procedure. It is believed that this may also serve as a means of further purification.

Complete inactivation of the purified phage occurred in 30 minutes by exposure to 65°C., whereas homologous broth phage of similar titer (10^{-7}) and pH (6.1 to 6.2 by the indicator method) required a temperature of 75°. The purified phage was completely inactivated by 50% C. P. acetone in 24 hours at room temperature, though broth phage of similar titer and pH still had a titer of 10^{-5} after the same period of time under the same conditions. After evaporation to dryness in a Freas vacuum oven at 22°C. and resuspension in one-fourth the original volume of water, the titer of the purified phage was 10^{-2}; that of the crude broth phage, 10^{-6}. These results appear to support the findings of other workers that purified phage is more susceptible to inactivation by physical and chemical agents than is crude broth phage.

Summary. Serological tests and nitrogen determinations indicate that bacteriophage prepared by repeated washing of lysogenic cultures of *B. coli* contains little bacterial or other protein. Methods of preparation and concentration of such purified phage are described and some of its properties compared with those of homologous crude broth phage of similar titer and pH.†

9133

Comparative Study of Water Metabolism in Amphibians Injected with Pituitrin.

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Since the work of Brunn[1] it has been shown by several other investigators that frogs *(Rana pipiens)* when injected with pituitrin, increase markedly in weight due to the absorption of water. This increase, occurring in the case of frogs, gave rise to the problem of comparing the amount of water uptake in different animals in the class Amphibia when similarly treated.

The animals selected for this comparative study were: the toad *(Bufo americanus)*, mud puppy *(Necturus maculatus)*, and two

† The collodion membranes used in this work were prepared by Dr. Evelyn B. Tilden of the Department of Research Bacteriology.

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

species of frogs *(Rana pipiens* and *Rana clamitans)*. This selection affords a wide variation in natural environmental conditions, the toad living in dry surroundings and the mud puppy constantly in water; as to the 2 species of frogs used, the pipiens reside primarily in damp, marshy fields some distance from the streams, while the clamitans are found more or less submerged in pools of water along the stream's edge. The experiments were carried out in the late summer and early fall.

The procedure was similar to that used in previous experiments.[2] The animals were nearly submerged in tap water at room temperature for at least 12 to 18 hours before the experiment. They were then removed from the water separately, dried with gauze as uniformly as possible, and weighed accurately to one-tenth of a gram. They were next injected subcutaneously with obstetrical pituitrin (Parke Davis), one-tenth cc. per 10 gm. of body weight (one international unit), and again returned to the water. Routine weigh-

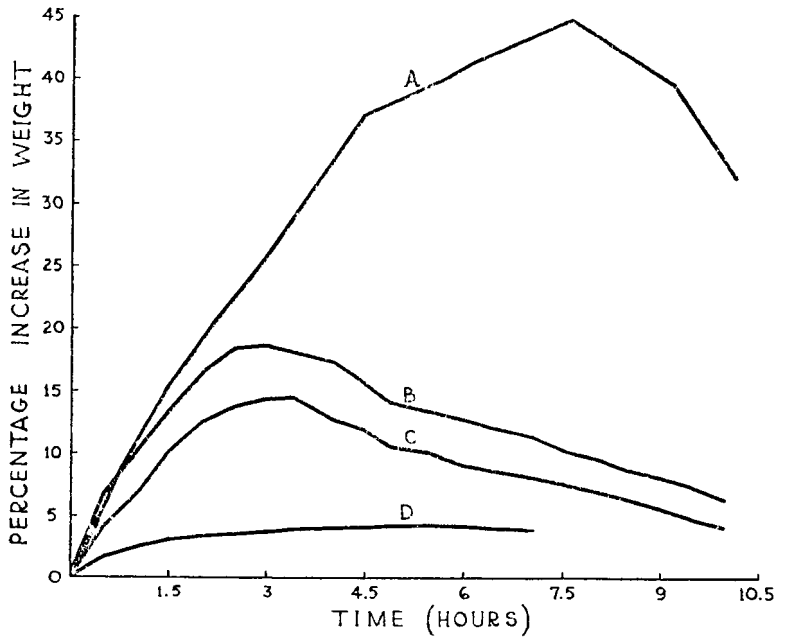

FIG. 1.

Each of the above curves represents the average percentage weight changes due to water absorption in 6 animals.

A. Toad *(Bufo americanus)*.
B. Frog *(Rana pipiens)*.
C. Frog *(Rana clamitans)*.
D. Mud Puppy *(Necturus maculatus)*.

[2] Steggerda, F. R., and Essex, H. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 425.

ings were made at one-half to one hour intervals for 7 to 10 hours. Any changes in weight were calculated on the basis of percentage variation from normal. In all cases the experiments were controlled with uninjected animals kept in the same environment and weighed at the same time intervals. These control animals never showed any appreciable changes in weight and therefore will not be referred to further.

The results of these experiments (see curve) show that the injection of pituitrin into the toad caused a very marked uptake of water with an average increase in weight of nearly 45% in 7½ hours, after which there was a gradual return to normal. On the other hand, the mud puppy, whose natural environment is water, showed an increase of only 4%. The *Rana pipiens* gave an increase of about 18% in weight in 3 hours, which is in agreement with our previous findings; the average increase in the clamitans, with the same dose of pituitrin, was consistently about 4% less than that of the pipiens. These observations indicate that the response of the animal to pituitrin may be related to its natural habitat.

Since the above results show so great a difference in the uptake of water following the injection of pituitrin, a limited number of experiments were carried out to find whether there was also a similar difference in the animals studied regarding the amount of water they could lose by dehydration before death. Six each of the *Rana clamitans* and *pipiens* and 4 toads were weighed and placed in separate cages in the breeze of an electric fan for 2½ hours. During this time the average percentage weight lost in the 2 species of frogs was approximately 22%, while that of the toads was 36%. The mud puppy was not studied in these experiments because they survive so short a time after being removed from water.

After this dehydration period the animals were placed in water to record their ability to recover. Four of the 6 clamitans died, whereas all the pipiens and toads recovered their lost weight and survived. This shows an interesting correlation with the previous experiments regarding the ability of animals to withstand environmental changes.

Regarding the exact nature of the comparative response of these animals to hydration or dehydration, little can be said at the present time. Earlier experiments[3] on frogs with cloaca tied would lead one to deduce that the hydration effect was due primarily to the increased skin permeability. Other experiments[4] indicate that the skin changes are not definitely related to a melanophore dilation

[3] Steggerda, F. R., *Am. J. Physiol.*, 1931, **98**, 255.

[4] Oldham, F. K., *Am. J. Physiol.*, 1936, **115**, 275.

which accompanies pituitrin injections. Recently, however, Rey[5] reported that the hydrating response in frogs to pituitrin is not alone due to the increased absorption through the skin, but that the decreased kidney output and an increased water retention by certain tissues play an important rôle in causing the increase in body weight. The work of Novelli[6] with skin pouches in which the circulation was left intact indicates that the skin permeability is in some way affected by pituitrin to allow an increase transfer of water.

Although it is admitted that the exact nature of the transfer of water in amphibians after the injection of pituitrin is still obscure, it can be concluded from the above experiments that there is a definite correlation between the amount of water interchange and the natural habitat of the animal.*

9134

An Ensemble for Determination of Circulation Time of Blood By an Ionization (Geiger Chamber) Method.

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A decade ago there appeared the first of a series of publications by Blumgart and his associates[1] concerning the use of injections of the disintegration products of radon (radium emanation) for the determination of the time elapsing between the introduction of the radioactive material at a given point in the blood-vascular system and its arrival at some other point in the system as detected by a shielded ionization chamber.

In the early investigations the radioactive material was injected into the cubital vein of one arm and detection of its arrival in the radial artery of the other arm was accomplished by means of a modi-

[5] Rey, P., *Comp. rend. Soc. de biol.*, 1935, **118**, 949.

[6] Novelli, A., *Rev. Soc. Argent. Biol.*, 1936, **12**, 163.

* These experiments were begun at the University of Michigan Biology Station at Douglas Lake.

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[1] Blumgart, H. L., and Yens, O. C., *J. Clin. Invest.*, 1927, **4**, 1. Blumgart, H. L., and Weiss, Soma, *J. Clin. Invest.*, 1927, **4**, 389.