VITAMIN D DEFICIENCY IN THE RAT

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In the study of the physiological consequences of vitamin deficiencies, the usual procedure is to maintain the experimental animals on a diet which lacks the particular vitamin concerned. Such experimental diets contain, however, all other factors essential in the diet. What is often referred to as vitamin D deficiency in the rat, does not conform to these simple conditions. When young growing rats are placed on a diet such as the Steenbock-Black rachitogenic diet 2965[1], the animals develop rickets in 20 to 30 days. The rachitic condition, which can be demonstrated by line tests or the X-ray technic, is then said to be due to the deficiency of vitamin D in the diet. Actually, rickets cannot be produced in the rat when **only** vitamin D is absent from the diet. The diet must also have a low phosphorus content, as is the case with the Steenbock-Black diet. Rickets, produced by the Steenbock-Black diet, can be cured by the administration of vitamin D. It can also be cured by the addition of sodium or potassium phosphate to the diet.

In spite of the fact that rickets in the rat is not a symptom of vitamin D deficiency alone, the rachitic rat has been used extensively in studies concerning the function of the vitamin. One of the most recent and significant viewpoints is that of Schneider and Steenbock[2] "that vitamin D induced the utilization of P by bone, thereby depriving the soft tissues of their supply of P, which in turn inhibited growth". This action of the vitamin, we must remember, is taking place when the supply of P in the diet is not sufficient to supply both hard and soft tissues, for example, when vitamin D cures the rickets induced by the Steenbock-Black diet. There is also epxerimental evidence to support the view that vitamin D facilitates the absorption and prevents losses of calcium and phosphorus from the intestinal tract.

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[1]Steenbock, H., and Black, A., J. Biol. Chem. **64**, 263, 1925.
[2]Schneider, H., and Steenbock, H., J. Biol. Chem. **128**, 159, 1939.

Another signicant finding is that of Day and McCollum[3]. These authors placed young rats on a diet deficient in phosphorus but adequate in other respects. They found a marked decrease in the growth rate and the animals died in 7 to 9 weeks. They noted progressive rarefaction of the skeleton and great losses of calcium and phosphorus in the urine and feces. The soft tissues retained phosphorus at the expense of the skeleton, this occurring in spite of the presence of vitamin D in the diet.

It seems to us that, in view of the complications which arise when inadequate phosphorus intake is superimposed on a lack of vitamin D in the diet, the study of a simple deficiency of vitamin D in the rat deserves some attention. The experimental work reported at the present time concerns the effects of a diet deficient only in vitamin D.

Experimental

In making up such a diet it was, of course, necessary to use constituents which were free of vitamin D, or which on suitable treatment could be freed of the vitamin. The diet formula which was used is as follows: Casein (alcohol extracted) 18, starch 65, lard 9, salt mixture 4, Vitab rice-bran concentrate 3, wheat germ oil 1. In addition carotene was fed by drop feeding every two days and amounting to 120 micrograms per rat per week. The salt mixture used was adequate in all respects. The animals were given the above diet ad lib and were given distilled water to drink.

Now this diet cannot be tested for the presence of vitamin D by the usual assay procedure since the phosphorus content is adequate and this brings about healing of rickets without vitamin D. The constituents can be tested separately. The lard is, however, the only constituent of the diet which is to be suspected of containing vitamin D. To test the lard, it was added to Steenbock-Black diet in a proportion of 9 per cent and fed to 2 rachitic animals for 8 days. X-rays taken before and after feeding the lard-containing diet showed no healing of the rickets. Thus we are reasonably certain tha our diet is deficient only in vitamin D.

[3]Day, H. G., and McCollum, E. V., J. Biol. Chem. **130**, 269, 1939.

The original plan of the experimental work was to maintain the rats on the D deficient diet through several generations and observe the effects on growth rate, and on the general condition of the animals. As will be explained later this plan had to be abandoned. However, the reason for its abandonment is of interest. In all, four groups, totalling 47 animals, have been maintained on the diet. Only three of these groups will be reported.

Group 1: This group includes 8 males and 8 females, started on the diet at an average body weight for the males of 54 gm. and the females 51 gm. Body weights were determined every 2 days at the time of the carotene feedings. After 57 days, when the females had attained an average weight of 144 gm. they were divided into 2 groups of 4 each, and one of the vitamin D deficient males put with each group. The females were with the males for 39 days and showed no signs of pregnancy in either group. Since this unhappy circumstance upset the original plan of the experiment we became interested in its possible cause.

The estrous cycle of all the females was followed for 6 days by taking vaginal smears and staining with hematoxylin and eosin. In none of the females did we find evidence of a normal estrous cycle. This abnormality was confirmed by a smear series between the 120th and the 135th day on the diet and again by a 6 day series starting on the 159th day.

Now although we cannot rule out the possibility that the fault may have been with the males, it is probable that the disturbed estrous cycle explains the inability of the females to become pregnant.

The growth curves of the animals in this group are worth reproducing because of the striking difference between the males and the females. In Figure 1 are shown the average growth curves of both sexes. The poor growth of the males had its counterpart in their physical appearance. There was a loss of hair from the abdomen and about the head. There were also sores about the head and neck. By contrast the females showed no defects in appearance although their growth attainment was not as great as that for rats on

an adequate diet. Bone and blood samples from these animals were taken for analysis when they were killed on the 165th and the results will be reported at some future date.

Group 2: This group consisted of 8 females of an average body weight of 142 gm. when put on the diet. By vaginal smear series normal estrous cycle was established before starting on the diet. After being on the diet for 34 days, another series was run and it was found that 6 of the 8 animals had lost their normal cycle. These females were not mated.

Group 3: This group consisted of 15 males maintained on the D deficient diet for 45 days. Then 7 of the animals were given viosterol by drop feeding daily in addition to the diet. Over the next 15 day period these 7 rats showed a significant increase in body weight as compared with those not getting viosterol. It was necessary to terminate this experiment on the 60th day, and tissues were saved for analysis.

Discussion

The results we have obtained so far using a diet deficient only in vitamin D lead us to believe that much profit can be derived from further study. Abnormalities in the estrous cycle of the rat occur under several nutritional disturbances, most notably in vitamin A deficiency. Vitamin D is, apparently, also one of the factors. It remains to be shown whether or not vitamin D will restore the normal estrous cycle in these animals. The difference in reaction of the males and females to the diet is, we believe, of some significance. Chemical study of the tissues of these animals may reveal some interesting changes.

Summary

In some preliminary experiments a relationship between the estrous cycle of the rat and vitamin D has been demonstrated. When only vitamin D is absent from the diet, normal estrous does not occur and the females will not become pregnant. The growth rate of the males is definitely retarded as compared with the females on a vitamin D deficient diet.

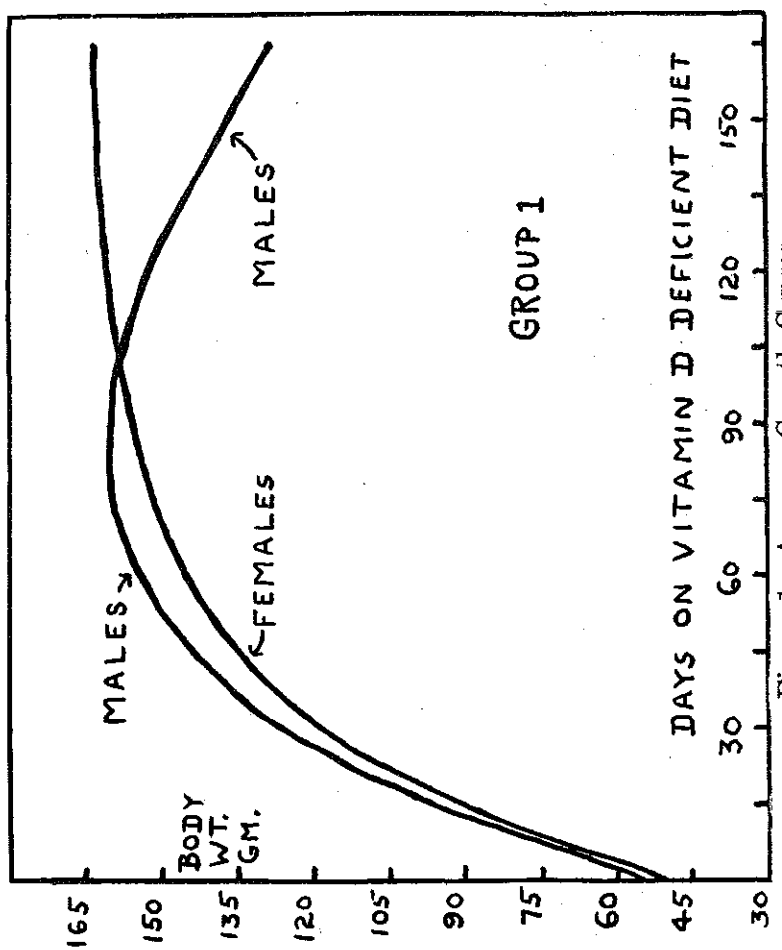

Figure 1—Average Growth Curves