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Manometric Studies on the Bio-oxidation of Histamine.

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(Introduced by C. J. Farmer.)

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Best and McHenry[1] reported observations on a histamine-inactivating substance or system which they denote as histaminase. They state that oxygen is consumed during this reaction but have postponed a detailed manometric study until obtaining a more concentrated histaminase preparation. We have found that a kidney powder, prepared according to their directions, when added to histamine gave sufficient gas changes to study the histamine-histaminase reaction from the standpoint of oxygen uptake.

The experiments were performed in the Barcroft-Warburg apparatus, with air in the gas space, phosphate buffer in the conical vessel, KOH in the well, and a measured amount of histamine in the side bulb. Since attempts at materially purifying the enzyme were unsuccessful, 200 mg. dry kidney powder from the dog were used in the phosphate solution as a source of histaminase. When the system was at a constant temperature, 37.5° C., the histamine was tipped into the vessel and manometric readings were made at regular intervals until the oxidation ran to completion. Parallel experiments were always made without histamine because the powder alone had a small oxygen uptake. (Ca 25% of that with histamine.) This was subtracted in every case from the values found with the addition of the base.

The rate of oxidation early in the experiments was constant. Ten minute observations of the histamine oxidation plus the gas change due to kidney powder averaged about 6 to 8 cmm. of oxygen. When the oxygen uptake had ceased, the histamine was inactivated as shown by its failure to contract guinea pig intestine *in vitro*. On the other hand, when the oxygen uptake was not complete, the substrate was capable of contracting smooth muscle. This was observed in several experiments. It was thought at first that an indication of the nature of the end products of the reaction could be obtained from the total amount of oxygen necessary to inactivate a given amount of histamine. This was not found to be the case. The first lot of kidney powder consumed an average of 41 cmm. oxygen per 0.5 mg.

[1] Best, C. H., and McHenry, E. W., *J. Phys.*, 1930, **70**, 349.

histamine dihydrochloride (neutralized) whereas the second lot consumed an average of 85 cmm. for an equal quantity. In the first case, the oxygen uptake with 0.5 mg. histamine was complete in 2 hours, while in the second, it required the same length of time for 0.25 mg. Apparently in neither case can the molar ratio between the amount of histamine inactivated and the total oxygen consumed be expressed in whole numbers.* It is possible that these discrepancies can be explained by concomitant or functional reactions not due to the histamine-histaminase oxidation or by varying degrees of inhibition by an unknown factor.

In connection with the question of the chemical fate of histamine, it was found that no CO_2 was given off during the reaction, *i. e.,* the respiratory quotient was zero. Furthermore there was no oxygen uptake when iminazole aldehyde, iminazole lactic acid, iminazole propionic acid, and histidine were added to active kidney powder.

When the kidney powder was kept constant at 200 mg., the rate of oxidation was independent of amounts of histamine varying from 0.25 to 2 mg., whereas the total oxygen uptake was directly proportional to these quantities. When the amount of histamine was kept constant at 1 mg., the rate of oxidation was roughly proportional to amounts of kidney powder, varying from 50 to 300 mg., whereas the total oxygen uptake was constant. At 9°C. there was no oxygen consumption in the histamine-histaminase system; at 55°C. the reaction was markedly accelerated, being complete in approximately one-third of the time required at 37.5°C. The rate of oxidation was the same when a buffer of pH 5.2, 7.4, and 10 was used. However, the total oxygen uptake of 0.5 mg. of histamine was 57.8 cmm., 87.4 and 127.5 cmm. for these respective buffers. A repetition of the experiment with 0.25 mg. histamine gave results of 30, 42.2, and 66.7 cmm. oxygen for the same pH concentrations. The histamine was inactivated in every instance after the oxygen consumption had ceased, as shown by its failure to contract smooth muscle.

Potassium cyanide, 0.002 M and 0.0002 M, inhibited the rate of oxidation 60% and 25% respectively during the first hour but had no influence on the total oxygen uptake. The histamine-histaminase reaction as revealed by the gas change was not influenced by either a 95% CO-5% O_2 mixture or by a 0.1 molar Na Pyrophosphate solution. This excludes certain forms of iron catalysis.

* Bernheim[2] has reported a simple molar relationship in the case of the tyramine-tyraminase reaction.

[2] Bernheim, M. L. C., *J. Biol. Chem.,* 1931, **93**, 299.

A preparation made from the small intestine of the dog contained almost as much histaminase as did kidney powder, while analogous preparations from the brain, spleen, and lung showed no oxygen uptake with histamine after 3 to 4 hours' observation. Kidney powder from the guinea pig, cat, human (obtained 2 hours after death), rat, and chicken with histamine showed such slight oxygen consumption that they were not adequate as a source of histaminase in these experiments. Fresh dog kidney in the form of a 200 mg. chunk showed about a 50% increase in oxygen consumption when 1 mg. of histamine was added. Freshly ground up kidney gave a much smaller histaminase effect.

An attempt to split histamine on a blood-charcoal model with the uptake of oxygen was unsuccessful.† Histidine, however, had a definite oxygen uptake on blood charcoal in phosphate buffer solution. Warburg and Negelein[3] in a similar type of experiment have observed the splitting of cystine, leucine, and tyrosine with an uptake of oxygen.

The manometric measurements reported above agree with the physiological and chemical observations of Best and McHenry in most respects. However, they observed (1) decreased inactivation of histamine at pH 10, (2) that the optimum inactivation of histamine occurred at 37° with a destruction of the inactivating enzyme at 60°, and (3) complete inhibition of the inactivating substance with KCN at 0.004, 0.002, 0.001, and 0.0005 molar concentrations.

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Reactions of Proteins in Liquid Ammonia with Metallic Sodium.

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McChesney and Miller[1] introduced the use of liquid ammonia as a medium for the study of the chemical properties of proteins. They found that proteins were acidic in liquid ammonia and that when they were treated with alkali metals at —33.5° C. or with ammono

† Choline, acetylcholine, neurine, tyramine, as well as betaine, showed no gas change with the charcoal model.

[3] Warburg, O., and Negelein, E., *Biochem. Z.*, 1921, **113**, 257.

[1] McChesney and Miller, *J. Am. Chem. Soc.* (1931), **53**, 3888.