LIV. STUDIES ON THE FAT-SOLUBLE GROWTH-PROMOTING FACTOR.

(I) STORAGE.

(II) SYNTHESIS.

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(I) STORAGE OF FAT-SOLUBLE *A*.

It has been known for some time that some land animals which receive the fat-soluble growth-promoting factor in their diet store a certain amount of it in their glandular organs [McCollum and Davis, 1915, 2; Osborne and Mendel, 1917, 1918; McCollum, Simmonds and Parsons, 1921; Zilva and Drummond, 1922] and a lesser amount in their body fat [Drummond, Golding, Zilva and Coward, 1920] and other tissues. Among the glandular organs the chief storehouse for this substance is the liver, but the kidneys and testes also contain a moderate amount.

The study here described is part of an investigation into the mode of action of certain forms of light in the promotion of growth and prevention and cure of experimental rickets in rats. In the course of this investigation it was found necessary to learn whether the liver of rats¹ also contains appreciable amounts of fat-soluble *A*, whether the quantity varies with that in the diet, and whether any is present in the liver of rats that have stopped growing after being fed on a diet as deficient as possible in that factor.

EXPERIMENTAL.

To test liver for the presence of the fat-soluble growth-promoting factor, the biological method described by Zilva and Miura [1921] was employed.

¹ Since this investigation was completed a communication has appeared on the subject of storage of fat-soluble *A* in the liver of rats [Coward, Lush and Palmer, 1923]. Their findings are very similar to those reported in Part I of this paper.

THE FAT-SOLUBLE GROWTH-PROMOTING FACTOR IN RATS 447

Rats were given a purified diet free from detectable amounts of fat-soluble *A*, but otherwise adequate when a sufficient amount of the above factor is present.

The diet was composed as follows:

Inactivated caseinogen	20 g.
Starch	50 g.
Cotton-seed oil (hardened)	15 g.
Salt mixture [McCollum and Davis, No. 185, 1915, 1]	5 g.
Marmite	5 g.
Decitrated lemon juice	5 cc.
Distilled water	50 cc.

When growth had ceased for about three weeks, or, after a shorter period if the animal was beginning to lose weight, the rat was considered prepared for the test and the liver feeding was begun. The liver was administered by hand to make sure that the entire amount was consumed. With few exceptions the animals ate it greedily. Liver feeding was continued for from four to six weeks, but for the sake of uniformity and of proper comparison the growth for the first four weeks only is illustrated in the charts.

The Effect of Feeding 0.5 g. of Liver from Rats receiving 3 % Cod-Liver Oil in their Diet.

Rats G. 122, G. 123, G. 126, G. 162, G. 163 and G. 165 were prepared for the test in the manner mentioned above. Of the six rats, five were actually losing weight when the special feeding was started and three had already developed varying degrees of xerophthalmia. To these six rats there was administered daily 0.5 g. of fresh liver from rats fed for from two to four months on a diet containing 5 % cod-liver oil as the main source of fat-soluble *A*. The effect can be seen in Table I and in Chart 1, in which the growth curves of rats G. 123 and G. 126 are illustrated as representative of the group¹.

During the first four weeks of liver feeding these rats gained on an average 15 g. a week, which is equal to the normal for animals of that age. The three rats (G. 122, G. 123 and G. 126) that had xerophthalmia when liver feeding was begun recovered from it in from two to four weeks. In one rat (G. 122) the cure was remarkable inasmuch as xerophthalmia was very far advanced when liver feeding was started.

In order to see whether an appreciable amount of the fat-soluble *A* contained in the liver fed to the above rats had been stored, 0.5 g. of their liver was administered to rats G. 174 and G. 175, previously prepared for the test. There was an appreciable response, but it was not nearly as great as in the case of the rats illustrated in Chart 1.

¹ In the charts only two typical representatives of all the groups are illustrated. The complete details for every group are given in Table I.

Table I. *Showing the total gain in weight of the test rats on — A diet after four weeks of additional feeding with the various amounts and kinds of liver.*

Rat No.	Type of liver tested	Amount of liver fed, g.	Total gain in weight during first four weeks of liver feeding, g.
G. 122	From rats on a diet containing 3 % cod-liver oil	0.5	59
G. 123	" " "	"	44
G. 126	" " "	"	68
G. 162	" " "	"	60
G. 165	" " "	"	71
G. 163	" " "	"	55
G. 174	From rats G. 122, G. 123, G. 126, G. 162, G. 163 and G. 165	"	22
G. 175	" " "	"	28
G. 159	From rats on a diet containing 3 % cod-liver oil	0.25	58
G. 169	" " "	"	73
G. 171	" " "	"	55
G. 170	" " "	"	45
G. 142	From rats G. 159, G. 169, G. 170 and G. 171	"	51
G. 144	" " "	"	36
G. 482	From rats on a diet containing 10 % butter	0.5	30
G. 160	" " "	"	17
G. 177	" " "	"	20
G. 178	" " "	"	18 (2 weeks)
G. 282	From rats on a diet very deficient in fat-soluble A	"	Lost weight
G. 263 a	" " "	"	"
G. 125	" " "	"	"
G. 264 a	" " "	"	3
G. 278	" " "	"	0
G. 314	" " "	1.0	Lost weight
G. 500	" " "	"	"
G. 498	" " "	"	8
G. 166	" " "	"	32
G. 418	From rats on — A diet irradiated for four weeks after they had definitely ceased gaining weight	0.5	23
G. 523	" " "	"	36
G. 525	" " "	"	22
G. 373	" " "	"	19
G. 520	" " "	"	19
G. 375	" " "	"	13

The Effect of Feeding daily 0.25 g. of Liver from rats whose diet contained 3 % Cod-liver Oil.

After preparation, rats G. 159, G. 169, G. 170 and G. 171 were given daily 0.25 g. of liver from rats whose diet had contained 3 % cod-liver oil. Resumption of growth was prompt, and during the first four weeks the gain in weight averaged 14 g. a week, which is equal to the normal for that age. (See Chart I and Table I.)

The amount of fat-soluble A stored by rats G. 159, G. 169, G. 170 and G. 171 was now tested by feeding their liver (0.25 g.) daily, to rats G. 142 and G. 144 which had been prepared for the test in the usual way. For the effect, see Table I. Although in these two rats the growth during the first four weeks of liver feeding was greater than in those which received 0.5 g. of more potent liver, yet this is probably accounted for by the fact that rats G. 142 and G. 144 were older and heavier when they were used for the test, and such animals, according to Zilva and Miura [1921], and others, require less fat-soluble A to induce growth. One rat (G. 142) had mild xerophthalmia at the time liver

feeding was begun, but in ten days this had completely disappeared. When liver was discontinued, growth ceased fairly promptly, which indicates that no great amount of fat-soluble *A* contained in the 0.25 g. of this liver fed daily had been stored. (See Table I.)

Chart 1.

G. 123 and G. 126. The effect of feeding daily 0.5 g. of the liver of rats whose main source of fat-soluble *A* was 3 % of cod-liver oil in their diet.

G. 159 and G. 169. The effect of feeding daily 0.25 g. of the liver of rats whose main source of fat-soluble *A* was 3 % of cod-liver oil in their diet.

G. 482 and G. 177. The effect of feeding daily 0.5 g. of the liver of rats whose main source of fat-soluble *A* was 10 % of butter in their diet.

Broken line - - - - = Preparatory period on - *A* diet.

Continuous line — = Period of liver feeding.

*The Effect of Feeding Liver from Rats whose Diet contained
10 % of Butter as the chief source of Fat-soluble A.*

Rats G. 160, G. 177, G. 178 and G. 482 were employed for this test and were given daily 0.5 g. of liver from rats fed during a period of from two to four months on a diet containing 10 % butter as the chief source of fat-soluble *A*. The result can be seen in Chart 1 and Table I. The difference between the growth of rats fed on the "butter liver" and that of animals fed on "cod-liver oil liver" is very striking.

Too few rats were available to permit the feeding of their liver to other rats for the detection of storage.

*The Effect of Feeding 0.5 g. and 1.0 g. of Liver from rats that have
ceased growing on a Diet very deficient in Fat-soluble A.*

For this test many rats were placed on the diet deficient in fat-soluble *A* described at the beginning of this communication. When they had definitely ceased growing (no gain in weight for three weeks), 0.5 g. of their liver was

fed to rats G. 282, G. 264 *a*, G. 263 *a*, G. 278 and G. 125 prepared for the test in the usual way. Chart 2 and Table I show definitely that no resumption of growth occurred as a result of this addition to the diet. More rats were tried, but they are not all charted, since some lived for only a week or slightly longer after liver feeding was begun, some developed acute entero-colitis, while others refused to eat the liver after a few days and had to be discarded. None of them showed any resumption of growth; in fact the loss of weight in some was very great on account of the intercurrent intestinal infection.

Chart 2.

G. 282 and G. 264 *a*. The effect of feeding daily 0.5 g. of the liver of rats that have ceased growing on the -A diet, very deficient in fat-soluble A.

G. 314 and G. 498. The effect of feeding daily 1.0 g. of the liver of rats that have ceased growing on the -A diet, very deficient in fat-soluble A.

G. 523 and G. 418. The effect of feeding daily 0.5 g. of the liver of rats on the -A diet which were irradiated for four weeks after they had definitely ceased gaining weight.

Broken line - - - - = Preparatory period on -A diet.

Continuous line — = Period of liver feeding.

One gram of similar liver was fed daily to rats G. 314, G. 501, G. 498 and G. 166. Rat G. 314 died after three weeks of liver feeding, having lost slightly in weight during the period. In the case of rat G. 501 liver feeding had to be discontinued after three weeks on account of shortage of material. During the period of liver feeding it did not gain weight. This rat remained on the -A diet and died one week after liver feeding was discontinued. Rat G. 498 gained 8 g. during a period of four weeks of liver feeding, while rat G. 166, receiving the same amount of similar liver, made a definite gain in weight during the first two weeks. After that growth ceased completely, the weight remained stationary, and during the last week the rat developed mild xerophthalmia. The explanation of this gain in weight during the first two weeks may lie in the fact that this rat's store of fat-soluble A was not completely exhausted and that the cessation of growth before the test was begun was not due to deficiency of fat-soluble A alone but to some complication; or, it is also possible that the liver used during that period was from rats which were on the deficient diet too short a time. However, since the deficient diet,

though very poor in fat-soluble *A* cannot be regarded as one containing no trace of that factor, it is hardly likely that the liver of animals fed on such a diet contains none of it. Therefore, it is possible that a rat stops gaining weight before the store of fat-soluble *A* in its body is completely exhausted.

SUMMARY AND CONCLUSIONS OF PART I.

The liver of rats on various diets has been tested by the biological method for the storage of fat-soluble *A*.

1. The results show that the fat-soluble growth-promoting factor, if present in the diet of rats, is stored in their liver, and that it is stored in that organ in rough proportion to the amount contained in the diet.

2. In an indirect way, the conclusion of Zilva and Miura that cod-liver oil is much more potent in fat-soluble *A* than butter is confirmed.

3. Half a gram of liver from rats that had stopped growing on a — *A* diet showed no appreciable traces of fat-soluble *A* when tested by the biological method. The effect of feeding 1 g. of such liver varied. Thus it would seem that a rat ceases growing before the store of fat-soluble *A* in its body is completely exhausted. Whether that means that this final reserve is used for some purpose other than growth cannot be deduced from these experiments.

(II) ON THE SYNTHESIS OF FAT-SOLUBLE *A* IN THE ANIMAL BODY.

It has been shown by Hume [1922] and the authors [1922] that rats on a diet very deficient in fat-soluble *A* if irradiated for about ten to twenty minutes daily by the mercury-vapour quartz lamp, grow very much better than non-irradiated rats on the same diet. Also, rats on a — *A* diet, if irradiated daily after they have ceased gaining weight, resume growth for a variable period. But in both cases gain in weight eventually ceases and the subsequent history is the same as that of non-irradiated rats, viz. decline in weight, development of xerophthalmia in some of the animals, and finally death with or without a complicating terminal infection. Because irradiation was unable to effect the continuance of growth on a — *A* diet, the assumption of a photosynthesis of fat-soluble *A* in the animal body to account for the stimulation of growth seemed unjustified. But it was suggested that the mode of action of light in stimulating growth was "by effecting an economy of the action of the fat-soluble factor still stored in the animal at the time irradiation was begun, as well as by making available the small quantities of organic factor which may still be present in the daily ration of deficient diet."

At the same time that the above investigation was being carried out another method was used to determine whether synthesis of fat-soluble *A* occurs in the body of a rat under the influence of rays from a mercury-vapour quartz lamp.

It has been shown in the first part of this communication that 0.5 g. of the liver of rats on a — *A* diet that have ceased gaining weight for about three weeks contains no fat-soluble *A* detectable by the biological method, and that

1 g. of such liver gave a positive result in only one out of four tests. Rats were therefore placed on the $-A$ diet, and when they had ceased gaining weight for three weeks, at which time but little fat-soluble A should have been left in the liver, irradiations by the mercury-vapour quartz lamp were begun. In most instances (as reported before) resumption of growth occurred promptly and lasted for a variable period, but rarely for longer than four weeks. Therefore, after four weeks of irradiation, when they had again stopped gaining weight, the rats were killed and their liver was tested in the usual way for fat-soluble A . In the case of rats G. 418, G. 523 and G. 525 the gain was as great or greater than that caused by the same amount of "butter liver" (see Chart 2). Rats G. 373, G. 520 and G. 375 did not show as great a gain (see Table I), but the liver feeding was carried out for only two weeks on account of shortage of material. In all three cases the rats ceased gaining weight quite promptly and died in from three to six weeks after the liver was discontinued. Rats G. 523 and G. 525 were kept on the $-A$ diet for about two weeks after liver feeding was discontinued, during which time they lost weight; and then 0.25 g. of liver from rats receiving cod-liver oil was administered to them. In both cases there was a very prompt gain in weight.

DISCUSSION.

The interpretation of the above results is difficult. For reasons already stated it is not justifiable to postulate actual synthesis of fat-soluble A in the body of these rats¹. If not from synthesis, then where did the vitamin in the liver of the irradiated animals come from? One possibility, which has not yet been tested experimentally, is that it is mobilised from the other parts of the body in which it occurs and is partly stored in the liver. Then why, despite the appreciable store of fat-soluble A still present in their liver, did the irradiated rats not continue to grow, while their liver, when fed to test animals, stimulated the growth of these? Apparently, in the irradiated rats, the vitamin, though still present in the liver cannot be liberated and so be made available to the organism, while that same liver when ingested by another rat becomes a direct source of supply of fat-soluble A immediately available by absorption from the intestinal tract. It must be remembered that during the experimental period of four weeks a test rat consumed about four livers from irradiated animals. But without definite knowledge of how and where in the body this vitamin acts one cannot state why the ingested liver should have this effect. The correct explanation must await the results of further studies on the nature of the fat-soluble growth-promoting factor as well as on the site and mode of its action.

¹ The photosynthetic production, however, of fat-soluble A from some stored material which is not renewed on the deficient diet, is not absolutely excluded.

SUMMARY AND CONCLUSIONS OF PART II.

1. Half a gram of liver from rats on a — *A* diet that have ceased gaining weight for at least three weeks contains no fat-soluble *A* detectable by the biological method.

2. Half a gram of liver from rats on the same — *A* diet irradiated by the mercury-vapour quartz lamp for four weeks after they have definitely ceased gaining weight does contain an appreciable amount of fat-soluble *A* detectable by the biological method.

3. The results of the above investigation are not interpreted as evidence of synthesis of fat-soluble *A* in the animal body.

(Note added on 23rd August, 1923.)

In a preliminary investigation by one of the authors (S.) on the storage of the *antirachitic* organic factor, definite protection against the development of rickets in rats on diet 3143 of McCollum, Simmonds, Shipley and Park [1921] was afforded by the daily administration of 1 g. of liver from rats on purified casein diet which were given 100 milligrams of crude cod-liver oil every day. Partial protection was obtained by 0.5 g. of liver.

But 1 g. of liver from rats, that had ceased growing on the purified diet alone, did not afford any protection against the development of rickets on diet 3143.

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