XXV. MILK AS A SOURCE OF WATER-SOLUBLE VITAMIN. III¹.

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THE question of the vitamin content of milk has, very properly, attained considerable prominence not only in its scientific aspects in relation to nutrition but also from the standpoint of public health. The classic experiments of F. G. Hopkins [1912] indicating an unexpectedly favourable effect upon health and growth secured by the addition of as little as 2 cc. of cow's milk per day to a "synthetic diet" fed to young rats have given the occasion for the current emphasis upon the richness of milk in that nutritive factor now commonly designated as vitamin *B*. Our own early experiments [1911] published in 1911 had indicated that what we originally termed "protein-free milk"² furnishes something without which rats cannot grow satisfactorily when they are kept upon "synthetic" diets consisting of mixtures of more or less isolated food substances. However, the quantities of "protein-free milk" which we have found necessary to supply the essential food factor to rats in numerous trials at various times have been decidedly larger than would correspond to 2 cc. of milk. This might have been due to a variety of obvious possibilities including the loss of vitamin in the process of preparation of the milk product. Consequently we [1918] undertook a re-investigation of the subject, using milk as the added source of vitamin *B* in the ration. The experiments indicated the necessity of feeding as much as 16 cc. of fresh cow's milk to secure the desired growth. Again [1920] we made further attempts to demonstrate a possible greater vitamin *B* potency by feeding unpasteurised milk of known origin. In some of these trials the diets used by Hopkins were imitated as closely as the materials at our disposal would permit. Again "the outcome

¹ The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, Washington, D.C.

² This product consists of the dried solids of skim milk from which the casein has been removed by precipitation with acid and the coagulable proteins removed by heat.

was in harmony with all our experience in showing that even additions of 10 cc. of fresh milk per day were insufficient to effect a food intake adequate for growth at a normal rate" [1920].

Neither seasonal variations, differences in the rations fed to the lactating cows, nor manipulations of the milk prior to marketing appeared to offer a satisfactory explanation of the differences between the results recorded by Hopkins and ourselves. He [1920]¹ has lately reported the outcome of a new series of tests of 2 cc. of milk per day as a source of vitamin *B* for rats. His experiences are related as follows:

"On receipt of Osborne and Mendel's private communication, I made a few experiments in the winter of 1919. At this time the results were frankly disappointing. When the animals receiving the 2 cc. of milk in addition to the synthetic diet were compared with others which had the latter alone, the favourable effect upon health and upon the survival periods of the animals was unmistakable. Growth, however, was very slow, and the death rate of the rats was higher than that of animals normally fed.

"In April of this year fresh experiments were begun and continued during the summer. The results now became such as to confirm entirely my earlier experiments. Out of 20 animals, each receiving daily 2 cc. of fresh cows' milk as an addition to a highly purified synthetic diet, not one failed to grow almost normally throughout the period of experiment. The observations were not extended beyond 60 days, as they were meant only for comparison with those described in my original paper, and not to determine the amount of milk necessary for continued growth. The technique was exactly that originally used by me. In each experiment control animals were fed upon the synthetic diet alone and the contrast between them and a corresponding set receiving 2 cc. of milk was just as marked as in my original experiments. There has been no selection of results."

It will be noted in most of Hopkins' experiments that he began the feedings when the rats were still quite young, often weighing not more than 40 g. We concluded to repeat our tests upon rats somewhat younger than those with which our previous experiments were begun. The standard diet consisted of

Casein ...	...	18 %	Butter fat	...	9 %
Salt mixture ² ...	...	4	Lard	...	20
Starch ...	...	54			

Each day 2 cc. of milk, supplied in a separate dish, were promptly consumed by the rats. The tests began October 19, 1921. The milk was obtained unpasteurised from a nearby supply. A bacterial count made in November on samples from the same source showed 230,000 bacteria per cc.

¹ In this paper a reference (p. 724) to previous experiments by Osborne and Mendel is erroneously cited as *Biochem. J.* **41**, 515. It should be *J. Biol. Chem.* **41**, 515.

² [Osborne and Mendel, 1919.]

The changes in body weight are shown graphically in the appended chart, the weekly food intakes (exclusive of milk solids) being indicated, where available, on the curve of growth. During the periods represented by the broken lines (-----) 40 mg. per day of a yeast fraction prepared by Osborne and Wakeman's method [1919] replaced the milk in the case of rats 7462, 7485, 7488, 7489. Rat 7506 received 0.1 g. dried brewery yeast per day. Rat 7460 died with symptoms characteristic of lack of vitamin *B*.

Fig. 1.

If variations in the vitamin *B* content of cow's milk from different sources actually reach the magnitude represented by the discrepancies between the English reports and our own they can scarcely be due to variations in the diet of the cows. The differences seem too large to be accounted for by such a probability. Hopkins himself states:

"The incomplete observation just mentioned, and the experience of Osborne and Mendel, suggest, at any rate, that the apparent seasonal variation in the results of my experiments was not likely to be due to differences in the milk. That there is a seasonal factor in the growth energy of rats is I think sure, but it is doubtful if it could account for the large difference in the experimental results now described. I am endeavouring to obtain further light on the matter."

In harmony with our own experiences alike with fresh cow's milk and "protein-free milk" prepared therefrom are reports from other investigators. Thus Johnson [1921], who studied the "growth-promoting properties" of milk and dried milk preparations used in the form of "reconstructed" milk in the Hygienic Laboratory of the U.S. Public Health Service, found that his animals required, as an adequate source of vitamin *B*, an amount of the milk equivalent to that reported by ourselves. Thus he states that "a food mixture consisting of purified foodstuffs plus milk as the sole source of water-soluble vitamines must contain at least $2\frac{1}{2}$ parts of milk to 1 part of the basal ration in order to produce normal growth. Since a full-grown male rat weighs about 280–300 grams, and a full-grown female about 180 grams, a rat upon such a diet consumes just about 16–18 cubic centimeters of milk daily. These figures agree with those of Osborne and Mendel. It is also seen that the rate of growth of rats receiving less than $2\frac{1}{2}$ parts of milk in their diet was accelerated after increasing the amount of milk in the diet."

According to Johnson and Hooper [1921] "Pigeons fed upon mixtures of spray process skim milk powder with polished rice require 30 per cent. of the food in skim milk powder in order to get full protection from polyneuritis. This corresponds to about 75 cc. daily liquid milk."

Dutcher [1921], Dutcher, Kennedy and Eckles [1920], Dutcher and colleagues [1920, 2], have found spring milk obtained after the cows were placed on green grass to be superior to winter milk in "antiscorbutic and nutritive properties." However, when the milk was at its best it required 10 cc. to furnish "sufficient water-soluble and fat-soluble vitamines for normal growth in the albino rat." Even the greatest reported increases in antiscorbutic potency in the milk of cows fed upon especially suitable rations [Hart, Steenbock and Ellis 1920; Dutcher and colleagues, 1920, 1; Hess, Unger and Supplee, 1920] do not parallel the increment which the differences represented by 2 cc. (Hopkins) and 10 cc. respectively per day denote. If the variations in the vitamin-content of the diet of cattle in different places will account for the marked differences in the vitamin *B* content of cow's milk they must be far more pronounced than has been suspected hitherto.

Through the cooperation of the Department of Obstetrics in the School of Medicine, Yale University, we have been enabled to test mixed samples of human milk for its content of vitamin *B* in the same way as has been reported for cow's milk. The product, which represented the secretion from several persons, was not essentially richer than we have found cow's milk

to be. 5 cc. of human milk per day added to the standard diet devoid of vitamin B was insufficient to secure continued increments of body weight. When the milk was desiccated and fed in the form of tablets the equivalent of 10 cc. sufficed, in addition to the standard food eaten, to secure growth at a fairly good rate. In no case was the human milk equivalent in its potency as a source of vitamin B to the cow's milk used in Hopkins' most successful tests.

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