

# HISTAMINE METABOLISM IN HUMAN DISEASE \* †

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The mode of histamine catabolism in various experimental animals has been carefully studied in recent years. Such studies have been facilitated by the availability of ring-labeled histamine- $C^{14}$ . Two main metabolic pathways have been found (Figure 1). In one, histamine is oxidized to imidazole-4(5)-acetic acid (ImAA) through imidazole-4(5)-acetaldehyde. This reaction *in vitro* can be catalyzed by a diamine oxidase found in kidney tissue of most species. A xanthine oxidase or an aldehyde dehydrogenase is needed to complete the reaction. Much of the ImAA is then conjugated with ribose and excreted in the urine as such (1).

A second pathway proceeds through methylation of the ring imino-nitrogen to form 1,4-methylhistamine followed by oxidation of the side chain, presumably through the corresponding aldehyde. *In vitro* reproduction of the methylation step has recently been accomplished with an imidazole-N-methyl transferase found in many tissues (2-5). The oxidation is catalyzed by a monoamine oxidase found in mouse and cat liver and in human kidney. Most of the radioactivity administered as histamine is excreted as 1,4-methylimidazoleacetic acid (meImAA) by mice and cats.

Studies on histamine- $C^{14}$  metabolism in humans have been published recently by Schayer and Cooper (6) and Nilsson, Lindell, Schayer and Westling (7), and indicate that both pathways of catabolism are involved, with the principal metabolites being meImAA and ImAA (free and conjugated). In pregnancy, little alteration in this pattern occurred; no disease states were reported.

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Abnormalities of histamine catabolism have been suspected in several diseases. Investigations designed to discover such abnormalities have not been particularly successful (8-13). Failure in some cases can be attributed to inappropriate measurements and in others to inadequate methods. The assay of whole blood histamine, for instance, reflects mainly the amount of histamine contained in the leukocytes, although assays on plasma should be more appropriate for determining the rate of release or catabolism of histamine. Studies on histamine excreted in the urine have given contradictory results; such assays are misleading and of questionable value since less than 5 per cent of intravenously or subcutaneously administered histamine is excreted unchanged (6, 14).

The objectives of this study were: 1) to establish the normal pattern of metabolism of exogenous histamine in man; 2) to look for an altered pattern of metabolism in disorders (asthma, histaminic cephalalgia) in which an altered reactivity to histamine is suspected clinically; and 3) to determine the pattern of metabolism in patients with insufficient liver or kidney function which conceivably could involve histamine-metabolizing enzymes.

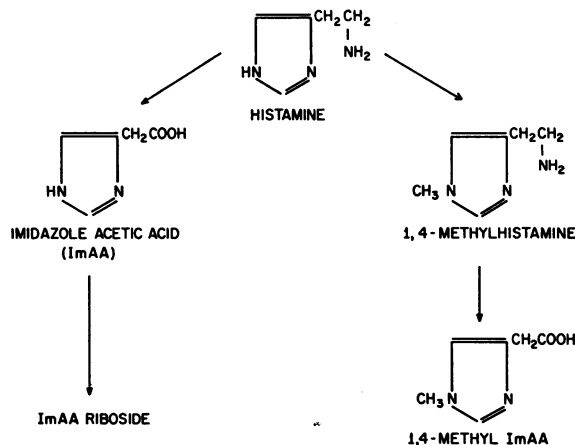


FIG. 1. PATHWAYS OF HISTAMINE CATABOLISM.

## METHODS AND MATERIALS

Nineteen subjects participated in the study. There were 3 with bronchial asthma, 1 with uremia from chronic glomerulonephritis, 5 with chronic liver insufficiency, and 3 with histaminic cephalgia. Seven control subjects included 2 healthy young men and 5 convalescent patients with presumably unrelated diseases. The patients with Laennec's cirrhosis had far-advanced disease with parenchymal damage and jaundice. Three of the 5 were dead within a year after the study. The 3 patients with histaminic cephalgia had typical attacks of unilateral throbbing headache, usually associated with ipsilateral nasal congestion and lacrimation. Two of the patients had previously had attempts at histamine "desensitization" with equivocal results.

The histamine- $C^{14}$ , labeled in the 2 position of the ring, had a specific activity of 4.52 mc per mmole.<sup>1</sup> Fifty to 100  $\mu$ g was injected intravenously in 30 seconds. Samples of venous blood were removed at intervals from 1 minute to 48 hours after the injection, and all urine was collected for at least 48 hours. Radioactivity of the plasma and urine samples was assayed with a liquid scintillation spectrometer by methods previously described (15). Samples containing more than 70 disintegrations per minute were counted long enough to provide an accuracy of  $\pm 5$  per cent at the 0.9 confidence limit. Samples with less than 70 disintegrations per minute were counted for 1 hour. The statistical limits were obtained from the nomograms of Jarrett (16).

The amount of unchanged histamine- $C^{14}$  in plasma was determined by subjecting some of the plasma samples to the concentrating and extracting steps of the chemical assay for histamine developed by Lowry and colleagues (17), followed by radioactive assay of the final extract. The remainder of the radioactivity was assumed to represent histamine metabolites.<sup>2</sup> The plasma radioactivity was concentrated 50- to 100-fold by this extraction procedure, thus providing sufficient radioactivity in the very small volume required for liquid scintillation assays (15).

Histamine- $C^{14}$  metabolism in a normal subject was studied during periods of administration of a potent antihistaminic drug, chlorphenpyridamine, and a histamine analog, 3- $\beta$ -aminoethylpyrazole.<sup>3</sup> The chlorphenpyridamine was given in doses of 8 mg every 4 hours, starting 12 hours before the study. The 3- $\beta$ -

aminoethylpyrazole was given subcutaneously in 3 doses of 50 mg 2 hours before, immediately after and 2 hours after the injection of histamine- $C^{14}$ .

One subject, GH, with histaminic cephalgia, had his studies repeated after "desensitization" to histamine. The "desensitization" was carried out by giving histamine subcutaneously 3 times per day for 8 days, increasing the dosage to a maximum of 200 mg.

Separation of the radioactive metabolites in the urine was accomplished by paper chromatography. A 10 or 20 ml aliquot of urine, voided during the first 3 hours after injection, was dried and extracted with methanol in a Soxhlet apparatus for 2 hours. A few samples obtained at later times were similarly treated. The methanol extract was concentrated *in vacuo* to a volume of 5 ml or less and the resulting precipitate discarded. The concentrated methanol solution was found to contain about 90 per cent of the radioactivity originally present in the urine specimen. (The range was 76 to 108 per cent in 18 extractions.)

Two-dimensional ascending paper chromatography was performed at room temperature with Whatman no. 1 paper (19 cm square). In one corner, 2 cm from either edge, 0.02 to 0.5 ml of the concentrated methanol extract was placed on the paper. Initial development was with *n*-butanol, concentrated  $NH_4OH$ , and 95 per cent ethanol in proportions of 8:3:1 (1). After the paper was rotated 90°, development was performed with *n*-butanol, glacial acetic acid, and water in proportions of 4:1:5. We found that two development "passes" of 3 to 4 hours each in each direction (with drying of the paper between each run) provided optimal separation of the radioactive areas.

Histamine- $C^{14}$  metabolites were detected by exposing Kodak no-screen X-ray film to the chromatogram for at least 2 weeks. Some chromatograms were exposed for as long as 3 months. Nonlabeled substances were used to aid identification of the radioactive metabolites. Histamine dihydrochloride, ImAA, and acetylhistamine were detected by spraying chromatograms with diazotized sulfanilic acid (19). Brom-cresol green (20) was used to detect spots of 1,4-meImAA.<sup>4</sup> Quantitation of the radioactive areas was attempted by elution with water followed by assay of the eluate with the liquid scintillation spectrometer.

## RESULTS

Histamine- $C^{14}$  was injected intravenously 23 times in 19 subjects. Ten to 15 seconds after the

<sup>1</sup> Obtained from the Nuclear-Chicago Corp., Chicago 10, Ill.

<sup>2</sup> Acetylhistamine and other derivatives of histamine with substitutions in the amino group will not behave as histamine in this procedure (17). Of the various histamine metabolites found by Schayer and Tabor, the only substance with a free amino group is 1,4-methylhistamine. We have not ruled out the possibility that this may mimic histamine in the chemical determination.

<sup>3</sup> Histalog; generously supplied by Eli Lilly & Co., Indianapolis, Ind. (18).

<sup>4</sup> Acetylhistamine was generously provided by Dr. H. Tabor, National Institutes of Health, Bethesda, Md. The 1,4-meImAA was generously donated by Dr. R. W. Schayer, Merck Institute for Therapeutic Research, Rahway, N. J. Histamine dihydrochloride was purchased from Eastman Organic Chemicals, Rochester, N. Y. ImAA (as the hydrochloride) was purchased from K & K Laboratories, Long Island City, N. Y.

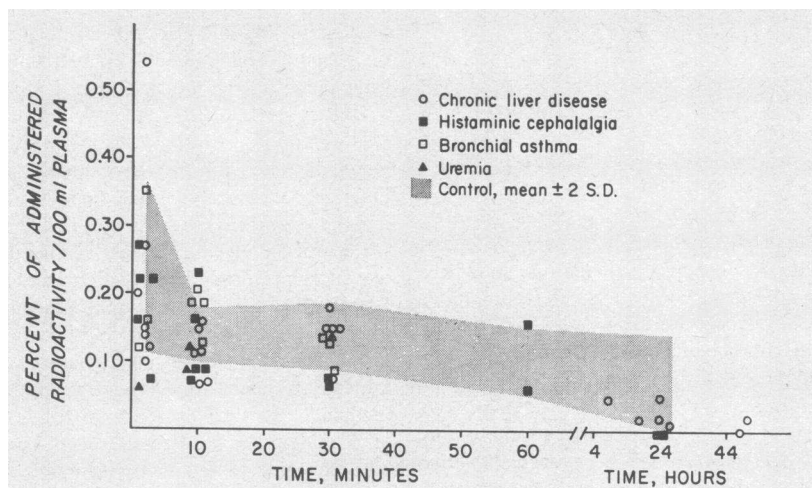


FIG. 2. TOTAL PLASMA RADIOACTIVITY EXPRESSED AS PERCENTAGE OF ADMINISTERED RADIOACTIVITY.

injection had begun most persons developed a flush, tachycardia, and mild hypotension. The duration and degree of these responses varied directly with the amount of histamine given; little clinical response occurred with less than 60  $\mu$ g. Blood pressure began to rise again 50 to 60 seconds after the injection had begun, at which time the pounding occipital headache usually started. All the symptoms but headache were gone three minutes after the injection. As expected, each of the three asthmatic patients experienced mild bronchospasm for about five minutes following histamine injection (21). Although histamine injection is known to provoke typical attacks of histaminic cephalalgia (22), none of our three patients had such a reaction.

*A. Studies on plasma.* Plasma radioactivity was expressed as the fraction (per cent) of the administered radioactivity present in 100 ml of plasma, and the values are presented in Figure 2. The mean of all samples obtained during the first three and one-half minutes after the injection was 0.24 per cent of the administered dose per 100 ml plasma ( $\pm 0.02$  at the 0.9 confidence limit). The concentration of radioactivity in the plasma decreased rapidly with the passage of time although a trace was present for many hours. No difference in the patterns of various groups emerged.

Not only was the radioactivity cleared rapidly from the plasma, but the plasma radioactivity that was present was largely due to metabolites of

histamine- $C^{14}$  and not to histamine- $C^{14}$  itself. Figure 3 compares the means of the points shown in Figure 2 with the means for true histamine- $C^{14}$ . Although some radioactivity persisted in the plasma for many hours, the "true" histamine radioactivity disappeared from the plasma promptly.

*B. Urinary excretion.* The radioactivity was excreted rapidly into the urine of all subjects except Patient AS. In most cases, 70 per cent or more of the administered radioactivity was excreted in 24 hours. Excretion was essentially complete in 30 hours.

Figure 4 shows the cumulative recovery of the

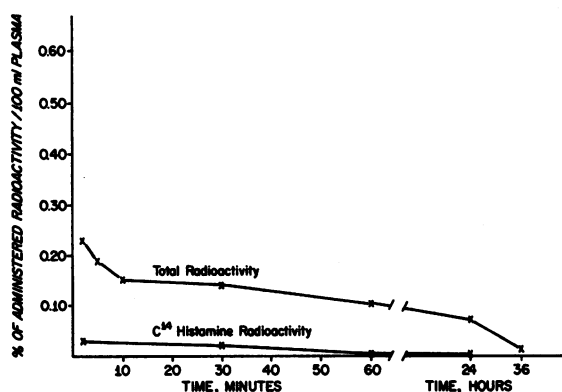


FIG. 3. MEANS OF DATA PLOTTED IN FIGURE 2 COMPARED WITH MEANS OF SPECIFIC HISTAMINE- $C^{14}$  RADIOACTIVITY IN PLASMA. An initial level of at least 0.5 per cent per 100 ml would be expected if the administered histamine were rapidly distributed in extracellular fluid, without being taken up by cells.

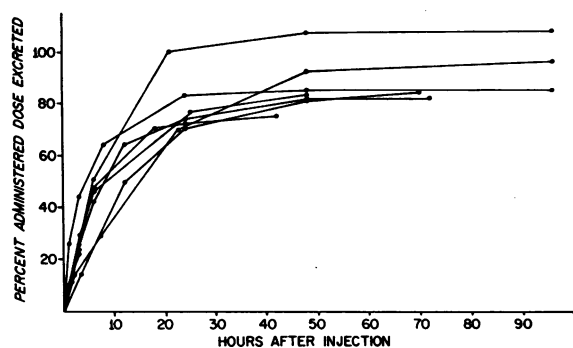


FIG. 4. CUMULATIVE RECOVERY OF ADMINISTERED RADIOACTIVITY FROM URINE OF SEVEN CONTROL SUBJECTS.

administered dose from the control subjects. Figure 5 shows the same data for the subjects with specific disorders. There was considerable variation in the initial rate of excretion. The urine collected from one asthmatic and two cirrhotic subjects contained only about 60 per cent of the injected radioactivity (Figure 5). Since the initial rate of excretion was rapid in all three of these subjects, the failure to recover more radioactivity was attributed to incomplete urine collection rather than to any metabolic abnormality. The patient with uremia and renal insufficiency (AS) excreted the radioactivity at a slow constant rate for at least 12 days.

**C. Chromatography.** Radioautography of the two-dimensional chromatograms of the control urines revealed three distinct radioactive areas (Figure 6). A fourth area was detected in the urine chromatograms of two of the five subjects. These were usually 3 to 4 sq cm in area and irregular in shape. The  $R_F$  values for each area

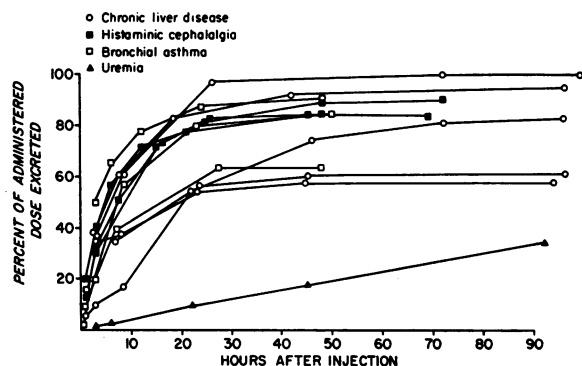


FIG. 5. CUMULATIVE RECOVERY OF ADMINISTERED RADIOACTIVITY FROM URINE OF SUBJECTS WITH VARIOUS DISEASES.

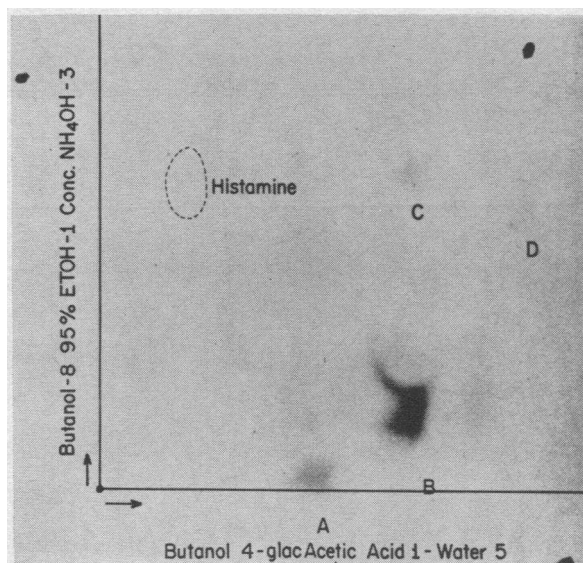


FIG. 6. TWO-DIMENSIONAL CHROMATOGRAM OF URINE EXTRACT. Three distinct areas are marked A, B and C. A fourth spot, D, was occasionally found. The dotted area indicates the locus of histamine when chromatographed in this fashion.

varied somewhat among the chromatograms, probably due to the varying amounts of interfering substances present in the concentrated methanol extracts of different urines. However, the darkened areas found were always similarly placed on the film in relation to each other. Elution of the radioactive areas followed by repeat chromatography of the eluate established the individuality of the three different substances and the identity of similar spots from different chromatograms. When eluates from spot B of two different chromatograms were combined with nonradioactive ImAA and subjected to chromatography, identical migration occurred ( $R_F$  0.21 in solvent 1 and 0.57 in solvent 2). This was regarded as reliable evidence that spot B was ImAA. Spot A was identified as ImAA-riboside on the basis of  $R_F$  values and chromatographic similarity to rat ImAA-riboside.<sup>5</sup> Spot C was identified as me-ImAA by comparison with migration of the non-labeled substance, but our efforts to elute enough of the radioactive material from a chromatogram for direct comparison of the mixture were un-

<sup>5</sup> The latter was obtained by elution from a paper chromatogram of urine from a rat given histamine- $C^{14}$  intravenously. It is the major metabolite in rat urine (23).

TABLE I  
*Recovery and distribution of urinary radioactivity after chromatography*

	Amount elutable	Distribution			
		A ImAA-* riboside	B ImAA	C 1,4-Methyl- ImAA	D 1,4-Methyl- histamine
Controls	%	%	%	%	%
KT	59	15	38	47	
CB	63	10	84	6	
RT	47	49	49	2	
JC	42	19	38	38	5
SN	60	45	35	8	12
Mean	54	28	49	20	
Cirrhotics†					
RS	56	25	65	6	4
JF	75	23	63	9	5
Mean	66	24	64	6	5
Asthmatics					
JL	90	17	63	20	
EV	39	33	62	5	
HL	44	27	66	7	
Mean	58	25	64	11	
Histamine cephalalgia					
JW	41	46	49	5	
RB	75	21	19	60	
GH	50	30	58	12	
Mean	56	32	42	26	
Uremia					
AS	63	100			

\* Imidazole-4(5)-acetic acid.

† Because of interfering substances, chromatographic patterns adequate for elution and quantitation could not be obtained for the other three patients.

successful. Similarly, unknown D could be identified as 1,4-methyl histamine only by comparison of  $R_F$  values. Chromatography and staining of nonlabeled histamine and acetylhistamine demonstrated that these substances moved to sites quite far removed from the radioactive areas.

Chromatography of urine samples collected at 6 and 12 hours was also carried out for two normal subjects and for one with cirrhosis. There were no differences in the metabolite distribution. Further studies were not done because the concentrations of radioactivity usually became too low for satisfactory chromatograms to be developed.

Table I illustrates the amounts of each of these metabolites that could be recovered from the paper chromatograms. In almost every case, ImAA

was the principal metabolite. No consistent variation in the types or amounts of metabolites excreted was found between the several groups studied. The chromatogram from the subject with uremia was interesting in that only one radioactive area was found. This was located at the site to which ImAA-riboside usually migrated. However, the low concentration of radioactivity in his urine made it difficult to confirm or amplify this finding.

No significant changes in plasma or urinary radioactivity, or in the chromatography of the urinary metabolites occurred after antihistamine or 3- $\beta$ -aminoethylpyrazole administration to Subject KT. Similarly, histamine "desensitization" did not alter the pattern of metabolism by Patient GH.

## DISCUSSION

The clearance of radioactive histamine from plasma was extremely rapid in all cases studied, and no differences among the various disease groups could be detected. The rate of urinary excretion of radioactive metabolites was also surprisingly constant in our studies. Only in the uremic patient was there an abnormality in urinary excretion; the radioactivity was excreted slowly at a relatively constant rate for at least 12 days. Since plasma levels of histamine- $C^{14}$  did not differ from the others, the deficiency in excretion of the radioactivity was probably due to impaired renal clearance rather than to a decrease in histamine-metabolizing activity.

Failure to find specific abnormalities in the overall rate of histamine- $C^{14}$  metabolism prompted us to search for more subtle alterations by paper chromatography of the urine. Imidazole-4(5)-acetic acid, ImAA-riboside and 1,4-meImAA were identified in the urine of all persons given histamine- $C^{14}$  except for the one with uremia, who excreted only ImAA-riboside. Another compound presumed to be 1,4-methylhistamine was sometimes found. Histamine- $C^{14}$  was never detected on the chromatograms, and no unusual or abnormal metabolites were discovered.<sup>6</sup> The absence of N-acetyl histamine was particularly significant. This substance is chromatographically identical with the so-called "conjugated histamine," which is so often measured in histamine studies of human urine (24). Although our chromatographic method is relatively insensitive, it should have detected any acetylation responsible for the large fraction of such "conjugated" histamine. Accordingly, this substance should not be used as an index of the metabolism of endogenous or parenterally administered histamine. In humans, it apparently represents mainly the action of intestinal bacteria (24).

In most of our chromatograms, ImAA was the principal urinary metabolite. Because the rela-

tive insensitivity of our chromatographic method might have led us to overlook histamine and other minor metabolites, exposure of some chromatograms to X-ray film for three months was particularly important. Significantly, this merely revealed further film reduction by radioactivity of previously identified metabolites. No new radioactive areas appeared.<sup>6</sup> Accordingly, our observations established with reasonable certainty that no qualitative abnormalities of histamine degradation exist in the disorders studied.

Quantitation of the radioactive metabolites was not so satisfactory. Even before development of the chromatogram, radioactive metabolites were irreversibly adsorbed by the filter paper so that only about 85 per cent of the radioactivity of a drop of a methanol extract applied to the paper could be eluted. Sensitivity of the method was also limited, due to the large amount of interfering substances which persist even after methanol extraction. Thus, only a few hundredths of a microgram of the substances of interest could be successfully chromatographed. In addition, the great variation from subject to subject (Table I) meant that any quantitative changes in disease would need to be appreciable before any reasonable inferences could be made. Detection of minor changes would require a prohibitive number of studies. Thus, although no measurable abnormalities were detected by our methods, subtle differences have not been ruled out.

The variations in metabolite distribution among individuals were noted also by Schayer and Nilsson and associates, who used isotopic dilution techniques to identify the metabolites of histamine- $C^{14}$  in human urine (6, 7). Our results do not differ qualitatively from theirs, although we found more ImAA and less meImAA in most cases. The reason for this difference is unclear, but is probably related to the method of metabolite identification rather than to the mode of histamine- $C^{14}$  administration (7).

Since injected histamine- $C^{14}$  is so rapidly metabolized and appears only in trace amounts in urine, it is questionable whether urinary assays of histamine in bound or unbound form really reflect the release of endogenous histamine. It is unlikely that urinary ImAA excretion will serve as a better index of histamine turnover, since

<sup>6</sup> When relatively large volumes of urine were passed through Decalco cation exchange columns, and the eluate extracted as in the chemical method for histamine determination (17). The "histamine" radioactivity thus isolated amounted to less than 5 per cent of the total radioactivity.

this substance is also derived directly from histidine catabolism (1, 25).

Precise measurements of plasma histamine levels may provide valuable information in those conditions such as asthma and anaphylaxis in which a high rate of histamine release might occur. However, our observations indicate that plasma levels would not change significantly unless a very large amount of free histamine were continually produced. Our curves of  $C^{14}$  plasma histamine clearance are not precise enough for any estimation of the amount required.

Elevated blood histamine levels were found in marked hepatic insufficiency by Mitchell, Butt and Code several years ago (13). Since plasma levels were not determined, it is difficult to interpret their findings, but we could establish no deficiency in the rate of histamine metabolism.

The nature of histamine production and release in disease is certainly of primary importance, and perhaps can be studied with more confidence now that a possible variable, namely, the mode and rate of histamine metabolism, appears to be surprisingly resistant to change.

#### SUMMARY

1. The metabolism of histamine- $C^{14}$  has been investigated in seven control subjects, and in five patients with Laennec's cirrhosis, three with bronchial asthma, three with histaminic cephalalgia, and one with uremia due to chronic glomerulonephritis.

2. The radioactive histamine was rapidly cleared from the blood, and excretion of radioactivity in the urine was usually complete within 30 hours.

3. Separation of the excreted radioactive metabolites by paper chromatography and radioautography showed imidazoleacetic acid to be the principal product of histamine metabolism. Imidazoleacetic acid-riboside and 1,4-methylimidazoleacetic acid were also consistently recovered in the urine, but histamine- $C^{14}$  was not detected.

4. Histamine inactivation and degradation in man is a rapid and complete process, and by our methods, no abnormalities were found in the diseases studied.

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