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Hypertensin, Hydroxytyramine and Action of Renal Hypertensor Extracts.

R. Croxatto, H. Croxatto and L. Marty. (Introduced by A. Lipschutz.)

From the Laboratory of Physiology, Catholic University, Santiago, Chile.

An hypothesis has been proposed to the effect that the hypertensor substance of renal origin may be a phenolic amine identical with hydroxytyramine (Bing,[1] Bing and Zucker,[2] Schroeder and Adams[3]). The destruction of hypertensin by tyrosinase (Schroeder and Adams[3]) and by an amine oxidase obtained from the liver of the cuttlefish (Croxatto and Croxatto[4]) spoke in favor of this supposition. Moreover, these data favored the idea that the enzyme in the kidney which destroys hypertensin may be an amine oxidase. Bing and coworkers,[5] however, showed that the destruction of angiotonin (hypertensin) by the kidney was not produced by an oxidation deamination and they presented evidence that this organ possesses an aerobic polyphenoloxidase which is capable of destroying specific pressor amines.

The results which are described below further weaken the supposition that hypertensin and hydroxytyramine are chemically identical; it is further established indirectly that hypertensinase (the enzyme destroying hypertensin) if of the polypeptidase type, as has been demonstrated by Croxatto and Croxatto.[6] In effect, the properties of the hypertensor principle derived from the action of renal decarboxylase anaerobically do not correspond to the properties of hypertensin when a comparative study is made of the arterial pressure of the cat and the vascular test of Loewen Trendelenburg (LT) carried out on *Caliptocephalus Gayi;* and, on the other hand, the condition for the destruction of each of these substances by renal extracts is almost the same.

Experimental. I. *Action of hypertensin, tyramine and hydroxytyramine in the LT test and on the arterial pressure of the cat.* Hypertensin was prepared according to the method of Braun Menendez and coworkers.[7] A solution of 0.05 cc of hypertensin diluted with 20 cc of Ringer elicits total vasoconstriction in the LT test. In the cat a dosage 50-100 times greater produces an increase in arterial pressure of 3-4 cm. Tyramine sulfate (Poulenc) at the 1-2 mg level injected in the femoral vein produces an appreciable hypertension in the cat (Fig. 1), but this

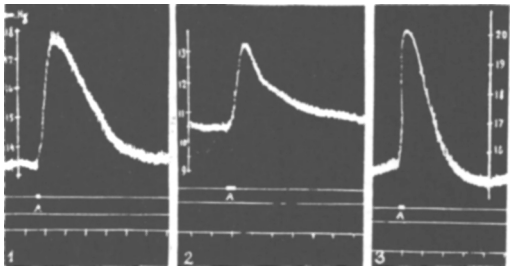


Fig. 1.
Arterial Pressure (Cat).
A. Injection of 0.001 gr tyramine sulfate.

Fig. 2.
At A, injection of 2 cc of renal extract incubated with dopa (8 hours) without deproteinization.

Fig. 3.
At A, same as Fig. 2, but deproteinized and concentrated.

dosage does not cause an appreciable vasoconstrictor effect in the vascular LT test when it is added to the perfusion fluid (Fig 4). Hydroxytyramine, obtained according to the directions of Bing from dopa in the presence of renal tissue under anaerobic conditions, in a dose which induces an ap-

1 Bing, R. J., *Am. J. Physiol.*, 1941, **132**, 497.

2 Bing, R. J., and Zucker, M. B., Proc. Soc. Exp. Biol. and Med., 1941, **46**, 343.

3 Schroeder, H., and Adams, M. H., *J. Exp. Med.*, 1941, **73**, 531.

4 Croxatto, H., and Croxatto, R., Proc. Soc. Exp. Biol. and Med., 1941, **48**, 392.

5 Bing, R. J., Zucker, M. B., and Perkins, W., Proc. Soc. Exp. Biol. and Med., 1941, **48**, 372.

6 Croxatto, H., and Croxatto, R., *Rev. Soc. Argent. Biol.*, 1941, **17**, 439.

7 Braun Menendez, E., Leloir, L. F., and Muñoz, J. E., *J. Physiol.*, 1940, **98**, 283.

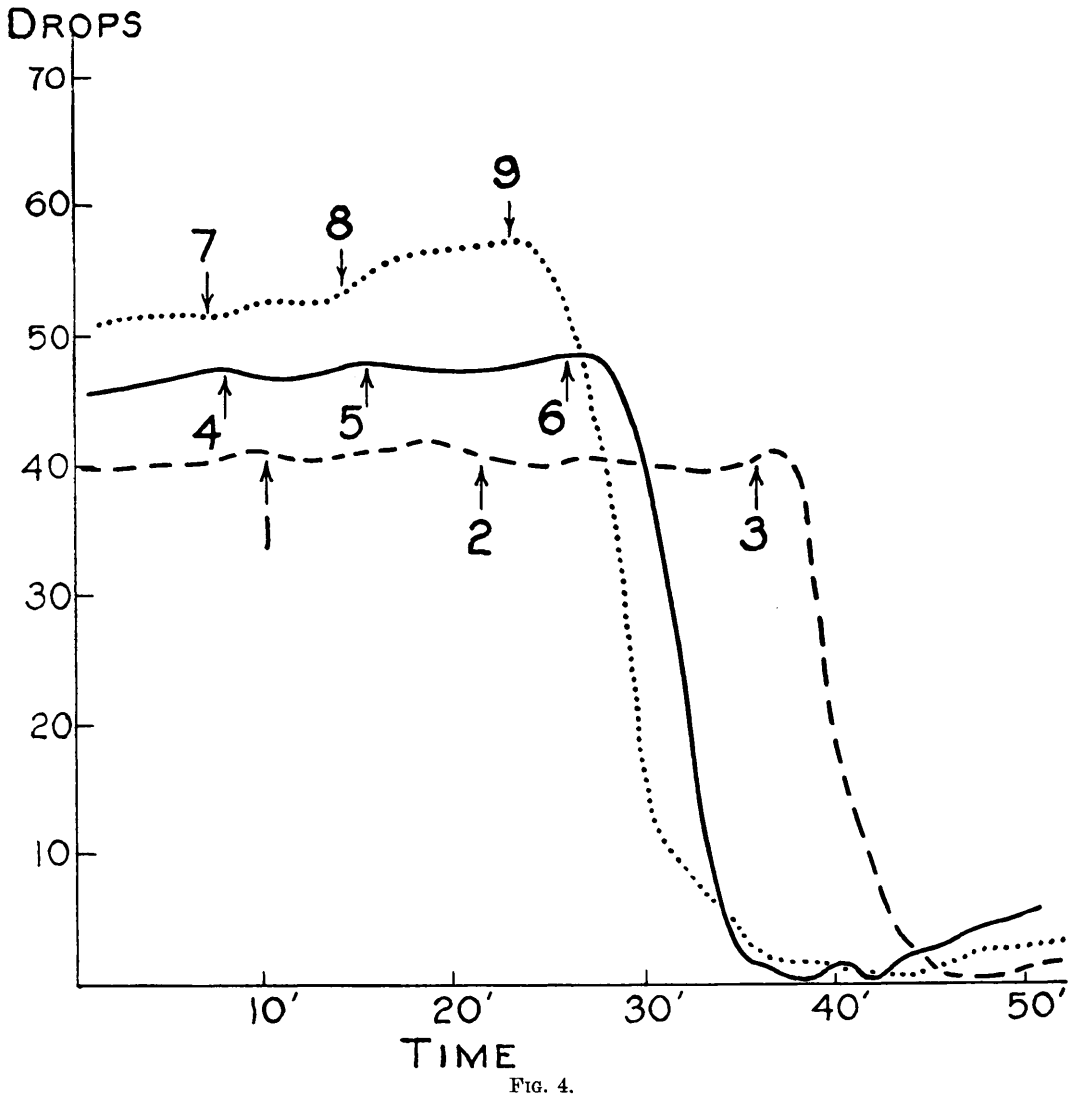


FIG. 4.

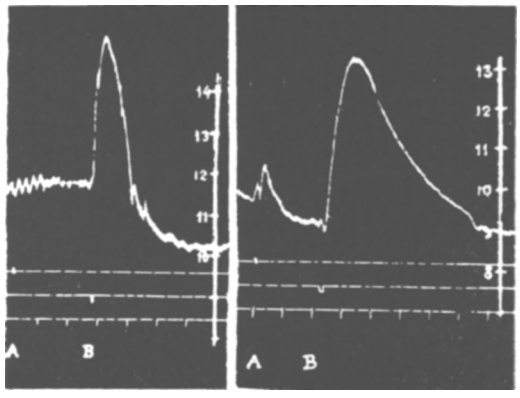
Flow of drops in the posterior ureter of the frog in three different preparations. Arrow 1 indicates the addition of 0.1 mg tyramine sulfate; arrow 2, 1 mg tyramine sulfate; 3, the addition of 1/50 the dose of hypertensin which produces a hypertension equal to 1 mg of tyramine in the cat; 4, addition of 0.2 cc of kidney extract using the cat (Fig. 2); 5, addition of 1 cc of the same extract; 6, same as arrow 3; 7, addition of 0.20 cc of the same extract used on the cat (Fig. 3); 8, addition of 1 cc of the latter extract; 9, same as 3.

preciable hypertension in the cat (Fig. 2 and 3) likewise produces but a very small decrease in the number of drops in the posterior ureter of the frog (Fig. 4). While we were not able to study the effect of crystalline hydroxytyramine, at least the hypertensor substance formed from dopa behaved differently in the frog from the hypertensin obtained by the action of renin on hypertensinogen.

II. *Enzymatic characteristics of hypertensive renal extracts.* An extract from hog liver capable of actively destroying hypertensin and pepsitensin may be prepared by extracting acetone-dried liver tissue with 2% NaCl, fractionally precipitating with acetone until the concentration is 1:1, and dissolving the precipitate in water. This property disappears on heating and has been attributed to the presence of a hypertensinase. This

FIG. 5.
Arterial Pressure (Cat).
A. Injection of 0.3 mg of epinephrine plus 0.5 cc of tyrosinase, incubated at pH 7.4 for 4 hours at 38°.
B. Injection of 0.15 mg of epinephrine kept at 38° at pH 7.4 for 4 hours.

FIG. 6.
A. 2 mg tyramine HCl plus 1 cc of tyrosinase, incubated for 3 hours at pH 7.4 at 38°.
B. Same as A, but without tyrosinase.



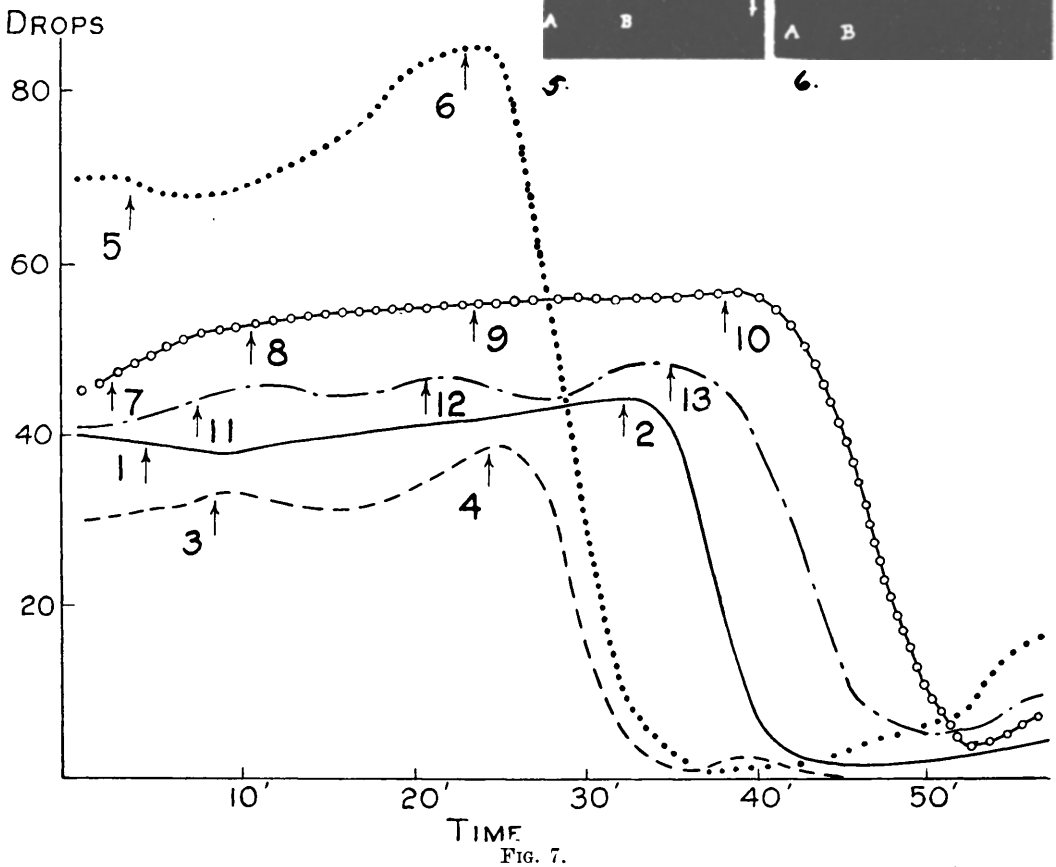


FIG. 7.
Flow of drops in the Loewen Trendelenburg preparation. Ordinate: No. drops per minute; abscissa: time in minutes.

1. Epinephrine 4 mg, incubated 25 min with 0.2 cc tyrosinase.
2. Epinephrine 4 mg.
3. Renin 0.1 cc, hypertensinogen 0.5 cc, renal extract 0.2 cc, incubated 15 min.
4. Same as 3, without renal extract.
5. Same as 3, incubated 10 min.
6. Epinephrine 4 mg, renal extract 0.2 cc, incubated 45 min.
7. Renin 0.1 cc, hypertensinogen 0.5 cc, renal extract 0.2 cc, incubated 20 min.
8. Same as 7, incubated anærobically 35 min.
9. Same as 7, incubated with 0.5 mg KCN.
10. Same as 7, but with the renal extract previously boiled for ½ min.
11. Renal extract 0.2 cc previously treated with octyl alcohol for 12 hours, then incubated 30 min with 0.1 cc renin and 0.5 cc hypertensinogen.
12. Same as 11, without octyl alcohol.
13. Renin 0.1 cc plus hypertensinogen 0.5 cc incubated 30 min.

enzyme destroys hypertensin after a short incubation period, but does not affect the integrity of tyramine and epinephrine, since both amines retain their vasoconstrictor action despite incubation for several hours at 37° at pH 6-8 in the presence of hypertensinase. In contrast, tyrosinase inactivates epinephrine and tyramine rapidly (Fig. 5 and 6). Besides, if hypertensin or epinephrine are placed in contact with tyrosinase under anaerobic conditions for a long period, both substrates are not attacked, while hypertensinase is equally active in the presence or absence of O_2 (Fasciolo and coworkers;[8] Bing and coworkers[5]). A similar difference between hypertensinase and tyrosinase may be demonstrated when these two enzymes act on hypertensin in the presence of cyanide (Fig. 7). Tyrosinase is totally inhibited while hypertensinase is unaffected. The amine oxidase obtained from cuttlefish liver is inhibited by octyl alcohol. Renal hypertensinase is unaffected by the presence of this alcohol (Fig. 7). The sensitivity of hypertensin in respect to tyrosinase and amine oxidase under anaerobic conditions has been interpreted as the possession of a common chemical grouping by tyramine and hypertensin. Hypertensin, like pepsitensin, is a polypeptide within all probability an end amino phenol group. Hypertensor extracts of the kidneys, even when highly purified, have been shown in previous investigations to behave like polypeptidases (Croxatto and Croxatto[6]).

Summary. The vasoconstrictor action of hypertensin and of the vasoconstrictor principle formed anaerobically by the action of renal tissue on dopa have been the subjects of study. It was shown that hypertensin has no vasoconstrictor effect in the Loewen-Trendelenburg test at a level producing a marked hypertension effect in the cat. Renal hypertensinase behaves similarly both aerobically and anaerobically, and its activity is not affected by cyanide or octyl alcohol, from which it is concluded that there is a clean-cut difference between this enzyme, amino oxidase and tyrosinase. These findings support the finding that hypertensinase has the characteristics of a polypeptidase (Croxatto and Croxatto[6]).

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

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Active Immunization of Rats Against *Nippostrongylus muris.*

JOHN Y. C. WATT. (Introduced by Morton C. Kahn.)

From the Department of Public Health and Preventive Medicine, Cornell University Medical College.

The fact that acquired immunity against the nematode, *Nippostrongylus muris,* could be induced in white rats by the injection of the homologous worm material was first demonstrated by Chandler.[1,2] This investigator employed vaccines prepared from larvae and adult worms killed by heat; 3% H_2O_2; saturated salt solution, and by drying, then suspending in saline. Therefore the vaccines were in the nature of a suspension of worm material.

The purpose of the present study was to test the effect of parenteral vaccination with two types of antigens, prepared by filtering aqueous extracts of the dried adult worms and of larvae of *N. muris,* upon the resistance of pied stock rats, in an effort to determine whether immunity could be developed in these animals with the particle-free filtrate.

Plan of Study. The strain of parasites was isolated from a single wild Norway rat captured in 1939, and maintained in this labo-

[1] Chandler, A. C., *Am. J. Hyg.*, 1932A, **16**, 750.
[2] Chandler, A. C., *Am. J. Hyg.*, 1936B, **24**, 129.