Summary. Mouse brains infected with the FA strain of Theiler's mouse encephalomyelitis virus have been diluted ten million times so that the solutions were no longer infective. By means of pervaporation it has been possible to concentrate 4000 cc samples of such inactive solutions into volumes of 40 to 80 cc. The virus present in the pervaporated solutions has been further concentrated by sedimentation in the ultracentrifuge into active fractions less than 1 cc in volume.

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Identification of Amines in Anaerobic Kidney Extracts.

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In recent studies on the formation of pressor amines by the kidney, Holz and Heise[1, 2] found that under anaerobic conditions, renal extracts could transform l-tyrosine and l-dihydroxyphenylalanine (l-dopa) into their corresponding amines. Bing and Zucker[3] reported that the decarboxylating enzymes in the kidney are specific for certain amino acids and that species differences exist. It was also found that the perfusion of kidneys with l-dopa, under conditions of oxygen lack, would result in the formation of a pressor substance, presumably hydroxytyramine.[4] Victor, Steiner and Weeks[5] prepared an anaerobic extract of kidneys that had marked pressor activity. Schroeder and Adams[6] found that tyrosinase would reduce the blood pressure of hypertensive dogs and rats, and would also abolish the pressor activity of Victor's extract, suggesting that the pressor activity of extract was due to amines. In view of this work it is of interest to determine the presence of amines in the extract of Victor, Steiner and Weeks, and to identify the amines if possible.

Methods. Extracts of ground hog kidneys were prepared ac-

1 Holz, P., *Klin. Woch.*, 1937, **16**, 1561.

2 Holz, P., and Heise, R., *Arch. Exp. Path. und Pharm.*, 1939, **191**, 87.

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

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

5 Victor, J., Steiner, A., and Weeks, D. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 767.

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

cording to the method of Victor, Steiner and Weeks,[5] being incubated under nitrogen at 37°C for 48 hours. Five kilograms of kidney were used in the preparation of each extract, but only 1 liter of Krebs bicarbonate buffer per kilogram of kidney was used in order to keep the final volume low. The extract was then treated according to the method of Barger and Walpole,[7] as follows: After incubation the extract was heated to 100°C with HCl to coagulate the proteins. The material was filtered by gravity and the filtrate evaporated to a thick syrup, which was then extracted with acetone, in which the hydrochlorides of the amines are soluble. After filtration the acetone was evaporated and the remaining liquid mixed with chloroform, any insoluble material being discarded. The chloroform solution was then extracted with dilute HCl. Four cc of the HCl extract (equivalent to 10 g of original kidney) were neutralized and injected intravenously into anesthetized dogs. The blood pressure rose an average of 100 mm of Hg in 3 minutes.

The acid solution of the bases was then made alkaline with NaOH, extracted 5 times with ether, and the ether solution dried with sodium sulphate. A solution of oxalic acid in dry ether was then added to the ether extract of the bases and a precipitate was obtained. The oxalate which precipitated was removed and crystallized from alcohol-acetone. After recrystallization the oxalate melted, with some decomposition, at 165°C. It gave a negative xanthroproteic and Millon test. Barger and Walpole[7] found the oxalate of isoamylamine to melt at 166°C. The free amine was obtained and converted to the hydrobromide which melted at 226°C. The amine was, therefore, isoamylamine.

After the ether extraction above, the solution was made N/2 to NaOH and extracted 4 times with amyl alcohol. The amyl alcohol extracts were treated according to Barger and Walpole's procedure[7] in an effort to obtain phenylethylamine. The final extract contained pressor activity, probably phenylethylamine, which was not positively identified. The solution gave a negative Millon test, showing the absence of p-hydroxyphenylethylamine.

The N/2 NaOH solution was then neutralized and made slightly alkaline with sodium carbonate, and again extracted with amyl alcohol. The amyl alcohol was removed under vacuum and after recrystallization as the hydrochloride the material melted at 268°C. Johnson and Daschavsky[8] give 269.5-270°C as the melting point of their tyramine hydrochloride and Barger[9] reports 268°C. The solu-

[7] Barger, G., and Walpole, G. S., *J. Physiol.*, 1908, **35**, 343.

[8] Johnson, T. R., and Daschavsky, P. G., *J. Biol. Chem.*, 1925, **62**, 725.

[9] *Cf.* reference 8.

tion gave a strong Millon reaction. The base was benzoylated (Shotten-Bauman process) and a benzoyl derivative obtained which melted at 168° C after recrystallization from alcohol-water. This is the melting point of dibenzoyl-p-hydroxyphenylethylamine and identifies the pressor material as tyramine (p-hydroxyphenylethylamine).

Discussion. Victor, Steiner, and Weeks[5] pointed out that the pressor material in their extract would pass through a collodion membrane, and would withstand 20 minutes of boiling. They also raised the question whether or not the active substance was a pressor amine. The analyses reported in this paper show the presence of isoamylamine and tyramine, with the probable presence of some phenylethylamine in the extracts. Thus anaerobic kidney extracts contain pressor amines whereas under aerobic conditions no pressor amines are produced.[5] Any amines present under aerobic conditions are probably oxidized by amino-oxidases.[10] This suggests that amines may be formed in the kidneys of dogs rendered hypertensive by the Goldblatt clamp. It has been observed that the release of a renal ischemia after ½ to 24 hours will produce a rise in blood pressure,[11-13] and Bing and Zucker[14] have produced acute renal hypertension by injecting l-dopa into the ischemic kidney of the cat. It is also of interest that tyrosinase will inactivate angiotonin under certain conditions.[6]

Summary. The pressor effects of anaerobic kidney extracts prepared according to the method of Victor, Steiner, and Weeks are probably due to pressor amines. Tyramine and isoamylamine have been identified in the extracts and the probable presence of phenylethylamine has been shown.

10 Holz, P., Credner, K., and Walter, H., *Z. physiol. Chem.*, 1939, **262**, 111.

11 Taquini, A. C., *Am. Heart J.*, 1940, **19**, 513.

12 Friedberg, L., Landowne, M., and Rodbard, S., *Am. J. Physiol.*, 1940, **129**, 358.

13 Collins, D. A., and Hamilton, A. S., *Am. J. Physiol.*, 1940, **130**, 784.

14 Bing, R. J., and Zucker, M. B., *J. Exp. Med.*, 1941, **74**, 235.