LXXVIII. THE FAT-SOLUBLE VITAMINS OF MILK.

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SOME years ago McCollum, Simmonds and Steenbock [1917] made the observation that fat-free milk, when included in a diet consisting of purified food substances, promoted growth and prevented decline of animals and, further, that melted butter-fat lost its growth-promoting capacity after being thoroughly agitated with twenty successive portions of water. This led them to suspect that the "fat-soluble A factor" might be appreciably soluble in water. Later, Steenbock, Boutwell and Kent [1918] were able to demonstrate that the "fat-soluble vitamin" was not actually removed from the butter by the water but that it was destroyed in the process of washing. Drummond, Coward and Watson [1921] using a basal diet free from both fat-soluble vitamins (the separate identity of the fat-soluble vitamins was not appreciated at that time) came to the conclusion that butter was less potent as a source of "fat-soluble A factor" than the same amount of fat supplied in the original milk and that the loss incurred was partly mechanical and partly due to destruction. As far as we are aware no strictly quantitative experiments have since been carried out to demonstrate whether the growth with the "fat-free" milk observed by McCollum, Simmonds and Steenbock was due to the presence of the fat-soluble vitamins present in the residual fat of the skim milk, which we presume their milk contained, or whether one or both of the fat-soluble vitamins of milk-fat is really appreciably soluble in water. The nature of an enquiry in which one of us (S.S.Z.) is at present engaged necessitated that this should be satisfactorily established and the experiments described in this communication were undertaken with this end in view.

METHODS.

The morning milk from a typical herd of nine short-horn cows, which are kept for the study of normal milk at the National Institute for Research in Dairying, Shinfield, Berks., was used in this experiment. The sample was collected on May 13th, 1929. The cows were on pasture day and night and in addition received a mixture consisting of dried grains two parts and yellow maize germ meal one part.

This concentrate mixture was given to six cows which were in full milk, in quantities which averaged 6.5 lb. per day per cow, varying from 0 to 12 lb. according to the milk yield.

The milk contained 3 % fat and 12.05 % total solids. 25.75 lb. of the whole milk was dried in a vacuum pan under diminished pressure (75.75 cm.) for 5 hours; the temperature of the milk never rose above 36°. When the milk had reached a suitable concentration, the drying was continued in an evacuated desiccator over P_2O_5 at 37° until the water content was 22.15 %. It was then transported to the Lister Institute, London, and kept there in the cold room during the entire testing period. Likewise the separated milk from 36.1 lb. of the original milk, containing 0.1 % of fat was concentrated to a water content of 32.75 %. The separated cream was churned and the butter-milk thus obtained concentrated. The latter concentrate was added in equivalent quantities to the dried separated milk during the tests. The butter contained 11.7 % water. The dried milks were acid and had an odour of butyric acid.

In the butter, vitamin A was determined as previously described [Golding, Soames and Zilva, 1926] by the modified Steenbock method, daily doses of 0.1 g., 0.2 g. and 0.6 g. being employed. The estimation of vitamin A in the whole and in the skim milk plus butter-milk was carried out by administering daily the dose of dried milk mixed with an equal quantity of the basal diet [see Golding, Soames and Zilva, 1926] from which the caseinogen had been eliminated. After the animals had consumed the dose, the basal diet was offered *ad lib*. In the case of the whole milk, doses approximately equivalent to 0.05 g., 0.1 g. and 0.2 g. and in that of the skim milk plus butter-milk to 0.2 g., 0.5 g. and 0.7 g. of butter were tested. The animals apart from their doses consumed their basal diet willingly and in ample quantities (12–15 g. *per diem*).

Vitamin D in the whole and in the skim milk could not be assayed by a method in which McCollum's diet No. 3143 was used, because the daily consumption of the necessary quantities of dried milk equivalent to the butter doses would have also entailed the consumption of significant quantities of phosphorus, in which this diet is characteristically deficient. In fact, preliminary tests have actually shown that no satisfactory results could be obtained under the circumstances. Recourse was had to another method, namely that of Soames and Leigh-Clare [1928]. This method is based in the main on the increase in weight in young rats produced by the addition of vitamin D to a diet free from this vitamin but otherwise adequate. The basal diet employed in this research was Soames and Leigh-Clare's diet No. 2 and was made up as follows:

	Parts by weight
Wheat starch	43.7
Inactivated caseinogen ...	14.3
Cotton seed oil (hardened)	12.4
Yeast	8.0
Salt mixture	5.0
Lemon juice	5.0
Wheat germ	20.0
Water	50.0

The daily doses of dried milk were given in equivalent quantities of the following mixture:

	Parts by weight
Starch... ..	79
Hardened cotton seed oil ...	21
Yeast	14
Salt mixture	9
Lemon juice	9
Wheat germ	36
The dried whole or skim milk	125
Water... ..	82

After the consumption of the test dose, the above basal diet was offered *ad lib.* and quantities adequate to induce good growth in presence of sufficient vitamin D were consumed by the animals. In addition to the growth index, the ratio (*A/R* ratio) of the amount of mineral ash to the amount of organic material contained in the fat-extracted bone [see Chick, Korenchevsky and Roscoe, 1926] was also determined as suggested by Soames and Leigh-Clare.

DISCUSSION OF RESULTS.

Vitamin A.

The skim milk to which, as already mentioned, the butter-milk from the churning of the butter was added, contained of course a certain amount of the milk-fat. Thus the highest dose of 2.8 g. which was equivalent to 0.68 g. of butter contained 0.066 g. of fat, the second dose of 2.1 g. which was equivalent to 0.51 g. of butter contained 0.050 g. of fat, and the lowest dose of 0.7 g. which was equivalent to 0.17 g. of butter contained 0.016 g. of fat. Were any appreciable quantity of vitamin A associated in the milk after separation or in the butter-milk after churning with constituents other than fat, or should the vitamin have been more concentrated in the residual fat than in the butter, it would have been reflected in the growth curves of the rats receiving the "skim milk." By examining the growth curves of these animals, however (Fig. 1), it is seen that this was not the case, but that the growth obtained was more or less proportional to the residual fat present in the doses. Thus the lowest dose containing 0.016 g. of residual fat induced no appreciable growth, but the higher doses gave much better growth. This is particularly marked in the case of the highest dose containing 0.066 g. of residual fat. In fact the growth obtained with this dose was almost as good as that obtained with 0.1 g. of the butter (group VI, Fig. 2), which suggests that the minimum dose of milk-fat lies between these quantities. The weight curves of the rats receiving the whole milk (Fig. 2) also indicate that the vitamin A content of the doses was proportional to their milk-fat content, since the daily minimum amount of milk-fat necessary to induce optimum growth lies in this case between 0.05 g. (group III, Fig. 2) and 0.10 g. (group II, Fig. 2), a minimum also indicated by the growth curves obtained with the "skim milk" and with the butter.

Fig. 1.

Group I. Daily dose 2.80 g. of dried skim milk and butter-milk, equivalent to 0.68 g. of butter and containing 0.066 g. of milk-fat.
 Group II. Daily dose 2.10 g. of dried skim milk and butter-milk, equivalent to 0.51 g. of butter and containing 0.050 g. of milk-fat.
 Group III. Daily dose 0.51 g. of butter and containing 0.016 g. of milk-fat.
 Group IV. Daily dose 0.17 g. of butter and containing 0.016 g. of milk-fat.
 Group V. Negative controls.

Fig. 2.

Group I. Daily dose 1.0 g. of dried whole milk, equivalent to 0.20 g. of butter and containing 0.21 g. of milk-fat.

Group II. Daily dose 0.50 g. of dried whole milk, equivalent to 0.10 g. of butter and containing 0.10 g. of milk-fat.

Group III. Daily dose 0.25 g. of dried whole milk, equivalent to 0.05 g. of butter and containing 0.05 g. of milk-fat.

Group IV. Daily dose 0.125 g. of dried whole milk, equivalent to 0.025 g. of butter and containing 0.025 g. of milk-fat.

Group V. Daily dose 0.06 g. of dried whole milk, equivalent to 0.012 g. of butter and containing 0.012 g. of milk-fat.

Group VI. Daily dose 0.03 g. of dried whole milk, equivalent to 0.006 g. of butter and containing 0.006 g. of milk-fat.

First arrow indicates beginning of the administration of irradiated cotton seed oil (vitamin D), second arrow that of the test dose.

+ denotes death.

Fig. 3.

Group I. Daily dose 1.0 g. of dried whole milk, equivalent to 0.20 g. of butter and containing 0.21 g. of milk-fat.
 Group II. Daily dose 0.50 g. of dried whole milk, equivalent to 0.10 g. of butter and containing 0.10 g. of milk-fat.
 Group III. Daily dose 0.25 g. of dried whole milk, equivalent to 0.05 g. of butter and containing 0.05 g. of milk-fat.

Fig. 4.

Group I. Daily dose 0.6 g. of butter.

Group II. Daily dose 0.2 g. of butter.

Group III. Daily dose 0.1 g. of butter.

Fig. 5.

Group I. Daily dose 2.80 g. of dried skim milk and butter-milk, equivalent to 0.68 g. of butter and containing 0.066 g. of milk-fat.

Group II. Daily dose 2.10 g. of dried skim milk and butter-milk, equivalent to 0.51 g. of butter and containing 0.050 g. of milk-fat.

Group III. Daily dose 0.70 g. of dried skim milk and butter-milk, equivalent to 0.17 g. of butter and containing 0.016 g. of milk-fat.

Vitamin D.

Although the limitations of the method for the determination of vitamin D employed in this research have not yet been fully studied, we are of the opinion that the results obtained leave little doubt that, as in the case of vitamin A, vitamin D is associated entirely with the milk-fat. The growth-promoting capacity of the skim milk was again proportional to the amount of residual fat present in the dose. In these tests the minimum daily dose which induced optimum growth (as compared with the positive control group) was between 0.05 g. and 0.066 g. (groups II and I, Fig. 5), which was also more or less the

Fig. 6.

Group I. Positive controls; 5 drops of cod-liver oil daily.

Group II. Negative controls.

minimum dose indicated by the whole milk and butter tests (Figs. 3, 4 and 6). The analysis of bones of these rats (Table I and Fig. 7) showed that the A/R ratios were also more or less proportional to the amount of milk-fat present in the dose. These experiments showed that an optimum average A/R ratio was attained only when the rats received a daily dose of 0.2–0.6 g. of butter, a dose higher than that required to induce optimum growth. The animals on a dose of dried “skim milk” containing 0.066 g. of milk-fat (group IX, Fig. 7) showed a somewhat higher average A/R ratio than the animals receiving 0.1 g. of butter equivalent to 0.088 g. of fat (group I, Fig. 7)¹. This, however, we consider as an error inherent to the method rather than an indication that the residual fat in the skim milk was more potent than the butter-fat.

¹ Also the animals on the dose 2.1 g. of dried skim milk containing 0.05 g. of milk-fat showed a slightly higher ratio than the animals receiving the dose 0.25 g. of whole dried milk containing 0.05 g. of fat.

Table I.

[illegible]

Fig. 7.

Percentage differences between average ratio of groups of animals and negative controls, ash/organic residue, not fat. (Difference between average ratio of positive group receiving daily 5 drops of cod-liver oil and negative group of each series = 100 %.)

Group I. Daily dose 0.1 g. of butter.

Group II. Daily dose 0.2 g. of butter.

Group III. Daily dose 0.6 g. of butter.

Group IV. Daily dose 0.25 g. of dried whole milk, equivalent to 0.05 g. of butter and containing 0.05 g. of milk-fat.

Group V. Daily dose 0.50 g. of dried whole milk, equivalent to 0.10 g. of butter and containing 0.10 g. of milk-fat.

Group VI. Daily dose 1.0 g. of dried whole milk, equivalent to 0.20 g. of butter and containing 0.21 g. of milk-fat.

Group VII. Daily dose 0.70 g. of dried skim milk and butter-milk, equivalent to 0.17 g. of butter and containing 0.016 g. of milk-fat.

Group VIII. Daily dose 2.10 g. of dried skim milk and butter-milk, equivalent to 0.51 g. of butter and containing 0.050 g. of milk-fat.

Group IX. Daily dose 2.80 g. of dried skim milk and butter-milk, equivalent to 0.68 g. of butter and containing 0.066 g. of milk-fat.

SUMMARY.

The results obtained in these experiments suggest definitely that the entire vitamin A and vitamin D content of milk is associated with the milk-fat. There are no indications that these vitamins are more concentrated in one fraction of the milk-fat than in another, nor that there is a significant loss in either vitamin due to separating or churning, and consequently by testing the butters it is possible to obtain the approximate relative potency of milks. These experiments further suggest the utility of the Soames and Leigh-Clare method for the determination of vitamin D. The limitations of this method are being studied further.

We are indebted to Miss Dearden for the churning of the milk.

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