

beneficial. On two occasions there was nausea and vomiting. There were no other evidences of untoward digitalis action. A detailed report of the clinical aspects of this study will shortly be made.

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Experimental production of rickets with diets of purified food substances.

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The possibility of producing experimentally in animals symptoms essentially identical with those associated with rickets in human beings promises to advance the study of this disease greatly. The efforts in this direction have hitherto been concerned with the effects of diets of varied composition—usually for the most part mixtures of natural foods or materials derived therefrom without much manipulation. This has made comparisons between different rations somewhat difficult and often unconvincing because with a change in the natural foods several chemical ingredients are altered at the same time and consequently the cause of any marked change induced thereby in the animal usually cannot be charged directly to changes in any one chemical factor. For example, Sherman and Pappenheimer have demonstrated that rickets is brought about in a few weeks in rats by a diet of patent flour 95 per cent plus a mixture of three inorganic salts (Ca lactate 2.9 per cent, NaCl 2.0 per cent, Fe citrate 0.1 per cent). In experimental feeding tests under otherwise comparable conditions we found that the introduction of 10 per cent of a protein (lactalbumin was used) to replace an equal weight of flour in the Sherman-Pappenheimer ration increased the severity of the symptoms. The calorie value of the

two foods was essentially alike. One diet was made far richer than the other in protein of good biological quality; but this result was attended with "dilution" of the flour so that the various food factors which it specifically introduced into the diet, viz., phosphates and certain other inorganic ingredients, vitamins, and carbohydrates, were decreased. Were the gains or the losses responsible for the increased severity of the rickets? Obviously the physiological "analysis" or interpretation becomes complicated where several food factors are altered by an even seemingly simple change in the diet.

This criticism applies to most of the investigations conducted hitherto on rickets from a dietary standpoint. It has therefore been difficult to point unmistakably to a single responsible feature in the midst of numerous simultaneously shifting variables. McCollum, Simmonds, Becker, and Shipley¹ realized this when they attempted to produce rickets in rats by the use of more refined food materials. Their diets still included such products as wheat germ, wheat gluten and agar-agar; and the animals "developed xerophthalmia notwithstanding the presence of sufficient fat-soluble A to cover the minimum nutritive needs of the rat."

In order to supply a diet in which, as nearly as the present state of our knowledge and the development of our technic will permit, one factor at a time can be modified, we have resorted to feeding tests with purified food materials and a salt mixture in which each element could be varied independently.² Although even this procedure does not entirely avoid all of the difficulties referred to, in so far as withdrawal or addition of one factor may alter the relative proportions though not the kinds of the remaining factors, it affects them far less than when natural products are used. This applies especially to the inorganic ingredients. In general the food mixtures have consisted of some or all of the following components: purified protein, starch, lard, salt mixture, Osborne-Wakeman yeast fraction in small quantity for vitamin B.

The "complete" salt mixture is composed of:

¹ McCollum, E. V., Simmonds, N., and Becker, J. E., and Shipley, P. G., *J. Biol. Chem.*, 1922, liv, 249.

² Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1918, xxxiv, 121.

	gm.
CaCO ₃	134.8
MgCO ₃	24.2
Na ₂ CO ₃	34.2
K ₂ CO ₃	141.3
H ₃ PO ₄	103.2
HCl	53.4
H ₂ SO ₄	9.2
Citric acid + H ₂ O	111.1
Fe citrate 1 1/2 H ₂ O	6.34
KI	0.020
MnSO ₄	0.079
NaF	0.248
K ₂ Al ₂ (SO ₄) ₄	0.0245

A comparable salt mixture can be prepared without calcium, magnesium, or phosphorus as may be desired. For example, the phosphorus-free mixture used was prepared from

	gm.
CaCO ₃	122.4
MgCO ₃	12.1
Na ₂ CO ₃	17.1
K ₂ CO ₃	35.3
HCl	53.4
H ₂ SO ₄	9.2
Citric acid H ₂ O	95.0
Fe citrate 1 1/2 H ₂ O	6.34
KI	0.020
MnSO ₄	0.079
NaF	0.248
K ₂ Al ₂ (SO ₄) ₄	0.0245

Young albino rats weighing about 50 grams were placed on selected diets for a period of 35 days whereupon they were killed and examined. When the food contains *all* the known essentials including protein of good quality, as indicated above, the male animals grow within this period to about 125 grams in body weight. Without vitamine A, growth is retarded; but histological examination of the bones fails to reveal any changes characteristic of rickets.

Marked rickets has appeared on diets consisting of

Protein (edestin or lactalbumin)	16-20 per cent	} With a daily addition of 40 mg. yeast fraction.
Starch	56-52 per cent	
Lard	0-24 per cent	
P-free salt mixture	4 per cent	

The skeletons of the rats showed the gross and microscopic changes characteristic of rickets. The costo-chondral junctions were greatly enlarged and deformed. The lower ends of the radius and ulna were much enlarged. The femur cut easily. Microscopic examinations taken through its lower end disclosed the characteristic rachitic metaphysis. The cartilage was broad-

ened, had grown in an irregular manner toward the shaft, and on the shaft side showed the finer metaplastic or degenerative changes found in human rickets. Calcification was either deficient or entirely lacking. The trabeculæ showed well-marked osteoid borders.

The foremost significance of these experiments seems to us to lie in the possibility that they present of modifying, independently, a number of factors that may influence the abnormal metabolism of the bones. The rôle of ions other than phosphate can be tested. The debated importance of carbohydrate in the genesis of rickets, the comparative importance of light versus the organic and inorganic factors, the calorie intake, the possible contributory significance of the protein element—these and other influences can perhaps better be evaluated experimentally in this way. We are engaged in the applications of the method to the problems suggested.

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On the feeding habits of oysters.

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Studies of the feeding habits of oysters have been in progress since the spring of 1919 to determine: (1) the duration of active filtration of water, and (2) the effects of salinity, temperature, light, food content, and turbidity of the water upon the rate and the extent of feeding. The animals were studied while lying on an oyster reef in their natural environment. By means of recording apparatus continuous kymograph records of all shell movements of the oysters for over 2 months were obtained. Water samples were taken at regular intervals of time and tide, and in addition whenever the animals closed completely or opened after a period of closure in excess of 2 minutes.¹

¹ Preliminary reports of the writer in *Anat. Rec.*, 1921, xx, 181; *Reports of the Department of Biology, N. J. Experiment Station* for 1920, p. 333, and for 1921, p. 293.