LXXXVI. MAMMARY SECRETION. IV.

THE RELATION OF PROTEIN TO OTHER DIETARY CONSTITUENTS.

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I. INTRODUCTORY AND HISTORICAL.

It is generally held that the diet of a lactating animal should contain a large amount of protein, and it seems quite obvious that a maximal growth of the young cannot be obtained if the mother's protein intake is low. On the other hand it has been demonstrated [Hartwell, 1921, 2] that large amounts of protein fed to the mother, result in harmful effects to the young. It has, however, been found [Hartwell, 1922] that large quantities of whole milk, and large amounts of yeast extract added to the mother's excess protein diet, prevent the bad symptoms in the litters. The obvious, though possibly not the correct explanation of such results, was that the important factor was vitamin B, since this is present in both milk and yeast. Also it was shown [Hartwell, 1922] that lactose, butter, milk-ash and calcium lactate were ineffective in improving the bad condition. Consequently the milk effect was not due to any obvious dietary factor.

The experiments described in this paper were started in the hope of throwing some light on the relation of vitamin B to protein in the metabolism of the lactating animal.

That there is a possible relation between vitamin B and protein is to be seen from the work of Karr [1920]. He found that the dog refused food unless vitamin B was included in the diet, and since this animal is a carnivore, it may be that the vitamin is in some way essential for protein metabolism.

In the experiments to be detailed here the mother rats were fed on an excess protein diet, to which were added various fruit and vegetable juices and extracts made from other foods said to contain vitamin B. From the results described, it appears that the addition to the mother's protein-rich diet of juices of, or extracts from, foods reported to contain vitamin B, is effective in rendering the nature of the milk more normal.

At the present time, the published results as to presence or absence of vitamins in foods show discrepancies. This is probably due to

- (1) The different methods used for testing the presence of the vitamins.
- (2) The lack of quantitative estimations.

It appears to be generally accepted that vitamin B is widely distributed in the plant kingdom, although there are differences of opinion as to the amount present.

Osborne and Mendel [1920, 2] state that the edible part of an orange contains vitamin B and that the potency is similar to that of comparable volumes of cow's milk. Grape juice they consider to be less potent than orange; apples contain the vitamin, but are not rich in it. These observers [1920, 1] also find that tomatoes and carrots contain appreciable amounts of the water-soluble vitamin; potato is quite a good source, but not so good as tomato. Beetroot contains less of the substance. The occurrence of vitamin B in animal tissues is possibly less extensive, but it may be present in smaller quantities. Cooper [1913] prepared a curative solution of the antineuritic substance from horse flesh and from beef.

According to Drummond [1919] animal tissues (with one or two exceptions) are deficient in the water-soluble antineuritic factor. This observer fed young rats on protein-free extracts of muscle from herring, cod and salmon and found that his solutions contained no vitamin B. When the extract was made from the whole herring, he obtained a slight growth and concludes that the source of the vitamin in this case was the ova and glands, shown to contain the antineuritic vitamin by Chick and Hume [1917]. Drummond does not explain how he prepared the protein-free solutions and it is possible that any vitamin present was adsorbed when the protein was precipitated. If the factor investigated in the experiments below be vitamin B the results obtained contradict those of Drummond.

II. METHODS.

The technique employed has been fully discussed in a previous paper [Hartwell, 1921, 1]. At least three mother rats were fed on the various diets and each attempted to rear six baby rats. One typical curve is shown as representative of the three. In some experiments where two or three members of one litter survived and another litter all died (*e.g.* cucumber juice, Fig. 1) two growth curves are given as showing the best and worst growth on the given diet.

The basal diet was:

- (i) *White bread,*
  - (ii) *Butter for fat and vitamin A,*
  - (iii) *Lemon juice for vitamin C,*
  - (iv) *Caseinogen*
  - (v) *Salt mixture*
- } [Hartwell, 1922],

to which was added one of the following as potential source of vitamin B:

(a) *Fruit or vegetable juices.* These were made as follows: the soft fruits were placed in muslin and the juice squeezed out by hand. The harder vegetables (e.g. potato, carrot) were grated and then squashed in a hand press. The juice was filtered through muslin and allowed to stand in a cylinder so that the starch (if present) settled at the bottom of the vessel and the juice was decanted. All juice was freshly prepared, just before being fed to the animals.

Mothers' diets									
526	x — x	15 g. bread, 5 g. caseinogen, 0.5 g. butter, 0.7 cc. lemon juice, 50 cc. apple juice							
541									
473	▲ — ▲	"	"	"	"	"	"	50 cc. tomato juice	
422	□ — □	"	"	"	"	"	"	50 cc. orange "	
437	• — •	"	"	"	"	"	"	50 cc. artichoke "	
421	• — •	"	"	"	"	"	"	50 cc. beetroot "	
382	○ — ○	"	"	"	"	"	"	50 cc. carrot "	
373	■ — ■	"	"	"	"	"	"	50 cc. potato "	
608	◇ — ◇	"	"	"	"	"	"	50 cc. marrow "	
495	△ — △	"	"	"	"	"	"	50 cc. cucumber "	
501	△ — △	"	"	"	"	"	"		
597	● — ●	"	"	"	"	"	"	50 cc. grape "	

(b) *Meat or fish extracts.* The meat was freed from fat as far as possible and minced finely. The fish muscle was removed from the bones (in the herring the backbone was taken out, but probably a few small bones were left) and minced. No roe was included.

The minced meat and fish were weighed, placed in a bottle, warm distilled water added (1 cc. water to 1 g. flesh) and the whole shaken on a mechanical shaker for $1\frac{1}{2}$ hours. The contents of the bottle were then transferred to a flask, which was placed in a boiling water-bath for some of the protein in the solution to coagulate. The whole was filtered through paper and the filtrate fed to the animals.

(c) *Wheat germ extract.* The wheat germ was added to warm distilled water in the proportion of 3 g. wheat to 50 cc. water. This mixture was shaken for $1\frac{1}{2}$ hours and then filtered through paper. The volume of the filtrate was measured and the liquid boiled to coagulate some of the protein. When cold the volume was made up with distilled water to what it was before boiling and the protein filtered off. This filtrate was fed to the animals.

(d) *Extract rich in vitamin B made from crude "marmite."* 250 g. "marmite" were dissolved in 400 cc. distilled water and transferred to a Winchester quart bottle. 1600 cc. absolute alcohol were added and the whole shaken for 3 hours. This liquid was decanted (filtered if necessary) and distilled *in vacuo* at 40° – 50° until all the alcohol had been distilled off. The remainder was left to cool, filtered, and the volume made up to 250 cc. with distilled water. It was boiled for 4 hours to sterilise.

(e) *Egg yolk solution.* The egg yolk was separated from the white as much as possible, 50 cc. warm distilled water added and the whole well mixed.

The diet given.

In all the experiments to be described in this paper the proportion of white bread to protein was the same as that previously used as an excess protein diet, *i.e.* 15 g. bread; 5 g. protein (caseinogen).

The initial basal ration consisted of: 15 g. bread, 5 g. protein (caseinogen), 0.7 g. salt mixture, 0.5 g. butter, 0.7 cc. lemon juice.

To this were added 50 cc. of fruit or vegetable juice, meat extract, or egg yolk solution etc.

When wheat germ or soya bean was given as solids, 3 g. were added to the above ration and water was used to mix the dry constituents.

All the constituents were increased proportionately when necessary.

III. RESULTS.

(i) *Vegetable and fruit juices.*

Jerusalem artichoke, carrot, tomato and potato gave excellent results. The baby rats were normal in all respects and were extraordinarily fit and healthy. The litters belonging to the mothers which were given tomato juice in their

diets, were, perhaps, just fitter than the others and had very thick coats. Representative growth curves are shown in Fig. 1, 437, 382, 473, 373.

Apple, orange, beetroot and vegetable marrow juices were also effective in preventing very bad symptoms in the young, but the babies were not so healthy and fit as were those whose mothers received artichoke, carrot, tomato or potato juice, although the "apple babies" grew at a greater rate.

In all these experiments slight spasms were seen in the young, but the condition of the babies was never very bad. The spasms were generally noticed for one day only, and the growth curve was not interfered with, *i.e.* no loss of weight was observed. In the case of the "apple babies" the rate of growth was practically maximal. From the 18th–20th day screaming fits [Hartwell, 1921, 2] were noticed, but in spite of this the babies seemed well and gained weight. All were successfully weaned at the 21st day (Fig. 1, 526, 541, 422, 421, 608).

Cucumber juice when fed to the mother was of no effect in relieving the bad symptoms in the young. All three litters suffered badly, the spasms were severe and only 4 out of the 18 young survived. These were weakly for about two weeks after weaning (Fig. 1, 495, 501).

Grape juice, gave even worse results than cucumber juice. All three litters suffered badly and none of the young survived (Fig. 1, 597).

(ii) *Wheat germ and soya bean.*

These two foods were tried because both are reported to be rich in vitamin B.

(a) *Wheat germ 3.0 g. and wheat germ extract 50 cc.* (preparation see p. 828) were most effective. The litters did well and showed none of the bad symptoms. In the first series of experiments, it is interesting to note that the mother was receiving still more protein, since wheat germ itself contains 24.3 % of protein [Plimmer, 1921] (Fig. 2, 405, 480).

(b) *Soya bean.* When 3 g. were added to the mother's basal diet the litters suffered severely; all the babies had bad spasms; 11 out of 16 survived, but were weakly for some time after weaning.

Soya bean contains 33.7 % protein [Plimmer, 1921] and therefore it seemed possible that the bad results of the above experiments might be explained by the increase of protein in the mother's diet. Accordingly three more experiments were tried in which the soya bean was increased from 3 to 6 g. in proportion to the 15 g. bread, 5 g. protein etc. In this case the babies all survived and were practically normal. Slight spasms were noticed, but only for one day. The results were comparable to those obtained when the mother received apple juice (Fig. 3).

(iii) *Fish and meat extracts. Egg yolk. Whey.*

Lean muscle (beef) and herring extracts when added to the mother's diet entirely protected the young from any harmful effects (Fig. 4, 390, 433).

Cod extract was nearly as good, but not quite. No spasms were noticed, but the baby rats were not quite so lively as normal animals. However, three days after weaning (on a bread and whole milk diet) they had recovered entirely (Fig. 4, 447).

- Fig. 2.
- Mothers' diets*
- 405 • — • 15 g. bread, 5 g. caseinogen, 0.5 g. butter, 0.7 g. salt mixture, 0.7 cc. lemon juice, 3.0 g. wheat germ.
  - 480 ■ — ■ 15 b., 5 c., 0.5 b., 0.7 salt, 0.7 lemon (see above), 50 cc. wheat germ extract.
  - 369 △ — △ " " " " 3 cc. extract from marmite.
  - 531 ■ -- ■ } 15 b., 5 c., 0.5 b., 0.7 salt, 0.7 lemon (see above), 50 cc. wheat extract previously
  - 527 □ -- □ } boiled with charcoal.
  - 533 ▲ — ▲ 15 b., 5 c., 0.5 b., 0.7 salt, 0.7 lemon (see above), 50 cc. marmite extract previously boiled with charcoal.

Egg yolk was extraordinarily good. The growth curve of the young was equal to that obtained on any diet (*i.e.* maximal) and the babies were absolutely fit in all respects. Their coats were especially thick and long. This is interesting because egg yolk itself contains 15 % of protein (Fig. 4, 464).

Whey. It has previously been shown that the whey from 100 cc. whole milk when added to the mother's excess protein diet was adequate in safeguarding the young. In these experiments 50 cc. were tried in order to compare these results with those obtained when the mothers received 50 cc. of fruit juice, etc.

The babies suffered badly; 13 out of 24 survived, but these were miserable specimens. They were weakly and their coats were thin and dirty (a typical sign that an animal is not fit). They were put on a bread and milk diet on weaning, but it was over a fortnight before they could be considered normal (Fig. 4, 515).

(iv) *Extract rich in vitamin B made from crude "marmite."*

(Preparation given on p. 827.)

3 cc. of the extract were added to the basal ration, and proved most effective. All three litters did well and were normal in all respects (Fig. 2, 369).

The results of the above experiments are tabulated on p. 832. The distribution of vitamin B as given in the report of the Medical Research Council [1919] and by Eddy [1921] is included.

(v) *Experiments with a diet physiologically incomplete.*

The rats were fed on a diet of bread and caseinogen, as before, in the proportion of 15 : 5 and to this was added tomato juice, wheat germ or marmite extract. Such diets were incomplete physiologically, since salt mixture and fat were omitted. Also vitamin A would be poorly represented, if present at all. As explained in a previous paper [Hartwell, 1922] the

caseinogen used was not pure and might therefore contain a small amount of vitamin A, as would also the wheat germ and tomato juice.

Three mothers were fed on each diet and in all nine experiments they remained fit and well. The young had a good growth curve and no spasms were seen. The baby rats appeared perfectly fit and healthy, and were quite equal to those whose mothers were getting a more complete diet (Fig. 5, 396, 519, 553).

Fruit and vegetables.

50 cc. juice	M.R.C. on 3 + system	Eddy on 4 + system	Condition of litters
Apple ¹	Not given	++	Slight spasms. Growth good
Artichoke	"	Not given	No spasms. Growth good
Beetroot ²	"	+	Spasms. Babies weakly, but all 18 survived
Carrot ³	+	+++	No spasms. Growth good
Cucumber	Not given	Not given	Bad spasms. 4 out of 18 young survived
Grapes ⁴	"	+	Bad spasms, all young died
Orange ⁴	"	+++	Slight spasms. Growth good
Potato	+	+++	No spasms. Slight jerky type of walking. Growth good
Soya bean (3 g.)	++	+++	Bad spasms, 11 out of 16 young survived, but were weakly
" " (6 g.)	++	+++	Very slight spasms. Babies normal and healthy
Tomato ⁵	Not given	+++	No spasms. Growth good. Babies coats very thick. Young very fit
Vegetable marrow	"	Not given	Slight spasms, but quite good growth
Wheat germ ⁶ 3 g.	+++	+++	No spasms. Very good growth
Wheat germ extract	—	—	" " "
Yeast 3 g. ⁶	+++	++++	" " "
Yeast extract "marmite"	+++	+++	" " "

Meat, fish, eggs, milk.

Cod ⁶	White fish very slight if any	+	No spasms. Good growth. Not quite fit
Egg yolk ⁶	Eggs +++	Eggs ++	No spasms. Very good growth, extraordinarily fit babies
Herring	Very slight if any	++	No spasms. Quite good growth
Meat extract ⁶ 1 g. 1 cc. water	Lean meat +	0	No spasms. Good growth
Whey	Milk +	+++	Bad spasms. 13 out of 24 young survived, but all were weakly

¹ Osborne and Mendel [1920, 2].

² ——— [1919].

³ Sugiura and Benedict [1918].

Denton and Kohman [1918].

⁴ Osborne and Mendel [1920, 1]. (Commercial grape juice.)

⁵ Chick and Hume [1917, 1, 2].

⁶ Cooper [1913].

From such a series of experiments it seems justifiable to conclude that protein and the substance supplied in tomato, marmite, wheat germ etc. (? vitamin B) are of primary importance in the diet of a lactating animal.

(vi) *Experiments demonstrating adsorption by charcoal of the accessory factor.*

From the experiments just described it is clear that the protective substance has a distribution similar to that of vitamin B. It has been shown by Chamberlain and Vedder [1911] that the active principle (prepared from rice

polishings) which prevents polyneuritis in fowls is adsorbed by filtering through animal charcoal.

Cooper [1913] found that a curative solution prepared from horse flesh lost its activity to the extent of 30 % when filtered six times through a bed of animal charcoal. It was therefore decided to try if the protective substance shared this property of vitamin B. Accordingly the following experiments were made in which the mother rats were fed on marmite and wheat germ extracts which had previously been boiled with animal charcoal.

Fig. 4.

Mothers' diets

- 464 □ — □ 15 g. bread, 5 g. caseinogen, 0.5 g. butter, 0.7 g. salt mixture, 0.7 cc. lemon juice, 50 cc. egg yolk solution.
- 433 △ — △ 15 b., 5 c., 0.5 b., 0.7 salt, 0.7 lemon (see above), 50 cc. herring extract.
- 390 ● — ● " " " " 50 cc. meat extract.
- 515 ■ — ■ " " " " 50 cc. whey.
- 447 ● - - ● " " " " 50 cc. cod extract.

Preparation of extracts.

(a) *Wheat germ.* The extract was prepared as previously described (p. 828). Animal charcoal was added and the whole boiled. When boiling, a little of the liquid was taken in a spoon; if the charcoal settled rapidly and left a clear liquid on top, the whole was allowed to cool. If the charcoal remained suspended in the liquid, more charcoal was added and the boiling continued until the clear liquid settled out when tested in the spoon. When cool the volume was made up with distilled water to what it was before boiling, and the whole filtered. This clear filtrate was added to the diet.

Mothers' diets

396	● — ●	15 g. bread, 5 g. caseinogen, 3 cc. extract from marmite.
519	○ — ○	" " 3 g. wheat germ.
553	▲ — ▲	" " 50 cc. tomato juice.
N	— ● —	" " 50 cc. water (taken from Hartwell (1921, 1)) for comparison.

(b) "*Marmite*" (yeast extract). 6 % marmite solution was made with hot water. This was boiled with charcoal (as described above), cooled, the volume made up and filtered. This process was repeated (usually three times) until a clear, colourless filtrate was obtained. This was used for the feeding.

The diet was the same as that used in the preceding experiments, *i.e.* 15 g. bread, 5 g. protein, 0.5 g. butter, 0.7 g. salt mixture, 0.7 cc. lemon juice + 50 cc. of one of the above extracts.

Three experiments were made with each diet.

Results. When the mothers received the marmite extract which had been boiled with animal charcoal, the babies suffered badly and none survived. A typical curve is shown in Fig. 2 with the yeast "charcoal" extract, two litters died and one survived, though all had bad spasms. The young which survived were weakly and were not normal for some time after weaning.

The slight difference in these results may be accounted for by the fact that the marmite extract was boiled three times with charcoal and the wheat extract, only once, so that in the former case there might be more complete adsorption (Fig. 2, 527, 531, 533).

IV. COMMENTS.

Many natural foods or extracts made from them, contain a factor which protects the suckling rats from the harmful effects of excess of protein in the mother rat's diet.

The distribution of this factor is similar to that of vitamin B. The foods reported to be especially rich in this vitamin, *e.g.* egg yolk, wheat germ, when added to the mother's diet not only prevent the bad symptoms, but provide for good growth in the young. Grape juice when fed to the mother was of no effect in relieving the symptoms in the baby rats; all the 18 young (3 litters) died. According to Eddy the vitamin B content of grapes is represented by + (see Table, p. 832).

There are, however, discrepancies between my findings, the work of other physiologists, and the tables published by the Medical Research Council and Eddy.

Drummond [1919] finds that there are no appreciable amounts of the water-soluble factor in the muscles of cod and herring. Eddy gives the vitamin B content of cod as + and herring ++. According to the experiments described in this paper, the extracts from cod and herring muscle had appreciable protective properties, since no spasms were seen in the young, when such extracts were added to the mother rat's diet.

Again Eddy represents orange and tomato as equal and containing +++ and apple as ++. My experiments show tomato to be very superior to orange and that apple and orange are about equal. When the mothers received a ration of 50 cc. orange juice per day, the litters developed spasms, while a ration of 25 cc. tomato juice added to the mother's diet was sufficient to give complete protection to the young.

Soya bean gave interesting results (Eddy + + +, M.R.C. Report + +). When 3 g. were fed to the mother rats, the babies developed spasms and were in a very poor condition; but with 6 g. instead of 3 g., the litters

were practically normal, only slight spasms were noticed and the growth was good. Soya bean contains 33.7 % of protein and hence adding 3 g. soya bean to the mothers' diet also increased the protein ration. With 6 g., however, the vitamin content of the diet was nearly doubled, while the percentage of protein was very little different from that of the diet with 3 g. protein. On the whole my results agree more closely with those of Eddy than with those published in the Medical Research Council's Report.

It is possible that the disagreement may be due to:

1. Method of investigation—some observers find the curative dose, while others merely add a definite amount of the food under investigation to a diet deficient in vitamin B.

2. The method of preparation of extracts used, since vitamins easily form adsorption compounds.

Further the protective factor is similar to vitamin B in its solubility and its property of adhering to charcoal. Consequently there is presumptive evidence that the protective factor is vitamin B. This is interesting as vitamin B is thus definitely linked with protein metabolism; the one gland that synthesises protein in large amount functions badly in the absence of vitamin B when there is excess of amino acids in the blood. The lactating animal, when there is excess of protein in the diet, needs much more vitamin B than normal, not for herself, but to regulate the activity of the mammary glands.

SUMMARY.

1. When 50 cc. tomato, artichoke or carrot juices are added to the mother's basal high protein diet, the litters do well and show no harmful effects, such as would otherwise be produced by such a diet.

3 g. wheat germ or 50 cc. wheat germ extract give equally good results. Soya bean, 3 g., are ineffective, but 6 g. added to the above diet greatly improve the condition of the litters.

2. Apple and orange juices give good results, but not quite equal to those obtained with potato etc. The litters show good growth, but slight spasms are noticed. These do not last long and the growth curve is not impaired.

3. Wheat germ extract and marmite extract after being boiled with animal charcoal have no beneficial effect.

4. Beef muscle, herring, and cod extracts, when added to the mother's diet have also the property of protecting the young from any bad effects. Egg yolk has a similar protective action and the growth curve of the young is exceptionally good.

5. The substance in foods which exercises this protective action resembles vitamin B in its distribution, its solubility and the ease with which it is adsorbed by charcoal.

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