LXVIII. NOTE ON THE VITAMINE CONTENT OF MILK.

By FREDERICK GOWLAND HOPKINS.

From the Biochemical Laboratory, Cambridge.

(Received November 8th, 1920.)

THE observations described in this note will, it is to be feared, have less interest for others than for myself.

Since, in the early part of 1912, I published a paper describing the startlingly favourable effects of adding minute amounts of milk to synthetic dietaries, incapable, by themselves, of maintaining the growth and health of rats [1912], the conception of Accessory Food Substances or Vitamines has become widely based upon a large mass of experimental evidence. My early experiments have now no more than historic interest.

Since, however, Osborne and Mendel [1920], during their studies of the water-soluble vitamine, have found themselves unable to maintain the growth of rats, when using milk as a source of vitamine in quantities so small as those used by me, I have been naturally anxious to clear up what seemed to be a discrepancy in experience.

I may be allowed the privilege of saying here that the experiments which I described in 1912 followed upon a long experience of the effects of adding tissue extracts, and especially fractionated yeast preparations, to purified diets. Looking back to one's experience in these years during which startling successes were mingled with puzzling failures—failures which led to delay in publication—I realise that the absence of all knowledge concerning the factor associated with fats was the cause of any experimental contradictions. Had I possessed the scientific vision which afterwards led McCollum and Osborne and Mendel to recognise the existence of this, I should have reached full conviction as to the reality of my results long before I did. In the synthetic diets employed by me, the protein and the carbohydrates were purified to the uttermost, but I used little or no discrimination with regard to the fats.

With milk, as an addendum to the purified foodstuffs, I got consistent results in all cases. In preparing at that time a paper for publication I naturally wished to bring out the characteristic quality of the "Accessory Growth Substances" (which in spite of all experimental difficulties had, by 1910-11, become very real to me) of exerting their influence when present in very small amount. I wished in particular to emphasise the fact that their effects had nothing to do with energy supply; hence I used the milk addendum in quantities made as small as possible.

The published experiments, in which quantities of milk as small as 2 cc. per animal per day were employed, were in no sense selected. They were few in number, but gave consistent results and I had no feeling at the time that they would offer any difficulties in repetition.

Some months before they published the paper referred to above, Professors Osborne and Mendel, with great courtesy, communicated the results privately to me.

Even earlier than this I had, as a result of some remarks previously published by Drummond, and by Osborne and Mendel themselves, made, on a small scale, an effort to repeat my original experiments. I became impressed by the apparent influence of seasons on the results.

On the 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. All the observations made from April onwards are here described.

It may be that such experiments, in which it must be supposed that the supply of vitamins (at any rate of the water-soluble substance) is minimal, are well calculated to bring out the minor variations in the quality of the milk, as it may depend, for instance, upon variations in the summer and winter feeding of cows.

Osborne and Mendel, however, were unable to show that this factor influenced their results. In connection with the experiments just described, I myself endeavoured to get information on the point. Goats were fed upon dietaries corresponding roughly to the winter and summer feed, respectively, of milch cows. Three goats received a mixed cereal diet, together with a small ration of hay and mangold. Three other animals had the same diet, but in addition took grass and green vegetables freely. Two of the animals in each set produced young and their milk was supplied to rats in quantities of 5 cc.

per rat per day. Growth was nearly normal in each case, though, doubtless as an accidental circumstance, the rats upon the "summer" dietary grew rather less well than the others. Analysis of the milk in each case showed that the total solids and fat content was, throughout, almost the same in both. The goats deprived of green stuff produced, however, considerably less milk than the others, a circumstance which would seem to make such comparisons very difficult. It may be when there is deficiency of some essential constituent in the food of a lactating animal that adjustment may occur by a limitation of the total quantity of the milk rather than by a falling off in its quality. 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. The purpose of this ^{note} is merely to point out that given the right conditions my original observations can be repeated.

Experiment 1, begun April 9, 1920. Eight rats were placed upon the synthetic diet. Four received this alone, and four were given 2 cc. of milk each before receiving their daily feed; great care being taken that the milk was completely consumed. The latter grew vigorously during the 60 days of the experiment, the former grew slowly for about 25 days, when their growth ceased and on the 35th day three out of the four died. The individual animals showed a strikingly uniform behaviour in each set. This experiment was supervised by Mrs E. C. Bulley (curves of average growth in Fig. 1).

Experiment 2, begun April, 1920. Of ten rats, five received the synthetic diet alone, and five had the addition of 2 cc. of milk, fed, as before, in advance of the ration. The latter grew normally for 35 days, then somewhat more slowly, but still vigorously, up to the 60th day, when the experiment was stopped. The former grew slowly for rather more than 20 days, after which their weight rapidly fell off, one dying on the 30th day, and the others soon after. For this experiment I have to thank Miss Killby (average curves of growth in Fig. 2).

Experiment 3, begun May 5, 1920. Of 18 rats, eight were upon the synthetic diet alone, and ten received 2 cc. of milk. This experiment, carried out by myself, almost exactly reproduced the results of Experiment 1. The growth curves of individual rats receiving the milk were nearly superimposable, so uniform was their behaviour (average curves in Fig. 3).

Experiment 4. This was the experiment referred to above in which rats were fed upon goats' milk. In Fig. 4 the upper curve shows the average growth of five animals receiving 5 cc. of milk from the "winter" fed goats, while the lower curve refers to five rats receiving the milk of goats supplied with abundant green stuff.

FIG. 1

FIG. 2

FIG. 3

FIG. 4

REFERENCES.

- Hopkins (1912). *J. Physiol.* **44**, 425.
 Osborne and Mendel (1920). *Biock. J.* **41**, 515.