2	19,20
Propionic	2	20
Butyric	2	20
Isocapric	2	20
Aldehydes		
Formaldehyde	2	20,22
Acetaldehyde	1,2,3,4	6,19,20,21,22
Propanal	1,2,3,4	6,19,20,21,22
Butanal	1,2,3	19,21,22,23
Pentanal	1,2	19,20,22
Hexanal	1,2,4	6,19,22
Heptanal	1,2	19,22
Octanal	1,2,4	6,19,22
Nonanal	1,2,4	6,19,22
Decanal	2	22
Undecanal	2,4	6,22
Dodecanal	1,2	19,22
Tridecanal	1	19
Tetradecanal	1	19
Pentadecanal	1	19
Hexadecanal	1	19
Heptadecanal	1	19
Octadecanal	1	19
Isobutyraldehyde	1,2,3	19,20,21
Isovaleraldehyde	1,2,3	19,20,21
2-Methylbutanal	1,2,3	19,20,21
2-Butenal	1	19
2-Methyl-2-butenal	1	19
2-Hexenal	2	22
2-Heptenal	1,2,4	6,19,22
2-Octenal	1,2,4	6,19,22
2-Nonenal	2,4	6,22
2-Decenal	2,4	6,22
2-Undecenal	2,4	6,22
2-Dodecenal	2	22
2,4-Heptadienal	2,4	6,22
2,4-Nonadienal	2,4	6,22
2,4-Decadienal	2,4	6,22
2,4-Undecadienal	2	22
2,4-Dodecadienal	2	22
2-Phenyl-2-butenal	1	19
5-Methyl-2-phenyl-2-hexenal	1	19
Benzaldehyde	1	19
Phenylacetaldehyde	1	19
Ketones		
Acetone	1,2,3,4	6,19,20,21
2-Butanone	1,2	19,20
Diacetyl	2	20
2-Heptanone	1	19
2-Octanone	1	19
2-Decanone	1	19

TABLE 2 (continued)

Volatile Compounds from Processed Pork and Ham

Compound	Meat sources and treatment*	References
Ketones (cont.)		
2-Tridecanone	1	19
2-Heptadecanone	1	19
3-Pentanone	1	19
3-Octanone	1	19
4-Methyl-2-pentanone	1	19
3-Buten-2-one	1	19
3-Methyl-3-buten-2-one	1	19
3-Penten-2-one	1	19
2,3-Butanedione	1	19
2,3-Pentanedione	1	19
Cyclopentanone	1	19
2-Cyclopenten-1-one	1	19
1-Phenyl-2-propanone	1	19
1-Hydroxy-2-propanone	1	19
3-Hydroxy-2-butanone	1	19
3-Hydroxy-2-pentanone	1	19
2-Hydroxy-3-pentanone	1	19
Esters		
Methyl formate	1	19
Ethyl formate	1	19
Ethyl acetate	1	19
Pentyl acetate	1	19
Acetol acetate	1	19
Ethyl propionate	1	19
Ethyl isovalerate	1	19
Ethyl dodecanoate	1	19
Ethyl tetradecanoate	1	19
Methyl hexadecanoate	1	19
Alcohols		
Methanol	1	19
Ethanol	1	19
2-Propanol	1	19
Butanol	1	19
2-Methyl-1-propanol	1	19
2-Butanol	1	19
3-Methylbutanol	1	19
2-Methyl-2-butanol	1	19
2-Methyl-3-buten-2-ol	1	19
3-Octenol	1	19
Hydrocarbons		
2-Pentene	1	19
Heptane	1	19
Pentadecene	1	19
Pentadecane	1	19
Hexadecene	1	19
Hexadecane	1	19
Heptadecane	1	19
Benzene	1	19
Ethylbenzene	1	19
Hexylbenzene	1	19
Toluene	1	19
m-Xylene	1	19
Limonene	1	19

TABLE 2 (continued)

Volatile Compounds from Processed Pork and Ham

Compound	Meat sources and treatment*	References
Lactones		
δ-Nonalactone	1	19
δ-Decalactone	1	19
Furans		
2-Methylfuran	1	19
2-Ethylfuran	1	19
2-Butylfuran	1	19
2-Pentylfuran	1	19
2-Furaldehyde	1	19
5-Methyl-2-furaldehyde	1	19
Furfuryl alcohol	1	19
2-Acetylfuran	1	19
Propionylfuran	1	19
Furfuryl methyl ketone	1	19
2-Methyltetrahydrofuran-3-one	1	19
Furfuryl formate	1	19
Furfuryl acetate	1	19
Furfuryl propionate	1	19
Furfuryl butyrate	1	19
Furfuryl pentanoate	1	19
Furfuryl hexanoate	1	19
Ethyl furoate	1	19
Ethyl furfuryl ether	1	19
Furfuryl ether	1	19
2,2′-Methylenedifuran	1	19
5-Methyl-2,2′-methylenedifuran	1	19
2(or 3)-Phenylfuran	1	19
Thiazoles		
Thiazole	1	19
4-Methylthiazole	1	19
2-Acetylthiazole	1	19
Thiophenes		
2-Methylthiophene	1	19
2-Thiophenecarboxaldehyde	1	19
3-Thiophenecarboxaldehyde	1	19
3-Methyl-2-thiophenecarboxaldehyde	1	19
5-Methyl-2-thiophenecarboxaldehyde	1	19
2,5-Dimethyl-3-thiophenecarboxaldehyde	1	19
2-Acetylthiophene	1	19
3-Acetylthiophene	1	19
5-Methyl-2-acetylthiophene	1	19
Thiopheneacrolein	1	19
Sulfur compounds		
Hydrogen sulfide	2,3	21
Methanethiol	1,2,3	19,21
Methyl sulfide	1	19
Methyl disulfide	1	19
Methyl sulfone	1	19
Methyl sulfoxide	1	19
Methyl thioacetate	1	19
Methyl thiopropionate	1	19

TABLE 2 (continued)

Volatile Compounds from Processed Pork and Ham

Compound	Meat sources and treatment*	References
Sulfur compounds (cont.)		
Ethyl thiopropionate	1	19
Benzyl methyl sulfide	1	19
Furfuryl methyl sulfide	1	19
Furfuryl methyl disulfide	1	19
Pyrroles		
Pyrrole-2-carboxaldehyde	1	19
5-Methylpyrrole-2-carboxaldehyde	1	19
Acetylpyrrole	1	19
2-Acetylpyrrole	1	19
2-Propionylpyrrole	1	19
Pyrazines		
Pyrazine	1	19
Methylpyrazine	1	19
Ethylpyrazine	1	19
2,3-Dimethylpyrazine	1	19
2,5-Diethylpyrazine	1	19
2,6-Dimethylpyrazine	1	19
2-Ethyl-5-methylpyrazine	1	19
2-Ethyl-6-methylpyrazine	1	19
2,5-Diethylpyrazine	1	19
Trimethylpyrazine	1	19
2-Ethyl-3,5-dimethylpyrazine	1	19
2-Ethyl-3,6-dimethylpyrazine	1	19
5-Ethyl-2,3-dimethylpyrazine	1	19
2,3-Diethyl-5-methylpyrazine	1	19
2,5-Diethyl-3-methylpyrazine	1	19
3,5-Diethyl-2-methylpyrazine	1	19
Triethylpyrazine	1	19
Tetramethylpyrazine	1	19
2-Methyl-3-vinylpyrazine	1	19
2-Methyl-5-vinylpyrazine	1	19
Acetylpyrazine	1	19
2-Methyl-5(or 6)-acetylpyrazine	1	19
2-Ethyl-3-acetylpyrazine	1	19
2-Ethyl-5(or 6)-acetylpyrazine	1	19
6,7-Dihydro-5H-cyclopentapyrazine	1	19
2-Methyl-6,7-dihydro-5H-cyclopentapyrazine	1	19
5-Methyl-6,7-dihydro-5H-cyclopentapyrazine	1	19
2(or 3),5-Dimethyl-6,7-dihydro-5H-cyclopentapyrazine	1	19
Quinoxaline	1	19
2-Methylquinoxaline	1	19
6-Methylquinoxaline	1	19
5,6,7,8-Tetrahydroquinoxaline	1	19
2-Methyl-5,6,7,8-tetrahydroquinoxaline	1	19
(2-Furyl)pyrazine	1	19
2-(2-Furyl)-5(or 6)-methylpyrazine	1	19
Miscellaneous compounds		
Ethyl vinyl ether	1	19
Diethyl ether	1	19
Acetaldehyde, diethyl acetal	1	19
Phenol	1	19

TABLE 2 (continued)

Volatile Compounds from Processed Pork and Ham

Compound	Meat sources and treatment*	References
Miscellaneous compounds (cont.)		
Trimethyloxazole	1	19
Vanillonitrile	1	19

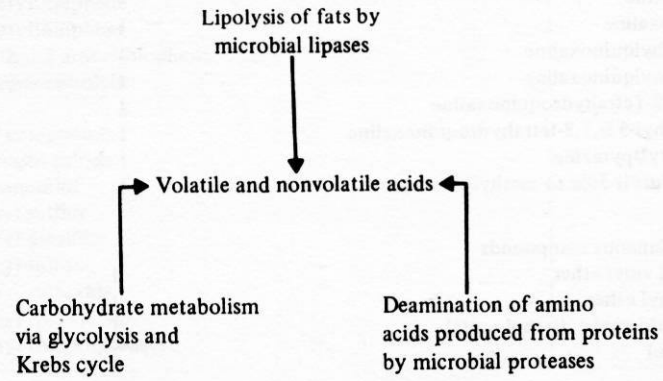
*Volatile compounds were isolated from the following sources:

1. Pressure cooked (157°C) pork liver.
2. Dry cured ham.
3. Uncured ham.
4. Pork lipids heated at 100°C.

by Demeyer and co-workers[40] that the lipases occurring in sausages have specificity for position 3 of triglycerides. The difference observed for linoleic and oleic acids may be related to specificity of the enzyme for the fatty acid structure, as both positional and structural specificity is known to occur in microbial lipases.[41] Since only unsaturated fatty acids undergo nonenzymatic oxidation and produce lipid peroxides and carbonyl compounds, the specificity of microbial lipases for position 3 of triglycerides, which in the case of pork lipids contains a greater percentage of unsaturated fatty acids than positions 1 and 2 of triglyceride molecule, appears to be an important factor in the development of ripened sausage flavor. Micrococci[32,33] and to a lesser degree lactobacilli[42,43] have been reported to be responsible for lipolysis in dry sausage.

During sausage ripening, the carbonyl concentration of the product increases severalfold. In a study carried out by Demeyer et al.,[40] carbonyl compounds increased in the sausage during the first week of ripening, decreased after smoking, and again increased to final values of about 300 μmol/100 g of dry matter. The initial increase was attributed to compounds formed in carbohydrate fermentation,[44] while the increase in the last stages might have been due to further metabolism of lipid peroxides.[39]

The volatile fatty acids produced by the microorganisms during sausage ripening are very important in the dry sausage aroma. Significant concentrations of acetic, propionic, and butyric acids have been reported in fermented sausages, along with large amounts of nonvolatile lactic acid which affects the taste and reduces the pH of sausage from an average of 5.8 to 4.8.[44] The homo- and hetero-fermentative metabolic pathways, which may be involved in the formation of volatile fatty acids and carbonyl compounds by the microorganisms during dry sausage ripening, were discussed in detail in the earlier review.[2] Among the volatile fatty acids mentioned above, acetic acid may be formed from aspartic acid, alanine, serine, glycine, cystine, threonine, and glutamic acid by the bacterial metabolism. Propionic acid may originate from alanine and serine. Butyric acid may be formed from threonine and glutamic acid. These fatty acids may also be synthesized from fats and carbohydrates as shown below:



Nonvolatile Flavor Compounds and Flavor Potentiators

The overall composition of meat extracts and the possible taste effect of various compounds identified have been extensively investigated.[45-62] Inosine-5′-monophosphate (IMP) is a major component of meat extracts. It is considered a key flavor component responsible for the strong meaty taste.[58,63,64] Other nonvolatile components with taste properties include amino acids, peptides, and other nucleotides. The high content of IMP in meat extracts is due to the presence of adenosine-5′-triphosphate (ATP) as a major mononucleotide in the muscle of living animals. After slaughter there is a rapid transformation of this nucleotide to adenosine-5′-monophosphate (AMP), which is then deaminated to IMP.[64] A partial list of compounds isolated from meat extracts is presented in Table 3. Several of the compounds listed in this table act as flavor precursors. The meat flavor precursors are discussed in detail later in this article. The contribution of peptides and amino acids to the taste of foodstuffs has been investigated by Kirimura and co-workers.[65] The organoleptic characteristics of amino acids reported by these workers are reproduced in Table 4.

TABLE 3

Potential Flavor Precursors Isolated from Meat Extracts

Alanine	Fructose-6′-phosphate	Lysine
β-Alanine	Glucose	Methionine
quaternary Amines	Glucose-6′-phosphate	Methylhistidine
Ammonia	Glutamic acid	Nicotinamide-adeninedinucleotide
Anserine	Glutamine	Ornithine
Arginine	Glutathione	Peptides
Asparagine	Glycerophosphoethanolamine	Phenylalanine
Aspartic acid	Glycine	Phosphoethanolamine
Carnitine	Glycoproteins	Phosphoserine
Carnosine	Histidine	Proline
Citrulline	Hydroxyproline	Purine-nucleosides
Creatine	Hypoxanthine	Purine-nucleotides
Creatinine	Inosine-5′-monophosphate	Ribose
Cysteine	and other nucleotides	Ribose-5′-phosphate
Cystine	Isoleucine	Serine
Fructose	Leucine	Taurine
		Threonine
		Tyrosine
		Urea
		Valine

TABLE 4

Organoleptic Characteristics of Amino Acids

Amino acid	Relative taste intensities[a]				
	Sweet	Salty	Sour	Bitter	MSG-like
Sweet amino acids					
Hydroxyproline	***			**	
Lysine·HCl	**			**	
Alanine	***				
Glycine	***				
Serine	***		*		*
Glutamine	*				*
Threonine	***		*	*	
Proline	***			***	

TABLE 4 (continued)

Organoleptic Characteristics of Amino Acids

Amino acid	Relative taste intensities[a]				
	Sweet	Salty	Sour	Bitter	MSG-like
Sour and MSG-like amino acids					
Aspartic acid			***		*
Glutamic acid			***		**
Histidine·HCl			***		
Asparagine			**	*	
Monosodium glutamate(MSG)	*	*			***
Na-aspartatate		**			**
Bitter amino acids					
Histidine				**	
Arginine·HCl				***	
Methionine				***	*
Valine				***	
Arginine				***	
Isoleucine				***	
Phenylalanine				***	
Tryptophan				***	
Leucine				***	

[a]Asterisks denote relative taste intensities.

From Kirimura, J., Shimizu, A., Kimizuka, A., Ninomiya, T., and Katsuya, N., *J. Agric. Food Chem.*, 17, 689, 1969. With permission.

ORIGIN OF MEAT FLAVOR

Patents on Synthetic Meat Flavors

The importance of the flavor precursors, listed in Table 3, in the formation of meat flavor may be easily recognized by the fact that a large number of patents on meat flavoring compositions, which are based on the heat treatment of flavor precursors, have been awarded in the last few years. Some of the patents are based on the Maillard reactions between sugars and amino compounds. Others make use of the strong flavor potentiation of nucleotide derivatives, along with sugar-amine interactions. A sample of the important patents issued on synthetic meat flavors, in the last few years, is summarized below:

A patent issued to Unilever[66] in 1966 details a method of preparing flavoring compounds by heating a mixture of carbohydrates containing at least one amino acid in the presence of a fatty acid. The carbohydrate may be a pentose or a hextose or a polysaccharide, or a mixture of these. There may be a single amino acid or a mixture of two or more amino acids. For example, a mixture of 480 g wheat gluten hydrolyzate, 56 g cysteine-HCl, 70 g xylose, 50 g oleic acid, and 1,500 g water was heated for 2 hr with stirring in an oil bath at 120°C, then cooled to 50°C, and a slurry of 400 g cornmeal in 600 g water was added with vigorous stirring. A yellowish brown powder was obtained that had the flavor and aroma of roast mutton when dispersed in a small quantity of water.

A similar Unilever patent[67] deals with mixtures of amino acids including glutamic acid, nucleotides (preferably GMP and IMP), succinic acid, and hydroxycarboxylic acids such as lactic acid in a defined ratio to have more pronounced meaty flavor.

Another Unilever patent[68] is concerned with the meat flavorings containing at least one of the optical antipodes of pyrrolidonecarboxylic acid. In flavorings also containing other additives, the preferred weight ratios were pyrrolidonecarboxylic acid/glutamine/succinic acid/5′-ribonucleotides = 1/(0–25)/(0–1)/(0–5).

In 1970, Van der Ouweland and Peer[69] were awarded a patent, assigned to Unilever, on a synthetic meat flavor utilizing the capability of certain heterocyclic ketones such as 4-hydroxy-

5-methyl-2,3-dihydrofuran-3-one, to react with inorganic or organic sulfur compounds, and to form compounds with an aroma similar to that of cooked or roast meat.

A recent patent awarded to Brinkman and Van der Heyden,[70] assigned to Lever Brothers Co., claims that the addition of thiaalkanethiols, where two aliphatic groups are methyl- or ethyl-radical, or their salts, in an amount such that the food contains 0.1 to 1,000 mg compound/1,000 kg product results in a rich meat flavor. 2-Thiabutane-3-thiol was cited as an example of thiaalkanethiol in the patent.

Kyowa Fermentation Industry was awarded a patent on meat flavoring composition[71] prepared by applying the Maillard reaction to ribose phosphate and an amino acid, such as lysine, hydrolyzed protein, or a casein hydrolyzate at 150 to 180°C.

Kitada et al.[72] were awarded a patent, assigned to Ajinomoto Co., on a Maillard reaction product having a meat-like flavor. Meat flavor was produced by heating a solution containing cystine and/or cysteine and a reducing sugar at 50 to 120°C at a pressure of 70 to 200 kg/cm^2.

Another Ajinomoto patent[73] discloses the synthetic compounds which enhance the meat flavor. Such compounds were obtained by mixing amino acids, organic acids, phosphates, 5′-nucleotides, and analogous compounds in water, adjusting pH of the solution to about 5 and then cooking it for a few minutes.

International Flavors & Fragrances patented a flavoring material[74] with a strong meat flavor by reacting thiamine or 3-acetyl-3-mercaptopropanol-1 in the presence of an organic acid.

Giacino patent,[75] assigned to International Flavors & Fragrances, discloses the preparation of meat-like flavors by heating a mixture comprised of taurine and thiamine and then adding it to a source of free amino acids.

Thomas patent,[76] assigned to Pfizer Inc., discloses synthetic ham- and bacon-flavored compositions prepared by heating an aqueous mixture of hexose or pentose, cystine or cysteine, and glycine. After cooling the mixture, monosodium glutamate, protein hydrolyzate, sucrose, edible fats, ribonucleotide, and hickory smoke flavor are incorporated to obtain the desired flavor.

Broderick and Marcus patent,[77] assigned to Kohnstamm and Co., discloses a procedure of preparing artificial flavoring substances with the aroma and taste of cooked meat by causing a ribose moiety-containing ribonucleotide to react at elevated temperature with cystine or cysteine in the presence of water.

The use of thiamine as one of the ingredients used in the preparation of synthetic meat flavors is not surprising. Thiamine, like cystine and cysteine, on heating produces a number of sulfur compounds that contribute significantly in meat-like synthetic flavor formulations. It should be noted here that meat flavor being a very characteristic and complex flavor has not yet lent itself to synthetic duplication. The patents on synthetic meat flavors discussed above and the others may be regarded as extenders of meat flavor, rather than the true meat flavor. However, the synthetic preparation of the characteristic flavors of different cuts of meat and species flavor, such as pork, lamb, and beef flavor, are within the realm of being feasible.

The Role of Enzymes in the Development of Flavor Precursors

The nature and concentration of meat flavor precursors depends, to a large extent, on the metabolic activities in the animal body and the post-mortem storage conditions. The biochemical reactions in a living system are catalyzed by enzymes. The nature and concentration of the enzymes found in different parts of an animal body are directly responsible for the accumulation or depletion of metabolites, some of which act as flavor precursors, in muscle. The enzymatic reactions as related to the development of flavor precursors have not been studied to any significant extent. However, a considerable amount of indirect evidence is available. This evidence is presented under the headings: ante-mortem and post-mortem factors.

Ante-mortem Factors

The following observations suggest the importance of enzymes in the development of flavor precursors:

Age of the animal at the time of slaughter is related to cooked meat flavor. Barbella et al.[78] and more recently Lowe and Kastelic[79] reported that young calves up to about 11 months lack the typical flavor quality and intensity of beef. The change in metabolism with age is well known and

it is an important factor in the development of a normal meat flavor. The change in the composition of muscle with age is another important factor. It is safe to assume that if flavor is related to the concentration of metabolites in muscle, an increase in intensity and a change in profile up to an age of a little metabolic change should be expected. A similar observation was made by Crocker[80] who reported that odor of cooked meat generally increases with age of the animal, as the distinctive taste of beef and insipid taste of veal testify. It is well known that myoglobin content of muscle increases with age. However, this increase is not likely to influence the flavor. It is more likely that this difference may be due to an increase in the concentration of certain amino acids; for example, Gruhm[81] has reported that the relative concentration of valine, methionine, leucine, and isoleucine increases with age, or to some constituents of intramuscular fat which alter with increasing maturity.

An interesting study made on 10 pairs of bull and steer twins by Bryce-Jones et al.[82] showed that steers were definitely more tender and also better than bulls in juiciness and flavor.

The effect of breed on flavor is less widely recognized, but scientific studies have revealed that this factor certainly has an influence. European-type cattle give a better flavored meat than do Brahmin cattle,[83] though it is not clear to what extent this difference may be affected by the undoubted toughness of the latter. Again, the introduction of Hampshire blood into a large white pig stock has been reported to favor an enhanced tendency to rancidity and thus to an off flavor development.

The sex of animal also influences the flavor of meat to some extent. According to Weir[84] mature males have a "staggy" odor. It is probably in regard to off flavor that the influence of the sex of an animal is most clearly noted. Thus, male pigs at maturity are frequently associated with an unpleasant "boar odor" on cooking. Patterson[85,86] has recently shown that the substance responsible is probably 5-androst-16-ene-3-one and, what is particularly interesting, this is closely related to a corresponding alcohol in the salivary gland of mature boar which acts as a stimulant to female at the time of mating.

Differences in meat flavor arising from the type of joint are widely recognized. Thus, the rump and topside yield roasts with markedly greater flavor though also greater toughness than those from the sirloin.[54]

Electric shock treatment during the 24 hr preceding slaughter at 20 min intervals invariably increases the pH of cooked steaks from Angus or Hereford steers, compared with control steaks from paired animals receiving no shock treatment.[87]

Feeding of antibiotics or tranquilizers could affect meat flavor. The effect on rumen or intestinal flora may be important in the case of antibiotics, because the digestion of ruminants relies on the action of bacteria, and the fatty acids produced by these microorganisms can modify the lipids of the animal. Harris et al.[88] reported that meat from conventionally raised chicken had a more characteristic flavor than meat from the chicken born and raised in a germfree environment.

A relatively high pH results from injecting adrenaline just ante-mortem.[89] Ante-mortem feeding of sucrose, on the other hand, lowers pH and increases lactate content of pork from pigs that had been transported 340 mi in 24 hr prior to slaughtering; the same effect was not noticed in animals transported only 3.1 mi.[90]

Proteolytic enzymes have been successfully injected ante-mortem into the bloodstream in order to tenderize the meat. Although no qualitative differences in lower boiling aroma volatiles of beef steak from the animal injected with papain were observed, some differences in quantities of the individual compounds were noticed.[91]

Biochemical state of the muscle is also important. Post-mortem all muscles convert their stores of glycogen into lactic acid causing the pH to fall to an ultimate value of about 5.5. If glycogen store is lower than 0.6% of the muscle weight, the extent of this post-mortem glycolysis results in an ultimate pH value between 5.5 and 7. A relationship between the ultimate pH of muscle and its flavor has been reported.[92]

Let us now examine how the flavor precursors are produced in an animal body. It is quite clear from the patent literature cited earlier and other reported findings that meat flavor precursors include amino acids, small peptides, carbohydrates, particularly hexoses and pentoses, and nucleotides (Table 3). As illustrated in Figure 1, amino acids found in blood and tissues may result from their absorption from intestines, breakdown

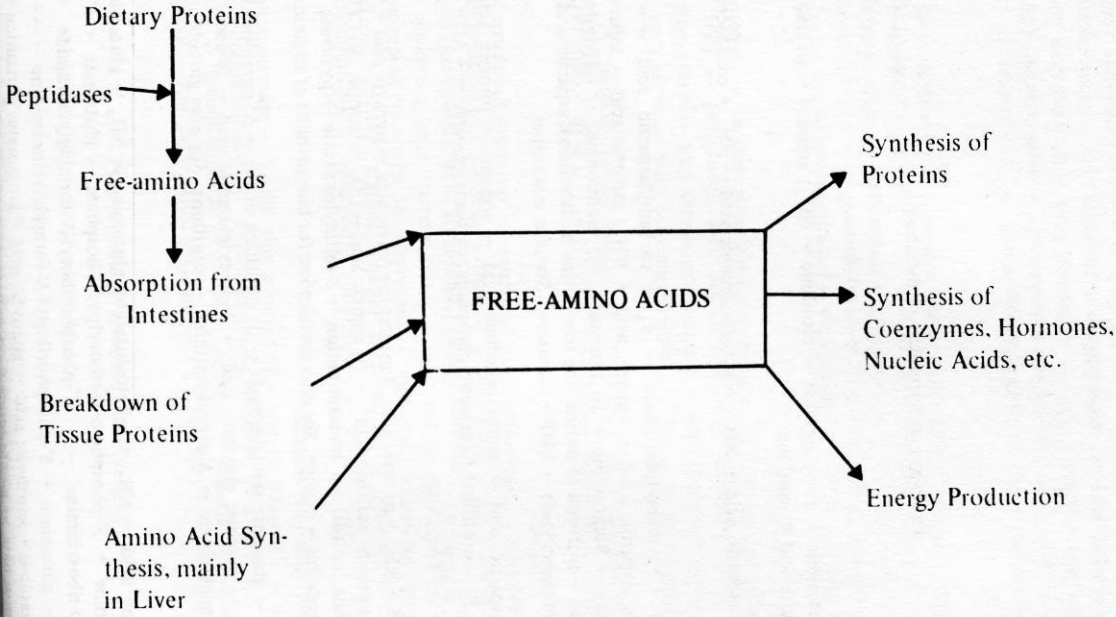


FIGURE 1. Fate of amino acids in mammalian body.

of tissue proteins, and synthesis of amino acids, particularly in the liver. These amino acids are used by the animal body in a number of ways. The major pathways and products of amino acid metabolism are given in Table 5. Considering various metabolic functions of different animal tissues, it appears likely that most of the amino acids found in meat are produced by the breakdown of tissue proteins. In that case, the nature and concentration of amino acids in the muscle would depend on the genetic make up and physiological state of the animal at the time of slaughter. Post-mortem, tissue proteins continue to degrade and release amino acids by the action of native muscle and microbial proteases.

Other metabolic pathways which may have a role in the development of meat flavor precursors are given in Figures 2 to 8.

Figure 2 refers to the mammalian fatty acid metabolism. The compounds isolated from meat extracts, which may originate from one or more of the metabolic schemes described, are acetoin, diacetyl, formic acid, acetic acid, hydroxybutyric acid, and succinic acid. It is widely recognized that the species flavor of meat develops from adipose tissues. The depot fat and water soluble constituents of adipose tissues probably interact to produce the species flavor. The nature of depot fat is governed by the genetic make up and diet of the animal. The various pathways involving fatty acid, glycerolipid, sphingolipid, and sterol metabolism, which are called upon by the living system and influence the make up of depot fat, are outlined in Figures 2 to 5. The role of depot lipids in the development of meat flavor is discussed later in this review.

The carbohydrates present in raw meat interact with amino compounds and produce volatile flavor compounds via Maillard reactions. These reactions are discussed in detail in a latter section in this article. Sugars and sugar phosphates identified from meat extracts include: fructose, fructose-6′-phosphate, fructose-1,6′-diphosphate, glucose, glucose-6′-phosphate, ribose, and ribose-5′-phosphate (Table 3). The pathways of carbohydrate metabolism and interrelationship of sugars and sugar phosphates discussed above are shown in Figure 6. These metabolic schemes also depict the synthesis of lactic acid from glycogen of the muscle. The amount of lactic acid present in meat is important in its characteristic taste.

Figure 7 gives the metabolic interrelationship of lipids, carbohydrates, and proteins. These metabolic schemes show how a metabolite such as acetate may originate from proteins, lipids, or carbohydrates.

Figure 8 details pathways of nucleoprotein catabolism. Nucleoproteins are composed of basic proteins (histones or protamines) associated with nucleic acids in a salt linkage. The reactions

TABLE 5

Pathways of Amino Acid Metabolism

Amino acid	Product of oxidative deamination or transamination	Product of decarboxylation	Pathways and products of metabolism
1. L-Alanine	Pyruvic acid		
2. L-Arginine	α-Keto-δ-guanidovaleric acid	Agmatine	Arginine → ornithine + urea; arginine → citrulline + NH_3; arginine + glycine ↔ guanidoacetic acid + ornithine
3. L-Asparagine	α-Ketosuccinamic acid		Asparagine ⇌ aspartic acid + NH_3; α-ketosuccinamic acid → NH_3 + oxaloacetic acid
4. L-Aspartic acid	Oxaloacetic acid	α-Alanine	Aspartic acid + carbamylphosphate → P_i + carbamylaspartic acid →→→ pyrimidines; aspartic acid ↔ fumaric acid + NH_3; aspartic acid → aspartic semialdehyde → homoserine → (i) threonine, (ii) methionine, or (iii) lysine; aspartic acid → nitrogen of purine ring (*see* purine metabolism); aspartic acid + IMP → adenylosuccinate → AMP + fumarate. *See also* asparagine.
5. L-Citrulline	α-Keto-δ-carbamidovaleric acid		Citrulline + aspartic acid → argininosuccinic acid ↔ arginine + fumaric acid; citrulline + P_i ↔ ornithine + carbamylphosphate; carbamylphosphate + ADP ↔ CO_2 + NH_3 + ATP
6. L-Cysteine and L-cystine	β-Mercaptopyruvic acid		Cysteine → H_2S + NH_3 + pyruvic acid; β-mercaptopyruvic acid → pyruvic acid + S; cysteine → cysteine sulfinic acid* → (i) cysteic acid → CO_2 + taurine, (ii) CO_2 + hypotaurine, or (iii) via transamination → β-sulfinylpyruvate → pyruvate + SO_3^{2-}; 2 cysteine ↔ cystine. *See also* methionine for biosynthesis of cysteine.
7. L-Glutamic acid	α-Ketoglutaric acid	γ-Aminobutyric acid	Glutamic acid ↔ glutamic semialdehyde ↔ (i) ornithine or (ii) → Δ′-pyrroline-5-carboxylate ↔ proline; glutamic acid → *N*-acetylglutamic acid ↔ *N*-acetylglutamic semialdehyde ↔ *N*-acetylornithine → ornithine. *See also* glutamine, histidine.
8. L-Glutamine	α-Ketoglutaramic acid		α-Ketoglutaramic acid → NH_3 + α-ketoglutarate; glutamine ⇌ NH_3 + glutamic acid; glutamine + 5′-phospho-α-D-ribosylpyrophosphate → glutamate + 5′-phospho-β-D-ribosylamine →→ 5′-phosphoribosyl-*N*-formylglycineamide + glutamine → glutamate + 5′-phosphoribosyl-*N*-formylglycineamidine →→→ purines; glutamine + xanthylic acid → guanylic acid + glutamate; glutamine + fructose-6-phosphate → glucosamine-6-phosphate + glutamate. *See also* histidine.

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U.S. Patent No. 11,224,241

Pathways of Amino Acid Metabolism

Amino acid	Product of oxidative deamination or transamination	Product of decarboxylation	Pathways and products of metabolism
9. Glycine	Glyoxylic acid	5,10-Methylene tetrahydrofolate + NH_3 + 2H	Glyoxylate → formate + CO_2; glycine + 5,10-methylene tetrahydrofolate ↔ serine + tetrahydrofolate; glycine + succinyl CoA → δ-aminolevulinate →→→ porphyrins; glycine + 5′-phospho-β-D-ribosylamine → 5-phosphoribosyl-glycineamide →→ purines; glycine → glycocholic acid; glycine → hippuric acid. *See also* serine, threonine, arginine.
10. L-Histidine	β-Imidazolepyruvic acid	Histamine	Histidine → NH_3 + urocanic acid → imidazolone propionate → *N*-formimino-glutamate → glutamate + 5-formimino tetrahydrofolate; histidine → (i) anserine, or (ii) carnosine; histamine → imidazoleacetic acid → (i) imidazole-acetic acid ribonucleoside or (ii) NH_3 + formylaspartic acid; histamine → methylhistamine; ATP + 5-phosphoribosyl-1-phosphate → phosphoribosyl-ATP → phosphoribosyl-AMP + glutamine → 5-amino-1-ribosyl-4-imidazolecarbox-amide-5′-phosphate + imidazoleglycerol phosphate → histidinol phosphate → histidinol → histidine.
11. Hydroxyproline	α-Keto-2-hydroxy-δ-aminovaleric acid		Hydroxyproline → Δ′-pyrroline-3-hydroxy-5-carboxylate → γ-hydroxyglutamate ↔ α-keto-2-hydroxyglutarate ↔ pyruvate + glyoxylate. *See also* proline.
12. L-Isoleucine	α-Keto-β-methylvaleric acid	2-Methylbutylamine	α-Keto-β-methylvaleric acid → CO_2 + α-methylbutyryl CoA ↔ tiglyl CoA ↔ α-methyl-β-hydroxybutyryl CoA ↔ α-methylacetoacetyl CoA ↔ acetyl CoA + propionyl CoA; pyruvate + α-ketobutyrate (*see* threonine) → CO_2 + α-aceto-α-hydroxybutyrate → α-β-dihydroxy-β-methylvalerate → α-keto-β-methylvalerate ↔ isoleucine.
13. L-Leucine	α-Ketoisocaproic acid	3-Methylbutylamine	α-Ketoisocaproic acid → CO_2 + isovaleryl CoA ↔ β-methylcrotonyl CoA + CO_2 ↔ β-methylglutaconyl CoA ↔ β-hydroxy-β-methylglutaryl CoA → (i) acetoacetic acid + acetyl CoA, or (ii) →→→ cholesterol; α-ketoisovaleric acid (*see* valine) + acetyl CoA → β-carboxy-β-hydroxyisocaproic acid → α-hydroxy-β-carboxyiso-caproic acid → α-keto-β-carboxyisocaproic acid ↔ α-ketoisocaproic acid ↔ leucine.
14. L-Lysine	α-Keto-ε-aminocaproic acid	Cadaverine	α-Keto-ε-aminocaproic acid → Δ′-dehydropipecolate → L-pipecolate → Δ^6-dehydropipecolate → α-aminoadipate semialdehyde ↔ α-aminoadipic acid ↔ α-ketoadipic acid → glutaryl CoA → glutaconyl CoA → crotonyl CoA (fatty acid degradation); aspartic semialdehyde + pyruvate →→→ diaminopimelic acid → lysine; α-ketoglutarate + acetyl CoA →→→→ α-ketoadipic acid → α-aminoadipic acid →→ saccharopine → lysine + α-ketoglutarate.

TABLE 5 (continued)

Pathways of Amino Acid Metabolism

Amino acid	Product of oxidative deamination or transamination	Product of decarboxylation	Pathways and products of metabolism
15. L-Methionine	α-Keto-γ-methiolbutyric acid		Methionine + ATP → PP_i + P_i + *S*-adenosylmethionine (active methyl donor); *S*-adenosylmethionine + guanidoacetate (*see* arginine) → creatine + *S*-adenosylhomocysteine; *S*-adenosylhomocysteine → adenosine + homocysteine; homocysteine + serine → cystathionine → cysteine + homoserine; homoserine → α-ketobutyrate + NH_3; α-ketobutyrate → CO_2 + propionyl CoA → methylmalonyl CoA → succinyl CoA; homoserine (*see* aspartic acid) + cysteine → cystathionine → serine + homocysteine; homocysteine + one C donor → methionine; homocysteine → (i) homocysteic acid or (ii) NH_3 + H_2S + α-ketobutyrate.
16. L-Ornithine	Glutamic semialdehyde, or α-keto-δ-aminovaleric acid	Putrescine	Ornithine ↔ proline; ornithine ↔ glutamic semialdehyde ↔ glutamate; ornithine + carbamylphosphate → citrulline. *See also* citrulline, glutamic acid.
17. L-Phenylalanine	Phenylpyruvic acid	Phenylethylamine	Phenylalanine → tyrosine; phenylpyruvic acid → (i) phenyllactic acid or (ii) CO_2 + phenylacetic acid; phosphoenolpyruvate + erythrose-4-phosphate → 3-deoxy-D-arabino-heptulosonic acid-7-phosphate →→→ 5-dehydroquinate → 5-dehydroshikimate → shikimate → shikimate-5-phosphate →→→ prephenate → (i) phenylpyruvate → phenylalanine or (ii) → *p*-hydroxyphenylpyruvate → tyrosine. *See also* tyrosine.
18. L-Proline	Glutamic semialdehyde or α-keto-δ-aminovaleric acid		Proline ↔ glutamate; proline ↔ ornithine; proline → hydroxyproline. *See also* glutamic acid, ornithine.
19. L-Serine	β-Hydroxypyruvic acid		Serine → pyruvate + NH_3; hydroxypyruvate ↔ D-glycerate ⇌ (i) 2-phosphoglycerate or (ii) 3-phosphoglycerate; 3-phosphoglycerate ↔ β-phosphohydroxypyruvate ↔ phosphoserine → serine; phosphatidyl ethanolamine + serine → ethanolamine + phosphatidyl serine; phosphatidyl serine → CO_2 + phosphatidyl ethanolamine; palmitaldehyde + serine → dihydrosphingosine. *See also* glycine, methionine, tryptophan.
20. L-Threonine	α-Keto-β-hydroxybutyric acid		Threonine → NH_3 + α-ketobutyrate; α-ketobutyrate → CO_2 + propionyl CoA → methylmalonyl CoA → succinyl CoA; threonine → (i) glycine + acetaldehyde or (ii) CO_2 + aminoacetone. *See also* aspartic acid.

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TABLE 3 (continued)

Pathways of Amino Acid Metabolism

Amino acid	Product of oxidative deamination or transamination	Product of decarboxylation	Pathways and products of metabolism
21. L-Tryptophan	β-Indolepyruvic acid	Tryptamine	Tryptophan → *N*-formylkynurenine → formate + kynurenine → 3-hydroxykynurenine → alanine + 3-hydroxyanthranilate → α-amino-β-carboxymuconate semialdehyde → CO_2 + α-aminomuconate semialdehyde → α-aminomuconate → α-ketoadipate (*see* lysine for further degradation); tryptophan → 5-hydroxytryptophan → CO_2 + 5-hydroxytryptamine (serotonin) → 5-hydroxyindoleacetic acid; indole-3-glycerol phosphate + serine → tryptophan.
22. L-Tyrosine	*p*-Hydroxyphenylpyruvic acid	Tyramine	*p*-Hydroxyphenylpyruvic acid → CO_2 + homogentisic acid → maleylacetoacetic acid → fumarylacetoacetic acid → fumarate + acetoacetate; tyrosine → 3,4-dihydroxyphenylalanine → CO_2 + 3,4-dihydroxyphenylethylamine → noradrenalin → adrenalin. *See also* phenylalanine.
23. L-Valine	α-Ketoisovaleric acid	2-Methylpropylamine	α-Ketoisovaleric acid → CO_2 + isobutyryl CoA ↔ methacrylyl CoA ↔ β-hydroxybutyryl CoA → β-hydroxyisobutyrate ↔ methylmalonic semialdehyde → (i) β-aminoisobutyric acid or (ii) methylmalonyl CoA (?); 2 pyruvic acid → α-acetolactate → α,β-dihydroxyisovaleric acid → α-ketoisovaleric acid ↔ valine.

*Desulfination pathway for cysteine sulfinic acid → alanine + SO_2. Abbreviations: CoA = coenzyme A; ADP = adenosine diphosphate; AMP = adenosine phosphate; ATP = adenosine triphosphate; IMP = inosine phosphate; P_i = inorganic orthophosphate; PP_i = inorganic pyrophosphate.

From Altman, P. L. and Dittmer, D. S., Eds., in *Metabolism*, Federation of American Societies for Experimental Biology, Bethesda, Maryland, 1968, 426. With permission.

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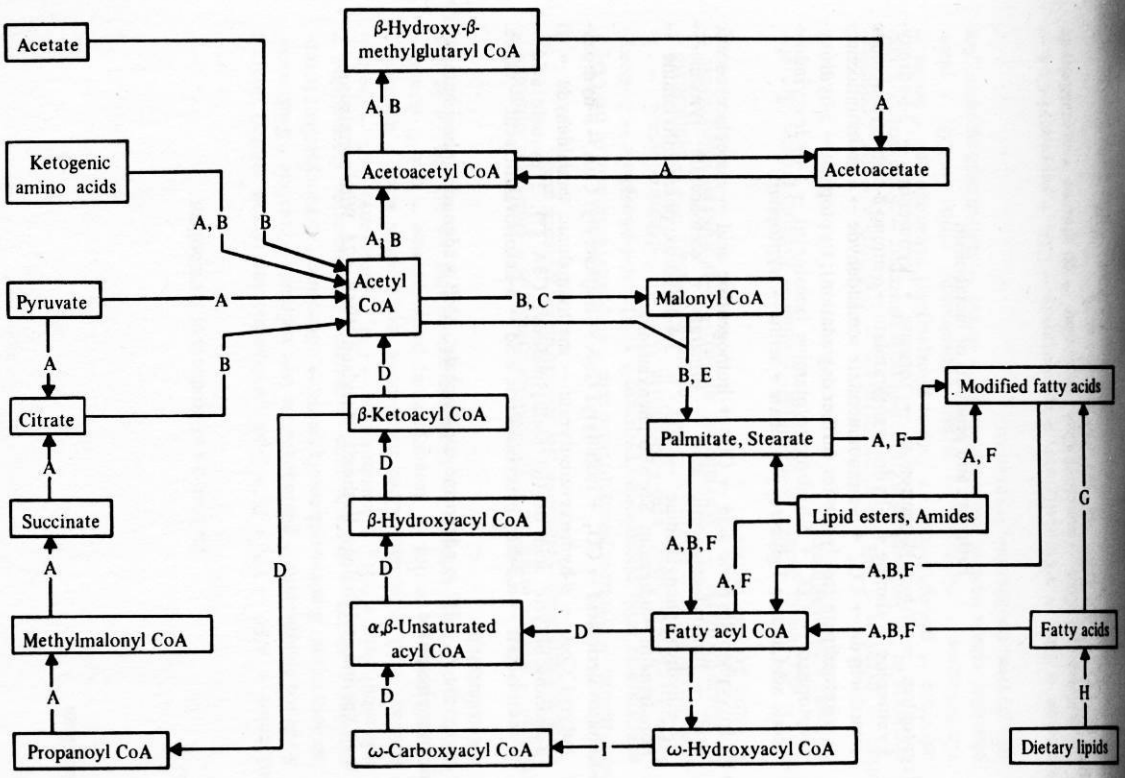
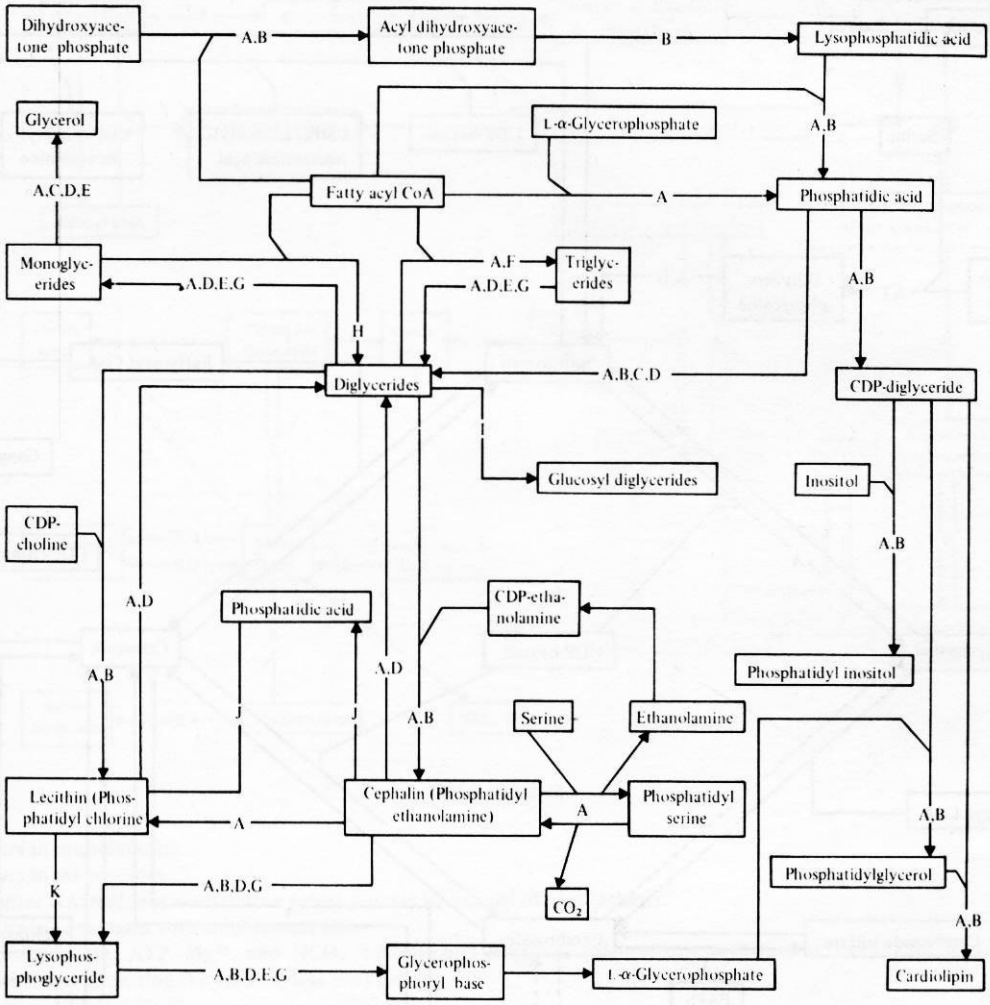


REACTIONS

A: Occurs in mitochondria.
B: Occurs in cytoplasm with soluble enzymes.
C: Requires biotin, ATP, Mg^{2+}, and HCO_3^-; citrate and isocitrate enhance carboxylation of acetyl CoA by complexing with and activating the carboxylase enzyme.
D: β-Oxidation in mitochondria; carnitine esters are involved in transporting fatty acids into mitochondria; requires FAD and NAD^+; ATP or GTP may participate in preliminary extramitochondrial formation of fatty acyl CoA.
E: Involves transacylases, ACP's β-ketoacyl-ACP synthetase, β-ketoacyl-ACP reductase, β-hydroxyacyl-ACP dehydrase, and enoyl-ACP reductase; requires NADPH (furnished mainly by pentose phosphate pathway and by oxidation of oxalacetate to pyruvate via malic acid; NADH required for the latter produced through glycolysis); yields mainly palmitic acid.
F: Occurs in microsomes.
G: Includes chain elongation in microsomes involving malonyl CoA and fatty acyl CoA (analogous to de novo synthesis), chain elongation in mitochondria involving acetyl CoA and fatty acyl CoA, shortening of carbon chain by 2 carbons in microsomes, and desaturation of fatty acyl CoA coupled with chain elongation to yield polyunsaturated acids (requires NADH or NADPH) in microsomes.
H: Involves mainly pancreatic and intestinal lipases and bile salts in intestinal lumen.
I: ω-Oxidation in intestinal lumen.

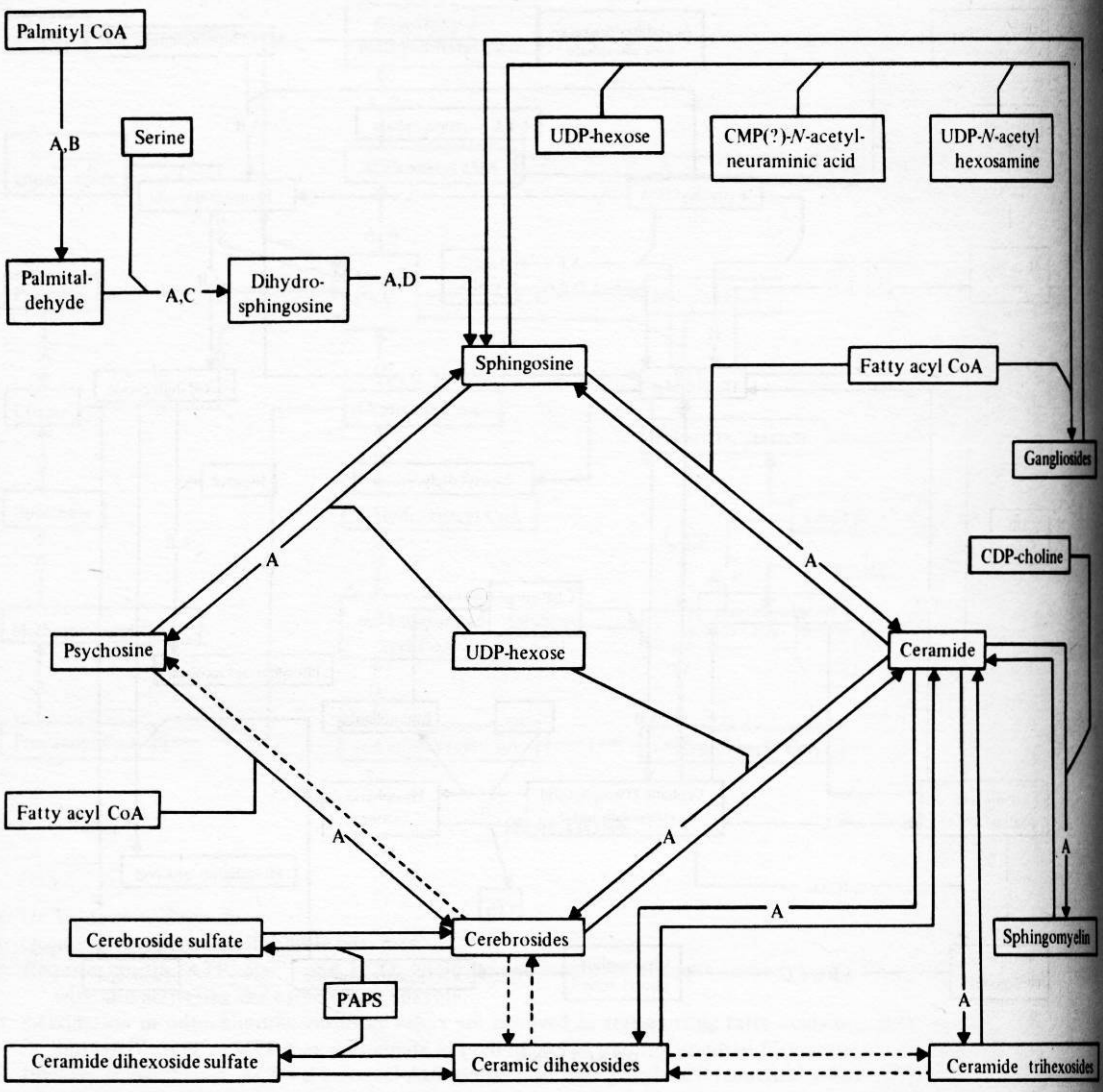
FIGURE 2. Mammalian fatty acid metabolism. (From Altman, P. L. and Dittmer, D. S., Eds., in *Biological Data Book*, Vol. III, Federation of American Societies for Experimental Biology, Bethesda, Maryland, 1973, 1538. With permission.)



REACTIONS

A: Occurs in microsomes.
B: Occurs in mitochondria.
C: Occurs in cytoplasm with soluble enzymes.
D: Occurs in lysosomes.
E: Occurs in cell membrane.
F: 1,2-Diglycerides, but not 1,3-diglycerides.
G: Occurs in the intestinal lumen.
H: Acylation involves mainly a primary hydroxyl group; occurs in microsomes of the intestinal mucosa and mammary glands.
I: Occurs in microsomes of brain.
J: Occurs in plants.
K: Requires cholesterol as a fatty acid receiver; occurs in microsomes, mitochondria, lysosomes, and cell membrane of intestinal lumen and plants, and in blood plasma.

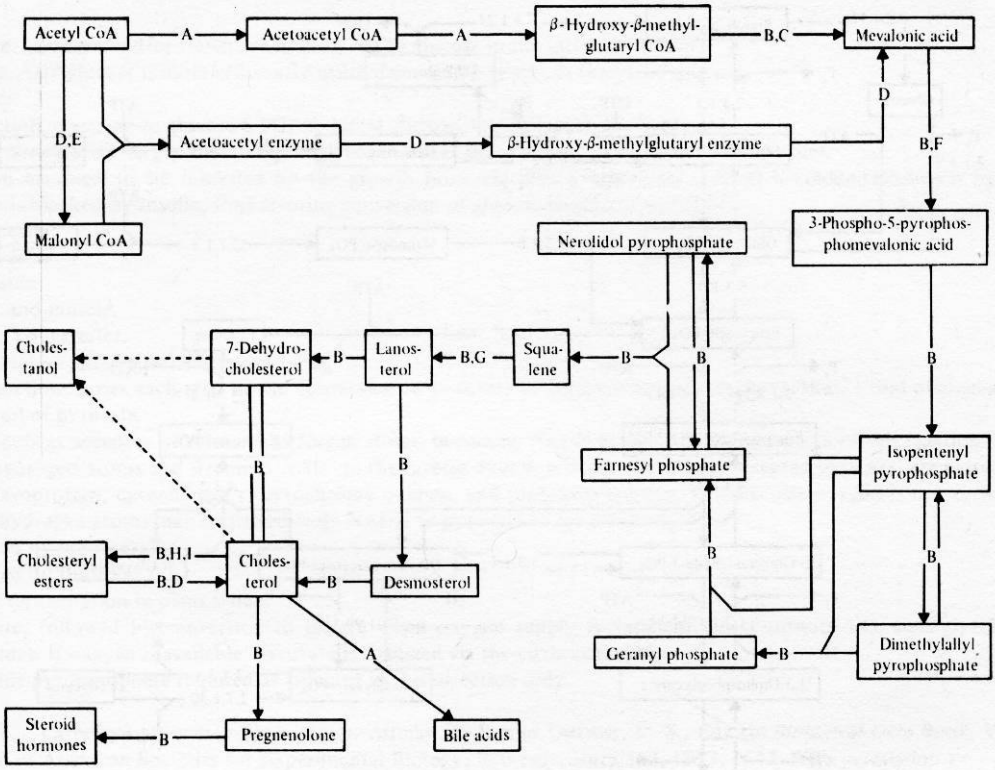
FIGURE 3. Mammalian glycerolipid metabolism. (From Altman, P. L. and Dittmer, D. S., Eds., in *Biological Data Book*, Vol. III, Federation of American Societies for Experimental Biology, Bethesda, Maryland, 1973, 1539. With permission.)



REACTIONS

A: Occurs in microsomes.
B: Requires NADPH.
C: Requires Mn^{2+} and pyridoxal 5-phosphate.
D: Requires a flavoprotein dehydrogenase.

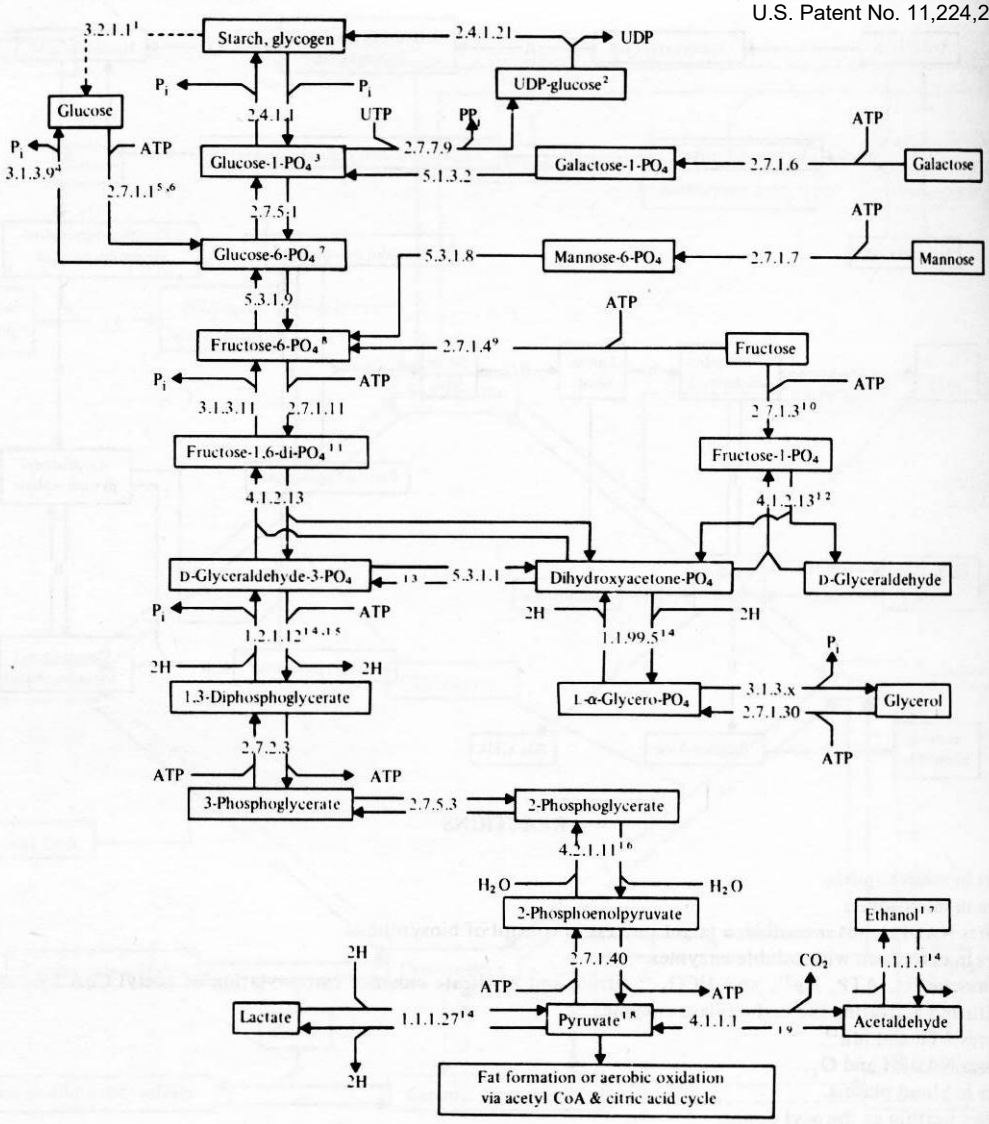
FIGURE 4. Mammalian sphingolipid metabolism. (From Altman, P. L. and Dittmer, D. S., Eds., in *Biological Data Book*, Vol. III, Federation of American Societies for Experimental Biology, Bethesda, Maryland, 1973, 1540. With permission.)



REACTIONS

A: Occurs in mitochondria.
B: Occurs in microsomes.
C: Requires NADPH; not reversible; a target process in control of biosynthesis.
D: Occurs in cytoplasm with soluble enzymes.
E: Requires biotin, ATP, Mg^{2+}, and HCO_3^-; citrate and isocitrate enhance carboxylation of acetyl CoA by complexing with and activating the carboxylase enzyme.
F: Requires ATP and Mn^{2+}.
G: Requires NADPH and O_2.
H: Occurs in blood plasma.
I: Requires lecithin as the acyl donor.

FIGURE 5. Mammalian sterol metabolism. (From Altman, P. L. and Dittmer, D. S., Eds., in *Biological Data Book*, Vol. III, Federation of American Societies for Experimental Biology, Bethesda, Maryland, 1973, 1541. With permission.)



ENZYME KEY [COFACTOR]

1.1.1.1 = alcohol dehydrogenase [NAD^+, NADH].
1.1.1.27 = L-lactate dehydrogenase [NAD^+, NADH].
1.1.99.5 = glycerolphosphate dehydrogenase [NAD^+, NADH].
1.2.1.12 = glyceraldehydephosphate dehydrogenase [NAD^+, NADH].
2.4.1.1 = α-glucan phosphorylase [AMP, Mg^{2+}, P_i].
2.4.1.21 = UDPglucose-starch glucosyltransferase.
2.7.1.1 = hexokinase [Mg^{2+}].
2.7.1.3 = ketohexokinase [Mg^{2+}].
2.7.1.4 = fructokinase [Mg^{2+}].
2.7.1.6 = galactokinase [Mg^{2+}].
2.7.1.7 = mannokinase [Mg^{2+}].
2.7.1.11 = phosphofructokinase [Mg^{2+}].
2.7.1.30 = glycerol kinase [Mg^{2+}].
2.7.1.40 = pyruvate kinase [K^+, Mg^{2+}].
2.7.2.3 = phosphoglycerate kinase [Mg^{2+}].
2.7.5.1 = phosphoglucomutase.
2.7.5.3 = phosphoglyceromutase.
2.7.7.9 = glucose-1-phosphate uridylyltransferase (UDPglucose pyrophosphorylase).
3.1.3.9 = glucose-6-phosphatase.
3.1.3.11 = hexosediphosphatase [Mg^{2+}].
3.1.3.X = phosphoglycerol phosphatase.
3.2.1.1 = α-amylase.
4.1.1.1 = pyruvate decarboxylase [Mg^{2+}].
4.1.2.13 = fructosediphosphate aldolase.
4.2.1.11 = phosphopyruvate hydratase [Mg^{2+}].
5.1.3.2 = UDPglucose epimerase [Mg^{2+}, UDP-glucose].
5.3.1.1 = triosephosphate isomerase.
5.3.1.8 = mannosephosphate isomerase.
5.3.1.9 = glucosephosphate isomerase.

FIGURE 6.

[1] Digestion; glycogen and/or starch are hydrolyzed to glucose in the intestinal lumen.
[2] In plants, ADP glucose is more efficiently utilized for starch synthesis than UDP glucose.
[3] Cori ester.
[4] The reaction, glycogen to glucose-6-PO_4 to blood glucose, takes place in the liver only.
[5] The reaction, glucose to glucose-6-PO_4 to glycogen, takes place in liver, muscle, and other tissues.
[6] Reaction assumed to be inhibited by the growth hormone plus a suprarenal cortical hormone; inhibition by these substances is blocked by insulin, thus favoring conversion of glucose to glucose-6-PO_4.
[7] Robison ester.
[8] Neuberg ester.
[9] In all tissues.
[10] In liver and muscle.
[11] Harden-Young ester.
[12] Liver aldolase also degrades fructose-1-PO_4^-.
[13] This reaction causes each step in the conversion to pyruvate to be doubled quantitatively; thus, 1 mol of glucose gives rise to 2 mol of pyruvate.
[14] NAD^+ acts as acceptor of released hydrogen atoms, becoming NADH in the oxidative direction of this reaction. NADH gives up hydrogen atoms and becomes NAD^+ in the reverse direction. Hydrogen atoms accepted by NAD^+ are passed on in turn to flavoprotein, cytochrome *c*, cytochrome oxidase, and molecular oxygen. If molecular oxygen is not sufficiently available, hydrogen atoms may be passed from NADH to pyruvate to form lactate.
[15] Inhibited by iodoacetate.
[16] Inhibited by fluoride.
[17] End of fermentation in plant tissue.
[18] Pyruvate, followed by conversion to lactate when oxygen supply is deficient (*see* Footnote 14), ends glycolysis in animal tissues. If oxygen is available, pyruvate is oxidized via the citric acid cycle.
[19] Thiamine pyrophosphate required as cofactor in this direction only.

FIGURE 6. Carbohydrate metabolism. (From Altman, P. L. and Dittmer, D. S., Eds., in *Biological Data Book*, Vol. III, Federation of American Societies for Experimental Biology, Bethesda, Maryland, 1973, 1543. With permission.)

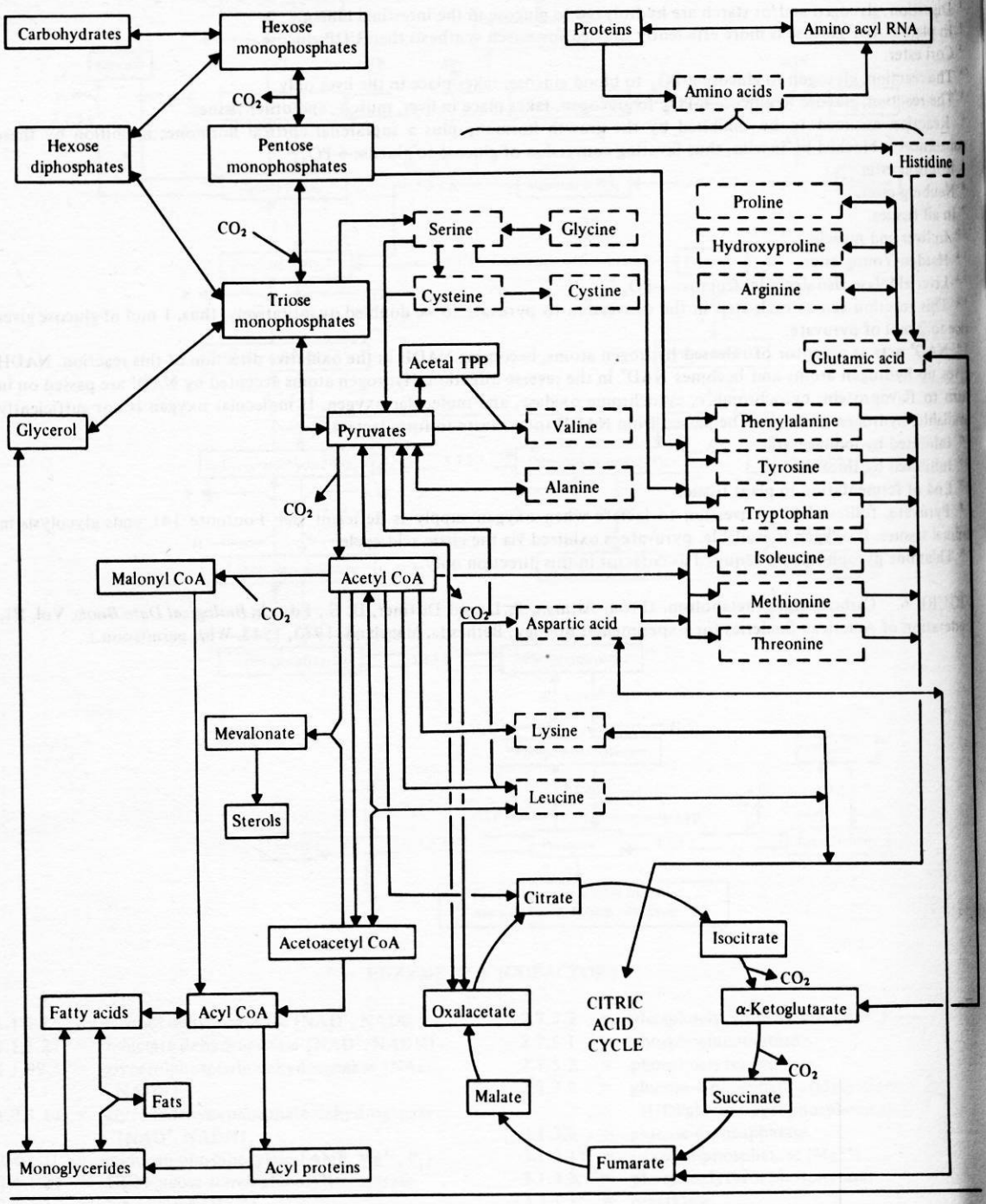


FIGURE 7. Metabolic interrelationship: Lipids, carbohydrates, and proteins. (From Altman, P. L. and Dittmer, D. S., Eds., in *Biological Data Book*, Vol. III, Federation of American Societies for Experimental Biology, Bethesda, Maryland, 1973, 1551. With permission.)

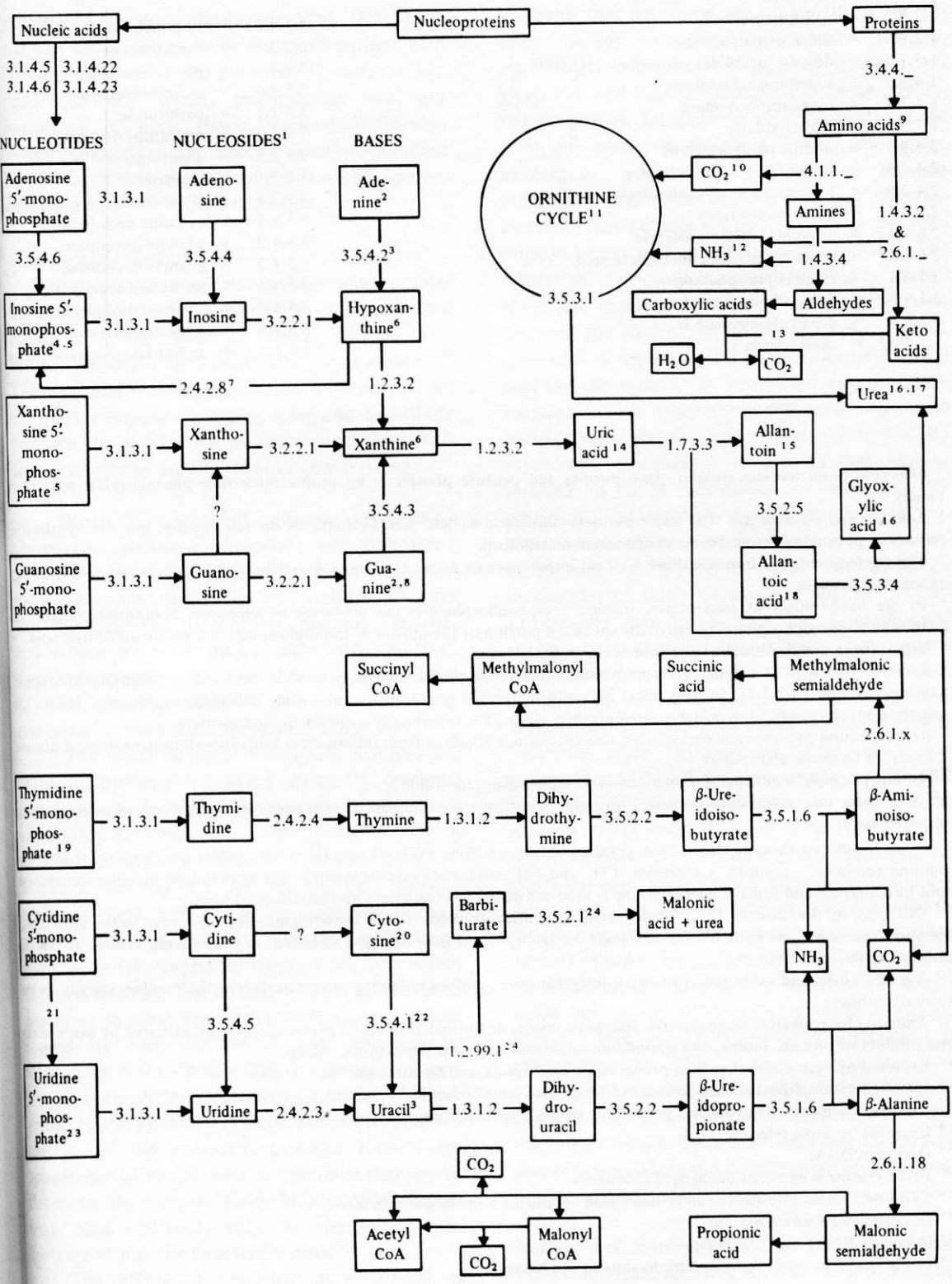

Nucleic acids
Nucleoproteins
Proteins
3.1.4.5
3.1.4.6
3.1.4.22
3.1.4.23
3.4.4.
NUCLEOTIDES
NUCLEOSIDES[1]
BASES
Amino acids[9]
Adenosine 5'-monophosphate
3.1.3.1
Adenosine
Adenine[2]
ORNITHINE CYCLE[11]
CO_2[10]
4.1.1.
Amines
1.4.3.2 & 2.6.1.
3.5.4.6
3.5.4.4
3.5.4.2[3]
NH_3[12]
1.4.3.4
3.5.3.1
Inosine 5'-monophosphate[4,5]
3.1.3.1
Inosine
3.2.2.1
Hypoxanthine[6]
Carboxylic acids
Aldehydes
13
Keto acids
H_2O
CO_2
2.4.2.8[7]
1.2.3.2
Urea[16,17]
Xanthosine 5'-monophosphate[5]
3.1.3.1
Xanthosine
3.2.2.1
Xanthine[6]
1.2.3.2
Uric acid[14]
1.7.3.3
Allantoin[15]
Glyoxylic acid[16]
?
3.5.4.3
3.5.2.5
Guanosine 5'-monophosphate
3.1.3.1
Guanosine
3.2.2.1
Guanine[2,8]
Allantoic acid[18]
3.5.3.4
Succinyl CoA
Methylmalonyl CoA
Succinic acid
Methylmalonic semialdehyde
2.6.1.x
Thymidine 5'-monophosphate[19]
3.1.3.1
Thymidine
2.4.2.4
Thymine
1.3.1.2
Dihydrothymine
3.5.2.2
β-Ureidoisobutyrate
3.5.1.6
β-Aminoisobutyrate
Cytidine 5'-monophosphate
3.1.3.1
Cytidine
?
Cytosine[20]
Barbiturate
3.5.2.1[24]
Malonic acid + urea
NH_3
CO_2
21
3.5.4.5
3.5.4.1[22]
1.2.99.1[24]
Uridine 5'-monophosphate[23]
3.1.3.1
Uridine
2.4.2.3
Uracil[3]
1.3.1.2
Dihydrouracil
3.5.2.2
β-Ureidopropionate
3.5.1.6
β-Alanine
CO_2
2.6.1.18
Acetyl CoA
CO_2
Malonyl CoA
Propionic acid
Malonic semialdehyde

FIGURE 8.

1.2.3.2 = xanthine oxidase.
1.2.99.1 = uracil dehydrogenase.
1.3.1.2 = dihydro-uracil dehydrogenase [$NADP^+$].
1.4.3.2 = L-amino-acid oxidase.
1.4.3.4 = monoamine oxidase.
1.7.3.3 = urate oxidase.
2.4.2.3 = uridine phosphorylase.
2.4.2.4 = thymidine phosphorylase
2.4.2.8 = hypoxanthine phosphoribosyltransferase.
2.6.1.– = aminotransferases.
2.6.1.18 = β-alanine aminotransferase.
2.6.1.x = aminotransferase, uncharacterized.
3.1.3.1 = alkaline phosphatase.
3.1.4.5 = deoxyribonuclease I.
3.1.4.6 = deoxyribonuclease II.
3.1.4.22 = ribonuclease I.
3.1.4.23 = ribonuclease II.
3.2.2.1 = nucleosidase.
3.4.4.– = proteases.
3.5.1.6 = β-ureidopropionase.
3.5.2.1 = barbiturase.
3.5.2.2 = dihydropyrimidinase.
3.5.2.5 = allantoinase.
3.5.3.1 = arginase.
3.5.3.4 = allantoicase.
3.5.4.1 = cytosine deaminase.
3.5.4.2 = adenine deaminase.
3.5.4.3 = guanine deaminase.
3.5.4.4 = adenosine deaminase.
3.5.4.5 = cytidine deaminase.
3.5.4.6 = AMP deaminase.
4.1.1.– = amino acid decarboxylases.

[1] 6-Oxypurine nucleotides are split into purines and pentose phosphate by purine nucleoside phosphorylase present in tissues.
[2] Adenine and guanine are the major purines occurring in nucleic acids. Mammals do not require, but can synthesize, purines or pyrimidines from products of protein metabolism.
[3] The route adenine → hypoxanthine is of no importance in animals; adenine deaminase [3.5.4.4] is not found to any extent in mammals.
[4] In the biosynthesis of nucleotides, inosine 5′-monophosphate is the precursor of adenosine 5′-monophosphate and guanosine 5′-monophosphate; in uricotelic species, a portion of the inosine 5′-monophosphate is a precursor of uric acid.
[5] Important in metabolism, but does not occur in nucleic acid.
[6] Excreted to a limited extent by all mammals; primary end product of subjects with xanthinuria, congenital absence of xanthine oxidase [1.2.3.2]. In man, most of xanthine formed is excreted as uric acid. This is the source of ∿ 75% of the urinary urate; hypoxanthine, which contributes the other 25%, is primarily a metabolic intermediate.
[7] Recycle found in all normal mammalian tissues; absence resulting from inborn error leads to serious neurological disease.
[8] Excreted by swine and spiders.
[9] For more complete information on amino acid metabolism, *see* Table 5.
[10] May enter into metabolic processes, into the ornithine cycle and be incorporated into and excreted as urea, or be excreted as CO_2.
[11] Urea formation in mammalian liver occurs via the ornithine cycle (Krebs-Henseleit cycle): ornithine → citrulline → arginine succinate → arginine → ornithine. CO_2 and NH_3 enter the cycle via aspartic acid at citrulline; arginine succinate is split into arginine and fumaric acid; arginine is then converted to ornithine with the release of urea.
[12] NH_3, as in the case of CO_2, is also used to synthesize many tissue constituents; hence, it may enter into many metabolic processes, be built into amino acids, be incorporated into urea and excreted, or be excreted as NH_3 across the kidney tubule.
[13] Via the citric acid cycle (prior to entry into this cycle, sulfur-containing amino acids lose their sulfur, usually in the form of sulfate).
[14] Excreted by primates, some reptiles, and some insects as the end product of purine catabolism; excreted by birds as the end product of protein, purine, and pyrimidine catabolism (no urea formation in birds).
[15] Excreted by mammals other than primates, by gastropods, and by some insects.
[16] Excreted by amphibians, most fishes, and freshwater lamellibranchs.
[17] Excreted by mammals as the end product of amino acid metabolism.
[18] Excreted by some teleosts.
[19] Occurs in DNA.
[20] Free cytosine is excreted unchanged in animals.
[21] Cytidine 5′-monophosphate can be converted to uridine 5′-monophosphate.
[22] In yeasts and *Escherichia coli*.
[23] Occurs in RNA.
[24] In *Corynebacterium*, *Mycobacterium*, and U-i soil bacterium.

FIGURE 8. Pathways of nucleoprotein catabolism. (From Altman, P. L. and Dittmer, D. S., Eds., in *Biological Data Book*, Vol. III, Federation of American Societies for Experimental Biology, Bethesda, Maryland, 1973, 1552. With permission.)

presented are for ribonucleic acid (RNA). A parallel series occurs with deoxyribonucleic acid (DNA). The breakdown products of nucleic acids, produced enzymatically post-mortem, are very important in meat flavor, particularly inosine 5′-monophosphate. The specific reactions involved in the formation of this important nucleotide are discussed in detail in a latter section.

Post-mortem Factors

Post-mortem factors, particularly aging, greatly influence the flavor of cooked meat. The desired flavor is not produced on cooking unaged beef, rather a metallic and astringent flavor results.[84] The enzymes present in raw meat continue to act on meat components during aging and contribute towards the production of flavor precursors. This fact is supported by a number of observations:

Wood[48] identified glucose, glucose-6′-phosphate, fructose-6′-phosphate, and diphosphopyridine nucleotide as reducing substances in raw meat. In an aged muscle, where autolytic processes had been allowed to continue, glucose, fructose, and ribose were the major sugars with traces of sugar phosphates. Apparently during aging, most of the sugar phosphates were enzymatically converted to sugars and inorganic phosphate.

The aging of meat principally involves the action of proteolytic enzymes on muscle proteins, which results in the tenderization of meat. It is commonly accomplished by letting beef cuts hang at temperatures of about 4°C for a period of 1 to 4 weeks. The extent of proteolysis during normal aging is slight but it can be definitely demonstrated. Aging of beef for 2 weeks results in an increase in free amino nitrogen of the nonprotein nitrogen fraction. The contribution of native tissue proteases in meat tenderization has been demonstrated by preparing rabbit and lamb muscles aseptically from freshly killed animals and preserving them under aseptic conditions at 25°C for about 150 days. After 21 days, the electrophoretic pattern of the extracted proteins showed the appearance of several new components that moved faster in the electric field. Microscopic changes were also observed, such as the longitudinal splitting of myofibrils after 50 days.[95]

The effect of post-mortem myotomy on glycolysis and ultimate qualitative characteristics of porcine longissimus and rectus femoris muscles was investigated by Koch et al.[96,97] It was observed that myotomy at or shortly after exsanguination stimulated contractile activity, significantly increased the rate of post-mortem glycolysis, and tended to decrease ultimate qualitative properties. Longissimus muscles incised at the time of exsanguination had lower pH values, glycogen, adenosine triphosphate, creatine phosphate, and higher lactate levels at all post-mortem time periods studied through 2 hr, than muscles not incised until 45 min post-mortem. The effects of myotomy were greater among normal than low quality muscles. The rates of post-mortem pH decline, levels of glycogen, lactate, glucose-6′-phosphate, adenosine triphosphate, and creatine phosphate, as influenced by the post-mortem muscle temperature and myotomy, were investigated. Muscle contraction stimulated by myotomy, particularly in the excised sample, resulted in rapid diminution of adenosine triphosphate and creatine phosphate, which in turn appeared to have contributed to the observed differences in glycolytic rate. An activating mechanism of this nature would not be expected to be as effective on a process that is already rapid as on one that is relatively slow. Thus, muscle contraction resulting from myotomy was more effective in increasing glycolytic rate of normal longissimus muscles than those of lower quality (inherent rapid glycolyzing). Also, since low temperatures (2 to 4°C) are normally used to chill pork carcasses and are known to exert an inhibitory effect upon glycolytic rate, the inhibition would be expected to have a greater effect on the faster process. Thus, the rapid heat dissipation of the rectus femoris muscles decreased glycolytic rate to a greater extent among low quality than normal muscles. Consequently, what appeared to be a discrepancy in response between the longissimus and rectus femoris muscles was in actuality a natural physiological response to differences in experimental procedure.

As has been discussed earlier, in the case of ripened sausages, proteases, lipases, and other enzymes released by micrococci and lactobacilli play a major role in the development of flavor compounds and some flavor precursors.

Nature and Concentration of Flavor Precursors Found in Meat and Their Role in the Development of Meat Flavor

During the last 2 decades, a large number of publications have dealt with the nature of flavor

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TABLE 6

Concentration of Various Amino Compounds and Carbohydrates in Lyophilized Diffusate from Beef before and after Heating

Compound	Concentration mg/100 g tissue	
	Unheated	Heated (boiling water for 1 hr)
Total amino compounds	161.53	71.45
Phosphoserine	0.36	0.29
Glycerophosphoethanolamine	0.02	0.01
Phosphoethanolamine	0.66	0.82
Taurine	9.05	4.02
Urea	0.01	–
Aspartic acid	0.82	0.32
Threonine	1.11	0.74
Serine + asparagine (as serine)	7.53	1.35
Glutamic acid	4.63	2.22
Glycine	2.40	1.32
Alanine	11.28	6.22
Cystine	4.37	–
Valine	2.99	1.47
Methionine	2.01	0.75
Isoleucine	2.04	0.87
Leucine	3.81	2.34
Tyrosine	1.85	0.80
Phenylalanine	1.36	0.97
NH_3 + lysine	6.19	4.11
Histidine	4.10	4.17
Anserine + carnosine (as carnosine)	90.14	38.17
1-Methylhistidine	4.80	0.49
Total carbohydrates	48.66	28.46
Glucose	43.86	25.25
Fructose	3.56	3.21
Ribose	1.09	Trace
Unknown nucleoside (as ribose)	0.15	–

From Macy, R. L., Naumann, D. H., and Bailey, M. E., *J. Food Sci.*, 29, 142, 1964. With permission.

precursors of various types of meat. Recently, Heath[1] reported that a cold water extract of raw beef, after freeze drying and counter current dialysis, showed that only those fractions having a molecular weight of less than 200 produced any beef flavor on heating, and that fractions were composed of 32 amino acids, small peptides, and carbohydrates such as glucose, glucosamine, fructose, and ribose. A partial list of water soluble compounds isolated from meat is given in Table 3.

The literature reports indicate that the mechanisms of meat flavor development include not only Maillard browning reactions and fatty acid oxidation but also inter- and intra-molecular cyclizations as well as numerous reactions which are made possible by the reactivity of ammonia, hydrogen sulfide, mercaptans, and other undefined intermediates, especially at elevated temperatures.[17] It is generally agreed that a major portion of meat volatiles is produced as a result of interaction between sugars and amino compounds. These flavor precursors, in heated and unheated meat samples, have been extensively investigated.[50,51,57] Quantities of individual amino compounds and sugars determined in heated and unheated lyophilized diffusate from beef, lamb, and pork are presented in Tables 6, 7, and 8. The amino compounds found in the meat of three species appear to be quite similar quantitatively as well as qualitatively, except that cysteic acid and ornithine were common only to pork and lamb, and that glutathione was present only in lamb.[51]

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TABLE 7

Concentration of Various Amino Compounds and Carbohydrates in Lyophilized Diffusate from Pork before and after Heating

Compound	Concentration mg/100 g tissue	
	Unheated	Heated (boiling water for 1 hr)
Total amino compounds	109.86	88.10
Phosphoserine	0.35	0.23
Glycerophosphoethanolamine	0.02	0.01
Phosphoethanolamine	0.45	1.89
Taurine	12.58	7.94
Aspartic acid	1.37	0.45
Threonine	0.48	0.45
Serine + asparagine (as serine)	2.95	0.53
Glutamic acid	1.95	1.19
Proline	0.64	0.24
Glycine	2.75	1.58
Alanine	4.19	2.80
Cystine	2.11	0.89
Valine	0.30	0.20
Methionine	0.69	1.00
Isoleucine	1.03	0.83
Leucine	1.68	1.00
Tyrosine	0.56	0.37
Phenylalanine	0.51	0.39
Ornithine	Trace	0.56
NH_3 + lysine	4.27	4.06
Histidine	2.55	3.11
Anserine + carnosine (as carnosine)	67.94	58.38
1-Methylhistidine	0.49	Trace
Total carbohydrates	45.90	30.04
Glucose	43.56	28.02
Fructose	2.08	2.02
Ribose	0.20	Trace
Unknown nucleoside (as ribose)	0.06	–

From Macy, R. L., Naumann, D. H., and Bailey, M. E., *J. Food Sci.*, 29, 142, 1964. With permission.

In all three cases, taurine, anserine-carnosine, and alanine were the major constituents of unheated samples, and losses during heating were relatively large. Decreases in these constituents accounted for 69, 72, and 45% of the total amount during heating of this type of compounds in beef, pork, and lamb, respectively. Other important constituents degraded during heating were glutamic acid, glycine, lysine, serine, cystine, methionine, leucine, isoleucine, and methyl histidine. The loss of essential amino acids ranged from approximately 20% in pork samples to approximately 55% in beef samples. Macy et al.[51] also reported that beef contained largest amount of amino compounds, and pork the least. The precentages of the total amino compounds lost during heating were proportional to initial quantities present. Greater amounts were lost from the beef samples than from the lamb and pork samples.

Macy et al.[50,51] reported the presence of glucose, fructose, ribose, and some unidentified carbohydrate material in beef, lamb, and pork extracts (Tables 6, 7, and 8). The concentrations of these sugars in three species were quite similar; glucose was present in greatest quantities followed by fructose, ribose, and the unknown fraction. Ribose appeared to be the most labile to heating, and fructose the most stable (Tables 6, 7, and 8).

Recently, Jarboe and Mabrouk[61] carried out an extensive investigation of free amino acids, sugars,

TABLE 8

Concentration of Various Amino Compounds and Carbohydrates in Lyophilized Diffusate from Lamb before and after Heating

	Concentration mg/100 g tissue	
	Unheated	Heated (boiling water for 1 hr)
Total amino compounds	130.01	81.46
Phosphoserine	0.30	0.22
Glycerophosphoethanolamine	0.01	–
Phosphoethanolamine	1.15	1.90
Taurine	26.25	16.47
Urea	Trace	–
Aspartic acid	1.52	1.36
Threonine	3.14	1.36
Serine + asparagine (as serine)	4.74	2.26
Glutamic acid	6.08	2.85
Proline	3.05	0.83
Glycine	4.30	2.25
Alanine	9.18	6.53
Cystine	6.46	5.24
Valine	0.44	0.18
Methionine	2.37	1.53
Isoleucine	2.79	1.87
Leucine	5.62	3.06
Tyrosine	1.98	1.67
Phenylalanine	1.85	1.12
Ornithine	0.95	0.65
NH_3 + lysine	10.51	5.59
Histidine	9.70	7.16
Anserine + carnosine (as carnosine)	25.55	16.28
1-Methylhistidine	2.07	1.08
γ-Amino-*n*-butyric acid	Trace	–
Total carbohydrates	36.42	23.94
Glucose	32.87	21.33
Fructose	2.68	2.61
Ribose	0.52	Trace
Unknown nucleoside (as ribose)	0.35	–

From Macy, R. L., Naumann, D. H., and Bailey, M. E., *J. Food Sci.*, 29, 142, 1964. With permission.

and organic acids in aqueous beef extracts. These workers confirmed the presence of glucose-6′-phosphate, fructose-6′-phosphate, fructose-1,6′-diphosphate, and adenosine-5′-monophosphate, reported earlier by Wood[48] and others, and provided quantitative data on these compounds. Data of Jarboe and Mabrouk are not in agreement with the earlier work of Macy et al.,[51] possibly due to differences in the state of muscle, and the analytical techniques used.

Batzer et al.[49] in 1962 identified inosinic acid, inorganic phosphate, and a glycoprotein of which the carbohydrate was glucose, as the principal components involved in the development of beef flavor on heating. The principal amino acids in the protein moiety were β-alanine and serine, with smaller quantities of glycine, glutamic acid, α-alanine, leucine and isoleucine. When mixtures of some of these amino acids were added to a solution of glucose, inosine, and inorganic phosphate, meaty flavor developed on heating in fat or water.

Comparatively, little work has been done on the contribution of water soluble components from adipose tissue in the development of meat flavor. The work done by Pepper and Pearson[58]

indicates that there are some differences in proteins present in lean muscle and adipose tissue. It was suggested, therefore, that the water soluble fraction from adipose tissue may make a distinct and characteristic contribution to meat flavor above and beyond that related to the lipid portion. The authors further suggest that an additional role of adipose tissue to meat flavor could be derived from the increased number of sulfhydryl groups due to the presence of salt. Since salt is commonly used in cooking, it is possible that it may retard oxidation of these groups, thereby promoting the formation of parent sulfur compounds, which are known to play a major role in the development of cooked meat aroma. An extension of this work was recently reported by Pepper and Pearson.[62] The dialyzable fraction from beef adipose tissue, either treated with salt or untreated and stored under either air or nitrogen, was lyophilized, fractionated by column chromatography, and analyzed by chemical procedures or thin-layer chromatographic techniques. The chromatographic profiles of dialyzable components were unaffected by salt treatments or by storage under air or nitrogen. Amino acid analysis revealed that glycine, glutamic acid, and proline comprised about 46% of the total amino acid residues for the untreated samples as compared to 31% for the sample treated with salt. The diffusate from the salt-treated sample contained more arginine, threonine, serine, isoleucine, leucine, tyrosine, and phenylalanine (22%) than the untreated sample (9%). Only traces of sulfhydryls were evident, but positive tests were obtained for aldose and free amino groups. The diffusates from untreated adipose tissue contained about five times as many free amino groups and ten times as many aldoses as that from the salt treatment. This suggests that salt stabilized the adipose tissue from breakdown.

Thin-layer chromatography of beef adipose tissue diffusate confirmed the presence of creatine, creatinine, creatine phosphate, cytosine, uracil, and several fluorescent and phosphate-containing compounds, but purine bases, nucleosides, nucleotides, and lactic acid were absent.

Wasserman and Spinelli[60] have recently published preliminary studies on the components of adipose tissue which may be involved in the development of meat aroma (Table 9). Precursors of the characteristic beef, lamb, and pork aromas were shown to be present in the lipids extracted from adipose tissue, with a chloroform-methanol mixture. Water washing of the extract removed components involved in the formation of these odors. Amino acids and glucose were identified in the wash which produced a nonspecific roast meat aroma on heating to dryness.

Since adipose tissue is composed of metabolically active cells, it is not surprising that a number of amino acids and at least one sugar component have been identified in tissue extracts. The absence of purine bases, nucleosides, and nucleotides in beef adipose tissue diffusate, reported by Pepper and Pearson,[62] may be due to inadequate detection techniques, as nucleic acids are essential cellular constituents and their metabolic and breakdown products are usually found in living cells.

As reported earlier, Batzer et al.[49] found that inosinic acid, along with glucose and unidentified glycoprotein, was necessary for the development of meaty flavor in beef. Inosinic acid is produced from adenosine 5′-triphosphate post-mortem. Nucleotides are also potential precursors of free ribose and ribose phosphate, which have been implicated in Maillard browning reactions. Macy et al.[56] investigated the effect of cooking on nucleotides, nucleosides, and bases of beef, pork, and lamb. The effect of cooking on beef and pork nucleotides is given in Table 10. These workers reported that inosinic acid was the prominent nucleotide in all three species and it was degraded by heating. Adenylic acid increased during cooking in meat from all three species, possibly due to the hydrolysis of adenosine di- and triphosphate and nucleic acids. Cytidylic, uridylic, and guanylic acids were present in relatively low concentrations in meat from all three species and changed little during cooking.

It is well known that stiffening of muscle post-mortem is related to decrease in adenosine-5′-triphosphate (ATP) content.[98-103] The formation of adenosine-5′-monophosphate (AMP) results from the removal of two terminal phosphate groups of ATP by the combined catalytic action of ATPase and myokinase. AMP is rapidly deaminated to inosinic acid (IMP) by the catalytic action of AMP deaminase. Further degradation of IMP into inosine and hypoxanthine occurs during prolonged storage. The details of these metabolic reactions may be found in Figure 8.

Howard et al.[104] studied nucleotide breakdown in beef tissue and found that with progressive aging the hypoxanthine levels rose to 1.5 to

TABLE 9

Amino Acid Composition of the Water-soluble Fraction from Chloroform:Methanol Extracts of Adipose Tissue[60]

Compound	Concentration μmol/100 g tissue		
	Pork	Beef	Lamb
Cysteic acid	2.35	1.95	1.50
Taurine	19.40	9.20	34.70
Urea	44.60	39.15	–
Aspartic acid	0.85	3.60	3.25
Asparagine + threonine	10.00	3.85	8.85
Serine	7.00	3.90	8.40
Glutamic acid	4.50	4.90	8.25
Proline	7.05	4.45	10.35
Glycine	16.85	13.20	35.25
Alanine	39.55	25.75	45.40
Valine	5.80	3.90	8.65
Methionine	1.65	0.90	2.30
Isoleucine	5.45	6.55	11.40
Leucine	8.10	4.35	11.30
Tyrosine	4.20	1.65	4.45
Phenylalanine	2.95	2.50	5.80
Ammonia	3.65	2.90	1.15
Lysine	0.75	0.95	1.90
Anserine	0.80	0.50	1.55
Carnosine	5.90	0.85	0.75
Arganine	1.70	0.80	0.11

TABLE 10

Ratios of Sums of Individual Nucleotides and Total Nucleotides of Cooked Relative to those of Raw Beef and Pork[55]

Constituent	Ratio of chemical constituents in cooked and raw meat (raw meat = 1.0)			
	Beef		Pork	
	49°C	71°C	49°C	71°C
Cytidylic acid	0.81	0.99	0.67	0.58
Adenylic acid	1.46	3.38	0.80	1.81
Uridylic acid	0.59	0.75	0.83	0.84
Inosinic acid	0.75	0.61	0.90	0.78
Guanylic acid	0.55	1.41	0.75	0.67
Sum of individual nucleotides	0.77	0.74	0.87	0.83

TABLE 11

Post-mortem Changes in the Concentrations of Nucleotides and Related Compounds in Porcine Longissimus Muscle[59]

Breeds	Post-mortem time (hr)	Concentration μmol/g wet tissue					
		Inosine + hypoxanthine	DPN^+ + DPNH	5′-IMP	5′-IDP	5′-ADP	5′-ATP
Poland China (n=2)	0	–	0.62	2.11	–	1.09	4.13
	3	1.65	0.10	5.48	0.59	0.38	0.16
	24	2.12	Trace	5.17	0.54	0.63	Trace
	72	2.75	Trace	5.03	0.69	0.54	Trace
	144	2.73	Trace	4.47	0.46	0.36	Trace
Chester White (n=2)	0	–	0.66	0.78	–	0.90	6.04
	3	1.26	0.49	5.67	0.42	0.33	0.38
	24	1.72	0.30	5.44	0.69	0.31	0.21
	72	2.07	0.17	5.20	0.79	0.37	0.23
	144	2.90	0.15	4.59	0.57	0.42	0.11

2.0 μmol/g of meat. Lee and Webster[105] studied the effect of temperature, freezing, frozen storage, thawing, and pH on hypoxanthine production in beef tissue and reported that an increase in temperature and a higher ultimate pH or thawing of frozen samples stimulated the rate of hypoxanthine production.

Tsai et al.[59] investigated the post-mortem metabolism of nucleotides in porcine longissimus muscle. The post-mortem changes in the concentrations of nucleotides and related compounds post-mortem are reported in Table 11. Tsai and co-workers observed that AMP deaminase activity decreased considerably during storage, whereas adenosine deaminase activity remained nearly the same during storage. On the other hand, 5′-nucleosidase activity seemed to increase during the first few hours of storage and then remained constant for several days before declining. The direct consequence of decrease in AMP deaminase activity during storage resulted in the stabilization of IMP concentration within the first few hours of post-mortem. On the other hand, inosine + hypoxanthine concentration in muscle continued to increase for several days, as would be expected from nearly constant adenosine deaminase activity during storage (Table 11).

During the ripening of dry sausage, the concentration of water soluble nitrogenous compounds increases and can reach values up to 25% of the total nitrogen.[106-108] The nature and concentration of these compounds such as amino acids, peptides, nucleotides, and nucleosides determine, to a large extent, the final dry sausage flavor.[109]

Pohja and Niinivaara[110] and Sajber et al.[111] reported that free amino acid production in dry sausage is at least partly due to bacterial protease activity. Although native muscle proteases may continue to hydrolyze proteins in dry sausage, if not inactivated during processing, their contribution in the liberation of free amino acids would be comparatively smaller to bacterial proteases. Cantoni et al.[112] investigated the fate of inosinic acid during dry sausage ripening and reported that inosinic acid was converted to inosine nucleoside and hypoxanthine, respectively, by the action of phosphomonoesterase and nucleosidase. Dierick et al.[113] investigated changes in nonprotein nitrogen compounds during dry sausage ripening. Two batches of sausages were prepared: Batch 1 was a normal sausage preparation;[114] in batch 2 a starter culture, containing lactobacilli and micrococci, and 1% less sugar were added. The concentration of nonprotein nitrogen compounds (Table 12) and free amino acids (Table 13) in the two batches were monitored during ripening. These data show that during the first 3 days of ripening, the rate of free amino acid production is maximal and exceeds the rate of ammonia production and peptide production from proteins. During this period intensive carbohydrate metabolism and bacterial growth also takes place.[114] Subsequent to this, the rate of ammonia production increases but remains lower than amino acid production, whereas the concentration of peptides decreases. The concentration changes for individual amino acids during ripening (Table 13) show that the major amino acids responsible for the increase in

TABLE 12

Concentration of NPN Compounds at Various Stages of Dry Sausage Ripening (mg N/100 g dry matter)

	Batch 1 Stage of ripening (days)				Batch 2 Stage of ripening (days)			
Compound	0–3	3–15	15–36	0–36	0–3	3–15	15–36	0–36
Ammonia	6	28	18	52	2	34	12	48
Free amino acids	47	46	21	114	45	30	72	147
Peptide-nitrogen	34	-43	-7	-16	10	-67	-55	-112
Nucleotide-nitrogen	-1	-20	-1	-22	-16	-5	-2	-23
Nucleoside-nitrogen	8	37	5	50	11	33	14	58

From Dierick, N., Vandekerckhove, P., and Demeyer, D., *J. Food Sci.*, 39, 301, 1974. With permission.

TABLE 13

Concentration of Free Amino Acids at Three Stages of Ripening (mgα-NH_2-N/100 g dry matter)

	Batch 1 Stage of ripening (days)			Batch 2 Stage of ripening (days)		
Free amino acid	0	15	36	0	15	36
Aspartic acid	0.74	1.90	5.30	0	3.79	7.25
Threonine	0.82	3.30	25.20	0	4.95	6.94
Serine	1.73	5.50	9.10	0	7.20	9.85
Glutamic acid	19.00	7.20	5.60	25.40	24.30	18.30
Proline	0	3.40	5.50	0	5.72	6.45
Glycine	3.00	6.15	8.80	0	7.65	14.20
Alanine	10.20	20.90	25.20	3.92	23.10	22.30
Valine	1.44	6.35	8.85	0	7.41	11.95
Methionine	0.56	2.72	3.84	1.60	3.38	5.26
Isoleucine	1.60	3.74	5.45	2.24	4.50	8.10
Leucine	1.06	11.50	13.30	8.30	13.20	17.60
Phenylalanine	0.93	4.50	5.25	2.98	5.00	7.15
Lysine	2.07	4.67	6.35	2.30	3.94	6.46
Histidine	0.73	1.46	0.01	2.77	2.20	0
Tyrosine	0.77	0	0	0	0	0.30
γ-Amino butyric acid	0	2.72	4.07	1.22	7.89	12.50
Ornithine	1.16	0	0	1.98	0	0.83

From Dierick, N., Vandekerckhove, P., and Demeyer, D., *J. Food Sci.*, 39, 301, 1974. With permission.

total α-amino nitrogen were alanine, leucine, threonine, valine, serine, glycine, and proline. It was also reported that during sausage ripening added glutamate disappeared, and at least partly decarboxylated to γ-amino butyric acid. Langner,[115] based on the observation that glutamate is metabolized during dry sausage ripening, questioned its incorporation as a flavor additive in dry sausage. Other amino acids may be decarboxylated during dry sausage ripening, as indicated by the disappearance of histidine, tyrosine, and ornithine. The decarboxylation products of these amino acids are histamine, tyramine, and putrescine, respectively. Data reported by Langner[116] and Reuter and Langner[117] are in contrast with those of Dierick et al.[113] These workers reported an increase in these amino acids. In order to prove the validity of their observation that histidine, tyrosine, and ornithine are indeed decarboxylated during sausage ripening, Dierick et al.[113] determined the concentration of highly basic amines in the ripened samples. Although very small amounts were detected, the concentration of histamine, tyramine, and putrescine was increased at least tenfold, the rate of increase being maximal during the first three days of ripening. Cadaverine, a decarboxylated product of lysine, was also detected in significant quantities. The presence of starter culture in batch 2 produced no striking differences in the nature and concentration of nonprotein nitrogen compounds during ripening, except for a higher final concentration of free amino acids, coupled to a lower peptide-nitrogen and lower increase in threonine concentration (Tables 12 and 13). The lower concentration of amino acids in batch 2 may suggest a higher exopeptidase activity in batch 2.

MECHANISMS OF MEAT FLAVOR DEVELOPMENT

During the heat processing of meat, the types of reactions which appear to be responsible for the development of flavor compounds include:

1. Autoxidation, hydrolysis, dehydration, and decarboxylation of fats giving rise to aldehydes, fatty acids, lactones, and ketones.
2. The nonfatty components may undergo glycoside splitting, oxidation and decomposition to yield volatile and nonvolatile compounds.
3. The complex degradation products of sugars and sugar-amine interactions.

These chemical reactions are discussed in detail in this section. The reactions are divided into subheadings: amino acids, carbohydrates, and lipids, depending on which is the substrate of the reaction.

Amino Acids

The thermal decomposition of amino acids has not been studied at temperatures normally attained in cooking of meat, but several investigations have been made at temperatures of 200°C and above. Based on the energies of activation of these reactions, it is conceivable that some of these reactions would take place at lower (cooking) temperatures.

The thermal decomposition products of alanine, β-alanine, valine, leucine, and isoleucine, which were heated at or above 220°C, were identified by Lien and Nawar (Table 14).[118,119] They have also proposed mechanisms of their formation (Figure 9).

Olefins are produced by the decarboxylation and deamination of aliphatic amino acids; the corresponding paraffins result upon reaction with hydrogen. By this process isobutylene and isobutane are produced from valine, 3-methyl-1-butene and isopentane from leucine, and 2-methyl-1-butene from isoleucine. In addition, the formation of propane and propene from valine, isobutane and isobutylene from leucine, and butane and butene from isoleucine, may be explained by cleavage of carbon-carbon bonds beta to carbonyl group of the amino acid to form a free radical which can then accept or lose a hydrogen atom.

Primary amines are produced by decarboxylation, while the corresponding aldehydes result from both deamination and decarbonylation of the amino acid. The formation of isobutyraldehyde and isobutylamine from leucine cannot be explained by the deamination and decarbonylation reactions.[119] The imines formed from the heat degradation of amino acids probably originate from the reaction of carbonyl compounds with primary amines. Aliphatic ketones react with amines more slowly than aldehydes to form imines which necessitates the use of higher reaction temperatures and longer reaction times than required for the aldehyde.[120] This is probably the reason why Lien and Nawar[118,119] found only aldimines and not ketimines in their investigation on thermal decomposition products of aliphatic amino acids. Thus the reaction of isobutyralde-

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TABLE 14

Thermal Decomposition Products from Alanine, β-Alanine, Valine, Leucine, and Isoleucine[118,119]

Alanine	β-Alanine	Valine	Leucine	Isoleucine
Ammonia	Ammonia	Ammonia	Ammonia	Ammonia
Carbon dioxide	Carbon dioxide	Carbon dioxide	Carbon dioxide	Carbon dioxide
Carbon monoxide	Carbon monoxide	Carbon monoxide	Carbon monoxide	Carbon monoxide
Ethane	Ethene	Propane	Isobutane	Butane
Ethene	Propene	Propene	Isobutylene	Butene
Propene	Acetonitrile	Isobutane	Isopentane	Isopentane
2-Butene	Acetone	Isobutylene	3-Methyl-1-butene	2-Methyl-1-butene
Acetaldehyde	Pyridine	Acetone	Acetone	2-Butanone
Ethylamine	3-Picoline	Isobutyraldehyde	Isobutyraldehyde	2-Methylbutyraldehyde
N-Ethylidene-ethylamine	2,4-Lutidine	Isobutylamine	Isovaleraldehyde	2-Methylbutylamine
2-Methyl-5-ethylpyridine	3,5-Lutidine	*N*-Isobutylideneisobutylamine	Isobutylamine	*N*-(2-methylbutylidene)-2-methylbutylamine
N-Ethylpropionamide	2,3,5-Trimethylpyridine	Diisobutylamine	Isoamylamine	bis(2-Methylbutyl)amine
Propionamide	Poly-β-alanine		*N*-Isobutylidene-isoamylamine	
			N-Isoamylidene-isoamylamine	
			Diisoamylamine	

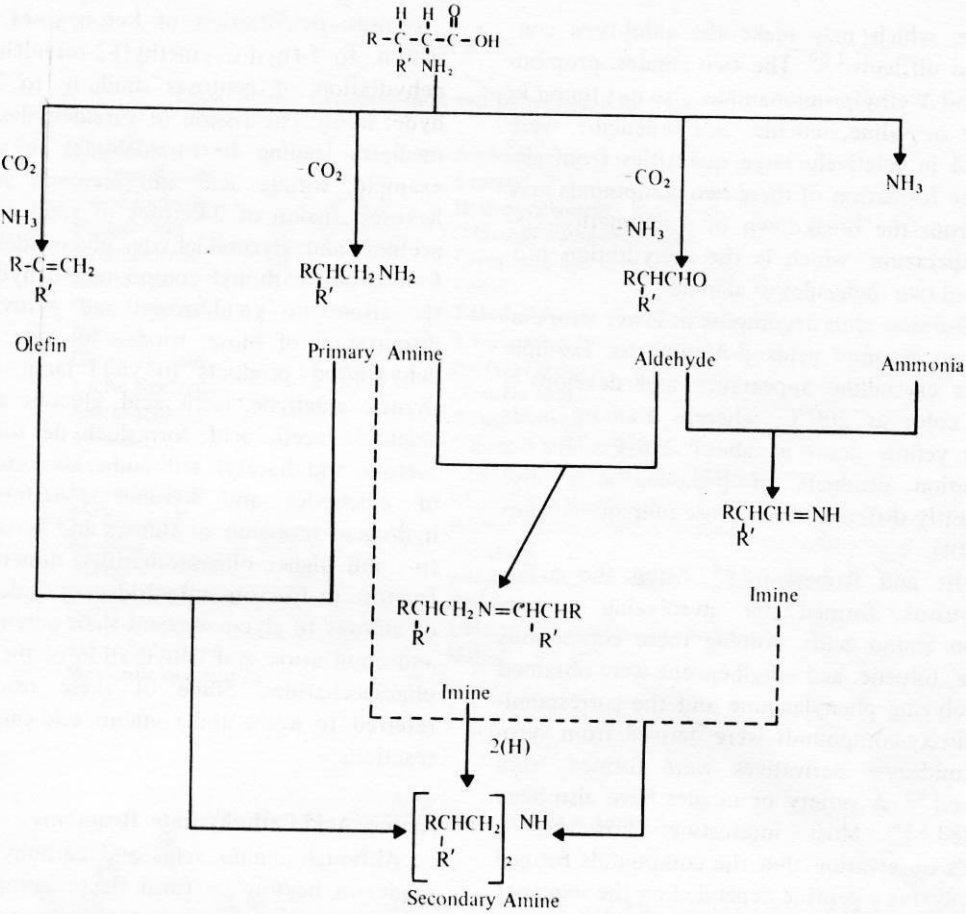


FIGURE 9. Suggested mechanisms for the production of heat degradation products from neutral alimatic amino acids (see Table 14). (From Lien, Y. C. and Nawar, W. W., *J. Food Sci.*, 39, 911, 1974. With permission.)

hyde and isobutylamine, both thermal decomposition products of valine, resulted in the formation of *N*-isobutylidene-isobutylamine. Similarly, *N*-isobutylidene-isoamylaminé and *N*-(2-methylbutylidene)2-methylbutylamine were produced from thermal decomposition of leucine and isoleucine, respectively. Aliphatic aldehydes can also react with ammonia to first form simple addition compounds called "aldehyde ammonias." These compounds are unstable and easily decompose to the original constituents, or lose water to form imines.[121] The secondary amines diisobutylamine, diisoamylamine, and bis(2-methylbutyl)-amine result from the addition of hydrogen to the above mentioned imines. An alternative pathway, however, may be the reaction of primary amines with imines to additional products which can be reduced to secondary amines.[122] A third possibility may result from the addition of primary amines to olefins.[123] A characteristic imine, *N*-ethylidene-ethylamine, was detected in the heat breakdown products of alanine. This imine probably resulted from the reaction of acetaldehyde and ethylamine;[119] however, the secondary amine, which was present in the degradation products of valine and leucine, was absent in alanine (Table 14).

The formation of 2-methyl-5-ethylpyridine in the case of alanine may arise from the aldol condensation of acetaldehyde to form crotonaldehyde, followed by dimerization in the presence of ammonia. The most probable reaction mechanism of pyridine synthesis is via Tschitschibabin reactions.[121] This mechanism involves the reaction of aldehyde with ammonia to form imine, followed by aldol-type condensation to produce pyridine derivatives. The absence of pyridine derivatives in pyrolyzed valine, leucine, or isoleucine is probably due to their branched chain

structure, which may make the aldol-type condensation difficult.[120] The two amides, propionamide and *N*-ethylpropionamide, also not found in the case of valine, leucine, and isoleucine, were produced in relatively large quantities from alanine. The formation of these two compounds may result from the breakdown of 3,6-dimethyl-2,5-diketopiperazine, which is the dehydration product from two molecules of alanine.

The β-amino acids decompose at lower temperatures than α-amino acids. β-Alanine for example loses its crystalline appearance and develops a yellow color at 200°C, whereas α-amino acids develop yellow color at about 250°C. The decomposition products of β-alanine were also significantly different from those of α-amino acids (Table 14).

Merritt and Robertson[124] listed the major compounds formed on pyrolyzing various common amino acids. Among these compounds benzene, toluene, and ethylbenzene were obtained on pyrolyzing phenylalanine and the corresponding hydroxy-compounds were derived from tyrosine. Imidazole derivatives were formed from histidine.[125] A variety of nitriles have also been reported.[126] Most interesting, however, is Merritt's observation that the compounds formed on pyrolyzing a peptide depended on the sequence of amino acids. Although only dipeptides were used in this study, this presumably holds true for longer peptides and may explain the formation of flavor compounds not obtained on heating model systems of single amino acids.[127]

Carbohydrates

The heat degradation products of carbohydrates, particularly sugars, are important flavoring ingredients, and have been studied extensively. On heating, sugars lose their bound water between 100 to 130°C, without alteration in molecular structure. With increasing temperature, 150 to 180°C, a molecule of water splits from sugars forming anhydrides, and further heating, 190 to 220°C, results in the removal of second water molecule with the formation of furfural from pentoses and hydroxymethylfurfural from hexoses.

Table 15 lists some compounds that have been reported as carbohydrate caramelization and pyrolysis products.[128] Following are likely reaction steps in sugar caramelization: enolization of aldoses with the production of more reactive 2-ketoses; dehydration of ketohexoses, without fission, to 5-(hydroxymethyl)-2-furaldehyde, and dehydration of pentoses similarly to 2-furaldehyde; hydrolytic fission of furaldehydes or intermediates leading to furaldehydes to yield, for example, formic acid and levulinic acid from hexoses, fission of 2-ketoses to yield dihydroxyacetone and glyceraldehyde, glycolaldehyde and four-carbon carbonyl compounds; dehydration of the trioses to yield acetol and pyruvaldehyde; dismutation of biose, trioses, tetroses, and their dehydration products to yield lactic aldehyde, pyruvic aldehyde, lactic acid, glycolic acid, acetaldehyde, acetic acid, formaldehyde, formic acid, acetoin, and diacetyl; self- and cross-condensations of aldehydes and ketones containing active hydrogen; reversion of aldoses and ketoses to di-, tri-, and higher oligosaccharides; dimerization of fructose to fructose anhydrides; cyclodehydration of aldoses to glycosans, and then polymerization; and enolization and dehydration of the synthetic oligosaccharides. Some of these reactions are referred to again under amino acid-carbohydrate reactions.

Amino Acid-Carbohydrate Reactions

Although amino acids and carbohydrates degrade on heating to form flavor compounds, in meat they exist in close proximity and interact with each other to form a variety of compounds.[127] Flavor production by nonenzymatic browning in foods proceeds mainly from reactions of reducing sugars with amines, amino acids, peptides, and proteins.

Aldoses react with primary or secondary amines to give aldosylamines. Under the influence of a suitable catalyst, aldosylamines rearrange to form ketoseamines. This reaction is known as the Amadori rearrangement. Ketoseamines can also be formed directly from aldoses and amines. Ketoseamines are distinguished from aldosylamines by their relatively strong reducing properties and by the formation of sugar degradation products, instead of parent hexoses, on acid hydrolysis. The chemical reactions leading to the formation of flavor compounds, i.e., furaldehydes, acetaldehyde, pyruvic aldehyde, diacetyl, acetic acid, and others, are outlined in Figure 10. These compounds are also produced as caramelization products of sugars. In the absence of amines it may be assumed that 1,2-enolization of sugars leads to the same transformations shown in Figure 10.[128] The

TABLE 15

Carbohydrate Caramelization and Dehydration Products[128]

Carbon monoxide	Acetoin
Carbon dioxide	Diacetyl
Formaldehyde	Hydroxydiacetyl
Formic acid	C-Methyl triose-reductone
Acetaldehyde	Levulinic acid
Acetic acid	Furan
Glycolaldehyde	2-Methyl furan
Glyoxal	2,5-dimethyl furan
Glyoxylic acid	Furfuryl alcohol
Lactic aldehyde	2-Furoic acid
Lactic acid	2-Furaldehyde
Acrolein	5-Methyl-2-furaldehyde
Acrylic acid	5-(Hydroxymethyl)-2-furaldehyde
Pyruvaldehyde	2-Furyl methyl ketone
Pyruvic acid	2-Furyl hydroxymethyl ketone
Acetone	Isomaltol
Acetol	4-Hydroxy-2,5-dimethyl-3-(2H)-furanone
Dihydroxyacetone	Reductic acid
Glyceraldehyde	1-Methylcyclopentenol(2)-one(3)
Triose-reductone	Maltol
Mesoxalic dialdehyde	

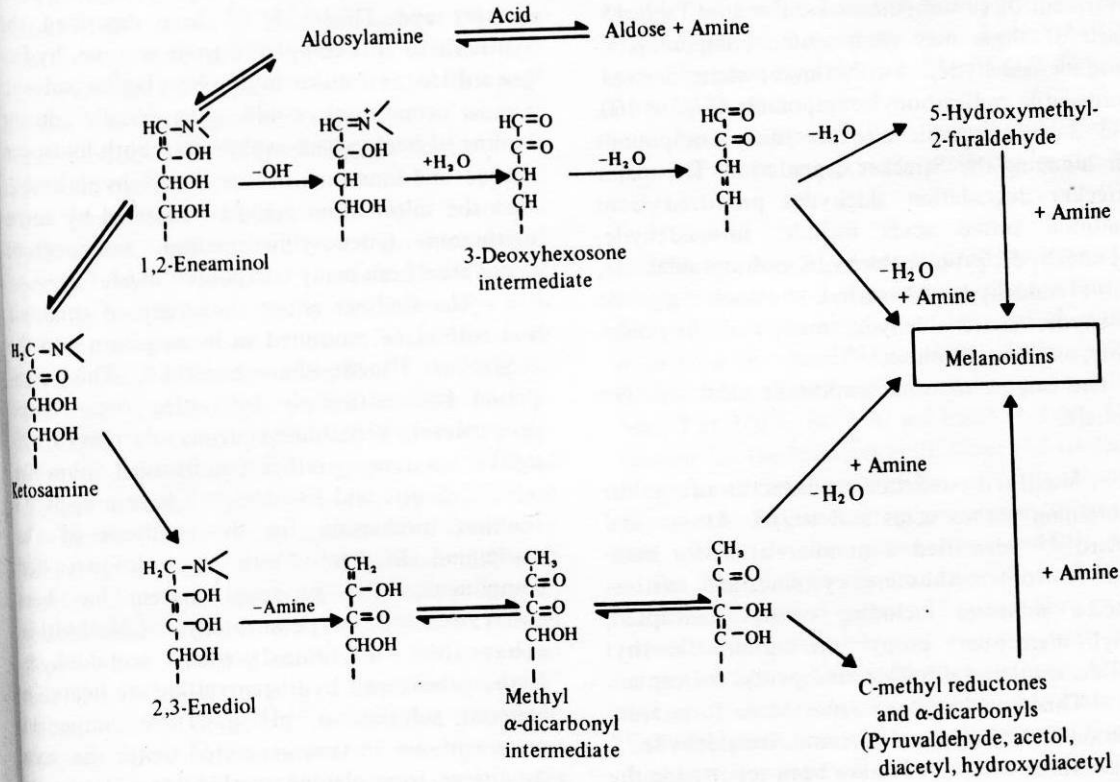


FIGURE 10. Mechanism of the Maillard reactions. (From Hodge, J. E., in *The Chemistry and Physiology of Flavors*, Schultz, H. W., Day, E. A., and Libbey, L. M., Eds., Avi Publishing, Westport, Conn., 1967, 465. With permission.)

hydroxyl group on C-3 and C-1 would be eliminated (at higher temperatures or under more acidic or alkaline reaction conditions) in the same way shown for glycosylamines and ketoseamines. For example, maltol is formed from maltose by heat alone,[131] and also in the presence of amino compounds;[129,132] but the condensation with amino compounds allows the enolizations and eliminations to take place near neutral pH and at much lower temperature.

The Maillard browning reactions have been reviewed by Reynolds[129,130] and Hodge.[129] Both aldosylamine and ketoseamine reaction schemes provide active compounds for the degradation of α-amino acids to aldehydes and ketones of one less carbon atom by the Strecker degradation mechanism. In this reaction it is required that the amino group must be α- to the carboxyl group and that the carbonyl compound must contain a –C:O–C:O– or a –C:O–(CH:CH)$_n$–C:O grouping. The reaction is illustrated by the following equation:

$$R.CO.CO.R' + R''\,CH(NH_2).COOH \longrightarrow R''.CHO + CO_2 + R.CH(NH_2).CO.R$$

If the hydrogen on the α-carbon of the amino acid is substituted, a ketone is produced. For example, acetone is formed from α-aminoisobutyric acid. 2-Furaldehyde produced in Maillard browning reactions participates in the Strecker degradation reaction because it yields a α-diketone and a conjugated dicarbonyl compound by hydrolytic ring opening. Several α-dicarbonyl compounds that are produced by simple heat treatment of carbohydrates are listed in Table 15. Each of these may react amino compounds to produce aldehydes. Furthermore, sugar derived, nonvolatile α-dicarbonyl compounds (Figure 10), and dehydroascorbic acid are likely compounds for inducing the Strecker degradation. The major Strecker degradation aldehydes produced from common amino acids include: formaldehyde, acetaldehyde, propionaldehyde, isobutyraldehyde, isovaleraldehyde, 2-methyl butanal, glycolic aldehyde, lactic aldehyde, methional, benzaldehyde, and several others.[128]

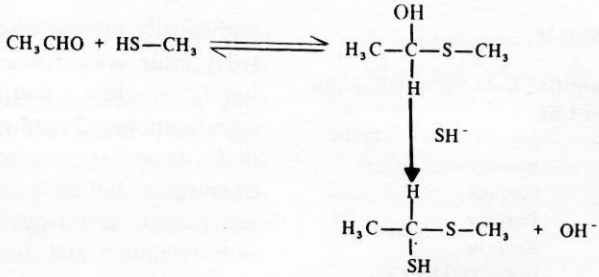
The origins of other important meat volatiles include:

Maillard reaction products of sulfur containing amino acids and sugars. Arroyo and Lillard[133] identified a number of sulfur compounds from methionine, cysteine, and cystine-glucose mixtures including methyl mercaptan, ethyl mercaptan, propyl mercaptan, dimethyl sulfide, dimethyl disulfide, and pentyl mercaptan.

Thialdine has been reported to form from ammonia, hydrogen sulfide, and acetaldehyde.[29] These three compounds have been reported in the headspace of beef broth. Brinkman et al.[29] believe that in view of the ease of thialdine formation from the above mentioned compounds, it is almost certainly present in the broth or the headspace and can be considered as a contributor to beef broth flavor.

Another interesting sulfur compound first identified by Herz[3] in cooked beef is 2,5-dimethyl-1,3,4-trithiolane. This compound has a sulfide odor in concentrated form, but when diluted gives a meaty aroma. It may well be an important contributor to the aroma of boiled beef. Asinger and Thiele[134,135] have described the synthesis of this compound from acetone, hydrogen sulfide, and sulfur in diisobutylamine solvent. Similar conditions could exist locally during boiling of beef, where evolution of both hydrogen sulfide and ammonia occurs. Herz[3] hypothesizes that the sulfur atom could be supplied by active methionine (adenosylmethionine), and acetone could arise from many compounds in beef.

The findings about the origin of thialdine and trithiolane prompted an investigation into the origin of 1-methylthio-ethanethiol. This compound has particularly interesting organoleptic properties; in very dilute aqueous solutions (1 to 5 μg/l) it has a meaty rather than its usual onion-like odor.[29] Scutte and Koenders[136] have proposed a reaction mechanism for the synthesis of this compound in beef broth from known beef components. The proposed system has been substantiated experimentally. 1-Methylthio-ethanethiol is formed when acetaldehyde, methanethiol, and hydrogen sulfide are heated in aqueous solution at pH 6. These immediate precursors are in turn generated under the same conditions from alanine, methionine and cysteine in the presence of Strecker degradation agent such as pyruvaldehyde. The proposed mechanism may be represented as follows:



1-Methylthio-ethanethiol

Another important class of compounds identified in cooked meat aroma are pyrazines. Pyrazines are known to arise from the condensation of sugars and amino acids. The necessary precursors, that is, amino acids and sugars, are available in uncooked meat and pyrazine formation should occur readily upon heating. Koehler and Odell[138] have recently investigated the factors affecting the formation of pyrazine compounds in sugar-amine interaction.

Lipids

The role of lipids in the development of the characteristic aroma of meat is a subject of some controversy. The ability of fat to dissolve and retain aroma that originates outside the fat is well known and demonstrated in the case of smoked meat products. Fat trimmed from uncooked meat has little characteristic flavor. It has been reported that rendering of fat trimmed from uncooked meat at low temperature does not produce species specific flavor. However, if rendered at normal cooking temperatures species flavor develops with little or no basic meat character.[98] In the work carried out at Kohnstamm and Co.,[98] rendered fat was steam distilled and distillate solvent extract. Gas-liquid chromatographic studies of meat fat volatiles showed that the characteristic flavor of cooked meat fat depended largely on unsaturated aldehydes. There was considerable qualitative similarity between meat fat volatiles of different species but the quantitative picture showed considerable differences. Fatty acids, methyl ketones and saturated aldehydes were also found and are believed to play a supporting role.[98] Wasserman and Spinelli[138] investigated the role of adipose tissue constituents in the development of species specific flavor. Adipose tissue, which yields the characteristic aroma of the species on heating, was extracted with chloroform-methanol (2:1), and the extract was washed with water. The original extract retained the species specific aroma but in the water-washed residue the characteristic aroma was either missing or greatly reduced. The water soluble material extracted from adipose tissue contained amino acids and at least glucose in the carbohydrate fraction. A typical lean meat aroma developed on heating this fraction.

Lipids may contribute to meat flavor in several ways:

Lipids may act as solvent, trapping aroma components produced elsewhere. Thus, the aroma of water-washed pork lipid extract still had a "piggy" note when heat was first applied.[138] This may be due to 5,α-androst-16-en-3-one, the boar odor identified by Patterson,[85] which is dissolved in the lipids of the animal.

Lipid components or degradation products may react with compounds produced in the lean meat.

Lien and Nawar[139] investigated the interaction of amino acids and triglycerides, using valine and tricaproin as a simple model system, under nonoxidative conditions. The decomposition products identified from valine-tricaproin mixtures heated at 270°C for 1 hr are listed in Table 16. A scheme for the interaction of valine and tricaproin as proposed by Lien and Nawar[139] is shown in Figure 11. Propane, propene, carbon monoxide, and carbon dioxide, which these workers previously identified in the heated triglyceride[141] as well as in heated valine,[118] were also present when both the amino acid and the triglyceride were heated together. Methane, ethane, ethene, butane, butene, pentane, pentene, hexanoic acid, and dicaproin, which were found to form in heated tricaproin, were also present in the heated mixture. Also, ammonia, acetone, isobutane, isobutylene, and isobutyraldehyde which were produced by heating the amino acid, valine, were

TABLE 16

Decomposition Products Identified from Valine-tricaproin Mixture Heated at 270°C for 1 hr

Carbon monoxide	Isobutylene
Carbon dioxide	Pentane
Ammonia	Pentene
Methane	Acetone
Ethane	Isobutyraldehyde
Ethene	Hexanoic acid
Propane	Caproic nitrile
Propene	Caproic amide
Butane	*N*-Isobutylcaproic amide
Butene	Dicaproin
Isobutane	

From Lien, Y. C. and Nawar, W. W., *J. Food Sci.*, 39, 917, 1974. With permission.

individually present when both the amino acid and triglyceride were heated together. On the other hand, acrolein, methyl hexanoate, hexanal, 6-undecanone, 2-oxoheptyl hexanoate, propanediol dicaproate, propenediol dicaproate and oxopropanediol dicaproate (all observed in heated tricaproin), and isobutylamine, *N*-isobutylidene-isobutylamine, and diisobutylamine (all observed in heated valine), could not be detected when valine and tricaproin were heated together. In addition, some compounds not previously observed when the individual substrates were heated have been identified as interaction products formed during heating of the substrate mixture. These are caproic nitrile, caproic amide, and *N*-isobutylcaproic amide. It is evident from the

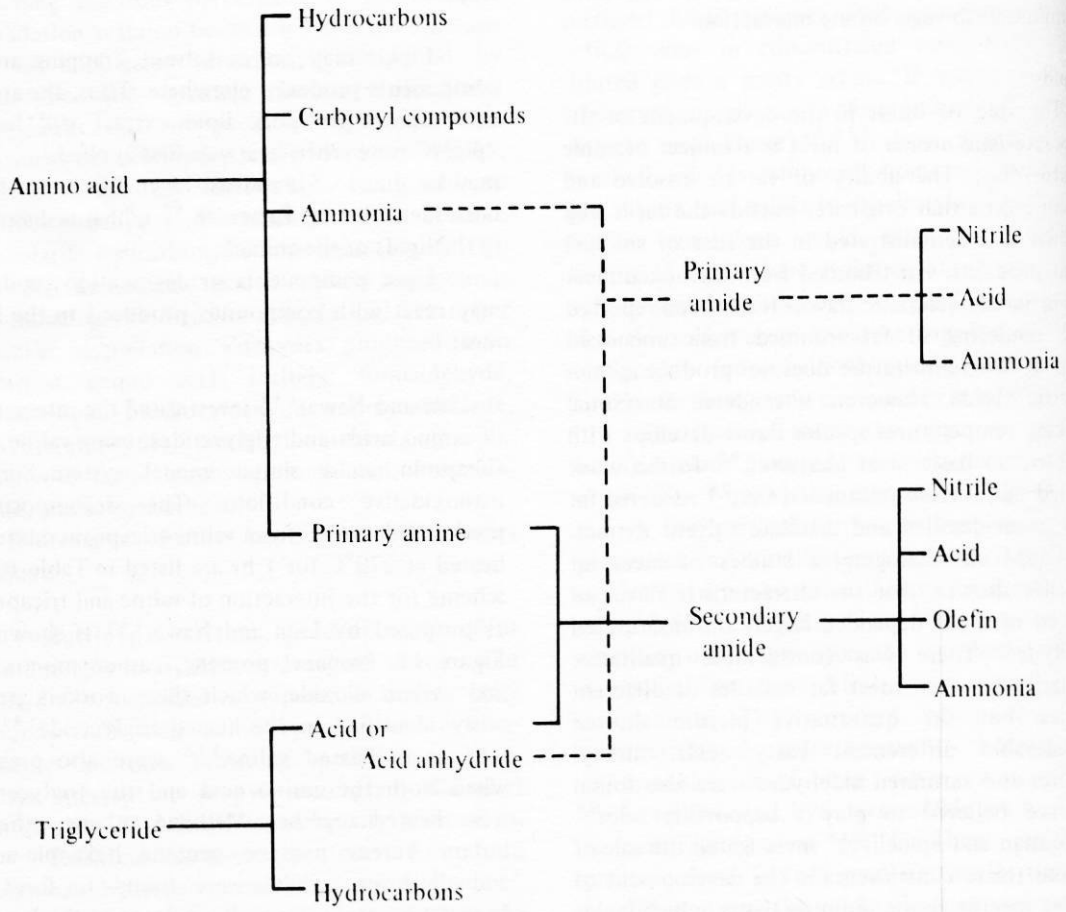


FIGURE 11. Scheme for interaction of amino acid and triglyceride upon heat treatment. (From Lien, Y. C. and Nawar, W. W., *J. Food Sci.*, 39, 917, 1974. With permission.)

work of Lien and Nawar[118,119,139] that in a simple system containing only one amino acid and one triglyceride, substantial interaction does take place during heating. It is also quite evident that studies on the heat decomposition products of individual components using model systems can aid significantly in unraveling thermal interaction mechanisms in more complex mixtures.

A large number of thermal oxidation products of lipids are formed, in keeping with the size of the molecules, degree of unsaturation, and type of substitution. Thermal oxidation of lipids can occur at 60°C in the presence of a few free radicals, but most of the degradation occurs at 200 to 300°C. Lactones, alcohols, ketones, and lower fatty acids are associated with the thermal oxidation of lipids.

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