On the Biological Methylation of Histamine. 4

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In the past few years Schayer and his coworkers have shown that methylation of histamine to 1,4-methylhistamine * and its further oxidation to 1-methylimidazole-4-acetic acid is the principal route of histamine inactivation in man, mouse, cat, and dog. The literature concerning the catabolism of histamine *in vivo* has recently been reviewed by Schayer [1].

In vitro studies concerning the histamine methylating enzyme system have been discussed in earlier communications under the above heading [2-4]. It was reported that the formation of 1,4-methylhistamine requires methionine plus an ATP generating system, this leading on to the finding that S-adenosylmethionine is a most effective methyl donor in this system. These results have later been confirmed in other laboratories [5].

The present communication deals with the influence of some commonly used SH-inhibitors on the histamine methylating enzyme in liver extract.

Enzyme preparation. Pig liver was homogenized in 0.1 M NaAc buffer pH 5.6, centrifuged at 10 000 *g*, heated to 47° for 7 min, and centrifuged at 20 000 *g*. The supernatant liquid was fractionated with $(NH_4)_2SO_4$ at pH 6. The precipitate obtained between 50 and 70 % saturation was dissolved in 0.05 M NaAc pH 5.6, dialyzed overnight, and the resulting solution used for the experiments after centrifugation.

Determination of enzyme activity. The method makes use of the fact that 1,4-methylhistamine is extracted by chloroform from an alkaline solution, while histamine is not [6].

25 μl of the enzyme preparation, containing 25 mg protein per ml, were incubated for 30 min at 37°C with 0.050 μC ^{14}C-histamine and 0.3 μmoles S-adenosylmethionine in 0.5 M Tris buffer of pH 8.0 to make 200 μl. The reaction was interrupted by dipping the test tubes in boiling water. After addition of 50 μl 10 N NaOH saturated with Na_2SO_4 at 100°C and cooling, the samples were extracted with 1 000 μl of chloroform by vigorous shaking for 10 min.

The phases were separated by rapid centrifugation. Samples of 200 μl were withdrawn from the chloroform layer, and evaporated on planchets. The radioactivity was counted under an end window Geiger-Müller tube. From these figures the percentage methylhistamine formed was obtained with the aid of a calibration curve. Details of the method will be published later.

Effect of inhibitors.

o-Iodosobenzoic acid in concentrations 1 to 25 × 10^{-4} M had no influence on the enzymic activity, when the inhibitor was brought in contact with the enzyme 10 min prior to the substrate.

p-Chloromercuribenzoic acid markedly inhibited the histamine methylation (Table 1). The concentration necessary for com-

* Correct name: 1-methyl-4-(β-aminoethyl) imidazole.

Table 1. The effect of *p*-chloromercuribenzoate on the histamine methylating enzyme in pig liver.

Conc. of *p*-chloromercuribenzoate M	No glutathione added		Glutathione added in conc. 5 × 10^{-4} M	
	Histamine methylated %	Inhibition %	Histamine methylated %	Inhibition %
—	70	—	70	0
5 × 10^{-6}	70	0		
5 × 10^{-5}	67	4.3		
1.25 × 10^{-4}	59	15.7		
2.5 × 10^{-4}	31	55.7	70	0
5 × 10^{-4}	0	100	62	11.4
12.5 × 10^{-4}	0	100		

plete inhibition is rather high, *viz.* 5×10^{-4} M, probably on account of the presence of comparatively large amounts of inactive protein in the enzyme preparation. The inhibition was not reversed by dialysis. However, glutathione in slightly higher than equimolar concentrations abolished the inhibition completely.

Chloroacetophenone in high concentrations inhibited the enzyme, to a certain extent, *viz.* 90 % at 2.5×10^{-3} M. *Iodoacetamide* had a similar effect.

Garlic extract contains allicin which is known as an inhibitor of SH-groups [7]. It may be noted that garlic extract, prepared in the proportion 1:200 w/v, inhibits the enzyme completely. Glutathione counteracts this inhibition.

The inhibition of the histamine methylation by *p*-chloromercuribenzoate and the counteraction of glutathione are taken as evidence that SH-groups might be essential for the activity of the methylating enzyme. The inefficiency of *o*-iodosobenzoate may be explained by a protecting effect of other proteins present.

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Long Range Coupling of Nuclear Spins in Some Olefinic and Acetylenic Compounds

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In view of the growing interest in the theory of the indirect coupling of nuclear spins [1-6] we wish to report some recent findings in connection with our study of long range couplings in proton magnetic resonance spectra.

In an earlier communication [7] one of us suggested that the coupling of methyl group protons over four bond distances with protons bonded to unsaturated carbon atoms should be understood in terms of hyperconjugation.

The need for new evidence for the existence of hyperconjugation in the ground state of unsaturated organic molecules has become obvious as Dewar and Schmeising have recently pointed out [8] that the earlier evidence is inconclusive. It is hoped that a continued investigation of the above mentioned spin couplings may provide such evidence.

In order to verify the interpretation given in Ref.[7], we have tested two predictions that follow from the discussion presented there.

1. The interaction of the π-electron, on an unsaturated carbon atom, with the protons of an attached methyl group is approximately equal to its interaction with a proton directly bound to the unsaturated carbon.

2. The coupling of methyl group protons (to other protons) over triple bonds should be considerably larger (about twice) than those over double bonds.

These predictions were tested in a number of substances. Thus we find, that the coupling between the two non-equivalent methyl groups in tiglaldehyde and in β-bromoangelic acid methylester are 1.0 c/s and 1.5 c/s, respectively *, or of the same order of magnitude as J^{I}_{ab} in *trans*-crotonaldehyde (I), which equals 1.6 c/s or the analogous coupling in *trans*-propenylbenzene, which is reported [9] to equal 1.8 c/s.

Since methyl acetylene is a gas at room temperature, we have instead studied propargyl alcohol and propargyl chloride. Here the coupling of the methylenic protons with the acetylenic proton equals 2.4 c/s and 2.6 c/s, resp., in agreement with the values 2.6—2.8 c/s for the propargyl halides reported by Whipple *et al.*[10]

When the acetylenic hydrogens are substituted by methyl groups, the long range coupling constants (here between the methyl and methylene protons) remain vir-

* All coupling constants reported here are given to $\pm$ 0.1 c/s. We use the symbol J^{I}_{ab} to denote the coupling constant J between groups a and b in compound I.