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Inhibitory Action of Amine-Oxidase and Tyrosinase upon the Vasoconstrictor Effect of Hypertensin.

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The publications of Holtz[1, 2] confirmed by Bing[3, 4] suggest that the vasoconstrictor principle which is found in the ischemic kidney is identical with hydroxytyramine. The nature of this pressor amine suggests that it may be destroyed enzymatically by means of two processes: by deamination of its side chain or by oxidation of its nucleus, since the latter possesses a phenolic character. Both Holtz and Bing favor the first mentioned view and regard the destruction as due to the action of amine-oxidase. Schroeder and Adams[5, 6] furnished experimental and clinical evidence showing that tyrosinase—a ferment which acts on the α-dihydrophenols and their corresponding α-quinones—decreases the blood pressure of hypertensive animals and inactivates angiotonin, tyramine and adrenalin *in vitro*.

The substance (hypertensin or angiotonin) which is formed through the interaction of renin and the pseudoglobulins of the serum, can readily be detected because of its direct vasoconstrictor action on Loewen Trendelenburg preparations of the posterior extremities of frogs.[7, 8] This test represents an adequate method for the investigation of hypertensin activity and thus may be used for the determination of any enzymatic action capable of destroying such pressor amines.

Our investigations revealed that hypertensin proves to be very labile if it is incubated at 20-39°C for a short time with extracts containing amine-oxidase. Blaschko[9, 10] reported that tissue extracts contain an amine-oxidase which destroys the vasoconstrictor proper-

1 Holtz, P., *Klin. Wschr.*, 1937, **16**, 1561.

2 Holtz, P., und Heise, R., *Arch. Exp. Path. u. Pharm.*, 1939, **191**, 87.

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

4 Bing, R. J., and Zucker, M. B., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 343.

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

6 Schroeder, H. A., *Science*, 1941, **93**, 116.

7 Houssay, B. A., et Taquini, A. C., *C. R. Soc. Biol.*, 1938, **128**, 1125.

8 Garreton, A., Croxatto, R., Fuenzalida, O., e Viveros, R., *Rev. Arg. de Card.*, 1941, **8**, 1.

9 Blaschko, H., and Richter, D., *J. Physiol.*, 1937, **90**, 1.

10 Blaschko, H., and Schlossmann, H., *J. Physiol.*, 1940, **98**, 130.

ties of adrenalin and tyramine. Since this enzyme is present in appreciable quantities in the liver of certain cephalopodes,[11] we decided to study the action of an extract prepared from the liver of the squid, *Sepia officinalis*.

The recent work of Schroeder[5] induced us to examine, furthermore, whether the vasoconstrictor action of hypertensin—tested on the hind legs of the frog—is inhibited by tyrosinase. The results of these experiments are in agreement with those obtained following the use of amine-oxidase. Probably tyrosinase inactivates the vasoconstrictor substances because it leads to the oxidation of their phenolic nucleus while amine-oxidase would inactivate the same substances by deaminating the side chain.

Experimental Findings. Previous tests proved that if a mixture of renin obtained from hog kidney by the method of Helmer and Page[12] is incubated during 10-15 minutes with hypertensinogen ("renin activator") of cattle serum, purified by the method of Page and Helmer,[13] it assumes the ability to cause intense vasoconstriction in the hind limbs of the Chilean frog *(Caliptocephalus gayii)* perfused at constant pressure. 0.2 mg of renin and 0.5 ml of hypertensinogen thus incubated for 10-15 minutes suffice to reduce by 100% the number of drops leaving the abdominal vein. The same mixture incubated either before or after the 15-minute period with an extract of the liver of the squid within a very short time completely loses its vasoconstrictor ability, indeed in many cases it assumes vasodilator properties. This extract obtained according to the specifications of Blaschko[11] is rich in amine-oxidase. The dose of 0.05 ml of the liver extract completely destroys the vasoconstrictor effect of hypertensin within 5-10 minutes. A tyrosinase-containing purified extract of the mushroom *(Psalliota campestris)* obtained by the method of Nelson[14] likewise possesses the ability to destroy the activity of hypertensin following a short period of incubation (Table I).

We are now engaged in a purification of the two enzymes but meanwhile our first concern was to identify them. A comparative study of various tissues of the squid showed that the muscles possess a much less pronounced hypertensin-destroying ability than the liver. In agreement with the findings of Blaschko,[11] this difference may be ascribed to the comparatively small quantity of amine-

[11] Blaschko, H., *J. Physiol.*, 1941, **99**, 364.

[12] Helmer, O. M., and Page, I. H., *J. Biol. Chem.*, 1939, **128**, 757.

[13] Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 29.

[14] Ludwig, B. J., and Nelson, J. M., *J. Am. Chem. Soc.*, 1939, **61**, 2601.

TABLE I.
Effect of Amine-oxidase and Tyrosinase on Vasoconstrictor Potency of Hypertensin as Tested on the Hind Leg of the Frog.

Protocol No.	Substances added following 15 min. incubation, 36°C	Initial output No. of drops per min. prior to extr. administration	Max. output (maximal No. of drops per min. after extr. administration)	Min. during which max. effect evident
68	.1 ml renin + .5 ml hypertensinogen	40	0	3
81	.1 ml renin + .5 ml hypert. + .1 ml. extr. of squid	46	50	—
82	.1 ml ren. + .5 ml hypert. + .1 ml liver extr. of squid boiled 2 min.	50	3	5
85	.1 ml ren. + .5 ml hypert. + .1 ml liver extr. of squid + urea 3/M	36	15	3
92	.1 ml ren. + .5 ml hypert. + .1 ml liver extr. of squid + octyl alcohol	31	0	6
94	.1 ml ren. + .5 ml hypert. + .1 ml liver extr. of squid + 2.5 mg NaCN*	35	33	10
189	.1 ml ren. + .5 ml hypert.	28	1	4
169	.1 ml ren. + .5 ml hypert. + .25 ml tyrosinase	50	63	9
173	.1 ml ren. + .5 ml hypert. + .25 ml tyrosinase boiled 5 min.	65	0	8
194	.1 ml ren. + .5 ml hypert. + .25 ml tyrosinase + .01 g sodium benzoate	35	0	4
191	.1 ml ren. + .5 ml hypert. + .25 ml tyrosinase + .003 g NaCN*	57	0	3
200	.1 ml renin + .5 ml hypert. + .25 ml tyrosinase + .001 g NaCN*	70	12	4
197	.1 ml renin + .5 ml hypert. + .25 ml tyrosinase + octyl alcohol*	45	70	8

*The NaCN and the octyl alcohol acted on the respective ferments during several hours before these were mixed with renin and hypertensinogen.
In order to save space only one experiment of each series is reported in this table.

oxidase present in muscular tissue. Boiling destroys the action of the extracts. The presence of octyl alcohol destroys 60-100% of the antihypertensin activity. Urea in a 3 times molar concentration appreciably reduces this activity when kept in contact with the extract during 24 hours. On the other hand, HCN does not suppress the activity of the extract. This furnishes an indirect proof that the enzyme is an amine-oxidase.

The mushroom extract appears to be very sensitive to HCN and sodium benzoate, especially if these act on the enzyme for several hours. Both of these substances separately can completely or almost completely inhibit the activity of the enzyme. Octyl alcohol, on the other hand, has no appreciable effect. The activity of the mushroom extracts (some obtained by the method of Keilin and Mann[15]) was measured in the Warburg apparatus using catechol

[15] Keilin, D., and Mann, T., *Proc. Roy. Soc. SB.*, 1938, **125**, 186.

as substrate. The results ran parallel with those obtained in the perfused hind legs of the frog. The oxygen consumption was completely inhibited by HCN but not affected by octyl alcohol.

Discussion. The inhibitory action of amine-oxidase and tyrosinase on the product of the interaction between renin and hypertensinogen constitutes an indirect proof that the vasoconstrictor substance is a phenolic pressor amine as has been suggested by Holtz, Bing and Schroeder. Even though the enzymes used in these experiments have not been completely purified up to the present, their different behavior towards HCN, octyl alcohol, etc., is in agreement with the conception that the activity of squid extracts is due to amine-oxidase and that of the mushroom extracts to tyrosinase.

Summary. The vasoconstrictor substance (hypertensin, angiotonin) obtained by the interaction of renin and hypertensinogen and tested on the Loewen-Trendelenburg preparation is destroyed (enzymatic destruction) in contact with amine-oxidase (extract of *Sepia officinalis* liver) or tyrosinase (extract of *Psalliota campestris* liver).

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Sex and Proteinuria of Mice.*

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While engaged in an investigation of possible systemic effects of methylcholanthrene on mice, many samples of their urine were analyzed. These urines frequently contained a surprisingly large amount of coagulable protein. This was regarded as due to the toxicity of the hydrocarbon until examination of normal, untreated and apparently healthy animals also revealed a high proteinuria. Further observations disclosed that this proteinuria is essentially sex-linked, for it was encountered very conspicuously in the males and usually only to a much smaller degree in females. The urine samples were from 9 pure strains (Swiss, A, New Buffalo, Old Buffalo, D, C_3H, CBA, AKA, C57 Black) and mixed stock mice. The mixed stock animals were of vague ancestry but definitely no

* Aided by a grant from the U. S. Public Health Service.