LXVIII. FEEDING EXPERIMENTS IN CONNECTION WITH VITAMINS A AND B.

III. MILK AND THE GROWTH-PROMOTING VITAMIN.

IV. THE VITAMIN A CONTENT OF REFINED COD-LIVER OIL.

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III.

DURING 1920 Osborne and Mendel [1920] published a paper entitled "Milk as a Source of Water-soluble Vitamin," in which they state that they are unable to produce results similar to those obtained by Hopkins in his classical experiments published in 1912 [Hopkins, 1912].

The administration of 2 cc. of milk per day in these experiments was sufficient to provide the accessory factors for growing rats, but the American authors did not obtain anything approaching normal growth until at least 16 cc. per day were supplied.

Osborne and Mendel's experiments included the use of winter milk (which was probably deficient in vitamins owing to stall-feeding of the cattle) and also milk from grass-fed animals. In each case comparable results were obtained. The authors also considered the possibility of an unsuspected inorganic substance in Hopkins' salt mixture, but experiments showed that this was not the cause of the discrepancy.

Subsequent to this, Hopkins [1920] repeated his experiments and confirmed his previous findings, without, however, being able to throw any light on the discrepancies of the American investigators. He suggests that a seasonal variation in the food value of milk may be a contributing cause, although an experiment upon goats' milk, secreted on winter and summer diets, planned to elucidate this point, failed to yield much evidence. He also suggested that there was a seasonal variation in the growth energy of rats.

In view of these conflicting results, the experiment now to be described may be of some interest, although the circumstances under which it was carried out were not comparable (as regards age of animals) to those in the experiments of Hopkins and of Osborne and Mendel referred to above.

The chief point of difference was that the animals used in the present work were the survivors from a previous experiment which had already

lasted for 101 days, the results of which showed conclusively that the materials tested were almost entirely deficient in vitamin *A*. The average weight of the animals at the commencement of this experimental period was 57 g. and at the termination 80 g.—an increase of only 23 g. as compared with the normal for this period, which is 142.

There were ten of these survivors and their average age when selected for use in this new experiment was 150 days. Eight of them were showing marked signs of keratomalacia and general lack of condition and the other two were also out of condition but not to the same extent.

They received throughout the experiment 2 cc. of cow's milk per animal per day and the only other food supplied was entirely deficient in vitamins and composed as follows:

Basal dietary	...	...	...	85 %
Steam distilled palm-kernel oil	...			15 %

[For method of preparation see Stammers, 1921.] No antineuritic or anti-scorbutic was given, the milk supplied being the only source of these factors available to the animals.

The experiment was carried on for 111 days, and during this period the animals were weighed twice a week, first thing in the morning, before feeding.

It should be stated that the experiment was carried out during the months of December, January, February and March, and the milk used was obtained from stall-fed cows. Butter-fat obtained from the same source during this period showed the effect of stall feeding in giving growth far below the normal (see graph) when used as a control in other experiments, so that the milk may be considered to have been of fairly poor quality as regards its vitamin content.

RESULTS.

As already mentioned, the average weight of ten animals at the commencement of the experiment was 80 g. and their average age was 150 days. In 111 days the increase in average weight was 38 g., *i.e.* 80–118.

The normal increase for this period and age is 43 g., *i.e.* 202–245; hence there is a deficit of only 5 g. in average weight over a period of nearly sixteen weeks.

The animals improved out of all knowledge in general condition, the keratomalacia disappearing and the only difference from normal animals was in their weight and size. Otherwise, they appeared to be in excellent health.

A graph is appended (Fig. 1) which shows

- A*, growth from milk,
- a*, normal for *A*,
- B*, *C*, growth from winter butter-fat,
- b*, *c*, normals for *B*, *C*.

It will be noticed that the butter-fat curves are almost super-imposable.

Fig. 1.

a, b, c, Donaldson's curves of normal growth.
A, growth from milk. B, C, growth from butter-fat.

CONCLUSIONS.

While it is not maintained that this experiment either definitely confirms the results obtained by Hopkins, or disproves the statements of Osborne and Mendel, it seems to throw some additional weight of evidence in favour of the former, although, for the reasons already mentioned, the conditions under which the various experiments were carried out are not comparable.

It is considered, however, that the probabilities are that if an attempt had been made to duplicate Hopkins's experiments by making the conditions similar, the results, even with the sample of winter milk used, would have confirmed the findings of this worker.

If young animals had been used, the initial "growth momentum," as it were, derived from the food consumed prior to weaning, plus the 2 cc. of milk administered daily during the experimental period, would probably have sufficed to give normal growth, having regard to the seasonal variation; in other words the growth from milk would have compared favourably, if nothing more, with that from winter butter-fat.

It may be urged that an adult requires less vitamins to keep it in condition than does a young animal and an explanation of the results of this experiment may be found in this fact: on the other hand, this hypothesis is, in the writer's opinion, only applicable in the case of an animal which has received a normal diet up to the age of maturity and not to animals such as those used in this work, which had been fed on a vitamin-deficient diet since they were seven weeks old.

A further point of interest is now touched upon. When an animal has been fed upon a deficient diet and is suddenly placed upon one containing a greater or less quantity of the growth-promoting vitamin, a considerable stimulus is usually applied by the latter to the growth impulse and this can generally be well seen on the graphic record of the growth. It is quite common for animals in such a case to put on a large increase in weight in the first week, but if this occurs there is usually an almost corresponding drop within the next week or so. It will be noticed that, in this case, the effect of any stimulus applied by the administration of the milk was not reflected in a sudden jump in weight; in fact, during the first week there was a drop and if the curve is smoothed out, it will be seen that it remains almost parallel to the normal.

A possible explanation is that the deficiency disease caused by the previous experiment had suppressed the growth impulse to such an extent that the latter could not immediately respond to the stimulus applied. It is also possible that the greater the age of the animal the greater must be the magnitude of the stimulus to produce this effect, even though this may appear, under certain circumstances, to contradict the axiom that the older an animal is, the less vitamin it requires to keep it in health.

A possible criticism may be applied (owing to the advanced age of the animals used) to the conclusions drawn and therefore attention is invited at this stage to the next paper (IV).

It will be observed that in four of the experiments there described, the animals were also of a mature age (varying from 130 to 160 days) and in three of the experiments had been previously fed upon a diet which had failed to maintain adequate growth, thereby approximating to the conditions under which the present experiments were carried out.

Cod-liver oil is well known to be a rich source of vitamin A, but in spite of this fact its administration failed to raise the weights of the animals concerned to the normal, but the curves ran roughly parallel to that of normal growth (Donaldson) without any marked approach to the latter.

It might have been expected that cod-liver oil would be sufficiently potent to overcome the stagnation of the growth impulse and raise the weights to somewhere near the normal, but this did not take place even in any individual rat.

It therefore seems that age is the important factor and that once an animal has reached a certain age, either (a) the amount of vitamin which would have been adequate to give normal growth in a young animal, is insufficient, or (b) no amount of vitamin can fully restore the growth impulse.

The writer's opinion is that the latter is probably the correct explanation.

A study of these results therefore would seem to indicate that the curve of normal growth remains constant, within limits, for age and not for weight, and, if for any reason the growth impulse has been inhibited, an attempt at restoration of the impulse by however powerful a stimulus cannot succeed to an extent greater than that which would be expected from the age of the animal.

IV.

Six experiments are described in this paper and two varieties of cod-liver oil were used. It has already been established that crude cod-liver oil is exceedingly rich in vitamin A [Zilva and Drummond, 1921]. Of the two varieties used in the experiments now described, one, A, was an ordinary sample procured from a pharmacist and of a type commonly supplied for medicinal purposes; the other, B, was more crude and a brand suitable for administration to cattle. Sample A was used in Exps. 1, 2, 4, 5, and 6, and sample B in Exp. 3.

The animals in Exp. 1 were 160 days old at the commencement and had been fed (for the previous 110 days) upon butter-fat and used as a control in another set of experiments. Their average weight was 148 g. Those in Exp. 2 were also 160 days old and had previously been fed (also for 110 days) upon a partially deficient diet. Their average weight was 130 g. In Exps. 3 and 4 the animals were also of mature age (135 days) and had also been fed (for the previous 90 days) upon a partially deficient diet. Their average weights were 100 and 90 g. respectively.

Exps. 5 and 6 were confined to young animals. In Exp. 5 they were 43 and in Exp. 6, 33 days old. Their average weights were 77 and 49 g. respec-

tively. The experimental technique was that usually adopted in this laboratory and has already been described in this journal [Stammers, 1921].

Exps. 1 and 2 were carried on for 101 days, Exps. 3 and 4 for 73 and Exps. 5 and 6 for 87 days.

Fig. 2.

a, Normal for Expts. 1 and 2. *b*, Normal for Expts. 3 and 4.

Expt. 1 ——— Expt. 2 - - - - Expt. 3 Expt. 4 - . - .

Fig. 3.

a, Normal for Exp. 5. *b*, Normal for Exp. 6.
 - - - - - Expt. 5 (cod-liver oil). ——— Expt. 6 (cod-liver oil).

RESULTS.

The results are given in tabular form and graphs are also appended (Figs. 2 and 3) which show Donaldson's normal curves in addition to the growth recorded.

Exp.	Previous treatment	Age at commencement	Weight at commencement	Weight at conclusion	Actual increase	Normal increase (for age at commencement)
		days	g.	g.	g.	g.
1	Butter-fat	160	148	180	32	37
2	Partially deficient	160	130	191	61	37
3	Partially deficient	135	100	133	33	41
4	Partially deficient	135	91	145	54	41
5	None	43	77	164	87	141
6	None	33	48	156	108	144

Taking the first four experiments, *i.e.* those in which mature animals were used, it will be seen that in Exps. 1 and 3 the growth was subnormal, while in Exps. 2 and 4, although it was supernormal (for age) the growth shown was not nearly sufficient to cause the curves to approximate to that of normal growth—in other words, although cod-liver oil is probably the richest source of vitamin *A* available, its administration in these cases was not attended by any very marked or maintained increase in weight. The less refined sample, used in Exp. 3, which, *ceteris paribus*, would be expected to give the best results, actually proved the least effective.

As regards Exps. 5 and 6 in which young animals just weaned were used, the growth was slightly subnormal in each case although the animals were considerably above normal weight at the commencement of the experiment: the curves in each case fell off gradually and crossed the normal between the seventh and eighth weeks. When the experiments were terminated, however, they were still on the up grade.

SUMMARY AND CONCLUSIONS.

Two samples of cod-liver oil, one more highly refined than the other, were tested upon rats of mature age, some of which had previously been fed on a diet partially deficient in vitamin *A*. The more highly refined oil was also tested on young animals.

In the latter case (Exps. 5 and 6) the growth registered was *slightly* subnormal and, in view of the potency of cod-liver oil as a source of vitamin *A*, expectations were hardly realised. The sample used was, however, highly refined and this may be responsible.

As regards the first four experiments, No. 3 may be considered first. Although the sample used in this case was fairly crude, it failed to restore deficiency animals to normal weight even after a period of 73 days, moreover there was no sharp initial rise in the growth curve as might have been expected.

In the other experiments an initial rise did take place, but after three weeks the curves flattened out and the growth recorded ran roughly parallel to the normal (for rats of that age). Exp. 2 showed the best results, but even after 101 days the animals averaged 54 g. below the normal.

The explanation offered for these results is (*a*) that the growth impulses of the animals concerned in these experiments had, owing to their ages (135 and 160 days), been deprived to a great extent of their power to react to the

stimuli applied even by a fat rich in vitamin A and consequently they failed to show substantially greater growth than the normal for their age, or (b) that the oil had in the process of refinement lost a large part of its original vitamin A.

Point is given to the former theory by the fact that in Exp. 1 the animals had previously been fed on a diet containing butter-fat, which would, in itself, not be expected to impair the growth impulse.

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