LXXIX. SOME EVIDENCE OF THE EXISTENCE OF A FURTHER FACTOR NECESSARY FOR GROWTH OF THE RAT.

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THE experiments described in this paper were designed to ascertain how far the vitamin A-free diet in use in this laboratory could, when supplemented by cod-liver oil, support normal growth. In the early work of this department (1927) there had been nothing in the behaviour of the rats used in assaying cod-liver oil to suggest any deficiency in the diet other than vitamin A, for the addition of this substance in a dose of 0.01 g. daily caused resumption of rapid growth in the test rats for a period of 9 weeks, after which the test was discontinued (Fig. 1). It was several months later that a

Fig. 1. A test for vitamin A in cod-liver oil. Rats given "vitamin-free casein" (Glaxo) throughout the experiment. Evidence that some rats, at least, can grow well when this casein is the source of protein.

deficiency other than vitamin A was suspected in the vitamin A-free diet and possibly also, though to a smaller extent, in the diet of the stock colony.

The history of the attempt to breed a colony of rats on some diet that should be uniform throughout the year may form the subject of a separate communication. The result of the first stage of it may be summarised as a progressive deterioration of the litters obtained from the imported mature animals, both in respect of their rate of growth when allowed to grow to maturity for breeding and later in their failure to rear the young they had borne. A progressive decrease in the periods of time required for the young

rats to become steady in weight when given the vitamin A-free diet, and a lower weight at which they ceased growth was also noticeable. The whole of these results could, so far, be attributed to a low vitamin A content in the diet of the stock rats with the consequent low reserves of this factor in the rats used for vitamin A assays. It became evident, however, that the responses to doses of cod-liver oil given by these particular rats were definitely less than the responses obtained in rats of different history. Moreover, increasing the dose of vitamin A gradually to a very high dose (0.2 g. daily) of a sample of cod-liver oil (which in other rats caused resumption of good growth in a dose of 0.01 g.) failed to bring about any increased rate of growth. It was evident that the diet was lacking in some factor other than vitamin A.

Coward, in 1926, discussed with Prof. Palmer his work on the growth-promoting powers of different samples of caseinogen prepared in different ways, and it appeared at once possible that the rats in the experiments described in this paper were suffering from a deficiency such as Palmer had noted in his work. The first substance examined for the unknown factor was therefore a fresh kind of caseinogen. The British Drug Houses "light-white casein" was substituted for the Glaxo "vitamin-free casein" of the diet previously used. The response was immediate and dramatic (Fig. 2). The rats grew rapidly and gave no further evidence of any deficiency in their diet. Although Palmer's work has finally indicated that his rats were suffering from a shortage of the vitamin B₂ [Palmer and Kennedy, 1927, 1, 2; 1928, 1, 2], yet further work in this laboratory has shown that the "light-white casein" (B.D.H.) contains neither B₁ nor B₂, but some growth-promoting property which is independent of both of these. The same factor has been found in various natural foodstuffs. It is at least partially destroyed by heat; it is extracted slightly from the casein by hot alcohol and by hot ether after cold alcohol. It seems to be more easily extracted by hot ether from wheat embryo. It is not vitamin E.

TECHNIQUE.

Rats from various colonies were used in the early tests carried out in this department. That they varied greatly in their reserves of vitamin A was evident from their varied behaviour when given the vitamin A-free diet of this laboratory. Litters were kept intact and members of one litter were, in general, exhausted of their reserves of vitamin A within a very few days of each other. There were much greater differences in vitamin A reserves between different litters than between different members of any one litter.

The vitamin A-free diet consisted of:

Dextrinised rice starch	...	...	...	71 %
"Vitamin-free casein" (Glaxo)	...	...	...	15
Agar-agar	...	...	...	2
Dried yeast	...	...	...	8
Salt mixture (Steenbock's)	...	...	...	4

The mixture was irradiated in very thin layers (200 g. over an area of 4 sq. ft.) in zinc trays for 30 minutes at a distance of 2 ft. from a quartz mercury vapour lamp, running at 2.5 amp., 130 v. It was stirred and re-spread after the first 15 minutes of irradiation. This method has been shown by Steenbock and Coward [1927] to generate ample supplies of vitamin D; and that it is also adequate with the materials available in this laboratory has been shown from time to time by experiment. Rats which had ceased growth on this diet have been given large daily doses of irradiated ergosterol and have not resumed growth.

Fig. 2. A test for vitamin A. Rats given "vitamin-free casein" (Glaxo) at first. Growth ceased in spite of the doses of cod-liver oil being greatly increased and in spite of the addition of watercress. Growth was only resumed when "light-white casein" (B.D.H.) was substituted for the "vitamin-free casein."

The rice starch was partially dextrinised by mixing it with enough water to make it firm, and then heating it in shallow trays in an ordinary gas cooking stove for 6 hours, which renders it crisp and easily ground. The dextrinisation of rice starch does not seem to be necessary for making it more easily digested, as with the corn starch used in American laboratories. Dextrinisation is necessary, however, to get the starch into a less fine state of division, for in

its original state the rats collect a great deal of it in their fur and the whole experiment is less clean than when dextrinised and ground starch is used.

The "vitamin-free casein" supplied by the Glaxo Company was guaranteed substantially free from vitamin A by tests which were until recently supervised by the Accessory Food Factors Committee of the Medical Research Council and Lister Institute.

Agar-agar was incorporated in the diet as a preventive of constipation, although, in view of the large percentage of dried yeast also used, it is very doubtful whether it was necessary. Sometimes the powdered form of agar-agar was used, sometimes the shredded. When the latter was used it was eaten as readily as the rest of the food. It was not washed or otherwise purified before use.

The dried yeast used in this diet was brewer's yeast. Every sample purchased for use in this laboratory is tested for its vitamin B complex and there is ample evidence that the inclusion of 8 % of it in the vitamin A-free diet is abundant for the vitamin B needs of the rat at all stages of growth. The evidence rests on the ability of rats, from some colonies at least, to grow rapidly (bucks to more than 200 g., does to about 180 g.) when this is the only source of the vitamin B complex in their diet.

The rats were kept on screens to prevent coprophagy only when they were being used for a vitamin B test. Otherwise they were kept on shavings or sawdust.

The evidence for the existence of a growth-promoting factor other than the vitamins A, B₁ and B₂, D and E in "light-white casein" (B.D.H.).

The general scheme of the experiments which led to the detection of the absence of this factor from the "vitamin-free casein" of the vitamin A-free diet and of its presence in the particular sample of untreated (*i.e.* "light-white," B.D.H.) casein examined later, was as follows. In addition to those experiments with rats which had failed to give the expected response to doses of cod-liver oil, others were performed with rats which had given a certain amount of response under the same condition. In the latter experiments the vitamin A tests of the samples of cod-liver oil were carried on for much longer periods than that of 5-6 weeks usually considered necessary for determining the potency of the oil. In general, the rats ceased to grow and would not resume growth on being given larger and larger doses of cod-liver oil, or if growth were resumed it was only for a short time. Watercress, as an additional and different source of vitamin A, was then tried on some of these rats. A dose of 0.5 g. of it caused no resumption of growth, although an independent test [Coward and Eggleton, 1928] had shown that in the normal way of testing, when the rats were only about 10 weeks old, a dose of 0.1 g. had been enough to restore growth. Unlimited supplies of watercress were then given to the rats which had failed to grow on 0.5 g. of it, and although growth was resumed for a time, it was only temporary. Then "light-white casein"

The distribution of the factor, so far studied, may be summarised thus:

Table I.

Substance	Dose tested	No. of rats used	Results
1. "Light-white casein" (B.D.H.) untreated, 4 different samples	15 % of diet	40	Rapid growth in all but 3 rats, slow growth in these
	10 % of diet	4	Good growth but less rapid than when 15 % was given
	5 % of diet	4	No growth
2. "Crude casein" (B.D.H.)	15 % of diet	4	1 died. 3 maintained weight for 75, 49, 30 days respectively*
3. Dairy butter	0.5 g. daily	3	Maintained weight for 34 days
4. Fresh milk (London supply)	10 cc. daily	4	Rapid growth in all
5. Milk boiled for 15 mins.	10 cc. daily (= 15 cc. fresh)	4	Moderate growth in all
6. Lettuce—fresh	1 g. daily	7	Maintained weight in 5 for 7–30 days. Fair growth in 2
	<i>Ad lib.</i> , often 40–50 g. per rat per day	7, the same animals that had 1 g.	Good growth
7. Etiolated wheat shoots, 10 days old (grown under laboratory conditions)	15 g. daily	3	Slight growth
8. Dried grass clippings	5 % of diet	3	Rapid growth in 2 rats. Slow growth in 1 rat increased by raising amount to 10 %
9. Fresh grass clippings	About 10 g. daily	2	Rapid growth
10. Extra dried yeast	10 % of diet	14	No influence*
11. Marmite	0.5 g. daily	13	Slight temporary increase in weight in 5. Maintained weight in others†
12. Autoclaved marmite	1.0 g. daily	3	No influence†
13. Watercress	<i>Ad lib.</i> , about 10 g. daily	3	Slight increase†
14. Ox muscle	1 g. daily	4	Good growth
	<i>Ad lib.</i> , 15– 20 g. daily	4	Very rapid growth
Ox liver	1 g. daily	4	Very rapid growth
15. Wheat embryo	5 %	3	Rapid growth in 2. Weight maintained in 1
	10 %	3	Rapid growth
16. Wheat germ oil, sample 0	Up to 0.08 g.	6	No influence†
	Up to 0.16 g.	4	1 maintained weight. 2 fair growth. 1 died
Wheat germ oil, sample 1	Up to 0.02 g.	3	No influence
	Up to 0.12 g.	3	No influence in 2. Fair growth in 1
Wheat germ oil, sample 2	0.12 g. daily	4	Fair growth in 3. Maintained weight in 1

* One of these rats was finally given "light-white casein" to which it responded with rapid growth.

† Two of these rats were finally given "light-white casein" to which they responded with rapid growth.

‡ One of these rats was finally given 10 % wheat germ to which it responded with rapid growth.

Similar tests were made with (a) liquid paraffin (0.06 g. daily for 21 days) and (b) 3 % extra agar-agar in the diet to see whether the active substances had merely been exerting an influence on peristalsis. Each substance was without effect on the growth of the test animals.

The palatability of the two diets containing the "Glaxo casein" and the "light-white casein" respectively.

A simple experiment was carried out to see whether possibly greater palatability of the "light-white casein" diet could account for the observed results. Six young rats weighing about 60 g. were divided into two groups of 3 each. One group was given the vitamin A-free diet containing the "vitamin-free casein" (Glaxo) and the other the diet containing "light-white casein." All the rats were given 0.02 g. of a good sample of cod-liver oil daily to make the same rate of growth possible in all of them. Records of food consumed each day were taken at 9 o'clock in the morning in order to get figures as accurate as possible. After 2 days the diets of the two groups were interchanged. This was repeated 5 times in all and then each group was kept to one diet for a period of 5 days, and the diets were again interchanged for a further period of 5 days. The amounts consumed in g. are given in Table II.

Table II.

Rat ...	M 347		348		349		350		351		352	
Casein in diet	G.	L.-W.	G.	L.-W.	G.	L.-W.	G.	L.-W.	G.	L.-W.	G.	L.-W.
27. iii. 28	7	—	5	—	7	—	7	—	3	—	3	—
28	6	—	7	—	5	—	7	—	5	—	5	—
29	—	7	—	5	—	7	9	—	7	—	8	—
30	—	7	—	5	—	7	9	—	7	—	8	—
31 (2 days)	13	—	14	—	17	—	19	—	16	—	17	—
2. iv. 28	—	5	—	2	—	8	10	—	8	—	9	—
3	—	5	—	2	—	7	10	—	8	—	8	—
4	5	—	6	—	9	—	9	—	9	—	8	—
5	6	—	6	—	9	—	9	—	8	—	9	—
6	—	5	—	7	—	8	11	—	9	—	11	—
7 (2 days)	—	10	—	10	—	12	14	—	14	—	14	—
9	—	2	—	4	—	(Spoilt) 12?	4	—	4	—	4	—
10 (2 days)	—	15	—	20	—	20	20	—	21	—	19	—
12	6	—	9	—	8	—	10	—	9	—	9	—
13	7	—	9	—	11	—	12	—	10	—	10	—
14 (2 days)	12	—	17	—	22	—	28	—	22	—	27	—
16	8	—	10	—	11	—	12	—	10	—	11	—
17	—	8	—	10	—	14	14	—	12	—	11	—
Total for 11 days	70	64	83	65	99	95	101	113	90	92	92	99

An examination of this table shows that

(a) there is no significant difference between the total amounts of the two diets eaten by any one rat during the experiment, and that

(b) there are practically no instances of a greater consumption of the "light-white casein" diet on the first day of receiving it than of the diet containing the "Glaxo casein." The evidence shows rather the reverse of this.

Hence the possibility of these results being due to the greater palatability of the "light-white casein" diet must be discarded.

Effect of heat on the growth-promoting power of "light-white casein."

I. "Light-white casein" was heated in very thin layers in open trays in an electric oven (300 g. over 8 sq. ft.) for 24 hours at 105°. It was raked over twice during this time. It was then given to rats (5) as 15 % of their diet. They grew as well as the rats receiving 15 % of untreated "light-white casein" and it was evident that there had been no appreciable diminution in the potency of the casein.

II. "Light-white casein" was heated as above for 7 days, and then tested in the same way. Again the response was good but definitely less than before.

III. Milk which had been simmered for 15 minutes in an open pan lost a great part, though not all, of its activity.

Attempts to extract the growth-promoting substance.

I. *From "light-white casein."*

Extracts of the "light-white casein" made with (a) cold 97 % alcohol (4 times), (b) cold ether (4 times) after cold alcohol (once) and evaporation *in vacuo* both failed to cause any resumption of growth, while the casein so extracted showed no impairment of its growth-promoting powers. Similar results were obtained with a 2 % acetic acid extract, neutralised with potassium hydroxide and evaporated on a water-bath, and with the casein thus extracted.

Rather more active extracts were obtained by the use of (a) hot alcohol (4 extractions of 6 hours each) and of (b) hot ether after drying overnight (4 extractions of 6 hours each). The residual casein showed somewhat impaired activity. The extracts described above were all tested in doses equivalent to the amount of casein eaten per rat per day. Slight activity was also shown by an ether extract of a solution of casein in sodium carbonate, which was tested in a dose equivalent to 5 times the daily intake of casein. The growth-promoting factor is therefore not easily removed from the casein by the usual fat solvents.

II. *From wheat embryo.*

(a) *Hot 90 % alcohol.* Extracting wheat embryo with 90 % alcohol 4 times by boiling for 6 hours each time removes the active factor almost completely and gives an active extract. The yield of this extract was about 30 % (95 g. and 87 g. respectively from two portions of 300 g. each). When given to two rats in doses of 0.05 g. daily (equivalent to 0.15 g. wheat embryo), there was no response. When given in larger doses (1 g. and later 2 g.), one rat resumed rapid growth immediately and another after a period of about 30 days. Another, having remained steady on about 1 g. per day for 80 days,

resumed rapid growth when the amount was doubled. Three rats given the extracted wheat embryo grew very slowly for about 60 days but resumed rapid growth immediately non-extracted wheat embryo was substituted for the extracted product (Fig. 4).

Fig. 4. A 90 % alcohol extract of wheat embryo. Definite activity of the extract and impairment of the activity of the wheat embryo.

(b) *Hot ether.* Wheat embryo was dried overnight in an oven at 105° and extracted 4 times with boiling ether. The ether was distilled off at atmospheric pressure. The extract was decidedly active in a dose of 0.16 g. and more so in one of 0.24 g. The extracted wheat embryo was definitely less active than the non-extracted product (Fig. 5).

Evidence from vitamin B testing of the existence of a growth-promoting factor in "light-white casein" (B.D.H.).

In view of the slight temporary response to extra doses of vitamin B in the form of marmite, which was occasionally experienced in the experiments

already described, "light-white casein" (B.D.H.) was tested first for the whole B complex, then for B₁ and B₂ independently. It was found to contain neither of these but to contain a third factor essential for growth and independent of B₁ and B₂.

Technique. The diet on which rats have been prepared for vitamin B testing in this laboratory (until a few months ago) was composed of:

Dextrinised rice starch	...	...	...	79 %
"Vitamin-free casein" (Glaxo)	...	...	...	15
Agar-agar	...	...	...	2
Salt mixture (Steenbock's)	...	...	...	4

Fig. 5. A hot ether extract of wheat embryo. Some activity of the extract and slight impairment of the activity of the wheat embryo.

Each rat was given separately 5-6 drops of a good sample of cod-liver oil twice a week. In 10-14 days on this diet, the rats became steady in weight or declined.

To test "light-white casein" for the full vitamin B complex, it was substituted for the "Glaxo casein" (15 %) in the diet of 5 rats prepared as just

described. None of the animals showed any tendency to resume growth. They all declined in weight steadily and died in 10–20 days. The “light-white casein” was therefore considered to be devoid of the vitamin B complex.

To test “light-white casein” for B_1 and B_2 independently of each other, 8 rats were prepared on the diet described above. The scheme of the experiment was to determine whether “light-white casein” could produce growth if the rats were given in addition (a) excess of B_1 , (b) excess of B_2 , (c) excess of both B_1 and B_2 .

The B_1 preparation used in this experiment was very kindly given to the authors by Prof. J. C. Drummond, who had found that the dose required to make a rat grow in the presence of ample supplies of B_2 was about four times the amount required to cure a pigeon of polyneuritis. The pigeon dose of this particular preparation was 0.1 cc. (confirmed in our own laboratories) and in order to ensure excessive doses of B_1 , 0.8 cc. was used for each rat.

The B_2 preparation was also supplied by Prof. Drummond. It was made by autoclaving marmite (decidedly alkaline) at 125° at 15 lbs. pressure for 3 hours. It was then made very slightly acid with HCl and the volume made up so that 1 cc. was equivalent to 0.4 g. of the original marmite.

The 8 rats were divided into four groups and given the following doses:

Group I.	0.8 cc. B_1 preparation per rat per day.
	1.0 cc. B_2 „ „
Group II.	0.8 cc. B_1 preparation per rat per day.
	“Light-white casein” in place of “vitamin-free casein.”
Group III.	1.0 cc. B_2 preparation.
	“Light-white casein” in place of “vitamin-free casein.”
Group IV.	0.8 cc. B_1 preparation.
	1.0 cc. B_2 „ „
	“Light-white casein” in place of “vitamin-free casein.”

After 16 days, the dose of B_2 was doubled, as several workers have declared lately that the vitamin B potency of marmite is somewhat less than formerly.

The result is seen in Fig. 6. Even the high doses of B_1 and B_2 have failed to produce normal growth in the animals of Group I. The animals of Group II have grown considerably less than those of Group I, thus showing that “light-white casein” supplies no appreciable quantity of B_2 . The animals of Group III died sooner than did those of Group II, thus showing that “light-white casein” does not contain B_1 . The animals of Group IV resumed rapid and prolonged growth till the experiment was terminated at the end of 8 weeks. It is therefore evident that “light-white casein” contains some factor necessary for growth which is not one of the vitamins A, B_1 , B_2 or D.

Two questions then presented themselves.

(a) Why was any growth obtained either by ourselves or by other workers when rats, prepared as these, were given preparations of B_1 and B_2 ?

(b) Is the factor here demonstrated the same one as was demonstrated by the experiments previously described in this paper?

The first question can apparently be answered (a) by assuming the rat's ability to store an unknown factor such as is contained in the "light-white casein" or (b) by assuming the presence of this growth-promoting factor in the vitamin A-free diet, but in a much smaller degree than in the "light-white casein."

The second question can at present only be answered by repeating the tests on the substances used in the first set of experiments (vitamin A technique), using now the technique described immediately above; *i.e.* testing each substance by the vitamin B technique in the presence of excess doses of B_1 and B_2 .

Fig. 6. To demonstrate the presence of a growth-promoting factor in "light-white casein" which is not in the "vitamin-free casein" and which is neither vitamin B_1 nor B_2 .

Two experiments on this latter point have been carried out.

In the first experiment, 4 rats were prepared on the "B" diet containing the "vitamin-free casein" (Glaxo). After 11 days, they were all given daily doses of 2 cc. of the same preparation of B_2 as in the last experiment and 0.1 cc. of a B_1 preparation very kindly given to the authors by Dr H. Chick, who had found this dose of her preparation ample for the growth needs of the rat. One of the 4 rats continued to receive the "Glaxo casein"; it grew slowly and 21 days after the beginning of the test period had gained 17 g. in weight. Two rats were given "crude casein" (B.D.H.) in place of the "vitamin-free casein" (Glaxo) and at the end of the experiment (21 days) had gained 2 and 7 g. respectively. The fourth rat was given "light-white

casein" (B.D.H.) and had gained at the end of the experiment (21 days) 52 g. in weight. This would appear to indicate that the "crude casein" (B.D.H.) was as nearly free from the unknown factor as the "Glaxo casein."

In the second experiment a comparison was made between (a) the "vitamin-free casein" (Glaxo), (b) the "light-white casein" extracted once with alcohol and four times with ether in the cold, and (c) untreated "light-white casein." The same doses of B_1 and B_2 as in the last experiment were given to all the rats. At the end of 31 days, the one rat given "vitamin-free casein" had gained 17 g. in weight, the two given the extracted "light-white casein" had gained 37 and 32 g. respectively, while the one given untreated "light-white casein" had gained 49 g. It was evident that some of the active factor, but by no means all of it, had been extracted from the casein by the method adopted.

These two experiments have given the same results for the "crude casein" and for the extracted "light-white casein" which were given in the previous method of testing.

It would appear, then, that this offers an alternative method of testing for the unknown factor. Actually, by the time the rats are ready for the test of the unknown substance, they are receiving the same diet in the two ways of testing ("vitamin-free casein," dextrinised rice starch, salt mixture, agar-agar, vitamins B_1 and B_2 as independent preparations or as yeast, and cod-liver oil) whether they began with a shortage of vitamin A as in a vitamin A test or of vitamin B as in a vitamin B test. The former, "vitamin A way," has given us more animals for this work because the tests have necessarily been extended over longer periods (preparatory and test) than in the "vitamin B way," and the rats have apparently exhausted their reserves of the unknown factor. In the "vitamin B way" the rats have a very short preparatory period (10 days) and often a short test period (3–4 weeks), so that the test is often completed before the animals' reserves of the factor are exhausted.

The importance of supplying this factor in the basal diet of all rats used for growth experiments is obvious. It is a simple matter to give "light-white" or a similar sample of casein in the diet of rats preparing for a vitamin B test, for this form of casein is free from either B_1 or B_2 . For vitamin A testing, however, a method of removing vitamin A without removing the unknown factor must be adopted. These points will be the subject of a later paper.

DISCUSSION.

Certain food substances have been found to restore growth in rats which had ceased to grow although receiving a diet considered adequate in all factors known to be essential for growth.

It would appear probable that these substances supplied some factor which was missing from the basal diet of the rat, and that those rats which had succeeded in growing to maturity on this diet had reserves of the unknown factor which they had stored from pre-experimental feeding. No satisfactory

extract has yet been obtained from "light-white casein" (B.D.H.), but alcohol and ether extracts of the factor have been prepared from wheat embryo. The fact that it can be so extracted is evidence of its character as a fat-soluble factor.

Another suggestion is that the casein in general use in the vitamin-free diet has, by the process of freeing it from vitamin A, been damaged in some way that has rendered it less digestible or less adequate for growth and that the substances which have restored growth contain "unspoilt" protein. That the active substance can be extracted at all seems to be against this explanation. The fact, too, that many rats grow rapidly on the diet containing the "vitamin-free casein" (Glaxo) shows that it is an adequate protein for some rats at least. Indeed, many rats of both the albino and the black and white strains obviously have been able to digest the protein all their lives. The possibility of racemisation of the protein must be discarded as the activity has been almost completely removed from the protein by hot alcoholic extraction. The fact that the response to the untreated protein is sometimes delayed for many days also seems to be indicative of some actual deficiency rather than of some inability to digest the protein available.

Distinction from vitamin E. At one time it began to appear probable that the factor causing the remarkable resumption of growth would prove to be vitamin E. Its distribution was apparently similar to that of the latter factor; but further work, particularly with wheat-germ oil, makes this at least doubtful. Evans [1928] has demonstrated the growth-promoting power of this factor, and reported that wheat-germ oil in general made a marked difference in growth rate, in the absence of other sources of vitamin E, in a dose of 0.12 g. daily. Three samples which were prepared specially for this laboratory by extraction of wheat germ with light petroleum and concentration *in vacuo* were found to have distinctly less growth-promoting activity than that reported by Evans for vitamin E. One sample prepared by ourselves also showed less activity than was generally experienced by Evans, a dose of 0.16–0.24 g. being necessary to cause a really definite resumption of growth. Moreover, Evans has shown that etiolated pea-shoots are as rich in vitamin E as green ones. Our experience of one species of etiolated shoot (wheat) shows it to be poor in the unknown factor. Further, the liver which we have examined is richer than ox muscle, a relationship which is the reverse of Evans's experience with vitamin E.

The fact that the unknown factor can be destroyed by heat also makes it clear that it is not vitamin E. Moreover, rats suffering from a shortage of it show none of the usual cyclical changes in the reproductive organs as seen by examining the vaginal smears (unpublished work), whereas a shortage of vitamin E has no influence on the oestrous cycle [Evans and Burr, 1927].

There has, moreover, been no indication of a shortage of vitamin E among the animals of the stock colony of this laboratory. (They have had liberal supplies of fresh watercress twice a week, for which we are indebted to

W. Bedford, Esq. of Bere Regis.) Every mating is recorded; each doe is weighed once a week, and oftener during the last week of pregnancy. In the history of some 200 breeding does the occurrence of resorptions has been only about 1 % of the matings which have themselves averaged 4-6 per animal. This factor, then, seems to bear no relation to vitamin E beyond a somewhat similar distribution.

SUMMARY.

Rats often cease to grow even though they are receiving a diet hitherto considered to be satisfactory in calorific value, salt content, and digestibility and also known to contain abundance of all recognised vitamins.

Resumption of growth in these animals was first obtained by the substitution of "light-white casein" (B.D.H.) for the "vitamin-free casein" (Glaxo) generally used in the basal diet.

Other substances which bring about rapid resumption are fresh milk, lettuce, fresh and dried grass, ox muscle, liver and wheat embryo.

Less rapid growth is brought about by watercress and milk which has been simmered for 15 minutes.

Butter and etiolated wheat shoots show very little influence on the growth of these rats.

The power to cause growth resumption is removed from "light-white casein" only with difficulty. Heat can destroy it, at least partially. Cold alcohol, ether, and 2 % acetic acid give inactive extracts and leave unimpaired casein. Boiling alcohol and ether give slightly active extracts and leave definitely impaired casein. The loss may be due to heat.

Boiling alcohol (90 %) and ether give extracts of wheat embryo of definite activity and leave the wheat embryo with only slight activity.

The results here described indicate either the existence of a growth-promoting factor hitherto unrecognised or a biological inadequacy of the protein supplied to the rats in the basal diet. The preparation of active alcohol and ether extracts supports the former view.

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Addendum.

Since this paper was written, we have read that Evans and Burr (*Proc. Soc. Exp. Biol. Med.* 1927, 24, 740) have demonstrated a deterioration in their 'casein' after long extraction with alcohol and ether.