Short Communications

On the Biological Methylation of Histamine

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For some time histamine has been known to be methylated and oxidatively deaminated prior to excretion¹. Recently the postulated intermediary metabolite 1-methyl-4-(β -aminoethyl) imidazole, hereafter referred to as methylhistamine, has been shown to appear in the urine following administration of an amine oxidase inhibitor².

In vitro experiments have also demonstrated that the methylation process precedes the deamination^{3,4} and may be separated from the following reaction steps⁵.

In the above mentioned *in vitro* experiments methionine was added to the enzyme extracts on the assumption that the methyl group is derived from methionine as in many other biological transmethylation. The experiments reported in this communication were undertaken in order to test this hypothesis.

A mouse liver extract, described previously⁵ in which methylation of histamine takes place but not deamination, was incubated from 1 to 24 h with nonisotopic histamine and methionine containing radio-carbon in the methyl group. The extract was dialysed prior to use.

Samples were withdrawn at the beginning and the end of the incubation, and analyzed for radioactive methylhistamine according to the isotope dilution method developed by Schayer and co-workers (for a description see Lindell and Schayer⁶).

The method briefly consists of the following steps: carrier methylhistamine* is added to the sample, extracted with chloroform after protein precipitation, and the picrate is recrystallized from water-ethanol using alternately different charcoal adsorbents until constant radioactivity is attained.

The zero-time samples were entirely inactive after two crystallizations, whereas the incubated ones retained a constant activity from the 2nd through the 4th crystallization.

* Methylhistamine was obtained from Dr. R. W. Schayer, Merck Institute for Therapeutic Research, which is hereby gratefully acknowledged.

Table 1.

	15.5 μ g ^{14} C histamine, 1.48 mC/mM; 100 μ g nonisotopic methionine	100 μ g ^{14} C methionine 2.49 mC/mM; 15.5 μ g nonisotopic histamine
^{14} C methylhistamine after 2 h incubation, cpm ¹	2 376	1 122
^{14} C histamine at zero time, cpm	5 816	—
^{14} C histamine after 2h in- cubation, cpm	3 464	—

¹ The samples were counted in a windowless flow counter, Tracerlab, SC-16, which was made available through the courtesy of Prof. A. Tiselius, Institute of Biochemistry, Uppsala.

In the same manner simultaneous incubates containing only enzyme extract and ^{14}C methionine were assayed for methylhistamine. Now the carrier contained no radioactivity after only two crystallizations. It was thus concluded that carrier methylhistamine can, by repeated crystallization of the dipicrate, be completely freed from contamination by ^{14}C methionine and its possible metabolites.

The destruction of histamine and the formation of methylhistamine was followed by simultaneous incubation of the crude enzyme extract with radiocarbon histamine labelled in the imidazole nucleus. At the end of the incubation remaining histamine and methylhistamine were assayed by isotope dilution technique. It has been shown that by repeated crystallization of the dipicrate carrier amounts of methylhistamine can be freed from contamination by ^{14}C histamine or its metabolic products. By conversion of carrier histamine to its pipsyl derivative the contaminating methylhistamine can be removed⁷. Therefore constant activity of the samples during three successive crystallizations was considered as a sufficient criterion of the purity.

The essential figures from an experiment are given in Table 1. It has been concluded from these results that the methyl group of methylhistamine can be transferred from methionine. Whether methionine is the immediate precursor of the methyl group or an intermediate step is involved cannot be concluded from this type of experiment.

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Phosphorus-32 Pentasulfide: Preparation by Isotopic Exchange and Conversion to Thiophosphoryl- 32 Chloride and Phosphorus-32 Trichloride

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Certain triesters of phosphoric and thiophosphoric acids are important as pesticides and potential chemical warfare agents. Adequate studies on the metabolism and mode of action of these organophosphorus compounds require their synthesis from high specific activity radio-labeled inorganic intermediates such as phosphorus pentasulfide (P_2S_5), thiophosphoryl chloride (PSCl_3), phosphorus trichloride (PCl_3), and phosphoryl chloride (POCl_3).

$^{32}\text{POCl}_3$ can be readily prepared in high specific activity from the reaction of $\text{H}_3^{32}\text{PO}_4$ with phosphorus pentachloride (PCl_5)¹. Neutron irradiation is commonly employed to label certain of the other inorganic phosphorus intermediates²⁻⁵. This method is often inconvenient and the specific activity of the organophosphorus compounds prepared from these irradiated intermediates is usually not greater than 5 mC/g. $^{32}\text{PSCl}_3$ can also be prepared from $\text{H}_3^{32}\text{PO}_4$ via $^{32}\text{POCl}_3$ and $^{32}\text{PCl}_3$ ^{6,7} or by an inefficient isotope exchange reaction⁸.

Various procedures were tried to achieve isotope exchange between $\text{H}_3^{32}\text{PO}_4$ or $\text{Na}_2\text{H}^{32}\text{PO}_4$ and red phosphorus, PSCl_3 or P_2S_5 . The products from each attempt were reacted with ethanol⁹ or converted to other known organic derivatives⁴ and then separated by paper and ion-exchange chromatography¹⁰ to determine the degree of exchange. After correction for the radioactivity remaining on the glassware, the

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