

Influence of Epinephrine on Vasoconstrictive Action of Organ Extracts.*

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Epinephrine quantitatively insufficient in itself to have any constrictor effect has been observed to activate various otherwise inert fractions of kidney extract as demonstrated by constriction of the vessels of the perfused rabbit's ear.¹ It has been shown that this effect is neither species nor organ specific. This evidence will now be presented together with such facts as have been secured related to the character of the agent in the tissue extracts.

Organ extracts of hog, dog, and rabbit including liver and heart as well as kidney have been prepared in the following several ways:

(1) Approximately 200 g of minced organ were thoroughly mixed with equal amounts of saline, kept at 5°C for 24 hours prior to removing the precipitate by centrifugation and dialysis of the supernatant fluid.

(2) Twenty g of the dry powder residue after acetone-ether extraction of minced organ, were treated with approximately 150 cc of 2% saline and centrifuged to secure the supernatant for dialysis.

(3) Many methods of fractionating the above more crude extracts were utilized. Only the one that yielded a product of greater interest is detailed. Approximately 5 kg of fresh hog liver were minced. The following procedures were carried out at 5°C: Extraction with 10 liters of distilled water for 24 hours and filtration through several layers of gauze; the careful adjustment of the filtrate to pH 2.9 by slow addition of 10% trichloroacetic acid. (The final concentration of the trichloroacetic acid was 1.4%.) Removal of the precipitate through fluted filter paper (Whatman No. 2) after 6 hours. The filtrate was

then fractionated with ammonium sulphate, the precipitates redissolved and dialyzed, re-fractionated with ammonium sulphate and alcohol, in general following the procedure described for the preparation of renin from kidney.² The obtained organ fractions are of protein nature and differ from the dialysates prepared by Cannon and Lissak,³ the alcohol extracts used by v. Euler⁴ and the preparations made by Raab and Humphreys.⁵

Results. No significant vasoconstriction resulted from the perfusion of the rabbit's ear with either the dialyzed saline extracts of hog heart or liver or with the saline preparation of the powders resulting from acetone-ether treatment of any of the three organs of the several animal species. Even addition of minimal quantities of epinephrine had reasonably little effect as supplement to the saline extract of minced organ. This latter finding contrasted with the result of similar addition of epinephrine to the saline preparation derived from treatment of organ-powder after acetone-ether extraction. Vasoconstriction was always evident and as a rule it was marked. This is illustrated in Fig. 1.

Marked vasoconstrictor effects resulted from the addition of epinephrine in minimal amounts to the last fraction of the liver designated as IV A. A typical experiment is illustrated in Fig. 2.

Throughout this study attention was directed to the ratio of the nitrogen content of the perfusate to the activity curve. The trend had been downward and with Fraction IV A reached the new low. Two cubic centimeters

² Katz, Y. J., and Goldblatt, H., *J. Exp. Med.*, 1943, **67**, 78.

³ Cannon, W. B., and Lissak, K., *Am. J. Physiol.*, 1939, **125**, 765.

⁴ v. Euler, U. S., *Nature*, **157**, 369, March 23, 1946.

⁵ Raab, W., and Humphreys, R. J., *Am. J. Physiol.*, 1944, **148**, 460.

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¹ Mylon, E., Horton, F. H., and Levy, R. P., *Proc. Soc. Exp. Biol. and Med.*, 1947, **66**, 375.

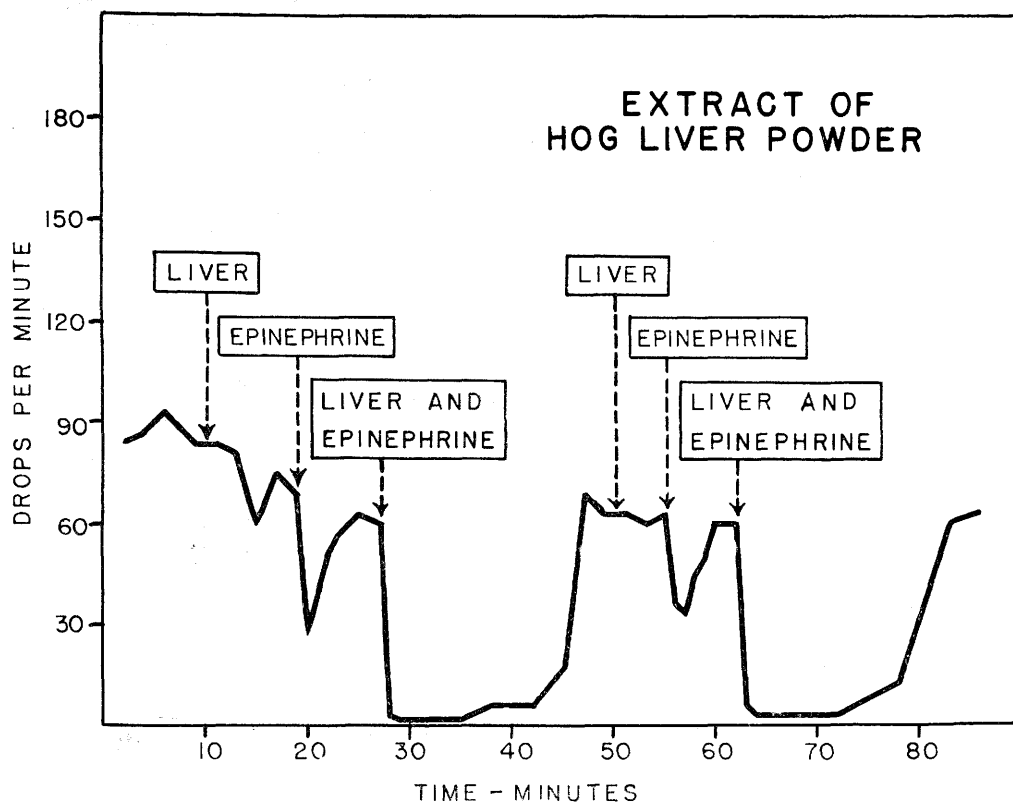


FIG. 1.

Vasoconstrictor action of a combination of an extract of hog liver powder and epinephrine. The epinephrine amount used was 1 cc of 1:2,500,000. It should be added that at least 5 times the amount of epinephrine alone is required to produce an equipressor effect.

of the extract with high activity had a total content of only 0.8 mg of protein.

To corroborate this result in part, 2 more lots of liver were processed after the same fashion detailed above. Despite rigid adherence to the prescribed method, the fractions of the two new lots with highest activity when supplemented with epinephrine were not the same. This is shown in Table I that represents 36 experiments with the various fractions of lots 2 and 3.

Analysis of Fraction IV A. This fraction

was lyophilized and no reduction of its vasoconstrictive resulted. Samples of the lyophilized fraction, a fluffy, light brown powder, were analyzed with the following results:

1. *Qualitative Tests:* The usual qualitative tests for protein were positive. 95% of the nitrogen was amino N. There was a positive test for sulfur.

2. *Quantitative Analysis:* All elements reported were calculated on a moisture and ash free basis.

%H	C	N	S	P*
6.98	49.18	15.18	0.95	0.06

* The phosphorus probably represents an impurity in the ash.

Discussion. Protein fractions of several organs of various species become vasoconstrictive when supplemented with subthreshold quantities of epinephrine just as such addition is known to effect otherwise inactive sympathomimetic amines.⁶ Whether this activation

TABLE I.

Activity	Fractions	Protein range, mg/cc	Lot No.
None	3	—	2
Moderate	10	0.12- 5.6	2
Marked	5	2.6 -13.2	2
None	4	0.5 - 1.7	3
Moderate	3	0.8 - 2.4	3
Marked	6	0.4 - 4.7	3

⁶ Morton, M. C., and Tainter, M. L., *J. Physiol.*, 1940, **98**, 264.

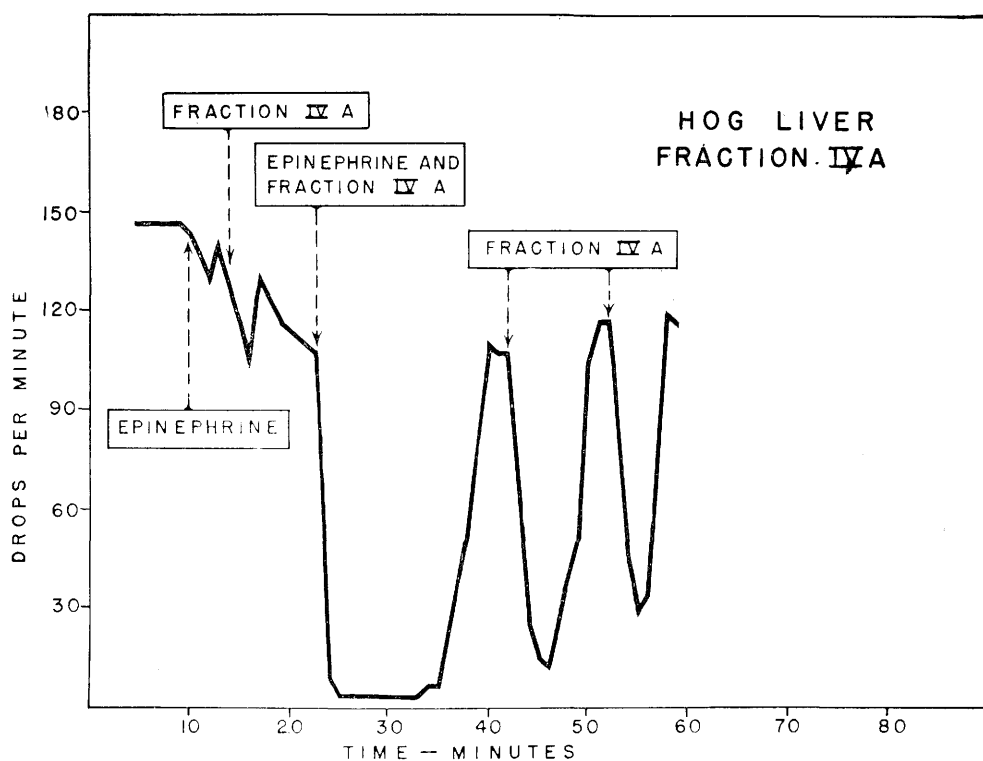


FIG. 2.

Vasoconstrictor action of a combination of the hog liver fraction IVA and epinephrine. Epinephrine amount used: 1:2,500,000. Note the slight constrictor effect of fraction IVA alone, and the marked constriction subsequent to the injection of the combination of fraction IVA and epinephrine. This marked constriction never occurred prior to the injection of the combination. No explanation is offered for this effect.

indicates a potentiation of epinephrine or a stimulation of effector cells normally responding to this compound remains unanswered.

Varying amounts of phospholipid were removed from the protein fraction by the different methods employed for separation. This may be associated with the change of the vasoconstrictor effect on blood vessels.

Summary. Many protein fractions isolated from kidney, liver and heart of hog, and from kidney and liver of dog and rabbit, inactive when perfused alone through the rabbit's ear, become vasoconstrictive at varying degrees

when combined with subthreshold quantities of epinephrine.

In contrast, dialyzed saline extracts of fresh minced organs were inactive with and without addition of epinephrine. After freezing and thawing or prolonged dialyzation against distilled water epinephrine addition resulted in some activity. 0.8 mg of protein or protein mixture secured by continued fractionation of hog liver and combined with subthreshold amounts of epinephrine, produced marked and long lasting vasoconstrictions.