TABLE III. DISTRIBUTION OF PHOSPHORIC ACID IN RAT BLOOD

Diet No.	Total acid sol. mg.	Inorganic mg.	Inorganic %	Org. Hydrolysable mg.	Org. Hydrolysable %	Non-Hydrolysable mg.	Non-Hydrolysable %
NON-RACHITIC							
303D	23.0	6.0	26	8.3	36	8.7	38
303	20.6	5.4	26	7.6	37	7.6	37
303A	18.7	4.3	23	6.0	32	8.4	45
302C	22.3	6.1	27	5.3	24	10.8	48
302B	22.6	6.2	27	5.4	24	10.9	48
302A	20.0	6.7	33	6.7	34	6.6	33
302	19.5	7.6	39	5.9	30	6.0	31
301E	28.0	5.9	21	7.5	27	14.6	52
301	30.0	8.0	27	7.6	25	14.4	48
Aver.	22.7	6.2	27		30		42
RACHITIC							
303C	17.5	2.4	14	4.3	25	10.8	62
303B	17.4	2.7	15	6.4	37	8.3	48
302D	18.7	2.6	14	4.1	22	11.9	64
301A	20.0	2.6	13	3.7	18	13.7	69
301B	20.0	3.6	18	4.0	20	12.2	61
301C	19.2	3.1	16	4.0	21	12.0	63
301D	17.1	3.4	20	3.8	22	9.9	58
Aver.	18.6	2.9	15		24		60

The nature of these diets will be discussed elsewhere. In this table they are simply grouped as rachitic and non-rachitic, according to the X-ray and histological findings.

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Observations on the distribution of anti-rachitic substances.

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When Mellanby found that the rickets produced in dogs was prevented or cured by cod liver oil, he thought that since cod liver oid is extremely rich in the fat-soluble A vitamin, that this substance was responsible for the cure and that the deficiency of

fat-soluble A is the cause of rickets. This assumption was soon proved to be erroneous, and a second natural assumption was made by McCollum that the deficiency of another vitamin was responsible for rickets production. No one doubts the existence in cod liver oil of a substance having a favorable influence on mineral metabolism. If this substance is concerned in the rickets of infants then we must be able to show that a normal diet for infants contains the substance in sufficient amount and that when rickets occurs the substance is deficient. It is perfectly well known, however, that rickets occurs quite freely in infants on mother's milk or on best grades of fresh cow's milk. It is, furthermore, a well established fact that rachitic infants are not cured by the addition of liberal amounts of cream to their diet.

Both Park and Howland believe that an anti-rachitic vitamin occurs in foods probably associated with the fat-soluble A in green leaves. Having found a method by which we can materially concentrate the active substance from cod liver oil, we investigated the occurrence of this material in various plant and animal substances. We have subjected the following materials to the processes similar to those by which we obtained a substance from cod liver oil one thousand times as active as cod liver oil itself: butter, cocoanut oil, spinach, carrots, pig's liver and sheep's adrenals. From none of these materials were we able to obtain an extract which even without dilution approached the action of cod liver oil, while from cod liver oil itself we obtained extracts at least one hundred and later more than one thousand times more active than the original cod liver oil. We cannot say that these preparations were entirely free from anti-rachitic substance, but the amounts, if there was any, were so small that they gave minimal and irregular results. Taking as an example the plant materials spinach and carrots—these were treated first with acetone, then with ether, then with alcohol and again ether. We believe that this gave us a thorough extraction of the fats contained in the plant tissues. This fat was then saponified and put through procedures previously described. The extraction of the fats must have concentrated the material at least ten times and the further processes at least one hundred times. On this basis, spinach or carrots if they contain any anti-rachitic material will require four kilogram to furnish as much as one teaspoonful of cod liver oil. We can safely say then that

by means of a method which can reasonably be expected to give results, we have been unable to confirm the assumption that green leaves and a number of other food materials contain a significant amount of anti-rachitic substance.

How then are we to explain the results of McCollum and others that butter and cocoanut oil produce partial cures. The following experiment, we think, throws light on the subject: When rats are kept for four weeks on a diet of flour and a salt mixture containing 2.9 per cent. of calcium lactate, rickets is regularly produced. When we use this diet with the addition of 20 per cent. of either cotton seed oil or crisco, a hydrogenated product from cotton seed oil, rickets is still produced. If, however, we lower the percentage of calcium lactate to 1.5 per cent., the diet without fat produces rickets, while the addition of 20 per cent. of crisco entirely prevents it and the cotton seed oil does not prevent it but merely gives a somewhat lesser degree of rickets. In this case vitamin action is excluded, but we find that the hydrogenated product prevents rickets. The hydrogenation has increased the melting point of the oil; and the soaps from crisco are more insoluble than those from cotton seed oil. Our interpretation is, that in the case of crisco more insoluble calcium soaps are formed in the intestines and by precipitating calcium soaps phosphate in soluble form which would otherwise be precipitated as calcium phosphate is made available for absorption. With a great excess of calcium in the diet containing 2.9 per cent. of calcium lactate, the precipitating action of crisco is, however, insufficient to prevent rickets. Now if we turn back to cocoanut oil, we can easily see how this fat whose soaps are notoriously insoluble will show such a marked rickets preventing action.

We also see from this experiment that the rickets producing qualities of a diet depend on the exact adjustment of quite a number of factors, and we are led to believe that the slight rickets curing action of butter recorded by McCollum is due to fatty acids in the butter rather than a vitamin, particularly since we are unable to obtain any concentrated active substance from butter, and since after all rickets in infants cannot be prevented or cured by either butter or cream.

While we cannot say that we have proved the non-existence of an anti-rachitic vitamin the facts adduced certainly detract

from the plausibility of the assumption that there is one. Until the presence of such a vitamin is established in natural infants' diets in cases where rickets is prevented and a deficiency in diets of cases where rickets occurs it will be safer to approach the subject with an open mind. The rickets-curing substances in cod liver oil and in egg yolk might just as well be looked upon as therapeutic agents, possibly internal secretions, which will prevent or cure rickets.

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On the nature of pneumococcus antigen.

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Various extracts of killed pneumococci have been studied for the purpose of determining their immunizing power and their freedom from toxic products. A non-toxic pneumococcus antigen of high potency can be made by suspending the sediment from a centrifuged pneumococcus broth culture in saline and digesting the pneumococci with 0.2 per cent. trypsin for 24 to 48 hours. The undigested portion is centrifuged off and the metaproteins in the supernatant fluid precipitated with acid. After filtration the filtrate is thrown into 7 volumes of 95 per cent. alcohol, the resulting precipitate filtered off and the alcoholic solution evaporated in vacuum. The residue in the flask is taken up in saline and made up to the original volume of the saline suspension. This solution contains the immunizing antigen of the pneumococcus. Mice which had received three subcutaneous injections of this antigen were protected against a hundred thousand lethal doses of a pneumococcus broth culture, injected intraperitoneally.

Human volunteers were injected subcutaneously with the alcohol soluble fractions of pneumococcus Type I, II, and III.