XIV. MAMMARY SECRETION. III.

1. THE QUALITY AND QUANTITY OF DIETARY PROTEIN.
2. THE RELATION OF PROTEIN TO OTHER DIETARY CONSTITUENTS.

By GLADYS ANNIE HARTWELL.

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

*Thesis approved for the Degree of Doctor of Science in the
University of London.*

(Received December 21th, 1921.)

INTRODUCTORY AND HISTORICAL.

In a previous paper [Hartwell, 1921, 2], it was shown that large quantities of protein in the diet of a lactating rat were detrimental to the young. The diet consisted of bread and protein in the proportion 15.0 g. bread to 5.0 g. protein. An obvious criticism was that such a diet is deficient in fat and vitamins, but it was pointed out that it was unlikely the bad effects were due to deficiency since the rat could bring up a healthy litter on a diet of white bread alone, a diet equally deficient.

It is generally held that the diet of a nursing mother should be rich in protein, because young growing animals need large amounts of protein in their diet. It is probable that if large quantities of protein are ingested, some other factor must be present in the diet to safeguard the young from such harmful effects as those previously noted. The object of the present investigation was, therefore:

1. To find out what proportion of protein can be fed to the mother without danger to the offspring.
2. To determine what other factor or factors should be included in the diet in order that the large quantity of protein may be effectively metabolised.

It is probable that not only should the diet be physiologically complete, but that a greater quantity of one or more constituents might be necessary when the intake of protein is high. For instance, when large quantities of fat are ingested, it is essential that carbohydrate should be included in the diet; otherwise the fat is not properly metabolised and acetone bodies are excreted.

It is also probable that the large amount of protein deemed necessary has been somewhat overestimated, and that less amounts would give equally good results provided that the diet was satisfactory in other respects.

That excess of protein in the mother's diet has detrimental effects on the young is evident from the experiments of other workers, but such results have been explained differently.

The experiments of McCollum and Simmonds [1918] are on almost identical lines with those described in this paper. These observers, however, were working with diets which were inadequate for growth of the young, and the main object of their research appears to be to show how far "the mother can serve as a protective agent in producing milk suitable for the nutrition of the young," when she herself is getting a deficient diet. A great difference between the work of McCollum and Simmonds and that of the research now being described is the duration of the experiments. From the former's curves it appears that the mother was left with the young for 40 days or more, which seems rather long, considering that a normal baby rat is perfectly capable of living alone from the 21st day. In one or two of their experiments the symptoms of the litter closely resemble those described in this series of experiments. In McCollum's experiment 738 the mother rat was fed on rolled oats alone, and certain symptoms were noted in the young. "On about the 20th day a few of the young would throw themselves about the cage, and scream during this performance. Several of the young went into coma and died."

In some experiments where the mother appeared to have a high tolerance for protein, the babies behaved in the way McCollum describes about the 20th-24th days; they had shown no symptoms at the earlier stage. Again in experiment 948 he says that "the young all died at a very early date. The intestines were filled with gas. We are unable to account for the high mortality of these young." The diet used in this case was rolled oats 82.0 and caseinogen 18.0, *i.e.* a greater proportion of protein than when the rats had rolled oats only. The young died at intervals from the 3rd to the 23rd day. Seven experiments were made.

The condition of the young certainly suggests a similar state to that found in this work [1921, 2], but only a few of the typical symptoms are recorded by McCollum and Simmonds. In their paper they state that the babies were weighed "frequently" and the curves are plotted at intervals of eight days. Unless daily weighings were made, and the babies carefully examined, it is highly probable that the spasms would be overlooked, since this stage is frequently of short duration, sometimes lasting only a few hours. The screaming fits exhibited by the older babies are much more obvious, the noise being distinctly heard in the next room.

It is possible that these results are due to a deficiency of some dietary factor, but this deficiency is only evident when the diet is too rich in protein and therefore it may be that they are directly due to the excess of protein. A similar diet, but with less protein, would not be deficient.

GENERAL CONDITION OF THE ANIMALS.

Only normal healthy animals were used in these experiments. If any rat became ill the results were discarded. There was no trouble with scab, pneumonia, or epidemic diseases. McCollum [1920] says that the "palatability of the ration is the most important factor in animal nutrition," and it is a well-known fact that many animals will starve rather than eat food which is distasteful to them. The rats used in this work readily ate any of the mixtures offered to them, and in some cases consumed enormous quantities. For instance, many rats were taking as much as 40.0 g. bread (+ added protein and other constituents) on the ninth day of lactation. The initial ration of 15.0 g. bread etc. was usually adequate for the first two days, after which the animals' appetites increased rapidly. The cages were examined for any hidden food, the "food-hoarding" instinct being especially marked in rats.

The males and females were kept together until a few days before the birth of the litters, when the mothers were removed and placed in separate cages. After lactation the females were kept from the males for about two weeks to avoid over-strain of the maternal organism, and to give the mother a chance of recovery, should the special diet have upset her in any way. This method of procedure was adopted merely as a safeguard, because even when the litters died, the mothers appeared fit and well. Except during the actual period of lactation (and with one exception pointed out later) the males and females were fed on a diet of kitchen scraps, supplemented with small quantities of whole milk and white bread. This mixture proved a very good one and, as is shown later, the growth curves of the young animals fed on such a diet, are better than those given by Donaldson [1906] as normal for the white rat.

Some white rats, and some white and black were used. It is possible that the latter should be somewhat heavier, since Donaldson states that black rats are heavier than white ones, yet there appeared practically no difference in size and weight.

METHODS.

The technique employed was the same as that previously described [Hartwell, 1921, 1].

The litters, as before, were reduced to six. In a few cases less than six were born, and this is noted in the text and on the curves. The mothers and babies were weighed daily and the weights recorded graphically. In the "foster" experiments to be described later, the number in the litter varies, but is always stated.

The foodstuffs used. (a) Pure caseinogen was not obtainable when these experiments were in progress, and a commercial form, "food casein,"¹ was therefore used. Experiments showed that the results obtained with "food casein" were exactly similar to those previously found with pure caseinogen.

¹ I am indebted to Casein, Ltd., for supplying me with the composition of "food casein."

The "food casein" was 83 % pure, and the amounts given were calculated in terms of pure protein.

(b) Pure egg albumin (as supplied by Baird and Tatlock) was used. It was very finely ground, in order to obtain an even mixture of the foodstuffs fed.

(c) A commercial preparation of edestin was used. It was tested at the beginning of the experiments and found to contain only slight amounts of impurities. A new sample was tested lately and found to contain cane sugar. Every sample was not analysed, and as that obtained lately was less pure, it is possible that the results obtained with 1 g. and 2 g. edestin are due to the fact that smaller amounts of protein were actually fed.

To find what amount of protein is really excess, it would be essential to work with absolutely pure foodstuffs.

(d) The lactose was pure, as supplied by Baird and Tatlock.

(e) The salt mixture was that recommended by Mottram, and is a modification of the original one used by Hopkins.

Sodium chloride NaCl	46.25 g.
Magnesium sulphate (anhyd.) MgSO_4 ...	71.20
Acid sod. phosphate $\text{NaH}_2\text{PO}_4, \text{H}_2\text{O}$ ...	92.68
Di. pot. phosphate K_2HPO_4	254.60
Calcium tetrahydrogen phos. $\text{CaH}_4(\text{PO}_4)_2\text{H}_2\text{O}$	144.20
Calcium lactate $\text{Ca}(\text{C}_3\text{H}_5\text{O}_3)_2 5\text{H}_2\text{O}$ ...	347.00
Ferric citrate $\text{Fe}(\text{C}_7\text{H}_6\text{O}_7) 1\frac{1}{2} \text{H}_2\text{O}$ ...	31.52
Sodium fluoride NaF	.55
Manganese sulphate $\text{MnSO}_4 5\text{H}_2\text{O}$ ...	2.00
Potassium iodide KI	10.00
Total...	1000.00

Water, *ad lib*, was always given unless the rats were having large quantities of milk.

1. THE QUALITY AND QUANTITY OF DIETARY PROTEIN.

In some recent experiments [Hartwell, 1921, 2] the initial diet consisted of 15.0 g. bread to 5.0 g. protein, both being increased proportionately. Only a very few babies survived and those which lived were not normal, therefore in these experiments less protein was given to the mother, in order to see if the litter would then survive. Small quantities of commercial yeast extract, marmite (about 0.2 g. per day) were added to the diet to make it more palatable. It has been found that small amounts make no difference to the final results and the animals ate such a mixture more readily.

Three experiments were made with each protein. This involved 9 mothers and 54 young. The diet was not physiologically complete except in two of the experiments, when 0.5 g. butter, 0.7 cc. lemon juice and 0.7 g. salt were added to the mixture; this made no difference to the final results.

(i) *Proportion of 15 g. bread to 1 g. protein.*

(a) Caseinogen. Fig. 1 a.

Exps. 160 and 173. 15.0 g. bread, 1.0 g. caseinogen, marmite.

Exp. 175. 15.0 g. bread, 1.0 g. caseinogen, marmite, butter, lemon juice and salt mixture.

Litter 160 were perfectly normal in every respect. One was weakly and died three days after weaning, but no typical symptoms (previously described [1921, 2]) were exhibited.

Fig. 1 a. Caseinogen.

Litter 173 exhibited slight symptoms. However they recovered rapidly, the mother was removed on the 21st day, when all the babies appeared normal. They all survived weaning. On the 21st day, the average weight of babies 160 was 30.6 g. and that of litter 173 was 26.0 g. From the normal curve according to Donaldson [1906] the weight at 21 days is about 21.0 g. Hewer [1914] states that she used for subsequent feeding experiments any young rats which weighed at least 23.0 g. at weaning, so it would appear that both litters described above were well above average weight, in spite of the fact that slight symptoms developed in 173. It is possible that "breed" and number in the litter are factors influencing the actual weight of the babies. This is discussed later in the paper.

Litter 175 showed bad symptoms and were all dead by the 19th day.

The mothers all remained well. They were not full grown at the beginning of the experiment and put on a considerable amount of weight.

(b) Edestin.

Exps. 141 and 195. 15.0 g. bread, 5.0 g. edestin, marmite.

Exp. 163. 15.0 g. bread, 5.0 g. edestin, marmite, butter, lemon juice and salt mixture.

All litters were normal in every respect, none of the typical symptoms were noticed in any one baby, not even excitability. The rate of growth in the middle part of lactation was extraordinarily good and apparently maximal (*i.e.* as good as when the mother is fed on bread and milk, a diet previously shown to give the best results).

Fig. 1 b. Egg albumin.

The mothers, however, showed a slight loss of weight during the first two weeks and this loss became very marked during the last week of lactation.

This seems rather extraordinary because during the last part the young were able to eat for themselves, and were not entirely dependent upon the mother.

(c) Egg albumin. Fig. 1 *b*.

Exps. 190, 206, 207. 15.0 g. bread, 1 g. egg albumin, marmite.

In these experiments no litter was quite normal, but the mothers were removed on the 21st day and all the babies were successfully weaned.

Litter 190 showed only slight spasms, and recovered rapidly. Two days after the mother was removed they were normal, but small and somewhat thin.

Litter 206 were very similar to 190, but their condition was a little worse and they were not normal till a week after weaning. It will be seen from Fig. 1 *b* that this litter lost weight for three days before they began to eat for themselves.

Litter 207 showed worse symptoms than 190 and 206 but eventually all the babies recovered.

At first the mothers maintained their weights or showed a slight loss, but from the 11th or 12th day they lost weight considerably. In ten days two of them lost approximately one-quarter of their body-weight and the third lost about one-fifth body-weight. The significance of this is discussed later (p. 86). Thus when 1.0 g. protein to 15.0 g. bread was fed to the mothers, slight symptoms developed in the litters, except with edestin. This is probably due to the fact that commercial edestin was used and, therefore, the mothers were receiving less amounts than with the other proteins.

(ii) *Proportion of 15.0 g. bread to 2.0 g. protein.*

(a) Caseinogen. Fig. 2.

Exps. 161 and 198. 15.0 g. bread, 2.0 g. caseinogen, marmite.

Exp. 176. 15.0 g. bread, 2.0 g. caseinogen, marmite, butter, lemon juice and salt mixture.

Litter 198 were normal in all respects except that they were somewhat excitable and, as will be seen from the curves, gained only small amounts the two days before they were able to eat for themselves.

Litter 161 showed the typical "excess protein" symptoms, but two lived till the 21st day and survived weaning, although they were not normal till two weeks after.

Litter 176 were in a worse state than 161 and were all dead by the 18th day.

The mothers 161 and 176 put on weight, whilst 198 lost weight. In this connection it is interesting to note that 198 litter were normal, but 161 and 176 suffered badly. This is in agreement with a suggestion previously made that there is some correlation between the weights of the mother and babies.

(b) Edestin.

Exps. 143 and 215. 15.0 g. bread, 2.0 g. edestin, marmite.

Exp. 197. 15.0 g. bread, 2.0 g. edestin, marmite, butter, lemon juice, salt mixture.

Litter 215 were normal except that they developed slight "toe walking" towards the end of lactation. They recovered rapidly and were successfully weaned. Two of the babies died the 2nd day and, therefore, the mother had

only four to suckle. Since the average weight of these (see table on p. 87) is so much better than that of the two other litters, it is possible that it can be accounted for in this way. The fact that this rat may have had a greater tolerance for protein is also a factor to be considered.

Litter 197 exhibited all the usual symptoms to some extent, but were never very bad. They soon recovered and were successfully weaned.

Litter 143 developed very bad spasms accompanied by the usual symptoms. One died the 19th day and the others were all dead two days after the mother was removed.

Fig. 2. Caseinogen.

The mothers' curves are somewhat irregular, but on the whole show a maintenance of weight.

(c) Egg albumin.

Exps. 189, 208, 209. 15.0 g. bread, 2.0 g. egg albumin, marmite.

All the litters survived and none showed any spasms.

On the other hand none of the babies was quite normal. Litter 189 did best, but were somewhat excitable. Litter 208 were very excitable, and showed "toe walking."

Litter 209 had similar symptoms to a greater degree and were cold and very weak.

The mothers appeared well, but, as in the case of those which received 1.0 g. egg albumin, they lost weight rapidly.

DISCUSSION OF RESULTS.

The average weights of the young rats at 21 days are given below:

Caseinogen	Edestin	Egg albumin
1.0 g. protein:		
30.6	31.16	27.33
26.0	31.4	24.66
All died	31.16	18.16
2.0 g. protein:		
30.33	33.0 (after 2nd day only four)	29.16
22.0 (only two survived)	24.33	23.5
All died	20.40	19.33

(i) *Irregular growth of the young.*

It has been previously pointed out [Hartwell, 1921, 1] that on any given diet for the mothers, similar growth curves could be obtained for the litters, and that such curves could be regarded as characteristic for that specific diet. This statement now needs some modification.

The experiments described in this paper show that on any given diet the growth curves of the sucklings are by no means identical. It seems probable that when sufficient protein is given to produce bad symptoms in the young, then the above statement no longer holds good. This is well illustrated by the figures above. The average weights of a 21 day rat when the mother received 1.0 g. edestin are practically constant. These litters were normal in every way, so it appears that this amount of protein was not excessive. It is possible that less than 1 g. protein was given in these experiments, since recent samples of the commercial protein have been shown to contain cane sugar as impurity.

In all the other experiments, varying degrees of abnormality were produced and the weights are by no means constant.

This point is again illustrated by experiments to be described later on in this paper.

Thus with sufficient protein in the mother's diet to produce bad symptoms in the young, the individual metabolism of the mother rat seems to play an important part.

The mother's initial weight is not a factor to be considered, since on any given diet the babies of a heavy rat may die while those of a lighter rat may survive, or *vice versa*.

(ii) *Correlation between weights of mothers and offspring.*

It was previously suggested that there was some correlation between the weight of the mother and condition of the offspring, and that if the mother lost weight she might be able to spare the babies some of the symptoms. This suggestion is confirmed by these experiments.

In Fig. 2 litter 198 have a fair growth curve, and showed no symptoms. The mother lost weight. Litters 176 and 161, on the other hand, exhibited

typical symptoms and only two of the 12 survived. These mothers put on weight.

In both sets of experiments (*i.e.* 1.0 g. and 2.0 g. protein) it is very obvious that the mothers fed on egg albumin lost weight considerably.

Since albumin is a less good protein than either edestin or caseinogen, it may be that the mother is supplying the necessary amino-acid constituents from her own tissues.

Even 5.0 g. egg albumin to 15.0 g. bread results in some loss in weight of the mother, in a deficient diet [Hartwell, 1921, 2], but practically no loss is found when that proportion is given in a physiologically complete diet (experiments described later).

From the following figures it can be seen that, in some cases, the mother rat lost as much as one-third of her body weight; moreover this loss was very rapid and occupied only ten days.

	Exps.	Weight at beginning of lactation. g.	Weight at end. g.	Loss g.
1.0 g. egg albumin:	190	221	164	57
	206	228	159	69
	207	168	142	26
2.0 g. egg albumin:	189	247	165	82
	208	214	174	40
	209	149	128	21

Rat 207 lost less than 190 and 206 and her litter were in a worse condition and gained less weight than those of 206 and 190.

Again, in the other series, rat 209 lost less and her litter were the worst.

(iii) *The amount of protein constituting excess.*

It is not possible to give a definite statement as to the proportion of protein which should be fed to a lactating animal, since with different proteins, a different amount will constitute excess. For instance, good growth curves were obtained when the mother was fed with 15.0 g. bread and 1.0 g. edestin, but in the three litters whose mothers had 1 g. egg albumin, there were no normal babies; with caseinogen, one litter was normal, one had slight symptoms and the babies of the third litter all developed bad symptoms and died. As previously explained (p. 81) there is a possibility of impurity in the edestin and hence the animals might be getting less than 1.0 g. of protein, which might account for the slightly better results obtained with the edestin.

It is probable that the amount of protein constituting excess will vary with the presence or absence of other dietary constituents, and that such constituents must be considered *quantitatively* in relation to the protein. This point will be dealt with later in the paper. With caseinogen and edestin, a diet which is complete physiologically from a qualitative point of view gives just as bad results as one not physiologically complete. For example rat 176 received butter, lemon juice and salt mixture in addition to bread, caseinogen and marmite, thus completing the diet qualitatively, yet her litter were worse

than those of 198 and 161, whose mother had only bread, caseinogen and marmite.

(iv) *The quality of the protein.*

Certain typical symptoms, already described, were characteristic of the litters, irrespective of the protein fed to the mothers. With egg albumin, however, some extra symptoms were noticed. The weakness appeared to come on at an earlier stage, the babies were thinner and were very cold to the touch. This coldness was observed at an early age and was frequently the first indication that something was wrong. It was evident even when the symptoms were slight and the babies recovered. It is possible that the thinness of the babies is due to less good milk being produced by the mother. It is suggested that egg albumin is deficient or poor in certain amino-acids which are essential for the production of milk of adequate nutritive value. To prove such a point it would be necessary to feed with pure amino-acids.

2. THE RELATION OF PROTEIN TO OTHER DIETARY CONSTITUENTS.

(i) *The effect of large quantities of protein in a physiologically complete diet.*

Hartwell [1921, 2] showed that an initial diet of 15.0 g. bread and 5.0 g. protein fed to nursing rats was detrimental to the young. It was, however, pointed out that such a diet was deficient physiologically, yet it was unlikely the bad symptoms were due to such deficiencies, since the rat can bring up a healthy litter when fed on white bread alone [Hartwell, 1921, 1], a diet also lacking in fat, vitamins and salts.

In these experiments the proportion of protein to bread was kept the same as that used in the previous investigation (*i.e.* 15.0 g. bread to 5.0 g. protein), but 0.5 g. butter (fat and vitamin *A*), 0.2 g. marmite (vitamin *B*), 0.7 cc. lemon juice (vitamin *C*) and 0.7 g. salt mixture (composition given on p. 81) were added to complete the diet.

Three proteins were tried, caseinogen, edestin, and egg albumin and in each case two series of experiments were done.

1. The diet started as soon as possible after the birth of the litter; never more than 24 hours.

2. Diet started before the birth of the litter, and at different stages of pregnancy.

The bread and protein were increased proportionately as required, but the other constituents remained constant.

(a) Caseinogen. Fig. 3.

Exps. 113, 194, 171. Diet started *at* birth of litter.

Exp. 126. Diet started 10 days *before* birth of litter.

"	133.	"	13	"	"
"	134.	"	16	"	"
"	166.	"	27	"	"

The gestation period in the rat is given by some observers as 21 and by others as 23 days, so in the second series of experiments the rats were getting the excess protein diet for at least half the gestation period. Rat 166 received this diet during the whole of gestation. In this animal, either the gestation period was longer than normal, or else fertilisation was delayed, because she was separated from the males when the special feeding was started and the litter were not born for 27 days.

A. Feeding started after the birth of the litter.

Litter 113 became excitable on the 9th day and from that time onwards showed signs of being hungry.

No bad symptoms became evident until the 19th day when four of the litter had very bad fits, screamed, rushed round their cage, bit each other and hung on to the cage with firmly fixed jaws. These fits lasted from one to five minutes. Afterwards the babies were very exhausted and would sprawl on the floor of the cage. At this stage the mother frequently removed them to the nest and covered them up. The mother was taken away on the 21st day and all the litter survived, in spite of the fact that bad screaming fits were again noticed on the 22nd day.

Litters 194 and 171 gave the typical "excess protein" curves and all died. The condition of the alimentary canals of the babies was exactly the same as previously described [Hartwell, 1921, 2].

B. Feeding started before the birth of the litter.

The actual duration of the feeding before birth seems to be an unimportant factor, since all four curves are similar and the babies were all dead either the 14th, 15th, or 16th day, irrespective of when the feeding was started. In some experiments to be described later (p. 92) the mothers were fed on the caseinogen diet from the time they themselves were weaned and had, therefore, been eating this mixture for 70 days before the birth of the litter.

(b) Edestin.

Exps. 177, 112.	Feeding started <i>at</i> birth of litter.			
Exp. 124.	Feeding started	2 days	<i>before</i>	birth of litter.
" 146.	"	11	"	"
" 132.	"	21	"	"

A. Feeding started at birth of litter.

Litter 177 showed quite good growth up to the 11th day, after which they only gained small amounts. They exhibited all the typical symptoms, though not so markedly as usual. They ate for themselves on the 16th day and were all successfully weaned. They were not normal for about ten days.

Litter 112 grew much more rapidly than did 177, but they developed bad symptoms earlier and were much worse in every way. Only one survived and it was weakly for a month after being taken from its mother.

B. Feeding started after the birth of the litters.

In these experiments the time of feeding before birth bears a definite and inverse relation to the time the babies lived, but this does not apply to the litters whose mothers were fed with caseinogen and egg albumin.

There is no reason to describe the experiments separately, or in detail, as the results are similar to those already described for caseinogen.

(c) Egg albumin.

Exps. 111, 193, 221.	Feeding started <i>at</i> birth of litter.			
Exp. 125.	Feeding started	4 days	<i>before</i>	birth of litter.
" 129.	"	11	"	"
" 145.	"	9	"	"
" 130.	"	15	"	"

A. Feeding started at birth.

Litter 111 had the best growth curve in this series, but bad spasms were seen on the 13th day and after this the usual bad symptoms were marked.

Only one of the litter survived and it was very weak and backward in every way. It did not eat for itself till the 21st day and therefore the mother was not removed until the 24th day. After this the young rat was weakly for some time, but survived.

Litter 193 were all dead the 17th day, having suffered from all typical symptoms, though not to a marked degree. It has been noticed that if extreme weakness comes on at an early stage, the acute spasms are frequently absent.

Litter 221 suffered badly. They showed spasms and all typical symptoms. They were all dead the 16th day.

B. Feeding started before birth of litter.

These results again agree with those obtained with edestin and caseinogen.

With all three proteins the mothers were very fit and in most cases put on weight.

From these experiments one would conclude that feeding the mother rat with protein in the proportion 15.0 g. bread to 5.0 g. protein, even in a physiologically complete diet is harmful to the litter. If the feeding is started before the birth of the litter, then no babies survive; also it appears to make very little difference if the feeding is started a few days before the birth or maintained during the greater part of gestation.

Failure in rearing the babies is of much more frequent occurrence when the mother is given an excess of protein in the diet (*at* birth of litter) than when she is fed on other mixtures. It is very easy to see if the young rats have been fed, and with an excess protein diet it was not infrequently found that the whole litter died in the first two or three days, and had hardly had any food.

The symptoms develop at approximately the same time and this seems to suggest a correlation with some stage of development of the young. This possibility is supported by the fact that in a foster litter the symptoms also develop about the same time (experiment to be described later).

If the feeding is started *after* the birth of the litter, some babies may survive, but they are never normal; the majority die.

The results with egg albumin are less good than those with caseinogen and edestin, but this as already suggested is probably due to the different composition of the proteins. Other differences will be commented on later (p. 98).

The fact that some litters do better than others (*e.g.* litter 113 show a better growth curve than any other on the same diet) is possibly due to the greater tolerance of certain rats for high proportions of protein. In this connection it is also probable that the loss of weight of the mother is a factor to be considered; rat 113 lost weight, while other rats on that diet gained and their litters died.

Thus the bad effects on the litters are obtained even when the diet is physiologically complete. It should be pointed out that the amounts of butter (fat and vitamin *A*), marmite (vitamin *B*), lemon juice (vitamin *C*) and salt mixture (inorganic constituents of diet) used were adequate for a normal diet

(see experiment described below, when weanlings were fed on a similar mixture).

The mothers remained well during the experiments and when the diet was started before the birth of the litter, the period of feeding lasted as long as five weeks.

Fig. 4. 1, 4, 6 caseinogen; 2, 3 control.

Growth of young rats on a diet similar to that fed to the mother. Fig. 4.

One criticism which might be offered is that although the mother rats appeared well, they might not have remained so had the feeding been continued longer. To test the adequacy of this diet, young rats were given a similar mixture. Two normal healthy litters were chosen and fed on bread and milk for two days after weaning. Then half of each litter was put on this diet, the protein used was caseinogen, and the other halves of the litters were used as controls, being fed on kitchen scraps supplemented with small quantities of bread and milk.

The young rats were weighed separately every day at first and later every four days. The increase in weight of the males is shown in Fig. 4.

The caseinogen-fed males did better than the controls, but the females grew equally on both diets.

The experiment lasted 90 days, which should prove a suitable length of time for testing the diet.

The protein-fed animals grew rapidly and were very fit, their coats were thick and silky and decidedly better than that of the average animal. The females became pregnant and the litters were born at about 13 weeks, which

is recognised as the normal time for the appearance of the first litter. In the controls the first litter appeared about two weeks later. The three caseinogen-fed rats produced healthy litters, which compared very favourably both in size and weight with the average first litter.

The litters were not reared. Their growth curves were typically those described previously when the mothers received excess protein in the diet.

The weights of the animals at 70 and 90 days are compared with those of Donaldson [1906] and Osborne and Mendel [1911].

The average weights given by Osborne and Mendel are slightly less than those of Donaldson. The authors suggest that this is due to differences of "breed."

The rats used in these experiments were black and white and as before suggested they may be of a bigger breed. In other experiments both white, and black and white animals were used, and practically no differences in weight were noticed. The only difference was that the white rats do not make such good mothers and, therefore, for the later experiments, they were rarely used.

	Weight of rat at 70 days		Weight at 90 days		
	Average weight		Average weight		
	Male	Female	Males		
	g.	g.	g.		
Donaldson	107	100	150		
Osborne and Mendel	120	100	135		
Caseinogen-fed	(3) { 198	(3) { 114	(3) { 231		Females not given because they were pregnant
	201	143	242		
	216	165	258		
Controls. Fed on kitchen scraps and small amount bread and milk	(2) { 178	(3) { 103	(2) { 204		
	182	158	223		
		162			

This experiment proves the diet to be adequate in all respects except during lactation. It is extraordinary that a diet, so obviously good for a growing animal, suitable for fertilisation and production of offspring, should cause such disastrous effects on the suckling babes.

Experiments with foster-mothers.

It was suggested on p. 91 that the bad symptoms in the babies were in some way connected with development, since the symptoms usually came on at about the same time. It does not seem at all feasible to suppose that the milk contained toxic substances at a definite time, and then lost these properties again. In support of this view it was found that if a rat could be induced to suckle more babies after her own had all died and the excess protein feeding continued, then the new litter gained for a time though not to any great extent, but finally died. The growth curve for the young is very similar to that obtained with the rat's own litter, except that the increase of weight of the babies is less than with the original litter. (This fact applies also to a good diet, see experiment 107.)

Several "foster" experiments were made. Rat 152 was fed on bread

edestin, butter, marmite, lemon juice and salt mixture (the same diet as previously used) while nursing her own babies (experiment 112), and the diet continued with the foster litter, five in number. The foster babies gained weight until the 9th day, then ceased to gain, finally lost weight and died. No spasms were noticed, but weakness was a prominent feature. It was frequently found that slower-growing babies developed weakness earlier and showed only slight spasms, or none at all; however, this was not always the case. The mother, 152, lost weight considerably, but in experiment 112 she had gained a good deal. At the beginning of experiment 112 she weighed 212 g. and at the end of experiment 152 she was 205 g. In this instance loss of weight of the mother did not spare the litter, but the mother's loss is hardly comparable to that of other experiments, because in this case the rat had just put on what she now lost.

Rat 107 brought up foster babies when she was having a diet of bread and milk. Previously she was fed on a diet of egg albumin and bread, and all her own litter had died, with typical symptoms. These two experiments are representative of others, and it is inferred that after her own babies have died as a result of too much protein in her diet, the rat is capable of bringing up another litter. If a good diet is given in the second experiment, the babies are normal but small. They do not develop so rapidly as a rat's own babies, and are not able to eat for themselves until about the 21st day. If the excess protein diet is continued, the babies gain small amounts for a time, but eventually die, having shown the usual symptoms, except that they do not suffer from spasms.

In the experiments in which the mothers received excess protein, the foster babies did not develop symptoms until a few days later than the original babies. It has been pointed out that on a good diet the foster babies do not eat till later. This is in agreement with the suggestion that the development of the young is a factor to be considered.

Quite a number of rats were unable to bring up a foster litter after their own had died as a result of too much protein in the diet. The rats took to the babies and mothered them, but were apparently unable to feed them. The babies lived from two to four days, during which time they lost weight. They did not appear to have been fed, although the rat took care of them in the nest. These babies were rarely eaten by the foster mothers.

It was previously suggested [Hartwell, 1921, 1] that excess protein caused an alteration in composition of the milk, and finally a cessation of the flow. From these experiments it would appear that in some rats the secretion could be re-started, but not in others.

It is possible in some cases to alter the diet, either by adding milk, omitting the excess protein, or entirely changing the food to bread and milk, and thus to obtain recovery of the babies.

Experiments on changing litters.

That the milk of the rat on ~~excess~~ protein contains some toxic substance is further demonstrated by changing the litters.

Rat 136 received bread and milk. Rat 135 had a diet with excess protein. ~~The litters~~ were born on the same day. As soon as the babies 135 developed spasms (the 12th day) the litters were changed. Litter 135 recovered rapidly when fed by rat 136, which was having bread and milk, and were successfully weaned at the normal time. Two of litter 136 developed screaming fits and bad spasms the 20th day, but recovered. In another exchange the normal babies given to a mother, whose own litter were showing bad symptoms, lost weight for three days, after which they ate for themselves. In this experiment no typical symptoms were noticed.

Thus it is evident that with large amounts of protein in the diet, which is complete physiologically, the milk is affected, and the babies show abnormal and typical symptoms. It is suggested that

(i) The *quality* of the milk is altered, in that it possesses some toxic properties responsible for the spasms. This may be due to excess of amino-acids, or possibly to some deficiency in the milk.

(ii) The *quantity* of the milk is inadequate, since the babies lose weight.

Since large quantities of protein are usually recommended for a lactating animal, it is probable that definite and large amounts of one or more other constituents should be included in the diet.

With a view to throwing some light on this, the following experiments were made.

(ii) *Effect of adding whole milk to a diet containing excess protein.*

The bread and protein were fed in the same proportion as in the last series of experiments. The milk was heated to 70° C. In these experiments the rats had no water to drink. (In some earlier experiments, when 100 cc. whole milk were given, it was noticed that the animals drank no water, and usually upset the containing vessel as soon as it was given to them.)

The control experiment D was done at the same time of the year as the other experiments, though not actually the same year. So far the growth curves of the young when the mother is fed on bread and milk are practically constant, irrespective of the time of year, so long as the animals are kept in a well-heated room. Other observers find different growth curves according to the time of year. It is possible that this difference is minimised when the diet is exceptionally good, *e.g.* bread and whole milk.

In these experiments no abnormal symptoms of any kind were shown, but in all cases the growth was depressed, as compared with the standard (bread and milk).

This is well shown by the following figures:

Average weight of young rat at twenty-one days.

Exp.	Diet	Weight in g.
D	Bread and milk	39.2
41 }	" " edestin	31.3
108 }		29.6
J }	" " caseinogen	27.8
C }		30.6
201 }	" " gelatin	30.4
202 }		26.0
203 }	" " egg albumin	29.0
204 }		34.3 (litter of three only)

In these experiments the rats were eating even larger quantities of protein, since milk contains from 2.5 %–4.0 % protein [Matthews, 1921]. The exact amount of milk required to prevent the babies from developing the typical symptoms has not been determined, but other experiments showed 50 cc. to be inadequate. In all probability the individuality of the rat would be a factor conditioning the amount required. Later in this paper (p. 101) it is demonstrated that the whey from 100 cc. milk is equally efficient in preventing bad results with caseinogen and edestin, and that the growth is equal to that obtained with bread and milk.

Thus milk contains some dietary constituents which render it possible for the rat to ingest even larger quantities of protein and yet the litter is not affected by bad symptoms. The only detrimental effect is that the babies do not grow at a maximal rate.

The next experiments were started with a view to finding the nature of such substance or substances.

Two experiments were made with each protein and the average weight of the two litters does not correspond very closely. In spite of the fact that no symptoms developed in the babies, the mothers were ingesting very large amounts of protein and it is probable (as before suggested) that the individual metabolism of the mother is a partial explanation of these discrepancies.

(iii) *Effect of adding other constituents to a diet containing excess protein.*

In this series the proportion of protein to bread was kept constant as in the previous experiments, *i.e.* 5 : 15.

(a) *Salts.* Calcium given as lactate, 1.0 g. per diem (Fig. 5).

Exps. 114, 172, 156. Bread, caseinogen, marmite, calcium lactate.

Exp. 138. Bread, egg albumin, marmite, calcium lactate.

„ 140. Bread, edestin, marmite, calcium lactate.

Litter 114 grew well the whole time; they were excitable and exhibited toe-walking, but otherwise no typical symptoms. It is probable that the mother had a greater tolerance for protein, as before suggested. Apart from this litter, the results were in agreement and the same for each protein tried,

i.e. caseinogen, edestin, and egg albumin. All the litters showed typical and very bad symptoms, but the babies lived longer than usual.

Thus the addition of calcium lactate to a diet containing excess protein does not alleviate the symptoms but causes a slight prolongation of the life of the sucklings.

(b) *Salt mixture.* Bread, caseinogen, marmite, salt mixture. No improvement in the litter was obtained when 1.0 g. salt mixture was added to the daily ration. The symptoms were bad, and the young were all dead on the 15th day.

Fig. 5. Calcium Lactate.

(c) *Milk ash*¹. The milk was evaporated to dryness, the residue scraped up and strongly heated in a crucible until a constant weight was obtained; 0.7 g. were given per day (*i.e.* the amount in 100 cc. milk).

These results were very similar to those obtained on adding calcium lactate to the excess protein diet. The symptoms were in no respect alleviated, but the life of the young was slightly prolonged.

(d) *Fat and vitamin A.* No beneficial effects were obtained on adding 3.5 g. butter per day to the excess protein diet. Edestin was used.

¹ I am indebted to Miss W. M. Clifford for the preparation of the milk ash.

(e) *Lactose*. 4.0 g. lactose were added to a diet containing excess edestin, but the spasms and bad effects were in no way improved.

(f) *Fat, vitamin A, and lactose*. Caseinogen was used and the diet was physiologically complete in all respects, fat and lactose being added in approximately the same amounts as found in 100 cc. milk.

The litter suffered very badly, two eventually survived, but they were weakly for some time and were not in a fit condition to leave the mother until the 26th day.

Vitamin C. The diet consisted of bread and egg albumin in the usual proportions and 0.7 cc. lemon juice per day. Great difficulty was experienced in getting a rat to bring up a litter on this diet. In many experiments the babies died within the first three days. The mothers were well, the babies were average weight, and apparently all right at birth. This may be pure coincidence. No explanation is offered to account for this failure.

Finally two experiments were successful. The litter all exhibited typical symptoms, and were dead by the 13th day.

(iv) *Effect of adding large quantities of yeast extract (commercial preparation, marmite) to a diet containing excess protein (Fig. 6).*

Two experiments were made with each protein (caseinogen, egg albumin and edestin).

1. The diet consisted of 15.0 g. bread, 5.0 g. protein, and 3.0 g. marmite, all constituents increased proportionately as necessary.

2. The bread, protein, and marmite were the same as above, but butter, lemon juice, and salt mixture were added to complete the diet.

These curves are very similar to those obtained when 100 cc. whole milk were added to the excess protein diet. To bring out this point curve D (bread and milk standard) is included in the figure. With edestin and caseinogen a depression of the growth curve was obtained, very similar to that obtained when the mother had these proteins + 100 cc. whole milk. The babies were practically normal, but slight toe-walking and excitability were noticed; these features, however, were very quickly recovered from. With egg albumin, on the other hand, the results were not so good. No spasms were noticed, but the babies were far from normal. They were all right at first, but on the 14th day they felt cold, were weak, inclined to roll over, thin and lethargic. They walked very badly and their movements were jerky and incoordinate. Two of the litter 180 died but the rest survived, as did all litter 182. The survivors were not normal for two weeks after weaning.

The average weights of the young rats are given below.

		Average weight at 21 days. g.	
Exp. D	Bread and milk (standard)		39.2
" 164.	" edestin, marmite		34.7
" 165.	" " " butter, etc.		25.7
" 179.	" caseinogen, marmite		34.0
" 181.	" " " butter, etc.		30.7
" 180.	" egg albumin, marmite		22.0
" 182.	" " " butter, etc.		26.3

From these experiments it is clear that "something" in marmite is capable of obviating the bad results in the litters usually obtained with excess of caseinogen or edestin in the mother's diet. Also the actual weights of the babies are better when the mother's diet is lacking in fat, vitamin *A*, salt mixture and the antiscorbutic vitamin. This may be merely coincidence; a far greater number of experiments would be required before any conclusions could be drawn. It is interesting to note that similar results were obtained

when less amounts of protein were given. Since the results are as good when the mother's diet is deficient in fat, vitamins *A* and *C* and salt mixture, it appears that the "something" in marmite is the primary factor. It may be of interest that the marmite contained only traces of calcium salts.

With egg albumin the improvement was not nearly so good. The litters certainly did better than when small amounts of marmite were fed to the

mother, but they could not compare at all favourably with the caseinogen and edestin babies. These differences are discussed later (p. 102).

The mothers maintained or increased their weights, except 180 (fed on egg albumin in a deficient diet) which lost appreciably.

(v) *The importance of protein.* (Fig. 7).

Exp. D.	Control.	Bread and milk (same as in Fig. 6).
Exps. 118, 120.	15.0 g. bread, 5.0 g. edestin	} + whey from 100 cc. milk.
Exp. 167.	" " 3.0 caseinogen	
" 168.	" " 3.0 edestin	
Exps. 159, 162.	" " 0.0 protein	
Exp. L.	Bread alone.	[Taken from Hartwell, 1921, 1].

Fig. 7.

Preparation of the whey. 100 cc. milk were warmed to 40° C., and a few drops of rennet added. When the junket was firm, it was hung up in a muslin bag and the whey separated by dripping through. Each rat was given daily as much as could be obtained from 100 cc. milk. The protein and bread were increased proportionately as before.

It has already been mentioned that the whey was as effective as milk in preventing the bad symptoms in the litters. Also when the proportion of bread to protein was 15 : 5 the growth of the litter was practically equivalent to that obtained with a bread and milk diet, which up to the present time is regarded as maximal. It is extraordinary that such good growth should be obtained when the mother's diet was so poor in fat and vitamin A¹.

In this connection it is interesting to note that all the mothers that were killed had large deposits of subcutaneous and peritoneal fat. It may be that with large reserves of body fat, the amount in the diet is immaterial from the point of view of milk production.

In experiments 159 and 162 in which the mothers were fed on bread and whey, the litters did very little better than in experiment L when the mother received bread alone (taken from Hartwell [1921, 1]). Comparison of the protein rations in these experiments shows that the bread and whey contains only slightly more protein than bread alone. When more protein was added to the diet (experiments 167 and 168) a considerable improvement in growth of the young was obtained, though all the other constituents were the same as before. A further addition of protein (experiments 118 and 120) gave a still greater improvement in the growth of the litters. In this way it is seen that adding protein to the diet can raise the growth curve from almost the minimal to the maximal. Thus protein itself does play a very important part in the production of milk of good nutritive value, yet from other experiments described in this paper, it is obvious that the diet must be rich in other constituents as well or else harmful results occur in the litter.

One of litter 118 was killed accidentally on the 7th day and the average weight of these babies is rather greater than that of 120, whose mother received a similar diet. It is quite possible that the actual number in the litter is a factor of importance. Experiments to investigate this point are now in progress.

DISCUSSION OF RESULTS.

(i) *Prevention of the bad symptoms in the young.*

It has been conclusively proved that large quantities of protein are by no means harmful to the rat itself, since the young and adult animals can live normally on a diet containing excess protein for considerable periods. The proportion of 15.0 g. bread to 5.0 g. protein proved an excellent diet for growing rats, but much less protein, even as little as 15.0 g. bread to 1.0 g. protein produced bad symptoms in the suckling litter. Therefore, it appears that the rat can metabolise the protein effectively from the point of view of its own growth, but is unable to produce normal milk, unless definite quantities of some other constituent (or constituents) are supplied. Our knowledge

¹ Since the caseinogen and edestin used were not absolutely pure, it is possible that traces of vitamin A may have been present.

of the metabolism of individual amino-acids is very limited, but the experiments described in this paper show that there is some quantitative relation between the amino-acids and other dietary constituents. What these constituents are is a very difficult problem. The fact that whole milk can prevent the bad symptoms throws very little light on the subject. Owing to the complicated nature of milk, it merely proves that large quantities of protein in the mother's diet produce no harmful effects in the litter, as long as "something" else is included. It is even possible that the necessary factor will vary with the nature of the protein.

It has been pointed out that 3.0 g. marmite to 5.0 g. protein is adequate in preventing spasms, etc., in the case of caseinogen and edestin, but with egg albumin, although the symptoms are somewhat reduced, the litter still suffers badly. Also with egg albumin much better results were found when small quantities of butter, lemon and salt mixture were added to the diet, but with caseinogen and edestin, it was immaterial if the diet was complete or not as long as large quantities of marmite were fed. Since caseinogen and edestin are somewhat similar in constitution, and egg albumin is different there seems some evidence for suggesting that the factor will vary with the constitution of the protein.

Some experiments with egg albumin now in progress show that the litter exhibit still greater improvements (but are *not* normal) when more butter is added to the ration, the diet being physiologically complete.

It is quite obvious that some other constituents are vitally essential for the production of normal milk, if large quantities of protein are ingested.

These are all present in milk, and in the case of caseinogen and edestin, present also in commercial yeast extract ("marmite"). In the light of present-day investigations the most obvious explanation is that vitamin *B* is the factor, but this conclusion is by no means justified from the experiments described here. Again "marmite" alone was not wholly effective with egg albumin. It might be that insufficient was given, but this is hardly likely, because 3.0 g. to 5.0 g. protein is a large proportion. A more probable explanation is that some other constituent is also needed for the proper metabolism of this protein in a lactating animal.

The fact that large quantities of milk and marmite were necessary suggests an actual chemical relation between the protein and some other factor, and not a catalytic or hormone action. It is possible that less marmite would be required if the right proportion of other constituents could be arrived at. Experiments have shown 1.0 g. marmite per day to be ineffective, yet the amount necessary to supply vitamin *B* to a rat is estimated as 0.2 g. per day.

It is possible that the animal needs a greater amount of vitamin *B* for the effective metabolism of large amounts of protein; hence it is used by the mother and she has none to spare for her milk. On the other hand the mother is usually regarded as being a safeguard for the young and it is thought that she can sacrifice her own tissues for the benefit of her offspring. Also it has

been proved that in a non-lactating animal quite small amounts of vitamin *B* are adequate even when the intake of protein is high.

The experiments of Karr suggest that vitamin *B* is an essential constituent of a dog's diet, otherwise the animal refuses its food. Since the dog is a carnivor, it might be assumed that this vitamin plays some part in connection with protein metabolism. Karr, however, states that the intermediary metabolism of nitrogen is unaltered by adding vitamin *B* to the diet, but he appears to deduce such a conclusion from analysis of excreta and food ingested. It is possible that the nitrogen excretion might remain constant and yet the intermediary metabolism be altered.

Thus there seems a certain amount of superficial evidence for associating vitamin *B* with protein metabolism. In the experiments described in this paper, all other recognised dietary constituents have been eliminated except vitamin *B*. This is only a negative proof and therefore of little value. The commercial preparation marmite is a somewhat crude product and contains many other substances besides vitamin *B*. It is even possible that extractives are of use in obviating the bad symptoms resulting from excessive amounts of protein in the mother's diet.

(ii) *The amount of protein.*

At present it is impossible to state what amount of protein is harmful and what amount is beneficial, because it appears to depend largely on the other dietary constituents. The experiments described in this paper show that too much protein depresses the growth curve, even if no bad symptoms are shown. For example when the mother is fed on bread and protein (15 : 5) and whole milk, the litter show a less good growth curve than when the mother is fed on bread and milk only, although the babies are normal in each case. In the former experiment the rat was taking at least 8.0 g. protein per day, but no bad symptoms occurred, yet in a less complete diet 1.0 g. protein can produce a very bad condition in the young.

As the results differ with different proteins it is probably certain amino-acids which are primarily responsible for the bad effects on the babies. If this is so, a diet of mixed proteins (such as taken by man) should prove less harmful, unless the protein is taken in very great excess. The actual composition of foods themselves is a safeguard, because it is rare to find only one protein.

(iii) *Development of the young.*

It has been pointed out that the time of onset of the symptoms is remarkably constant and it is therefore suggested that the stage of development of the young rat is a factor to be considered. In foster litters, the babies develop a little more slowly and their eyes open about two days later than those of normal litters. They do not eat for themselves until a few days later than the original babies and therefore cannot be weaned at the normal time.

(It has been found advisable to leave the mother with the young for at least three days after they can eat for themselves.) In general they seem more backward and less developed than normal babies of the same age. In foster litters, when the mother is fed on an excess protein diet, the symptoms usually develop about 2-3 days later, than in a litter left with its own mother. Also the symptoms come on at the same time, even if the mother has been fed with excess protein during gestation, or if she has first fed her own litter when she has had the excess protein diet. If the composition of the milk changed towards the middle and end of lactation, then the symptoms in the foster-litter should come on at an earlier stage, provided that the excess protein diet were continued throughout. But this is not so.

(iv) *The value of milk in the diet of a lactating animal.*

"Milk in *liberal amounts* should always be included in the diet of the lactating mother." This statement is made by McCollum [1920] and is probably a very true one. It was shown [Hartwell, 1921, 1] that bread and milk fed to the mother produced maximal growth of the young. Various other diets have been tried but as yet no curves have been obtained which are as good (except 15.0 g. bread to 5.0 g. protein + whey from 100 cc. milk—and this is a milk derivative). It has been proved in this paper that large quantities of milk entirely obviate the bad symptoms produced by excess protein in the diet. It is, therefore, possible that by taking large amounts of milk, the mother safeguards herself from any errors in diet.

The value of milk is, no doubt, due to the fact that it contains all the necessary constituents of a good diet, and not excessive amounts of any. It has been suggested by some physiologists that milk contains a specific galactagogue. Some experiments now in progress show that there are other diets which will give as good results as bread and milk (at any rate with rats) provided that the *right proportion* of the various constituents can be ascertained.

Another advantage of milk is that the proteins are adequate and it is quite obvious that the quality of the protein fed to a lactating animal is of primary importance.

SUMMARY.

1. In a lactating rat, the amount of dietary protein constituting excess varies with the type of protein and with the individuality of the rat. The proportion 15.0 g. bread to 1.0 g. protein (egg albumin or caseinogen) fed to the mother results in abnormal symptoms in the litters.

2. On a diet of bread and egg albumin (either 1.0 g. or 2.0 g. egg albumin to 15 g. bread) the mother loses a considerable amount, in one case as much as one-third of her body weight.

3. When excess protein is fed to the mother, the growth curves of the litters are not constant. The variations obtained are probably due to the individual metabolism of the different rats.

4. Large quantities of protein fed to a nursing rat are detrimental to the young, even when the diet contains all the essential constituents.

5. If such a diet is started at birth, some babies may survive, but they are not normal; the majority die.

6. If the diet is begun before or during gestation, none of the young survive.

7. The litters die at approximately the same time, irrespective of the length of time the mother has been fed on the excess protein diet.

8. Foster babies suffer in exactly the same way as the original family if the same diet is continued.

9. The typical symptoms can be induced in a healthy litter by giving them to a mother whose own litter has developed this condition.

10. It is suggested that the toxic substances in the milk affect the baby rat at some special stage of development.

11. A diet containing large amounts of protein (15.0 g. bread to 5.0 g. protein) in a physiologically complete mixture is adequate for growth, fertilisation and reproduction in the rat, and is unsuitable only during lactation.

12. The bad effects are entirely obviated by adding 100 cc. whole milk to the mother's diet, but the growth of the litter is not maximal. This applies to caseinogen, edestin, egg albumin and gelatin. With caseinogen and edestin, 100 cc. whey are equally effective.

13. With caseinogen, edestin and egg albumin the addition of calcium lactate to the diet prolongs the life of the young, but the symptoms are just as severe. Milk ash has a similar effect when added to a diet containing excess egg albumin or edestin.

14. The addition of butter and lactose causes no improvement in the young when the mother is eating large quantities of edestin or caseinogen.

15. Yeast extract (marmite) in large amounts added to the excess protein diet prevents all bad symptoms in the case of caseinogen and edestin; with egg albumin the bad condition is improved, but not entirely cured.

I wish to express my thanks to Prof. V. H. Mottram for his interest in this work, the expenses of which were defrayed by a grant from the Medical Research Council.

REFERENCES.

- Donaldson (1906). *Boas Anniv. Vol.*, 5.
Hartwell (1921, 1). *Biochem. J.* 15, 140.
Hartwell (1921, 2). *Biochem. J.* 15, 563.
Hewer (1914). *J. Physiol.* 47, 480.
McCollum and Simmonds (1918). *Amer. J. Physiol.* 46, 275.
McCollum (1920). *Newer Knowledge of Nutrition*, 129.
Matthews (1921). *Physiological Chemistry*, 306.
Osborne and Mendel (1911). *Feeding experiments*.