LVII. FEEDING EXPERIMENTS IN CONNECTION WITH VITAMINS A AND B:

(I) THE VALUE OF STEAM-DISTILLED PALM KERNEL OIL AS A CONTROL FAT.

(II) WHEAT BRAN AS A SOURCE OF VITAMINS A AND B.

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(I) SOME difficulty has been experienced in the past in obtaining a material, deficient in vitamin A, which can be relied upon to give a minimum of growth and thus furnish a control in feeding experiments upon other products.

It has been found that palm kernel oil, steam-distilled for a period of three or four hours at a temperature of 230° to 260° C., is entirely deprived of any vitamin A which it may originally have possessed. It is probable if not certain that any vegetable oil, if treated in this manner, would give the same results; the writer's experience, however, is confined to palm kernel oil.

Two curves are appended (Fig. 1) which show the growth obtained from this material. It will be observed from these that the total gain in weight recorded was in one case 22 g., and in the other 20 g. This, for all practical purposes, may be regarded as a minimum, and is borne out by other experiments now in progress in the writer's laboratory.

The experiments in each case extended over a period of three months, and 16 animals were involved, only four of which survived until the end of the experiment—these four showing marked signs of deficiency disease.

Donaldson's curves of normal growth are shown on the graph, and lettered *A* and *B* to correspond with the experiment to which they refer.

(II) The objects of the experiments to which this part of the paper relates were:

(a) to decide whether the administration of bran in the daily food of experimental animals would take the place of the antineuritic usually supplied; and

(b) to decide whether the sample used contained vitamin A.

The Medical Research Council's report makes it clear that bran is satisfactory as a source of vitamin B, but it is not so definite as regards vitamin A. On page 22, it is observed that an inconclusive result was obtained, while on page 102 a definite indication is given that bran is deficient in vitamin A.

Fig. 1.

A, B. Donaldson's curves of normal growth.

a, b. Growth from steam-distilled palm kernel oil.

The experiments now to be described took place during the winter and the butter control which was used was therefore somewhat unsatisfactory; however, for purposes of comparison the results may be of interest, inasmuch as they bear out the hypothesis that bran does contain vitamin A and are mutually supporting in this respect.

TECHNIQUE.

Twenty-four animals (rats) were used in all. Three sets of eight were fed upon:

1. Basal diet, steam-distilled palm kernel oil and wheat bran.
2. Basal diet, butter fat and wheat bran.
3. Basal diet and butter fat.

The bran used was an ordinary sample, obtained locally. In 1 and 2 no anti-neuritic was given, but 3 received the usual allowance.

The basal dietary used in this laboratory is as follows:

Purified caseinogen ...	26 %
Starch	54 %
Cane sugar	15 %
Salts	5 %

Ordinary commercial casein is used and treated by being spread out in thin layers in a hot-air chamber for some hours, subsequently being extracted with hot alcohol.

The salt mixture is based on McCollum's formula, with extensions suggested by Hopkins. It consists of:

NaCl	46.25
MgSO ₄	71.20
NaH ₂ PO ₄	92.68
K ₂ HPO ₄	254.60
CaH ₄ (PO ₄) ₂ .H ₂ O ...	144.20
Ca lactate	347.00
Ferric citrate	31.52
Sodium fluoride	55
Manganese sulphate ...	2.00
Potassium iodide	10.00
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	1000.00

Before feeding 85 parts of basal diet are taken and 15 parts of fat (appropriate to the experiment) are rubbed into the mixture. To this is added the antineuritic, which is prepared from marmite, in the following manner:

The crude marmite (250 g.) is shaken with 2 litres of 80 % alcohol for two hours on a shaker. The liquid is then decanted and filtered into a distilling flask, and the alcohol evaporated off under reduced pressure. The resulting fluid is filtered and made up to 250 cc. with distilled water.

Each animal receives 2 cc. of this per day. 1 cc. of lemon juice per day is also given, not that it is required for antiscorbutic purposes, but because the animals appear to grow better if this forms a constituent of the diet.

The animals receiving bran were given 2 g. each per day, and suitable allowance for the composition of this was made when preparing the food.

Fig. 2.

a. Bran + butter fat.
c. Bran + palm kernel oil.

b. Butter fat.
x. Donaldson's curve of normal growth.

Each animal receives, nominally, $1\frac{1}{2}$ g. of fat per day, with $8\frac{1}{2}$ g. of basal diet, but in practice it is given as much as it will eat, and it is endeavoured to give just such an amount as will cause a small quantity to be left. It is found that the actual amount eaten varies from 8 to 12 g. per day.

The animals were weighed twice a week, first thing in the morning before feeding and the experiment lasted for 101 days (from Oct. 19th 1920 to Jan. 28th 1921).

RESULTS.

The growth curves are shown in the graph appended (Fig. 2). It will be seen that the average weight at the commencement was between 45 and 47 g. At the conclusion, the combination of bran and butter fat averaged 133 g., butter fat alone 120.5 g., and bran and palm kernel oil 112 g.

It will be seen, by reference to the curve of normal growth (Donaldson), which has been included in the graph, that the growth recorded was far below the normal. The heaviest animal at the conclusion of the experiment weighed only 150 g., whereas the normal for the age reached is 210 g.

None of the animals fed upon bran and palm kernel oil exhibited any traces of deficiency disease, and hence it appears certain that the sample of bran used contained sufficient vitamin A to give adequate resistance to disease if not to give normal growth. The palm kernel oil, being steam distilled, for four hours at 230° C., was beyond suspicion.

The curve denoting butter fat alone is a fair sample of those obtained in this laboratory during the winter and is probably, if not certainly, due to the butter being manufactured from cream obtained from stall-fed cows. Evidence is to hand that this was the case.

Throughout the experiment no trouble was experienced of such a kind as to indicate that the antineuritic properties of the bran were not adequate.

SUMMARY.

Experiments made upon rats fed with mixtures of bran and butter fat and bran and palm kernel oil (steam-distilled) show that the sample of bran used undoubtedly contained vitamin A, the growth from the latter mixture being little inferior to that from butter fat.

The animals fed upon bran and palm kernel oil displayed no signs of deficiency disease although their growth was subnormal.

The butter fat control animals also showed a markedly subnormal growth and this is probably due to the cream from which the butter was made being obtained from stall-fed cows.

The bran and butter fat animals support the view that bran contains vitamin A by exhibiting a better growth than those fed on butter fat alone.

Throughout the experiment there were no symptoms which would give rise to the opinion that the antineuritic properties of the bran were inadequate.