on the 28th day.

The above experiment with pantothenic acid-deficient rations was repeated with a group of 24 mice with similar results.

For the experiments with pyridine-3-sulfonic acid, a group of 12 weanling mice was fed the basal ration described at the beginning of this paper, minus nicotinic acid. Four mice received in addition 5% of the ration as pyridine-3-sulfonic acid, and 2.5% as $NaHCO_3$. Four were fed the ration plus 5% sulfonic acid, 2.5% $NaHCO_3$, and 0.5% nicotinic acid. As may be seen from the data in the table, those mice getting pyridine-3-sulfonic acid grew as well as did the controls. Furthermore, during the 4 weeks that the rations were fed, no symptoms of disease were seen in any of the animals.

Summary. In contrast to the findings with bacteria, pantothenic acid deficiency of mice and of hamsters was not produced by the feeding of the sulfur analog of pantothenic acid (N-(β,β-dimethyl-α,γ-dihydroxybutyryl)-taurine). Similarly, the feeding of pyridine-3-sulfonic acid did not produce symptoms of nicotinic acid deficiency.

14047

Effect of Posterior Pituitary Extract on Water Up-Take in Frogs After Hypophysectomy and Infundibular Lesions.*†

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It has been known for the last 20 years that injection of the posterior pituitary extract causes a weight increase in frogs due to the up-take of water.[1] However, very little is understood in regard to the pharmacological mechanism involved in this action. The experiments to be reported were performed in order to shed some light on the problem.

Material and Method. Male frogs weighing between 30 to 40 g were used. The procedure for total hypophysectomy has been previously described.[2] For partial hypophysectomy, the cartilage lining the base of the skull was incised along the border of the gland, leaving part of it intact so that it may be placed back in its original position following the removal of the gland. The anterior lobe was removed with forceps after it had been freed from the neighboring structures with a fine dental hook. For removal of the neural and intermediate lobes, suction was applied, the anterior lobe being kept away from the suction with the tip of a retractor.

The infundibular lesions were made in two ways: (1) a small puncture in the midline of the base of the third ventricle and (2) a wide incision made at the base of the third ventricle between the pituitary body and the tuber cinerium. After the removal of the anterior pituitary or with a wide lesion at the base of the third ventricle, the frogs became permanently dark and lost their ability of adaptation to light and darkness.

The posterior pituitary extracts were prepared according to the directions given in U.S.P. XI. They contain 2 or 4 units of the international standard powder per cc. The amount of extract given was in proportion to the body weight; each frog received not more than 0.5 cc. The solution was injected into the ventral lymph sac through the mouth.

The frogs were kept in separate cages in running water at a temperature between 15°C

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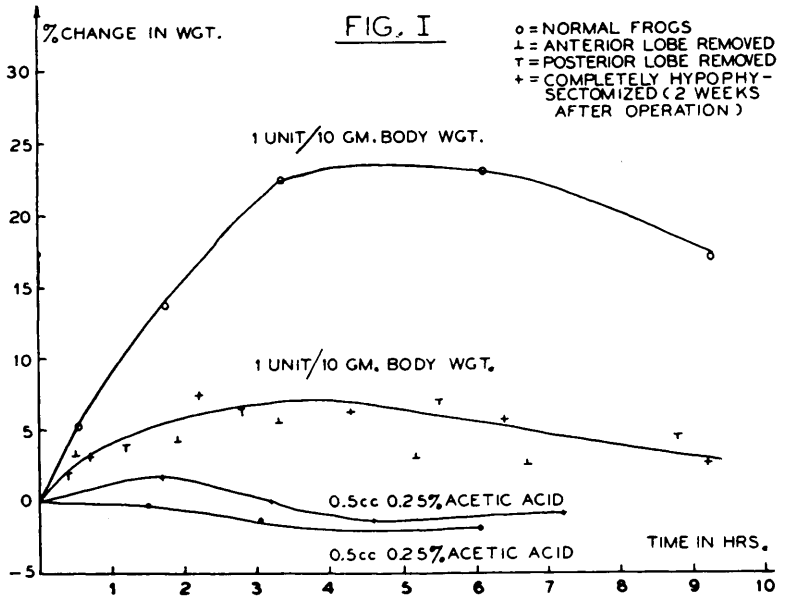
† A preliminary report of this work was given at the 1941 meetings of the Federation of American Societies for Experimental Biology.

1 Brunn, F., *Z. ges. exp. Med.*, 1921, **25**, 170.

2 Hogben, L. T., *Quart. J. Exp. Physiol.*, 1922, **13**, 177.



to 18°C. Twenty frogs were used for each experiment except in cases where a series of experiments were conducted on the same animals over a period of 3 or 4 weeks and death occurred in some of them. However, even in these cases, at least 12 to 16 animals were available toward the latter part of the experiment. At half hour to hour intervals, the urine was expelled by pressure, the animal was dried with a towel and weighed.

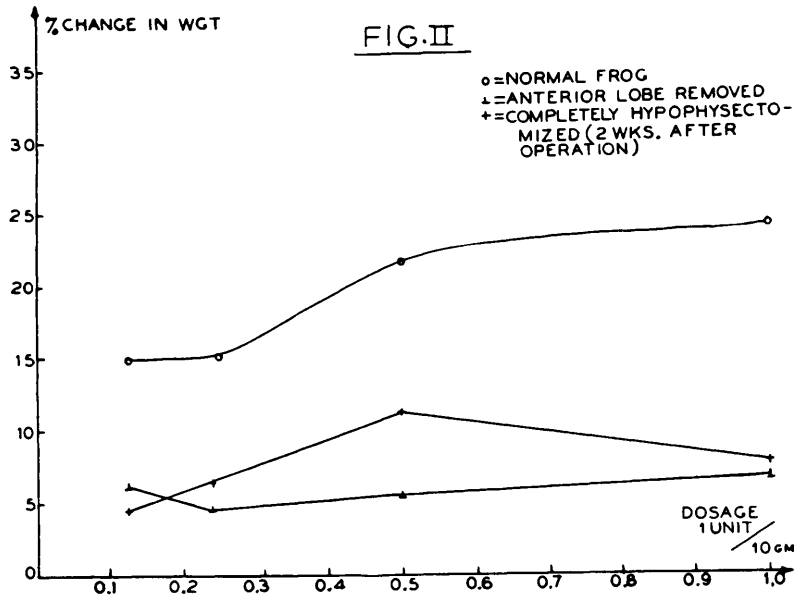
In comparative studies on partially or totally hypophysectomized frogs or animals with infundibular lesions, the experiments were conducted on the same day and under the identical conditions.

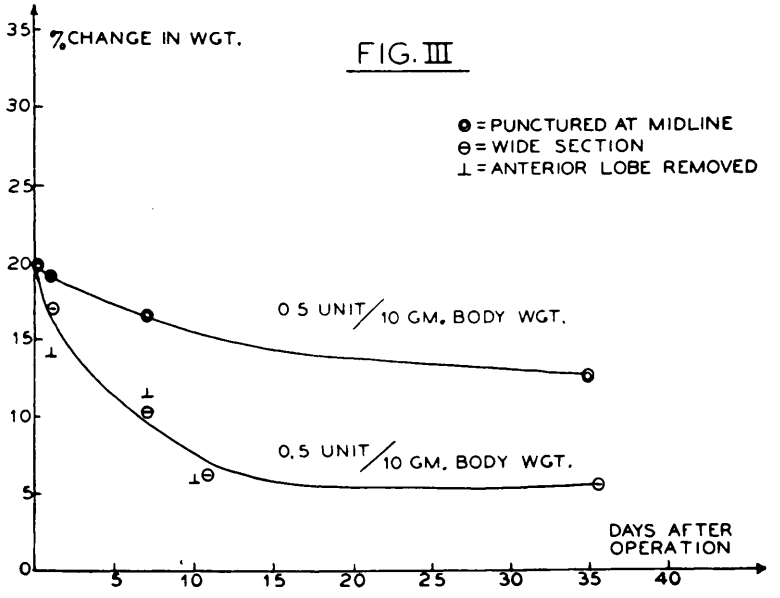
Results and Observations. The changes in weight of the normal and hypophysectomized frogs, following the injection of a pituitary solution, are shown by graphs in Fig. 1. The ordinate represents the changes in weight, expressed in per cent of the initial weight, and the abscissa the time in hours after the injection. Each point on the curve is an average value at each weighing, calculated from the individual weights of 20 frogs. The control graphs obtained with frogs injected with 0.5 cc of 0.25% acetic acid, the maximal volume of solution injected into each frog in these experiments, indicate a variation of $\pm 2\%$ in body weight. Therefore, the small quantity of solution injected is immaterial in calculations of the weight increase if the weight before injection is taken as the initial weight.

Two important facts may be observed from the graphs representing the responses of the normal and hypophysectomized frogs to the posterior pituitary extract. First, there is a difference of 18% in the maximal up-take of water between the normal and hypophysectomized animals. This is in agreement with the findings of Steggerda and Jones.[3] On the other hand, no difference in response is indicated between animals after the removal of the anterior or the neural and intermediate lobes, or following total hypophysectomy.

A striking difference in response between the normal and hypophysectomized animals may be seen from the graphs in Fig. 2, in which the maximum increases of body weight are plotted against the quantities of the pituitary solution injected. The average of the maximum increase in weight was calculated from the maximum individual weights observed at different weighings. The characteristics of the graphs indicate that in normal frogs the maximum increase in weight is exponentially proportional to the quantity injected. However, such a relation no longer

[3] Steggerda, F. R., and Jones, M. E., *Am. J. Physiol.*, 1935, **112**, 397.





holds true after partial or total hypophysectomy; 0.1 unit of the extract is as effective as an amount 10 times greater.

These observations led us to investigate what would be the effect of a lesion at the base of the third ventricle on the water imbibition by frogs. The results are represented by graphs in Fig. 3. The maximum increases of body weight are plotted against the days after the operation. The following features may be brought out from the graphs: The "punctured" frogs, during the first few days after the operation, responded to the posterior pituitary principles as did the normal frogs. Later they gradually showed a decrease in response, a reduction about 10% at the end of 35 days. On the other hand, the decrease in response is abrupt in frogs in which a wide lesion was made at the base of the third ventricle. This decrease in response is similar to that following the removal of the anterior pituitary.

Summary. The effect of posterior pituitary principles on the water up-take in hypophysectomized frogs is lower than in the normal. This applies not only to totally hypophysectomized frogs but also to those in which only the anterior or the posterior lobe is removed.

After a wide lesion at the base of the third ventricle, the response to posterior pituitary principles falls off very sharply. When the third ventricle is merely punctured in the midline, the response decreases somewhat but not to the same extent as in the case of a wide lesion. A wide lesion at the base of the third ventricle results in a reduction of the same magnitude in response to posterior pituitary principles as does hypophysectomy.

14048

Ichthyometric Comparison of Cobra Venom, Morphine and Saponins.

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While the mechanism of analgesia produced by cobra venom and morphine is similar, since both act centrally on the brain, extensive clinical experimentation has revealed a marked difference between the two drugs with regard to their effect on the neuromuscular system.[1] It has been found that morphine markedly depresses the neuromuscular apparatus and relaxes the skeletal muscles while cobra venom does not. In order to investigate this phenomenon more critically and quantitatively, kymographic studies were made of the behavior and neuromuscular activity of goldfish, *Carassius auratus*. The contrivance employed, called an ichthyometer, has been described in detail elsewhere.[2] Fig. 1 illustrates the technic. Studies were made on goldfish measuring two inches from snout to root of tail. A silk thread was drawn through the muscles just in front of the dorsal fin and connected to one arm of the lever, while the other arm, suitably weighted, registered the movements of the fish on a slow-moving kymograph. The speeds employed with the kymograph vary from one revolution in one hour, which in this paper is described as "fast," to the much slower speeds of one revolution in 15 and 16 hours, respectively. The normal movements of each fish in ordinary tap water were first registered on the kymograph and then their activity in tap water solutions of the various drugs was carefully recorded. Fifty experiments were made with morphine sulphate in various concentrations and 60 with solutions either of crude cobra venom or of that from which proteins, hemotoxins and other poisonous ingredients had been removed, leaving for all practical purposes a solution of cobra neurotoxin. All cobra venom solutions were assayed by the mouse method described elsewhere.[3]

It was found that morphine solutions produced a gradual depression of the fish movements, its time of onset and intensity depending on concentration of the narcotic. Solutions of morphine, 1:500, killed the goldfish in a few hours but even more dilute solutions, 1:1,000 to 1:2,000, usually paralyzed and frequently killed the fish in 12 hours.

The effect of cobra venom on the activity of goldfish was quite different. Cobra venom in concentrations of 1:100,000 produced a transient primary sedation of movement which was followed by return to normal activity or, more often, by definite stimulation of the neuromuscular responses. Occasionally solutions even as strong as 1:80,000 exerted no other deleterious effect on goldfish kept therein for 24 hours than a slight depression from which they recovered on being placed in fresh water. Higher concentrations of from 1:80,000

[1] Macht, D. I., *Med. Rec.*, 1941, **153**, 369.

[2] Macht, D. I., *Physiol. Zool.*, 1930, **3**, 412.

[3] Macht, D. I., *Proc. Nat. Acad. Sc.*, 1936, **22**, 61.