in these studies, although we have observed it in normal uncentrifuged erythrocytes treated by other methods.[6]

Summary. Three distinct substances which differ in their relative specific gravities have been stratified by high centrifugal force in the erythrocytes of man. These substances seem to differ chemically for they may be differentially stained by certain dyes. The stretching of certain of the erythrocytes by centrifugal force indicates that they possess a membrane of considerable elasticity.

[6] Jordan, H. E., Kindred, J. E., and Beams, H. W., *Anat. Rec.*, 1930, **46**, 139.

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Influence of Epinephrine on Vasoconstrictor Action of Kidney Extracts.*

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Perfusion of rabbit's ears with saline extracts of minced kidney is usually associated with vasoconstriction. Minute amounts of histamine or histamine-like compounds present in the minced organ are thought to be responsible for this effect. When these are removed by dialysis, acetone-ether treatment and other methods involved in fractionation of the crude preparation, the vasoconstrictive action as determined with the rabbit's ear or the dog's tail, is lost.

It is reported, further, that the vasoconstrictive action of some kidney fractions as demonstrated by intravenous injection in the living animal may be absent when tested by the perfusion method.[1,2,3] Important conclusions, it will be recalled, have been drawn from this discrepancy as determined by the two methods of testing the same extract.[2,3] Little if any attention on the other hand has been given the reports of Schaumann,[4] Burn and Tainter[5] and more recently of Morton and Tainter.[6] These authors observed that various sympathomimetic amines are vaso-inactive when perfused through isolated peripheral blood vessels. When, however, subthreshold amounts of epinephrine are added to the perfusate, strong vasoconstriction follows.

In the light of these findings the vasoactivity of various kidney extracts has been re-examined.

As test object the vessels of the isolated rabbit's ear were selected. This is a technique devised by Pisemski and Krawkow,[7] modified by Schlossman,[8] Kahlson and von Werz,[9] and further by Brandt and Katz.[10] More recently it has been extensively employed by Page[11] who incorporated pulsating pressure. It is the general opinion that this method adequately permits measurement of vasoconstrictive substances in perfusion fluids.

Materials and Methods. In general, the experiments followed the technique described by Landis[12] and by Morton and Tainter.[6] A constant, non-pulsating perfusing pressure

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[1] Hessel, G., *Klin. Wchnschr.*, 1938, **17**, 843.

[2] Braun-Menendez *et al.*, *Renal Hypertension*, translated by Dexter, L., Chas. C. Thomas, Springfield, Ill., 1946.

[3] Page, T. H., and Helmer, P. M., *J. Exp. Med.*, 1940, **71**, 29, 495.

[4] Schaumann, O., *Arch. Path. Pharmacol.*, 1928, **138**, 208.

[5] Burn, J. H., and Tainter, M. L., *J. Physiol.*, 1931, **71**, 169.

[6] Morton, M. C., and Tainter, M. L., *J. Physiol.*, 1940, **98**, 263.

[7] Krawkow, N. P. *et al.*, *Z. f. die ges Exp. Med.*, 1922, **27**, 127.

[8] Schlossman, E., *Arch. f. Exp. Path. u. Pharmacol.*, 1927, **121**, 160.

[9] Kahlson, G., and von Werz, R., *ibid.*, 1930, **148**, 173.

[10] Brandt, G., and Katz, G., *Z. f. Klin. Med.*, 1933, **123**, 23.

[11] Page, T. H., *Am. Heart J.*, 1942, **23**, 336.

[12] Landis, E. M., *et al.*, *Am. J. Physiol.*, 1943, **139**, 26.

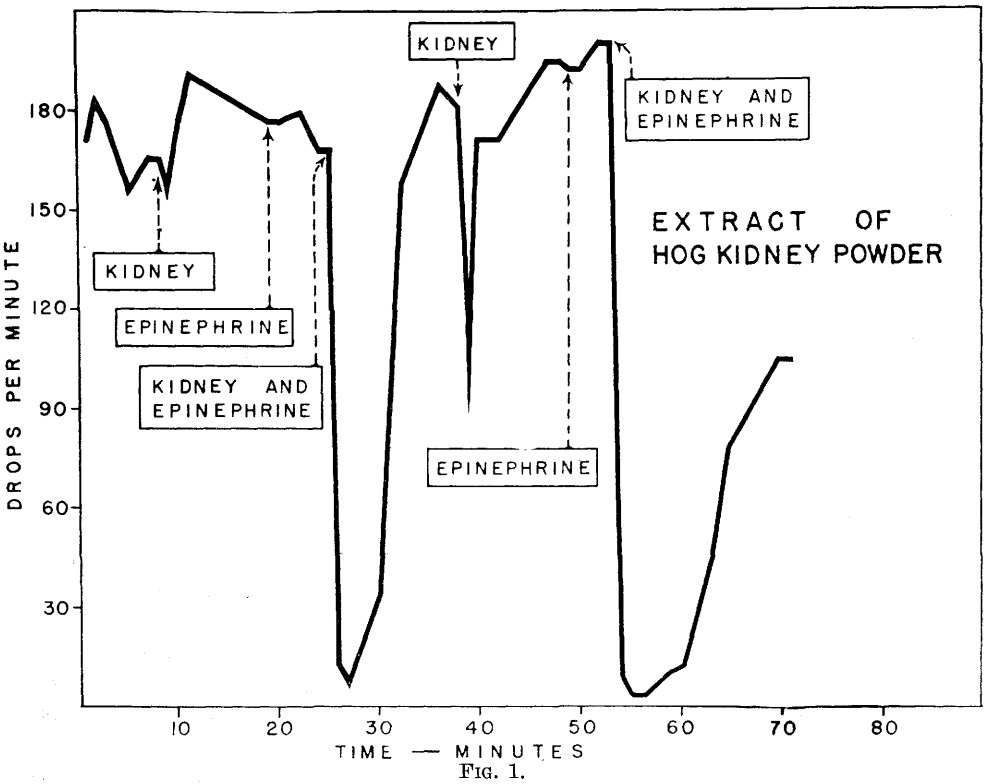

FIG. 1.

Vasoconstrictor action of a combination of an extract of hog kidney powder and epinephrine. Epinephrine amount used: 1 cc of 1:2,500,000.

was employed since Morton and Tainter[6] did not observe any significant differences between constant and pulsating pressure as tested on the hind leg of the cat. Careful washing out of the ear with Ringer-Locke solution preceded each injection of test material. Epinephrine (Parke, Davis 1:1000) was diluted with distilled water and finally 0.5 cc, 1.0 cc or 1.5 cc of this 1:2,500,000 or 1:5,000,000 solution was made up to 8 cc with Ringer-Locke. The subthreshold dose was determined for each ear and was found to range between .2 and .6 μg. When a mixture of epinephrine and a kidney extract was to be tested, an appropriate amount of Ringer-Locke solution was replaced by kidney extract, the total volume remaining 8 cc.

Hog Kidney Fractions. The simplest of these preparations resulted from the extraction of the dry powder secured by acetone-ether treatment of fresh minced hog kidney. Twenty grams of the powder were extracted with approximately 150 cc of 2% saline; this suspension was then centrifuged and the supernatant dialyzed.

A second preparation made in this laboratory in 1939[13] resulted from the further treatment of the above saline extract with mono- and dipotassium phosphate and then with 10% sodium chloride at pH 4.0.[14] On intravenous injection 3.6 mg of the resulting protein raised the blood pressure of a 10 kilo dog 30-40 mm of Hg.

A third preparation was made recently by a different method. Aqueous extracts of hog kidney brought to pH 2.9 with 10% trichloracetic acid were filtered, neutralized and fractionated with ammonium-sulfate and alcohol. The procedure is similar to one described for the preparation of renin.[15] 0.36 mg of the

[13] Winternitz, M. C., Mylon, E., Waters, L. L., and Katzenstein, R., *Yale J. Biol. and Med.*, 1940, **12**, 623.

[14] Helmer, P. M., and Page, I. H., *J. Biol. Chem.*, 1939, **127**, 757.

[15] Katz, Y. I., and Goldblatt, H., *J. Exp. Med.*, 1943, **78**, 67.

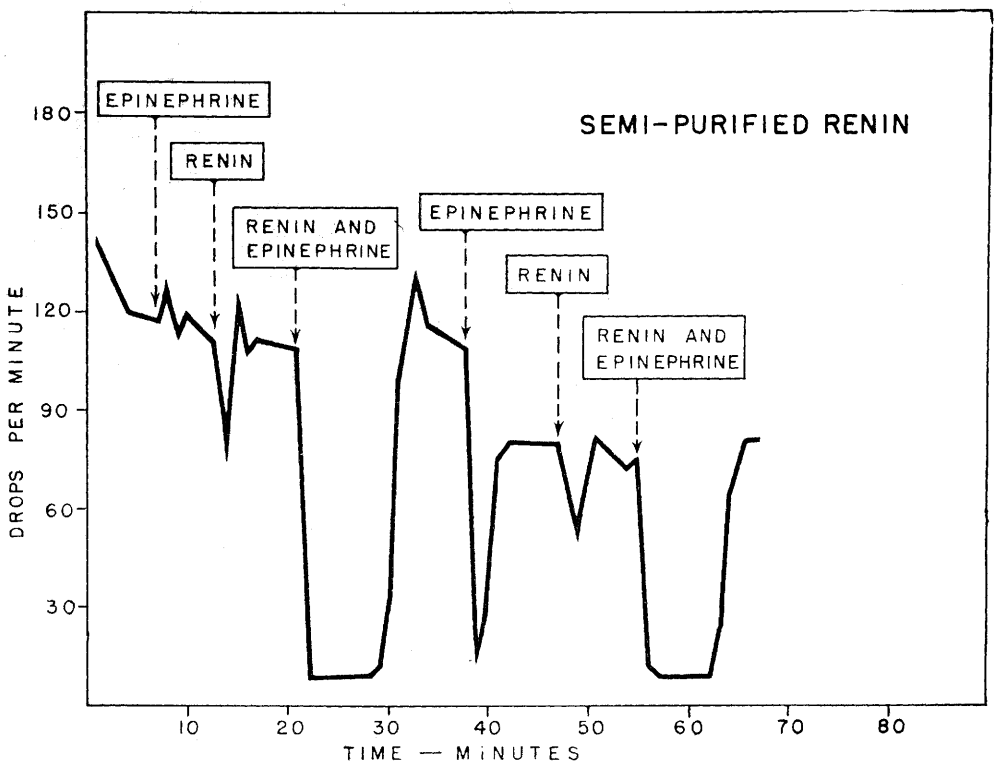

FIG. 2.
Vasoconstrictor action of a combination of semi-purified renin and epinephrine. Epinephrine amount used: 1 cc of 1:2,500,000.

resulting lyophilized protein mixture injected intravenously raised the blood pressure of a 10 kilo dog 30-40 mm of Hg.

Results. The saline extract of the dry powder produced no significant effect on the flow of the perfusate through the rabbit's ear in 10 trials. When combined with a quantity of epinephrine, in itself without effect on the perfused vessels, marked vasoconstriction followed. A typical experiment is recorded in Fig. 1.

A short constrictor effect was occasionally encountered on the second or third injection of amounts of epinephrine that were "subthreshold" on the first injection. This contrasted with the prolonged constriction that followed the combination of epinephrine and renin.

The second preparation gave equivocal results. In itself inactive on perfusion, vasoconstriction followed with addition of a subthreshold quantity of epinephrine in 16 of the 26 trials. The preparation was found to be only relatively soluble, as might have been anticipated after years of storage and this is thought to be associated with the inconstant results secured.

The findings were uniform with the recently prepared and quite soluble third preparation. No vasoconstriction followed when it was added to the isolated rabbit ear perfusate in many trials. When, however, it was supplemented with the above described minimal amounts of epinephrine marked vasoconstriction invariably resulted. This is illustrated in Fig. 2.

Summary. Protein fractions of hog kidney, in themselves inactive on perfusion, become strongly vasoconstrictive when supplemented with traces of epinephrine. While a similar influence of minute quantities of epinephrine on vasoconstriction is well known to occur with some sympathomimetic amines, insufficient information is at hand to clarify the pharmacological nature of the observed synergistic action.