CXLIII. GROWTH AND REPRODUCTION ON SYNTHETIC DIETS. II.

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(Received August 5th, 1927.)

IN an earlier paper [Hartwell, 1926] a description was given of various synthetic diets which produced good but not maximal growth in young rats. The importance of a really good synthetic diet for use as a standard in nutrition experiments is self evident, and certain modifications of the foods used previously [Hartwell, 1926] have yielded satisfactory results, although, as yet, no diet has been found which will produce growth equal in rate to that obtained on kitchen scraps. The improvement in reproduction and lactation on these modified diets is even more marked than with growth, and therefore it is thought advisable to publish these results briefly in that they may be of use to others working on similar lines.

The difficulty of obtaining normal reproduction on synthetic diets is commented on by many researchers, while the actual rearing of the young when the mothers are living on such foods is almost impossible. Nelson, Jones, Heller, Parks and Fulmer [1926] write that "one thing is certain, that reproduction is not normal on certain synthetic diets and diets involving restricted food materials." They find that the number of young born may be comparatively few and that the majority die before weaning, although they have carried their experiments through several generations.

Using a basal synthetic diet of caseinogen, salt mixture, butter fat and dextrin, with wheat, rye, barley or corn as sources of vitamin B, Guest, Nelson, Parks and Fulmer [1926] found that rats reproduced normally, but that there was a high mortality among the young, due in their opinion to failure of milk secretion. Hogan and Harshaw [1924] reared four generations on a synthetic diet, but found that the number of litters was subnormal, the mortality of the young high and that any young which survived grew very slowly during the suckling period. Anderegg [1924] found a high mortality among the young even when the protein was given as whole milk powder, and Murphy and Jones [1926] state that on a diet of bran, salts, crisco and starch the rats produced offspring, but had little success in rearing them. Kennedy and Palmer [1926] used crisco instead of lard in the basal diet shown by Evans and Bishop [1923, 1] to be inadequate for reproduction, and

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obtained litters in three successive generations of rats. They found, however, that the mother rats had great difficulty in rearing their young even when the diet of each rat was supplemented by 15 cc. of whole milk throughout the lactation period.

Thus the failure to rear their sucklings when the mother is fed entirely on a synthetic diet appears to be universally recognised.

EXPERIMENTAL.

The preparation of the food, weighing the animals, etc., has been described previously [Hartwell, 1926]. The composition of the diets is given in the following table.

	Diets				
	<i>A</i>	<i>E</i>	<i>CE</i>	<i>CL</i>	<i>CLO</i>
Caseinogen	20 g.	16 g.	8 g.	14 g.	20 g.
Egg-albumin	—	—	8 g.	—	—
Lactalbumin	—	—	—	6 g.	—
Potato starch	64 g.	64 g.	64 g.	60 g.	64 g.
Butter	12 g.	16 g.	16 g.	16 g.	—
Cod-liver oil	—	4 cc.*	4 cc.†	4 cc.	14 cc.
Salt mixture (Hartwell)	4 g.	4 g.	4 g.	4 g.	4 g.
Marmite‡	5 g.	5 g.	5 g.	5 g.	5 g.
Water	300 cc.	300 cc.	300 cc.	300 cc.	300 cc.

* After first six weeks of experiment.

† After first four weeks of experiment.

‡ During lactation 10 g. of marmite were used instead of 5.

Three generations of rats were reared on diet *A*, but slight alterations in the fat were made from time to time. It was noticed that a few of the young rats developed slightly sore eyes at about 17–18 days of age and as this was first seen in the winter it was thought advisable to increase the butter to 16 g. and decrease the starch to 60 g., thus keeping the percentage composition of the other dietary constituents constant. The increase in butter caused no improvement in the eye condition of the suckling young and therefore 4 cc. of cod-liver oil were added to the diet. This alteration, apparently, led to disastrous results in that the mother frequently died when the young were about to be born (this is described later in the paper) and so the cod-liver oil was omitted from the diet. The duration of the experiment and the alterations in diet are given below.

	Duration of experiment	Alterations in diet
1st generation	June 15, 1926–April 5, 1927	4 cc. cod-liver oil added to diet from March 5, 1927
2nd generation, from first litters	Sept. 9, 1926–March 17, 1927	Butter increased to 16 g. on Feb. 3, and 4 cc. cod-liver oil added March 4
2nd generation, from second litters	Oct. 12, 1926–March 16, 1927	Butter increased to 16 g. on Feb. 3, and 4 cc. cod-liver oil added March 4
3rd generation	Feb. 3, 1927–July 15, 1927	Expt. started with 16 g. butter, March 4 4 cc. cod-liver oil were added; May 4 cod-liver oil discontinued and butter reduced to 12 g.

RESULTS AND DISCUSSION.

All the rats fed on the synthetic diets described in this paper were in good condition throughout the experiments. The animals were firm to feel and their muscles were fit; there were no signs of general flabbiness, and except that the rats were slightly smaller at any given age, they could not be distinguished from stock animals fed on kitchen scraps supplemented by bread and milk.

Growth.

The rate of growth on diets *A*, *E*, *CE*, and *CL* was good, though not equal to that of stock animals fed on the kitchen scrap mixture (see Fig. 1). Diets *E*, *CE*, and *CL* produced slightly quicker growth than diet *A*. It is impossible

Fig. 1. Growth curves of male rats. Each curve is taken from the average of six animals.

to say what was the cause of this improvement since several factors were varied in each diet. It seems unlikely that the differences in the protein ration are of primary importance here, because diet *E* containing only 16 % of caseinogen produced better growth than diet *A* with 20 % of this protein,

and diets *CE* and *CL* containing two proteins at the level of 20 % produced no better growth than diet *E* with 16 % of one protein. The main difference between diets *E*, *CE*, and *CL* on the one hand and diet *A* on the other is the increased fat content of the former diets, diet *A* containing 12 % of butter, while diets *E*, *CE* and *CL* had not only an increase in butter to 16 % but also 4 cc. of cod-liver oil as well. This involves not only an increase of fat, but also of vitamins A and D in the diet, and possibly vitamin E.

Diet *CLO* gave comparatively poor results for growth, and the curve flattened early as seen in Fig. 1; the rats, however, were in good condition throughout the experiment. Diets *A* and *CLO* are similar in all respects except the fat, the former containing 12 % of butter and the latter 14 cc. of cod-liver oil in place of the 12 g. of butter. It is extraordinary that diet *CLO* produced such poor growth for it *must* have been adequate as regards vitamins A and D, and 5 g. of marmite were used while 2 g. have been found to produce good growth in young rats. It may be that there is an excess of vitamins A and D in this diet and that the poorer growth is due to upsetting the balance of the dietary constituents, or that vitamin E, stated by Evans and Bishop [1923, 1, 2] and Sure [1927] to be deficient, or present in only small quantities, in cod-liver oil, is essential for growth as well as for reproduction. If vitamin E can be stored in the animal body as can vitamin A, the good growth produced in the first few weeks of the experiment and the early flattening of the curve are easily understood.

Reproduction.

(a) *Diet CLO.* The fat in this diet was cod-liver oil, which it was hoped to use in place of butter in synthetic diets, in order to have a constant supply of vitamins A and D throughout the experiments, it having been shown by Luce [1924] that the antirachitic value of cow's milk varies with the season of the year. The six does in this experiment produced no litters, though the feeding was continued for 21 weeks, during which time other rats on synthetic diets would each have had two or three families. *Post mortem* examinations showed abnormal uteri and mammary glands, the histological details of which will be published later. The males varied, some appearing almost normal, while others had testes of very low weight. When it was found that this variation occurred, two males with apparently well formed testes were kept for a further period and mated with stock does which had previously reared litters, but no families were born. This experiment appears to confirm the work of Evans and Bishop [1923, 1] that cod-liver oil does not contain vitamin E, regarded by these workers as essential for reproduction. It should however be pointed out that the methods of investigation are not similar; Evans and Bishop used what might be termed a "curative method," while the experiments described in this paper are of the "preventive" type. The assumed absence of a special reproductive dietary factor is a very obvious explanation of these results, but it must be recognised that in both series of experiments the diets contained

large amounts of cod-liver oil and therefore it is possible that an upset of the balance of the various dietary constituents may be a factor of importance. Evans and Bishop [1923, 1] used as much as 24 % of cod-liver oil in one experiment, and they considered that one sample of their oil was at least eleven times as rich in vitamin A as the milk fat of their standard diet.

(b) *Diet E*. All six does became pregnant at the normal time, but no young were reared. One doe was killed because of a cancerous growth; three does had first litters either born dead, or the young were eaten immediately after birth, and all three died with their second litters; the other two does died with their first litters. The does appeared perfectly fit and well throughout gestation, but they became comatose and eventually died with the young unborn. Before death they frequently had brief convulsions at intervals during the comatose period. The mothers apparently died from internal haemorrhage; their paws and tongue were quite white; their muscles very anaemic and the liver appeared bloodless. The young seemed normal in all respects. Some of the animals were killed while in the comatose condition, so that the *post mortem* observations should be of more value. As a rule the rats died about two days after the young were due to be born. (This statement is based on general observation of pregnant does; over 2700 litters have been born during the author's research, and by experience it is easy to tell when to expect the birth of the young.) When the six does all died, five other stock females, which had previously reared litters, were put with the males. Four of these had litters, born normally, and three of them reared some of their young.

(c) *Diet CE*. This diet gave better results in that some litters were reared, but three out of the six does died, showing the same symptoms as described under diet *E*.

Details of the breeding are as follows:

Number of litter	Number of young born	Average weight in g. day after birth	Number reared	Average weight in g. at weaning (21 days)
Rat 1				
—	5 dead			
2624	7 + 2 dead	5.3	4	31.5
2679	8	6.25	5	30.4
Rat 2				
—	Born dead, rat died the next day			
Rat 3				
—	5. Eaten second day			
—	Born dead			
Rat 4				
—	Died at birth of first litter			
Rat 5				
2589	3. Dead second day.			
2642	1 + 1 dead	4. Eaten third day		
2680	4 + 3 dead	6	4	28.75
Rat 6				
2590	7	6.3	5	30.6
—	Died when litter born			

(d) *Diet CL*. The results of this experiment are very similar to those obtained with diet *CE*, three does dying at, or soon after, the birth of a litter.

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The few litters born to animals fed on diets *CE* and *CL* compared with those born to rats on diet *A* must not be taken as evidence that the diets *CE* and *CL* are less good for reproduction because these two experiments were of much shorter duration, each being discontinued after a third doe had died.

Number of litter	Number of young born	Average weight in g. day after birth	Number reared	Average weight in g. at weaning (21 days)
Rat 1				
2643	8 + 2 dead	4.6	3	23
2702	9	6	5	42
Rat 2				
—	Died with first litter			
Rat 3				
2635	2 + 1 dead	5. Eaten sixth day		
2677	4 + 2 dead	4.75	2	23.5
Rat 4				
2673	8	5.5	4	29.75
Rat 5				
2638	8	5	5	29.2
—	Died at birth of litter			
Rat 6				
—	Born dead, rat died a few days after			

(e) *Diet A*. This diet was by far the most satisfactory for reproduction and three generations were successfully reared. The rate of growth of the second and third generations was slightly slower than that of the first (see Figs. 2 and 3), but all the rats were in good health throughout the experiment and no dietetic disturbances, such as the hair falling out, etc., were ever seen. The slower growth of the second generation is in agreement with the results of Evans and Bishop [1923, 1]. The does in all three generations were fertile and litters were produced as frequently as in animals fed on a mixed diet of kitchen scraps. Two cages of second generation animals were kept, one from first litters and one from second; it was thought possible that if the diet were lacking in any way the second litters might show some effects not found in the first litters, which might have had the advantage of any reserve stores of the mother. However, no such differences appeared, and the two cages of rats were similar in all respects. The rearing of the young was by no means up to standard, although those which survived were usually fit and healthy, but below average weight at weaning. The first generation does reared 85 young out of a possible 156 (all litters were reduced to six on the day after birth); the second generation does from first litters reared 51 out of 100, the second generation from second litters brought up 30 out of 57 and the third generation reared 25 out of 52. This is a great improvement on the results obtained with synthetic diet 1 [Hartwell, 1926] on which only 10 young were reared out of 59. The number of young born to the does on synthetic diet *A* was not up to the controls, 10 being the largest litter, while stock animals produce as many as 18. There was little difference between the numbers born to the first and second generation rats, but there was a considerable drop in numbers per litter of the third generation families.

Details of the reproduction in the first generation are given in the following table:

Number of litter	Number of young born	Average weight in g. day after birth	Number reared	Average weight in g. at weaning (21 days)
Rat 1				
2348	6 + 3 dead	6	3	22.3
2388	6	5.7	3	30
2442	8	5.7	5	31.4
2483	3	5.6	2	30.5
2527	7	6	6	29.7
—	5. Born dead			
Rat 2				
2347	5	6. Eaten 15th day		
2383	5	5	2	14.5
2432	4	5	4	25.8
2472	1 + 2 dead	6. Eaten 5th day		
2498	3	5	3	24
2561	2	5.5. Eaten 6th day		
Rat 3				
2352	2	4. Eaten 5th day		
2382	7	5.7	5	28
2437	7	5.7	5	27.6
2475	5	4.2. Eaten 4th day		
2566	4	4.3. Eaten 6th day		
2587	4	4.5. Eaten 19th day		
Rat 4				
2345	3	4.6. Eaten 4th day		
2377	10	5.5	5	35
2406	7	6	6	29.3
2453	4	6.3	4	34
2500	7	5.4	6	29.3
Rat 5				
2346	5	5.6. Eaten 14th day		
2381	9	5.4	5	26.6
2430	4	5	1	38
2470	4	5.3	2	26.5
2514	3. Eaten 2nd day			
2555	1	6. Eaten 5th day		
2580	5	6	5	28.4
Rat 6				
2351	7	5.2	3	29.3
2396	4	6. Eaten 6th day		
2426	7	6.2	6	27.8
2469	4	5.5. Eaten 8th day		
—	Eaten at birth			
2524	6	6	4	30.8
2579	3	5.3	2	21.5

The results of breeding in the second and third generations are not given in detail, but the main points are shown in this table:

	Duration of expt. in weeks	Number of does	Number of litters born	Weight of young on second day (g.)		Average weight of young at weaning (g.) (21 days)
				Lowest	Highest	
1st generation	63	6	37	4.2	6.5	28.4
2nd generation						
From first litters	27	9	28	4.6	6.3	29.1
From second litters	23	7	15	4.7	6.3	26.9
3rd generation	23	8*	15	4.7	6.3	32.5

* Two of these does died at the birth of their first litters.

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It is interesting to note that at 21 days the average weight of 87 young from the first generation is 28.4 g., while the corresponding figure from 81 young from the second generation is 28.3 g.

When an experiment was finished a *post mortem* examination was made on each parent animal, and from this examination, all organs appeared to be in a normal condition. This is especially interesting in connection with the third generation rats, where evidence of any dietary deficiencies might have been more marked. The third generation animals had very thick and strong abdominal muscles, the difference being as marked as in the wild rabbit compared with the hutch animal.

Fig. 2. Growth curves of some of the female rats fed on diet A. L=litter born.

One of the most striking features of these experiments is the death of the mother rat coincident with the failure of the young to be born. This appears to have a direct connection with the alteration of the fat content of the diet. With diet A, the first generation rats were receiving 12 g. of butter and each

doe had 5, 6 or 7 litters, all born normally. In the second generation the fat was altered, but near the end of the experiment, and no catastrophes occurred. The third generation were started from weaning on the increased fat, *i.e.* 16 g. of butter and 4 cc. of cod-liver oil and two does died with their first litters. Consequently the fat ration was immediately changed to 12 g. butter and no more does died. With diets *E*, *CE* and *CL* the fat consisted of 16 g. of butter and 4 cc. cod-liver oil, and 12 of the 18 does died. An experiment is now in progress with a diet similar in composition to diet *A* but with 20 g. butter and no cod-liver oil, to see if the oil is the primary factor or if the actual amount of fat is also of importance. It may be significant that with the fat ration entirely cod-liver oil, no reproduction occurs and that the addition of 4 cc. of the oil to the diet results in a high mortality of the does. It is possible

Fig. 3. Growth curves of some of the male rats fed on diet *A*.

that vitamin E is as essential for normal birth as for effective placentation [Evans and Bishop, 1923, 1]. If the cod-liver oil has some inactivating action on the butter, similar to that suggested by Fridericia [1924] for certain oils, it might conceivably diminish the vitamin E content of the diet. Although this is a possible explanation of the results described in this paper, it is merely theoretical at the moment and the problem obviously awaits further elucidation. The evil effects consequent on the addition of cod-liver oil to the diet may be due to some upset of balance of the dietary constituents.

The cod-liver oil was added in the hopes of preventing the sore eyes noticed in the suckling young, but no such improvement was obtained. More recently a similar condition has been found in suckling rats belonging to stock does

fed on synthetic diets during lactation only. These mothers obviously cannot lack vitamin A as they have large reserves of body fat, so it is probable that the eye trouble is the "salt ophthalmia" described by McCollum, Simmonds and Becker [1922; 1925], although the conditions are not absolutely comparable, as these observers were feeding young rats on the diet, while in my experiments the eye trouble developed in the suckling young and was therefore caused indirectly by poor feeding of the mother rat. The soreness was observed as soon as the eyes opened (usually late on synthetic diets compared with stock animals). In some litters no bad eyes were seen, while in others two or three of the young showed the symptoms; in no case was the whole of the litter affected. In several experiments the eye trouble was noticed at the 17th day, and if the young ate for themselves on that day or the next, there was often complete recovery by weaning, *i.e.* the 21st day. This suggests that the diet was deficient for lactation though adequate for growth; it also emphasises the different needs of the rat during lactation and growth, because the young recovered when eating the diet for themselves.

It is possible that lactation throws an even greater strain on the mother than does gestation. In the rat, even if the litter be reduced to six, the combined weight of the young at weaning is sometimes equal to that of the mother, or in the case of a young animal, even greater, while the weight of a new-born litter is small compared with that of the mother, and yet gestation lasts for 23 and lactation 21 days. Thus a litter of six rats at 21 days of age would weigh from 240–300 g., while a new-born litter would rarely weigh over 70–75 g. It seems probable therefore that in a diet suitable for lactation, the carbohydrate should be decreased and other dietary factors increased as compared with the needs for growth.

Diet *A* is probably a good basis, which with some slight modifications, would make a suitable standard for nutrition work. This diet provides for good growth and reproduction. It is not satisfactory for lactation, but is fairly good for the gestation period as the young at birth were either of normal weight or not far below. The fact that the second and third generation animals grew rather less well than the first suggests that diet *A* is not complete, but as three generations were reared on the food it seems probable that all the necessary constituents are present, but that the balance of the various dietary factors needs adjustment. It is possible that a slight increase in mineral salts and perhaps vitamins as well (with a corresponding decrease of starch) is required during gestation, and a larger though similar increase for lactation. Only a few families were reared on diets *CE* and *CL*, but there is some evidence that this mixture of proteins gives better results during lactation, though for growth there seems to be no advantage in supplementing the caseinogen by other proteins. In fact diet *E* with only 16 % of caseinogen produced as good growth as diets *CE* and *CL* which contained 20 % of protein.

SUMMARY.

1. Three generations of rats have been reared on a synthetic diet of butter, caseinogen, potato starch, salt mixture, marmite and distilled water.
2. The use of cod-liver oil instead of butter in this synthetic diet produces less good growth and causes sterility.
3. Synthetic diets are described which produce good growth in young rats, but cause inability of the young to be born and consequent death of the doe.
4. Further evidence is given that the dietary requirements of the rat vary at different phases of its existence.

The expenses of this research were defrayed by a grant from the Medical Research Council. I am indebted to Miss W. M. Clifford for making the *post mortem* examinations in connection with this research.

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