

house and with him dug out four bags of soil at a depth of about two to eight inches from unexcavated parts of the cellar. From these soil samples more than 500 *Datura* plants, together with 168 plants of other genera, chiefly *Acalypha* and *Chenopodium*, were grown at Cold Spring Harbor. There seems to have been no possibility of seeds being carried into this location after the building of the house and its kitchen. The foundation walls (brick for the house and concrete for the kitchen) extend below the ground surface and also about two feet above this level to the floor of the house. A small, externally shielded ventilator was well above the ground line; the outside cellar door was covered, and the closed windows were under the porch. The soil was a hard, dry clay.

Controls were grown from seed of wild plants on Mr. Derr's farm, harvested in 1933, and from the standard inbred Line 1 which furnished test and control seed for our aged seed experiments. Because of a change in the sampling method a higher proportion of the pollen abortion mutations that actually occurred may have been found in the 1934 experiments than was found in 1933. In 1934 a flower from each of the two main forks of each plant was examined, while in the 1933 season the two flowers were taken at random and were, no doubt, in many cases from the same main branch. Since most of the mutations involve sectors of half or less than half of a plant, more may be expected to be found by the method of sampling both main forks. The results from these 22-year-old seeds and their controls are shown in Table I.

TABLE I  
POLLEN ABORTION MUTATIONS (TWO-FLOWER SAMPLES)

From seeds—	Plants	Mutations	Percentage
RECORDS OF 1934			
Buried 22 years	427	8	1.8
1-year-old, control .....	47	0	0.0
1-year-old, Line 1 control .....	261	3	1.1
RECORDS OF 1933			
Aged 5 to 10 years in lab. (Line 1) .....	405	32	7.9
1-year-old, Line 1 control .....	331	2	0.6

One of the eight mutations found seemed to involve the whole plant; the others occurred in sectors of the plants. Five had the appearance of chromosomal mutations and three of the gene mutation type.

Heat treatment of barley<sup>4</sup> and *Crepis*<sup>5</sup> seeds is re-

ported to have given effects like those of aging seeds. Line 1 plants from seeds that were treated at the Boyce Thompson Institute, through the cooperation of Dr. Wm. Crocker and Miss L. V. Barton, are now being grown for mutation rate studies. Preliminary tests have shown that mutations may be induced in *Datura* seeds by heat treatment and that the moisture content of the seeds subjected to heat is of great importance in this connection.

Plants from the buried seeds under discussion are not strictly comparable with our standard Line 1 used as a control, although there is no evidence that the two races would be expected to differ in mutability. The rate of pollen abortion mutations found in the 22-year-old seed plants is scarcely higher than that in the controls, and is much lower than that found in 1933 for seeds aged five to ten years in the laboratory. Taken as they stand, these results indicate that seeds buried in the soil under these more or less natural conditions were unaffected by whatever influences there may be to induce mutations, and that age alone does not greatly, if at all, increase the mutation rate.

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## THE RELATION OF WATER AND ELECTROLYTES TO METABOLISM

A CONSIDERATION of the views held regarding the regulation of water by the living organism reveals an interesting fact. Most workers in this field have stressed the physico-chemical aspects of the problem and have adopted what is essentially a non-vitalistic concept of water balance. Thus Fischer<sup>1</sup> regarded acidosis as being the primary factor in water retention. Schade<sup>2</sup> has emphasized alkalosis. Gamble<sup>3</sup> and his co-workers considered that the total amount of water was determined by the total electrolytes present in the organisms. Starling<sup>4</sup> pointed out the importance of the colloid osmotic pressure of the blood. Davis and Dragstedt<sup>5</sup> have shown how acidosis diminished the ability of the body to retain water. All this evidence points to a mechanistic concept of water regulation. In order to examine further the truth of this purely physico-chemical explanation of

<sup>6</sup> Aided by a research grant from the Penrose Fund of the American Philosophical Society.

<sup>1</sup> N. H. Fischer, "Oedema," pp. 209, New York, 1910.

<sup>2</sup> H. Schade, *Ergebn. inn. Med.*, 32: 425, 1927.

<sup>3</sup> J. L. Gamble, N. C. Putnam and C. F. McKhann, *Am. Jour. Physiol.*, Vol. 88, p. 571.

<sup>4</sup> E. H. Starling, "Fluids of the Body," pp. 186, 1909.

<sup>5</sup> H. A. Davis and L. R. Dragstedt, *Am. Jour. Physiol.*, 109: 88, 1934.

<sup>4</sup> F. Peto, *Can. Jour. Res.*, 29: 349-362, 1933.

<sup>5</sup> M. Navashin and P. Shkvarnikov, *Nature*, 132: 482, 1933.

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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