cholesterol fell 56 mg. After 12 days on carotene, during which time the animals ate well and the symptoms improved markedly, the cholesterol fell 101 mg. In this animal reduction of the amount of carotene was accompanied by a rise in blood cholesterol. The hypercholesteremia apparently occurs while the animal is being depleted of its vitamin stores. When this depletion is advanced the blood cholesterol falls. It may well be that when the animal reaches a state of nutritive failure due to the deficiency and enhanced by the refusal to eat, the blood cholesterol falls because the animal is utilizing its body stores as a source of food. In young animals, dying of an intercurrent infection before profound nutritive failure can occur, the fall in blood cholesterol may not manifest itself. It seemed significant to us that the blood cholesterol was reduced rapidly to normal limits by the administration of the vitamin and the general condition of the animal improved at the same time.

*Summary.* Observations are reported on the level of the blood cholesterol in 4 dogs while on an A deficient diet. There is a rise in the blood cholesterol at the time when the animals developed the symptoms of A deficiency. In the young dogs this was maintained at a higher level than in the 2 older dogs. The feeding of vitamin A or its precursor caused a drop in the cholesterol. When the source of the vitamin was diminished the blood cholesterol rose. While the amount of the vitamin was adequate the blood cholesterol remained at the normal level.

*Conclusion.* These observations tend to substantiate the suggestion of other[2, 3] investigators that the metabolism of cholesterol is affected by the amount of vitamin A in the body.

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## Growth-Promoting Rachitogenic Diets for Rats.

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In the Medical Research Council's 1924 "Report on the Present State of Knowledge of Food Accessory Factors" emphasis is laid on the fact that rickets develops in well nourished children and that rachitic dogs also grow at a normal rate while the disease develops.

They call attention to the lack of good growth in rats on all commonly used rachitic diets and say: "It is manifest that experimental rickets in rats as at present produced fails to fulfill the condition of being a disease occurring in well growing and apparently healthy animals." Some of the early diets, made up for other purposes, were highly deficient and gave little growth. Diets designed later on for rickets production were much better in this respect, but even Steenbock's diet No. 2965 falls short of normal growth. This lack of growth cannot be looked upon as a "Vitamin D" deficiency since the addition of cod liver oil in liberal amounts will not materially improve the condition.

Several years ago we began a survey of feeding materials suitable for rickets production with a view to finding a more rational procedure for studies in experimental rickets and avoiding the obviously correct criticism of the British investigators. Numerous experiments were done with a view to satisfying the various aminoacid, vitamin and mineral requirements and it became apparent that Vitamin B (complex) was the most important factor. It was shown that by means of rice polishings a diet otherwise poor in growth and only slightly rachitogenic could be made to produce practically normal growth with a greater degree of rickets. Yeast and yeast extracts gave a marked improvement in growth but led to other complications to be discussed later. Observations on diets with yeast extracts, stressing growth, have been reported by Osborne, Mendel, and Park,[1] and previous to that the subject of growth and rickets has been extensively discussed by Shipley, Park, McCollum, and Simmonds.[2]

The diet we used in the above experiments was:

DIET 746

| | | | |
|---|---|---|---|
| Casein | 5 | Rice polishings | 7 |
| Egg albumen | 5 | Milk concentrate | 1 |
| Patent flour | 76 | Cotton seed oil | 2 |
| NaCl | 2 | $CaCO_3$ | 1 |
| Spintrate | 1 | | |

During the 3 weeks of the experiment this gave growth quite comparable to that of a good stock diet.

It will be seen from this that the requirements for growth in rat rickets can be satisfied fully as well as in dog rickets, thereby removing the objections cited. The amount of cod liver oil required

[1] Osborne, T. B., Mendel, L. B., and Park, E. A., PROC. SOC. EXP. BIOL. AND MED., 1923, **21**, 87.

[2] Shipley, P. G., Park, E. A., McCollum, E. V., and Simmonds, N., *Johns Hopkins Hospital Bulletin*, 1921, **32**, 159.

for prevention on these more rapidly growing rats with comparatively mild rickets, was the same as in the Steenbock diet for the prevention of extremely marked rickets. The cod liver oil requirement is not *per se* a measure of the severity of rickets. We believe that the conditions are more nearly such that, irrespective of the diet, a certain amount of antirachitic substance is required to turn the metabolism from a rachitic to a non-rachitic state. The amount of phosphate, however, which has to be added to various types of rickets-producing diets to prevent the rachitic changes varies anywhere from about 0.5% of primary potassium phosphate to 2%, depending only in part on the level of calcium in the diet.

Certain practical applications have also been made of this new type of growth-promoting rachitogenic diet. The rickets production is much more rapid and it is possible to produce in 7 days, or less time, an amount of rickets perfectly satisfactory for purposes of "Vitamin D" assay. With one of these diets having Ca/P ratio of $.713/.154 = 4.6$ the amount of rickets produced in one week as judged by the split, silver-stained tibia is not as much as is obtained on the Steenbock No. 2965, but still is a very suitable degree of rickets to work with.

The diet used had the following composition:

DIET 728

| | | | |
|---|---|---|---|
| Egg albumen | 10.0 | Rice polishings | 7.0 |
| Wheat gluten | 10.0 | Milk concentrate | 1.0 |
| Corn starch | 65.5 | Cotton seed oil | 2.0 |
| NaCl | 2.0 | $CaCO_3$ | 1.5 |
| Spintrate | 1.0 | | |

This diet is very suitable for 7-day tests, but does not, necessarily, support growth ideally during a longer experimental period.

In these one-week experiments determinations of inorganic blood phosphate show a mean of 3.7 mg. % for rachitic animals, 4.7 mg. % with partial, and 7.3 mg. % with complete prevention by cod liver oil. The bone ash figures obtained on fat-extracted, dried femurs gave averages of 42% for the rachitic and 46% for the non-rachitic.

We have successfully carried out assays in 7 days, by prevention method, in good agreement with the results obtained by 21-day prevention tests and 31-day line tests (the last 10 days being the test period). We are now engaged in studies looking towards further reduction of time necessary for assay.