C. MAMMARY SECRETION. V.

I. FURTHER RESEARCH ON THE THRESHOLD AND EFFECTS OF PROTEIN "EXCESS."

II. THE QUANTITATIVE RELATION OF VITAMIN B TO PROTEIN.

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IN a previous paper [Hartwell, 1922, 1] it was shown that the proportion of 1 g. protein (egg albumin or caseinogen) to 15 g. bread, in a mother rat's diet, was sufficient to cause abnormal symptoms in the sucklings. In these experiments small quantities of yeast extract (marmite) were added to improve the palatability of the diet. At that time the significance of vitamin B was not understood, and therefore no effort was made to use the same supply of yeast extract throughout the work. In the light of more recent experiments, therefore, it was thought advisable to repeat some of this earlier work, omitting yeast extract entirely. It was also hoped by a longer series of experiments, using other proportions of protein as well, to ascertain where the greatest danger level lies.

The second group of experiments described in this paper was started with a view to finding if a quantitative relation existed between the protein and vitamin B in the lactating rat's diet. In some previous experiments [Hartwell, 1922, 2] it was shown that 50 cc. of various fruit (except grapes) and vegetable juices, when added to the mother's "excess" protein diet, prevented any bad symptoms in the sucklings. The different juices did not give equally good results, and as fruits and vegetables are recognised as containing varying quantities of vitamin B, it seemed possible that some quantitative relation might exist between the protein and the vitamin B. In these experiments [Hartwell, 1922, 2] the amount of caseinogen was kept constant and it was noticeable that the best results were obtained by the addition of juices (or other foods, *e.g.* egg yolk) shown by other workers to be especially rich in vitamin B.

With our present state of knowledge, any quantitative work on vitamins is very difficult, especially so, as no methods for the complete isolation of these

food factors are available. Various assumptions as to amounts needed, etc. are made, but there is little actual proof, and practically no accurate work on the subject.

Osborne and Mendel [1922] suggest that there is a correlation between the size (and therefore the age) of the animal (rat) and its vitamin B requirement. They describe experiments indicating that on a definite and low vitamin B intake the heavier animals fare worse than those of lighter weight. These workers used one diet throughout, and themselves point out that their experiments throw no light on the question as to whether the composition of the food has any direct relation to the vitamin B intake.

Cowgill [1923], working on dogs, found a similar relation between the vitamin B intake and the animal's body weight.

Following along these lines of argument it might be expected that the requirements of the lactating animal, in respect of vitamin B, would exceed those of the non-lactating creature. Other workers have assigned to vitamin B an important rôle in connection with glandular secretion, and, if this is correct, an extra supply of this vitamin would be needed by the active mammary gland.

EXPERIMENTAL DATA.

(a) *Method.* During gestation the mother rats received a good mixed diet consisting of kitchen scraps with a small amount of bread and milk per day. At least three animals were fed on each diet, and each attempted to rear six babies (with the exception of 1177 and 1199, where the litters consisted of five only). In previous experiments the litters have been reduced to six on the first day. A modification of this procedure has recently been adopted, viz. the litter was not reduced in number until the second day. It was noticed that large litters (12 or more) frequently contained one or two weakly members which either died or were eaten by the mother within 24 hours; hence a better selection could be made on the second day and there was less risk of losing a baby during the first few days of lactation. The mothers and litters were weighed every day as previously described [Hartwell, 1921, 1], and the foods were given in the form of a paste, all constituents being well mixed and increased proportionately when necessary. The tomato juice and the extract from marmite rich in vitamin B were prepared as described in an earlier paper [Hartwell, 1922, 2].

(b) *Experiments.* A brief summary of the experiments is given below:

1. *Experiments with bread, "food casein" (82.86 % protein) [Hartwell, 1922, 1] and water (Fig. 1).*

Diet. 15 g. bread to x g. food casein mixed to a paste with water.

Fig. 1.

Fig. 2.

Proportion of food casein to 15 g. bread	Litter numbers	Results
<i>Expt. A.</i>		
0.6 g.	962 968 975	No spasms or screaming fits—babies not quite normal—17th–18th days they appeared frightened and slightly jerky in their movements—3 out of 18 died.
<i>Expt. B.</i>		
0.9 g.	969 970 971	All 3 litters showed spasms—one lot had screaming fits and one litter could not be weaned until 27th day, instead of the 21st as usual—3 out of 18 died—rate of growth of young about the same as when the mother received 0.6 g.
<i>Expt. C.</i>		
1.2 g.	963 964 965	All 3 litters exhibited spasms and screaming fits—3 out of 18 died—rate of growth of young slightly better than when the mother had less protein, but general condition of litters worse.
<i>Expt. D.</i>		
1.5 g.	991 994 995	Condition practically the same as with 1.2 g. but rate of growth less good at end of lactation—3 out of 18 died.
<i>Expt. E.</i>		
3.0 g.	1070 1084 1085 1087 1088 1089 1157 1159 1164	Condition rather better than with lower proportions of protein—4 out of 54 died.
<i>Expt. F.</i>		
3.9 g.	1091 1092 1093	All 3 litters showed typical symptoms and one whole litter died, <i>i.e.</i> 6 out of 18—growth about the same as before.
<i>Expt. G.</i>		
5.1 g.	1165 1166 1173	Condition much worse—11 out of 18 died (this involves one entire litter).
<i>Expt. H.</i>		
6.0 g.	1111 1177 1199	None survived.

In all these experiments the rate of growth of the litters for the first 12 days was quite good (except when the mother had the proportion 6 g. food casein) and fairly constant in spite of the variation in the amount of protein fed to the mother. The six young on the 12th day weighed approximately 110–120 g. With the lower proportions of protein the babies weighed about 30 g. each at weaning on the 21st day, while with the higher proportions they were less, showing only slight gains after the 12th day. The controls usually weigh 40–45 g. at weaning. The mothers remained well throughout in spite of the fact that many of them lost weight. It was noticeable that an appreciable loss in weight of the mother was usually correlated with a better condition in the sucklings. This point has been commented upon in a previous communication [Hartwell, 1921, 2].

From these experiments it is obvious that even small quantities of protein

(0.6 to 15 g. bread) added to a bread diet result in the suckling rats exhibiting an abnormal condition. With an increase in the protein ration the symptoms became gradually worse, and when the mother rat was given 6 g. food casein to 15 g. bread, none of the babies survived. The supply of vitamin B was practically constant and therefore it seemed probable that if the vitamin B were increased with the protein, the bad effects might be obviated. The following experiments were started with a view to testing this hypothesis.

2. *Quantitative relation of protein to vitamin B.*

(a) Experiments using tomato juice as a source of vitamin B.

Expts. I and J. These are comparable with Expt. C (p. 788) for the animals were receiving the same proportion of bread and food casein, but tomato juice was added.

Diet. 15 g. bread, 1.2 g. food casein, 1.5 cc. tomato juice, 31.5 cc. water.

972 One litter showed spasms. All 3 litters grew well and their coats were good, but from
973 about the 18th day (i.e. when the baby rats ran about and ate for themselves) they
974 appeared frightened and exhibited slight incoordination.

Thus the condition of the sucklings was improved by adding 1.5 cc. of tomato juice to the mother's initial diet, but the bad effects were by no means obviated.

Diet. 15 g. bread, 1.2 g. food casein, 3 cc. tomato juice, 30 cc. water.

979 Growth of the young was rather better than in Exp. I but spasms and incoordination
980 were still exhibited, although not quite so markedly.
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Expt. K. Comparable with Expt. D (p. 788).

Diet. 15 g. bread, 1.5 g. food casein, 6 cc. tomato juice, 27 cc. water.

1151 The baby rats were normal in all respects, and the growth curve was quite good. Their
1153 fur was especially thick.
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Hence it appears that 6 cc. of tomato juice when added to the mother's diet of 15 g. bread and 1.5 g. food casein contained sufficient vitamin B to prevent any abnormal symptoms in the suckling rats.

Expts. L, M, N, O. Comparable with Expt. H (p. 788).

Diet. 15 g. bread, 6 g. food casein, 6 cc. tomato juice, 45 cc. water.

1345 The growth of the young was less good than when a lower proportion of protein was fed
1352 to the mother rat. Spasms etc. were evident from the 10th day and onwards and the
1353 baby rats were not normal for some days after weaning.

Diet. 15 g. bread, 6 g. food casein, 12.5 cc. tomato juice, 37.5 cc. water (Fig. 2).

512 The results were similar to those described for Expt. L. It might be expected that
516 Expt. M should afford better results than Expt. L, but in the latter the mothers lost weight
517 and in the former they did not. It has been pointed out previously [Hartwell, 1921, 2]
that a loss in weight of the mother frequently means a better condition in the offspring.

Diet. 15 g. bread, 6 g. food casein, 25 cc. tomato juice, 25 cc. water (Fig. 2).

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Diet. 15 g. bread, 6 g. food casein, 50 cc. tomato juice, 25 cc. water (Fig. 2).

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In both cases the young were normal in all respects and the growth was good, being practically the same as that obtained in Expt. K.

Expt. P.

Diet. 15 g. bread, 21 g. food casein, 111 cc. tomato juice.

1013 No bad symptoms were seen in any litter; the growth was not quite so good as in
1014 Expts. K, N, O.
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(b) Experiments using extract from "marmite" as a source of vitamin B.

Expts. Q and R to compare with Expts. C and K.

Diet. 15 g. bread, 1.5 g. food casein, 0.6 cc. extract containing vitamin B, 33 cc. water (Fig. 3).

1244 Growth of the young rats was good and their condition normal.
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Diet. As above, but 0.9 cc. extract instead of 0.6 cc. (Fig. 3).

1238 The results were the same as those in Expt. Q, therefore the increase in the vitamin B
1242 content of the mothers' diet had no apparent effect.
1279

Expts. S and T to compare with Expt. H (p. 788).

Diet. 15 g. bread, 6 g. food casein, 0.9 cc. extract, 48 cc. water (Fig. 3).

1307 All 3 litters suffered badly, and spasms etc. were noted from the 13th day and onwards.
1308 The growth of the sucklings was fairly good up to the 12th day, but after that it was
1309 very slow.

Therefore it may be concluded that 0.9 cc. of the extract was not sufficient when the protein ration was increased.

Diet. 15 g. bread, 6 g. food casein, 3 cc. extract, 45 cc. water.

Condition normal [see Hartwell, 1922, 2, litter 396].

Expt. U to compare with Expt. P.

Diet. 15 g. bread, 21 g. food casein, 12 cc. extract, 99 cc. water.

1147 The sucklings grew very slowly, but no abnormal symptoms were observed.
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(c) Experiments with bread and tomato juice.

Expt. V.

Diet. 15 g. bread, 33 cc. tomato juice (Fig. 4).

1019 A slow, but steady growth curve was given by the sucklings, but the mothers' curves
1020 show an appreciable loss of weight, probably due to the low protein content of the diet.
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(d) Experiments with bread, starch and salt mixture (Fig. 4).

Diet. 15 g. bread, 5 g. starch, 0.7 g. salt mixture [Hartwell, 1922, 1].

1002 The results were practically the same as described for Expt. V, i.e. bread + excess
1003 vitamin B; therefore starch does not need extra vitamin B, whereas protein does.
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CONSIDERATION OF RESULTS.

In the first series of experiments the diet consisted of bread, food casein and water. Such a diet is low in fat and probably also in vitamin A, since these constituents were only present (if at all) in small quantities in the food casein. Vitamin B might also be present in the protein used, but the chief source of

Fig. 4.

Fig. 3.

it is the baker's bread, which has been shown to contain enough vitamin B to provide for the growth of the rat for several months ($7\frac{1}{2}$ months in one experiment) [Hartwell, 1924]. When 0.6 g. of food casein was added to 15 g. of bread in the mother's diet, the babies were not quite normal, while the addition of 0.9 g. food casein to 15 g. of bread led to the sucklings exhibiting spasms and screaming fits. With further increases in the protein in the mother's diet, the babies became gradually worse, until finally, when 6 g. of food casein were added, none survived.

Drummond, Crowdon and Hill [1922] state that they have observed symptoms in suckling rats similar to those described by me, but "only in the case of those being reared by mothers on diets deficient in vitamin B."

In some unpublished work of the author's, rats were kept for 9 months on a diet of 30 g. bread, 3 g. food casein, 3 g. butter, 0.7 g. salt mixture, and water; in another experiment rats were kept for 4 months on a similar diet but with a higher proportion of protein, viz. 12 g. food casein instead of 3 g. In both experiments the animals remained fit and healthy and never exhibited any symptoms indicative of lack of vitamin B. Therefore such diets cannot be said to be deficient in vitamin B. It might be suggested that there is not much excess of vitamin B in bread and that the addition of other constituents to a bread diet would necessarily dilute the vitamin B in the diet, and thus put the vitamin B content below the limit required for health. That this is not so is shown by the experiments described in this paper in which the mother rats received a ration of 15 g. bread, 5 g. starch, 0.7 g. salt mixture, and water, and the babies were successfully reared and showed no spasms. The mother rats also remained in good condition. Hence a diet of 15 g. bread + 5.7 g. extra solid is *not* deficient in vitamin B; further, the spasms were noticed when the mother rat received only 0.9 g. of food casein to 15 g. bread.

Two sets of mother rats were fed respectively on 15 g. bread, 5 g. starch, 0.7 g. salt mixture, and 15 g. bread and 33 cc. tomato juice. Examples of the weight curves of the mothers and litters are shown in Fig. 4, and it is interesting to note how nearly they coincide. In both, the growth of the baby rats is slow, owing to the low proportion of protein in the mothers' diets. Since the mothers receiving tomato juice must have had an excess of vitamin B, and the growth curves of the sucklings are practically equal, it does not appear that there is any lack of vitamin B in the diet of bread, starch and salt mixture.

Again Drummond, Crowdon and Hill state that "A high protein diet can be adequate for lactating female rats...provided that it is also adequate in components serving as sources of the vitamins." It is difficult to know what is meant by "adequate" and also although these authors say vitamins, they suggest no possible function for either vitamin A or vitamin C in this connection.

The experiments described in this paper afford evidence that there is a distinct *quantitative* relation between protein and vitamin B in the diet of a lactating rat, i.e. the more protein in the diet, the more vitamin B must be added for the offspring to be successfully reared. An excess of vitamin B has

no effect, for the addition of 50 cc. of tomato juice instead of 25 cc. to a diet of 15 g. bread and 6 g. food casein made no apparent difference either to the growth curve or general condition of the young. Similar results were obtained when 0.6 cc. or 0.9 cc. of extract of vitamin B were added to a diet of 15 g. bread and 1.5 g. food casein.

It is interesting that the growth curves of the young are practically the same when the mother receives 1.5 g. or 6 g. food casein to 15 g. bread provided that more vitamin B is added in the latter instance.

The exact amounts of vitamin B necessary for different levels of protein intake have not yet been determined, but experiments are in progress to elucidate this point. It is quite possible, almost probable, in fact, that the amount of vitamin B will not only vary with the protein intake, but will be affected by other dietary constituents.

In the experiments described in this paper the diets were very low in fat and vitamin A. If larger amounts of fat were present it is conceivable that even more vitamin B might be needed to prevent spasms. This low proportion of fat did not seem to affect either the mothers or the offspring adversely, possibly because the mother rats were in very good condition and had fairly large amounts of depôt fat.

Drummond, Crowdon and Hill [1922] state also that there is some evidence for believing that vitamin B is concerned with carbohydrate metabolism, since the non-nitrogenous residue from amino acids is said to be metabolised along the same path as carbohydrates. They suggest therefore that this explanation might be applied to my results. That this is not so, is quite obvious from my earlier work [Hartwell, 1921, 1]. No spasms were developed when the mother rats received 15 g. bread and 5 g. dextrin, and if extra vitamin B were needed for the proper metabolism of the carbohydrate, surely spasms should have resulted from this large addition of carbohydrate to the diet. These results have been confirmed by feeding the mother rats on 15 g. bread, 5 g. starch and 0.7 g. salt mixture, when no spasms developed in the baby rats. In this earlier paper [Hartwell, 1921, 1] it was also shown that adding 5 g. butter to 15 g. bread produced no harmful results in the suckling babies, which proves that vitamin B is not specially concerned, in the lactating animal, with fat metabolism; hence it appears fairly clear that the rôle of vitamin B in the metabolism of protein is concerned with the nitrogenous part of the protein molecule or with protein *quâ* protein, and not with any fatty acid or carbohydrate residue.

CONCLUSIONS.

1. In the lactating rat, when protein is added to a diet of bread, as little as 0.6 g. food casein (82.86 % protein) to 15 g. bread is sufficient to produce an abnormal condition in the young. Increasing the proportion of protein in the mother's diet leads to worse symptoms in the sucklings, and when 6 g. of this protein are added to 15 g. bread none of the young survives.

2. It is impossible to state what constitutes an excess of protein in a lactating rat's diet because there is a *quantitative* relation between protein and vitamin B. Thus, provided the vitamin B is increased when the protein is increased there should be no evil effects.

3. In the lactating rat the function of vitamin B is primarily concerned with the metabolism of protein *quâ* protein or with the nitrogen part of the protein molecule; for to a diet of 15 g. bread and 5 g. protein must be added far more vitamin B than to a diet of 15 g. bread and 5 g. starch.

4. A high proportion of protein (15 g. bread to 21 g. food casein) in the lactating rat's diet produces no harmful effects in the offspring, provided the diet is very rich in vitamin B, but the rate of growth of the sucklings is impaired.

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