THE NECESSITY OF CERTAIN LIPINS IN THE DIET DURING GROWTH.

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Whether or not the growing mammal must obtain certain lipins preformed in the diet, has up to the present time not been definitely determined. That bodies of this class can be dispensed with for a considerable period without interfering with normal growth, is now well established. Osborne and Mendel[1] have recently published data showing that young rats can grow normally during sixty days on rations containing but insignificant traces of ether-soluble matter.

That lipins of several kinds can be synthesized in large quantities in the animal body, is apparent from the experiments of McCollum, Halpin and Drescher,[2] who secured normal egg production in hens on a ration containing but very small amounts of ether-soluble substances. This observation has been fully confirmed by Fingerling[3] with ducks.

During the past year we have been engaged in a study of the influence of the composition and quantity of the inorganic content of the ration on growth in the rat.[4] In this work we have employed rations compounded of pure casein, carbohydrates, and salt mixtures made up of pure reagents, and the same rations in which a part of the carbohydrates was replaced by lard, with a considerable degree of success. Young rats have been found to be very sensitive to variations in the character of the salt mix-

[1] This *Journal*, xii, p. 81, 1912.

[2] McCollum, Halpin and Drescher: Proceedings of the American Society of Biological Chemists, this *Journal*, xi, p. xiii, 1912; also *ibid.*, xiii, p. 219, 1912.

[3] Fingerling: *Biochem. Zeitschr.*, xxxviii, p. 448, 1912.

[4] See McCollum and Davis: Proceedings of the American Society of Biological Chemists, this *Journal*, xiv, p. xl, 1913.

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tures supplied, but with certain mixtures we have been able to obtain practically normal growth for periods varying from 70 to 120 days. Beyond that time little or no increase in body weight can be induced with such rations. These rats may remain in an apparently good nutritive condition on these rations for many weeks after growth ceases. That they are still capable of growth has been repeatedly demonstrated by changing to naturally occurring food-stuffs. That our animals, during their period of growth or during the period of suspension of growth which always accompanies long continued feeding of purified food substances, are in a physiological state which is nearly normal is evident from the fact that we have had three female rats produce young after being fed only casein, carbohydrates, lard and salt mixtures, for periods of 108, 127 and 142 days, respectively. These rats had made approximately normal growth for about eighty days on this ration. In none of these cases did the mothers produce enough milk to properly nourish the young, so that they were found to be decidedly undersized when seven to eighteen days old.

The fact that a rat of 40 to 50 grams in weight can grow normally during three months or more on such rations, then cease to grow but maintain its weight and a well nourished appearance for weeks and then resume growth on a ration containing certain naturally occurring food-stuffs would lead one to the belief that on these mixtures of purified food substances the animals run out of some organic complex which is indispensable for further growth but without which maintenance in a fairly good nutritive state is possible.

After numerous attempts to prevent the occurrence of growth suspension by nice adjustments between the various ingredients of our diets, we have found that the failure of rats to make further growth, after being brought to this "critical" point on mixtures of isolated food substances, is due to a lack of certain ether-soluble substances in the diet. These can be supplied by the ether extract of egg or of butter. The curves of a number of rats are presented to illustrate how a resumption of growth at about the normal rate results from the introduction of such ether-soluble substances into the diet after growth has ceased. It should be borne in mind that these experiments were intended primarily to furnish data as to the values of the salt mixtures

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supplied by the rations and do not in all cases represent the highest degree of success attained by feeding these rations. The curves are selected entirely with a view to illustrating our almost invariable success in inducing a resumption of growth after complete suspension for a time on a ration which during eight weeks or more had sufficed for growth somewhat closely approximating the normal rate. The ether extracts employed were freed from ether *in vacuo* at temperatures below 60°C.

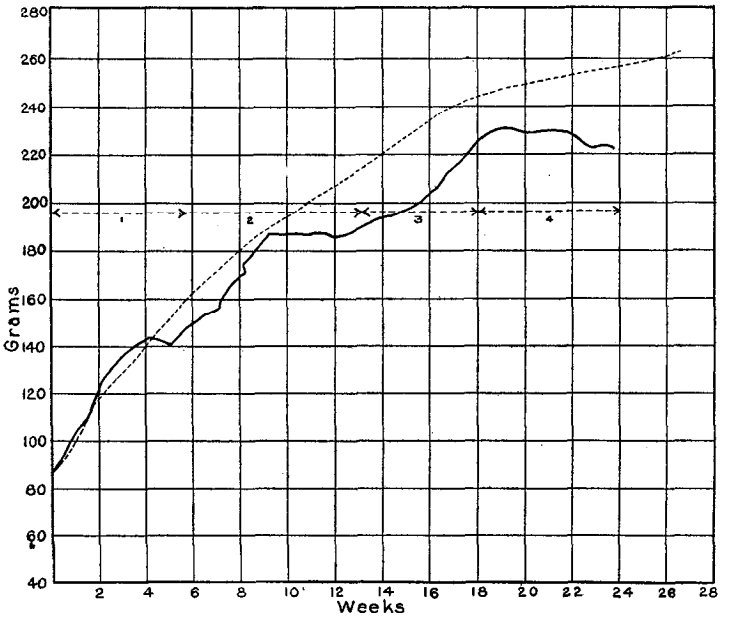

CHART I. *Rat 104* (male) shows the record of a rat which grew in a nearly normal manner during sixty-five days on a ration of purified food substances. The broken line represents the normal curve of growth. A suspension of growth occurred at this time and after four weeks without increase in body weight, 1 gram of ether extract of egg was added to the ration every other day, with the result that the animal gained 35 grams during the following forty-two days. The rations employed during the four periods of the experiment were as follows:

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Period I.	per cent	Period II.	per cent	Period III.	per cent	Period IV.
Salt mixt.	6	Salt mixt.	6	Salt mixt.	6	Same as III but without the ether extract of egg.
Casein.	18	Casein.	18	Casein.	18	
Lard.	20	Lactose.	15	Dextrine.	74	
Lactose.	20	Dextrine.	59	Agar-agar.	2	
Starch.	31	Agar-agar.	2	Ether extract of egg, 1 gm. every other day.		
Agar-agar.	5					

The salt mixture employed in these rations consisted of:

	grams		grams
Calcium lactate.	3.785	Dipotassium phosphate.	3.648
Sodium citrate (anhydrous).	3.296	Sodium chloride.	3.430
Magnesium citrate (10.2% Mg)	1.298	Ferric citrate.	1.000
Potassium citrate.	3.118		

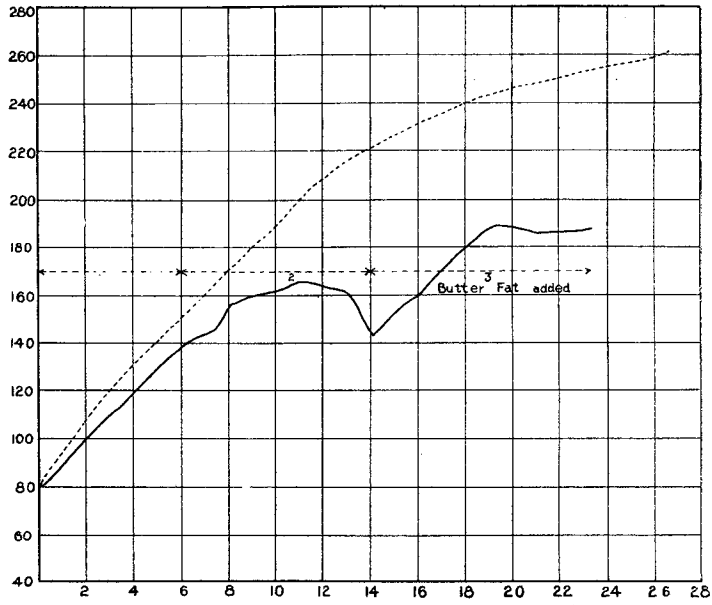

Chart II. *Rat 141* (male) shows the record of a rat which grew continuously although slightly under normal rate during eighty days on a ration of purified food substances. There was at this time a complete suspension of growth and a rapid decline in body weight. The addition of 10 per cent of ether-soluble butter fat to the diet led to a prompt resumption of growth during the following thirty-five days, when the rat gained 50 grams.

The rations employed were as follows:

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Period I.	per cent	Period II.	per cent	Period III.
Salt mixture	6	Salt mixture	5	Same as II with butter fat replacing part of dextrine.
Casein	12	Casein	12	
Lard	20	Lactose	20	
Lactose	15	Dextrine	61	
Starch	42	Agar-agar	2	
Agar-agar	5			

The salt mixture employed consisted of:

	grams		grams
Sodium chloride	0.61	Calcium lactate	11.38
Dipotassium phosphate	17.00	Magnesium citrate(10.2%Mg)	23.42
Monocalcium phosphate	1.63	Ferric citrate	1.00

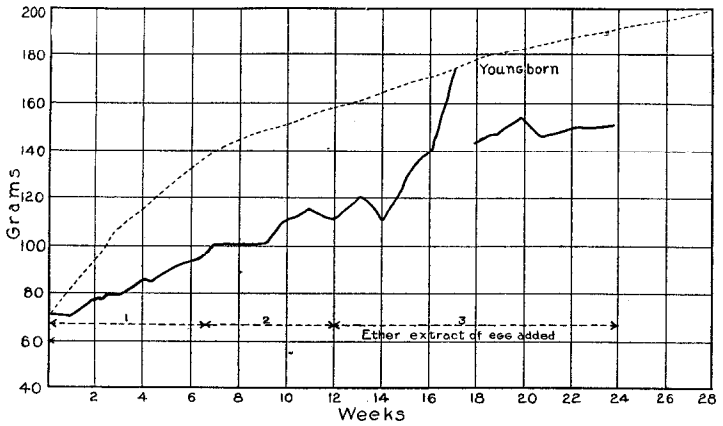

Chart III. *Rat 138* (female). This rat received a salt mixture which has induced much better growth in other rats. Her growth was but little over half normal during the first eighty-five days on a ration of purified food substances. She was with a male during this time but did not become pregnant. From the 86th day she was given 1 gram of ether extract of egg every other day. Growth was at once resumed and she became pregnant on the 14th day after the addition was made. On the 119th day of "artificial" feeding she gave birth to eight young which she suckled normally during twenty days. The aggregate weight of the young at the age of twenty days was 162 grams, the weight of the mother at this time being 155 grams. She is still in an excellent nutritive condition after one hundred and sixty-eight days and is continuing on the diet.

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The rations employed were as follows:

Period I.	per cent	Period II.	per cent	Period III.
Salt mixture	6	Salt mixture	8	Same as II with ether extract of egg.
Casein	18	Casein	18	
Lard	20	Lactose	20	
Lactose	15	Dextrine	52	
Starch	36	Agar	2	
Agar-agar	5			

The salt mixture employed was composed of:

	grams		grams
Calcium lactate	3.78	Potassium citrate	3.12
Sodium citrate	3.29	Magnesium citrate(10.2% Mg)	1.30
Sodium chloride	3.43	Ferric citrate	1.00
Dipotassium phosphate	3.65		

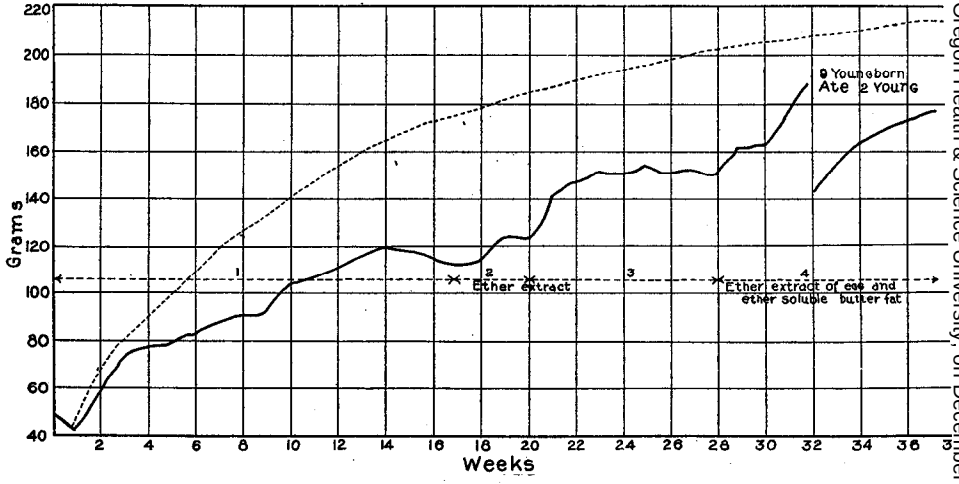

Chart IV. *Rat 104a* (female) shows the record of a rat during 257 days of nearly continuous growth. In period I the ration contained lard up to the 15th week. There was a suspension of growth near the end of this period. During period II the ration contained 5 per cent of ether extract of egg-yolk. No extract of egg was fed in period III which resulted in a second cessation of growth. In period IV growth was resumed and reproduction was attained. She ate two of the nine young produced and the others were removed. She is still growing on this ration and has attained about 80 per cent of the normal adult weight.

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The rations employed in feeding this rat were as follows:

Period I.	per cent	Period II.	per cent	Period III.	per cent	Period IV.	per cent
Casein	18	Casein	18	Casein	25	Casein	18
Lard	25	Lactose	10	Lactose	20	Lactose	20
Lactose	10	Starch	62	Dextrine	40	Dextrine	40
Starch	37	Agar	5	Agar	5	Agar	2
Agar	5	Salt mixt.	5	Salt mixt.	10	Ether extract of egg and of butter	10
Salt mixt.	5	Ether extract of egg added.				Salt mixt.	8

There have been many slight modifications in the character of the inorganic content of the rations of this rat.

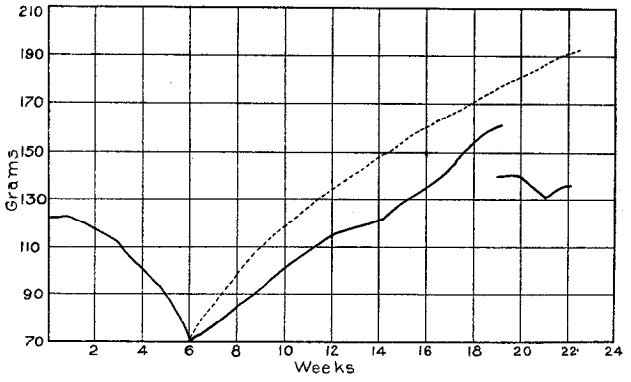

Chart V. *Rat 50* (female) is introduced to show what degree of success can be attained on diets containing no fats and but insignificant amounts of ether-soluble substances. The experiment covered one hundred and fifty-four days. During the first forty-two days the ration contained lard. There was a steady and rapid decline due apparently to the unsatisfactory character of the salt mixture fed, for on changing this there was a rapid recovery and nearly normal growth with the production of young after ninety-one days on a fat-free ration. The experiment was terminated because the mother ate two of her young and the third was only about half normal size on the 18th day (weight 10 grams). This one grew normally when placed with another mother suckling young of the same size.

The ration consisted of:

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Period I.	per cent	Period II.	per cent		grams
Casein	18	Casein	18	NaCl	1.022
Lard	25	Lactose	20	$MgSO_4$(anhydrous)	3.865
Starch	33.5	Dextrine	51	Mg citrate	7.000
Lactose	10	Agar-agar	5	K_2HPO_4	0.168
Agar-agar	5	Salt mixt. used in period I, Chart II	6	KH_2PO_4	12.795
Salt mixt.	7.5				

The salt mixture employed in period I consisted of:

	grams		grams
$Na_2HPO_4 \cdot 12H_2O$	5.052	$CaH_4(PO_4)_2H_2O$	1.880
Na citrate (anhydrous)	2.866	$CaSO_4$ (anhydrous)	0.168
Calcium lactate	25.377	Ferric citrate	1.000

We have seen this prompt resumption of growth after a period of suspension result from the introduction of ether extract of butter or of egg in about thirty animals and are convinced that these extracts contain some organic complex without which the animals cannot make further increase in body weight, but may maintain themselves in a fairly good nutritive state for a prolonged period. In no instance have we obtained such a result by the feeding of lard, or of olive oil. It is therefore not merely the absence of fats from the diet which causes the suspension of growth.

Whether the resumption of growth is the result of supplying in the ether extract of egg or of butter, some indispensable organic complex of the chemical nature of the lipins, or is the result of a stimulating action of some substance accompanying the lipins, cannot be decided from the data at present available. In a considerable number of instances we have fed lecithin or cholesterin with very doubtful results. Hopkins[5] observed that small quantities of milk added to rations of purified food substances, exert an influence on the growth of rats, which is out of all proportion to the added nitrogen or calorific value. Funk[6] and Suzuki, Shimamura and Odaki have isolated substances from rice polishings which exert a remarkable curative effect on birds suffering from polyneuritis as a result of exclusive feeding on polished rice.

[5] Hopkins: *Journ. of Physiol.*, xliv, p. 425, 1912.

[6] Funk: *Journ. of Physiol.*, xliv, p. 50, 1912; Suzuki, Shimamura and Odaki: *Biochem. Zeitschr.*, xliii, p. 89, 1912.

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Funk has also obtained preparations from brain, yeast, and milk which have the same power.

The extensive literature on the remarkable physiological properties of certain fresh food-stuffs as contrasted with the cooked or preserved materials, in preventing or curing scurvy and beriberi, diseases arising from unsatisfactory diets, has been recently summarized by Cooper.[7] From the experimental data available it seems apparent that very young animals cannot be made to complete their growth on rations supplying only purified proteins, carbohydrates, fats, and salts. Our observation that ether extracts from certain sources improve the condition of animals on such rations, strongly supports the belief that there are certain accessory articles in certain food-stuffs which are essential for normal growth for extended periods.

It is interesting in this connection to correlate our observation on the physiological properties of ether extracts of butter or eggs, with those of Osborne and Mendel on the power of an animal to maintain itself on a ration containing gliadin as the only protein. While no growth is possible on this ration, notable increase in weight due to the building of young, can take place and a milk supply capable of normally nourishing the young can be produced. Through the agency of the ovary in egg production, or the mammary glands in milk production, the necessary accessory bodies essential to the proper nourishment of the young are readily synthesized by the animal cell. The young themselves have not the power to produce these syntheses for their own preservation when these unknown substances are lacking in the diet.

The further study of the nature of the "active" bodies in these extracts must of necessity require a great deal of time and labor, since preparations from butter or eggs made with solvents poorer than ether, and of ether extracts from other sources, the examination of their stability, etc., can be tested only on animals which have been grown as far as possible on rations of purified food substances, and have reached the stage of suspension of growth.

This work will be carried on as rapidly as circumstances will permit.

[7] Cooper: *British Med. Journ.*, No. 2727, p 722, 1913.

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