60 (1807)

A delicate biological test for calcium-depositing substances.

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Some time ago we described two diets, Nos. 2638 and 2677, which we used with a view to the development of a biological test which would show the calcium-depositing power of any given substance.[1] The test was carried out as follows: The faulty diet was first fed to a group of young rats for the purpose of making the epiphyseal cartilage free from calcium, and producing a rachitic metaphysis. After a sufficiently long period had elapsed, the test substance was added to the diet of those animals which were to serve as test subjects, while the faulty diet without the test substance was continued in the case of the control rats. Substances, which when added to the faulty diets enabled the organism to deposit lime salts, caused the reappearance of the provisional zone of calcification in the bones. This biological test we called the "line test," because the new provisional zone of calcification appeared as a line of calcium salts extending transversely across the bone with a limeless cartilage on one side of it and a limeless metaphysis on the other.

The success of this test depends on the use of a diet which uniformly causes the epiphyseal cartilage and the metaphysis to be free from calcium salts. It is not sufficient that a diet should merely produce rickets. The rickets which it produces must be of so severe a type that no vestige of calcium remains in the cartilage, and a wide metaphysis is formed. Moreover, the diet must be so constituted that the animals restricted to it will grow and maintain a fair state of general health and nutrition. The diets which we earlier described were not satisfactory, since they did not invariably produce typical rickets. Now and again an animal which was restricted to one of them would be found whose

[1] Shipley, P. G., Park, E. A., McCollum, E. V., Simmonds, Nina, and Parsons, H. T. *Jour. Biol. Chem.*, 1921, xlv, 343.

cartilages contained deposits of calcium salts. We have finally worked out a diet which fulfills the requirements for the test.[1]

DIET (LOT) 3143.

Wheat	33.0
Maize	33.0
Gelatin	15.0
Wheat gluten	15.0
NaCl	1.0
$CaCO_3$	3.0

This diet is faulty because it contains only 0.3019 gram of phosphorus in 100 grams of the mixture, while calcium is present to the extent of 1.221 grams per 100 grams of food. We have found that about 0.40 gram of phosphorus per 100 grams of food represents the lowest concentration of this element on which optimal nutrition can be secured when 0.641 gram of calcium are present in each 100 grams of ration. The fat-soluble A is low in this diet (Lot 3143), but it is sufficient to allow growth and to protect the rat against xerophthalmia. The diet also contains very little of an uncharacterized organic substance present in certain fats. We have previously discussed in detail the results of restricting rats to it.

TECHNIC OF TEST.

A group of young rats is placed on diet 3143 for 35–45 days, or until they begin to lose control of their hind legs. They are then divided into two groups, a control group which continues to receive diet 3143 unchanged, and a test group, which is given for the number of days deemed necessary the faulty diet plus the substance which is to be tested.

When a sufficient number of days have elapsed for the test substance to have produced its effects, the animals are killed and the bones which are to be studied are split longitudinally. The proximal end of the tibia is best for the purpose. One half of the bone is immersed in a dilute silver nitrate solution and exposed to light. It is then examined in the solution through a binocular microscope for the presence of a newly formed line of calcification in the proliferative cartilage. This line of calcium, which looks like the cross section of a honeycomb under strong magnification, is blackened by exposure to light. If this line is present, the test

[1] Shipley, P. G., Park, A. E., McCollum, E. V., and Simmonds, Nina. PROC. SOC. EXPER. BIOL. AND MED., 1921, xviii, 277.

is positive. The line may be visible to the unaided eye in untreated bones. The results of the examination of the gross specimen should be confirmed by study of celloidin or frozen sections from the other half of the bone or other bones. Control rats and rats which fail to give the test do not show the line of calcification. The new line of calcification may extend completely across the bone or may be incomplete or fragmentary, according to the extent of the deposition of the lime salts induced by the substance which is under examination. Since complete starvation also causes the typical linear deposit of lime salts to appear in the cartilage of rachitic animals, the food intake of both test and control rats must be carefully watched during the course of the experiment. Control and test animals must be kept under identical conditions.

This method is applicable to the study of the calcium depositing-power of any chemical substance or physical force.

61 (1808)

The effects of pituitary extract on the body temperature of animals rendered poikilothermous by destruction of the optic thalamus.

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In earlier work the writer has shown that destruction of the cerebral hemispheres and the optic thalamus of birds reduces the animal permanently to a poikilothermous condition. In birds this is not an operation that leads to immediate death for they may be kept alive for one to three months by keeping them constantly at an atmospheric temperature of 30° to 35° C. The routine procedure was to remove the cerebral hemispheres in toto by the scalpel and then destroy the optic thalamus with an electro-cautery.

It has been pointed out elsewhere that to produce the poikilothermous condition there must be extensive destruction of the thalamus and that localized injuries did not appreciably change