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CATABOLISM OF C¹⁴-LABELLED HISTAMINE IN PATIENTS WITH ALLERGIC DISEASES¹

By

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The role of histamine in the development of allergic symptoms in man is rather obscure in spite of several investigations, (for references, see: *Rocha e Silva*, 1955, *Halpern & Holtzer*, 1958, and *Dunér & Pernow*, 1960). So far, it seems fairly well established that histamine is released during urticarial attacks and may cause at least part of the reactions in the skin. If bronchial tissue from a patient with a specific asthma is excised and incubated with the specific allergen, this tissue releases histamine and contracts. (*Schild, Hawkins, Mongar & Herzheimer*, 1951), but the bronchial constriction *in vivo* during asthma may be caused by other substances than histamine (*Brocklehurst*, 1958).

A person who tends to develop allergic reactions might have primary changes in the histamine metabolism. The allergic symptoms might thus be due to i.a. an accelerated synthesis of histamine, a larger release of histamine or a failure to inactivate the histamine. It has in fact been suggested by *Gaddum* (1951) that patients with allergic diseases might have a defect in the histamine catabolism. *Lecomte* (1959) recently quoted evidence that the elimination of histamine was delayed in allergic conditions.

¹ Parts of the results were communicated to the Swedish Society for Clinical Physiology in Göteborg in March 1959.

In the present study C^{14} -labelled histamine was injected subcutaneously or intravenously to patients with acute urticaria or bronchial asthma. For comparison, most of the patients were also studied when free from symptoms. The catabolism of the injected histamine was measured by analysis of the urinary excretion of C^{14} -histamine and its known radioactive metabolites.

MATERIAL AND METHODS

Patients studied.

Group I. All patients received C^{14} -histamine subcutaneously and urine was collected during the following 12 hours.

Case 1. Female, 60 years. Thyroidectomized 1936. Chronic asthmatic bronchitis since 1933. Since 1945 more severe asthmatic attacks, hospitalized several times. Given C^{14} -histamine on June 2, 1958 during a severe asthmatic attack and on April 15, 1959 during a period without asthmatic symptoms.

Case 2. Female, 27 years. Recurrent urticaria since childhood. Asthma of intrinsic type started 1958. Pronounced eosinophilia ($1000-3000/\text{mm}^3$). C^{14} -histamine given on June 4, 1958 during a severe asthmatic attack and on December 1, 1958 when the patient was free from symptoms.

Case 3. Female, 51 years. Since several years asthma of the intrinsic type. More symptoms at the menopause in 1956. Hospitalized 7 times during 1956-1958. Treated with small daily doses of corticosteroids. Injected with C^{14} -histamine on Sept. 28, 1958 during severe asthma in spite of corticosteroid therapy.

Case 4. Female, 42 years. Chronic bronchial asthma since 1949. Treated with small daily doses of cortisone since 1953. Injected with C^{14} -histamine on June 9, 1958, when she was symptom-free.

Case 5. Male, 62 years. Chronic asthmatic bronchitis with obstructive emphysema since 1919. Treated with small, daily doses of hydrocortisone since 1954. Injected with C^{14} -histamine on June 12, 1958 during slight asthmatic symptoms.

Case 6. Female, 29 years. 1953 urticaria due to lobster allergy. New attacks 1957 and early 1958 of unknown cause. Injected with C^{14} -histamine on July 11, 1958 during severe urticaria.

Case 7. Female, 51 years. Thyroidectomized 1954. Severe urticaria since August 1958. Etiology unknown. Given C^{14} -histamine Oct. 8, 1958 during severe symptoms.

Case 8. Female, 39 years. Recurring attacks of urticaria and angioneurotic edema of unknown cause since 1956. Injected with C^{14} -histamine on December 16, 1958 during a severe attack.

In addition to the patients with allergic symptoms C^{14} -histamine was given to a patient with agammaglobulinemia.

Case 9. Male, 31 years. Agammaglobulinemia, probably since childhood. Treated since 1955 with gammaglobulin. No allergic symptoms. C^{14} -histamine was injected on June 12, 1958.

Group II. The two patients in this group were given C^{14} -histamine intravenously and urine was collected through a catheter in the bladder. Urine was collected in 7 portions, 0-20, 20-40, 40-60, 60-90, 90-120, 120-180, and 180-240 min. after the injection.

Case 10. Female, 42 years. Asthma of the intrinsic type since 1932. At intervals serious attacks, especially in connection with respiratory infections. Injected with C^{14} -histamine intravenously 3 times in 1959, on July 16, during moderate asthmatic symptoms, on July 20 during a very severe asthmatic attack and on July 30 when she was free from symptoms.

Case 11. Female, 21½ years. Acute urticaria and angioneurotic edema due to penicillin allergy. C^{14} -histamine was injected on June 30, 1959 during severe symptoms and on July 8, 1959, when the symptoms had disappeared.

Injections of C^{14} -labelled histamine.

Histamine labelled with C^{14} in the 2 position of the imidazole ring was purchased from the Radiochemical Centre, Amersham, England. The specific activity was such that the injected amount (approximately 10 μ g C^{14} -histamine base) contained about 0.5 mikroCurie.¹ C^{14} -histamine was dissolved in 0.9 % NaCl with 0.2 % glucose and sterilized by boiling. C^{14} -histamine injections were given either subcutaneously or intravenously and the collection of urine started immediately after injection. All urine samples were immediately acidified to pH 1-2 with HCl and stored at -20° C.

Analysis of C^{14} -containing compounds in urine.

The methods used have been developed by Schayer and described by e.g. Nilsson, Lindell, Schayer & Westling (1959) and Lindell, Nilsson, Roos & Westling (1960). The following substances were determined: Histamine, methylhistamine (4-

¹ In the previous paper (Nilsson, Lindell, Schayer & Westling, 1959) a misprint occurred. The specific activity of the C^{14} -histamine then used was about the same as now, i.e. 47.7 mikroCurie per mg histamine base, and *not* as stated in that paper 42.7 milli Curie/mg base.

(β -aminoethyl)-1-methylimidazole) total imidazoleacetic acid (imidazol-4(5)-ylacetic acid). Briefly, the analytical procedure is as follows: a carrier amount (non radioactive) of the substance to be determined is added to an aliquot of the urine specimen; a preliminary extraction is made (butanol extraction for histamine, chloroform extraction for methylhistamine, and absorption on an anion-exchange resin for the acids); the compounds are crystallized as picrates, and the picrates of histamine and imidazoleacetic acid are then converted to derivatives of p-iodophenylsulphonyl chloride (pipsyl chloride). The picrates or pipsyl compounds are counted on standard plates at infinite thickness in a gas flow counter (background 20–23 c.p.m.). Repeated recrystallizations and treatments with activated charcoal are then performed. When the radioactivity is stable through three recrystallizations purity is considered to have been achieved. The samples to be assayed for total imidazoleacetic acid are first hydrolysed in a sealed glass tube for 5 hr. at 160° in N hydrochloric acid with the carrier included. This hydrolysis removes the ribose that is conjugated to part of the imidazoleacetic acid.

The excretion of total C^{14} was measured as described by Schayer & Cooper (1956).

RESULTS

Group I. Subcutaneous injections of C^{14} -histamine. Table I.

In the first series of experiments C^{14} -histamine was injected subcutaneously to patients (No. 1–9). The catabolism of histamine as reflected by the urinary excretion of its metabolic products was compared with previously reported results on non-allergic subjects, examined under similar conditions. In addition, two patients with bronchial asthma were examined when entirely free from asthmatic symptoms. It may be seen from Tables I and II that there were no conspicuous differences between the allergic and the non-allergic subjects, or between the results in one and the same patient when studied with and without asthmatic symptoms. The mean values for

TABLE I

Catabolism of C¹⁴-histamine in patients with allergic diseases and one case of agammaglobulinemia.
 Values refer to urinary excretion of "total" radioactivity, in per cent of the amount injected, and the excretion of C¹⁴-histamine and its main radioactive metabolites, in per cent of the excreted "total" radioactivity.
 C¹⁴-histamine injected subcutaneously and urine collected for 12 hours.

Case no.	Sex	Diagnosis	Excreted radio-activity	Per cent of excreted radioactivity appearing as				
				Hista- mine	Methyl- hista- mine	Methyl- imida- zole acetic acid	Imidazole- acetic acid	Sum of meta- bolites
1	F	Bronchial asthma; during symptoms	63	0	6	70	26	102
1	F	Bronchial asthma; no symptoms	63	2	9	61	20	92
2	F	Bronchial asthma; during symptoms	67	3	10	60	40	113
2	F	Bronchial asthma; no symptoms	42	3	6	49	27	85
3	F	Bronchial asthma; during symptoms and treat- ment with corticosteroids	63	3	4	60	32	99
4	F	Bronchial asthma; no symptoms during treatment with corticosteroids	47	2	5	59	29	95
5	M	Bronchial asthma; no symptoms during treat- ment with corticosteroids	52	0	8	61	28	97
6	F	Urticaria	97	1	6	45	33	85
7	F	Urticaria	50	1	4	53	37	95
8	F	Urticaria	65	2	5	40	38	85
9	M	Agammaglobulinemia. No allergic symptoms ...	68	0	8	53	25	87

TABLE II
Catabolism of C¹⁴-histamine in man.
 Mean values for urinary excretion of "total" radioactivity, unchanged C¹⁴-histamine and its main metabolites during 0-12 hr after
 a subcutaneous injection of about 10 μ g C¹⁴-histamine.

Diagnosis	No. of cases	Excreted radio-activity in % of injected	Per cent of excreted radioactivity appearing as				Sum of metabolites
			Histamine	Methyl-histamine	Methyl-imidazole-acetic acid	Imidazole-acetic acid	
Non-allergic*	4	82	2	5	43	30	84
Status asthmaticus	3	64	2	7	63	33	105
Bronchial asthma; no symptoms	2	53	2	7	55	24	88
Bronchial asthma; steroid treatment	2	50	1	7	60	28	96
Urticaria	3	71	1	5	46	36	88
Psychiatric disease**	3	68	2	8	63	25	98

* Case 9 from the present study + 3 normal females reported by Nilsson, Lindell, Schayer & Westling (1958).

** 3 women studied by Lindell, Nilsson, Roos & Westling (1960).

the different categories of patients examined are given in Table II.

The excretion of total radioactivity during 12 hours was slightly lower in the cases of bronchial asthma, both when acutely ill and when symptom-free, as well as during treatment with corticosteroids. We would not consider this as a significant difference. The urinary pattern of C^{14} -histamine metabolites in per cent of excreted radioactivity was also about the same in the different groups. There was no evidence for the presence of significant amounts of unknown radioactive metabolites in the material, the sum of the known metabolites accounting for 85–113 % of the excreted radioactivity.

It may also be seen from Tables I and II that the patient with agammaglobulinemia had a "normal" histamine catabolism.

Thus the initial experiments provided no evidence for a change in the catabolism and urinary excretion of injected C^{14} -histamine in asthma and urticaria. It was felt, however, that analysis of a single 12 hour urine portion might not give sufficient information about the rate of histamine catabolism and urinary excretion. This problem was therefore examined in the second series of experiments.

Group II. Intravenous injection of C^{14} -histamine. Fig. 1 and 2.

Observations on one female patient with bronchial asthma and one woman with wide-spread acute urticaria are given in Fig. 1 and 2. Both cases were given C^{14} -histamine intravenously and the urine collected in portions so that the rate of catabolism and urinary excretion of C^{14} -histamine could be followed. The patient with bronchial asthma was given 3 intravenous injections of C^{14} -histamine, the first one during asthma of moderate severity, the second one during very severe asthma and the last one when free from symptoms. The asthma during the second injection was so severe that it was necessary to give theophylline and corticosteroids intravenously during the last two urine collection periods.

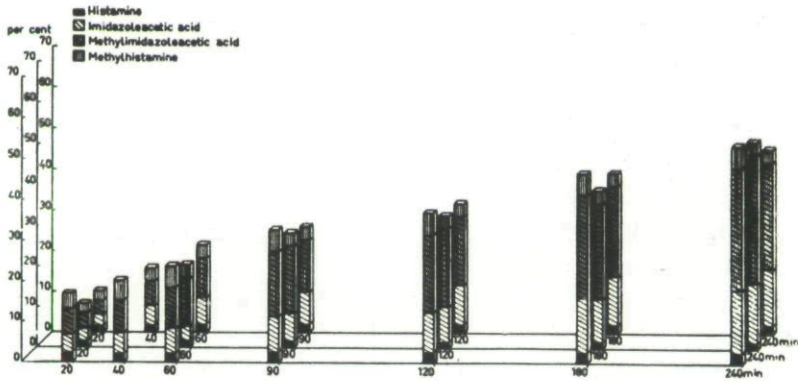


Fig. 1.

Case 10. Cumulative excretion of metabolites of C^{14} -histamine in per cent of intravenously injected amount of C^{14} -histamine.

Front row: During moderate asthma.
 Middle row: During severe asthma.
 Hind row: No symptoms.

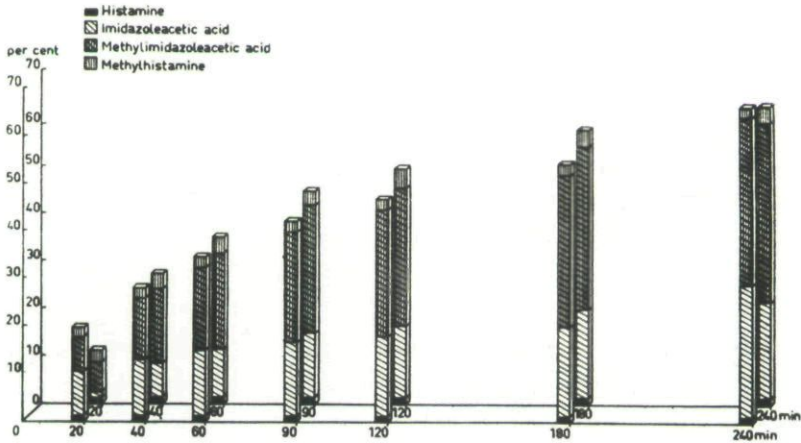


Fig. 2.

Case 11. Cumulative excretion of metabolites of C^{14} -histamine in per cent of intravenously injected amount of C^{14} -histamine.

Front row: During urticarial attack.
 Hind row: No symptoms.

From Fig. 1 it is apparent that the rates of excretion of total radioactivity, of histamine as well as of the different metabolites are similar during all three experiments on this patient, and thus independent of whether she had asthmatic symptoms or not. (Table III).

In Fig. 2 shows the constancy in histamine catabolism also in the patient with urticaria, both when acutely ill and when free from skin lesions.

The rate of excretion and the pattern of urinary metabolites shown in Fig. 1 and 2 and Table III are similar to those in two normal controls, reported by *Lindell, Nilsson & Rorsman*, (1961).

TABLE III

Cumulative excretion of total radioactivity after intravenous injection of C¹⁴-histamine. Values refer to per cent of injected amount. Case 10 is also shown in Fig 1, and case 11 in Fig. 2.

Case no.	Diagnosis	Total radioactivity excreted during						
		20	40	60	90	120	180	240
		min.						
10	Bronchial asthma; moderate symptoms ...	30	32	37	45	49	58	63
	Bronchial asthma: severe symptoms	19	—*	37	46	50	57	68
	Bronchial asthma; no symptoms	12	17	24	29	34	42	50
11	Acute urticaria, during symptoms	18	25	30	39	43	58	63
	No symptoms	18	32	39	48	53	61	67

* Combined with the 60 min. sample.

DISCUSSION

The results obtained give no support for the belief the development of allergic symptoms is dependent upon changes in the enzymatic catabolism or urinary elimination of histamine delivered to the blood stream directly or after absorption from the site of a subcutaneous injection. Particularly important are the observations after intravenous injection. (Group II). The patient with bronchial asthma had the same urinary pattern of histamine metabolites, whether severely ill or free from symptoms. The same holds for the patient with urticaria. More-

over, the pattern of urinary metabolites of C^{14} -histamine was about the same in patients with asthma and urticaria as in healthy individuals and patients with psychiatric disease.

Beall & VanArsdel (1960) recently studied the plasma and urine radioactivity after injection of C^{14} -histamine intravenously to normal individuals and among others 3 patients with bronchial asthma. The patients developed a mild bronchospasm for 5 min. after injection of histamine, but were otherwise presumably symptom-free. These authors found no difference between the asthmatic subjects and the normal controls, neither as regards rate of decline of plasma radioactivity nor in the rate of urinary excretion of radioactivity. The pattern of urinary metabolites of histamine was also roughly the same in the asthmatics and the controls, as judged by elution of radioactive spots from bi-dimensional paper chromatograms of concentrated urine.

As regards the lack of difference in urinary excretory products after injection of C^{14} -histamine to normal and asthmatic subjects our results agree with those of *Beall & VanArsdel*, but there is a conspicuous difference between their results and ours as regards the "normal" pattern of urinary metabolites of histamine. Thus *Beall & VanArsdel* find that the major histamine metabolite in man is imidazoleacetic acid, which in its free and conjugated forms (imidazoleacetic acid riboside) accounted for 77 % of the radioactivity that was elutable from paper chromatograms of urine. Methylimidazoleacetic acid accounted for only 20 % of the elutable radioactivity. The results of *Schayer & Cooper* (1956) and those given in Table II of the present paper indicate that in man, as in most other species, methylimidazoleacetic acid is the main histamine metabolite. (*Schayer*, 1959). This finding is based upon rigorous purification of urinary C^{14} -methylimidazoleacetic acid with the isotope dilution technique and measurements of the total C^{14} activity excreted in the urine. The divergence in relative amounts of methylimidazoleacetic acid in our studies and those of *Beall & VanArsdel* was considered by these authors to be related to the method of metabolite identification.

The urinary excretion of free, non-isotopic histamine decreases during symptoms of bronchial asthma (*Mitchell, Logan, Peters & Henderson, 1954; Dieckhoff, 1957; and Dunér & Pernow, 1958*) and increases during acute urticarial attacks (*Adam, Hunter & Kinneir, 1951*). The results of the present study indicate that these changes in the excretion of endogenous histamine are not due to an altered rate of excretion or enzymatic catabolism of histamine delivered to the blood stream. It would therefore seem fairly certain that acute urticaria is accompanied by a liberation of histamine, whereas the role of histamine in bronchial asthma is enigmatic. The findings of decreased urinary histamine during asthmatic symptoms is difficult to explain at the present state of knowledge.

Several studies indicate a relationship between histamine catabolism and the adrenal cortex, (e.g. *Rose, 1954, and Kahlson, 1956*) and the beneficial effects of corticosteroids in allergic diseases have been proposed to be at least in part due to changes in the histamine catabolism. The present results do not support this theory, since there were no obvious changes in histamine catabolism in the two asthmatic patients who were kept symptom-free by treatment with adrenocortical hormones.

SUMMARY

C¹⁴-histamine was injected subcutaneously or intravenously to patients with bronchial asthma and urticaria. The urinary excretion of total radioactivity, C¹⁴-histamine and its radioactive metabolites was measured. The results were compared with similar studies in the same subjects when free from symptoms, and with results from non-allergic patients.

The observations did not show any alterations in the catabolism or urinary excretion of C¹⁴-histamine during asthmatic and urticarial reactions, or during treatment with adrenocortical steroids. The catabolism of C¹⁴-histamine was found to be normal in a case of agammaglobulinemia.

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