one whose irreversible culmination requires other syntheses prevented by the uracil deficiency. The occurrence of an alteration in the structure of DNA in itself may be quite sufficient to establish the observed genotypic effect. In any case, the possibility that thymine starvation may in some way labilize the DNA structure warrants a closer chemical examination.

SUMMARY

The mutagenicity of thymine starvation was studied with several auxotrophic strains of a thymine-requiring bacterium. Some apparently mutagenic effects were due to increased survival of the revertants. Using mutants whose revertants did not possess this selective advantage, a study was made of the time of the appearance of revertants. During the first 30 minutes of starvation under conditions in which cells did not die, the number of revertants increased several fold. Revertants also accumulated under conditions of continued starvation.

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Received February 26th, 1958

SYNTHESIS AND METABOLISM OF A HISTAMINE METABOLITE, 1-METHYL-4-(β-AMINOETHYL)-IMIDAZOLE

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One of the major pathways of histamine metabolism involves methylation of the ring nitrogen remote from the side chain to give 1-methyl-4-(β-aminoethyl)-imidazole[1] (hereafter called methylhistamine).

Methylhistamine had previously been prepared only by a method devised by PYMAN[2]. This procedure was long and gave poor yields. It was unsatisfactory for large scale syntheses or for preparation of ^{14}C-labeled methylhistamine.

The present paper describes (a) the biosynthesis of ^{14}C-methylhistamine from ^{14}C-histamine (b) a study of some of its properties (c) a new chemical synthesis for preparing large quantities of methylhistamine (d) the synthesis of methylhistamine labeled with ^{14}C (e) methods for the determination of ^{14}C-methylhistamine and its oxidation product, ^{14}C-1-methylimidazole-4-acetic acid, by isotope dilution, (f) a study of the enzymic oxidation of methylhistamine to methylimidazoleacetic acid in intact mice, and (g) evidence that imidazoleacetic acid is not methylated directly in intact mice to give methylimidazoleacetic acid.

* This work was started at the Rheumatic Fever Research Institute, Chicago, Ill.

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Biosynthesis of methylhistamine

Six female mice were injected with 10 γ/g aminoguanidine[3] and 70 γ/g 1-isobutyl-2-isonicotinylhydrazine[3] (IBINH). In mice treated with these two inhibitors of histamine-metabolizing enzymes, a large percentage of injected ^{14}C-histamine appears in the urine either unchanged, or as ^{14}C-methylhistamine[1]. The mice were then injected subcutaneously with 40 γ ^{14}C-histamine (0.14 μC) each, and urine was collected for 7 ½ h.

^{14}C-histamine and methylhistamine were extracted into butanol from alkaline solution, re-extracted into acid, and the acid solution freeze-dried. The residue was dissolved in hot ethanol, filtered and paper chromatograms of the filtrate prepared using as solvent ethanol 95: *N*/10 hydrochloric acid 5. Under these conditions histamine and methylhistamine can be separated[1]. The radioactivity in the methylhistamine peak was eluted and the chromatography repeated. After a second elution the radioactive material was assayed by isotope dilution. When carrier histamine was added to an aliquot, the isolated pipsyl derivative[4] was practically devoid of radioactivity indicating that if any ^{14}C-histamine remained it constituted less than one-half of 1 % of the total radioactivity. When carrier methylhistamine was added to another aliquot it retained all the radioactivity throughout several recrystallizations.

Solubility properties of methylhistamine

Solubility tests were made on biosynthetic ^{14}C-methylhistamine in an attempt to find a satisfactory method for the isolation of carrier in the course of isotope dilution assays, particularly to insure separation from histamine. Of several organic solvents tested, chloroform was found to be excellent for these purposes. Methylhistamine is removed almost quantitatively from strongly alkaline aqueous solution, saturated with sodium sulfate, by three chloroform extractions. ^{14}C-histamine, under comparable conditions, was extracted only to the extent of 5 to 6 %. Furthermore, since chloroform is a very poor solvent for most urinary constituents, carrier methylhistamine can be recovered from large volumes of urine in excellent purity. A combination of chloroform extraction of methylhistamine, followed by recrystallization of the picrate eliminates significant contamination by histamine. Use was made of the chloroform extractibility in the new synthetic procedure described in the following paragraph.

Synthesis of 1-methyl-4-(β-aminoethyl)-imidazole

Histamine dihydrochloride, 70 g, was converted to acetylhistamine, 84 g, by the method of VAN DER MERWE[5]. The crude acetylhistamine, 35 g, was dissolved in 280 ml 10 % sodium hydroxide solution, 26 ml dimethylsulfate added and the mixture shaken while being cooled. After a few minutes, additional portions of sodium hydroxide and dimethyl sulfate (the same amounts as above) were added and shaking with cooling repeated. The solution was heated for 30 min on a steam bath, cooled, saturated with sodium sulfate, and then extracted three times with equal volumes of chloroform. The chloroform was removed by evaporation on a steam bath and the crude acetylmethylhistamines hydrolyzed by boiling for 2 h with 200 ml 6 *N* HCl. The solution was saturated with sodium sulfate, made strongly alkaline and extracted three times with equal volumes of chloroform. As the stability of the free amines

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was not known, the hydrochlorides were produced by adding to the chloroform a solution of hydrogen chloride in ethanol. The mixture was evaporated to dryness on a steam bath, the residue dissolved in 250 ml water, and a solution of 35 g picric acid (dry weight) in 160 ml absolute ethanol added. The desired isomer, 1,4-methylhistamine, crystallizes as the dipicrate in warm solution while the nonphysiological 1,5-isomer remains in solution. When the temperature dropped to 50° the crystals were collected and recrystallized from 50 % alcohol. 16.8 g of the pure dipicrate was obtained, a yield of 18 % from histamine, m.p. 216.5–217.5°. PYMAN[2] reported 217°. Analysis, calculated for $C_6H_{11}N_3(C_6H_3O_7N_3)_2$ C, 37.0, H, 2.9, N, 21.6; Found C, 37.2, H, 3.1, N, 21.5.

The hydrochloride was prepared by removing picric acid on Dowex-1 (chloride form) and recrystallizing from ethanol.

The dipicrate of the 1,5-isomer was never obtained pure from the mother liquors; it was always contaminated by the less soluble 1,4-isomer.

Using the same procedure on a small scale, methylhistamine dipicrate labeled in the 2-position of the imidazole ring was prepared from commercial ^{14}C-histamine, m.p. 216.5–217.5°. The activity was 1.6 μC/mg base.

Identification of the enzyme which oxidizes methylhistamine to methylimidazoleacetic acid in intact mice

Recent publications from other laboratories[6,7] indicate that methylhistamine is oxidized *in vitro* by diamine oxidase (histaminase) approximately as easily as is histamine itself. These findings conflict with the speculation of one of the present authors (R.W.S.) that diamine oxidase is probably not involved in the oxidation of methylhistamine *in vivo*[1]. However, this speculation was based on observations of chromatograms of urine of mice injected with ^{14}C histamine*, and not on direct measurements of the substrate and end product. With the development of methods for the determination of ^{14}C-methylhistamine and ^{14}C-methylimidazoleacetic acid, a more rigorous study could be made.

Aminoguanidine was chosen as a specific inhibitor for diamine oxidase as it is the most powerful one known *in vitro*[8] and *in vivo*[9].

Marsilid (1-isopropyl-2-isonicotinylhydrazine) is a strong inhibitor of monoamine oxidase[10]. However, because it has some inhibitory effect on diamine oxidase**, a more highly substituted isonicotinylhydrazine, 1-isobutyl-2-isonicotinylhydrazine (IBINH) was selected as the inhibitor for monoamine oxidase.

Female white mice were divided into three groups of 3 mice each. One group

* As histamine is metabolized by (a) methylation, and (b) oxidation to imidazoleacetic acid, the possibility that methylimidazoleacetic acid was formed by methylation of imidazoleacetic acid was not excluded by the earlier experiments. That direct methylation of imidazoleacetic acid does not occur to a significant extent in living mice is shown by the following experiment:

Five female mice were injected with 40γ each of ^{14}C imidazoleacetic acid (activity 1.0 μC/mg). Urine was collected for 24 h. To a one-fourth aliquot of the highly radioactive urine was added carrier methylimidazoleacetic acid. This was purified as described above and after the fifth recrystallization as the picrate was devoid of significant radioactivity.

** Isonicotinylhydrazine is a strong inhibitor if diamine oxidase; Marsilid is less active[14]. It was presumed that a more highly substituted derivative would be even less inhibitory to diamine oxidase. Furthermore, use of IBINH was desirable since we had earlier found it to be the most effective member of a series of substituted isonicotinylhydrazines in blocking the production of ^{14}C-methylimidazoleacetic acid from ^{14}C-histamine in mice[1].

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was injected subcutaneously with 20 γ/g aminoguanidine. The second group was injected subcutaneously with 70 γ/g IBINH. The third group, controls, received saline only. After 20 min all mice were injected subcutaneously with 1.0 γ/g ^{14}C-methylhistamine. The mice were placed on a wire screen in the bottom of a jar and urine collected in dilute hydrochloric acid for 22 h. Another identical experiment was performed except that the dose of ^{14}C-methylhistamine was 0.10 γ/g.

Assay for ^{14}C-methylhistamine was made by adding 70 mg nonisotopic methylhistamine dihydrochloride (equivalent to 206 mg of the dipicrate) to a one-third aliquot of urine, isolating by chloroform extraction, converting to the dipicrate, and counting at infinite thickness in a flow counter. There was no significant drop in activity after recrystallization. This count, corrected for background, was compared to the count obtained by adding the same amount of carrier methylhistamine to the same amount of ^{14}C-methylhistamine as used for the injection, converting to the dipicrate, and counting under identical conditions.

Assay for ^{14}C-methylimidazoleacetic acid was made by adding 99 mg non-isotopic methylimidazoleacetic acid hydrochloride (equivalent to 206 mg of the picrate) to a one-third aliquot of urine. It was purified by adsorption on Dowex-50 from acid solution, elution with ammonia, and conversion to the picrate. Counting was done as described above. There was no drop in activity following recrystallization.

Results are shown in Table I.

TABLE I

EFFECT OF INHIBITORS ON OXIDATION OF METHYLHISTAMINE TO METHYLIMIDAZOLEACETIC ACID IN INTACT MICE

Conc. methylhistamine injected, γ/g	*Inhibitor*	*Counts/min*		*Ratio Methylhistamine/ Methylimidazole-acetic acid*
		Methylhistamine	*Methylimidazole-acetic acid*	
1.0	None	314	600	0.52
1.0	Aminoguanidine	423	591	0.72
1.0	IBINH	887	158	5.6
0.10	None	38	64	0.59
0.10	Aminoguanidine	42	58	0.73
0.10	IBINH	62	10	6.2

DISCUSSION

The data of Table I show that aminoguanidine had only a slight inhibitory effect on the oxidation of methylhistamine to methylimidazoleacetic acid in intact mice. IBINH had a pronounced inhibitory effect. Although there are many points of similarity between monoamine oxidase and diamine oxidase[11], in the authors' opinion sensitivity to aminoguanidine is the best criterion of diamine oxidase activity *in vivo*. We have shown earlier, in intact animals, that aminoguanidine blocks the oxidation of histamine to imidazoleacetic acid[3] and that it also strongly inhibits the oxidation of ^{14}C-cadaverine, a model substrate for diamine oxidase[9]. From these facts we conclude that diamine oxidase plays a minor role, if any, in the physiological oxidation of methylhistamine to methylimidazoleacetic acid.

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Since IBINH does inhibit this oxidative step, it may be that the enzyme involved is monoamine oxidase, or a related enzyme system. IBINH is structurally very similar to Marsilid and the latter is widely used as an inhibitor of monoamine oxidase *in vitro* and *in vivo*[12].

There is some evidence that there is a pathway of metabolism of methylhistamine other than oxidation to methylimidazoleacetic acid. In most of the groups the urinary ^{14}C which could be accounted for as methylhistamine plus methylimidazoleacetic acid was about 65 % of the ^{14}C injected. Although the urine collection was not rigorously quantitative, these yields were lower than anticipated. A quantitative determination was made of the total ^{14}C present in the urine of one group (the mice treated with IBINH and 1.0 γ/g ^{14}C-methylhistamine). A recovery of 83 % was found. In this group the ^{14}C due to the two metabolites accounted for only 67 %. The discrepancy may be due to an unidentified metabolite. There was no increase in ^{14}C-methylhistamine after boiling with 3 *N* HCl. Thus there is probably no acetylation or other conjugation. We had earlier shown that there was no conjugate of methylimidazoleacetic acid in man[13]. No other work was done to account for the observed discrepancy.

No significant amount of methylimidazoleacetic acid is formed in mice by direct methylation of imidazoleacetic acid. There is no evidence at present that methylimidazoleacetic acid is formed by any route other than oxidation of methylhistamine.

SUMMARY

A new synthesis is described for 1-methyl-4-(β-aminoethyl)-imidazole, a major metabolite of histamine. This compound was also prepared in radioactive form by biosynthesis and by chemical synthesis.

When 1-methyl-4-(β-aminoethyl)-imidazole is injected into mice, a major portion is oxidized to 1-methylimidazole-4-acetic acid. Both compounds were determined quantitatively in urine using isotope dilution procedure. A study of the effect of inhibitors on this oxidation in intact mice indicated that diamine oxidase plays little or no role. The enzyme involved was inhibited by a monoamine oxidase inhibitor.

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Received April 3rd, 1958