

do not metamorphose. Animals fed on beef muscle will not metamorphose without addition of vitamins or minerals to their diet. Metamorphosis occurs successfully with liver and with earthworms, as is true with a mixed diet of the meats and worms. The latter, started in later larval life, produces the best growth of all diets. All of the reactions to foods are modified by the quality, quantity, frequency, and duration of the initial diet.

Use of synthetic diets emphasized the need for minerals and vitamins, the greater growth with vitamin A, showed greater growth with deprim present, the value of low fat content, the preference for beef muscle powder as protein basis rather than the powdered liver, egg-white, egg-yolk, casein, or klim—though growth was made with all of these. The diets were more useful with older, larger larvae. Younger larvae showed different relative reactions.

During larval life the size relations of *Amblystoma punctatum* and *tigrinum* were reversed, so that at the time for metamorphosis the ratio was approximately 1:2 in the order named. Larval life gave another S-cycle to the growth curve, this cycle terminating at the time of metamorphosis. A third life cycle, beginning at this point, is probably related to the development of sexual maturity. Plotting of the cube of length against weight, for measurements made on adults, shows the first quantity to be a linear function of the second.

Hibernation of adults was followed, on return to warm temperature and food, by a remarkable acceleration of growth until the size characteristic for the stage of development was reached or surpassed. The tail became proportionally shorter during the hibernation period, with return to normal proportion after renewal of growth. The index of build; weight/length, (Bardeen *et al*), was slowly decreased with increase of size.

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### The Effect of Glucose on Ketosis.

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During a study of the influence of insulin on fat and ketone bodies in the blood of depancreatized dogs, several observations were made on the effect of glucose, without insulin, on ketosis. Since clinical

experience has shown that glucose given to patients in diabetic coma often ameliorates the symptoms of ketonuria, it is of interest to determine whether or not the same effect can be demonstrated on animals made diabetic by removal of the pancreas.

Observations on animals under the influence of phlorhizin show that the administration of glucose is followed by a decrease or disappearance of ketone bodies, a phenomenon similar to that noted by the clinician.

Whatever may be the effect of glucose on animals rendered diabetic by the drug phlorhizin, a similar effect is not necessarily to be expected on depancreatized animals, for the physiological conditions of the two experimental animals are quite unlike. Although the exact effect of phlorhizin on the animal has not been definitely established, recent investigations indicate that there is no impairment in the ability of the tissues to metabolize glucose when present in normal quantities, and that its action is largely renal. In the depancreatized animal the tissues lose their power of metabolism because of the lack of insulin. Therefore studies of the ketolytic influence of glucose on phlorhizinized animals throw little or no light on any effect, or lack of effect, that it may have on depancreatized animals.

The present observations were made on 4 totally depancreatized dogs kept alive by the administration of insulin twice daily until recovery from the operation and resulting complications. Results of the operation were confirmed at autopsy. The degree of ketosis developed in the animals after the withdrawal of insulin varied, depending largely on the nutritive condition of the animal and the time lapsing between the withdrawal of insulin and the beginning of the experiment. As the animals were starved for 3 or 4 days prior to the administration of glucose, the ketone bodies formed are assumed to be derived entirely from the breakdown of the animals' tissues. Only 2 animals were allowed to develop a severe ketosis (3 mg. or more of total ketone bodies per cc. blood), the other 2 had a pronounced but not advanced ketosis (both registered between 1.5 mg. and 2 mg. of total ketone bodies per cc. blood). In 3 of the 4 experiments, acetone plus aceto-acetic acid was higher at the beginning of the experiments, and remained higher throughout, than hydroxy-butyric acid. In the fourth, hydroxy-butyric was slightly higher than the combined acetone and aceto-acetic acid.

After the initial blood samples had been taken, glucose was given, either by mouth (2 cases) or hypodermically (2 cases), and blood was drawn thereafter at 2 or 3 hour intervals for a period of 8 or

10 hours. The amount of glucose given by mouth was not the same for the two animals; one received 75 gm. and the other 60 gm. The animals receiving glucose hypodermically each received 16 gm. of a 20% solution injected subcutaneously.

In no case did any of the ketone bodies diminish after glucose was given. In two cases an increase was observed in the acetoacetic acid during the experiment; in one case, hydroxybutyric acid also increased.

Since these observations indicate that glucose has no effect on ketonemia of animals deprived of their pancreas, it is interesting to account for its effect on the diabetic patient. Dr. J. J. R. Macleod suggests that it may be due to stimulation of islets of the pancreas not yet involved in the disease process; the resulting increased production of insulin is assumed to more completely metabolize the ketones. Since no islets are left in the depancreatized animal, this theory seems in harmony with the results here obtained.

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#### Expulsion of Injected Solute by Contractile Vacuole of Amoeba.

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Although it has long been the presumption that the contractile vacuole expels substances in solution in the endoplasm of the protozoon, the actual taking up and expulsion of a definite solute by the organoid has never been demonstrated. This now has been accomplished by means of the micrurgical apparatus.

If a moderate amount of saturated aqueous solution of picric acid is injected into an ameba (*Amoeba dubia*) the course taken by the solute may be traced by its yellow color. The effect on the cytoplasm has already been described by Pollack.<sup>1</sup> Though a part of the colored region is often injured by the pipette and thereupon pinched off by the ameba, a certain quantity of the solute diffuses into the remaining endoplasm before this occurs. This is taken up by the vacuole, the intensity of the yellow color of the vacuolar fluid increasing in proportion to the fading out of color in the endoplasm.

When a 2% solution of picric acid in 95% alcohol is injected, diffusion throughout the endoplasm is much more rapid, and the yellow color appears more quickly in the vacuolar fluid. In the