LXIX. EXCESS PROTEIN AND MAMMARY SECRETION.

By GLADYS ANNIE HARTWELL.

*From the Physiological Laboratory, Household and Social Science Department,
King's College for Women, Kensington, London.*

(Received June 17th, 1921.)

INTRODUCTION AND HISTORICAL.

THE experimental work relating to the effect of diet on mammary secretion is not extensive and what knowledge is available is of an indirect nature. Practically all the literature on this subject emphasises the fact that plenty of protein in the mother's diet is essential, but in a recently published paper [Hartwell, 1921] it was shown that excess of caseinogen in the mother's diet produced typical symptoms followed by death of the offspring, although the mother remained apparently healthy. The object of the present investigation was to find if this result were due to caseinogen specifically or to proteins in general.

Klein [1917] states that the same constituents are always present in cows' milk, but that the proportions can vary considerably. The caseinogen may be from 2 % to 5 % and the lactalbumin from 0.39 % to 0.95 %. Such variations are attributed partly to the nature of the feed and partly to external conditions, such as weather, change of milkers etc. Klein further suggests that sudden changes of feed and over-feeding should be avoided, and that milk from cows under such conditions frequently causes digestive and intestinal disturbances in infants. Larson and Putney [1917] quote an experiment performed at the Experimental Station, Wisconsin, in which some cows were fed on a heavy ration and others on a normal ration. The former did not maintain the full flow of milk during the experimental time any better than the latter, in fact "there was even a small amount in favour of the normally fed animals."

A diet of bananas, purified caseinogen and yeast or carrot-extract was fed to albino rats by Sugiura and Benedict [1918]. They found this mixture entirely suitable for the animals *except during lactation*. The mothers did well and the young flourished on the diet as soon as they could eat for themselves. No explanation of this strange result is offered, but in a later paper [1919] these observers find that adding 10 cc. of whole milk (or protein-free milk) to the above diet renders it adequate for perfect milk production of

albino rats. It seems possible in view of work quoted above [Hartwell, 1921] that this failure of the milk supply may be due to an excess of protein in the food of the mother. The proportion of protein in Sugiura and Benedict's diet based on dry constituents is 46.0 % and in experiments to be described in this paper 34.0 % protein in the mother's food is sufficient to prevent successful lactation.

Sugiura and Benedict further suggest that milk contains a new accessory substance which is needed for "suitable milk production by the mother."

Some investigations now in progress and to be published at a later date, also show that whole milk can antagonise the harmful effects produced by an excess of protein, but so far there seems no reason for postulating a new vitamin of the nature of a lactagogue.

Steenbock and Boutwell [1920] fed rats on a diet of maize, caseinogen and salts. Nine pregnancies occurred, but not one of the offspring was successfully reared. Some of the symptoms described, such as the lethargic state of the young, are similar to those to be described later, and hence it seems possible that the failure in rearing the young might be due to the mother's too great intake of protein.

The percentage of protein used by Steenbock and Boutwell is less than that of Sugiura and Benedict, but investigations have not yet gone far enough to allow of a definite statement with regard to the minimum of protein which constitutes an excess. Mitchell [1918] showed that if a large amount of protein was fed to rats, the tissues of the adult animals contained only a slight excess of amino-acids, while the tissues of the young animals contained a considerably greater proportion of amino-acids than on a normal diet.

If feeding an excess of protein to a lactating animal results in the milk having a higher proportion of protein, then the above results of Mitchell may throw some light on the fact that the mother remains apparently well, while the offspring exhibit certain typical and abnormal symptoms.

EXPERIMENTAL.

I. *Methods employed.*

The technique was the same as that described in a previous paper. A mixed diet of kitchen scraps and a little whole milk was given to the rats during gestation. The mothers and litters (reduced to six) were weighed daily and the protein rations started as soon as possible after the birth of the litter. Protein was given in the proportion 15.0 g. bread to 5.0 g. protein. In a few experiments a small quantity of a commercial preparation of meat extract (Jardox) was given to make the food more palatable, previous work having shown that this makes no difference to the ultimate results. The rats ate as much as they liked, the bread and protein being increased proportionately when necessary. The protein was finely ground so that the animals could not pick out and discard any of it. Water was given *ad lib.*

II. *Results.*

1. (a) *Diet of bread and egg albumin.* Water to drink. Fig. 1, 19. Four rats were fed on this diet. A typical curve of the weights of mother and babies is shown in Fig. 1, 19. In two of the experiments the mothers were given bread and milk for three days and the protein feeding was started on the 4th day. However, this made no difference to the final results, the babies eventually died, although for the first few days of protein feeding a better rate of growth was obtained than when the albumin and bread were given from the first day. Some of litter 19 (see curve) survived till the 17th day, another litter were all dead on the 16th day, some members of the third survived till the 20th day and three of another lived till the 26th day. These three were able to eat on the 18th day and although they were given bread and milk, they remained weak and developed bad diarrhoea.

The litters gained weight for about 12 days and up to the 10th day appeared normal, after which they became excitable. This stage of excitability lasted from two to five days and was followed by extreme weakness characterised by inability to crawl properly, a tendency to drag one hind leg and roll over towards that side. The babies were very weak and emaciated, and post mortems showed that the stomach was practically empty and the intestines had only a small amount of contents, largely mixed with gas.

Typical spasms were noticed, like those previously described when the mothers were fed with caseinogen. The babies which survived longest were sometimes able to walk though not normally. They walked on the tips of their toes (especially with the hind legs), seemed to lack co-ordinated movement, and frequently lost their balance and rolled over.

In one experiment the excitable period was very short, only one baby developed spasms and extreme weakness was evident at an early stage.

On the whole the spasms and squeaking fits were not so bad as in those litters whose mothers received caseinogen.

The mothers appeared well throughout the experiment and were able to maintain their weights, except one rat which lost slightly.

The time of onset and severity of the symptoms seem to bear no relation to the amount of food eaten by the mother.

(b) *Diet of bread and blood albumin.* Water to drink. Fig. 1, 15. Two rats were used in this experiment. One litter were all dead on the 16th day and the other on the 20th day. The results were identical with those described for egg albumin, except that there was a tendency in the mothers to put on weight.

2. *Diet of bread and gelatin.* Water to drink. Fig. 1, 47. Three experiments were made, and in two of them a small amount of a commercial preparation of meat extract (Jardox) was added because the animals did not eat the food very well. All three experiments, however, gave similar curves, which are represented by Fig. 1, 47. Two litters survived till the 16th day and the third

thinner than normal ones, and compared unfavourably with those whose mothers were fed on bread alone. The tendency to drag one hind leg and roll over towards that side was very marked. The mothers lost weight considerably, one rat lost as much as 36 g. in 15 days. The significance of this is discussed in another part of the paper (p. 572).

3. *Diet of bread and gluten.* Water to drink. Fig. 1, 88. The gluten was prepared from flour by removing the starch in the usual way by kneading in muslin. When all the starch had been removed the protein was dried in an air oven and then finely ground up. Three experiments were done and the litters all died. One survived only till the 9th day, another till the 16th, and the third till the 24th day. The babies became excitable and very restless, but no actual spasms were noticed. As with gelatin extreme weakness was characteristic, and post mortems likewise showed the stomach and intestines to be practically devoid of contents.

Two of the three mothers lost weight, but the loss was not so great as with gelatin.

4. *Diet of bread and edestin.* Water to drink. Fig. 1, 17. Lactagol was used as a source of edestin. Three experiments were made and at first, in all three, a very good growth curve was obtained for the young, a greater rate of growth being shown than with any other protein (except caseinogen). The typical symptoms in the babies developed slightly later, the excitability not being shown till about the 12th day. In one experiment four of the litter survived (bad symptoms were not exhibited till the 15th day) and were successfully weaned. However they were not normal till about eight weeks old. At seven weeks they still showed the typical toe-walking (previously described) and exhibited in co-ordinated and jerky movements of their hind legs.

In the other litters the spasms were very bad, and were especially marked when the babies tried to eat. As soon as a baby took food in its mouth, the head and neck went rigid, the jaws remained open for a time, then slowly relaxed, but the rigidity returned as soon as another mouthful was taken. Post mortems showed the typical absence of contents in the alimentary canals.

In one experiment the mother was killed and the mammary glands, when cut, contained little or no milk. In all cases the mothers appeared well and either maintained or increased their weight, but the condition of the litters was as bad as that of the babies of the caseinogen-fed mothers.

5. *Diet of bread and peptone.* Water to drink. Fig. 1, 13. These experiments were not very satisfactory, because after about four days' feeding with peptone the mother had diarrhoea, possibly due to the toxic effects of the peptone. When peptone was fed to the mother immediately after the birth of the litter, the babies died within a week. In two experiments they died on the 3rd day and in another they survived a week. In this last experiment the litter gained only about 2 g. per day. The usual typical symptoms were absent, the babies merely became very weak and died. Post mortems showed absence of contents in the alimentary canals. In another experiment it was possible to continue the peptone feeding for nine days, but the mother had bread and milk for a week first, *i.e.* the litter were eight days old when the peptone feeding was started. These babies gained a small amount the first day of the peptone diet, and then lost a little each day. Two days after the peptone was given to the mother, they showed slight excitability, but no spasms. Later they

became weak, but soon ate for themselves and so survived but were weakly for some time.

6. *Diet of bread and boiled meat.* Water to drink. Fig. 2. Lean beef was used in these experiments. It was minced, extracted with distilled water in

Fig. 2. Bread and boiled meat.

66	65	15 bread	5 meat	49	15 bread	15 meat
50	15	"	10 "	51	15	" 45 "

* Babies eating.

a boiling water-bath for half an hour, strained and again extracted with more distilled water for three quarters of an hour. The protein was then strained through muslin and squeezed as dry as possible.

(a) *Experiments 66 and 65.* 15.0 g. bread; 5.0 g. meat protein. Rat 65 had two babies of her own and five foster babies. The former were five days old when the experiment was started. They survived and were successfully weaned, but were weakly and exhibited the typical toe-walking already described. The foster babies all developed typical symptoms, had bad spasms and squeaking fits and all died.

Litter 66 all had spasms, but survived and could eat for themselves. They never became normal¹.

(b) *Experiment 50.* 15.0 g. bread; 10.0 g. meat protein. The litter were all dead on the 10th day, they had no spasms, but were very weak. Post mortems proved the alimentary canals to be empty.

(c) *Experiment 49.* 15.0 g. bread; 15.0 g. meat. The babies were never excitable and had no spasms. About the 15th day they seemed weak and walked badly. However, they recovered, ate at the usual time and were successfully weaned.

(d) *Experiment 51.* 15.0 g. bread. 45.0 g. meat. The litter were absolutely normal in all respects and exhibited none of the typical symptoms at any stage.

From Fig. 2 it is easily seen that the rats 49 and 51 (whose litters did best) lost a considerable amount of weight during lactation. This is not fully understood, but in several experiments when some litters survived and others did not, the survival was coincident with a big loss in weight of the mother. Also from other experiments rats 49 and 51 are to be regarded as particularly "good milkers." Further the loss in weight of the mothers might be explained by assuming that they are mobilising an antagonistic factor from their own tissues.

7. *Diet of bread and dried meat.* Water to drink. Fig. 3. The meat was prepared as in the last series of experiments then completely dried in an air oven. The ration consisted of 15.0 g. bread and 5.0 g. dried meat. As can be seen from Fig. 3 the growth of the litters was at first fairly good, but after the 12th day there was practically no gain in weight. From this time, also, the mothers showed considerable loss in weight. The usual symptoms were absent, a few babies showed excitability and some weakness, but only to a slight degree. In all three experiments the young were not normal, but were lethargic and disinclined to move or crawl about. When taken from the cage and put on the table, they had no inclination to sniff around as would a normal rat, but remained almost motionless where they were put. In one litter three died the week after they were weaned, though fed on bread and milk. The others survived, but were weakly for some time. It is probably signi-

¹ They were all killed by the male, put in when the mother was removed. It was suggested to me by Professor Mottram that an old male is very useful for keeping with the young when they are weaned. (Technique used in Cambridge Laboratories.) As a rule it works extremely well and the male is very good to the young. He will even allow them to take food out of his mouth.

ificant that the large loss of weight of the mother coincides with the maintenance of the litters at a period when, in other experiments, the babies would have lost weight and died. As a rule when the mother is fed with protein, she either maintains or puts on weight. Whether the observed loss indicates

that the mother is supplying some essential constituent to the milk, or that she is producing some substance which inhibits the spasms, or antagonises the toxic substance responsible for this condition in the babies, remains to be proved.

8. *Diet of bread and dried fish.* Water to drink. Fig. 4. Cod was used for these experiments and the dried protein prepared in the same way as the dried meat protein. The ration consisted of 15.0 g. bread and 5.0 g. dried fish.

These results are very similar to those obtained when the mothers were fed with boiled meat. Since the individual results differ, it would perhaps be better to describe each experiment separately.

Fig. 4. Bread and dried fish protein.

* Babies eating.

Experiment 79. The growth of the litter shows a typical "protein curve" and the usual symptoms were noticed during the course of the experiment. The spasms were marked and affected all the babies at different times.

Experiment 82. The babies were normal up to the 12th day, when they were excitable, but developed no spasms.

They still gained weight until the 16th day, after which they lost small amounts each day. They were decidedly weakly, two died, and the others did not eat till the 26th day (a normal baby eats about the 18th day). Two more died on the 27th day and only one survived. This was not normal for three weeks after weaning.

Experiments 67 and 81. The litters appeared normal in every respect, except that they were slightly excitable and only gained small amounts towards the end of lactation.

Experiment 69. This litter resembled litters 67 and 81 except that they were more excitable and one exhibited slight spasms.

In all the experiments the mothers remained well, their weights varied somewhat but there was no great loss.

Experiment 96. 15.0 g. bread; 6.0 g. dried fish. The litter were all dead the 13th day, but the curve is by no means typical, compared with other experiments. The litter gained weight up till the 12th day, when they appeared very weak. No spasms were noticed and the usual stage of excitability was absent.

DISCUSSION OF RESULTS.

1. *The amount of protein.* From these experiments it is clear that a lactating rat should not have a large proportion of protein in the diet. If too much protein is given, the litter rarely survive. The exact amount of protein which constitutes excess has not been determined, but it is in the neighbourhood of 33 %. It is probable that with different proteins a different proportion will constitute excess. A rat can suckle her young satisfactorily (*i.e.* the babies are normal in all respects and are successfully weaned) when fed on white bread alone [Hartwell, 1921]. Such a diet contains a very low proportion of protein, about 14 %. It is therefore possible for the rat to bring up a healthy litter when she is getting only a small amount of protein. However it is certain that a greater proportion promotes better growth, but it is obvious that such addition constitutes a danger. Where the danger line is, has to be determined by future experiments.

2. *Biological value of the proteins.* That there is some relation between the biological value of the proteins and

- (i) the growth of the young,
- (ii) the occurrence of spasms,
- (iii) the ultimate loss of weight and death of the young, and
- (iv) the mother's weight during lactation

is clear. Edestin and caseinogen were the best proteins given [see analyses by Matthews, 1916] and the young grew, showed worse spasms, and were more certain to die, while the mothers put on weight. On the most defective protein (gelatin), the young rats grew slowly for a time, became weak, showed no spasms and died. The mothers lost weight rapidly. This decrease could not be stopped by meat extract, so it is suggested that it is not due to loss

of water, but an actual tissue breakdown. There is too with other proteins a correlation, though by no means complete, between the loss of weight by the mother and the evil effects on the young. One cannot help thinking that the mammary gland cannot function properly when flooded with an excess of amino-acids and that, up to a point, the mother can antagonise that effect by mobilising something from her own tissues, perhaps vitamin, perhaps carbohydrates, perhaps salts, but improbably fats or proteins.

3. *Origin of protein.* The edestin and gelatin were commercial products, the former being used in the form of "lactagol." Hence it is possible that the results might to some extent be due to their method of preparation, or to some impurity, such as acid. Since the home-made gluten, fish and meat proteins give similar results, it is improbable that the effects are due to the "denaturing" of the proteins by commercial methods of preparation.

4. *Deficiencies in the diet.* An obvious criticism of these experiments is that a deficient diet was given, fat and vitamins being almost entirely absent. That the results are not due to the absence of these constituents is suggested by the fact referred to above, *i.e.* that a rat can bring up a healthy litter when fed with white bread alone, a diet equally deficient in fat and vitamins. But perhaps the animal may be able to tolerate a slightly greater proportion of protein when the diet is physiologically complete; the greater the amount of food metabolised the greater the need for vitamins¹.

The addition of protein makes the diet less deficient in amino-acids, except when gluten is added. Gluten with albumin forms a more balanced diet than gluten alone. None the less gluten alone is good, but gluten with other proteins, or in simple excess, is bad, therefore the bad effects on the litters must be due to the excess of protein.

5. *Variations in results caused by meat and fish proteins.* The results obtained with meat and fish proteins are obviously different from those of caseinogen, edestin, albumins, gelatin and gluten. In some experiments the litters die, exhibiting typical symptoms, exactly comparable to those seen when the mothers eat caseinogen etc. In others the litters do not suffer so badly and in a few the babies appear almost normal. Two explanations are suggested:

(i) The proteins may not be pure and consequently a smaller quantity is really being given than with pure caseinogen etc. The exact amount constituting excess has not been determined. Since the proportions used do not seem very excessive in comparison with a normal diet, it may be that the border line of excess is about the level used. If so, then the individual metabolism of the rats is a factor to be considered and what constitutes excess for one might be more easily metabolised by another. Certainly some very fine rats can tolerate more caseinogen than others.

(ii) Experiments now in progress show that the harmful effects of excess

¹ Experiments to elucidate this point are now in progress; up to the present time, no normal litters have been reared on any diet containing large amounts of protein.

protein can be antagonised by whole milk. If some specific substance in milk is responsible for this, then such a substance might be found in animal tissues, and might, therefore, be present in the meat and fish dried proteins.

SUMMARY.

1. The observation published in an earlier paper, *i.e.* that a diet containing 40 % protein (by dried weight) fed to nursing rats, is detrimental to the young, has been confirmed and extended to commercial edestin, blood and egg albumin, gelatin and home-made gluten. Such dietaries apparently render the milk poisonous and finally check its flow.

2. Home-made fish and meat proteins do not always cause such harmful results as edestin, albumin and gelatin, but in some cases the litters die, having exhibited typical symptoms. In others the babies survive, but only a few are normal.

3. In spite of the ultimate ill effect of edestin (and caseinogen) the immediate effect of its addition to bread is to produce a maximal growth curve of the young, *i.e.* as good as when the mother's diet is bread and milk [Hartwell, 1921]. Other proteins used produce slower growth. Toxicity is not shown till the 10th or 11th day.

4. The toxicity of the mother's milk is demonstrated by the spasms etc. The cessation of the flow of milk is shown by the litter's loss of weight and the emptiness of their alimentary tracts, and this is not due to absence of suction power, because the babies are found sucking each day while they are losing weight.

5. There is some correlation between increase in the weight of the mother and danger to the offspring; if the mother puts on weight, the suckling rats suffer; if she loses weight, she may be able to spare them some of the symptoms. Gelatin, however, is an exception, because the mother loses a considerable amount of weight and yet the babies all die.

6. It is unlikely that the effect is due to an absence of vitamin, because a rat can bring up a healthy litter when she is eating white bread alone, but it is probable that it is rather due to an excess of protein in the diet. This is being worked out.

Part of the cost of these experiments was borne by the Medical Research Council to whom my best thanks are due.

I have also to acknowledge my indebtedness to Professors E. Mellanby and Mottram for criticism and encouragement.

REFERENCES.

- Hartwell (1921). *Biochem. J.* 15, 140.
Klein (1917). Principles and Practice of Milk Hygiene.
Larson and Putney (1917). Dairy Cattle Feeding and Management.
Matthews (1916). Physiological Chemistry, 129.
Mitchell (1918). *J. Biol. Chem.* 36, 501.
Steenbock and Boutwell (1920). *J. Biol. Chem.* 41, 81.
Sugura and Benedict (1918). *J. Biol. Chem.* 36, 171.
— (1919). *J. Biol. Chem.* 40, 449.