

generation in a family confined to this diet showed no signs of physical deterioration. Without the calcium addition, but with 3 per cent. of butter fat, very little growth can take place.

The striking differences between the nutritive properties of cod liver oil as contrasted with butter fat we have up to the present time been able to demonstrate in so satisfactory a manner only with diets in which the phosphorus content is approximately the optimum, and with the calcium content distinctly below the optimum. The results of feeding these two fats with a diet similar to that described but in which the phosphorus content is low and the calcium varies from low to high, we shall discuss in a forthcoming paper.

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**Studies on experimental rickets, V.
The production of rickets by means of a diet
faulty in only two respects.**

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The following diet, when fed to the young rat, in a comparatively short time (three to five weeks) produces rickets. The diet is composed of:

Rolled oats.....	40.0
Gelatin.....	10.0
Wheat gluten.....	7.0
Sodium chloride.....	1.0
Potassium chloride.....	1.0
Calcium carbonate.....	2.0
Dextrin.....	39.0

This diet is extremely poor in fat-soluble A, the anti-xerophthalmic substance. Young rats develop xerophthalmia when placed upon it in from four to five weeks. Its proteins are of good quality and are supplied in abundance (21 per cent.). The phosphorus content is relatively low (0.209 gms. per 100 gms. of the food mixture). The calcium content is approximately the optimum.

The gross evidences of rickets in the rats eating the faulty ration were briefly these: deformities of the thorax consisting in flattening or hollowing along the line of costochondral junctions; pigeon breast; enlargement and distortion of the costochondral junctions; fractures of the ribs; enlargements of the ends of all the long bones; diminished resistance of the bone to cutting; great diminution in the tensile strength; and finally, the presence of a zone of a white or pale yellow color, between the cartilage and the shaft, visible to the naked eye, the rachitic metaphysis.

The microscopic evidences of rickets in the skeleton of the rats receiving the diet were convincing. There was increased thickness and great irregularity of the proliferative cartilage which extended in irregular prolongations toward the shaft, complete absence of calcium deposition in the cartilage or great defects in calcification, the presence of an intermediate zone between cartilage and shaft, the zone already alluded to as the rachitic metaphysis, composed of cartilage in all stages in the process of metaplasia into osteoid, osteoid trabeculæ, blood vessels accompanied by marrow elements, irregular deposits of calcified intercellular substance encased in osteoid and, finally, by broad osteoid investments of the trabeculæ of the shaft. The condition in the bone produced by the faulty diet in question may be said to have been at all points identical with that seen in advanced rachitis of human beings.

When now butter fat was added to the faulty diet just discussed in the proportion of 0.5 per cent. of the total ration, the occurrence of the xerophthalmia was somewhat postponed and the life of the animal lengthened. The pathological condition of the skeleton, however, remained essentially unmodified. If anything, it was intensified, probably as the result of the slight stimulation given by the butter fat to the growth of the bone.

When butter fat was added in the proportion of 10 per cent. of the total ration, the occurrence of the ophthalmia was effectually prevented, life was prolonged for a number of months and the pathological condition in the skeleton was somewhat modified but still remained rickets (observations on four rats). In one animal, light but uniform calcification in the zone of cartilage nearest the shaft was present. In all four rats the metaphysis was exceedingly deep and composed in greater part of interlacing osteoid trabeculæ

separated by blood vessels, the cartilage exceedingly irregular, the trabeculæ of the shaft very numerous and surrounded by osteoid. Even the addition to the faulty ration of a large quantity of butter fat did not, therefore, prevent the development of the rickets.

When, on the other hand, cod liver oil was added to the diet in the proportion of 2 per cent. of the total ration, no pathological condition of any sort developed in the skeleton. The addition of cod liver oil to the faulty diet, therefore, completely prevented the development in the skeleton, not only of changes of a rachitic nature but of all changes of a pathological nature.

When the faulty diet was still further modified by the subtraction of the added calcium carbonate, so that calcium was deficient as well as the phosphorus and the organic factor, a pathological condition developed in the skeleton which, however, was not rickets.

When the calcium carbonate of the faulty diet was replaced by 2 per cent. CaHPO_4 , so that the diet was deficient in the organic factor alone, the pathological condition which developed in the skeleton was not rickets but osteoporosis.

Conclusions: 1. When the content of the diet in the anti-rachitic organic factor or factors is sufficiently low, the protein of the diet and the quantities of Mg, Na, K, Cl, Fe and I being optimal or nearly optimal, a disproportion in the quantities of calcium and phosphorus present of such nature that the calcium is in optimal or above the optimal quantity but the phosphorus low, gives rise to rickets.

2. When the level of the phosphorus in the faulty diet is raised, so that both calcium and phosphorus are present in presumably adequate amounts, and the fault in the diet, therefore, becomes limited to a deficiency in the organic factor, a rachitic condition does not develop. A deficiency in the organic factor alone does not produce rickets, as our experiments have attested with other diets.

3. When no calcium carbonate is added to the diet, so that the quantities of both calcium and phosphorus are low, rachitis does not develop.

4. Butter fat is feeble in its anti-rachitic properties. This fact, repeatedly confirmed in our experiments, is in our opinion of

great practical importance in so far as the explanation of the occurrence of rickets in the human being is concerned.

5. Cod liver oil, on the contrary, as our experiments have repeatedly shown, is powerful in its anti-rachitic properties.

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Subcutaneous tubes for chemotactic studies and leucocyte collection.

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For the study of the chemotactic influences of various substances, including bacteria and their products, and for the collection of phagocytes and other wandering cells, small glass test tubes of about 5 c.c. capacity have been placed in the loose subcutaneous tissues of the rabbit. The procedure is as follows.

The mouths of test tubes, 1.2×5 cm., are covered with drum heads of fine bolting silk, tied on with thread, which permit free interchange of liquids with the subcutaneous tissues and the migration of cells and bacteria. The chemotactic material or the organism to be studied, either in agar (1 c.c.) overlaid with Ringer's solution, or in fluid form is then added to the sterilized tubes through a hollow needle. A tube may be placed in each flank through a single dorsal incision, after blunt dissection of the loose subcutaneous tissue. Surgical anesthesia and asepsis are required for the operation. Removal of the tube contents is accomplished by hypodermic puncture through the cloth heads of the tubes, after careful cleansing of the skin with iodine and alcohol. The needles of two 20 c.c. syringes are plunged into the tube. One syringe is already filled with Ringer's solution or other liquid to replace the tube contents as it is drawn into the second syringe. Thus the tube is washed out and left full of fresh material. The tubes themselves are practically non-irritating and their contents may be recovered daily, or at longer intervals, for weeks or months.