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THE EFFECT OF COPPER ON THE ENZYMIC OXIDATION OF HISTAMINE AND ALIPHATIC DIAMINES

BY

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The mechanism of oxidation of histamine by histaminase is not yet fully understood. Zeller (1938) described the enzymic oxidation of short-chain diamines, such as putrescine and cadaverine, and suggested that this reaction was catalysed by histaminase; the reaction was considered to be an oxidative removal of the amino-group with the formation of the corresponding aldehyde. For histamine this aldehyde would be imidazoleacetaldehyde.

There are indications that the enzymic oxidation of putrescine and cadaverine differs in certain respects from that of histamine. According to Kapeller-Adler (1949), both dialysis and addition of flavine-adenine dinucleotide (FAD) have different effects on the enzymic reaction according to whether the substrate is histamine or an aliphatic diamine. Differences in the action of steroid hormones and gonadotrophins on the enzymic oxidation of histamine on the one hand and of cadaverine on the other have also been described (Kapeller-Adler, 1951). Tabor (1951), using a purified preparation of histaminase from pig's kidney, has obtained evidence that aldehyde oxidase will oxidize the primary product of the enzymic oxidation of histamine but not of cadaverine.

Holmberg and Laurell (1948) reported that blood plasma contains a copper-protein which actively destroys histamine, but this has not been confirmed (see Zeller, 1951). The effect of copper on the enzymic oxidation of histamine by aqueous extracts of human placenta has recently been examined (Bruns and Stüttgen, 1951). Oxygen was found to be taken up when copper was added to preparations of histaminase plus histamine even after the histaminase reaction had been completed.

In the present work the action of copper on the enzymic oxidation of both histamine and aliphatic diamines by a preparation of pig kidney has been studied. A few experiments on the action of other metals will also be reported.

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METHODS

Enzyme Preparations.—The starting material in all experiments was an extract of acetone-dried powder of pig's kidney. The kidneys were usually frozen at $-10°$ C. overnight and then worked up with acetone. The powder thus obtained was extracted with 0.067 M sodium phosphate buffer, *p*H 7.4, 10 ml. of buffer to each g. of powder. The extract was stirred and left standing for 1 hour and then centrifuged. The sediment was washed with more buffer and the second supernatant was added to the first.

This crude extract was fractionated according to Tabor (1951). In this procedure the enzyme is precipitated when the concentration of Na_2SO_4 is increased from 14.5 to 21.5% (w/v). The precipitate was dissolved in a small volume of 0.067 M sodium phosphate buffer, *p*H 7.4, and dialysed against running tap water for 1 hour. One-half volume of 0.2 M sodium phosphate buffer *p*H 7.4 was added. This solution contained all the activity of the crude extract and the purification on the basis of non-dialysable nitrogen was about fourfold. This material was used in most of the experiments.

Manometric Experiments.—1.6 ml. of the enzyme solution was used in the main compartment of the manometer flask. 0.2 ml. of water or 5×10^{-2} M substrate solution was in the side bulb. The side bulb also contained 0.2 ml. water or 0.2 ml. of a solution of a metal salt, e.g., $CuSO_4$. The total volume was 2 ml. The central tube contained 0.3 ml. N KOH. The gas phase was oxygen and the temperature 37.5° C.

RESULTS

When histamine was incubated with histaminase in the presence of copper as $CuSO_4$ an increase in the rate of oxygen consumption was regularly observed. With cadaverine as substrate there was no similar increase and the rate of oxidation in the presence of copper was often slightly decreased. A typical experiment is reproduced in Fig. 1, in which the rate of oxygen uptake in the presence of histamine was approximately doubled by the addition of 1.5×10^{-3} M $CuSO_4$, whereas the copper salt had no similar effect upon the rate of oxidation of cadaverine.

With histamine as substrate the increase in the rate of oxygen consumption was greatest when

the concentration of $CuSO_4$ was between 2 and 5×10^{-3} M. With cadaverine the oxygen consumption was not increased by $CuSO_4$ at any concentration. The only effect of copper on the rate of oxidation was a decrease. This decrease was about 50% in 5×10^{-3} M $CuSO_4$. These results are shown in Fig. 2. In subsequent experiments the concentration of copper used was 1.5×10^{-3} M. At that concentration the increase in the rate of oxygen consumption with histamine was almost maximal, whereas the oxidation of cadaverine was slowed only slightly.

It was necessary to find out whether the increased consumption of oxygen in the presence of copper was accompanied by a similar increase in the rate of inactivation of histamine. Histamine was therefore incubated with histaminase in the presence and absence of copper in a number of manometer flasks. While the oxidation proceeded, the histamine remaining in the flasks was determined by biological assay.

Six manometers were set up. Three of the flasks contained copper and three did not. The initial concentration of $CuSO_4$ was 5×10^{-3} M and the concentration of $CuSO_4$ was 1.5×10^{-3} M. After 0, 90, and 240 minutes the incubation of one pair of flasks, one without copper and one with copper, was terminated. One ml. of the content was withdrawn and added to 1 ml. of 12.5% (w/v) trichloracetic acid. The mixture was filtered and 1 ml. of the filtrate was prepared for histamine assay by Code's method (1937). The procedure differed from Code's procedure in that the samples were not boiled but heated on the boiling water bath. The histamine assay was carried out on the isolated ileum of the guinea-pig. The results are set out in Table I. They showed clearly that in spite of the different amounts of oxygen consumed the rate of inactivation of histamine was the same in the presence and absence of copper. This meant that the additional oxygen taken up in the presence of copper was used in an oxidation reaction other than the oxidation of histamine by histaminase.

This interpretation was supported by experiments in which copper was added when the oxygen uptake in the histaminase reaction had ceased. The result of such an experiment is shown in Fig. 3. In this experiment the histaminase reaction came to a standstill with the uptake of 71 μl. of oxygen. This is 1.27 atoms of oxygen per mole of added histamine. Copper was then added from a second

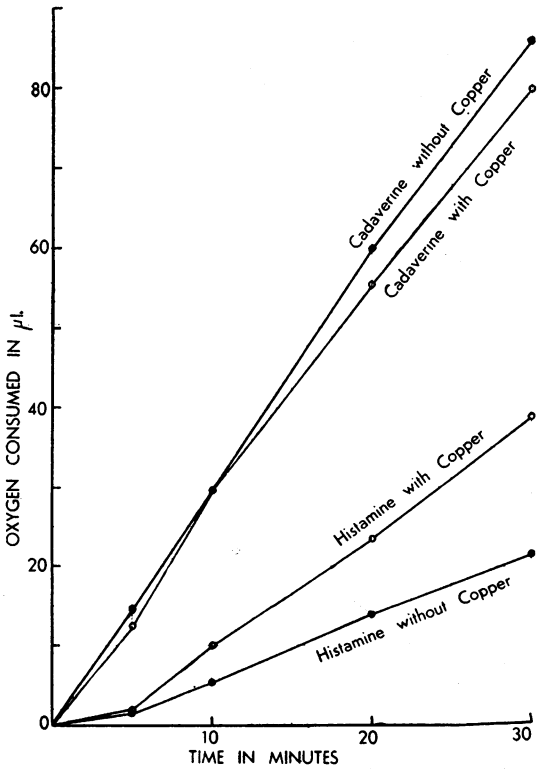

FIG. 1.—Effect of $CuSO_4$ on the enzymic oxidation of histamine and cadaverine. Abscissa: time in minutes. Ordinate: oxygen consumed in excess of blank. Initial substrate concentration: 5×10^{-3}M. Concentration of $CuSO_4$: 1.5×10^{-3}M.

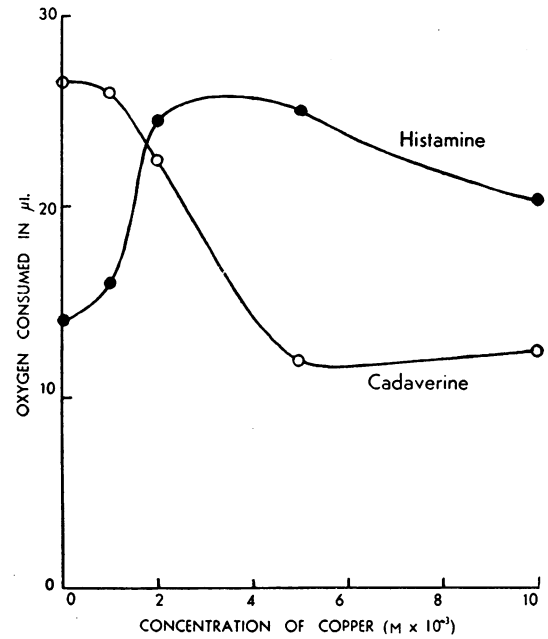

FIG. 2.—Effect of different concentrations of $CuSO_4$ on oxygen consumption with histamine and cadaverine. Initial substrate concentration: 5×10^{-3}M.

TABLE I
THE RATES OF INACTIVATION OF HISTAMINE BY HISTAMINASE IN THE ABSENCE AND IN THE PRESENCE OF COPPER

Time (min.)	Oxygen Consumed (μl.)		Total Mean Histamine in Flask (μg.)		
	Without $CuSO_4$	With $CuSO_4$ (1.5×10^{-3}M)	Calculated from Oxygen Consumption	Without $CuSO_4$ Found	With $CuSO_4$ (1.3×10^{-3}M) Found
0	0	0	1,110	972	1,075
90	46	126	655	590	618
240	121	268	0	0	43

side bulb of the manometer flask. This caused a further rapid uptake of oxygen until the total consumption was approximately doubled. This result agrees with similar observations of Bruns and Stüttgen (1951) on extracts of human placenta.

Effect of Copper on the Enzymic Oxidation of Aliphatic Diamines

With cadaverine as substrate, copper did not increase the rate of oxygen uptake. Also, when copper was added after the enzymic oxidation of cadaverine had come to a standstill, no further oxygen uptake occurred. This experiment was repeated with other diamines known to be oxidized by the pig's kidney preparation: trimethylene diamine, tetramethylene diamine, hexamethylene diamine, heptamethylene diamine, and octamethylene diamine.

In each experiment the diamine was added at zero time and when the uptake of oxygen due to the oxidation of the diamine had come to a standstill $CuSO_4$ was added from the side bulb. As expected, all the diamines from C_3 to C_8 were oxidized; but the addition of copper did not cause oxidation to be resumed. An experiment with octamethylene diamine is shown in Fig. 4. The oxygen uptake was followed until it came to a standstill when one atom per mole of substrate had been taken up. In one flask, $CuSO_4$ was then added without causing any uptake of oxygen. In another flask putrescine was added. This caused a renewed oxidation, indicating that the enzyme was still active.

Agmatine ($NH_2C(:NH)NH(CH_2)_4NH_2$) was also oxidized by the preparation. Addition of $CuSO_4$ did not cause a renewed oxygen uptake.

A few salts of other metals were also tested with histamine as substrate and it was found that cobalt caused an increased rate of oxygen consumption. This is shown in Fig. 5, where the effect of 1.5×10^{-3} M $CoCl_2$ was compared with that of 1.5×10^{-3} M $CuSO_4$. $FeCl_3$, $ZnSO_4$, and $MnCl_2$ at the same concentration slightly reduced the oxygen consumption.

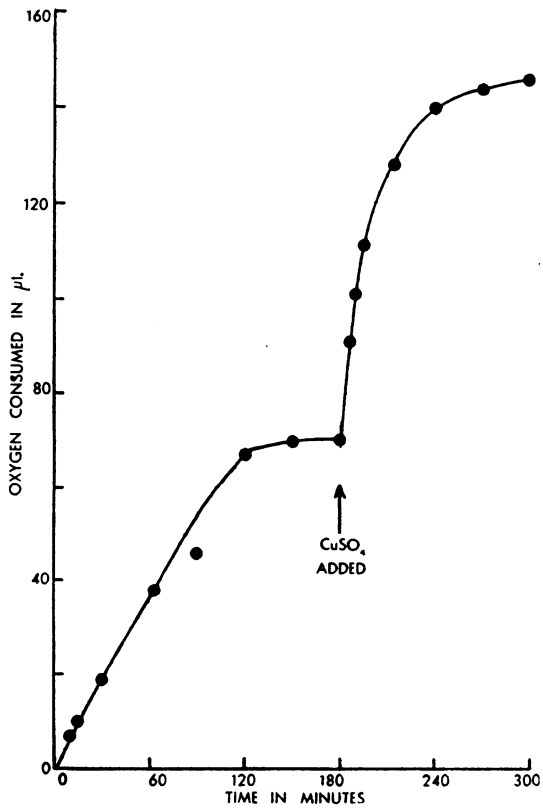

FIG. 3.—Enzymic oxidation of histamine and subsequent addition of $CuSO_4$. 1.5×10^{-3}M $CuSO_4$ added after 180 min.

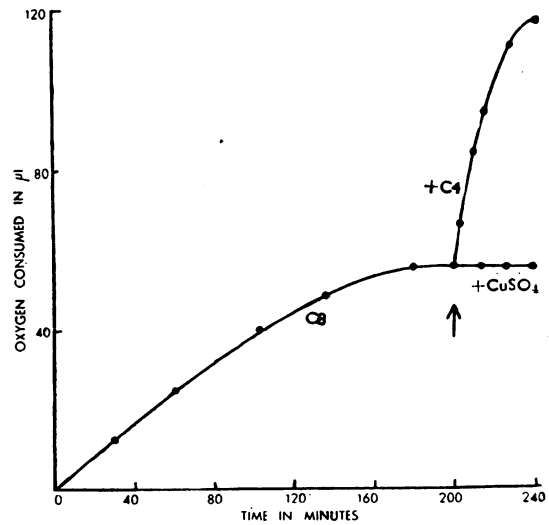

FIG. 4.—Enzymic oxidation of octamethylene diamine and effects of subsequent addition of (*a*) $CuSO_4$ and (*b*) putrescine (C_4). Initial substrate concentration: 2.5×10^{-3}M. Concentration of $CuSO_4$: 1.5×10^{-3}M. Concentration of putrescine: 2.27×10^{-3}M.

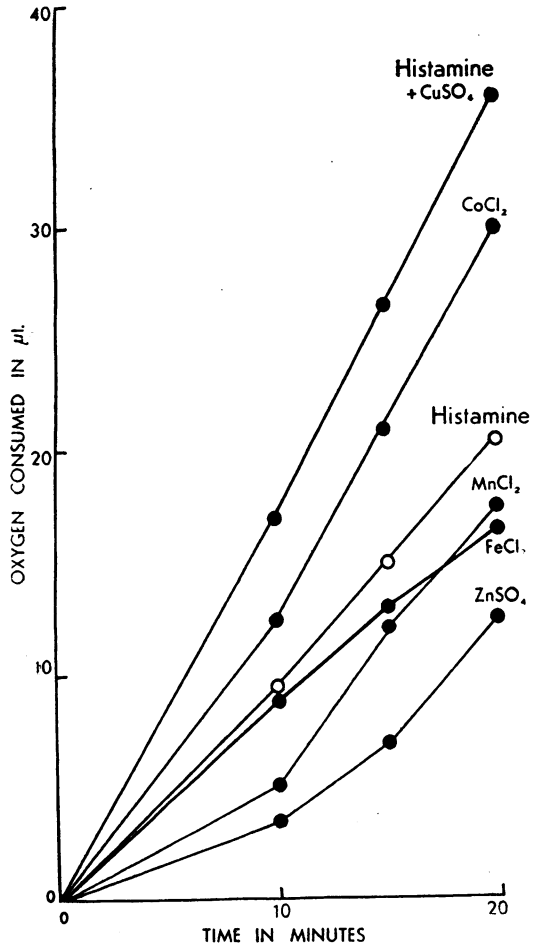

FIG. 5.—Effect of different metals on the enzymic oxidation of histamine. Initial substrate concentration: 5×10^{-3}M. Concentration of metal: 1.5×10^{-3}M.

DISCUSSION

The experiments with histamine show clearly that the increased oxygen uptake in the presence of copper is not due to an oxidation of histamine but of a product of its primary oxidation by histaminase. This is in complete agreement with the observations of Bruns and Stüttgen (1951) on human placenta.

There is still some doubt about the initial point of attack by histaminase on the histamine molecule, but the suggestion that imidazoleacetaldehyde is the first oxidation product has received support from Tabor's (1951) observation that aldehyde oxidase causes a further oxidation after the histaminase reaction is over. Thus it seems that copper catalyses the same reaction as aldehyde oxidase, i.e. the formation of imidazoleacetic acid from imidazoleacetaldehyde.

Copper did not cause an additional oxygen uptake when the aliphatic diamines were the substrates. This is analogous to the observation by Tabor (1951) that the aldehyde oxidase does not act on the primary products formed in the enzymic oxidation of putrescine and cadaverine. He suggests that this is due to the cyclization of the ω-amino-aldehydes formed. Cyclization would probably occur less readily with the shorter and longer amino-aldehydes. It is interesting, therefore, that all the diamines tested gave rise to oxidation products which were not further oxidized by copper.

These observations show that differences in the rates of oxygen uptake when histamine is oxidized by histaminase do not always indicate that the inactivation of histamine proceeds at a different rate. They also show that differences in the oxidation of histamine and aliphatic diamines need not be explained by assuming that two different enzymes are concerned unless differences in the secondary reactions of the products of the enzymic reaction are excluded.

SUMMARY

When the oxidation of histamine is catalysed by histaminase prepared from pig's kidney, the presence of 1.5×10^{-3} M copper increases the rate of oxygen consumption, but the rate of inactivation of histamine is not increased. When copper is added after the histaminase reaction has stopped there is an additional uptake of about one atom of oxygen per molecule of histamine. Copper has no similar effect when aliphatic diamines are oxidized by the pig kidney preparation. The evidence suggests that copper catalyses the further oxidation of the primary oxidation product of histamine but not that of the aliphatic diamines.

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