CLIII. MAMMARY SECRETION. VI. VITAMIN B AND THE LACTATING RAT'S DIET.

1. THE QUANTITATIVE RELATION OF VITAMIN B TO PROTEIN (*continued*).

2. VITAMIN B REQUIREMENT OF THE LACTATING AND NON-LACTATING RAT. (PRELIMINARY NOTE.)

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1. THE QUANTITATIVE RELATION OF VITAMIN B TO PROTEIN (*continued*).

INTRODUCTION.

At the present time there appears to be no definite knowledge as to the minimum amount of any vitamin required for growth and the maintenance of health. Quantitative work is, as yet, very much in its infancy (especially in respect of vitamin B); probably our technique is not sufficiently good to permit of any very accurate work in this field of research, and such work as there is lies along other lines. For example, the distribution of the various vitamins in different foods has been the subject of much research, whilst very few observers have attempted to ascertain the animals' actual needs in respect of these elusive factors (except the antiscorbutic requirements of the guinea-pig and monkey). Many dietetic workers (using rats) give as much as 5.0 g. marmite for every 100 g. (approximately) of synthetic food. It is probable that this figure is adopted because it is the amount used in the diet laid down by the Accessory Food Factors Committee when testing for the presence of vitamin A [Medical Research Council, 1924]. It obviously supplies the needs of the rat, but does it not also provide excess of the vitamin? For example, a young rat (about 60 g.) bred in this laboratory and fed on a synthetic diet from weaning (21 days of age) would eat approximately 0.6 g. marmite per day, whilst Osborne and Mendel [1922] find that a daily ration of 200 mg. of their yeast is enough to provide for the rat to attain "the average adult size within the average normal time." One cannot assume that the English yeast extract is absolutely equivalent to the American yeast, but allowing for some variation there is a big difference between 0.6 g.

and 0.2 g. From this it appears that the 5.0 g. marmite to 100 g. synthetic diet may provide an excess of vitamin B. There is so far no really good method for estimating vitamins, and therefore even the synthetic diet which is spoken of as "vitamin-free" may be only relatively free, for there is no proof that the constituents of that diet are *absolutely* vitamin-free. The only fact proved by experiments is that such a diet does not contain sufficient of any vitamin to support life, and hence all vitamin experiments can only be regarded as comparative.

In a previous communication [Hartwell, 1924, 2] it was shown that a definite quantitative relation exists between the amount of protein and the need for vitamin B in a lactating rat's diet. In this work, bread, food casein, and tomato juice were used, and it was thought advisable to make more experiments along similar lines using synthetic diets in which the protein content could be more accurately estimated. With this object in view the experiments to be described in this paper were carried out.

EXPERIMENTAL DATA.

During gestation the mother rats received a good mixed diet of kitchen scraps, supplemented by bread and milk. The synthetic diet was started as soon as possible after the birth of the litter (never more than 18 hours) and continued for 21 days, at which time the young were weaned. In one or two experiments, when the diet was low in protein (10 %), the baby rats were small and weakly, and would not eat for themselves at the usual time (16th–18th day). In such cases the young were left longer with the mother and the diet was continued for three days after the young were seen to be eating from the mother's food.

The general method of experiment (daily weighings of mother and babies, etc.) has been fully explained in previous papers [Hartwell, 1921, 1; 1924, 2].

Foods. The dry constituents (caseinogen, potato starch and salt mixture) were mixed in bulk; the butter and marmite, with water, were added daily to weighed amounts of the dry mixture. Three different proportions of caseinogen were used, as follows:

	Diet A	Diet B	Diet C
	g.	g.	g.
Caseinogen	20	10	30
Potato starch	64	74	54
Salt mixture	4	4	4
Washed butter [Hartwell, 1924, 1]	12	12	12

Vitamin B. Marmite was used as a source of this factor, the same sample being used for all the experiments. The marmite was accurately weighed in a beaker, dissolved in warm water, and by this means transferred to a graduated flask. When cold the volume was made up to the mark. The marmite solution was made daily in hot weather, and every other day when it was cold; the required amount was pipetted out and mixed evenly with the food.

Experiments.

1. *Diet A* (20 % caseinogen). A series of experiments was made in which decreasing strengths of marmite were used, viz. 20, 15, 10, 5 %, and no marmite at all. The mothers and young were watched carefully for any abnormal symptoms.

The results are given in the following table:

Number of exps.	G. of marmite per 100 g. diet	<i>Condition of young rats</i>	<i>Condition of mother rats</i>
2	8	Normal	Very slight loss in weight, otherwise normal
9	6	8 litters normal; 1 litter slight spasms	Slight loss in weight, otherwise normal
4	4	All 4 litters had spasms, and 3 litters screaming fits. 9 out of 24 died. Fur in good condition, but no young normal	Mothers lost weight, but appeared to be well
3	3	All 3 litters spasms and screaming fits; whole litter died	„ „
7	2	Condition very poor; 4 whole litters died. Babies which survived were weakly for at least 10 days after weaning	Great loss in weight (in the worst case a rat which weighed 286 g. at birth of litter weighed only 173 g. at weaning)
7	0	Condition of young very poor indeed; 26 out of 40 died, and the survivors were not normal for some time after weaning	Great loss in weight (the worst case being a loss of from 306 g. to 210 g.)

Thus it appears that with diet A (20 % caseinogen) 6 g. of marmite to 100 g. diet in the mother's food is generally enough to protect the young from bad effects, and 8 g. is a complete safeguard.

2. *Diet B* (10 % caseinogen). Three series of experiments were made, using 15, 5 and 2.5 % marmite solutions respectively. This diet was by no means satisfactory; the mothers lost weight badly and the young were of small size, and some were weakly. In spite of these drawbacks, no spasms or abnormal symptoms of any kind were seen in the suckling rats when the mothers were receiving only 5 % marmite, and with 2.5 % no spasms occurred, but some of the young showed slight incoordination of movement.

3. *Diet C* (30 % caseinogen). One series of experiments only was made and the strength of marmite used was 15 %. All three litters were abnormal and exhibited typical spasms and screaming fits, so it is obvious that with a higher percentage of protein, the 15 % marmite is no longer sufficient to afford protection to the young.

4. *Test of diet A for freedom from vitamin B.* This synthetic diet was expected to be free from vitamin B, but it was thought advisable to make a test on animals from the same stock as were being used for the other experiments. Accordingly 12 young rats (6 of each sex) were fed on diet A + water; the results were quite in agreement with those of other workers using a vitamin B-free diet.

RESULTS AND COMMENTS.

The experiments described in this paper afford evidence that there is a definite relation between the protein content and the vitamin B of the lactating rat's diet. Thus 15 % marmite afforded complete protection to the young when the mother's diet contained 10 % of protein, almost complete protection when the mother rat was eating 20 % protein, but was quite inadequate for the higher protein intake (30 %). With a diet containing 20 % of protein, 20 % marmite afforded complete protection, and 15 % was almost enough, but with lower percentages of marmite the young became correspondingly worse (see Fig. 1) until with 5 % many died.

It is rather extraordinary that there appeared little difference in the condition of the young when the mother received 5 % or no marmite, especially since the 5 % level of intake appears to meet the needs of the young growing rat in respect of vitamin B (see second part of paper). A possible explanation may be that the 5 % is so little compared with what she really needs that it makes only a slight difference if she receives none. It is also remarkable that the rat can rear any young at all on a diet free from vitamin B. Presumably she does so at great expense to herself, for it is a well-known fact that the

mother safeguards her young by sacrificing her own tissues, as shown by great loss of weight. The young of mothers fed on a diet lacking in vitamin B are by no means normal, but 14 out of 40 survived weaning and eventually became healthy rats, though they were by no means fit for about two weeks after weaning (Fig. 2).

Another point which is well illustrated in the experiments described here is that a considerable loss in weight of the mother rat frequently results in the survival of her offspring, while another mother on the same diet maintains her weight (or loses slightly) and her offspring all die (Fig. 2). This point has been alluded to in previous papers [Hartwell, 1921, 2, and Hartwell, 1924, 2].

Diet B (10 % protein) gave very poor results.

The mothers lost weight, and the young were undersized and weakly. It is probable that the low percentage of protein is responsible for these bad effects. With 15 % marmite the young were still of low weight, though in rather better condition. It may be that the yeast protein is responsible for the improved condition. The mothers, on an average, ate 25 g. food, *i.e.*

2.5 g. caseinogen per day. With this they would receive 1.5 g. marmite, so it is conceivable that the protein ration of the synthetic diet was supplemented by the yeast protein.

In spite of the young being weakly, no spasms were observed even with as little as 2.5 % marmite, and since it is quite possible that weakly babies might be more susceptible to any deficiency in the mother's milk, this may be an added proof of the proportional relation of vitamin B to protein in the lactating rat's diet. The addition of large quantities of vitamin B to this low protein diet were ineffective in saving the mothers' weights to any great extent. This is shown in the following table:

Strength of marmite %	Average loss in weight of mother rat g.
15	61
5	70
2.5	80

2. VITAMIN B REQUIREMENT OF THE LACTATING AND NON-LACTATING RAT (PRELIMINARY NOTE.)

In a previous communication [Hartwell, 1924, 3] it was shown that, with diets rich in protein, the lactating rat must have a greater supply of vitamin B than at other periods of her existence, otherwise she is unable to rear her young. As a result of this work, the problem arose as to how much more vitamin B is required by the lactating than non-lactating rat.

Fig. 3.

- - - - Control rats—diet of kitchen scraps and bread and milk
- . — . Rats fed on diet A + 5 % marmite (25 g. diet A to 10 cc. of 5 % marmite solution)

Twelve young rats were fed on a diet consisting of 20 g. caseinogen, 64 g. potato starch, 4 g. salt mixture, 12 g. butter (washed), 2 g. marmite (which contain 27.35 % water)¹. At the end of 6 months they were fit and healthy. Some of their growth curves are given as examples (Fig. 3). The rate of growth is better than that described by Donaldson [1924] as the normal curve for the rat.

It was found with this diet that the mother rat required 6–8 g. marmite to enable her to rear her young successfully.

This work proves that on an average diet (such as 20 % protein, etc.) the lactating rat needs at least 3–4 times as much vitamin B as does the non-lactating animal, otherwise she cannot rear her young. These results are in agreement with the work of Grant [1924] who found that a similar ratio existed between the needs of the lactating and non-lactating rat.

SUMMARY.

1. There is a quantitative relation between the protein and vitamin B in the lactating rat's diet. On a synthetic diet containing 20 % caseinogen, 6–8 g. marmite per 20 g. caseinogen are needed for the mother rat to rear her young; with 4 g. marmite the babies exhibit spasms and screaming fits, while with 2 g. the young are in a still worse condition and the majority of them die.

2. On a synthetic diet containing 20 % caseinogen the lactating rat requires at least four times as much vitamin B as does the young growing animal, otherwise she cannot rear her young successfully.

3. The lactating rat can rear some of her young on a synthetic diet free from vitamin B, but she loses weight during the lactation period. Her young are weakly when weaned, but eventually recover.

4. On a synthetic diet containing 10 % of caseinogen the lactating rat loses weight and her young are under-sized.

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¹ I am indebted to the Marmite Food Extract Co. for supplying me with this figure.