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

(V) ORANGE JUICE AS A SOURCE OF VITAMIN B.

(VI) OPHTHALMIA IN RATS AFFECTED WITH AVITAMINOSIS.

(VII) THE VITAMIN CONTENT OF COD-LIVER OIL
AND MALT EXTRACT.

By ARTHUR DIGHTON STAMMERS.

From the Nutrition Laboratory, Port Sunlight, Cheshire.

(Received August 13th, 1923.)

V.

OSBORNE AND MENDEL [1920] found that the fresh juice of the orange contained vitamin B in an amount equivalent to that in an equal volume of milk. The experiment to which the present paper relates was carried out with a view to confirming this finding and rats of somewhat mature age were used. Four males and four females, averaging 150 g. in weight and 112 days in age, were fed upon the usual basal dietary with the addition of butter. The amount of the latter was such that each animal received the equivalent of 1.5 g. of butter fat per day. In addition, each animal received 10 cc. of orange juice per day.

The technique of feeding, weighing, etc., was as previously described [Stammers, 1921] and the experiment was carried on for 91 days.

As a control, a comparable set of animals of the same age and average weight was fed upon basal diet plus butter, to which the usual allowance of antineuritic (2 cc. marmite extract) was added. In neither case did the animals receive any lemon juice, which is the usual antiscorbutic supplied, so that the orange juice animals had the advantage over the controls in respect of the antiscorbutic contained in the orange juice.

The average weight of all the animals was about 15 g. subnormal for their age and in the case of the orange juice experiment a progressive decline in weight was observed until the 45th day when the curve of growth began to rise. This rise proved to be only temporary and at the conclusion of the experiment the average weight was only 125 g., the normal for this age being 223 g. These animals had lost 25 g. over the period of the experiment. The

controls also showed a loss of weight at the beginning and this also proved to be temporary, for, after the 14th day they began to gain and continued to do so until the conclusion of the experiment when the average weight was 183 g. They thus gained 33 g. during the course of the experiment.

A graph is appended (Fig. 1) showing the growth recorded in each case and Donaldson's normal curve for the age of the animals under observation.

SUMMARY AND CONCLUSIONS.

Eight rats, each supplied with 10 cc. per day of orange juice in addition to the usual basal ration plus butter, failed to maintain their initial weight, whereas eight others given 2 cc. of marmite extract in addition to the basal ration plus butter exhibited growth which, while not normal, yet indicated that the vitamins in the diet supplied were adequate.

The source of vitamin A in the diet was the same in both cases so that it appears that the marmite extract in the latter was the factor concerned in the superior growth shown by the control animals. It is pointed out that, whereas the controls got no antiscorbutic, the orange juice animals derived vitamin C from the fruit juice.

Looking upon orange juice, therefore, merely as a source of vitamin B, it appears that 10 cc. of this per day are not sufficient to promote adequate

growth even though there is a sufficiency of vitamin A in the diet. This quantity, however, appears to be protective against the disease associated with deficiency of vitamin B, no symptoms having been noticed during the course of the experiment, and the animals, apart from their loss of weight, appearing healthy.

VI.

Xerophthalmia or keratomalacia has been observed to develop in rats as a result of deprivation of vitamin A. It is not however maintained that this is the sole cause of the disease since it has been noted as a result of unsatisfactory relations in the inorganic portion of the diet [McCollum, Simmonds and Becker, 1922]. It has been well known for some time that a primary symptom of malnutrition among children is an ophthalmia, due to lowered resistance, which offers a focus for bacterial invasion at one of the weakest points in the body.

Histologically, it has been shown that the condition, among rats fed on a diet deficient in vitamin A, results from a degeneration of corneal tissue caused by bacterial invasion, but that this only occurs after a prolonged course of the deficient diet [Stephenson and Clark, 1920]. Yudkin and Lambert [1922] find that the eyelid is primarily affected and this is also the experience of the author. Further, they observe a degeneration of the epidermis with cellular infiltration, which, in some cases, extends into the subepidermal tissue. Osborne and Mendel [1921] have shown that, in experiments on a thousand unselected rats, xerophthalmia was only seen in animals supplied with food deficient in fat-soluble vitamin; amongst the latter—the number of animals involved is not stated—the incidence was 50 % and most of the cases developed characteristic symptoms before any marked decrease in body weight had occurred. The administration of a small amount of vitamin A, *e.g.* 0.1 g. of butter fat per animal per day caused a great improvement in, if not the disappearance of, the eye disorder, even though, in some cases, the animal subsequently died.

Another worker [Emmett, 1920] had previously described experiments on 122 rats, 120 of which, or 98 %, developed xerophthalmia.

In view of the discrepancy in the results obtained by these workers, the following details, collected by the author during the course of thirteen experiments upon the vitamin content of various oils and fats, are presented in the hope that they may be of interest. One hundred animals were involved in these experiments: 44 were fed with a fat known to have been deprived of vitamin A by steam-distillation [Stammers, 1921] and the remaining 56 upon fats with varying degrees of deficiency of this vitamin. Of the 44 on the totally deficient diet, 40 were young animals (33–49 days old) at the commencement of the experiments and of these 35 developed keratomalacia between the 31st and 80th days of the experiment. The remaining four were adults (145 days old) when they were used for experiment and they all developed the condition between the 104th and 129th days of the investigation.

Those animals, 56 in number, which were fed upon the partially deficient diets were not so susceptible, 24 only being affected and these between the 35th and 98th days.

From an examination of the appended table, which gives details of the experiments so far as they relate to the incidence of keratomalacia, the following deductions may be made.

Type of diet	No. of animals in expt.	Average weight and age at commencement	Average weight and age at conclusion	Days of expt. on which keratomalacia appeared	Total affected	Days of expt. on which animals died	Total mortality
1. Totally deficient	8	77 g. 42 days	82 g. 112 days	35, 45, 49, 52 (2), 56 (3)	8	56, 59, 63 (2), 66 (2), 70 (2)	8
2. " "	8	48 g. 33 days	86 g. 124 days	31, 38, 42 (2), 45, 63	6	52, 56 (2), 66, 70, 73, 80	7
3. " "	8	58 g. 36 days	70 g. 127 days	38, 42, 45, 49, 59, 66	6	49, 59, 63, 73 (2), 84	6
4. " "	8	60 g. 49 days	65 g. 150 days	31, 35, 42, 52, 66, 70, 77 (2)	8	63, 80, 87, 101	4
5. " "	8	55 g. 35 days	53 g. 136 days	35, 42, 45, 59, 66, 73, 80	7	66 (2), 84, 87	4
6. " (adults)	4	214 g. 145 days	174 g. 297 days	104, 108, 122, 129	4	143, 150	2
Total in experiments	44			Total affected	39	Total mortality	31
7. Partially deficient	8	77 g. 42 days	94 g. 129 days	77	1	—	0
8. " "	8	48 g. 33 days	104 g. 124 days	73, 84 (2)	3	—	0
9. " "	8	44 g. 43 days	112 g. 144 days	—	0	—	0
10. " "	8	53 g. 49 days	74 g. 150 days	35, 49, 56, 63, 66, 80, 98	7	70, 84, 91 (2), 94	5
11. " "	8	54 g. 49 days	61 g. 150 days	66, 77, 80, 94	4	63, 87, 98	3
12. " "	8	59 g. 45 days	79 g. 146 days	45, 52, 66, 73, 80	5	87, 98	2
13. " "	8	59 g. 45 days	90 g. 146 days	38, 63, 73, 77	4	84, 98	2
Total in experiments	56			Total affected	24	Total mortality	12

The figures in brackets refer to the number of animals.

In the totally deficient group, *i.e.* in the first six experiments, ophthalmic symptoms supervened in 88.6 % of the animals under observation, two-thirds of the cases appearing *before* the 60th day of the experiment, except in the case of the adult animals. In the other group, *i.e.* those on the partially deficient diets, and detailed in the last seven experiments, 42.8 % were affected and two-thirds of the cases appeared *after* the 60th day.

It therefore appears that the susceptibility of the animals and the time of incidence of the disease vary directly with the amount of vitamin A in the fat under investigation, and further that the first factor (susceptibility) is modified by the age of the animal. Thus, adult animals are less susceptible to a deficiency of vitamin A in the diet than are young animals, since the symptoms of the disease were not exhibited in the former until the period between the 104th and the 129th days, but, nevertheless, all four animals were affected and two subsequently died, during the progress of the experiment.

Further, it is interesting to notice the mortality among the two groups. In the first, 31 of the 39 affected animals died and in the second, 12 out of 24. These deaths all occurred during the course of the experiments. No further records were kept of those animals which survived until after the termination of the experiments, so that it is possible that the mortality may have been higher still.

Attention is also invited to the fact that, in the partially deficient group, most deaths occurred among the animals which were provided with the least amount of vitamin A in the diet as evidenced by the growth shown. Thus five deaths occurred in Exp. 10. The average gain in weight in this experiment was only 21 g. over a period of 101 days. Three deaths occurred in Exp. 11. The average gain in weight here was 7 g. over a period of 101 days.

There is one exception to this, viz. Exp. 7, where the average gain over the experimental period of 87 days was only 17 g., but only one animal was affected.

In this latter and in the other experiments on those fats in which a fair quantity of vitamin A was present, no deaths took place and only four cases of ophthalmia, all occurring late in the experimental period, supervened among the 24 animals involved.

VII.

Both cod-liver oil and malt extract have been shown to vary in vitamin content. The following details of experiments carried out upon these products, therefore, may be of interest.

An ordinary commercial sample of combined oil and malt extract was used in conjunction with the basal dietary and suitable allowance was made for the carbohydrate portion of the malt in preparing the food, so that the proper proportions of the dietary constituents should be maintained. The technique of feeding, dosage, weighing, etc., was as previously described in this Journal [Stammers, 1921]. Thus, each animal received about 1.5 g. of fat per day.

Two experiments were undertaken, one (*a*) comparing basal diet plus cod-liver oil and malt extract with basal diet plus a compound consisting of cod-liver oil and malt to which was added a certain quantity of an emulsion of sterilised baker's yeast and some neutralised lemon juice. This is spoken of as "compound V." The other experiment (*b*) is a comparison of basal diet plus compound V with basal diet plus steam-distilled palm-kernel oil (*i.e.* a diet deprived of vitamins).

Sixteen animals were used in each test, eight for each preparation and the technique was as previously described.

The first experiment consisted of two sets of eight rats fed upon (*a*) basal diet plus compound V and (*b*) basal diet plus cod-liver oil and malt. No controls were used, as it was merely desired to make a comparison between the two preparations. The animals, in this case, averaged 48 g. at the beginning of the experiment and were 36 days old. The test was continued for

91 days and, during this time, the compound V animals gained 141 g. and the cod-liver oil and malt animals 105 g. The normal gain for this period and age is 146 g.

In the second experiment, the animals used were 45 days old at the commencement, but they were exceptionally well grown and averaged 79 g. in weight. This test was carried on for 70 days and, during this time, the gain in weight was 146 g. The normal gain over this period for animals of this age is 123 g. The animals deprived of vitamins gained slightly during the first ten days, but afterwards they lost weight rapidly and at the 31st day had all succumbed.

The accompanying graphs (Fig. 2) show the growth recorded and Donaldson's normal curves corresponding to the ages of the animals used.

Fig. 2.

SUMMARY AND CONCLUSIONS.

Cod-liver oil and malt extract gave very satisfactory growth which was improved by the addition of yeast and lemon juice. It appears certain that "cod-liver oil and malt extract" contain sufficient vitamins A and B to provide the necessary stimulus to growth when the animals receive 1.5 g. of oil per day. (This represents about 8 g. of malt in the combined extract, which was substituted, in making up the dietary, for the starch and sugar usually supplied.)

If further supplies of vitamins B and C are added, in the forms of yeast and lemon juice respectively, better growth still is recorded. It is concluded that this may be due to the increase in vitamin B supplied by the yeast.

I am indebted to Messrs Lever Brothers, Limited, for permission to publish these researches.

REFERENCES.

- Emmett (1920). *Science*, **52**, 157.
McCollum, Simmonds and Becker (1922). *J. Biol. Chem.* **53**, 313.
Osborne and Mendel (1920). *J. Biol. Chem.* **42**, 465.
—— — (1921). *J. Amer. Med. Assoc.* **76**, 905.
Stammers (1921). *Biochem. J.* **15**, 489, 491.
Stephenson and Clark (1920). *Biochem. J.* **14**, 502.
Yudkin and Lambert (1922). *Proc. Soc. Exp. Biol. Med.* **19**, 375.