CXVI. EVIDENCE OF THE EXISTENCE OF A DIETARY PRINCIPLE STIMULATING GENERAL GROWTH AND LACTATION.

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THIS work was originally begun to investigate the possible advantages or disadvantages of liver therapy in connection with the nutritional requirements of the growing mammalian organism, and as such the first investigation was undertaken to see if an animal fed on a basal diet containing all the known vitamins and accessory food factors showed any extra growth response when small amounts of fresh liver were fed in addition. There were a few references in the literature which indicated a beneficial effect of feeding whole liver on the growth rate of the animal.

Osborne and Mendel [1926] showed that stock rats fed on a basal ration of caseinogen as protein, starch and salts, yeast and cod-liver oil as a source of vitamins A, B and D, when fed in addition 40 g. of fresh lettuce daily or 1 g. fresh liver daily, showed an accelerated growth response compared with control animals fed on the basal ration alone.

They concluded their paper with the significant remark: "Possibly the acceleration in growth noted is the outcome of stimulation or promotion of activities of cell groups which preside over growth."

Smith and Bing [1929] attempted to improve the growing rate of their stock rats, which received a stock diet consisting of 2 parts whole wheat, 1 part whole dried milk, and some yeast given daily. They found that the addition of fresh lettuce greatly augmented the growth rate of their animals.

Macy, Outhouse, Long and Graham [1927] fed rats on a diet comprising milk powder, whole wheat and salts. Such a ration when supplemented with fresh lettuce daily gave a growth response greater than that obtained by them using other synthetic diets of whole wheat, caseinogen as protein, salts, yeast and cod-liver oil.

More recently Gregg-Smith [1931] has reported that an accelerated growth response may be obtained by the feeding of dried whole liver to rats on a basal diet of starch, cottonseed oil and salts; vitamins were added as yeast and cod-liver oil. The protein in these experiments was given in the form of various tissues which had first been extracted with alcohol till free from fat. No

growth rates are given by the author, but it is stated that the accelerated growth rate obtained is not due to any of the known vitamins.

This evidence, although indirect and, with the exception of Osborne and Mendel's work, somewhat unsatisfactory, since in many cases no carefully controlled experiments were done, pointed nevertheless to a beneficial effect of feeding whole liver on the growth of young rats maintained on synthetic diets.

This influence of fresh liver on the growth rate of the young animal has not only been fully substantiated but now appears to be vastly greater than has been previously supposed. An unidentified substance has been shown to exist in liver capable of intensifying the growth rate to a supernormal degree.

After the completion of the experimental work my attention was drawn to a paper by Daggs [1931]. This worker has investigated the effect of feeding to pregnant bitches on a synthetic diet the following tissues; whole liver, kidney, muscle and eggs. These tissues were fed separately as supplements to the basal rations. He found a much better growth rate of the young puppies and a beneficial influence on the lactation of the mother when liver was fed. The author states that the effect of the liver is due either to a better source of protein or to a more plentiful supply of amino-acids. He, however, suggests the possibility of the existence of a growth-promoting hormone in liver. The results published in the present paper were obtained independently of Daggs's work.

EXPERIMENTAL.

Albino and piebald rats were used. The preliminary experiments were all carried out on piebald rats bred in this laboratory. In the experiments which involved direct feeding of fresh liver to the young animals, the latter were weaned at the age of three weeks and placed on the full synthetic basal diet for a few days before actually starting the experiment.

The basal synthetic diet which has been used throughout the work in the main is:

				Parts by wt.
Rice starch	...	...	...	40
"Light white casein"	B.D.H.	...	...	23
Cane-sugar	...	...	...	17
Palm kernel oil	...	...	...	15
Salt mixture	...	...	...	5
Cod-liver oil	...	...	...	30
Wheat germ	...	...	...	10
Dried yeast	...	...	...	8

This diet has been modified in several experiments which will be mentioned in the text.

The cod-liver oil was incorporated with the basal diet, the latter only being

made up for three days in advance to obviate any possible destruction of vitamin A or other vitamins.

All rats had the synthetic ration *ad lib*. The salt mixture incorporated to the extent of 5 % in the basal ration was as follows:

Sodium chloride	51.8
Magnesium sulphate	79.8
Sodium dihydrogen phosphate ...	104.1
Dipotassium hydrogen phosphate	286
Calcium dihydrogen phosphate	162
Calcium lactate	390
Iron citrate	35.4
Sodium fluoride	0.04
Manganese sulphate	0.2
Potassium iodide	1.00

Although not listed, sufficient copper was present in this salt mixture as an unavoidable constituent.

Experiments have now been completed using both the piebald and albino strain of rat, and the result has been substantially the same in both cases.

STIMULATION OF GROWTH.

A. *The effect of feeding fresh liver to young rats.*

At the commencement of the experiment the rats were approximately 40 to 50 g. in weight. Ox-liver alone has been used; this was obtained fresh from the abattoir, and was fed at levels of 0.5 to 1 g. of fresh liver *per rat per diem*. A smaller dosage of liver has not been given and the question as to the minimum dose to give the maximum effect remains to be determined.

Fig. 1.

Fig. 2.

Fig. 1. Growth response on feeding 0.5 g. fresh ox-liver as supplement to basal diet to young ♂ rats.

A, liver-fed ♂. B, control ♂.

Fig. 2. A. Growth response on feeding 0.5 g. liver daily } as supplement to basal diet.
B. Growth response on feeding 1.00 g. liver daily }
C. Control.

Fig. 1 shows the growth response on feeding fresh liver to young male rats. That the effect is not increased by feeding liver at a higher level than 0.5 g. is seen

in Fig. 2. Here it may be pointed out that the possibility exists that the livers themselves may show a seasonal or individual variation and in order to minimise this effect the liver was only obtained in small quantities from the abattoir; by this means fresh liver was always at hand and an average sample of liver tissue was thus fed to an animal over a period of time.

The curves indicate that the growth response to the feeding of ox-liver occurs rapidly. A marked acceleration of growth rate is usually seen for a period of from five to six weeks when compared with the growth rate of the control animals.

The animals fed on liver have been killed and autopsied but no significant increase of adipose tissue which could account for the increase of weight has been detected. The animals appear larger in every respect than their controls of the corresponding age, an increase lying far beyond the range of biological variation.

In the case of the female animal the growth response is much less marked than is the case with the male. Fig. 3 shows the accelerated growth response

Fig. 3.

Fig. 3. Growth response on feeding 0.5 g. fresh liver as supplement to basal diet to young ♀ rats.

A, liver fed ♀.

B, control ♀.

Fig. 4.

Fig. 4. Average growth rate curves of male offspring of parents fed:

A, basal diet + 1.0 g. of liver *per rat per diem*.

B, basal diet alone.

of the female animals when fed fresh liver. The average of values used for growth curves lie only just outside the individual variation of the control animals.

It will be noticed from the above results that the direct feeding of fresh liver to young rats led to an immediate response in their growth rates. This impetus to growth was very marked with young animals. The response produced in older animals under such conditions was less marked, indeed the older the animal used, within limits, the less chance was there of obtaining the growth response. Whatever the process was, which had thus been stimulated in the organism by the feeding of raw liver, it was one which, in the young animal, had a much greater effect on the growth rate than in an animal some weeks older.

It therefore seemed possible that feeding fresh liver to male and female animals before mating them might lead to an even greater effect in the case of the offspring. This expectation has been fulfilled.

B. *Transmission from parents.*

Both piebald and albino rats have been used in the breeding experiments. In the mating of parents great care was taken to insure that the various litters were properly distributed in each batch, liver-fed and control. As a rule a batch was made up from two to three litters, brother serving as control to brother, sister to sister. Several batches of rats have now been bred and the results have always been consistent.

The basal diet for these animals was the full synthetic diet containing 10 % wheat germ. In the first experiments whole liver was fed from an early age to both male and female rats; the feeding of the liver to the animals was continued until the end of the lactation period (the male being separated from the female half-way through the gestation period).

The mother received 1 g. of fresh liver daily during the preliminary growth and gestation periods and about double this throughout lactation. The male received 0.5 g. of liver daily. On the day of birth of the litters both control and liver animals were given milk *ad lib*. Weaning occurred in both the control and liver litters after the expiration of 28 days from birth.

The young were allowed no liver and no supplement other than the basal diet mentioned above.

Milk, however, was given them during a period of five weeks after birth. At the age of 21 days the offspring began eating for themselves, and their growth rates were carefully followed.

The weight of the young rat at birth lies between 4.5 and 5.5 g.: no difference could be detected between liver and control litters in this respect. The young were weighed every week, and at the end of 7 and 14 days after birth no significant difference in weight could be detected in the offspring from liver-fed and control-fed parents. During the following week, however, some litters derived from liver-fed parents showed a slight appreciable increase in weight over the control litters, but in the majority of the litters no difference in weight even at the end of 21 days could be detected. From this point, however, the rates and growth curves rapidly diverged, the offspring from liver-fed parents growing much more quickly than the control offspring. The curves in Fig. 4 illustrate these facts.

The time at which the growth response manifests itself in the liver-bred offspring is approximately that at which they begin eating for themselves. The growth curves of the liver-bred offspring are such as might indicate a restricted food supply during the preliminary period of lactation.

Turning to Fig. 5, it is seen that here, as when liver was fed direct to the young animals, the male animal is more greatly affected than the female. It is noteworthy that in the growth curve of the female offspring from liver-

fed parents a distinct break occurs at or about the eighth week; this retardation of growth probably occurs with both groups of offspring but owing to the greater rate of growth of the liver-bred animals it is more marked in their case. This point at which marked retardation occurs corresponds to the onset of puberty. From this stage the growth rate of offspring from liver-fed parents and control parents is practically identical. The female offspring of liver-fed animals thus become larger by a margin equivalent to the accelerated growth obtained during a period from the end of lactation to about the seventh or eighth week.

Fig. 5.

Fig. 6.

Fig. 5. Average growth rate curves of female offspring born to parents fed:

A, 1.00 g. fresh liver *per rat per diem* + basal diet.
B, basal diet alone.

Fig. 6. Average growth curves of

A, offspring from parents fed fresh liver;
B, offspring from control animals;
C, second generation offspring from grandparents fed liver.

Final adult weight.

The data at hand indicate a more prolonged growth acceleration in the case of young whose mother has received liver than in the case of direct liver feeding to the young animal. Thus the weight ultimately attained by animals which had been fed liver from an age of 4 weeks throughout their growing period was in the majority of cases only very slightly heavier than that reached by the control animals.

Animals, however, which only received this growth stimulant by transmission from the parent organism, finally attained a weight which ranged from 70–100 g. heavier than that of control animals. Thus the weight generally attained by control animals on the full synthetic diet at an age of approximately twelve months was of the order of 350 to 370 g., whereas the weight of animals receiving the growth stimulant was of the order of 450 to 480 g. Some of these animals are still being kept on the full synthetic diet in order to find if duration of life is affected.

The question as to whether this growth stimulation is prolonged into the second generation has also been studied. The offspring of parent rats which

had been fed on a liver ration as a supplement to the synthetic diet were bred again and the growth rate of their offspring determined. Such second generation rats showed a growth rate practically identical with that given by the control animals (see Fig. 6).

Size of litters.

One further effect of liver feeding, apart from the question of lactation which is dealt with later in this paper, has been noted. This is in connection with the size of the litters born. Table I shows the numbers in the litters from a batch of female rats. This question cannot be definitely answered until large numbers of rats have been studied, since the individual variation in this respect is very great. However, the results at present in hand indicate a larger number of offspring born in the majority of cases to animals which have been fed on liver. The average size of the litter in 20 controls was 7; that in 13 liver-fed cases was 10.3. This means an average increase in the number of young born of 47 %, which is probably too large a figure to be without significance.

In this work no attempt has been made to cut litters down to a maximum of six, and as can be seen from Table I, which is a fair picture of the general

Table I.

Diet of parents	Diet of offspring	Lactation	Number in litter	Average weight of ♂ offspring at 9 weeks g.
Basal + liver	Basal	+	11	246
"	"	+	11	255
"	"	+	13	235
"	"	+	11	280
"	"	+	9	270
"	"	+	10	295
"	"	+	8	230
"	"	+	7	260
"	"	+	12	240
"	"	+	10	270
"	"	+	12	275
"	"	+	9	245
"	"	+	11	265
Basal	Basal	+	8	165
"	"	—	7	—
"	"	—	6	—
"	"	—	6	—
"	"	—	10	—
"	"	+	8	186
"	"	—	6	—
"	"	+	7	179
"	"	—	9	—
"	"	—	7	—
Basal + 8 % dried yeast	Basal	—	7	—
"	"	—	5	—
"	"	—	8	—
"	"	—	8	—
"	"	—	4	—
"	"	+	6	197
"	"	—	9	—
"	"	+	9	190
"	"	+	5	185
"	"	—	6	—

results, despite the higher numbers in the case of the liver-fed litters, the growth rate of the offspring is very much increased after the 4th week. Whether the growth acceleration would have been even greater if the numbers in the individual liver-fed litters had been cut down to the average numbers usually occurring in the control litters remains to be determined. In any case the growth rates of the offspring given above are those from liver-bred litters which contained a larger number of young than the control litters, the advantage, if any, resting with the control litters in this respect.

A larger statistical study is being made with regard to the question of fertility and these results will be reported later.

Period of feeding.

In all the preliminary experiments liver was fed to the parent animals over a long period, from an age of four to five weeks to an age of sixteen to twenty weeks, before the animals were used for breeding. It was therefore important to ascertain whether to obtain this growth response in the offspring it was necessary to feed liver from an early age to the parent animal over a prolonged period. It was conceivable that a greater effect on the offspring might be obtained if liver was fed to the parents during their growing period.

Experiments involving feeding of liver over varying periods of time and at different times in the life history of the animal have been made. Male and female albino and piebald rats were divided into the following groups.

Group (1). Parents which received liver (0.5 g. *per rat per diem*) from the age of weaning until the end of the lactation period: mating occurred at the age of sixteen weeks, hence the group received liver during a period of some sixteen weeks.

Group (2). Adult male and female rats which received liver for a period of four to five weeks from the time of mating, the lactation period of the females being thus included.

Group (3). Male and female rats which have been fed on liver, after attaining adult weight, for a period varying from four to five weeks before mating, with a continuation of it during the periods of gestation and lactation.

The data indicate no extra beneficial effect of feeding fresh liver over a prolonged preliminary period before breeding is commenced.

Lactation.

Many workers have reported the failure of mothers to rear and wean their young when fed purified synthetic diets. Some of the earlier work in connection with the failure of lactation is explicable in the light of recent work in that the diets fed were deficient in or had an inadequate supply of the vitamin B complex. Sure [1929] and other workers previously referred to have shown the necessity for an adequate supply of the vitamin B complex if effective lactation is to be obtained. Evans and Burr [1928] found that the diet required as much

as 15 % of brewer's yeast for the supply of B-vitamins if good lactation was to be obtained. Clayton [1930] found, using synthetic rations of milk powder, starch, lard and salts, that lactation failure was largely prevented if the mothers had an abundant supply of the antineuritic vitamin B₁. Evans, working with synthetic diets, maintained that the high "vitamin B" requirements needed for lactation are due entirely to lack of vitamin B₁ in the diet, since normal lactation occurred in diets in which extracts containing vitamin B₁ were added.

The synthetic diet used in these experiments has been shown to be adequate in the vitamin B complex for normal growth. The diet contained all the known vitamin and accessory food factors which have been shown to be necessary for the growth, maintenance and life of the rat. Hence with these considerations in mind it was unlooked for that such a diet should be insufficient for the needs of the lactating female. However, female rats which had been reared and bred on the full synthetic diet for any length of time failed in a large percentage of the cases studied to feed their offspring effectively. This phenomenon was entirely a question of lactation failure. The litters born to such mothers were to all appearances normal in weight and condition, they commonly lived for a period of 1-3 days and eventually died through inanition. In the majority of these cases no attempt was made by the female animal to feed her offspring.

This failure of the lactating power in the case of the control females was common to 70 % of the animals. The litters of the control rats which succeeded in the feeding of their young, were to all appearance in no better condition than those in the mothers of which failure of lactation occurred, yet in the former cases no suspicion of failure to rear and wean the offspring could be detected. A curious feature of these lactation experiments has been the tendency for lactation to proceed in an all or none manner. Only a very small number of cases have been noted in which the parent started to feed her young and subsequently failed before the lactation period elapsed. In the vast majority of cases, if the parent commenced to feed her young, she maintained this throughout lactation.

No such failure of lactation has occurred with those litters whose parents received fresh liver in addition to the full synthetic diet.

The question as to whether the control female animals which failed to feed their young were in any way deficient in vitamin B is answered by the experiments which are more fully dealt with in the next section of this paper.

Manganese consumption.

Orent and McCollum [1931] have recently published work indicating the need of manganese for the proper functioning of the lactating powers of the female. Manganese incorporated in the basal diet at a concentration of 0.005% was found to be sufficient for normal lactation. It was therefore of interest to determine whether a manganese deficiency was the reason for the failure of lactation on the full synthetic diet used in this work.

According to the analytical figures of the manganese contents of various

tissues and plant products given by Reiman and Minot [1920] and more recently by Richards [1930], fresh ox-liver contains 0.170–0.250 mg. manganese per 100 g. fresh weight of tissue. On this basis the liver-fed females were receiving at the maximum only 0.01 mg. more of manganese than the control females *per diem*. The diet used in the early experiments contained, apart from what was present in the wheat embryo, dried yeast and ferric citrate used, 1 mg. manganese per 100 g. On the assumption of a consumption of 10–15 g. of the basal diet *per rat per diem* the control animals were receiving a minimum of 0.1 mg. of manganese *per diem*. It seemed hardly conceivable that the addition of 0.002–0.01 mg. to an intake of 0.1 mg. of Mn could remedy the lactation.

Attempts have been made to test this point more completely. The manganese content of the basal diet has been increased to 0.01 % which gives an intake of at least 1.0 mg. *per rat per diem* in addition to the manganese occurring in the basal diet. The preliminary results from such an increase of the manganese concentration have shown no beneficial effect. These results will be published in due course.

To eliminate the possibility of there being other unknown essential inorganic constituents in which the diet may have been deficient, fresh liver has been ashed, and the resulting inorganic material incorporated to the extent of 1–5 g. equivalent of fresh liver in the diet and fed to female rats over a period beginning 3–4 weeks prior to mating and continuing throughout the gestation and lactation periods. No beneficial effect was observed.

It is conceivable that the manganese present in fresh liver is in a form more readily utilised than inorganic manganese and the present results may become reconciled to the work of Orent and McCollum in this respect.

Effect of the condition of the mother during lactation.

One further problem has arisen in connection with this lactation study. Turning to those control animals which succeeded in rearing their young, the question arose as to whether the better growth obtained in the young offspring by the feeding of liver to the parents was not due to a more efficient maternal organism, better nutrition and better maternal care during the lactation period. Some evidence with respect to this question has been collected.

In the first place, if this were the case one might expect that it would be during that period when the young were entirely dependent on their mother for nutrition that a difference in the growth rate between the control rats and liver-fed rats would become apparent. One would thus expect on this hypothesis the difference in weight to show itself at the end of that period in which the babies are completely dependent on their parent. The data obtained, however, show at the end of 14–21 days no significant difference in weight between the two groups of offspring.

Secondly, a comparison of the growth of offspring bred on a stock diet used in this laboratory (which is adequate for normal lactation of the rat) with the

growth of offspring bred exclusively on the full synthetic diet used in these experiments supports the view that lactation was fully adequate in this latter case.

Litters bred on the stock diet were transferred to the full synthetic diet at the birth of the young, their growth rates being compared with those of offspring from litters bred entirely on the synthetic diet. No difference in the growth rates of these two groups could be detected. This confirms the observations made earlier that the offspring born to those parent control rats which succeeded in rearing their young on the synthetic diet were normal in all respects.

NATURE OF THE GROWTH PRINCIPLE.

Distinction from known dietary factors.

The first problem which presented itself was the question whether the diet, although it contained adequate amounts of the known vitamins, might yet fail to contain them in sufficient quantity to give the maximum growth response in the young animal. With this viewpoint in mind the basal diet has been altered in various respects and the growth rates on such diets have been studied.

The curves in Fig. 7 indicate the effect of increasing vitamins A and D simultaneously as supplements to the basal diet. These were given as 2-4 extra drops of cod-liver oil *per rat per diem*, equivalent to 20-40 mg. of cod-liver oil. It is clearly seen that increase of such vitamins has had no effect on the growth rate of the young animals.

Differentiation from the Coward factor.

Coward, Key and Morgan [1929] showed that rats on a diet consisting of caseinogen, yeast, salts and carbohydrate grew to a weight of approximately 200 g. and ceased growth at this stage, growth only being resumed when certain dietary principles were added, amongst which wheat germ at a level of 5 % of the basal diet was found to be adequate.

Fresh ox-liver was also found to have an effect, hence it was natural to suspect that control animals receiving 2 % wheat germ in the basal diet had not sufficient of these principles for maximum growth, and the extra growth response seen in the animals fed liver in addition may have been due to an increased intake of them. Accordingly wheat germ, up to as much as 10 %, has been incorporated into the basal synthetic diet. Fig. 7 shows that increasing the concentration of wheat germ has had no effect on the growth rate.

Fig. 7. A. Growth rate obtained on full synthetic fresh diet containing 2 % wheat germ + 0.5 g. fresh liver *per rat per diem*. B and C. Growth rate of control animals on basal synthetic diet (2 % wheat germ). ↑ 2 % wheat germ increased to 10 %. ↑ Cod-liver oil increased here.

Miss Coward's work has been repeated and confirmed in its main features, but although additions of wheat germ to diets deficient in this were effective, no effect could be obtained with fresh ox-liver. No explanation of this discrepancy has been obtained except in so far as Dr Guha informed me that he obtained a positive result using liver taken from animals killed during the summer months. Winter liver was used in these experiments and there is the possibility of a seasonal variation in this factor.

In order to eliminate any possible chance of the control animals being deficient in Coward's principle, I have conducted all subsequent experiments on basal synthetic diets containing 8 % of wheat germ. In the results so far recorded the synthetic diet has contained this 8 % wheat germ.

Vitamin B complex.

A third possibility accounting for the increased growth response of the liver-fed animals was a relative deficiency of the vitamin B complex in the basal diet. Many workers have shown in recent years the need for ample supplies of vitamin B for the young animal if good growth is to be obtained.

Macy *et al.* [1927] showed that rats during the lactation period need more vitamin B complex than non-lactating rats. If the female is deprived of vitamin B the young fail to grow and may even die. They find that, if the young are to grow at their maximum rate, three to five times the normal amount of the vitamin B complex is needed by the mother during the lactation period. They define a diet adequate in the vitamin B complex as a basal diet which enables the young to reach a weight of 34–45 g. in weight at the age of twenty-one days.

Sure [1929] has shown that for rats to rear and wean their young a certain minimum amount of the vitamin B complex is required. He also states that this minimum amount is several times greater than the amount required for normal growth of the rat. According to this author the parent has difficulty in passing adequate supplies of B-vitamins to the young during the lactation period unless it is receiving ample amounts of these substances during this time. Under conditions of inadequate vitamin B nutrition the young frequently die of convulsions before the fourteenth day of lactation.

It became necessary therefore to determine whether the active principle in liver yielding the increased growth rate of the offspring of liver-fed mothers and the increased growth rate of the young animal directly fed were not due to an increased supply of vitamins B₁ and B₂. This question has been examined in the following ways.

1. At the age of 4–5 weeks offspring from both control and liver-fed parents were placed on a diet deficient in the vitamin B complex and the time taken to deplete both litters was determined. Now it will be remembered that it is just at this age that the very rapid growth response induced by liver feeding is occurring. Hence if the growth acceleration be due to a better and

higher supply of B-vitamins obtained from the parent animal one might expect the control rats to become depleted far more rapidly than those whose parents had received fresh liver as supplement to the basal diet.

Figs. 8 and 8a show, however, that there is no difference in the times at

Fig. 8.

Fig. 8. Growth curves of offspring from liver-fed parents, placed on a B-vitamins deficient diet. ↑ vitamin B₂ given. ↑ vitamin B₁ given.

Fig. 8a.

Fig. 8a. Growth curves of offspring from control parents placed on a B-vitamins deficient diet. ↑ vitamin B₂ given. ↑ vitamin B₁ given.

which both sets of rats become deficient in B-vitamins. It should be noted that both batches run out of vitamin B₁ before B₂.

That the growth response is not due to vitamin B₁ is also seen from the experiments involving the increase of wheat germ in the basal diet, which had no effect on the growth rate of the young animal.

2. There yet remains the possibility that vitamin B₂ is relatively deficient in the control diet. Some evidence with regard to this question has been obtained by feeding extracts from liver in which vitamin B₂ has or has not been destroyed. Lilly's extract No. 343 and liver extract B.D.H. have both been fed to (a) young growing rats, (b) parent rats for a period before mating and during the gestation and lactation periods.

Both these extracts contain large amounts of vitamin B₂. The results obtained from such experiments show that both by direct feeding, and by feeding the pregnant female a slightly increased growth rate only is obtained over the control animals. These results are indicated in Fig. 9.

The slightly increased growth rate obtained in these experiments was in all probability occasioned by small amounts of the growth principle adsorbed by the precipitate thrown down by the high alcohol concentrations used in the preparation of the extracts. This will be returned to later.

Further indication that vitamin B₂ is not concerned is given by the preparation of an autoclaved liver extract made according to the method of Guha and Mapson [1931]. This extract contains no vitamin B₁ or B₂. The growth rate given by feeding such an extract was greater than that given by control diets, though not as great as that given by feeding fresh liver.

Such considerations, although partially indirect, indicate that the growth-promoting substance in liver is distinct from vitamins B₁ and B₂.

3. Further direct evidence is at hand. Male and female animals have been fed on the basal synthetic diet containing 7.5 % dried yeast in which the yeast concentration has been raised to 12–16 %. Increase of the vitamin B complex

Fig. 9.

Fig. 9. Average growth curves of offspring from parents fed:

A, basal diet and liver.

B, basal diet and extra vitamin B₂ fed as 4 g. liver equivalent of the Lilly ext. no. 343.

C, basal diet alone.

Fig. 10.

Fig. 10. Average growth curves of offspring from parents fed:

A, basal synthetic and liver.

B, basal synthetic + 8 % dried yeast.

C, basal synthetic.

occasioned by this procedure had only a slight effect in increasing the rate of growth of the offspring (Fig. 10).

The experimental evidence described above negatives the theory that the growth response is due to any of the vitamins of the B-complex.

Protein.

The possibility that the control animals were suffering from any inadequacy of protein or amino-acids seemed unlikely when the high biological value of caseinogen as a protein [cf. Hartwell, 1926] was considered. That the active substance is not a protein is seen from the fractionation described below. Whether it is a protein degradation product is uncertain at the present.

Preliminary attempts at fractionation.

The first step undertaken was to ascertain whether absolutely fresh liver was essential in order to obtain this growth acceleration. In these experiments the effect has been tested by the direct feeding of the liver fractions to young animals of an age of approximately 21 days.

Fresh liver was finely minced, brought to a p_H of 4.5–5.0, whereby autolysis is facilitated and bacterial growth inhibited, and allowed to autolyse for 24 hours at 37–40°. After such treatment the autolysate was fed in amounts equivalent to 0.5 g. of fresh liver. Two series of controls have been made in

such experiments: animals fed the equivalent of fresh liver and animals fed the basal ration alone. The growth curves obtained are shown in Fig. 11. It is apparent that the autolysis has had no detrimental effects on the growth stimulating principle. This result was to be anticipated since fresh liver when fed suffers, presumably, a digestion as thorough as that which results from autolysis.

When ox-liver is ashed in a muffle furnace and the resulting ash fed in equivalents of 1.5 g. of the fresh tissue no stimulating effect on the growth of young is produced.

Preparation of active extracts.

Aqueous extracts were made from fresh ox-liver by first mincing the liver and heating the mass to 70° to coagulate the liver-protein and subsequently filtering. The minced material was slightly acidified to a p_H of 5.5–6.0 before heating. Such aqueous extracts when fed in the equivalent of 1.0 g. fresh liver to young rats showed a growth response above that of the control animals.

An extract made by first autolysing the liver for 24–28 hours and then extracting with water, when fed in the equivalent of 1.0 g. of fresh liver gave a response which was equivalent to that obtained by feeding fresh ox-liver, and which was twice as great as the response obtained by feeding the ordinary aqueous extract. The only apparent difference in the manufacture of the two extracts was a process of autolysis prior to the aqueous extraction in the second case; the p_H of extraction was practically the same in each case. Fig. 11 shows the effects of these extracts on the growth rates of young animals.

This beneficial effect of autolysis prior to extraction is open to two possible explanations:

(1) that the active principle in liver is an integral part of a large molecule and that enzymic change liberates the active smaller molecule, which is then easily extracted;

(2) that the active principle is contained intracellularly in the tissue, and that autolysis by destruction of the cell membrane facilitates its removal.

Fractional separation of the active principle from aqueous liver extracts by means of alcohol has been attempted. Fresh liver was minced, brought to a p_H of 4.5–5.5 with dilute hydrochloric acid, and an equal volume of distilled water added. The temperature of the mass was raised to 70° whereby the liver-protein was coagulated and filtration facilitated. The aqueous extract was concentrated *in vacuo* to a thin syrup. Alcohol was added to make a concentra-

Fig. 11. Growth curves of young male rats fed.

A. Basal synthetic + 1.00 g. fresh liver per diem.

B. Basal synthetic + 1.00 g. equivalent of an autolysed liver extract.

C. Basal synthetic + 1.00 g. equivalent of an aqueous extract.

D. Basal synthetic alone.

tion of 60 % and the precipitate produced tested on young growing rats was found to be inactive. To the filtrate alcohol was then added to make a concentration of 90 %, and the precipitate was again inactive or only slightly active; the activity remained in the alcohol-soluble fraction. It has been found advantageous in order to obtain the maximum effect to ensure a partial digestion of the liver before making the aqueous extract. This has been effected by, (a) autolysis, as previously described; (b) digesting the tissue with papain at 60–70° for 10–20 minutes before making the aqueous extract.

Preliminary experiments have also shown that the active substance is extracted from fresh liver by alcohol of a concentration of 90 %¹.

DISCUSSION.

The results recorded in this paper indicate the presence in liver of a substance capable of considerably augmenting the growth rate of the young mammalian organism, when the latter is fed on a full synthetic diet containing all the known essential dietary principles. The growth curves studied, and the general condition and characteristics of the animals which have received such a stimulus to growth also indicate that we are dealing here with a normal physiological process or processes which have been stimulated to an increased activity. The animals have shown no retrogression in weight as frequently occurs when extracts made from certain endocrine organs are injected. Under such conditions a pathological condition of growth is set up, an immense acceleration, frequently followed at the cessation of the injections by a resultant fall in weight. Growth of this type is evidently far from normal in the physiological sense.

It is premature at this stage to say whether the substance active in stimulating the general growth is identical with that stimulating the lactating powers of the female. Further work in this connection is in progress.

The facts described in this paper are suggestive in the light of recent knowledge of a primary stimulation of the anterior pituitary gland. Whilst at this stage any theory advanced as to the physiological cause of these effects must of necessity be merely of a suggestive nature, yet the hypothesis is being borne in mind in this work that we are dealing with some such stimulation of endocrine function.

I have provisionally named the substance promoting growth “*physin*” until its chemical nature is more clearly defined.

¹ For some of the material used in testing the activity of substances soluble in 90 % alcohol I am indebted to Dr F. H. Carr of British Drug Houses. I am permitted to state that such an extract may be obtained from British Drug Houses, Ltd. by physicians wishing to make clinical experiments on human lactation, *etc.*

SUMMARY.

1. A substance present in fresh ox-liver has been shown to increase very markedly the growth of rats maintained on a diet containing all the hitherto known dietary principles in amount approximately twice the normal.

2. This substance has been shown to have a much more marked effect on the growth rate of the male than the female.

3. A stimulating effect on the growth rate after weaning is transmitted from a parent, which has received the active substance in its diet, to its offspring fed solely on the synthetic diet.

4. It has been demonstrated that liver has also a stimulating effect on lactation; whether the substances stimulating growth and lactation are identical remains to be determined.

5. The average number born to parents fed on liver has been definitely larger than the average number in control litters.

6. The growth-promoting substance has been shown to be distinct from hitherto known accessory food factors.

7. Preliminary investigations into the nature of the active substance are reported.

8. The name "physin" has been tentatively advanced for this substance.

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REFERENCES.

- Clayton (1930). *J. Nutrition*, **3**, 23.
Coward, Key and Morgan (1929). *Biochem. J.* **23**, 695.
Daggs (1931). *J. Nutrition*, **4**, 443.
Evans and Burr (1928). *J. Biol. Chem.* **76**, 263.
Gregg-Smith (1931). *Proc. Soc. Exp. Biol. Med.* **28**, 597.
Guha and Mapson (1931). *Biochem. J.* **25**, 1674.
Hartwell (1926). *Biochem. J.* **20**, 751.
Macy, Outhouse, Long and Graham (1927). *J. Biol. Chem.* **73**, 189.
Orent and McCollum (1931). *J. Biol. Chem.* **92**, 651.
Osborne and Mendel (1926). *J. Biol. Chem.* **69**, 661.
Reiman and Minot (1920). *J. Biol. Chem.* **42**, 329.
Richards (1930). *Biochem. J.* **24**, 1572.
Smith and Bing (1929). *J. Nutrition*, **1**, 179.
Sure (1929). *J. Nutrition*, **1**, 139.