In the rat, the mean value of $7 \cdot 0 \pm 0 \cdot 5 \times 10^{-4}$/min. in 20 controls reaches $7 \cdot 9 \pm 0 \cdot 4$ (17 animals) after treatment with thyroid extract for 30 days and decreases to $5 \cdot 7 \pm 0 \cdot 5$ (20 animals) after continuous administration of methyl-mercapto-imidazol for the same period of time. In these various conditions there was no significant modification of the soluble phosphate concentration of the red blood cells. It was possible to establish a high correlation between the variations of the s^{32}P coefficient and the basal oxygen consumption measurements.

These findings imply a close relationship between thyroid activity and the turnover-rate of acid-soluble phosphate in the red blood cells. From preliminary *in vitro* experiments, it seems that the thyroid hormone acts directly on phosphate transfer: *in vitro* addition of *l*-triiodothyronine (5 µgm. per cent) to labelled red blood cells, incubated in non-radioactive plasma of myxœdematous patients, is followed by an increase of the outflow-rate of phosphorus-32.

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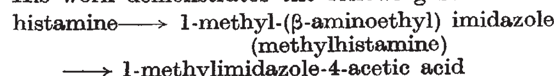
[1] Ermans, A. M., *C.R. Soc. Biol.*, **147**, 1525 (1952).
[2] Ermans, A. M., "Influence de l'activité thyroïdienne sur la vitesse d'échange du phosphore globulaire. Bases physiologiques et applications cliniques" (Editions Acta Clinica Belgica, 1958).

Enzymatic N-Methylation of Histamine

THE *in vivo* studies of Schayer[1] have shown ring N-methylation to be the principal pathway of histamine metabolism in a variety of mammalian species. His work demonstrates the following scheme:



Kobayashi[2] found that incubation of histamine with whole-liver homogenates resulted in the formation of 1-methylimidazole-4-acetic acid. These observations reflect the summation of at least three enzymatic processes, one of which is concerned with the synthesis of a co-factor needed for histamine methylation. This report describes the isolation and properties of an enzyme, imidazole N-methyl transferase, which catalyses the ring-N-methylation of histamine requiring S-adenosylmethionine as the methyl donor.

The enzyme was purified approximately eight-fold in two steps from the soluble supernatant fraction of mouse liver. After homogenization of mouse liver with 4 vol. of isotonic sucrose and centrifugation at 78,000*g*, the supernatant fraction was adjusted to *p*H 5·0 with dilute acetic acid and heated for 5 min. at 50° C. After centrifugation, the precipitate was discarded and the supernatant fluid fractionated with ammonium sulphate. The protein fraction precipitated between 50 and 70 per cent saturation was centrifuged, dissolved in 0·01 *M* phosphate buffer *p*H 7·4 and dialysed against 0·001 *M* phosphate buffer *p*H 7·4 overnight. The partly purified enzyme can be stored frozen for several weeks at − 10° C. without loss of activity.

Enzymatically formed methylhistamine was extracted with chloroform at alkaline *p*H[3]. Under these conditions, 88 per cent of the methylhistamine, but only 7 per cent of the histamine, are extracted into the chloroform.

Table 1 shows the absolute requirement by the purified enzyme for S-adenosylmethionine. Magnesium ions and reduced glutathione are not necessary in this system. Furthermore, dialysis of the enzyme against versene or the addition of versene to the reaction mixture does not alter the enzymatic activity. This indicates that imidazole-N-methyl transferase differs from catechol-O-methyl transferase[4]. Enzymatic activity is not altered by pretreatment of animals with 'Marsilid', a monoamine oxidase inhibitor which blocks the conversion of methylhistamine to 1-methylimidazole-4-acetic acid[3].

Table 1. REQUIREMENTS FOR RING-N-METHYLATION OF HISTAMINE BY PURIFIED MOUSE LIVER ENZYME

Reaction mixture*	Methylhistamine formed (µmoles)
Whole system	0·041
Whole system + 2 mgm. reduced glutathione	0·043
Omit magnesium chloride	0·044
Omit S-adenosylmethionine	0·000
Preheated enzyme	0·000

* Reaction mixture containing 2 ml. 0·1 *M* phosphate buffer *p*H 7·4, 0·5 ml. purified mouse-liver enzyme (0·22 mgm. protein), 0·4 µmoles S-adenosylmethionine, 20 µmoles magnesium chloride, 0·05 µmoles histamine dihydrochloride labelled with carbon-14 (Nuclear Chicago) (12,500 c.p.m.), in a final volume of 3·0 ml. was incubated at 37° C. for 4 hr.

The reaction mixture was saturated with sodium sulphate after the addition of 0·5 ml. of 5 *N* sodium hydroxide and the methylhistamine extracted with 15 ml. chloroform. An aliquot of chloroform was evaporated to dryness, the residue eluted with 0·5 ml. of hyamine (ref. 8) and phosphor and counted in a 'Tri Carb' liquid scintillation counter.

The enzymatic product was proved to be methylhistamine by three techniques. After cochromatography in ethanol/0·1 *N* hydrochloric acid (95 : 5) of the enzymatic product and synthetic methylhistamine[3], a radioautograph demonstrated a single spot which exactly coincided in R_F and shape with the ninhydrin-stained area. The specific activity remained constant after four recrystallizations as the dipicrate[5] with added carrier. Finally, incubation with mouse liver homogenate formed radioactive 1-methylimidazole-4-acetic acid. This was proved as follows: Enzymatically formed methylhistamine was incubated with whole mouse liver homogenate for 4 hr. The mixture was boiled, filtered and passed through a 'Dowex-1'-acetate 100–200 mesh column (10 cm. × 1 cm.). The column was washed and then eluted with 0·5 *N* acetic acid. After concentration of the eluate, it was recrystallized from acetone/water with carrier 1-methylimidazole-4-acetic acid (synthesized from cyanomethylimidazole[6] by Pyman's method[7]). There was no decrease in specific activity after four recrystallizations while carrier imidazole acetic acid could readily be freed of radioactivity by this technique.

Imidazole-N-methyl transferase has been found to be present in most tissues of all species studied so far, and appears to be of paramount importance in histamine metabolism. A more detailed study of this enzyme will be the subject of a future communication.

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[1] Schayer, R. W., *Brit. J. Pharmacol.*, **11**, 472 (1956).
[2] Kobayashi, Y., *Arch. Biochem. Biophys.*, **77**, 275 (1958).
[3] Rothschild, Z., and Schayer, R. W., *Biochim. Biophys. Acta*, **30**, 23 (1958).
[4] Axelrod, J., and Tomchick, R., *J. Biol. Chem.*, **233**, 702 (1958).
[5] Schayer, R. W., and Karjala, S. A., *J. Biol. Chem.*, **221**, 307 (1956).
[6] Bauer, H., and Tabor, H., *Biochem. Preparations*, **5**, 97 (1957).
[7] Pyman, F. L., *J. Chem. Soc.*, **99**, 2172 (1911).
[8] Passmann, J. M., Radin, N. S., and Cooper, J. A. D., *Anal. Chem.*, **28**, 484 (1956).

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