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.

DAVID I. MACHT.

From the Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md.

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.

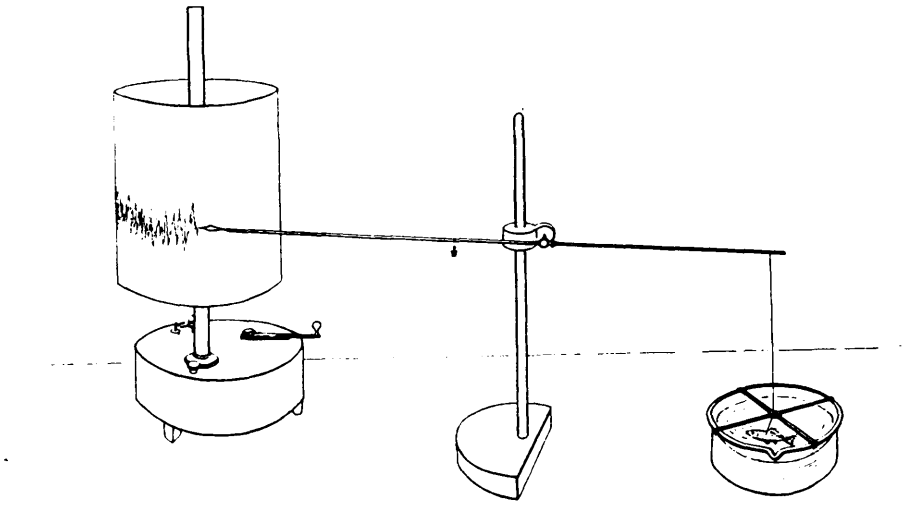


FIG. 1.
Ichthyometer.

to 1:20,000 definitely depressed fish activity and concentrations of from 1:20,000 to 1:10,000 usually killed the goldfish. To appreciate the magnitude of these concentrations we should remember that on biological standardization of various batches of cobra venom, one mouse unit equals from 0.01 to 0.005 mg by weight. The toxic effect of stronger concentrations leaves no doubt as to the fishes' absorption of the drug though the quantity of the venom by weight was too slight to demonstrate its absorption chemically. The transient primary depression noted after use of crude venom did not occur after use of neurotoxin solutions, which did not depress and usually stimulated goldfish activity but were toxic in concentrations of 1:50,000 to 1:10,000. For comparative purposes it should be borne in mind that the usual therapeutic dose of cobra venom solution for human beings is 10 mouse units, which corresponds to the therapeutic dose of morphine, 15 mg.

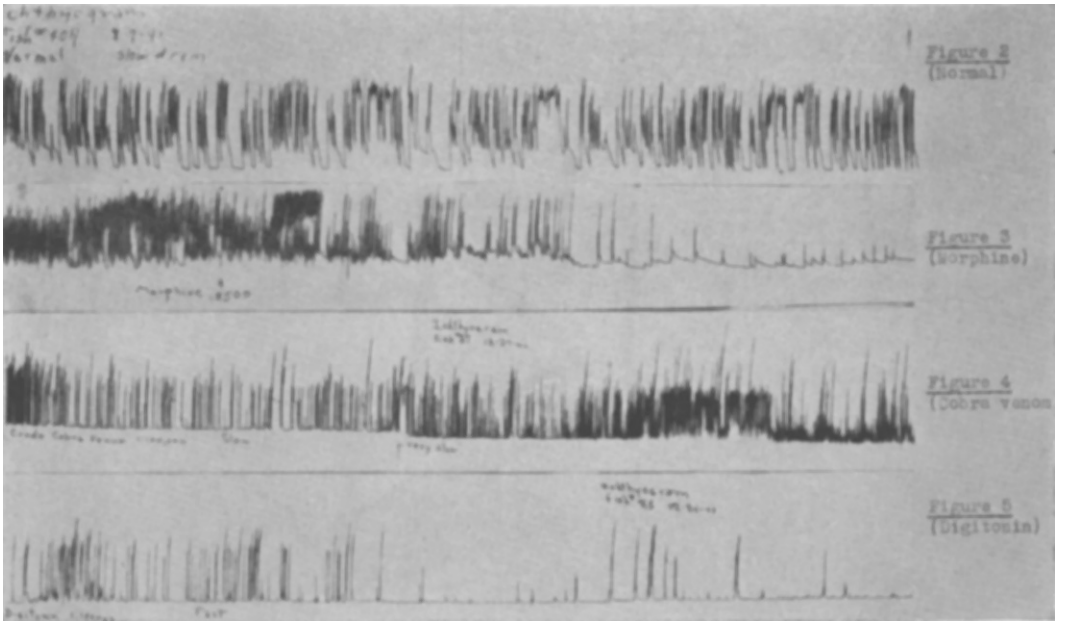
The writer employed an ichthyometric study of goldfish activity to throw some light on the chemical nature of cobra venom. Faust and other older writers on snake venoms held the view that they are closely related to sapotoxins.[4] This opinion was probably due to the powerful hemolytic properties of crude cobra venom which, in addition to neurotoxin, contains various hemotoxic principles such as hemolysins, precipitins, and hemorrhagins, coagulants and anticoagulants. The most recent work on the chemistry of cobra venom claims that its neurotoxin is neither an alkaloid nor a saponin but closely related to the glucosides.[5] It is therefore surprising that certain German writers claiming that cobra venom is an animal saponin have attempted to introduce clinically preparations of plant saponins fortified by vegetable proteins and carbohydrates.[6] An ichthyometric comparison of cobra venom and various saponins conclusively disproves the sapotoxin hypothesis with regard to the chemical nature of cobra neurotoxin. The present writer has performed numerous experiments on goldfish with saponins, and particularly with *saponin* (Merck) and with digitonin, a powerful saponin of the cholan group,[7] and found that the former in concentration of 1:2,000 poisoned the fish while the latter, even in concentrations of 1:100,000 usually killed them. These saponins were also tested for any possible analgesic

[4] Faust, E. S., *Heffters Handb. d. exp. Pharmakol.*, 1924, II/**2**, 426.

[5] Micheel, F., and Bode, G., *Ber. d. deutsch. chem. Gesellsch.*, 1938, **71**, 1302.

[6] Loewenstein, W., *Wien. klin. Wchnschr.*, 1938, **51**, 302.

[7] Tschesche, R., *Ergebnisse der Physiol.*, 1936, **38**, 172.



action on rats by the Macht method, described in these PROCEEDINGS,[8] and the results uniformly yielded by such experiments were negative. Figs. 2, 3, 4 and 5 show a normal ichthyogram and ichthyograms exhibiting the effects of morphine, 1:2,500, introduced at the point of the arrow, and of 1:100,000 solutions of cobra venom and digitonin, respectively.

Summary. 1. An ichthyometric study of the effects of cobra venom and morphine on goldfish revealed a marked difference between the two drugs, morphine in certain concentrations depressing and killing the fish in 12 hours while both crude cobra venom and cobra neurotoxin solutions, far from inducing death in 24 hours, depressed but little and usually stimulated goldfish activity. 2. An ichthyometric comparison of cobra venom solutions with solutions of *saponin* (Merck), and digitonin reveals that while the saponins are extremely toxic for goldfish, cobra venom is not, a finding which discountenances the sapotoxin hypothesis with regard to its chemistry.

[8] Macht, D. I., and Macht, M. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 428.