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Hypertensinase Activity of an L-Aminoacid Oxidase Preparation and of the Venom of *Bothrops neuwidii.*

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There is a certain degree of agreement concerning the possible hydrolytic nature of hypertensinase as an enzyme. Nevertheless its inactivation by tyrosinase (Schroeder and Adams,[1] Croxatto *et al.*[2]), by oxidized cytochrome, and by the oxidizing effect of certain reagents (Cruz Coke[3]) suggest that anoxemia might impair the ability of tissues to destroy the vasoconstrictor properties of hypertensin, as in the case of kidney ischemia. Furthermore, Raska[4] reported that oxygen consumption and the oxidative deamination of amino acids by slices and extracts of kidneys from hypertensive dogs are markedly decreased as compared to kidneys from normal animals.

The fact that the kidney is one of the richest sources of hypertensinase and also has a high *d*- and *l*-amino-acid oxidase activity led us to study the action of preparations of the latter enzyme on hypertensin. As aminopolypeptidase hydrolyzes hypertensin, (Croxatto and Croxatto[5]) this substance must contain free amino groups. Blanchard *et al.*[6] were able to separate *l*-amino-acid oxidase from rat kidney and a similar enzyme was recently shown to be present in snake venom by Zeller and Maritz.[7] Croxatto and Croxatto[8] have shown a high hypertensinase activity in several of these venoms. We attribute this activity to proteolytic enzymes, in spite of the fact that the activity is higher than that of any single crystallized proteolytic enzyme.

In the following experiments, we have used an *l*-amino acid oxidase preparation obtained from rat kidneys by the method of Blanchard *et al.* and the dry venom of a South American snake, *Bothrops neuwidii.*‡ Hypertensin was prepared by the method of Braun Menendez *et al.*[9]

Experiments. Kidney l-amino-acid oxidase. A series of tubes containing 10-20 U of hypertensin per ml, 0.2 M acetate or phosphate buffer (pH 5, 7.4, and 10.4) and different concentrations of *l*-amino-acid oxidase were incubated at 37.5° with aseptic precautions. Tubes containing all the other elements but lacking the enzyme preparation were treated in the same way and served as controls. The rate of hypertensin inactivation was determined by the method of Fasciolo *et al.*[10] by injecting aliquots of the incubated mixture into the femoral vein of an anesthetized cat ("Dial") and comparing the effect on blood pressure with that of the control. The sample used for determination was previously deproteinized by boiling in a water bath and centrifuging to separate the precipitate; the

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1 Schroeder, H., and Adams, N., *J. Exp. Med.*, 1941, **73**, 531.

2 Croxatto, R., Croxatto, H., and Marti, L., Proc. Soc. Exp. Biol. and Med., 1943, **52**, 64.

3 Cruz Coke, E., Conference on Experimental Hypertension, The New York Academy of Science, 1945.

4 Raska, S. B., *J. Exp. Med.*, 1943, **78**, 75.

5 Croxatto, R., and Croxatto, H., *Science*, 1942, **96**, 519.

6 Blanchard, M., Green, D. E., Nocito, V., and Ratner, J., *J. Biol. Chem.*, 1944, **155**, 421.

7 Zeller, E. A., and Maritz, A., *Helvetica Chem. Acta*, 1945, **28**, 365.

8 Croxatto, H., Marsano, A., and Croxatto, R., *Anales Soc. Biol. Bogotá*, 1944, **1**, 147.

‡ A high *l*-amino-acid oxidase activity in this venom was recently found by D. Green (personal communication).

9 Braun Menendez, E., Fasciolo, J. C., Leloir, L. F., and Muñoz, J. M., *J. Physiol.*, 1940, **98**, 283.

10 Fasciolo, J. C., Leloir, L. F., Muñoz, J. M., and Braun Menendez, E., *Rev. Soc. Argent. Biol.*, 1940, **16**, 643.

TABLE I.
Hypertensinase Activity of *l*-aminoxidase Extract.

Enzyme ml*	Time of incubation min	pH	Oxygen†	Inhibitor	Conc. M	Hypertensin inactivation %
0.02	400	7.4	+	—		60
0.02	400	"	—	—		60
0.05	60	"	+	—		50
0.05	60	"	—	—		50
0.05	180	"	+	—		100
0.1	100	"	+	—		80
0.1	100	"	—	—		80
0.1	60	5.4	+	—		70
0.1	60	10.5	+	—		80
0.1	60	7.4	+	—		100
0.02	400	7.4	+	Salicylic acid	0.4	60
0.05	60	"	—	Ammonium sulphate	0.25	50
0.05	180	"	+	"	"	100
0.1	100	"	+	"	"	80
0.05	180	"	+	*l*-leucine	0.1	100
0.05	180	"	—	"	"	100

* Three units of hypertensin were incubated at 37.5°C with the amount of enzyme indicated in this column.
† (+) Indicates presence of oxygen and (—) absence of oxygen in the mixture.

TABLE II.
Hypertensinase Effect of *Bothrops neuwidii* Venom.

Dried venom µg*	Time min	Oxygen†	Inhibitor	Conc. M	Hypertensin inactivation %
1	90	+	—		100
1	90	—	—		100
1	60	—	—		80
1	60	+	—		80
2.5	15	—	—		40
2.5	15	+	—		40
2.5	20	+	—		40
2.5	30	+	—		60
1	60	—	Salicylic acid	0.4	80
1	60	+	"	"	80
2.5	30	+	"	"	60
2.5	30	—	"	"	60
2.5	20	+	4 nitrotoluen sulf.	0.15	40
2.5	20	—	"	"	40

* Three units of hypertensin were incubated at 37.5°C with the amount of the venom indicated in this column.
† (+) Indicates presence of oxygen and (—) absence of oxygen in the mixture.

same procedure was applied to the control. Additional experiments were performed in Thunberg tubes, one series in anaerobic conditions obtained by high vacuum and another in pure oxygen.

Table I shows that hypertensin was inactivated by *l*-amino-acid oxidase preparation and that a complete inactivation of 3 U of hypertensin was achieved by 0.05 cc in 3 hours at pH of 7.4. A lower activity was observed at pH 5.4 and 10.4.

The rate of hypertensin inactivation was the same under aerobic and anaerobic conditions.

The same table shows that 0.25 M ammonium sulfate does not inhibit the inactivating action on hypertensin, contrary to the effect shown on amino acid deamination by Blanchard *et al.* No competitive effect of 0.1 M *l*-leucine was observed; inactivation went on at the same rate as in its absence.

Bothrops neuwidii venom. Applying the technic already described, we studied the effect of this venom on hypertensin. Amounts

of dried venom ranging from 1 to 5 μg were added to 3 U of hypertensin in a medium buffered to pH 7.4 where the venom shows maximum hypertensinase activity.

The results obtained are summarized in Table II. There was no difference in the rate of inactivation whether oxygen was present or absent. The effect of 4-nitrotoluene sulphonic acid and of salicylic acid was studied by addition of 10 mg of the first compound or 20 mg of the second (adjusted to pH 7.4) for every 3 U of hypertensin. Since there was no change in the course of the inactivation, this process is probably not due to the action of the *l*-amino-acid oxidase, as Zeller *et al.*[7] have reported inhibition of this enzyme by these 2 substances.

There are further differences between the inactivation of hypertensin and the deamination of amino acids by *l*-amino-acid oxidase extract. The hypertensinase activity is not affected by absence of oxygen or presence of ammonium sulfate. The inactivation of hypertensin is at a maximum at pH 7.5 and does not decrease sharply when the pH is decreased or increased. According to Blanchard *et al.* the rate of oxygen uptake and ammonia production from amino acids has a pH maximum about 10. Above and below this pH the velocity of oxidation declines sharply, being negligible at pH 7.

Conclusion. The hypertensinase activity shown in the same preparation of *l*-amino-acid oxidase used by Blanchard *et al.* may be attributed to another enzyme, associated as an impurity. The results obtained with the venom of *Bothrops neuwidii* support our previous view that the hypertensinase activity of this venom is due to the presence of a proteolytic agent, and do not support the hypothesis that the *l*-amino-acid oxidase participates in the inactivation of hypertensin by the same venom.

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Effect of Steroid Sex Hormones on Distribution of Alkaline Phosphatase in Uterus of Mouse.*†

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The distribution of alkaline phosphatase in the uterus during the reproductive cycle and pregnancy has been described in several species[1-3] following the development by Gomori[1,4] of methods for demonstrating the presence of this enzyme in tissue sections. It is generally agreed that alkaline phosphatase may be found in any of the component tissues of the endometrium, the exact distribution varying with the physiological state and the species studied. Although the specific function of phosphatase in the physiology of reproduction is not clear, there is reason to believe that

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1 Gomori, G., *J. Cell. and Comp. Physiol.*, 1941, **17**, 71.

2 Kabat, E. A., and Furth, J., *Am. J. Path.*, 1941, **17**, 303.

3 Wislocki, G. B., and Dempsey, E. W., *Am. J. Anat.*, 1945, **77**, 365.

4 Gomori, G., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 23.