CLXI. GROWTH AND REPRODUCTION ON SYNTHETIC DIETS. PART I.

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(Received October 29th, 1926.)

THE use of synthetic diets in nutrition experiments occupies a prominent place in modern research. For many years American workers have used what may be termed "semi-synthetic" diets in which such constituents as rolled oats, milk powder, etc., have formed a large bulk of the food, but experiments with *true* synthetic diets are not so numerous. It is of great importance to the nutrition worker to have a good standard synthetic diet, but at the present time such a food is not available. Various workers claim normal growth for animals on synthetic diets, but no control curve is given [*e.g.* Reader and Drummond, 1925]. In many instances the growth curve of Donaldson [1924] is taken as the standard, and it is quite true that rats on synthetic diets frequently show better growth than this, though not nearly so good as on a mixed diet of natural foods. The recent work of Osborne and Mendel [1926] shows that an improvement in the diet of the rat leads to a better rate of growth. These workers also found that the best growth was obtained on synthetic diets plus small additions of "natural foods," *e.g.* lettuce or liver. This obviously suggests that our synthetic diets are by no means perfect, and research work has yet to discover a synthetic diet which will produce as good growth as does the ordinary mixed diet of natural foods. Sherman and Campbell [1924] found that increasing the proportion of milk in an already adequate diet led to improved nutrition. Similar progress in true synthetic diets is badly needed. Mitchell [1922] added agar-agar to a synthetic diet and found that this led to the females breeding and rearing young, when they had previously failed. She suggests that bulk in the diet is a factor of importance. Previous work of the author [Hartwell, 1925, 2] also shows that the usual synthetic diets failed in this respect, and a method was described for overcoming this difficulty in another way. It seems useless to discuss the amounts of the various vitamins necessary for normal growth when we are using a diet which in itself only permits submaximal growth. Therefore, at the present time, it appears to be of vital importance for us to have a true synthetic diet which will provide for growth equal to that produced on ordinary mixed food. The experiments described in this paper were started in the hope of obtaining an improved synthetic diet.

EXPERIMENTAL.

Animals and method.

Piebald rats bred in the laboratory were used. For the majority of the experiments 12 animals, 6♀ and 6♂ were taken; they were kept in a large cage together so that breeding should proceed normally. The synthetic diet was started at the age of 23 days, food and water being given *ad lib.* The rats were weighed daily for 4 weeks, after which the weighings were omitted on Saturdays and Sundays. Later, when the gain was less, the animals were weighed weekly. Pregnant does were removed from the large cage a few days before the birth of the litter. The young and the mother were weighed every day. After suckling her babies for 21 days, the doe was returned to the big cage (except in one case where the young were not very fit and could not eat for themselves at the usual time).

Diets.

All diets were prepared according to the "cooked" method [Hartwell, 1925, 2].

Diets of the following composition were given:

	Diet					
	1	2	3	4	5	6
Caseinogen (purified; free from vitamins A and B) ...	20 g.	20 g.	20 g.	—	10 g.	10 g.
Food casein [Hartwell, 1922] ...	—	—	—	20 g.	—	—
Edestin* (94.36 % protein) ...	—	—	—	—	10 g.	10 g.
Gluten [Hartwell, 1925, 1] ...	—	—	—	4 g.	—	—
Starch (potato, pure) ...	64 g.	64 g.	64 g.	60 g.	64 g.	64 g.
Butter ...	12 g.	12 g.†	12 g.	—	12 g.	12 g.†
		or	or			or
Cod-liver oil ...	—	{ 4 cc. }	{ 4 cc. } ‡	4 cc.	—	{ 4 cc. }
Cotton-seed oil ...	—	{ 10 cc. }	{ 10 cc. }	10 cc.	—	{ 10 cc. }
Salt mixture [Hartwell, 1922, 1] ...	4 g.	4 g.	4 g.	4 g.	4 g.	4 g.
Marmite ...	2 g.	2 g.	5 g.	2 g.	2 g.	2 g.
Extract from marmite ...	—	3 cc.	—	3 cc.	—	3 cc.
(1 cc. = 1 g. marmite)						
Water	300 cc.	297 cc.	300 cc.	297 cc.	300 cc.	297 cc.

* The edestin used in this work was made for me by Messrs. E. T. Pearson and Co., Ltd. (Mitcham), to whom I am indebted for the above analysis.

† For 3 weeks and then cod-liver oil 4 cc., cotton-seed oil 10 cc.

‡ For 4 weeks and then 12 g. butter.

The changing of the fats in the above diets needs explanation. The experiment with diet 1 was made first, after which it was decided to use oils instead of butter, because cod-liver oil is regarded as a more constant source of vitamins A and D than is butter, since large quantities of the oil can be obtained at once and the vitamin content of butter varies with the feed of the cow, and also gives definite seasonal variations. When it was discovered that the does produced no litters on the oil diets, butter was substituted because the fertility on diet 1 was satisfactory, hence the change from oils to butter in experiment with diet 3. These changes in the fat ration in the early weeks of the experiments appear to make no difference to the final results, because breeding took place on diets 1 and 3 and not on diets 2 and 4.

RESULTS.

(a) *Growth.*

All six diets provided for growth, but the rate differed somewhat as shown in Fig. 1. This point will be referred to later. In each experiment the rats were in excellent condition; they were firm to feel and their coats were thick and sleek.

Fig. 1. Growth curves of male rats. Each curve is taken from the average of 6 animals (except with diet IV when only 5 were used).

(b) Reproduction.

The different experiments gave interesting results as regards reproduction. A summary of the breeding on the 6 diets is given below.

Diet 1. This food was given to two groups of does, one consisting of six animals which received the synthetic diet from 23 days of age (their weights ranging from 35–41 g.) and the other, a group of four does, which when the experiment was started weighed 57, 60, 63 and 68 g. respectively. During gestation, the diet contained 6 g. marmite instead of 2 g.

Group A. Duration of experiment: April 24, 1925—March 1, 1926. One doe died when her first litter was born, the other five animals produced 8, 6, 5, 8 and 9 litters respectively.

Group B. Experiment from April 17, 1925—March 1, 1926. One doe developed a prolapsed uterus at the birth of her second litter, the other three had 8, 6 and 6 litters respectively.

In both groups there was noticeable failure in rearing the young, but Group B gave decidedly better results than Group A. In both groups the litters were of small size and the young of low weight; frequently they were devoured by their mother soon after birth. From Group A 14 young were reared, while from Group B 28 were successfully weaned.

Diet 2. Experiment from June 29, 1925—August 9, 1926. During this time none of the does showed any signs of pregnancy.

Diet 3. This experiment is still in progress; so far each doe has produced 2 litters.

Diet 4. Experiment from August 29, 1925—August 9, 1926. There were five does on this diet and no litters were born.

Diets 5 and 6. These experiments were not continued long enough for testing the fertility of the rats.

DISCUSSION.

(a) The composition of the diets.

(1) *Protein.* It is customary to use caseinogen as the sole source of protein in synthetic diets, probably because there is so little choice of purified proteins. Man does not live on one protein only, and the inefficiency of a synthetic diet may be due to poorness in this respect. In milk, which is regarded as a perfect food for the young animal, the caseinogen is supplemented by lactalbumin. An attempt was made to remedy this possible protein deficiency by supplementing the caseinogen with edestin (diets 5 and 6), but the growth curve showed no indication of improvement, and after 12 weeks the experiments were discontinued. In diet 4 the protein was given in the form of food casein and gluten, thus providing a mixture of proteins. In this experiment the growth was good, though not quite up to that obtained when the rats had a mixed diet, and no better than with other diets containing pure caseinogen only. Diet 3 produced a slightly better growth curve than either of the other

synthetic diets. This food contained more marmite than any of the other diets and it is just possible that the yeast proteins (or protein products) were effective supplements to the caseinogen.

(2) *Vitamin B*. Diet 1 contained only 2 g. marmite per 100 g. solid, which may be regarded as rather little. This amount provided for quite good growth, though the rate was somewhat slower than when more vitamin B was given. Also this lower level of intake was sufficient for reproduction, because the does on diet 1 bred well. This is in agreement with the findings of Guest, Nelson, Parks and Fulmer [1926, 1], who state that the amount of vitamin B required for reproduction is not much greater than that required for normal growth. It is probable that 2 g. marmite is almost enough for maximal growth, and 5 g. is excess, because there is only a slight difference between the growth curves obtained with these two diets.

(b) *Growth and reproduction.*

It is evident from the experiments described in this paper that dietary requirements for growth and reproduction are totally different. The growth on diet 1 was less good than that on diets 2 and 4, yet the does receiving diet 1 produced numerous litters, while those fed on diets 2 and 4 gave birth to no young at all. From an analysis of the composition of the different diets, it appears that the fat is the primary factor in this respect. When butter was given reproduction occurred, but when cod-liver oil and cotton-seed oil constituted the fat ration, no young were born. According to Sure [1924, 1] the absence of vitamin E from the diet would explain these results. Butter is considered rich in this new vitamin, while cod-liver oil is low or lacking in it [Evans and Bishop, 1923]. On the other hand, Sure [1924, 2] obtained fertility in his rats with cotton-seed oil provided it was present to the extent of 5 %, and in the experiments described here the sterile rats were getting 10 % of cotton-seed oil. Mattill and Clayton [1926] also consider that vitamin E is necessary for fertility in both male and female rats, but Anderegg [1924], from similar experiments, concludes that it is unnecessary to assume the existence of a new vitamin for reproduction. Beard [1925] confirms the work of Evans and Bishop that a new factor, vitamin E, is necessary for reproduction.

The amounts of the fat-soluble vitamins in the diets described in this paper vary, and it may be that in diets 2 and 4 there is enough of these factors for growth, but insufficient for reproduction. The amount of cod-liver oil used should provide more vitamin A than is needed for growth, unless the cotton-seed oil possesses some inactivating action, as suggested by Fridericia [1924] for other oils.

Sherman and MacLeod [1925] found that rats on a diet containing relatively little vitamin A grew nearly to adult size, but were shorter lived, and their reproduction was poor compared with those on a similar diet, but containing about seven times as much vitamin A. It is difficult to reconcile the general fitness and good growth of the rats with an actual dietary deficiency, and it

seems probable that a more correct explanation is that offered by Nelson, Jones, Heller, Parks and Fulmer [1926] who suggest that failure in reproduction and lactation is more likely due to inadequate quantitative relations between the known constituents of the diet. It is now recognised that the amount of vitamin B necessary for normal lactation is much greater than that required for normal growth and reproduction [Guest, Nelson, Parks and Fulmer, 1926, 2]. Grant [1924] and Hartwell [1925, 3] find that the lactating rat needs four times as much as the non-lactating rat. It may be that a correspondingly greater quantity of vitamin A is needed for reproduction as compared with growth. The rats fed on diets 2 and 4 produced no litters, although they were in excellent condition. Post-mortem examinations showed that the testes of these animals were of low weight; the uteri also were abnormal. Histological preparations are now being made and will be discussed in a later paper. Experiments are also in progress to determine whether there is a failure in development of the reproductive systems or degeneration of the organs as soon as they mature.

SUMMARY.

1. Descriptions are given of various synthetic diets which produce good but not maximal growth; in one experiment, the growth curve approximates closely but is not quite equal to that obtained from control rats fed on a mixed diet.

2. The dietary requirements of the rat vary considerably at different phases of its existence. Two diets are described which provide for good growth (almost maximal), but no young were born. Another diet which gave less good results for growth was adequate for reproduction.

The expenses of this research were defrayed by a grant from the Medical Research Council.

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