the vaccine each showed an appreciable increase. As far as the tests have been completed the blood serums of 6 children neutralized between 100 and 200 and those of the other 6 between 200 and 500 additional infective doses of virus.

Of course we have no index as to the level of immunity required to protect the children against the natural infection, nor whether the immunity obtained in the present series is sufficient. However, inasmuch as the humans responded better to the antigen than did the experimental animals and inasmuch as the monkeys injected with formalized virus showed a relatively high tissue immunity, sufficient to withstand in most cases intracerebral inoculation of virus, it is quite likely that the humans have an appreciable tissue immunity. By analogy with louping-ill,[2] a disease of sheep that is quite analogous to poliomyelitis, it should be ample to protect against natural exposure to the virus, for formalized louping-ill virus was unable to protect against intracerebral inoculation, yet it did so against the natural disease. However, the ultimate proof of the protective value of the vaccine must be established in a properly controlled series vaccinated in an epidemic area.

7654 P

Vascular Action of Fresh Urine and Extracts Thereof.

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Human urine and extracts of urine have been known for some time to contain both pressor and depressor substances.[1] Attempts have been made to associate them with the presence of arterial hypertension with small success. Since the properties of these substances are little known, we have undertaken a study of them.

Cats anesthetized with ethyl urethane (7-10 cc. of a 25% solution given subcutaneously) or ether were employed as test animals. Blood pressure was recorded from the femoral artery and the vagus nerves were severed. Injections of the warmed fluid were made

[2] Gordon, W. S., *Proc. Roy. Soc. Med.*, 1934, **27**, 11; *Veterinary Rec.*, 1934, **14**, 1.

[1] Abelous, J. F., and Bardier, E., *J. d. Physiol. et path. gén.*, 1908, **10**, 627; 1909, **11**, 34.

into a femoral vein. Forty-one animals have been employed for the experiments.

Injections of 5 cc. of fresh normal urine usually produced elevation of blood pressure of from 6 to 20 mm. of Hg. Boiling the urine for 1-3 minutes did not affect the pressor action, nor did preliminary atropinization of the animal. Mixtures of urea and sodium chloride, or sodium acid phosphate of the same specific gravity as the urine, did not appear to duplicate the vascular action of urine.

Often the same test animal which yielded a pressor response to normal urine exhibited depression of the blood pressure on injecting the same urine at another time during the course of the experiment. Some animals exhibited only depressor responses. We have been unable to predict which response will occur, though the pressor was the more common. At times the animals became entirely refractory to urine injections. The variability of response led us to suspect that the functional intactness of the central nervous system was essential for a vascular response to be elicited. Decerebrate and pithed cats were found to be refractory to urine injections, whereas the same animals before pithing had responded actively. It appeared, then, that the vascular action of urine was not peripheral, but central.

An attempt has been made to separate the pressor and depressor substances from urine by extraction methods. It was found that ethyl acetate extracts much of the pressor substance from urine. One liter lots of urine were extracted with about 200 cc. of freshly distilled solvent and the solvent removed under vacuum. The residue was extracted with (a) ether (50 cc.) followed by, (b) alcohol (50 cc.) and the remaining insoluble material dissolved, (c) in water (10 cc.). The ether and alcohol were removed under vacuum and the residues dissolved in water (10 cc.). Such extracts (a and b) were usually found to depress the blood pressure from 12 to 40 mm. Hg., while the water solution of the residue (c) elevated the pressure 20 mm. or more. Thirty-two urine specimens have been examined in this manner.

Acetone extracts of urine have been prepared by saturating urine with salt and layering with acetone. After removal of the acetone the residue was separated into ether, alcohol and water soluble fractions. Essentially the same vascular responses were observed as when ethyl acetate was used as solvent. Adjusting the pH of the urine to acid and alkaline reactions did not appear to aid in the extraction.

Capps, Ferris, Taylor and Weiss (personal communication) have studied extracts of urine prepared by adsorption on Norit and elu-

tion with acetone and alcohol. They found that acetone removed from the Norit more pressor substance than did alcohol extraction. The pressor substance apparently acted centrally because its action paralleled that of alpha lobelin, a central acting drug. Contrary to Bohn and Hahn[2] they found no relationship between the amount of pressor substance in the urine and the presence or absence of hypertension in the patient.

7655 C

Potentials in Embryo Rat Heart Muscle Cultures.

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As far as we have been able to discover, there have been no published investigations of action potentials in heart muscle cultures. This is a report on a method which it is hoped will furnish information on the mechanism of irritable tissues in general and particularly those which are excited spontaneously.

In general, the potential difference between the micro-electrodes making contact with a muscle culture is impressed on the grid of a vacuum tube and measured by the deflection of a string galvanometer in the plate circuit.

The cultures were prepared by the usual hanging drop technique. Small pieces of the ventricle of 16-day rat embryos were explanted in a medium of rat blood plasma and rat embryo extract. The ages of the cultures varied from 2 days to 2 months. The present records were taken from the older cultures containing outgrowths of muscle which had differentiated in the manner described by Goss[1] and were presumably without nervous tissue elements. Spontaneous contraction occurs in practically all cultures at one time or another and may be rhythmic or irregular. There is a great variation in the duration of the resting and acting periods and also in the frequency of rhythmic activity.

The electrode systems are micro-pipettes, 2-5 mu in diameter at the tip, with chloride coated silver wire coils placed in the large ends and the whole filled with mammalian Ringer solution. These pipette

[2] Bohn, H., and Hahn, F., *Z. f. klin. Med.*, 1933, **123**, 558.

[1] Goss, C. M., *Arch. f. Exp. Zellforsch.*, 1932, **12**, 233.