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Aspects of histamine metabolism *

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The subject of the relationship of histamine to disease has a history that is nearly coincident with the discovery of histamine. Since its isolation from animal tissue (Barger and Dale, 1911) and the appraisal of its actions (Dale and Laidlaw, 1910, 1911), including its stimulation of gastric acid secretion (Popielski, 1920), histamine has been postulated to function in disease. In this regard, histamine differs from most other endogenous substances, specifically from other biogenic amines. The work leading to the discoveries of other biogenic amines – e.g., acetylcholine, norepinephrine, dopamine – and the work after their discoveries were aimed at learning their roles in normal physiology; subsequently, attention was given to their roles in disease. Research on histamine followed an opposite sequence. As Dale (1966) noted, "It cannot, indeed, be doubted that is has been with this mainly pathological aspect of its significance, that an overwhelming majority of the experiments dealing with the functions of histamine have hitherto been concerned. There is already, on the other hand, a great accumulation of evidence concerning the origin, distribution and metabolism of histamine, as a natural constituent of many organs and tissues; and such knowledge, whatever may have been the nature of the interest prompting the researchers which have produced it, will obviously be available for the consideration of other possible functions of histamine, in connexion with normal, physiological phenomena . . ." It now seems clear that histamine functions as a neuroregulator in brain (Prell and Green, 1986). What specific functions it regulates is not certain (Hough and Green, 1984). Learning the *physiological* functions of histamine and its contribution to homeostasis in man will almost certainly yield additional understanding of its roles in disease.

There are difficulties in unequivocally establishing a causal relationship between an endogenous chemical and a physiological or a pathological event, a subject analyzed by Lorenz et al. (1984). In this goal, the recent work on biogenic amines tends to follow a similar progression. The pharmacological activities of the substance are assessed. Analogs of the substance are synthesized. [Histamine itself, like some other biogenic amines, was synthesized (Windaus and Vogt, 1907) before it was known to exist in nature.] The analogs are examined for their effects on tissues, and from the relative agonist activities, the receptors are tentatively classified. Antagonists are then made. The antagonist not only may become a drug to benefit the sick, but it serves as a tool to probe and classify receptors more reliably than agonists can (see Green, 1983). The antagonists are then used to expolore the roles of the biogenic amine in the body. In this goal, the effects of the

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antagonist can provoke wrong inferences because at the concentrations that it may attain after systemic administration, the antagonist may block other receptors. For example, many H_1-antagonists block muscarinic receptors and the uptake of norepinephrine and 5-hydroxytryptamine (see Hough and Green, 1984; Prell and Green, 1986). Therefore, a pharmacological effect of an H_1-antagonist (or several of them) does not necessarily imply that the H_1-receptor is associated with the effect (see Hough and Green, 1984; Prell and Green, 1986). Analogously, the H_2-antagonist cimetidine was observed to antagonize, in a dose-dependent manner, the behavioral effects of a dopaminergic agonist in rats. Another H_2-antagonist ranitidine, failed to antagonize the effects, but imidazole acted like cimetidine (an imidazolyl derivative, unlike ranitidine). These observations suggest that the antagonist effect is not due to blockade of the H_2-receptor but may rest instead on the imidazoyl group (Ferrari and Baggio, 1985).

Inferences made on the effects of histamine antagonists can gain assurance if the effect or effects, before administration of the antagonist, is consonant with an effect of histamine; and, then, that the effect is prevented or its intensity reduced after administration of the antagonist. The demonstration that plasma histamine levels rise after administration of some drugs, that the rise is accompanied by physiological responses that are known to occur after histamine injections, and that the responses are reduced by prior administration of antagonists of histamine, are observations establishing a causal connection between histamine and a syndrome (see Doenicke and Lorenz, 1982; Moss and Rosow, 1983; Lorenz et al., 1984; Lorenz and Doenicke, 1985), thus fullfilling the criteria (Dale, 1929) that an endogenous chemical is related to an effect.

Only showing a rise in the plasma level of a mediator during a response is not sufficient to establish a causal relationship between the mediator and the signs and symptoms. The risk of doing so was shown by the early and erroneous attribution of the whole carcinoid syndrome to 5-hydroxytryptamine: although carcinoid tumors contain 5-hydroxytryptamine, and during the syndrome, plasma levels of 5-hydroxytryptamine are increased (see Page, 1968; Sokoloff, 1968), there was no correlation between the levels of 5-hydroxytryptamine and, at least, the flush which is the most conspicuous clinical sign (Robertson et al., 1962). This and other observations prompted Page (1968) to caution: "Another lesson is that it is always dangerous to attribute an entire syndrome to one substance or mechanism. While serotonin is doubtless intimately involved in the carcinoid system, there is good evidence that bradykinin is also concerned, probably both to varying degrees. But there may be a third and fourth substance as well." Analogously, both leukotrienes (lipoxygenase products of arachidonate) and histamine are released, apparently independently, from human bronchi and especially from human lung parenchyma by immunologic and non-immunologic (e.g., by an ionophore) stimulation (Salari et al., 1985). The likelihood of more than one substance being liberated in injury was suggested by Dale (1929) and amplified by Lorenz et al. (1984). Another circumstance that may obscure simple attribution is the interactions among mediators. For example, stimulation of the H_2-receptor in the myenteric plexus releases acetylcholine, 5-hydroxytryptamine, a peptide(s), and a cyclooxygenase-product(s) of arachidonate (Barker and Ebersole, 1982), implying that impingement of released histamine on the H_2-receptor can produce effects that are more complex than those commonly ascribed to histamine; but all would be reduced or prevented by an H_2-antagonist.

Metabolism of histamine

Histamine in tissues is believed to derive mostly from decarboxylation of histidine, (see Schayer, 1978), yet injections of histidine do not increase histamine levels in all tissues of mouse and rat containing decarboxylases (Taylor and Snyder, 1972; Schwartz et al., 1972; Huszti et al., 1977; Endo, 1979). This is a surprising finding, for histidine can be decarboxylated by both histidine decarboxylase, i.e., E.C. 4.1.1.22, and L-aromatic amino acid decarboxylase, i.e., E.C. 4.1.1.26 (see Aures et al., 1970). Furthermore, the concentrations of histidine in plasma and in tissues is less than the K_m of histidine for histidine decarboxylase which implies that the enzyme is not normally saturated and hence that the administration of histidine should raise tissue levels of histamine. Among the possible explanations for the failure of

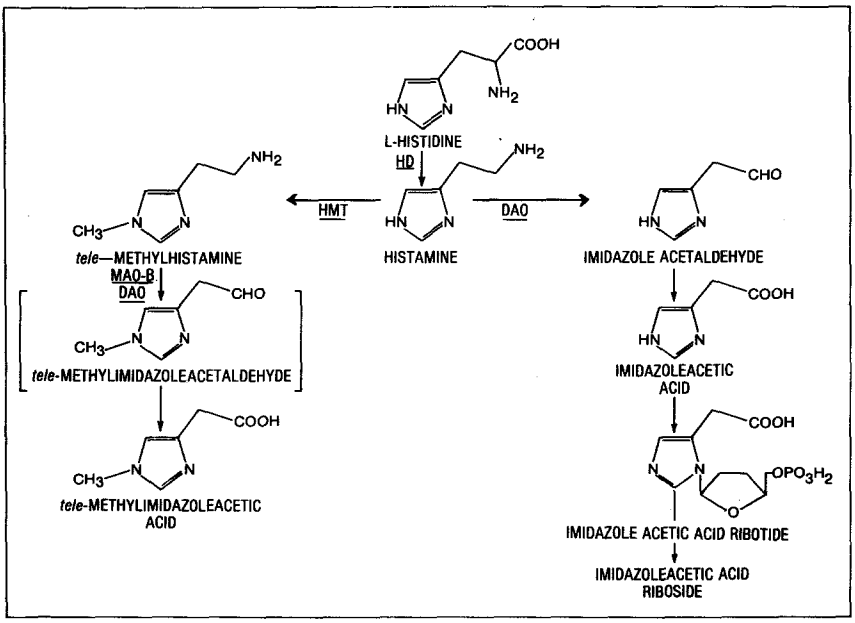

Figure 1
The two major metabolic pathways of histamine. HD = histidine decarboxylase; HMT = histamine methyltransferase; DAO = diamine oxidase; MAO-B = monoamine oxidase B.

histidine to raise levels of histamine in all tissues is that the transport system for histidine is already saturated or that other pathways of histidine metabolism are more active than histidine decarboxylation or that the histamine that is formed is rapidly metabolized.

A full and fair review of histamine metabolism would be voluminous, hence the discussion here is confined to some recent work and to work that shows questions that need to be answered. Reviews have appeared on the metabolism of histamine (e.g., Schayer, 1978; Wetterqvist, 1978; Beaven, 1982; Hough and Green, 1984; Code, 1985). As shown in Fig. 1, a major pathway is direct oxidative deamination, carried out predominantly by diamine oxidase to yield imidazoleacetic acid, i.e., IAA. IAA can arise from histidine without histamine as an intermediate by transamination of histidine to imidazolepyruvic acid which is oxidized to IAA (see Stifel and Herman, 1971).

Like other imidazoles (see Alivisatos, 1966), IAA can be converted to a riboside and ribotide, probably substituted on the *tele*-nitrogen (see below), both of which were shown in rat brain by chromatography after injection of radioactive histidine (Robinson and Green, 1964). The ribosyl derivative was shown in the urine of man after oral administration of radioactive histidine (Brown et al., 1960) and in the urine of goat (Eliassen, 1969) and in rat tissues (Snyder et al., 1964) after intravenous injection of radioactive histamine.

Also present in human urine and plasma and in rat organs is ribosylhistidine which is formed by an enzymatic pathway different from the one that forms ribosylimidazoleacetic acid (Imamura et al., 1984 a). The levels of ribosylhistidine in urine of histidinemic patients correlated with the levels of histidine, and the levels in rodent urine, plasma and organs increased after the diet was supplemented with histidine (Imamura et al., 1984 b). It is not known whether ribosylhistidine can be enzymatically decarboxylated to *t*-ribosylhistamine and whether the latter can then be oxidized to ribosylimidazoleacetic acid. If these enzymatic reactions can occur, the ribosylhistidine, which is found in brain (Imamura et al., 1984 a), offers an alternative source of ribosylimidazoleacetic acid (and ribotylimidazoleacetic acid) in brain (Robinson and Green, 1964).

The immediate precursor of IAA and the immediate oxidation product of histamine is imida-

zoleacetaldehyde (Fig. 1) which can be reduced to imidazolylethanol, i.e., histaminol. After administration of labeled histamine to goats (Eliassen, 1969) and man (Bergmark and Granerus, 1974), histaminol was found in urine. Histaminol was present in gastric juice and mucosa of dog after infusion of labeled histamine and treatment with pentagastrin (Code et al., 1976; see Code, 1985). Homogenates of goat kidney and liver produced histaminol from labeled histamine (Eliassen, 1969). Endogenous histaminol was found in rat and human urine, and its concentration increased after rats were treated with disulfiram to inhibit aldehyde dehydrogenase (Nakajima and Sano, 1964).

A major pathway of histamine metabolism is methylation of the pyrrole-like nitrogen (Fig. 1) by histamine methyltransferase. As histamine exists in two tautomeric forms (Fig. 2), it is feasible that methylation (or any substitution) could occur on either pyrrole-like nitrogen, thus giving rise to two isomers. Fig. 3 shows two methylhistamines that could, in principle, be formed by metabolism: *tele*-methylhistamine, also named N^{τ}-methylhistamine (i.e., *t*-MH) and *pros*-methylhistamine or N^{π}-methylhistamine (i.e., *p*-MH).

t-MH is by far the dominant methylated metabolite formed (see Schayer, 1978; Wetterqvist, 1978; Beaven, 1982; Hough and Green, 1984; Code, 1985). Endogenous *t*-MH has been found in human body fluids (see Keyzer et al., 1981; Swahn and Sedvall, 1981; Kahndelwal et al., 1982a; Imamura et al., 1984b) and in tissues (Green, 1970; Hough et al., 1979, 1981; Oishi et al., 1984; Imamura et al., 1984b).

The ability of tissues to form *p*-MH and its presence in tissues have been controversial issues. Recently, an enzyme purified from rabbit lung has been shown to methylate histamine to *p*-MH (Herman et al., 1985). The apparent K_m of this enzyme, indolethylamine *N*-methyltranserase, for histamine is 5.4 mM, which compares with a K_m of 11.0 μM of histamine N-methyltransferase from the same source. The high K_m suggests that *p*-MH could be formed only at very high concentrations of histamine, concentrations that could be attained after administration of exogenous histamine or after massive release of endogenous histamine or in circumstances that bring about a high local concentration of histamine. One such circumstance could be bacterial infection, e.g., it has been shown that *Haemophilis influenzae* isolated from the sputum of patients synthesizes high concentrations of histamine (Sheinman et al., 1986) which would add to the endogenous histamine found in the pulmonary tree (e.g., Agius et al., 1985).

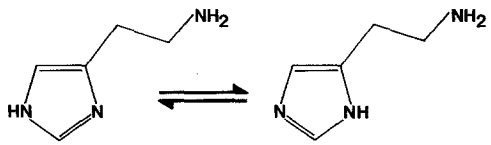

Figure 2
The two tautomers of histamine.

Injecting mice with labeled histamine for several days resulted in the appearance in urine of *pros*-methylimidazoleacetic acid (i.e., *p*-MIAA), demonstrated by chromatography, implying *p*-MH as an intermediate (Karjala and Turnquest, 1955). No *p*-MH has been detected in human body fluids by a method that readily showed *t*-MH (Khandelwal et al., 1982a). In rat brain no *p*-MH could be detected even after treatment with an irreversible inhibitor of monoamine oxidase (Hough et al., 1984), which raised levels of *t*-MH (Hough et al., 1984; Oishi et al., 1984). However, *p*-MIAA was found in human body fluids and rat brain (see Khandelwal et al., 1982b), perhaps arising from endogenous *pros*-methylhistidine by transamination followed by oxidation. *pros*-Methylhistidine as well as *tele*-methylhistidine have been found in human plasma (Stein and Moore, 1954) and urine (Imamura, 1984a).

About three hours after feeding, the levels of both *t*-MH and histamine increased in the plasma of man and in the urine of man and mouse (Imamura et al., 1984b). The increase in human urinary levels occurred after ingestion of either protein or non-proteinaceous mannan. These food-induced rises in urinary levels in rat were accompanied by a rise in *t*-MH and a slight fall in histamine concentrations in stomach and an increase in histidine decarboxylase activity in stomach. Intravenous alimentation reduced urinary levels of both histamine and *t*-MH in man. After gastrectomy, the human urinary levels of both compounds were reduced to less than one-third of the pre-operative levels. These observations suggest that ingestion of food stimulates histamine release and that the stomach makes considerable

contribution to the urinary levels of histamine and *t*-MH (Imamura et al., 1984b). The changes in *t*-MH were paralleled by changes in a compound stated to be IAA (Imamura et al., 1984b). In dog, the administration of pentagastrin increased, in gastric juice, the amount of labeled *t*-MH arising from intravenously infused labeled histamine with little change in the amount of labeled histamine, leading to the inference that gastrin increases histamine metabolism (see Code, 1985).

t-MH is oxidatively deaminated, primarily by monoamine oxidase B, to *tele*-methylimidazoleacetic acid (*t*-MIAA) which has been found in body fluids of man (Khandelwal, 1982b; Keyzer et al., 1982; Swahn and Sedvall, 1983) and in tissues (Khandelwal, 1982b). Its levels fall in the brain of rat treated with a monoamine oxidase inhibitor, paralleling the rise in *t*-MH (Khandelwal et al., 1984). These observations on turnover of histamine in rat brain (Hough et al., 1984; Oishi et al., 1984) and the fall in *t*-MH and *t*-MIAA in rat brain after treatment with an inhibitor of histamine methyltransferase (Hough et al., 1986) suggest precursor-product relationships among histamine, *t*-MH, and *t*-MIAA in rat brain.

Although the *tele*-nitrogen is the major recipient of the methyl group in histamine metabolism, the side chain amino group, N^{α}, may also be methylated. Small amounts of N^{α}-methylhistamine and N^{α},N^{α}-dimethylhistamine have been shown in gastric juice and gastric mucosa after intravenous infusion of labeled histamine (Navert et al., 1969; see Code, 1985). Neither compound was formed from labeled histamine by gastric mucosal homogenates of rat, guinea pig, cat, dog or pig (Maslinski et al., 1977). The indolethylamine *N*-methyltransferase in rabbit lung that formed *p*-MH, described above, also formed N^{α}-methylhistamine (Herman et al., 1985). Only the endogenous dimethylated derivative was shown in human gastric juice and urine (not serum) by gas chromatography (Navert et al., 1985); but small amounts of N^{α}-methylhistamine were detected by radioenzymatic assay in human gastric juice but not in human skin, blood, urine (Haimart et al., 1985) or brain of rodents (Nilam and Smith, 1981).

γ-Glutamylhistamine, a major product of histamine in *Aplysia californica* (Weinreich, 1979), can be formed by mammalian tissues. Labeled histamine (in analogy with other amines) injected into the ventricles of rats, was converted to γ-glutamylhistamine (Konishi and Kakimoto, 1976; Tsuji et al., 1977), a reaction that was carried out by γ-glutamyltranspeptidase from both brain and kidney of rat (Tsuji et al., 1977). γ-Glutamylhistamine, containing labeled histamine, was found in protein linkage after neoplastic murine mast cells were grown with labeled histidine or histamine, but no free γ-glutamylhistamine could be detected (Fesus et al., 1985). The amount of protein-bound γ-glutamylhistamine paralleled the increase in transglutaminase activity, and both were increased when the cells released histamine in an IgE-dependent mechanism or on exposure to an ionophore (Fesus et al., 1985). These authors recalled earlier work: after treatment of mice with the endotoxin of *Salmonella typhosa* or the vaccine for *Haemophilus pertussis,* histamine was incorporated into proteins in the liver. The incorporation, which was not seen in untreated mice, was also accompanied by increased transglutaminase activity (Ginsburg et al., 1963). Since mast cells, including those in liver (Haas et al., 1979), have proteolytic enzymes (Lagunoff and Benditt, 1963; Ende et al., 1964; see Mundy and Strittmatter, 1985; Wintroub et al., 1986), a peptide or peptides containing γ-glutamylhistamine might be released under special circumstances. Other peptides of histamine may exist. The supernatant fraction of pig hypothalamus was shown to incorporate histamine into complexes of *N*-acetylaspartate, glycine, glutamic acid, and γ-aminobutyric acid (Reichelt et al., 1976).

After ingestion of labeled histamine, labeled *N*-acetylhistamine was found in the urine (Urbach, 1949; Tabor and Mosettig, 1949), most of which is a product of bacterial flora (Urbach, 1949). There is no question of the presence of *N*-acetylhistamine, for it was crystalized from urine (Tabor and Mosettig, 1949). After intravenous administration of labeled histamine to dogs, pentagastrin infusion resulted in a rise in labeled *N*-acetylhistamine (see Code, 1985). Small amounts of endogenous *N*-acetylhistamine have been suggested by chromatography in some tissues of rat (Endo, 1979). *N*-acetylhistamine is deacetylated by an enzyme present in some tissues of rat, mouse, and guinea pig (Endo, 1979; Hegstrand and Kalinke, 1985). The deacetylase must be present in man too, for administration of

N-acetylhistamine increased human urinary levels of histamine (Sjaastad, 1967).

There is evidence that in different species and in different organs, the dominant pathways may differ (see Wetterqvist, 1978). For example, in mammalian brain, the formation of *t*-MH is the major, perhaps only, pathway (see Hough and Green, 1984) whereas in the nervous system of some inverterbrates, IAA is the main metabolite of histamine (see Prell and Green, 1986). A major impediment to confident conclusions about the importance of the various pathways is that many observations were made after administration of exogenous histamine which, as emphasized before (see Furano and Green, 1963) and discussed below, does not mix with the endogenous cellular pool of histamine. Another factor, also often ignored, that could confuse interpretation is the rate of egress of the metabolites from tissues. For example, it cannot be presumed that *t*-MIAA and IAA leave a tissue at the same rate. *t*-MIAA (Schwartz et al., 1971; Khandelwal et al., 1984), like dihydroxypenylacetic acid (Wilk et al., 1975) and unlike homovanillic acid and 5-hydroxyindoleacetic acid (Wilk et al., 1975; Westerink and Korf, 1976), does not increase in brain after treatment with probenecid. *t*-MIAA, and IAA as well, could be substrates for another transport system in tissues, perhaps the one for monocarboxylic acids (Miller and Oldendorf, 1986). The rates of transport of *t*-MIAA and IAA out of a tissue may differ, thereby influencing their relative rates of accumulation, thus complicating te interpretation of which catabolic pathway of histamine dominates in a tissue.

Issues in the study of histamine metabolism

Methods

Not all the methods used to measure histamine metabolites (on which the summary, above, is based) have been shown to be specific. For some of the putative metabolites, even their qualitative identification is not certain. The shortcomings of methods and the inappropriate use of them are problems that have long beset and beclouded research on histamine and on its metabolism. Work on histamine has confirmed the truism that a method that specifically measures a substance in one biological sample may not be specific when applied to a different biological sample, as observed in some applications of the original fluorometric method for measuring histamine (Carlini and Green, 1963; Lorenz et al., 1972). Analogous problems exist in the radioenzymatic method for measuring histamine (Hegstrand and Hine, 1985; Roberts et al., 1985), e.g., the radioenzymatic method missed a large increase in urinary histamine levels that was clearly revealed by gas chromatography-mass spectrometry (Roberts et al., 1985).

Methods to measure metabolites of histamine have been recently summarized (Navert et al., 1985). Methods using combined gas chromatography-mass spectrometry, which requires a relatively expensive instrument and a specialized operator, have been applied to the measurement of histamine (Mita et al., 1980; Keyzer et al., 1983a; 1984a; Roberts and Oates, 1984), *t*-MH (Hough et al., 1979, 1981; Keyzer et al., 1981; Swahn and Sedvall, 1981), *t*-MIAA (Khandelwal et al., 1982b; Swan and Sedvall, 1983), IAA (Khandelwal et al., 1985), and other imidazole acids, e.g., *p*-MIAA and urocanic acid (Khandelwal et al., 1982b). One major advantage of methods based on this instrumentation is that specificity can be precisely and swiftly tested. For example, in the measurement of *t*-MH by gas chromatography-mass spectrometry, the derivative of *t*-MH yields, on electron impact, two fragment ions having masses of 304 and 291 (Fig. 3) (Hough et al., 1979, 1981). Both ions must be seen at the same retention time; and the relative abundance of each ion is a characteristic of the molecule. The gas chromatographic-mass spectrometric assay for *t*-MH was shown to be applicable to brain (Hough et al., 1979, 1981), plasma, urine, cerebrospinal fluid (Khandelwal et al., 1982a), and some peripheral tissues. But when this method of selected ion monitoring was applied to lymph (Fig. 3), only one of the two mass ions, 304, ap-

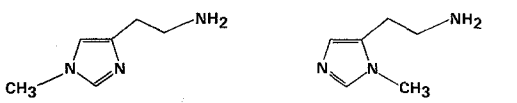

Figure 3
Two methylated isomers of histamine.

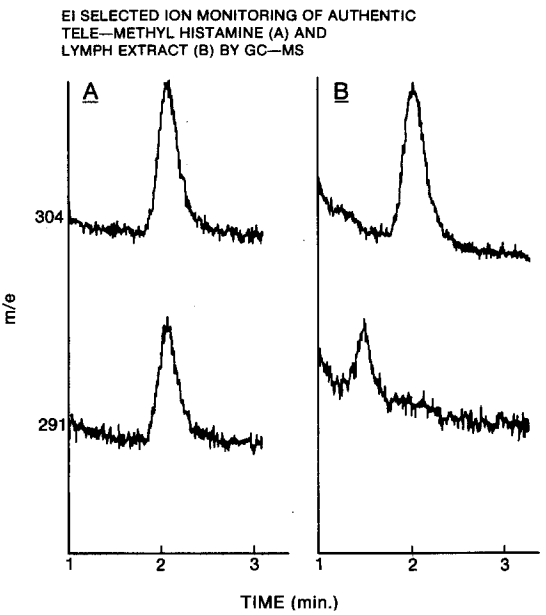

Figure 4
Electron ionization selected ion monitoring scans of bis-heptafluorobutyric anhydride derivatives of authentic *tele*-methylhistamine (A) and of a lymph (1 ml) extract (B) by the method of gas chromatography-mass spectrometry (Hough et al., 1981). Two prominent mass ions in the mass spectrum of the *t*-MH derivative, 291 and 304, were simultaneously monitored to identify the presence of *t*-MH in lymph extract by comparing the retention time and the ratio of the areas of the two ion peaks. Unlike authentic *t*-MH, the lymph extract did not show the concomitant 291 ion peak at the correct retention time.

peared at the appropriate retention time. The absence of mass ion 291 at that retention time shows that this sample of lymph has no detectable *t*-MH. If instead of using a mass spectrometer as a detector, we had measured the compound with a flame-ionization detector or nitrogen detector or electron capture detector instead of a mass spectrometer as a detector, none of which alone offers the extraordinary parameters for specificity that are offered by mass spectrometry, we could have been led to conclude that the derivative from lymph, having the precise retention time of the *t*-MH derivative, was *t*-MH. Additional confirmation of the identity of the compound being measured and hence the specificity of the method can be obtained with the use of chemical ionization by which the whole compound is protonated, and the product, at the appropriate retention time, has a mass equal to one unit higher than its molecular weight. Thus, the mass spectrometer can provide a rigorous proof of specificity that other methods can provide only after tedium.

The burden as well as the success of demonstrating the presence of an endogenous substance by gas chromatography without benefit of mass spectrometry are exemplified by work on a compound not related to histamine, the work showing the presence of pyrrolidone carboxylic acid in body fluids and tissues (Wilk and Orlowski, 1973). A perchloric acid extract of the biological sample was passed through two ion exchange columns and a DEAE Sephadex column, and the effluent was derivatized. The derivative had the same retention times as the derivative of pyrrolidone carboxylic acid on gas chromatography on eight different columns coated with liquid phases of varying polarities. As an additional probe, before derivatization, aliquots were removed and subjected to acid hydrolysis to convert pyrrolidone carboxylic acid to glutamate. The acid hydrolysate was derivatized. Coincident with the loss of the pyrrolidone carboxylic acid peak was the appearance of the glutamate peak. As yet another probe, the product resulting from acid hydrolysis, postulated to be glutamate, was incubated with L-glutamic acid decarboxylase; as postulated, γ-aminobutyrate was formed, as shown by derivatization and gas chromatography. The use of the decarboxylase also revealed that all the pyrrolidone carboxylic acid in cerebrospinal fluid was of the L-configuration whereas in urine about 40 percent was of the D-configuration. To learn if pyrrolidone carboxylic acid could have arisen as an artifact of the method – since glutamine can nonenzymically cyclize to pyrrolidone carboxylic acid – glutamine was carried through the whole procedure and shown to make negligible contribution to measurements of endogenous pyrrolidone carboxylic acid (Wilk and Orlowski, 1973). In a similar study showing the absence in brain of a compound that had been stated to be present at a level of over 100 ng/g of tissue, the variables of gas chromatography that can be explored to probe a qualitative or quantitative assertion were again demonstrated and described (Wilk and Zimmerberg, 1973). Though these are examples of the scrupulous use of gas chromatographic methods, they can serve as paradigms of the rigor essential to any method used to identify and to quantify a substance. A similarly probing skepti-

cism should be applied to the identification and quantification of histamine metabolites.

Endogenous and exogenous histamine

Many of the studies on histamine metabolism were done by identifying the metabolites after administration of *exogenous* histamine. The yields of insight and metabolites have been bountiful, and the results, at the least, depict the metabolism of histamine released into the circulation, as for example, after release from mast cells by IgE or drugs.

But the metabolism of exogenous histamine may not quantitatively or even qualitatively reflect the normal metabolism of *endogenous* histamine. In human urine, the ratio of the levels of endogenous t-MIAA to endogenous histamine is about 100 (Khandelwal et al., 1982b); after administration of labeled histamine, the ratio of exogenous (i.e., labeled) *t*-MIAA to exogenous histamine in urine is about 17 (Schayer and Cooper, 1956).

On systemic administration, histamine, like any substance, is distributed according to the blood flow to, and uptake by, the organs. Hence, the liver, and kidney, which have high capacity for histamine uptake (Snyder et al., 1964), could make high contribution to the pool of metabolites measured in blood or urine. In most species, these tissues make relatively slight contribution to endogenous histamine. In contrast, the brain takes up little or no exogenous histamine (Robinson and Green, 1964; Schayer and Reilly, 1970), but it metabolizes endogenous histamine (see Hough et al., 1984; Oishi et al., 1984).

A determinant of the metabolism of exogenous histamine is the uptake of histamine by different cells. There is no reason to presume that the rate and capacity of uptake of histamine by a cell is in any way reflective of the capacity of that cell to form histamine and to metabolize the histamine that it forms. There is, in fact, evidence from studies on vascular tissue that the uptake of labeled histamine diminishes as the amount of histamine stored in the tissue increases (Adams and Hudgins, 1976; Holcslaw et al., 1984).

Once inside the cell, exogenous histamine is exposed to both histamine methyltransferase and diamine oxidase, both of which are present in the soluble fraction of the cell, presumably the cytosol (Brown et al., 1959; Snyder et al., 1974; Taylor and Liebert, 1979), en route to being bound to particulate fractions (Robinson et al., 1965). It is not known that endogenously formed histamine has similar vulnerability; it is known (see below) that endogenous histamine is largely bound to particulate material.

Within the same cell, it canot be presumed that exogenous histamine mixes with the pool of endogenous histamine. There is, in fact, evidence to the contrary. Neoplastic mast cells in culture, like normal mast cells (Furano and Green, 1964a), take up exogenous histamine and 5-hydroxytryptamine (Day and Green, 1962a) as well as synthesize these amines (Day and Green, 1962b; Green, 1966). The endogenous and exogenous amines, neither of which is catabolized by these cells, are extruded from the cells at markedly different rates: endogenous histamine had a half-life of 27 hours but exogenous histamine, after an initial loss, persisted unchanged in these cells for at least 72 hours; endogenous 5-hydroxytryptamine had a half-life of 18 hours whereas exogenous 5-hydroxytryptamine had a half-life of 60 hours (Day and Green, 1962c). The two pools were physically separable. Density gradient centrifugation of homogenates of these cells, grown in a medium containing labeled histamine or 5-hydroxytryptamine, showed that the labeled, exogenous amines were found in fractions sedimenting at different densities from the particulate fractions containing the endogenous amines (Green and Furano, 1962). The different compartments for the endogenous and exogenous amines could explain the differences in the rate of release from the cell.

Further experiments (Furano and Green, 1964b) showed that when the mast cells had low levels of endogenous histamine [the levels of which spontaneously fluctuate in these cells (also see Green, 1968)], exogenous histamine entered the endogenous, particulate pool (Furano and Green, 1964b). When these same cells were pre-incubated with unlabeled histamine to saturate the particulate pool, the additional exogenous histamine was found mainly in the non-particulate pool in the cytosol (Furano and Green, 1964b). These observations are in accord with the idea that the binding site for endogenous amines, when not occupied by these amines, are accessible to exogenous amines.

The implications of these experiments are probably not restricted to neoplastic mast cells or confined to an explanation of the kinetics of egress of a substance. Perfusion of the cerebral ventricles of the cat with labeled histamine or labeled histidine revealed that the yield of labeled methylhistamine, relative to labeled histamine, was much higher from labeled histamine than from labeled histidine, fostering the suggestion the "endogenous brain histamine has a fate different from histamine introduced into the brain" (White, 1960). In brain, endogenous histamine is found in particulate fractions (Carlini and Green, 1963; see Hough and Green, 1984) while histamine methyltransferase is found in the cytosol (Brown et al., 1959; Snyder et al., 1974). In rat brain slices, the spontaneous efflux of exogenous histamine was six-fold higher than that of endogenous histamine, and the release of exogenous histamine was not influenced by conditions that modulated the release of endogenous histamine (Arrang et al., 1985). Human lung minces readily formed *t*-MH from exogenous histamine; but no labeled *t*-MH was formed from endogenous histamine (Lilja et al., 1960). These authors suggested that the labeled endogenous histamine may have been "bound in (mast?) cells" and inaccessible to the methylating enzyme. In lung, endogenous histamine is bound to particulate material in mast cells (see Lagunoff and Benditt, 1963). It should be noted that these experiments on the methylation of histamine were carried out on lung minces and whole brain, in both of which cellular integrity is preserved. Other experiments have shown that the organization of the cell must be retained to reveal the true kinetics of histamine-methylation. *In vivo,* male rats have a greater capacity to methylate histamine than do female rats (Westling and Wetterqvist, 1962). This difference is manifest by kidney minces (Westling and Wetterqvist, 1962), and slices (Aziz, 1961), but not by kidney homogenates (Netter et al., 1961). In the intact cell, a rate-limiting reaction may exist that is not apparent in homogenized cells.

Differences in the disposition of endogenous and exogenous histamine were, as noted above, also described for 5-hydroxytryptamine in neoplastic mast cells. Recently analogous differences in the disposition of endogenous and exogenous catecholamines in the brain have been emphasized (e.g., Herdon et al., 1985; see Kopin, 1985). In addition, differences in the metabolism of endogenous and exogenous amino acids, purines and pyrimidines, both in mammalian cells and bacteria, were emphasized in an early review and attributed to differences in their compartmentation and access to the metabolizing systems (Furano and Green, 1963).

There are problems too with the use of radioactive histidine in studying metabolism. Among the problems is that not all metabolites that have been characterized in studies with exogenous histamine *in vitro* and *in vivo* were found in studies with radioactive histidine. For example, labeled *t*-MH is found in urine after administration of labeled histamine (Snyder et al., 1964) but no labeled methylated histamine metabolites in urine were found after administration of labeled histidine (Brown et al., 1960). Random samples of urine have high concentration of the methylated histamine metabolites (as measured without use of isotopes, as noted above). Table 1 shows examples of some of the human urinary levels of histamine and its main metabolites.

The measurement of the steady-state levels of the histamine metabolites as well as of histamine provides an additional and critical tool to evaluate the contribution of different pathways to hista-

Table 1
Examples of normal human urinary levels (nmol/mg creatinine ±S.E.M.) of histamine (HA), *tele*-methylhistamine (t-MH), *tele*-methylimidazoleacetic acid (t-MIAA) and imidazoleacetic acid (IAA).

HA	t-MH	t-MIAA	IAA
0.047±0.007[a]	1.03±0.25[a]	20.8±1.3[b]	8.0±1.1[c]
0.148±0.015[d]	1.10±0.09[d]	13.8±0.8[d]	–
0.132±0.067[f]	0.81±0.51[f]	–	6.7±0.3[e]
0.236[g]	0.759[h]	–	–

[a] Random urine samples ($n=7$) collected in the morning (Khandelwal et al., 1982a).
[b] Random urine samples ($n=9$) collected in the morning (Khandelwal et al., 1982b).
[c] Random urine samples ($n=4$) collected in the morning (Khandelwal et al., 1985).
[d] 24 hr specimens ($n=20$) (Granerus, 1968).
[e] 24 hr specimens ($n=10$) (Imamura et al., 1984c).
[f] One-hour collection before feeding ($n=5$) (Imamura et al., 1984b).
[g] 24 hr specimens ($n=10$) (Keyzer et al., 1983a).
[h] 24 hr specimens ($n=8$) (Keyzer et al., 1983b).
[d–h] Based on an average daily urine volume of 1100 ml and creatinine excretion of 12 mmol.

mine metabolism, as has been exemplified in early studies of human urine (e.g., Green et al., 1964; Fram and Green, 1965; Granerus, 1968) and in recent studies of rat brain. Treatment of rats with an irreversible inhibitor of monoamine oxidase produced an increase in levels of *t*-MH in different regions of the brain, increases that were used to estimate the turnover of histamine in these regions, with the assumption of a one-compartment model (Hough et al., 1984; Oishi et al., 1984). The increase in *t*-MH in these brain regions (Hough et al., 1984) was highly correlated, i.e., $r=0.915$, with the fall in *t*-MIAA (Khandelwal et al. 1984), a correlation that is consonant with a precursor-product relationship. After treatment of rats with metoprine (Hough et al., 1986), which inhibits histamine methyltransferase, there was an equivalent decrease in the levels of *t*-MH and *t*-MIAA in brain. These observations suggest that *t*-MIAA may arise only from *t*-MH. As metoprine is a competitive (not irreversible) inhibitor of histamine methyltransferase, the drug did not eliminate *t*-MH but reduced it by 75 per cent; but the remaining *t*-MH in brain was highly correlated, i.e., $r=0.88$, with the residual histamine methyltransferase activity in the individual brains (Hough et al., 1986). In concert, these and other observations (see Hough and Green, 1984) show that in brain, *t*-MH and *t*-MIAA arise from histamine and the rate of *t*-MH formation equals histamine methylation. Thus, the use of methods to measure nonradioactive metabolites, combined with the use of enzyme inhibitors, provided additional insights into histamine metabolism.

It should be emphasized that the different data that are obtained with different methods are all of interest. In fact, any paradoxical or contradictory data that different methods produce may be exploited to reveal regulatory mechanisms that could elude any single method.

The value of studying metabolites

In work of great rigor showing the role of histamine release in reactions to drugs used in anesthesia (see Lorenz and Doenicke, 1985) and in food allergy (Reimann et al., 1985), histamine was measured in the plasma and, in the latter study, in the gastric mucosa as well. In most clinical research projects designed to learn the role of a mediator in a physiological or pathological process, the investigator does not have opportunity to take plasma samples while the patient is having a reaction, especially if the reaction is unexpected. And not in all studies is the reactive tissue as readily accessible as gastric mucosa. The study is often then restricted to an examination of urine. Under these circumstances, measurements of metabolites are essential because histamine is metabolized. Aside from the contribution the kidney and urinary tract may make to urinary histamine levels, its levels in urine represent residual histamine, i.e., histamine that has escaped metabolism.

The urinary levels of *t*-MH (Berg et al., 1971; Keyzer et al., 1983 b) and *t*-MIAA (Berg et al., 1971) are abnormally high in patients with chronic myelocytic anemia. Of nine patients, two had urinary histamine levels within the normal range, but all had abnormally high levels of *t*-MH and *t*-MIAA (Berg et al., 1971). Moreover, the mean elevation of histamine was less than six-fold whereas the respective elevations of *t*-MH and *t*-MIAA were 21-fold and 17-fold.

Of eight patients with mastocytosis, five had urinary levels of histamine within the normal range, but all had levels of *t*-MH and *t*-MIAA that were 17-fold higher than normal (Keyzer et al., 1983 c). Analogous findings were obtained in measurements of histamine, t-MH, and t-MIAA in the urine of patients who experienced adverse reactions to a radiographic contrast media (Keyzer et al., 1984 b). The value of measuring urinary metabolites of a biogenic amine that is suspected of a role in diesease was established many years ago. For example, in a study of 45 patients with pheochromocytoma, six had normal urinary levels of norepinephrine but all had abnormally high levels of a norepinephrine metabolite, vanillylmandelic acid (Gitlow et al., 1961). Without the luxury of having a needle in the vein of a patient experiencing a reaction to an endogenous substance, the investigator is reduced to looking at metabolites to make a diagnosis.

Measurements of metabolites in plasma will also prove useful. At the least, they supplement measurements of histamine in plasma. In an anaphylactoid reaction, a rise in *t*-MH in plasma reached its peak three minutes after the peak in histamine levels and persisted for longer than the elevated levels of histamine (Keyzer et al., 1985). Measuring plasma levels of histamine metabolites

can be revealing in other ways. If the histamine released into plasma is metabolized, as could occur to histamine released into the portal circulation, then histamine metabolites may be more reflective of the release than the amount of histamine. If histamine turnover is increased, measurements of the metabolites are essential, e.g., pentagastrin appeared to increase histamine metabolites in gastric fluid, not the amount of histamine (Code, 1985). If a drug inhibits the enzymes that metabolize histamine and also releases histamine (Sattler et al., 1985; Harle et al., 1985), then the relative contributions of enzyme inhibition and of histamine release to an adverse reaction can be accurately assesed only by measuring both histamine and its metabolites.

For studies of organs and tissues, to test, for example, hypotheses that drugs owe their actions to effects on biogenic amines, it is essential that metabolites of the amines be measured. Irreversible inhibition of monoamine oxidase activity by pargyline significantly altered the levels of the metabolite of 5-hydroxytryptamine and two metabolites of dopamine in all regions of the rat brain, but in two regions, the levels of dopamine were unchanged: furthermore, even in those regions where dopamine and 5-hydroxytryptamine were increased, the increase was less, usually far less, than the decreases in the metabolites (Nielsen and Johnston, 1982). Similarly, treatment of rats with estradiol produced an increase in the metabolites of dopamine in brain without an increase in dopamine (Di Paolo et al., 1985). These findings are in accord with the well established principle that the turnover of a substance may be altered without change in the concentration of the substance. It is plausible to suggest that the measurements of histamine metabolites as well as of histamine in the gastric mucosa during an allergic response (Reimann et al., 1985) may contribute to the understanding of the response. Studies on the concentration of histamine in gastric mucosal biopsies of patients with peptic ulcer showed that the rate of loss of histamine in the biopsies from control subjects was faster than in those from patients with duodenal ulcer (Thon et al., 1985). Measuring the metabolites that could contribute to the loss of histamine could be revealing and may enhance the understanding of peptic ulcer disease, especially in view of work suggesting that gastric acid secretion is accompanied by increased metabolism of histamine (Code, 1985).

Yet another reason to measure metabolites is that the metabolites themselves may be active and may therefore contribute to a syndrome accompanying altered levels of the parent amine. It was shown that *t*-MIAA reduces the binding of benzodiazepine to its receptors (Blandina et al., 1985). Therefore, drugs that reduce the levels of *t*-MIAA, such as drugs that inhibit monoamine oxidase (Khandelwal et al., 1984), may owe some of their effects not only to the accumulation of biogenic amines but to the fall in the metabolites. IAA has activities like those of γ-aminobutyric acid and at similar concentrations (see Green, 1970; Hough and Green, 1984). The presumption that metabolites are "inactivation" products clearly needs examination.

Conclusions

Progress in learning the role of histamine in physiology and pathology has been impeded by difficulties in accurately measuring histamine and by the deficiencies of methods to measure its metabolites. The availability of specific, sensitive and rapid methods to measure histamine has helped in understanding the role of histamine in disease. Measuring histamine alone may provide an incomplete indication of the role of histamine in disease or in any other process. For histamine is metabolized by multiple pathways, and the kinetics of these enzymatic activities (as well as the rate of synthesis of histamine) determine the steady-state levels of histamine in tissue and in body fluids. Measurements of both histamine and its metabolites would contribute, and may be essential, to the understanding of the role of histamine in disease, just as measurements of the metabolites of other biogenic amines have been critical to understanding of their roles in diseases. Yet another reason that compels measurements of metabolites is evidence that some of the metabolites of histamine are pharmacologically, perhaps physiologically, active.

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