water balance, this investigation was undertaken. The very essence of the non-vitalistic theories is exemplified by the fact that salt water is retained much better by the body than is salt-free water.

A preliminary series of over 25 dogs was used. Isotonic solutions of 0.9 per cent. NaCl solution and 5 per cent. glucose solutions were injected intravenously in amounts from 2,500 cc to 4,000 cc. The oxygen consumption of the animal was measured before, during and after the injection at standard intervals. In all the animals a considerable increase in the oxygen consumption occurred from 100 per cent. to 500 per cent. above the basal level. The increase in O_2 consumption produced by equal amounts of 5 per cent. glucose solution was greater than that produced by 0.9 per cent. sodium chloride solutions. The amount of water lost was proportional to the increase in the oxygen consumption and metabolic rate. In 3 animals in whom an increased oxygen consumption did not occur during the injection death occurred. Water given by the alimentary tract increased the consumption but slightly. This is interesting in view of the fact that fluid by mouth is retained for longer periods than is fluid given by vein. Conversely, it was found that repeated blood-letting lowered the metabolic rate. In such animals, isotonic solutions were retained for longer periods.

Moreover, the diuresis was quite limited. In the light of these findings, it might be suggested that one of the fundamental factors concerned in water regulation is the oxygen consumption rate of the body tissues. Further work is proceeding along these lines.

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CALCIFYING FACTORS IN THE DIET OF SALAMANDER LARVAE

THE degree of calcification in salamander larvae was controlled by different calcifying agents, by variations in the Ca : P ratio or level and by use of diets with unlike growth-promoting properties. The results, recorded by x-rays, showed that salamander larvae do not differ widely from higher animal forms in their responses to calcification factors in the diet.

Larvae of *Triturus viridescens* fed on a synthetic diet of powdered beef muscle with cod-liver oil but without mineral addition were almost devoid of skeletal ossification. In contrast, a good calcification of skull and vertebral column appeared in other larvae, of the same age and origin, growing at like rate on a diet of milk powder combined with crude casein and containing the above vitamin D supplement. A poorer bone deposit in the limbs suggested that the ratio of calcium to phosphorus or the level of these minerals in the diet was not optimum for the contained vitamin D. Analyses for the calcium in the total ash of animals from the two diet series gave figures forming a ratio slightly less than that of the calcium in their diets.

The availability of vitamin D from the three common sources and the relative quantities needed for calcification in salamanders were indicated in feeding larvae of *Amblystoma tigrinum* on a synthetic milk diet. During a period of two weeks, a more extensive calcification was produced by exposure to ultra-violet light (2 or more minutes on alternate days at 112 cm from a Burdick mercury vapor quartz lamp) or by feeding viosterol (1 R.U. to 10 gm of ration) than by cod-liver oil (3 Steenbock R.U. to 10 gm of ration). Use of the milk diet without vitamin D supplement was not followed by as complete a lack of bone minerals as resulted when other larvae from the same lot of *A. tigrinum* were fed on the low-calcium meat diet with the cod-liver oil.. This may have been due to the presence of some of the calcifying factor in the concentrated powdered form of milk or to the fact that the high degree of purification of the casein in this milk diet caused a lower growth rate and consequently a smaller rachitic tendency.

Larvae of *A. tigrinum* fed on the beef muscle diet with cod-liver oil but without mineral addition developed a bowing of the limbs typical of rickets, and this disease was sometimes indicated also by weakness at the limb attachment or by abnormal contour of jaws or of spine. Yet, even on the extremely low calcium content of the muscle diet, when the vitamin D supply was raised by irradiation (seven minutes on alternate days during two weeks), a fair bone deposit was made. On the beef muscle diet with cod-liver oil the bone deposit increased as the calcium was raised, until the phosphorus became the limiting factor, and an indication was given that the range of the Ca : P ratio required for salamanders is very like that needed by mammals and by the chick.

The sensitivity of response to variations in calcifying factors shown by these small urodeles, as well as the ease and practicability with which they serve for x-ray studies, points to a convenient means for biological assay of calcifying agents.

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