METABOLIC STUDIES ON HISTIDINE, HISTAMINE, AND RELATED IMIDAZOLES

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I. INTRODUCTION

The physiological and pharmacological significance of histidine and histamine has interested many investigators since the biological occurrence and activity of these imidazole compounds were first discovered over 40 years ago. A substantial part of these investigations has been devoted to metabolic studies, in an attempt to elucidate the pathway of synthesis as well as the pathways of degradation and conjugation. More extensive investigations of these metabolic aspects have been carried on in various laboratories during the past several years, using many of the technics developed in various other fields of intermediary metabolism.

This review presents our current knowledge of the metabolism of histidine, histamine, and other closely related imidazoles, summarizing the data obtained in animal, plant, and microbiological experiments. Figure 1 represents a schematic formulation of some of these metabolic steps; those pathways are included which seem most likely on the basis of the available data, even though our knowledge of some of the steps is very fragmentary at present. Due to the limitations of space this review is limited to certain metabolic aspects of the pharmacology and physiology of the imidazoles. Reviews on other aspects of the subject have already been presented (92, 93, 94, 127, 142, 143, 155, 159, 188, 273, 395).

II. HISTIDINE

A. BIOLOGICAL ORIGIN OF HISTIDINE

Essentially all of the histidine in animals is derived from the histidine present in the dietary proteins. The original source of this dietary histidine is the histidine produced by plants and microorganisms, most of which are capable of synthesizing histidine at rates adequate for their needs. Studies on the various steps in the biosynthetic pathway for histidine have been most conveniently carried out in microbiological experiments, and have mainly depended on the use of isotopic and mutant technics.

1. *Histidine Biosynthesis in Microorganisms*

Formate Incorporation Studies. The early steps in the biosynthesis of L-histidine are essentially unknown. The only information available concerns the incorporation of labeled formate into the C-2 position of the imidazole ring. In experiments with *Saccharomyces cerevisiae* (216a) and *Torulopsis utilis* (333) in which the culture medium contained $HC^{14}OOH$, a high concentration of isotope was

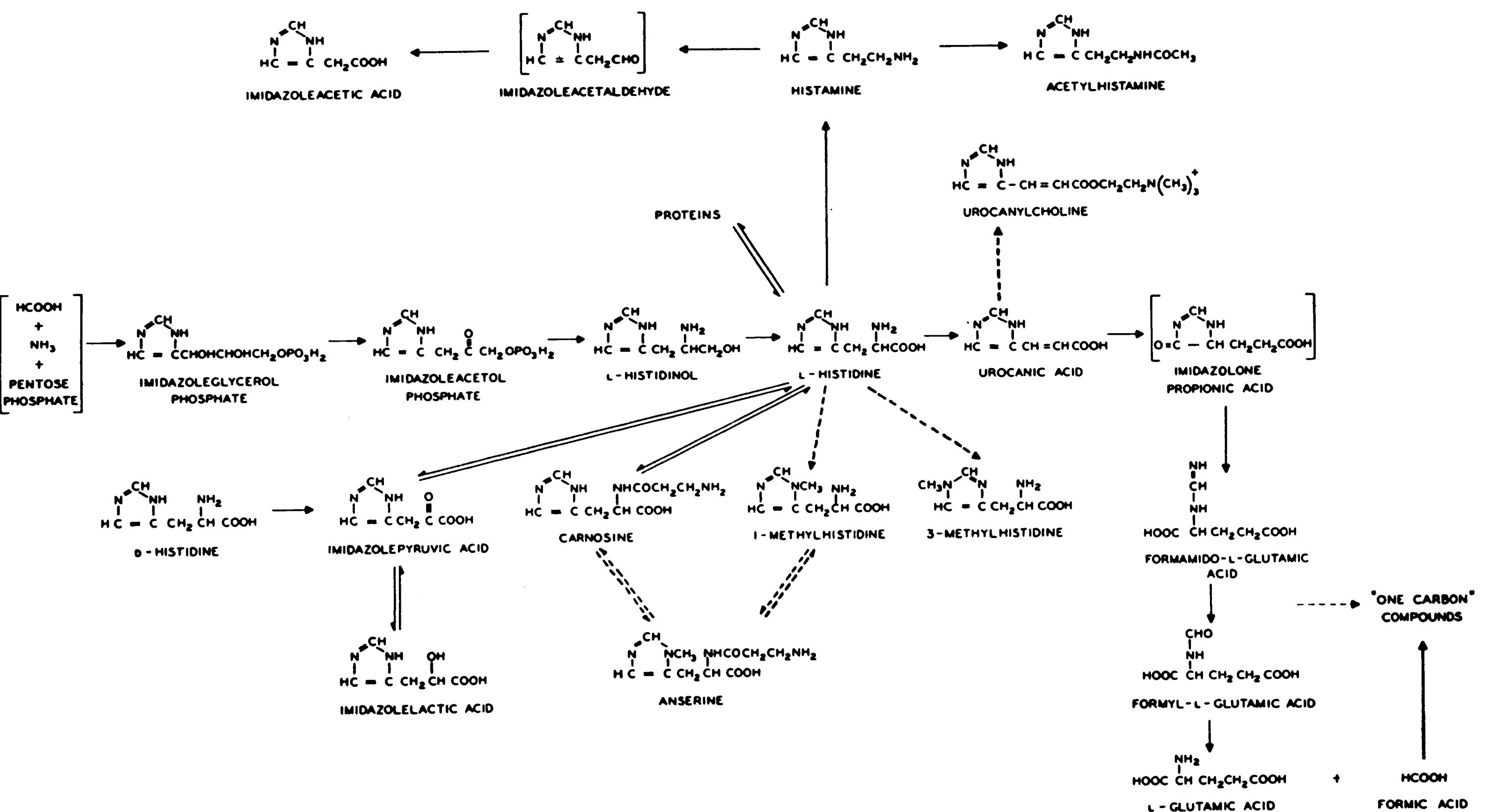


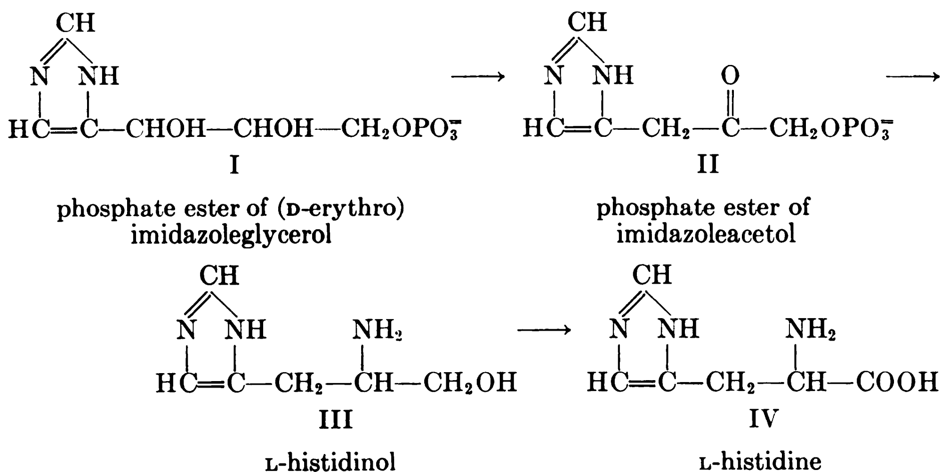
FIG. 1. *Metabolic Interrelations of Histidine and Related Imidazoles.*

found in the C-2 position of the imidazole ring. Little or no C^{14} was incorporated into the rest of the histidine molecule, indicating that formate or a closely related one-carbon fragment is specifically utilized for the C-2 position. This finding is similar to the observation that $HC^{14}OOH$ is incorporated into the C-2 and C-8 positions of purines (310).

Mutant Studies on Histidine Biosynthesis. Except for these $HC^{14}OOH$ incorporation experiments, no data are available on the mechanism of the formate fixation, or on the other steps leading to the synthesis of the imidazole moiety of histidine. Information on the later steps in L-histidine biosynthesis has recently been acquired from studies involving histidine-requiring mutants of *Escherichia coli* and of *Neurospora crassa*.

Vogel, Davis, and Mingioli (358) demonstrated that cultures of one of their histidine-requiring *E. coli* mutants accumulated large amounts of L-histidinol, indicating that L-histidinol is a precursor of L-histidine, and that the biosynthetic pathway is blocked between histidinol and histidine. A second histidine-requiring mutant grew adequately when either L-histidinol or L-histidine was added to the medium; no histidinol accumulated in these cultures. In this mutant, therefore, the synthetic pathway is blocked before the synthesis of histidinol.

Similarly Ames, Mitchell, and Mitchell (21, 22) were able to show that L-histidinol is accumulated by a histidine-requiring *Neurospora* mutant (158). Two other genetically-different histidine-requiring *Neurospora* mutants were also studied, and shown to accumulate the phosphate esters of imidazoleacetol and of (D-erythro) imidazoleglycerol respectively. On the basis of these studies, Ames, Mitchell, and Mitchell postulated that the biosynthetic pathway is:



The demonstration that phosphorylated intermediates are probably involved in histidine synthesis represents a hitherto unreported reaction mechanism in amino acid synthesis and strongly suggests the possibility that the imidazole ring arises by a condensation of a phosphorylated pentose with a one-carbon moiety and a nitrogen source. Three possibilities which are consistent with the optical

configurations involved are D-ribulose phosphate, D-ribose phosphate, and D-arabinose phosphate (21, 23). The difference between this biosynthetic pathway for histidine and those known for other amino acids offers a possible explanation for the apparently anomalous results obtained by Ehrensvärd et al. (79, 117), in studies on the incorporation of C^{14}-labeled acetate into histidine in experiments with *T. utilis* and *E. coli*. In most amino acids the carboxyl group was derived from the acetate carboxyl directly or via CO_2; the carboxyl of histidine, on the other hand, was derived from the methyl group of acetate.

It is of interest to point out that these schemes for the biosynthesis of the imidazole ring are very similar to the commonly used chemical syntheses of imidazoles, which involve the condensation of a sugar, ammonia, and formaldehyde in the presence of a metal catalyst (21, 22, 86, 87, 349a). This type of imidazole biosynthesis was often discussed in the older literature (155).

The enzymatic steps in the conversion of I → II → III → L-histidine are still unknown. No data are available on the mechanism of the dephosphorylation, nor is it known at which step the dephosphorylation occurs; consequently there is still some question as to whether the structure of III should be written as L-histidinol or as L-histidinol phosphate. The conversion of the keto compound to histidinol possibly involves a reaction of the transamination type.

The mechanism of the conversion of L-histidinol to L-histidine has recently been studied by Adams (13b) in extracts of *E. coli*. In these extracts L-histidinol is converted to L-histidine in a system requiring diphosphopyridine nucleotide. The enzyme is not found in the *E. coli* mutant in which the biosynthetic pathway is blocked between histidinol and histidine, although it is present in other *E. coli* mutants as well as in wild-type *E. coli*. Similar activity is found in autolysates of yeast and in extracts of an histidinol-adapted *Corynebacterium*.

Miscellaneous Pathways Producing Histidine. Although the above mechanism appears to represent the only *de novo* pathway for L-histidine biosynthesis, there are also a number of other ways in which histidine is produced from closely related compounds. Histidine, for example, is released during proteolysis. Imidazolepyruvic acid is converted to L-histidine by transaminases found both in animal and microbiological sources; the imidazolepyruvic acid may be formed from imidazolelactic acid by oxidation or from exogenous D-histidine by oxidative deamination, as well as from L-histidine by transamination or oxidative deamination. (The oxidative deamination and transamination reactions of amino acids have been reviewed recently by Krebs (207) and Gunsalus (156)). Since all of the naturally occurring compounds in this group were originally derived from L-histidine, these pathways do not represent any net synthesis.

The presence of an imidazole ring in both histidine and purines has resulted in considerable speculation about a possible purine → histidine conversion. Evidence for such a relationship was reported by Broquist and Snell (60). In their studies on the purine and histidine requirements of *Lactobacillus arabinosus*, *Streptococcus faecalis*, and *Lactobacillus casei*, growth experiments indicated that purines could serve as precursors of L-histidine, although no evidence was found for the reverse conversion of L-histidine to purines. The experiments of Levy and

Coon (216a) with *S. cerevisiae*, on the other hand, exclude a conversion of purines to L-histidine in this organism. When this yeast was grown in a medium supplemented with glycine-1-C^{14}, the purines became highly radioactive (310), but only traces of the isotope were found in the histidine isolated from a hydrolysate of the yeast protein. It has been suggested (216a) that the contrary results of Broquist and Snell may be attributed to an indirect utilization of the C-2 and C-8 carbons of the purines for the C-2 position of the imidazole ring of histidine via a one-carbon fragment, rather than a direct purine → histidine conversion.

2. Histidine Requirements in Animals

Growing Animals. Growing animals are not able to carry out the net synthesis of histidine, and require this amino acid in their diet. This was shown by the early rat nutrition experiments of Ackroyd and Hopkins (9), Rose and Cox (74, 75, 275, 276) and Harrow and Sherwin (169), and has been confirmed by more recent investigators. Weanling rats do not grow unless the diet contains an adequate amount of L-histidine or the closely related imidazolelactic acid, imidazolepyruvic acid, and D-histidine. Other imidazole compounds including L-histidinol and urocanic acid (imidazoleacrylic acid) are inactive (13a, 36, 68, 71, 75, 76, 297). Arginine does not substitute for histidine (276); the contrary results of Ackroyd and Hopkins (9) were apparently associated with the relatively impure diets available at that time.

Similar studies in mice have been carried out over many years by Berg and his associates (36, 67). As contrasted with the rat the L-histidine requirement of the mouse can only be satisfied by L-histidine or imidazolepyruvic acid; both D-histidine and imidazolelactic acid are inactive. However, Celander and Berg (67) have recently reported the interesting finding that these two compounds are active if the diet is supplemented with very small amounts of L-histidine. The significance of this observation is not clear at present, and further data are required before it can be adequately understood.

The inability of the rat to synthesize histidine has been confirmed in feeding experiments with N^{15}-ammonia, N^{15}-tyrosine, and N^{15}-glycine (31, 294). Incorporation of these labeled compounds in the diet results in the rapid labeling of the α-amino group of histidine and other amino acids, but no labeling of the imidazole ring. Since the N^{15} isotope is found in both purines and in arginine, these experiments also confirm the inability of these compounds to be converted into histidine. Similarly (253), administration of $HC^{14}OOH$ to rats resulted in good labeling of the purines but no labeling of the imidazole ring of histidine. Recently, however, $HC^{14}OOH$ and C^{14}-glucose have been found to be incorporated into the histidine of human liver slices (216b) and minced mouse brain (256) respectively. The experiments, however, were based only on the results of chromatographic separations, and further purification of the histidine obtained is required before these data are accepted as indicating the synthesis of histidine by mammalian tissues.

Adult Animals. Although the requirements of growing animals for an adequate histidine intake is well-accepted, in adult animals the situation is less clear. Wolf

and Corley (393) with rats and Rose and Rice (277) with dogs demonstrated that an adequate histidine intake is necessary for the maintenance of a positive nitrogen balance in the adult. Burroughs *et al.* (63), on the other hand, reported that nitrogen balance can be maintained in the adult rat in the absence of histidine. More recently the requirements of the adult rat have been reinvestigated by Benditt *et al.* (34, 35), Frazier *et al.* (138) and Wissler *et al.* (391) who reported that an adequate histidine intake is necessary for the maintenance of weight, well-being, and nitrogen balance. These findings cannot be attributed to inanition since similar results were obtained when the diets were force-fed. It is significant in evaluating other data on this subject, that the histidine-deficient animals in these experiments exhibited a negative nitrogen balance only after the deficient diet was fed for over a week. Although recently Bothwell and Williams (57) with younger rats reported that nitrogen balance can be maintained on a histidine-free diet with force-feeding, the rats lost weight and showed a high mortality. Thus, it seems likely at present that histidine is an essential amino acid for the adult rat and dog, even though the data are not unequivocal at this time.

Adult Man. In the adult human, on the other hand, both Rose *et al.* (278) and Albanese *et al.* (16) reported that nitrogen balance can be maintained on a histidine-deficient diet. Although weight loss was noted in the experiments of Albanese *et al.*, Rose *et al.* reported no weight loss in their subjects, and attributed to this the higher caloric content of their diets. These data appear to indicate that the histidine requirement in the adult human is different from that of the adult rat and dog. However, final conclusions cannot be drawn at this time since such factors as synthesis of L-histidine by intestinal bacteria, the possible role of hemoglobin as a storehouse of histidine, and the duration of the experimental period must be evaluated.

B. L-HISTIDINE IN PROTEINS

Although some free L-histidine can be found in the blood and various other tissues, the L-histidine of proteins constitutes the major part of the naturally occurring histidine. L-Histidine is widely distributed in plants, animals, and microorganisms, where it accounts for from <1 per cent to approximately 8 per cent of the various proteins (47a). In animal proteins the histidine content averages approximately 2–4 per cent, although hemoglobin contains about 8 per cent histidine. In electrometric titrations of proteins essentially all of the groups which are titratable in the neutral range are due to the imidazole moiety of histidine (115).

Certain characteristics of some proteins have been attributed to the histidine moiety. For example, on the basis of titration data and magnetic moment determinations, the ability of hemoglobin to undergo reversible oxygenation and deoxygenation without valence change, as well as the associated Bohr effect, is attributed to coordination complexes of histidine (in the protein), heme iron, and oxygen (394). A non-protein model for some of these reactions is given by the cobalt-histidine chelation complexes of Hearon, Burk, and Schade (174).

Histidine complexes have also been implicated in other reactions. Wagner-

Jauregg *et al.* (360b), for example, found that various mixtures of heavy metals and histidine or other chelating agents accelerated the hydrolysis of diisopropylfluorophosphate. These authors speculated that the reaction mechanism involves the formation of an active intermediate containing the central metal atom in coordination with diisopropylfluorophosphate and the chelating agent, and suggested that it might be considered as a model for the action of the enzyme fluorophosphatase.

Mention should also be made of the possible role of the weakly basic imidazole groups of the protein in the binding of substrates in various enzymatic reactions. This has been discussed recently by Wilson and Bergmann (388) in their report on the enzyme-substrate complexes of acetylcholinesterase and the effects of pH.

Incorporation of N^{15}- and C^{14}-Histidine into Proteins. Estimates of the rate of incorporation of dietary histidine (labeled in the imidazole ring with N^{15}) into rat tissue proteins have been reported by Tesar and Rittenberg (344, 345). In 3 days 29 per cent of the total histidine of the liver, 8 per cent of the carcass histidine, and 3 per cent of the erythrocyte histidine are replaced by the isotopically-labeled histidine. These results are essentially the same as those reported for L-leucine incorporation, and agree with the liver protein half-life of 7 days and the red blood cell half-life of 127 days previously reported by Shemin and Rittenberg (303). These results are consistent with the short term experiments of Wolf (392) with α-C^{14}-histidine; within four hours after the intraperitoneal injection of the isotopic histidine, 2.7 per cent of the histidine of the rat visceral proteins was labeled.

A somewhat different type of incorporation experiment was carried out by Borsook *et al.* (54–56) who measured the *total* C^{14} incorporation from L-histidine into mice and guinea pigs *in vivo*. These experiments showed that 30 minutes after an intravenous injection of labeled L-histidine about 40 per cent of the administered isotope could be found in the visceral protein. Since some of the histidine is metabolized during the experiment, and the isotope is secondarily incorporated into serine and other compounds (see page 313 below), these data represent the incorporation into the proteins of other amino acids as well as histidine. Similar experiments with carboxyl-labeled histidine have been reported by Novak (247) in mice. Recently Borsook *et al.* (55) have also demonstrated the incorporation of carboxyl-labeled histidine into the histidine of rabbit reticulocytes *in vitro*. In these *in vitro* experiments no significant incorporation via other amino acids occurred, since histidine isolated from the reticulocyte protein accounted for all of the incorporated radioactivity.

C. DEGRADATION OF L-HISTIDINE

The major pathways of L-histidine utilization are incorporation into proteins (discussed above), excretion into the urine (see page 314), and degradation. Of these the degradative pathway is quantitatively most significant. This can be clearly seen, for example, in the experiments of Borsook *et al.* and of Novak (54, 56, 247) quoted above; within 1 hour after the intravenous injection of iso-

topic L-histidine into mice, about one-third of the radioactivity is found in the expired CO_2. D'Iorio and Bouthillier likewise found a rapid excretion of $C^{14}O_2$ after histidine-$C^{14}OOH$ administration in the rat (90).

Deamination and Urocanic Acid Formation. Numerous investigators (215) have studied the enzymatic degradation of L-histidine since the early descriptions of this activity in liver preparations by Edlbacher (97), György and Röthler (157), Kauffmann and Mislowitzer (200), and Abderhalden and Buadze (2). Most of these investigators postulated that the first step in histidine degradation involves the rupture of the imidazole ring, and this formed the basis for the commonly-accepted schemes of histidine metabolism (Figure 2A, B). More recent experiments, however, have demonstrated that the first step in the degradation of histidine does not involve the imidazole ring, but proceeds via a non-oxidative deamination of the α-amino group; the product of this reaction is urocanic acid (imidazoleacrylic acid). (Figure 2C–F).

Urocanic acid was first described in 1874 by Jaffe (189, 189a) who isolated this compound from the urine of a dog; the structure of urocanic acid was shown to be the same as imidazoleacrylic acid by Hunter (186a) in 1912. Although the isolation of urocanic acid was confirmed by Siegfried (304) in the dog and by Swain (325) in the coyote, these were only occasional findings which could not be repeated consistently.

The relationship between urocanic acid and histidine was demonstrated by the bacteriological experiments of Raistrick (257, 258) in 1917 in which bacteria of the enteric group converted histidine into urocanic acid. In 1922 Kotake and Konishi (205) showed that urocanic acid could be isolated from dog urine after the oral or subcutaneous administration of histidine, and postulated the *in vivo* conversion of histidine to urocanic acid. Similar results were reported for rabbits by Kiyokawa (201). On the other hand, Edlbacher *et al.* (109, 110, 114) could not isolate urocanic acid after histidine administration to dogs, rabbits, and guinea pigs. Although Darby and Lewis (86, 87) failed to isolate urocanic acid from the urine of rabbits after the subcutaneous administration of histidine, it could be isolated in 5 out of 8 rabbits when the histidine was administered orally. These rabbits however, exhibited severe toxic manifestations, and the authors postulated that the formation of urocanic acid might represent an abnormal pathway; urocanic acid, itself, whether administered orally or subcutaneously, was not toxic.

The enzymatic conversion of L-histidine to urocanic acid with liver preparations was described in 1939 by Sera and Yada (204, 298, 300), and has been confirmed in a number of laboratories (151a, 163, 209, 225, 230, 231, 249, 251, 252, 355). The failure to demonstrate urocanic acid in the older experiments was presumably largely due to the presence of contaminating urocanic-splitting enzymes in the liver preparations. With aged or partially purified preparations a quantitative conversion of L-histidine to urocanic acid can be attained (231, 251, 338). In animal tissues the *in vitro* degradation of histidine through urocanic acid has been reported for the livers of rats, guinea pigs, rabbits, cats, and dogs (52, 163, 231, 249, 300, 338a, 341). Although insufficient data are available regarding the mech-

anism of the histidine degradation reported with the livers of man, cattle, mouse, chicken, pigeon, and frog (104, 114, 215), it seems likely that a similar pathway is involved.

Very active bacterial preparations have been obtained by Tabor and Hayaishi (332) by utilizing the technics of bacterial adaptation (172, 314, 321). If *Pseudomonas fluorescens* cells are grown on media containing histidine as the major carbon and nitrogen source, adaptive enzymes are developed for the degradation of histidine, which are not found to any extent in cells grown on other media. The adapted cells oxidatively degrade histidine to CO_2. Cell-free extracts, which have been subjected to high speed centrifugation, however, do not carry out the oxidative steps, and quantitatively convert L-histidine to ammonia, L-glutamic acid, and formic acid (322, 324, 332). These extracts have approximately 100 times more L-histidine degrading activity (per mg. of protein) than liver extracts (231, 338).

The enzyme catalyzing the histidine → urocanic acid reaction has been referred to as "histidine deaminase", "histidine-α-desaminase", "histidine-α-deaminase", "desamino-histidase", and "histidase" by various investigators. In this laboratory the term "histidase" has been retained because this is the term first introduced by Edlbacher to describe the enzyme degrading L-histidine, even though the present formulation of the reaction is different.

Histidase assays have usually been carried out by histidine or ammonia determinations (107, 151b, 215, 243, 300). Recent studies have been facilitated by utilizing the high ultraviolet absorption of urocanic acid (124, 163, 231). The molar extinction coefficient is 18,800 at a wavelength of 277 mμ at pH 7.4–10 (230, 231). This permits the direct observation of the L-histidine → urocanic acid conversion in the spectrophotometer. This conversion can be carried out quantitatively, and appears to be essentially irreversible (231, 338), in contrast to the aspartic ⇌ fumaric conversion by aspartase (123). This irreversibility is presumably due to the stability conferred on urocanic acid by its resonating structure. This may explain the inability of urocanic acid to substitute for L-histidine in the rat nutritional experiments discussed above.

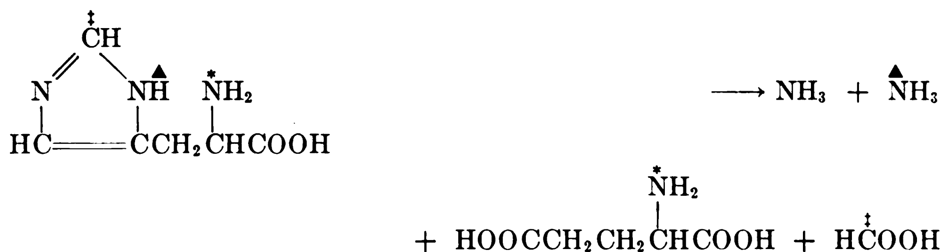
Histidase has been partially purified from liver and bacterial preparations (114, 225, 232, 243, 298, 300, 323, 340, 341). The *Pseudomonas fluorescens* preparation has been purified 100 fold (340). The enzyme is specific for L-histidine, and does not degrade D-histidine, α-N-methyl histidine, L-imidazolelactic acid, histamine and a number of other imidazoles (98, 106, 114, 340). The pH optimum for the enzyme is about 9. Aged or partially purified preparations show a requirement for glutathione or thioglycolate (209, 225, 231). Claims have been made for such cofactor requirements as Hg^{++}, Cd^{++}, Zn^{++} (323) and folic acid (225). The enzyme is inhibited by cysteine, glycine, BAL, and ethylenediaminetetraacetate (52, 231). Although these results indicate that histidine may react with the enzyme through a chelation mechanism, the cofactor and inhibitor data are still too fragmentary to permit anything more than speculation concerning the mechanism of the deamination process. The activity of histidase has been reported by Kapeller-Adler (190) to be decreased in the livers of pregnant women but these

findings could not be confirmed by Edlbacher and Heitz (112). Goryukhina (151b) has recently reported an increase in histidase activity in the livers of rabbits inoculated with a Brown-Pearce carcinoma, but not in animals with a methylcholanthrene tumor.

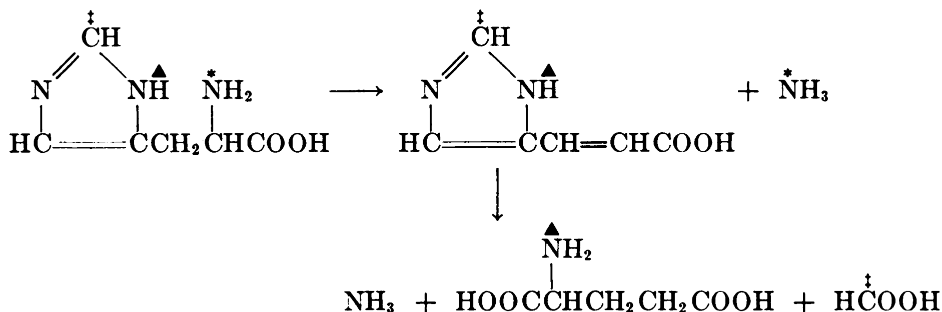
Although the enzymatic data demonstrated that histidine could be quantitatively converted to urocanic acid in purified enzyme preparations, there was still some question whether this pathway or a pathway involving a primary loss of ammonia from the imidazole ring represented the major path in crude unfractionated preparations and in the intact animal. This problem has been resolved in favor of the urocanic acid pathway by *in vitro* and *in vivo* experiments with histidine containing N^{15} and C^{14}.

The *in vitro* isotope experiments were first carried out with *Pseudomonas* extracts by Tabor, Mehler, Hayaishi, and White (333). As mentioned above, the *Pseudomonas* extracts quantitatively convert L-histidine to L-glutamic acid, formic acid, and ammonia. Since the L-glutamic acid can be readily isolated and crystallized, this preparation affords a convenient way to distinguish between rupture of the imidazole ring (Formulation 1) and α-amino deamination (Formulation 2), by using N^{15}-labeled histidine as the substrate:

Formulation 1



Formulation 2



The L-glutamic acid formed from histidine had the isotope distribution expected according to formulation 2, and thus indicates that essentially all of the L-histidine degraded by *Pseudomonas* extracts proceeds along this pathway. An isotope trapping experiment with C^{14}-labeled histidine and excess unlabeled urocanic acid confirmed this conclusion (333).

A comparable isotope trapping experiment with unfractionated liver homogenate (guinea pig) gave essentially the same results (231), and demonstrated that in liver also the urocanic pathway is the major (>85 per cent) pathway of L-histidine degradation. Similar support for formulation 2 is afforded by the isotope distribution data obtained after incubation of C^{14}-histidine with guinea pig liver slices (4) or rat liver homogenates (137). Confirmation of formulation 2 has also been obtained *in vivo* by similar isotope experiments. Following the administration of isotopically-labeled L-histidine to rats, L-glutamic acid was isolated from hydrolysates of protein (392), urine (334), and a liver non-protein filtrate (4), and was found to have the expected isotope distribution.

Although these data demonstrate that urocanic acid represents a major pathway of histidine degradation in both liver and *Pseudomonas* preparations, an apparent inconsistency is the observation of Edlbacher (112), Darby and Lewis (86, 87), and Celander and Berg (68), that there is a large excretion of urocanic acid in the urine in animals treated with urocanic acid, as contrasted with the small excretion of either histidine or urocanic acid after comparable doses of L-histidine. Similarly Celander and Berg (68) found that, in contrast to histidine, urocanic acid was not glycogenic in rats. These results, however, cannot be adequately evaluated at this time, since such considerations as cell permeability and renal clearance may account for some of the findings. The importance of renal factors in interpretation of balance data for amino acids has recently been demonstrated by Crampton and Smyth (77) (see page 314).

Urocanylcholine. Of considerable interest in discussing the physiological role of urocanic acid is the recent work of Erspamer and Benati (124c, d), who identified murexine as imidazoleacrylcholine (urocanylcholine). This compound, found normally in the hypobranchial glands of the snail, *Murex trunculus*, has strong nicotine-like and curare-like actions, with almost no muscarine-like activity. It is not hydrolyzed by cholinesterase (124a, 124b). The mechanism of synthesis is not known, but may involve a coenzyme A-activated acylating system similar to those described for choline acetylation and other acylation reactions (203, 217).

Urocanic Acid Degradation. The enzyme degrading urocanic acid has been found in various liver and bacterial preparations (110–112, 231, 249, 252, 298, 299, 300, 340–341, 350), and is usually referred to as "urocanase" or "urocanicase." The activity is followed easily by measuring the disappearance of the urocanic acid spectrum at 277 mμ directly in the spectrophotometer (see page 308). The pH optimum is near 7. The enzyme has been partially purified (<50 fold) from both liver and bacterial sources (300, 340, 341, 350).

When urocanic acid is degraded by liver homogenates or purified *Pseudomonas* extracts a product is obtained which contains bound forms of ammonia, L-glutamic acid and formic acid, which can be released on alkaline hydrolysis (112, 249, 299, 300, 337, 339). A similar product is obtained from the incubation mixture of crude liver preparations and histidine (3, 50, 51, 52, 99, 100). This reaction product has been isolated in crystalline form in a number of laboratories (50–52, 249, 299, 300, 334, 339). Although all of these compounds are described

as containing bound ammonia, and formic and glutamic acids, there has been considerable disagreement on the identity of the various compounds isolated in different laboratories and on their structural configuration (Figure 2). Although Sera *et al.* (299–300) and Oyamada (249) postulated N-formyl-DL-isoglutamine, for the structure of the compounds isolated in their laboratories, synthetic and enzymatic studies by Borek and Waelsch (52) and by Tabor and Mehler (339) have demonstrated that this structure is not applicable to the optically-active compounds isolated by them. Borek and Waelsch (52) have recently postulated formamido-L-glutamic acid as the structure on the basis of titration data, showing two acidic groups (pK′ 2.4 and 4.7) and a basic group (pK′ 11.1). Similar titration data have also been obtained by Tabor and Mehler (339). Carbamylglutamic acid and hydantoinpropionic acid (Figure 2-scheme F) are excluded as possible structures, since these do not contain a bound formic acid moiety.

Neither formamido-L-glutamic acid nor its postulated precursor, imidazalone-propionic acid, has been prepared synthetically. Both structures, however, are consistent with the experiments summarized in the next section involving the isolation of formyl-L-glutamic acid during the degradation of the above isolated material with *Pseudomonas* enzymes and the isolation of formamide during the degradation of histidine by *Clostridium cylindrosporum* and *Aerobacter aerogenes*. On the other hand, formamido-L-glutamic acid cannot be an immediate precursor of isoglutamine, which has been reported as a degradation product with *B. subtilis* and with liver preparations. Thus, a definite assignment of structure is not possible at this time, and will probably await the chemical synthesis of the various postulated compounds.

L-Glutamic Acid and Formic Acid Formation from L-Histidine and Urocanic Acid. Several different metabolic pathways have been described for the further degradation of the above intermediate product:

In *Pseudomonas* extracts L-histidine and urocanic acid, as well as the intermediate compound discussed in the previous section, are rapidly hydrolyzed to ammonia and L-glutamic and formic acids (332, 333, 334, 339). The formation of formyl-L-glutamic acid can be demonstrated as the penultimate step in these extracts (324, 339). The conversion of L-histidine to L-glutamic acid, formic acid, and ammonia is carried out by *Pseudomonas* extracts without any net oxidation.

Current work by Wachsman and Barker (360a) and by Magasanik (220) indicates that another pathway is present in *Clostridium cylindrosporum* and in *Aerobacter aerogenes*. In these organisms formamide is one of the products of histidine degradation. In *Bacillus subtilis* cultures, on the other hand, isoglutamine has been isolated as an intermediate by Nishizawa *et al.* (244, 245) in their studies on the conversion of histidine to glutamic acid.

With liver preparations the nature of the steps involved in the later stages of histidine and urocanic acid breakdown is not certain. With most preparations the reaction appears to stop with the formation of the intermediate compound discussed above. Although the formation of DL-isoglutamine, as well as L-glutamic acid, has been reported with liver preparations (4, 137, 300, 341, 350), unequivocal evaluation of these findings is difficult at present because of the marked la-

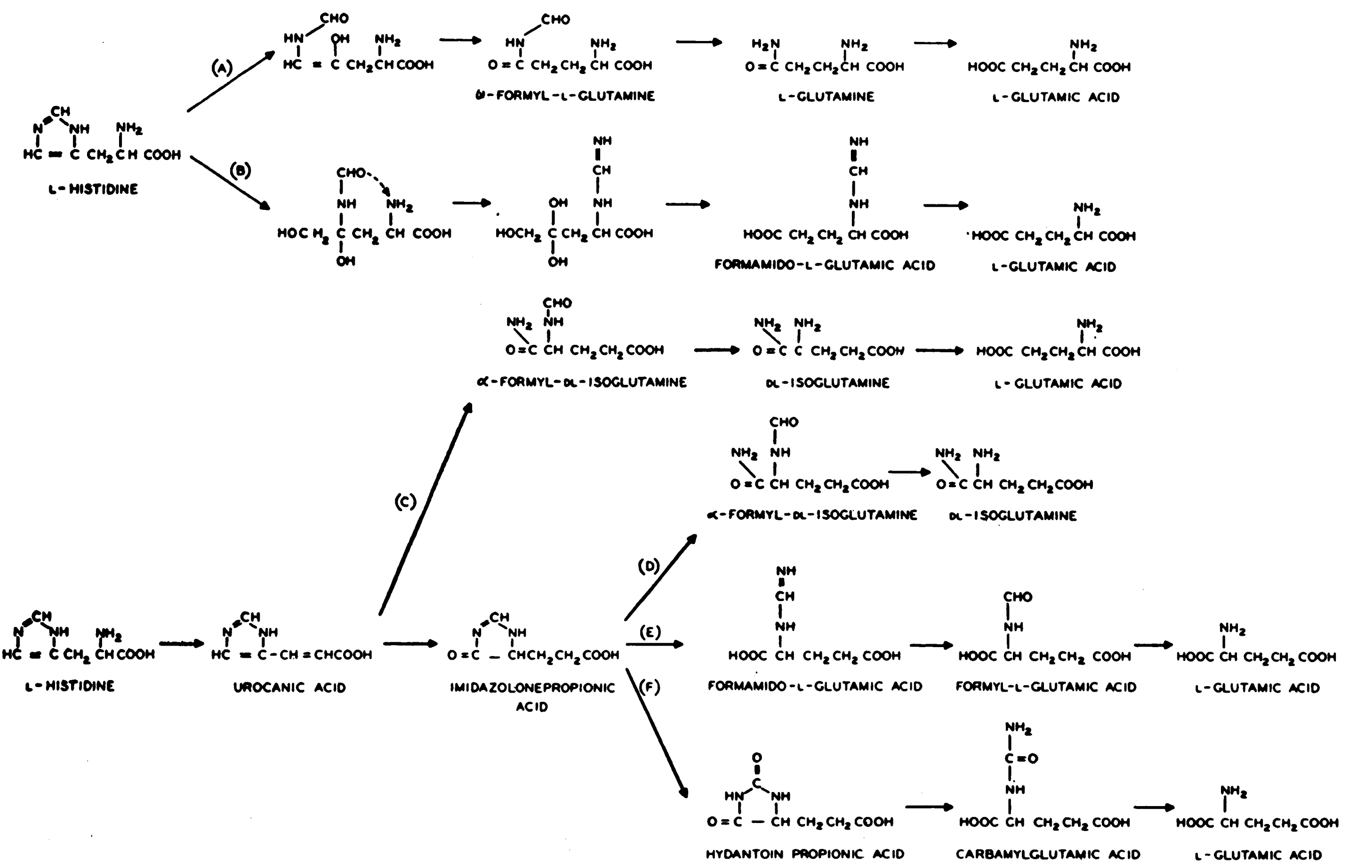


FIG. 2. *Pathways postulated for histidine degradation by various authors.* (A) proposed by Edlbacher (114), (B) proposed by Walker and Schmidt (361), (C) proposed by Sera (300), (D) proposed by Oyamada (249) and Akamatsu (15), (E) proposed by Suda *et al.* (322–324) and Tabor and Mehler (337, 339); steps up to formamido-L glutamic acid also included in formulation of Borek and Waelsch (50, 52), (F) proposed by Ichihara *et al.* (quoted by Suda *et al.* (324)). The intermediate role of hydantoinpropionic acid has also been suggested by Matsuda *et al.* (225). This figure represents a summary of the various postulated formulations; only the principal compounds for each pathway have been included.

bility of the probable precursors. It is probable that the later stages of histidine degradation are different in the various bacterial extracts and in liver; possibly in the latter glutamic acid is released by a more indirect transfer reaction.

In vivo conversion of L-histidine to L-glutamic acid is supported by the observations of Wolf (392) that, following the subcutaneous administration of C^{14}-histidine to rats, the isotope was found to be incorporated into the L-glutamic acid which was isolated from hydrolysates of the body protein. The formation of L-glutamic acid (and hence of α-ketoglutaric acid) from L-histidine furnishes an example of the interrelationship between amino acid degradation and the pathways of carbohydrate metabolism.

Relation of Histidine Metabolism to One-Carbon Metabolism. Recent studies in a variety of fields have demonstrated the metabolic importance of "one-carbon compounds" and their relationship to the folic-acid group of vitamins (reviewed in references 364, 365). Experiments from several laboratories have demonstrated the close relationship between L-histidine metabolism and "one-carbon" metabolism. $HC^{14}OOH$ is incorporated into the C-2 position of the imidazole ring of histidine in yeast (see page 300), and the C-2 of histidine is converted to formic acid by *Pseudomonas* extracts (see page 309). A number of different investigators (259, 260, 311, 313, 347, 348) have also shown by C^{14}-experiments in pigeons and rats *in vivo* that the C-2 carbon is incorporated into various "formate-fixers", such as serine, purines, uric acid, and choline.

The close relationship between histidine metabolism and folic acid has also been demonstrated by the recent studies of Tabor, Silverman, Mehler, Daft and Bauer (334). Folic acid deficient rats excrete in their urine a labile compound containing bound forms of ammonia, formic acid, and L-glutamic acid (29, 305). This compound has been isolated in crystalline form (306), and appears to be identical with the compound obtained by the degradation of L-histidine with liver homogenates (see page 310). The excretion of this derivative is increased by increasing the histidine content of the diet (334). Following the administration of L-histidine, labeled with N^{15} in the γ-nitrogen, a major part of the administered N^{15} is found in this urinary derivative, demonstrating that the latter is a product of L-histidine degradation. The mechanism by which the C-2 position of L-histidine is transferred to "one-carbon" acceptors is not known, but it is very tempting to speculate that *L. citrovorum* factor could act as a formate-transfer agent from the histidine degradation product to the acceptor compound. A similar function as a formate-transfer agent in other systems has already been postulated to explain the mechanism of action of folic acid and *L. citrovorum* factor (see review by Welch and Nichol (364)).

The synthesis and degradation of the purines and of certain amino acids, including methionine, histidine, and tryptophane, are known to be closely related to "one-carbon" metabolism (364). In addition to the above experiments the relationship of histidine and folic acid is indicated by microbiological growth experiments of Cutts and Rainbow (80), using a strain of yeast which requires p-aminobenzoic acid. Relatively large quantities of adenine, together with certain

amino acids, of which methionine and histidine are of prime importance, can substitute for the p-aminobenzoic acid growth requirement.

Similarly Broquist (61) found the inhibition of *Torula cremoris* by aminopterin to be reversed by either *L. citrovorum* factor (competitively) or by a mixture of methionine, purine bases, and histidine (non-competitively). In animal experiments Daft (81a) has found that tryptophan, methionine, and histidine are of particular importance in the prevention and treatment of blood dyscrasias and other signs of folic acid deficiency produced by amino acid mixtures (81).

D. URINARY EXCRETION OF HISTIDINE

Although the amount of histidine found in the urine is usually larger than that of most of the other amino acids, the quantities excreted are small relative to the histidine content of the diet (17, 33a, 49, 141, 168, 170, 211, 317). The urinary excretion rises after the oral or parenteral administration of L-histidine, but the increased excretion only represents a small fraction of the amount administered (3, 109, 153, 184, 315). With D-histidine, on the other hand, the percentage excreted varies with the species. In rats (184), for example, only a small percentage is recovered, while essentially all of the D-histidine is excreted in the urine of guinea pigs (109, 184), rabbits (1, 3, 201), dogs (3) and humans (17, 33a). Although these differences in the excretion of L- and D-histidine have usually been considered as indicating differences in the rate of their metabolic utilization, Crampton and Smyth (77) have recently demonstrated that this is the result of the different behavior of the renal tubules to the enantiomorphs of the amino-acids. In their experiments only the L-isomers are actively reabsorbed by the renal tubules. These experiments are similar to those of Wiseman (390), who showed that only the L-amino acids are transported against a gradient in the small intestine.

In pregnancy in humans the urinary L-histidine excretion rises. This was first described by Voge (357), and has been extensively studied by Kapeller-Adler and numerous other investigators (27, 113, 190, 193, 211, 254, 255, 366). Increased histidine excretion, however, cannot be demonstrated in gravid guinea pigs (109). The mechanism of this histidinuria is not clear. Kapeller-Adler considered that this might be a result of a hormonal inhibition of liver histidase (190), but experiments by Edlbacher and Heitz (112), using human autopsy material, did not confirm this. According to Page (250) simultaneous measurements of histidine blood levels and urinary excretion after the administration of histidine to pregnant and non-pregnant women do not indicate any defect in histidase activity, but suggest the possibility of changes in the rates of renal excretion.

It seems likely that the histidinuria of pregnancy is closely related to the increased amino acid excretion noted in certain other hormonal studies. In pregnancy, not only histidine but several other amino acids (362) show an increased urinary excretion. Similar increases in amino acid excretion have been reported following ACTH and cortisone therapy in rheumatoid arthritis and in asthma (49, 153, 274). On the other hand massive doses of progesterone did not affect the urinary histidine excretion (396); the histidine excretion of normal women is

not affected by the menstrual cycle (69). A renal explanation for the increased excretion of histidine in human urine after ACTH or cortisone administration has been presented by Grob (153); his studies indicate that the increased histidine excretion is due chiefly to a decrease in tubular reabsorption.

Although the findings of histidinuria in normal pregnancy have been confirmed in a number of laboratories, these findings have recently been questioned by Gabrawy. In a preliminary communication (140) he recently reported that there is no difference in the histidine excretion of pregnant and non-pregnant cases if the dietary intake was adequately controlled. Gabrawy also could not confirm the decreased histidine excretion reported by Kapeller-Adler as occurring in certain cases of pre-eclampsia. Further evaluation of these findings must await the publication of the complete report.

E. MISCELLANEOUS REACTIONS AND DERIVATIVES

Other Pathways of Histidine Metabolism. Although the various steps discussed above represent the major metabolic pathway for histidine, there are also several other pathways associated with histidine metabolism. One of these, L-histidine decarboxylase, will be discussed below under "histamine synthesis." The oxidation of D-histidine by D-amino acid oxidase (207) has been referred to already, as well as the transamination reactions of L-histidine (64, 156, 284); L-histidine is oxidatively deaminated by L-amino acid oxidase (41–43, 320, 346). Imidazolelactic (177) and imidazolepropionic acids (5, 201) have been described as metabolic products of L-histidine in bacterial cultures, but the reaction mechanisms have not been worked out. Imidazolelactic acid probably arises from imidazolepyruvic acid, while imidazolepropionic acid could arise by reduction of urocanic acid. The preliminary report of F. Ehrlich (118b) that histidine is converted to histidol (imidazolylethanol) by yeast extracts was not confirmed in his later experiments (118c). An oxidative pathway for histidine degradation in bacteria has been mentioned in a preliminary report by Edlbacher and Litvan (108); the data, however, are too fragmentary to permit adequate evaluation without further studies.

Relation to Creatine Metabolism. A relationship between histidine and creatine metabolism has been postulated by several investigators. Abderhalden and Buadze (2) showed an increase in the creatinine excretion of dogs, for example, after the administration of histidine. More recently Steensholt (316a-c) has reported the conversion of L-histidine to creatine by normal muscle *in vitro*. On the other hand, the N^{15} and C^{14} experiments quoted above (260, 344, 345) offer no support for the direct conversion of histidine to creatine.

Effect of Ascorbic Acid, Irradiation, etc. A number of papers have been published by Edlbacher, Holtz, and others (101, 181) on the *in vitro* degradation of histidine by ascorbic acid nonenzymatically. The reaction requires oxygen, and is catalyzed by traces of iron. Ammonia, CO_2, and a nitrogen-containing product are formed; the mechanism of the reaction is presumed to involve a peroxide intermediate. Similar studies have also been carried out on the degradation of histidine by sulfhydryl compounds, hydrogen peroxide, ozone, and ultraviolet irradiation

(pages 430–432, ref. 155). In some of these reactions a small amount of histamine has also been demonstrated as a byproduct. Although attempts have been made to apply these chemical findings *in vivo* (389), there is very little evidence that these reactions apply in biological systems. Related to these findings is the report of Galston (147) that histidine can be destroyed by irradiation with visible light in the presence of riboflavin. These observations have been confirmed by Tabor (unpublished data) and have been extended to include the degradation of histamine under similar conditions. Although Galston postulated that the riboflavin served as a hydrogen transport agent, it seems more likely that this oxidation is merely another example of photosensitization by fluorescent dyes (48, 363). The decolorization noted by Galston in anaerobic mixtures of riboflavin and substrate upon irradiation can probably be attributed to the formation of deuteroleuco-riboflavin (208), and can be demonstrated also in the absence of substrate.

Carnosine. Carnosine is a peptide of histidine and β-alanine (β-alanyl-L-histidine). Carnosine is found in muscle in concentrations of 100–1000 micrograms per gram of wet tissue, representing the second highest non-protein nitrogenous material. The earlier literature has been thoroughly reviewed by duVigneaud and Behrens (96a). Data involving carnosine in certain phosphorylation and decarboxylation reactions have been reported by Severin and Meshkova and others (301, 302), but these findings have not been adequately evaluated at this time. The biological synthesis of carnosine from L-histidine and β-alanine has not been thoroughly proven, although the recent experiments of Williams and Krehl (387) are strongly suggestive. These investigators showed that liver preparations could synthesize an acid-hydrolyzable histidine derivative in the presence of added L-histidine and β-alanine. Less direct evidence was presented by Goryukhina (151b), who showed that administration of histidine reversed the fall in carnosine and anserine observed in rabbits inoculated with a Brown-Pearce carcinoma. The hydrolysis of carnosine to L-histidine and β-alanine by an enzyme from hog kidney has been extensively studied by Hanson and Smith (167).

Anserine. Anserine, which is a methyl substituted carnosine (Figure 1), occurs in muscle (96a). The proportion of anserine: carnosine varies in muscles from different species. Little is known about the synthesis of anserine; i.e., whether the imidazole ring is methylated before or after the peptide is formed. Isotope experiments of Schenck *et al.* (292) have demonstrated that the methyl group of methionine is incorporated into anserine, but the incorporation is slower than into choline or creatine. The mechanism of the methylation probably involves a transmethylation reaction similar to that described by Cantoni (65).

Related to this discussion is the occurrence of methylhistidine in human and cat urine (88, 296, 317, 342a). The methyl substitution is on the nitrogen of the imidazole ring, and both possible isomers have been described. These have been called 1-methylhistidine and 3-methylhistidine, although this terminology is somewhat confusing. 1-Methylhistidine refers to α-amino-β(1-methyl-5-imidazole)-propionic acid, and has the same structure as the methylhistidine moiety of anserine (Figure 1). Essentially nothing is known about the function or metabolic behaviour of these compounds.

Ergothioneine. Another imidazole compound of considerable physiological in-

terest is ergothioneine (thiohistidine betaine), even though there is no evidence that this is derived from histidine. Ergothioneine was first isolated from ergot in 1909 (342b) and from red blood cells in 1925 (33c, 96b, 186b). Until recently the distribution of ergothioneine in animal tissues was considered to be restricted to the erythrocytes, but recent studies by Melville, Homer, and Lubschez (234b) with rat tissues have demonstrated that ergothioneine is widely distributed. The highest concentration was found in the liver (13.3 mg./100 g. fresh tissue); rat erythrocytes contained 10.4 mg. per 100 g. cells. A high concentration of ergothioneine has recently been reported for boar seminal fluid (221).

A close relationship between ergothioneine and the diet was demonstrated by Spicer, Wooley, and Kessler (312), who found that ergothioneine disappeared from rabbit erythrocytes if the animals are fed a purified diet. Similar observations have been reported for rat tissues (234b). It is not known definitely whether the effect of diet in these experiments is related to its ergothioneine content or to some precursor material. Feeding experiments with S^{35}-methionine and S^{35}-ergothioneine resulted in labeling of the ergothioneine isolated from the animal, but no incorporation was observed with S^{35}-thiohistidine, C^{14}-methionine, or C^{14}-histidine (175, 176, 234a).

The only experiments published on the possible functional significance of ergothioneine are those of Spicer et al (312), who observed that erythrocytes with a low ergothioneine content developed more methemoglobin upon exposure to nitrite than erythrocytes with high ergothioneine content, suggesting that the ergothioneine may be active in some way as an intracellular reducing agent.

Miscellaneous. A number of other imidazole compounds are of considerable physiologic interest, such as 4-amino-imidazole-5-carboxamide, pilocarpine, and others. These are not being reviewed here and reference is made to the books of Guggenheim (155) and Hofmann (178) and to the review of Battersby and Openshaw (32).

III. HISTAMINE

Of particular interest to the field of pharmacology is that aspect of histidine metabolism which is concerned with the synthesis and degradation of histamine. This base was first isolated by Barger and Dale (30) and Kutscher (210) from ergot extracts and by Ackermann (5) from the bacterial decomposition of histidine. The potent pharmacologic actions of histamine were immediately observed (6, 30, 82–84, 125a, 155, 159, 210), and, shortly thereafter, the presence of a histamine-like material in normal animal tissues was described. Although some of the latter studies were complicated by the possibility of secondary histamine formation resulting from bacterial action and by inadequate identification of the histamine, these variables were controlled in later studies, and the normal presence of histamine in animal tissues appears to be unequivocal (7, 38).

A. BIOLOGICAL OCCURRENCE

Distribution. Histamine is widely distributed in many species. The exact quantitation in the various tissues, however, is subject to some disagreement, mainly because of the different assays used by various investigators. All the bioassay

and colorimetric procedures (155) used have limitations of specificity and accuracy. Various other pharmacologically active compounds, for example, can interfere with the bioassay procedures. Although the modified bioassay procedure of Code (70a) eliminates the interferences due to most of these, the method does not completely differentiate histamine from such closely related active compounds as N-methylhistamine. The use of antihistaminics in the assay has improved the specificity of the bioassay, but these also do not differentiate histamine and N-methylhistamine (384). In only occasional cases have combinations of several bioassay procedures been utilized to ensure more complete identification. Recently, combined chromatographic procedures and colorimetric determinations or bioassays have also been used for this purpose (218a, 228, 236, 279, 384).

Within these various limitations, however, there is a very considerable body of data on the distribution of histamine in the various tissues. Histamine is widespread in many tissues of a variety of species, and usually varies in concentration from 0 to 75 micrograms per gram of wet tissue. The concentration varies with age, and is very low in fetal tissues (349b). The highest concentrations (261) are found in ox pleura (200–280 micrograms per gram of wet tissue). A summary of the histamine concentrations in various tissues is given in Guggenheim's book (155), and in Code's recent review on blood histamine (70b). In addition to this work on the normal distribution of histamine there have been some studies (155) on histamine concentrations in various pathological conditions, including malignant tissues (47b, 280). The highest histamine concentration reported in animal tissues has been found (261) in a human mast cell tumor (950 micrograms per gram). Changes in histamine levels have been attributed to such factors as sensitization, adrenalectomy, pregnancy, age etc. (116, 160–162, 195, 222, 271–273, 349b). These changes in histamine concentrations are covered in the recent reviews of Rose (271, 273) and of Code (70b) and therefore will not be reviewed here.

Although N-methylhistamine has not been reported as a naturally occurring substance, it is possible that some of the reported histamine values contain an N-methylhistamine component. This postulation is suggested by the close similarity of histamine and N-methylhistamine in both bioassay (356) and colorimetric determinations, as well as by the recent discovery of the epinephrine/norepinephrine interrelationships.

Localization. Since the quantities of histamine normally present in certain tissues represent toxic doses for the animal, it is clear that there is some binding or localization of histamine. This distribution is most readily studied in the blood, where it is generally agreed that the histamine is contained in the formed elements. A review of this work, as well as the studies of the concentration of histamine in the different blood cells, can be found in Code's extensive review on "Histamine in Blood" (70b).

Much less work has been carried out on the histamine content of the various cells in the tissues. Recently Graham, Lowry, Wahl, and Priebat (152) and Riley and West (261) have presented evidence for the localization of histamine within

the mast cells. The intracellular distribution of histamine has been studied by Copenhaver, Nagler, and Goth in dog liver homogenates (72). 15% of the histamine is contained in the nuclei, 52% in the mitochondria, 13% in the microsomes, and 17% in the supernatant fraction. The binding of histamine by tissue components is also indicated by the isotope findings of Schayer discussed below (page 322). Essentially nothing, however, is known about the mechanism responsible for the localization of histamine. Although there have been reports that histamine is bound by proteins *in vitro*, this could not be confirmed by Kaplan and Davis (198).

"Histamine-releasers". Closely related to the subject of bound histamine in the tissues are those experiments concerned with the study of the various agents which release histamine from the tissues. Although this aspect of the histamine problem is not being reviewed at length here, a short summary of this work is included because of the many interesting results which are being currently obtained in a number of laboratories. Histamine release from the formed elements of the blood by a variety of agents has been extensively studied by Code, McIntire, Rocha e Silva, Dragstedt and others. These studies on the effects in blood are summarized in Code's recent review (70b).

Considerable work has also been carried out on the release of histamine from tissues, both *in vitro* and *in vivo*. Some of the agents studied have been ammonia (293), anaphylatoxin (264, 265, 266), antihistaminics (28), atropine (286), bile salts (26, 286), curare (20, 26), diamines (219, 242), 5-hydroxytryptamine (134), muscular contraction (20), neoarsphenamine (286), peptone (130), pethidine (286), polylysine (148), primary amines (219, 238, 240), priscol (286), protamine (148), snake poisons (95, 128), stilbamidine (219), staphylococcal toxin (129), trypsin (264), *d*-tubocurarine (239, 241), tryptamine (134), "48/80" (132, 133, 219, 235, 238, 239, 308, 309), egg white (132, 287), and horse serum (131), as well as antigen-antibody reactions (92–94, 131, 239, 353).

In skin and cornea, histamine is released by various electrical, physical, and chemical stimuli (282). The liberation of histamine during the stimulation of isolated nerves has been reported by v. Euler and Astrom (359). These findings, together with other work on the histamine content of nerves, have contributed to the theory of histaminergic nerves (359, 360, 382, 384), and to the postulation that histamine is involved in sensory pain, both direct and referred (281, 282).

In discussing "histamine-releasers" it is of interest to refer again to the experiments of Riley and West (261), which indicate that these agents act by causing disruption of the histamine-rich mast cells. Graham, Lowry, Wahl, and Priebat (152) have discussed the possible advantage of having the histamine located in these fragile mast cells, where various stimuli can easily release the bound histamine, and permit it to exert its physiological activity.

B. HISTAMINE SYNTHESIS

1. Microbiological Synthesis of Histamine

The earliest knowledge of the origin of histamine developed from various bacterial experiments. The work of Ackermann (5) showing the bacterial conversion

of histidine to histamine was confirmed by a number of investigators (37, 233). This decarboxylase reaction was extensively studied by Hanke and Koessler (164–166, 202), and more recently by Epps and Gale (122, 144–146). Most of the investigations on L-histidine decarboxylase have been carried out with *Escherichia coli* and *Clostridium welchii*, although this activity is also found in a number of other organisms (118a, 150, 268, 269, 373). Hanke and Koessler (165) noted that the decarboxylase activity developed only if the pH of the culture medium was acid (usually about 5). They speculated "that the production of amines from amino acids by microorganisms seems to be a protective mechanism and is resorted to when the accumulation of H ions within the organism's protoplasm is incompatible with its normal life processes. The amines can be thought of as reaction buffers". Although this viewpoint has been commonly accepted, Gale (144) suggested that possibly the formation of decarboxylase activity in acid media may be due to the organism's inability to utilize carbohydrate and other substrates at this pH. Another possibility suggested by Gale (146) is that the decarboxylation of the amino acids affords an important source of CO_2 for the organism, since at an acid pH very little dissolved CO_2 would be present in the medium.

By using acetone powders of a selected strain of *Cl. welchii*, Epps and Gale (122, 145) were able to prepare a decarboxylase preparation which was specific for L-histidine. This has been used as an analytical tool for histidine determinations in proteins, etc., since the L-histidine is quantitatively converted to histamine plus CO_2. The enzyme was purified 50 fold by adsorption and ammonium sulfate technics. Although the pH optimum of the whole cells is 2.5, the pH optimum of the cell-free preparations is 4.5. The enzyme is inhibited by $AgNO_3$, $HgCl_2$, $CuSO_4$, and $KMnO_4$, as well as hydroxylamine and semicarbazide.

There is still some disagreement on the demonstration of a coenzyme in bacterial histidine decarboxylase. Gale (122, 146) not only failed to separate the *Cl. welchii* decarboxylase into a coenzyme and apoenzyme, but could not demonstrate any codecarboxylase activity (as measured with tyrosine and lysine decarboxylases) in boiled preparations of histidine decarboxylase. Rodwell (269) reached similar conslusions in his studies on histidine decarboxylase from *Lactobacilli*. Werle and Koch, on the other hand, reported a pronounced activation of the histidine decarboxylase of washed *E. coli* when pyridoxal-5-phosphate was added (381).

2. Histamine Synthesis in Insects and Plants

Interest in the histamine content and histamine formation in insects has been generated by studies on the possible role of histamine and acetylcholine in the swelling, itching, and erythema associated with insect bites. Evidence for a histidine decarboxylase in bees has been reported by Werle and Gleissner (383) to account for the high histamine concentrations found in bee sting (224). Histamine has also been reported in the common nettle, together with acetylcholine, by Emmelin and Feldberg (120) and in the gnat by Eckert, Passonen, and Vartiainen (96c). Histamine is present in the black fly with the highest concentration

in the thorax of the female. The amounts of histamine found, however, are not sufficient to account for all the symptoms of black-fly bites (187), or of bee stings (224).

The occurrence of histidine decarboxylase in plants has been reported by Werle and Raub (378).

3. Histamine Synthesis in Animal Tissues

The origin of the histamine found in animal tissues is more obscure than that found in bacterial cultures. Although dietary histamine has been considered as a possible source of tissue histamine, it has frequently been proposed that histamine is produced from histidine by a decarboxylation reaction in the animal, carried out by either the tissue decarboxylases or by the intestinal bacteria.

In vitro Studies. Studies on the *in vitro* formation of histamine from L-histidine in animal tissues (288) are complicated by the simultaneous destruction of histamine by diamine oxidase, as well as by the necessity of avoiding any bacterial contamination. The first demonstration of histidine decarboxylase in animal tissues was reported by Werle (367, 368) and by Holtz *et al.* (180) in the livers and kidneys of various animals. Since the amount of histamine formed is very small, bioassay procedures were used for the analyses.

Further studies on animal histidine decarboxylase (182, 185, 369, 370, 372, 375, 376, 381) were facilitated by the observations that, while diamine oxidase requires an aerobic environment, histidine decarboxylase is active both aerobically and anaerobically. Thus, by using anaerobic conditions, as well as partially purified preparations and, in a few cases, diamine oxidase inhibitors, histidine decarboxylase could be studied in tissues where it was present in low concentrations. The activity is widespread, and has been detected in such tissues as kidney, liver, pancreas, small intestine, and skin. The enzyme is specific for L-histidine; with D-histidine no histamine is formed. In contrast to the bacterial preparations the pH optimum of the enzyme is 8.6–9.0. The decarboxylase activity is strikingly inhibited by hydroxylamine (10^{-5} to 10^{-6}M) and semicarbazide; this inhibition can be reversed by dialysis or by pyruvic acid additions. Dinitrophenylhydrazine, Girard's reagent, and dimedon also inhibit (372). These inhibition studies suggested the possibility that a pyridoxal cofactor is involved, and more recently both Werle and Koch (381) and Holtz, Engelhardt, and Thielecke (185) reported that guinea pig kidney decarboxylase is activated by pyridoxal-5-phosphate. The data, however, are not so conclusive as those obtained with other enzymes containing a pyridoxal-phosphate coenzyme.

The absolute activity of the histidine decarboxylase preparations is rather small, and quantitatively represents only a minor pathway of histidine degradation (see histidine section above). 400 mg. of rabbit kidney, for example, produce only 8.5 micrograms of histamine from 133 mg. of histidine in 2.5 hours at 37.5°C (367). However, since only a very small amount of histamine can produce a very powerful pharmacological response, this activity represents a considerable production of histamine.

In Vivo Studies. Although these experiments demonstrate the production of

histamine from histidine by tissue decarboxylase activity *in vitro*, until recently there was very little to indicate that this process occurred *in vivo*. In 1936 Bloch and Pinösch (46) showed that the histamine content of the guinea pig lung doubled after the injection of 100 mg. of L-histidine monohydrochloride per 100 g. body weight. These results were confirmed by Edlbacher, Simon, and Becker (105). Holtz and Credner (183) demonstrated in guinea pigs an increase in the histamine content of the urine after the intramuscular injection of histidine.

In all these experiments the quantities of histidine administered were very large. Recently Schayer (290) reinvestigated this problem of histamine synthesis in guinea pigs *in vivo*, using small injections of radioactive compounds. Following the subcutaneous injection of radioactive L-histidine, C^{14}-histamine is found in the urine and internal organs for many days, indicating that the histidine is decarboxylated *in vivo* to histamine. If, on the other hand, C^{14}-labeled histamine is injected, no radioactive histamine is detected in the guinea pig's organs 4 hours after the injection; essentially all of the C^{14} is recovered in the urine (see page 331). Since exogenous C^{14}-histamine is excreted so much more rapidly than histamine formed in the animal from C^{14} histidine, these data indicate both that tissue histamine is formed *in situ* in the tissues, and that these cells contain a mechanism for binding the histamine which is formed endogenously. On the basis of his radioactivity data, Schayer estimates that the half-life of the histamine in the guinea pig organs is of the order of 50 days.

C. HISTAMINE METABOLISM IN VITRO

Numerous investigators have demonstrated *in vivo* the ability of the animal to dispose of relatively large quantities of histamine (18, 121, 202, 236, 270). The most important metabolic pathway quantitatively appears to be oxidative deamination by the enzyme diamine oxidase (histaminase). Other pathways include binding of histamine by the tissues and acetylation, as well as excretion into the urine.

1. *Diamine Oxidase* (*Histaminase*)

Dale and Laidlaw (83) in 1912 reported some evidence for the slow disappearance of histamine in liver perfusion experiments. In 1915 (125b) Eustis reported that histamine could be inactivated by incubation with the ground liver of the turkey buzzard. Beginning in 1929 the inactivation of histamine by various animal tissues was extensively studied by Best, McHenry and Gavin (39, 40, 226, 227), and by Gebauer-Fuelnegg (149), and shown to involve an oxidative deamination; the term *histaminase* was introduced by Best and McHenry (40). In 1938 Zeller (397) reported that this enzyme also degrades putrescine, cadaverine, and agmatine, and therefore called it *diamine oxidase*. Subsequently these studies have been extended by numerous investigators with animal, plant, and microbiological material (62, 194, 212, 213, 214, 285, 318, 328, 329, 373, 374, 379, 398–413). The terms *histaminase* and *diamine oxidase* are used interchangeably in this review, even though there is considerable disagreement on whether a single enzyme carries out both types of activity in all preparations (143, 197, 285).

Diamine oxidase is found in a variety of animal tissues (40, 102, 410). The

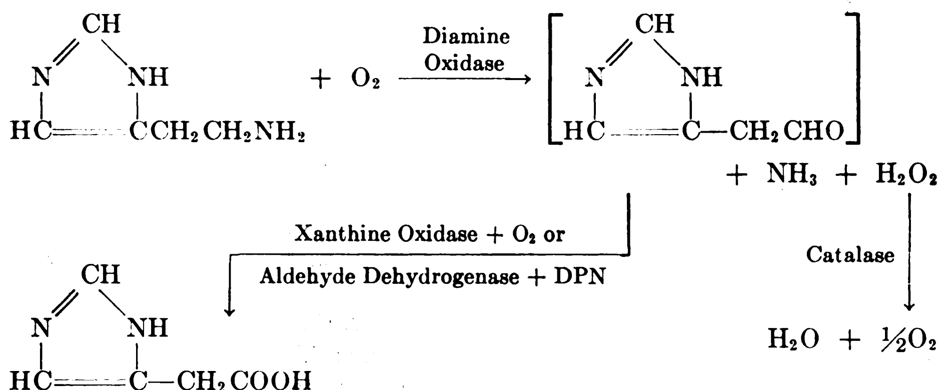
concentration of diamine oxidase in each tissue, however, varies considerably in different species; *e.g.*, in many species the kidney has the highest diamine oxidase concentration, followed by the intestines and liver; in the rat, however, the kidney and liver have essentially no diamine oxidase activity, although an active diamine oxidase is found in the intestine. A number of workers have paid particular attention to the distribution of diamine oxidase in the various parts of the gastrointestinal tract. In the cat (161) the largest amount of diamine oxidase is in the small intestine; no diamine oxidase could be detected in the gastric mucosa. The intracellular localization of diamine oxidase has been studied by Cotzias and Dole (73); in rabbit liver the enzyme is predominantly associated with the particulate components of the cell. Muscle and normal blood plasma have an extremely low diamine oxidase content, although histaminolytic activity has been reported in lymph (66). Tables of the relative activities in different organs may be found in references 182 and 410. Although many of the tissues with high diamine oxidase activity also have a high histamine content, several investigators (25, 66) have observed that intrinsic histamine is not attacked by intrinsic diamine oxidase until the tissue is well-ground or autolyzed (39). Most of the procedures for purification (329, 331) of diamine oxidase have utilized hog or beef kidney as the starting material. Several hundred fold purification can be obtained by conventional fractionating procedures (331).

Werle *et al.* (377, 378, 380) have surveyed a variety of plants, and demonstrated the widespread occurrence of diamine oxidase. Although none of the enzyme is found in the seeds, the seedlings have very high values, which later decline. Diamine oxidase is also found in a number of bacteria (283, 285, 380, 411), and appears to belong to the class of adaptive enzymes.

Substrates and Products. The substrates attacked by diamine oxidase (410) include histamine, agmatine, and such diamines as putrescine and cadaverine. Propylenediamine and hexamethylenediamine are oxidized more slowly, and there is essentially no activity with ethylenediamine. The higher homologues are attacked more slowly, and there is essentially no activity with the C14–C18 diamines (44). The lower homologue of histamine (aminomethylimidazol) is not degraded by the enzyme (338b). Spermine is not oxidized by the partially purified enzyme (194). As with monoamine oxidase, compounds with substitution on the α-carbon, as β-(imidazolyl-4-)isopropylamine, are not attacked (19). A methyl substitution in the 5 position of the imidazole ring, on the other hand, does not affect the enzymatic action. A number of other substrates, such as pyridylethylamine (385), are weakly attacked by crude preparations; until these are studied with more purified preparations one cannot evaluate whether they are really substrates of diamine oxidase.

The results of Zeller and others (reviewed in reference 410) indicate that the products of histamine oxidation are imidazoleacetaldehyde, ammonia, and H_2O_2. Although the imidazoleacetaldehyde has been accumulated, it has never been isolated. The accumulated imidazoleacetaldehyde has been oxidized by oxygen in the presence of xanthine oxidase (62, 331), by diphosphopyridine nucleotide in the presence of liver aldehyde dehydrogenase (331), and nonenzymatically by cupric and cobaltic ions (53, 62). Imidazoleacetic acid has been isolated following

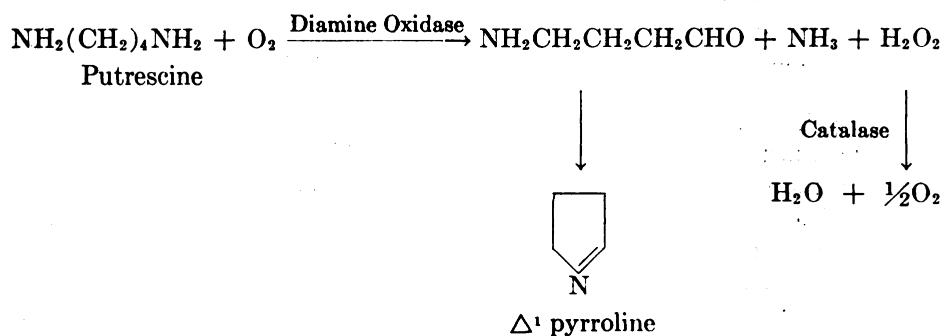
the oxidation of histamine by the combined action of diamine oxidase and xanthine oxidase (331) as well as by the adaptive bacterial enzyme of Satake and Fujita (285b).



There have been a number of reports indicating that the imidazole ring of histamine is opened during histaminase action (40, 194, 329). The significance of these findings cannot be evaluated unequivocally at this time. These results might be due to the presence of other enzymes responsible for later steps involving ring splitting, or to alternate degradative pathways. Although there is no enzymatic evidence, the known sensitivity of the imidazole ring to oxidation by H_2O_2 leads to speculation concerning a peroxidative rupture of this ring. A further complication in these experiments is the use of some modification of the Pauly diazotization reaction for the determination of the imidazole ring. The results of this assay vary with each imidazole, and hence are very difficult to interpret as a general quantitative test for the imidazole ring.

There is general agreement that destruction of the pharmacologic activity of histamine parallels its oxidative deamination, although careful quantitative comparisons have not been made with the more purified diamine oxidase preparations. Stern (319) has suggested that an early product of histamine breakdown is the pharmacologically active agent rather than histamine itself.

With the diamines, as putrescine or cadaverine, the corresponding aminoaldehydes are formed in the first step (331). These cyclize to the corresponding cyclic product Δ^1 pyrroline or Δ^1 piperideine.



Similarly the six-membered ring, Δ^1 piperideine, is formed from cadaverine. This type of amino aldehyde condensation has been extensively studied chemically by Schöpf and his collaborators (295) as part of their studies in alkaloid synthesis. Similar types of ring closure have recently been shown *in vitro* in proline synthesis from glutamic semialdehyde (358), and are postulated for the biosynthesis of pipecolic acid (218) from an oxidation product of lysine. It is of interest to speculate that this type of reaction may also occur in the biosynthesis of atropine, nicotine, coniine, and other alkaloids (78, 89, 206). This formulation probably accounts for the findings of Nordström (246) that the piperidine excretion of the urine increases after the administration of cadaverine, since piperidine would be formed by the reduction of Δ^1 piperideine.

As indicated by the above formulation the ratio of $\frac{\text{moles of ammonia}}{\text{atoms of oxygen}}$ is 1, and this has been found by a number of investigators. In preliminary communications, however, Zeller *et al.* have reported some very interesting discrepancies in this ratio if the reaction is interrupted before completion. In *Nocardia* preparations a ratio <1 was found (409), while in certain partially purified kidney preparations a ratio >1 was found (412, 413).

Inhibition Studies. Diamine oxidase is inhibited (410) by cyanide, semicarbazide, hydroxylamine, phenylhydrazine, and a number of other carbonyl-reagents. The cyanide inhibition has been studied extensively, and has been shown to be easily reversible. The enzyme is also strongly inhibited by isonicotinic acid hydrazide (isoniazid) (33b, 412). The inhibition of diamine oxidase by various amino and amidine compounds has been studied by Zeller, Blaschko and others (45, 410). The enzyme is not inhibited by pyrophosphate, fluoride, carbon monoxide, p-chloromercuribenzoate, or heparin (316d, 408, 410). The results with antihistaminic drugs vary with the specific compound, and do not correlate with the antihistaminic properties (194, 410). The competitive inhibition of histamine oxidation by putresine and other diamines has been studied by Zeller and others (139, 194, 397, 410).

An interesting series of observations has been reported by Zeller and his group (248, 411) on the inhibition of diamine oxidase activity of *Mycobacteria* and other bacteria by streptomycin and dihydrostreptomycin. The inhibition was only observed with streptomycin-sensitive cells, and not with streptomycin-resistant strains. All of the work was carried out with whole cell preparations, since no diamine oxidase activity could be obtained in extracts. The streptomycin effects were not observed if the cells had been preincubated with putrescine; no inhibitory effects could be observed with animal preparations. These data indicate that the diamine oxidase enzyme might be different in the animal and bacterial preparations. Recently, however, Hayaishi *et al.* (173, 321), Fitzgerald, Bernheim, and Bernheim (135, 136), and others have reported inhibitory effects of streptomycin on the oxidation of various substrates by bacterial cells, and have attributed their findings to inhibition of adaptive enzyme formation by streptomycin, *i.e.*, the inhibition by streptomycin of the synthesis of the corresponding degradative enzyme in response to the presence of a substrate. Since there is some evidence that bacterial diamine oxidase is an adaptive enzyme (283, 285, 380), this

explanation could explain some of the findings of Zeller *et al.* However, this does not account for all of their findings, (*e.g.*, Figure 1 in ref. 411), and therefore a final evaluation of the mechanism of the streptomycin inhibition is not possible at present.

Cofactors and Mechanism of Diamine Oxidase Reaction. The nature of the prosthetic group of diamine oxidase is still unclear. Zeller, Stern, and Wenk (401), Swedin (328, 329), and Kapeller-Adler (194) have presented suggestive evidence for the involvement of a flavin, such as flavin-adenine-dinucleotide. However, as Zeller (410) recently pointed out, there is still no conclusive proof available that flavin-adenine-dinucleotide is part of the diamine oxidase molecule itself. No evidence for a flavin was found by Leloir and Green (214) or Laskowski (213). Evidence that flavin-adenine-dinucleotide is a cofactor for the bacterial histaminase obtained from *Achromobacter sp.* has been presented by Satake *et al.* (285b) by demonstrating that it will reactivate a thoroughly dialyzed preparation of the enzyme.

The inhibitory action of cyanide and other carbonyl binders has resulted in considerable speculation that a carbonyl group is present on the enzyme, or that a carbonyl group is present on a coenzyme such as pyridoxal phosphate. Werle and Pechmann (380) have presented evidence that pyridoxal can stimulate the diamine oxidase activity of plant extracts; this was somewhat surprising since in other reactions the phosphorylated form of the coenzyme is required. In a preliminary note Sinclair (307) has presented further evidence for the involvement of a pyridoxal type of cofactor in animal diamine oxidase; histaminase is diminished in pyridoxine deficient rats, and some restoration can be accomplished with pyridoxal-5-phosphate. Copper has been suggested as a cofactor by Holmberg and Laurel (179), but the present status of this postulation is somewhat unclear (410).

Associated with this lack of definite conclusions on the nature of the cofactor(s) is a lack of any definitive knowledge on the mechanism of the hydrogen transfers involved. Zeller, Fouts, and Voegtli (413) postulate that the reaction involves a transfer of the hydrogen from the diamine to an acceptor group on diamine oxidase, followed by the transfer of this hydrogen to molecular oxygen under the influence of a second enzyme.

The Identity of Diamine Oxidase and Histaminase. The postulation of Zeller that histaminase and diamine oxidase are a single enzyme is supported by considerable work from his laboratory and others. This assumption is supported (410) by the somewhat parallel distribution of both activities in various biological forms, by the simultaneous formation of both activities when bacteria are grown on one substrate, by competitive inhibition experiments, and by the relative constancy of the histaminase: diamine oxidase ratio over a 300 fold purification of the enzyme from hog kidney (331).

Kapeller-Adler (196, 197b), however, has reported that the relative rates of oxidation with cadaverine, putrescine, agmatine, and histamine vary considerably with different preparations of the enzyme. The relative rates in blood samples from certain cases of pre-eclamptic toxemia were different from those found in

normal pregnancy. Very marked changes were also observed during the purification of the hog kidney enzyme. These results, however, were mainly obtained with an assay procedure which depends on the secondary oxidation of a dye, indigo disulfonate, by the peroxide formed during a 24 hour incubation period. These interesting experiments, therefore, should be repeated with a shorter assay period and with more direct measurements. Kapeller-Adler (194) has also reported that the oxidation of the various substrates is affected differently by dialysis and by reactivation with a flavin-adenine-dinucleotide preparation. Although these data have been presented as indicating the presence of separate enzymes for histamine and the other diamines, such factors as substrate affinities, the presence of competitive inhibitors, enzyme stability, and the presence of other enzymes for the further metabolism of the products (53, 62) must be considered further before a final evaluation is possible.

In addition to these experiments of Kapeller-Adler, Satake *et al.* (285a, 285b) have demonstrated the existence of separate enzymes for the oxidation of histamine, putrescine, and amylamine in *Achromobacter sp.* cultures, which had been grown on the respective substrates. Cell-free preparations were obtained which were specific for the respective substrate. These enzymes no longer reacted with oxygen, and the enzymatic reactions were studied by measuring the decolorization of 2,6-dichlorophenolindophenol. The relationship between these bacterial preparations and other bacterial and mammalian amine oxidases remains to be elucidated.

Changes in Diamine Oxidase in Pregnancy. A marked rise in diamine oxidase levels in the blood plasma of pregnant women has been reported by numerous investigators (14, 25, 91, 116, 195–197, 223, 326, 327, 371, 386, 404, 410). Although there is disagreement concerning some of the details, it is generally agreed that plasma diamine oxidase levels normally are very low, but start increasing during the second and third month of pregnancy. The levels attained (in the 6th–7th month) are about 400–1000 times the normal value. Within three days after delivery the level has fallen to its normal low value. There is some indication that the diamine oxidase level is low in certain pathological conditions, such as pre-eclamptic toxemia of pregnancy. High levels are reported in cases of chorioepithelioma.

This marked increase in blood diamine oxidase level is not observed in any of the laboratory animals tested (14, 25, 327). The placentae, however, in both the human and in certain animals, have a very high diamine oxidase content (25, 62, 263, 327, 386). The diamine oxidase content of the placenta has been exhaustively studied by Swanberg (326, 327), who showed by dissection studies that the diamine oxidase of the human placenta was concentrated in the maternal decidua. Those animals in which the placenta had a substantial maternal component also had high diamine oxidase levels. The dispensability of the fetus for the production of the placental enzyme was further shown by the high levels of diamine oxidase produced in experimental deciduomas in the non-pregnant uterus of rabbits, as well as the high levels found in pseudopregnancy. Similarly high levels could be produced in the endometrium of progesterone-treated rabbits.

The increase in diamine oxidase levels in pregnancy has led to the reasonable speculation that its function is to protect the uterus from any circulatory histamine. However, as discussed above, Werle showed that germinating plants also exhibit a dramatic increase in diamine oxidase content. Thus, it is possible that the presence of diamine oxidase is related to a synthetic function, perhaps associated with the formation of nitrogen-containing ring structures, as discussed above (p. 325).

Miscellaneous Factors Affecting the Concentration of Diamine Oxidase. The markedly increased susceptibility of adrenalectomized animals to histamine administration has led to considerable speculation that this might be the result of a decreased histaminase activity. Some of the studies on this subject have been reviewed by Rose (271, 273), who found that the histaminase content of rat tissues was lowered after adrenalectomy. Similar studies in the cat have been carried out by Haeger, Jacobson, and Kahlson (160–162) who found a marked decrease in the histaminase content of the kidney and small intestine after adrenalectomy or hypophysectomy. Although these data demonstrate that adrenalectomy does cause a marked decrease in histaminase levels, this is not necessarily the cause of the increased toxicity of histamine after adrenalectomy, since it is well-known that similar increases in toxicity can be observed with many other unrelated compounds when tested in adrenalectomized animals. (For data on the effect of adrenalectomy on the *in vivo* metabolism of histamine see page 330.)

Similarly there has been considerable interest in the possible changes in the concentration of histaminase in sensitized animals and in anaphylactic shock. Rose and Leger (272) for example reported an increase in serum histaminase levels in rabbits during anaphylaxis. On the other hand, Eisen (119) was unable to find any change in the histaminase content of the kidney of the dog, cat, rabbit, and guinea pig despite sensitization with horse serum or anaphylactic shock.

The effect of age has been reported by Zeller (410) and by Haeger and Kahlson (162). In these experiments the diamine oxidase levels were lower in young animals than in older animals.

Therapeutic Use of Histaminase. Although the administration of histaminase preparations for the therapy of allergy and related disorders has been frequently recommended, the results have been unsuccessful (see review by Feinberg (126)). Most of the preparations used have been too impure to permit the administration of adequate quantities, but the recent preparation of more purified enzyme preparations may permit reinvestigation of its *in vivo* use. The use of a partially purified histaminase as an experimental tool for *in vivo* experiments has recently been demonstrated by Grossman and Robertson (154). These authors found that the gastric secretion of HCl resulting from a subcutaneous histamine injection was absent if the dog was treated with an intravenous injection of histaminase. The histaminase preparation also abolished the gastric secretion due to mecholyl, urecholine, or a test meal. Although these results indicate the possibility that histamine is involved in the response to all of these agents, the reservation must be made that the specificity of the enzyme preparation used is not adequately

known. Nevertheless, it is evident that further experiments of this type with increasingly pure preparations of histaminase will be very useful in various physiological and pharmacological studies on histamine action.

2. Acetylation of Histamine

In Vitro Studies. As discussed further below, *in vivo* studies by Anrep *et al.* (24), in 1943, showed that histamine can be excreted both in the free form and in a conjugated form. Studies in this and other laboratories (referred to below) demonstrated that this conjugated form was acetylhistamine. This pathway has been demonstrated *in vitro* (236, 336) by the action of pigeon liver extracts on histamine in the presence of a suitable source of acetylcoenzyme A, such as coenzyme A plus either acetate and adenosinetriphosphate or acetylphosphate and bacterial transacetylase. The acetylating enzyme can be partially purified (30 fold), and at present cannot be distinguished from the enzyme described by Kaplan and Lipmann for the acetylation of sulfanilamide and other aromatic amines (217). The pharmacologic activity of histamine is lost after acetylation (12, 24, 236).

D. HISTAMINE METABOLISM IN VIVO

Acetylation and Excretion. Normal urine contains small amounts of free histamine (8, 10–12, 24, 153, 262, 352) as well as an inactive conjugate, which reacts like histamine in the usual bioassays only after vigorous acid hydrolysis. This conjugate was first described by Anrep *et al.* (24) and their findings have been confirmed in a number of laboratories, both with normal urines and with urines obtained from animals following histamine administration (10–12, 236, 237, 262, 279, 289, 291, 354). Following histamine administration to various species Millican, Rosenthal, and Tabor (236, 237) found that approximately 3–15% of the dose can be recovered from the urine in this conjugated form, and a variable additional amount as free histamine. The quantities of both forms recovered vary with the dosage and the species. The excretion of free histamine is highest in mice and rats (24, 236). The conjugate has been isolated and crystallized from the urine of dogs after the oral administration of histamine, and has been identified as N-acetylhistamine (330). These findings have been confirmed by Schayer in rats, using tracer doses of C^{14}-histamine (289).

It should be pointed out, however, that the isolation of acetylhistamine from the urine after the administration of histamine does not necessarily mean that this is the structure of the conjugate reported by Anrep *et al.* as normally occurring in the urine. Since Anrep *et al.* found the conjugate only after the ingestion of a meat diet, it is possible, for example, that the inactive conjugate could be the decarboxylation product of carnosine, namely, β-alanylhistamine.

Although *in vitro* experiments (see above) indicated that both liver extracts (236, 336) and intestinal bacteria (354) can acetylate histamine, there has been some disagreement on the site of the acetylation *in vivo*. To clarify this question, Millican (237) studied the acetylation of histamine in enterectomized rats, and showed that acetylation was unimpaired. These data demonstrate that acetyla-

tion can occur outside the intestinal tract, although they do not exclude the possibility that additional acetylation can occur in the intestinal tract in normal rats. In dogs and humans, however, Anrep *et al.* (24) and Adam *et al.* (10) reported that acetylation only occurs after the oral administration of histamine, and not after parenteral administration. Although on the basis of their data these authors postulate that acetylation takes place only in the gastrointestinal tract, the data are inconclusive since much smaller doses of histamine were administered in the parenteral experiments than in the oral experiments.

Adam and Mitchell (12) reported that children excrete histamine in free and conjugated forms. The rate of free histamine excretion is approximately proportional to the body weight, and varies from 0.3–0.4 microgram/kg./24 hours. The amount of conjugate excreted is more variable, with essentially no excretion of conjugate in infants. No significant difference in the urinary excretion of histamine was observed in 10 infants with gastroenteritis. An increase in the histamine content of the urine was reported in certain cases of preeclamptic toxemia of pregnancy by Kapeller-Adler (191–193) and Ungar (351), but not by Gabrawy (140) or Rockenschaub (267).

In addition to these urinary findings, acetylhistamine has been reported in small amounts in nerve (384), but has not been found in a number of other organs in normal animals (237). After histamine administration acetylhistamine has been found in the liver, lung, and spleen (237).

Degradation. In vivo confirmation of the diamine oxidase pathway was obtained by Mehler, Tabor, and Bauer (229) by the isolation of imidazoleacetic acid from the urine after the administration of histamine to rats. These results were confirmed by Bouthillier and Goldner (58) using C^{14}-labeled histamine. In addition an *in vivo* trapping experiment, in which tracer amounts of C^{14}-histamine and a relatively large amount of unlabeled imidazoleacetic acid were administered to rats, resulted in the excretion of labeled imidazoleacetic acid, indicating that this pathway occurred even when very small quantities of histamine are administered (335).

An interesting series of experiments has been reported by Schayer *et al.* (289, 291) on the degradation of histamine in mice, rats, guinea pigs, and cats. These experiments utilized isotope technics and highly radioactive histamine to study the *in vivo* metabolism of histamine with small doses. Following the injection of C^{14}-histamine (labeled in the C-2 position of the imidazole ring) the urine was collected and analyzed by paper chromatography. Three radioactive areas were present; one representing histamine plus acetylhistamine, and two representing unknown materials. The quantities of C^{14} in the different areas were not affected by adrenalectomy, in contrast to the *in vitro* experiments reported above (page 328). No effect was noted with pregnancy or with administered estradiol or progesterone; cortisone caused a marked increase in the histamine-acetylhistamine region. Considerable changes in the distribution occurred when isonicotinic acid hydrazide or aminoguanidine was administered, consistent with an inhibition of diamine oxidase (see page 325). Results with other inhibitors, such as 1-isonicotinoyl-2-isopropylhydrazine, affected the distribution differently, and the

authors postulated two pathways for the degradation of histamine. The interpretation of these interesting *in vivo* results is still tentative, however, and further work *in vivo* and *in vitro* is necessary for eventual clarification. The urinary excretion of C^{14}-compounds (58, 289) accounts for essentially all of the administered isotope in these experiments with ring-labeled histamine, as well as with side-chain labeled histamine. Essentially no isotope is found in the respiratory CO_2 in either case.

Only preliminary findings are available on the further metabolism of imidazoleacetic acid. When C^{14}-imidazoleacetic acid is administered to rats, both imidazoleacetic acid (59, 229) and an imidazoleacetic acid pentoside (171) can be isolated from the urine. No isotope is found in the respiratory CO_2 (59). With bacterial preparations (171, 285) further degradation of the imidazoleacetic acid occurs, and recent experiments (171) with *Pseudomonas* preparations have demonstrated formylaspartic acid as an intermediary product in this degradation.

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