XI. RESEARCHES ON THE FAT-SOLUBLE ACCESSORY SUBSTANCE. II. OBSERVATIONS ON ITS RÔLE IN NUTRITION AND INFLUENCE ON FAT METABOLISM.

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IS FAT-SOLUBLE *A* NECESSARY FOR THE ADULT?

FOR some years past it has been accepted that the fat-soluble accessory factor plays an important part in the nutrition and growth of young animals, but information regarding the requirements after maturity has been reached is scanty. A powerful stimulus to research along these lines was provided by the necessity for a drastic reduction of the fat-ration imposed during the war by the shortage of edible fats, and it at once became a matter of some urgency to determine whether fat-soluble *A* is only required during the period of growth or whether it is equally necessary for the maintenance of health in the adult.

In one particular case it has been experimentally proved that the adult animal requires a regular supply of accessory factors, and that is the case of the pregnant or lactating female [McCollum, Simmonds and Pitz, 1916, 1; Drummond, 1918, 1]. But the demand for the factors in this case might be accounted for by the requirements of the growing young, and might have no reference to the direct nutrition of the mother's body.

During the course of a large number of experiments carried out in this laboratory during the last few years it has been observed that as young rats approach maturity their requirements for fat-soluble *A* become markedly less. A rat which is nearly full grown can successfully complete its development upon an allowance of fat-soluble *A* which would be insufficient to permit any growth whatever in a much younger animal.

It was therefore possible that fat-soluble *A* is essentially a "growth" factor and might be dispensed with by the adult. Such was not, however, found to be the case.

A number of young full grown rats both male and female were fed upon a dietary deficient in fat-soluble *A* composed as follows:

Purified caseinogen	20 parts
„ starch	50 „
Salt mixture	5 „
Yeast extract	5 „
Hardened linseed oil	20 „

By experiments on young rats it has been ascertained that this ration contains no detectable traces of fat-soluble *A*.

Many of the adult animals nourished upon this dietary were able to live in apparently good health for considerable periods of time, but sooner or later they all showed a decline in body weight accompanied by an obvious decreased resistance to disease.

Of fourteen control male rats which received an adequate dietary con-

Table I. *Weights of eight adult male Rats maintained on Diet deficient in Fat-soluble A.*

No. of rat	Weight in g. every seven days															
	0	7	14	21	28	35	42	49	56	63	70	77	84	91	98	105
1	255	260	260	260	260	280	280	275	275	270	255	240	270	260	280	280
2	220	210	220	216	223	235	232	225	228	224	223	219	205	201	214	233
3	290	292	300	310	310	310	315	320	330	310	310	300	280	280	270	290
4	297	305	300	309	315	300	300	310	315	320	310	300	290	285	300	300
5	299	300	295	300	300	310	295	305	295	285	250	255	240	240	240	218
6	306	310	310	320	310	305	300	297	300	296	290	299	296	282	290	292
7	350	350	350	350	347	356	350	346	346	340	339	345	335	330	329	326
8	268	270	282	296	290	295	290	286	288	290	290	290	296	300	294	290

No. of rat	Weight in g. every seven days										External eye disease	Cause of death
	112	119	126	133	140	147	154	161	168	175		
1	280	270	250	255	260	266	250	245	235	230	Slight 140th day Severe 150th day	Died 182nd day, bronchopneumonia
2	227	225	233	225	220	197	165	140	—	—	Severe 143rd day	Died 162nd day, cause not clear
3	305	313	320	320	315	310	295	295	300	285	Slight 160th day	—
4	300	305	300	290	295	290	285	280	275	250	Slight 150th day Severe 160th day	—
5	—	—	—	—	—	—	—	—	—	—	—	Died 107th day, lung disease
6	276	260	—	—	—	—	—	—	—	—	Slight 90th day Severe 102nd day	Died 119th day, bronchopneumonia
7	325	316	305	300	297	290	287	262	—	—	Slight 122nd day Severe 129th day	Died 163rd day, cause not ascertained
8	290	280	286	280	270	260	262	260	250	242	Slight 150th day Severe 155th day	Dying with lung disease at end of experiment

taining butter fat instead of hardened linseed oil twelve were alive and in excellent health at the end of the thirty weeks period of observation. One died from bronchopneumonia while another succumbed to sepsis consequent on injuries received in fighting.

Eight adult male rats were fed upon the deficient ration and their subsequent behaviour is recorded in Table I. Only three survived the experimental period, and they were all in a very poor nutritive condition. Many deaths were due to lung diseases, whilst practically every animal showed the characteristic external eye disease at some time during the experiment.

A number of young adult females were also kept under observation and fed upon the fat-soluble deficient dietary. Breeding with the males upon the same diet was permitted. The females themselves behaved very much as did the males described in Table I. After a period during which they maintained more or less good health they began to lose weight and to present an impoverished condition. Many deaths from infective diseases occurred and cases of external eye disease were frequent.

A number of litters were born, particularly during the first few weeks of the experiment, when the bad effects of the diet had scarcely begun to be felt. These early litters were usually of normal weight when delivered, but failed to grow at a normal rate owing to the deficient nutritive value of the mothers' milk [compare McCollum, Simmonds and Pitz, 1916, 1; Drummond, 1918, 1].

The majority were reared until able to look after themselves, but they all appeared puny and very quickly contracted infections, particularly the eye disease, and the mortality amongst them was very high.

A few litters were born after the females had been on the deficient ration for many weeks, but most of the young which comprised these litters were devoured by the mothers shortly after delivery, and any which escaped that fate seldom survived for more than a day or two. They were in nearly every case undersized when born.

It may therefore be taken as proved that the adult animal organism requires a regular supply of fat-soluble *A*. This daily requirement is of a much smaller order than that of the young growing animal, but nevertheless an important factor in the maintenance of health. It appears probable that the resistance to diseases of bacterial origin is seriously impaired by a failure of the animal to obtain a sufficient supply of the fat-soluble factor. There is therefore every reason that great care should be taken to ensure that dietaries of adults contain an adequate supply of foodstuffs in which fat-soluble *A* is present.

IS FAT-SOLUBLE *A* CONCERNED WITH THE METABOLISM OF FATS?

Up to the present we are in complete ignorance of the function of fat-soluble *A* in the body. The modern theories regard fat-soluble *A* as synthesised by the plant, in the tissues of which it may be present in a loosely combined

form which is insoluble in fat solvents. It is conceived that in the alimentary tract of animals this complex is broken down and the factor *A*, now in a form soluble in fats and fat solvents, forthwith accompanies them during absorption [McCollum, Simmonds and Pitz, 1916, 2].

Its fate after absorption into the animal body is as yet unknown. Its presence in certain depôt fats, such as beef fat, suggests that it may be in part stored in company with the reserve fat.

In view of the possible connection between fat-soluble *A* and some phase of the metabolism of fats a few experiments have been made which will be described in this paper.

It was first decided to test whether the animal organism is able to survive as well on a diet free from both fat and the fat-soluble factor as when the latter factor alone is absent.

Osborne and Mendel [1912] have reported the ability of young rats to show considerable development upon diets containing, at the most, very small traces of fat. It is probable however that the fat-soluble factor was present as an impurity in certain of the food components.

Three batches of six nearly full grown male rats of approximately the same age and weight were selected from the stock. Each lot was then given one of the three following dietaries.

	A	B	C
Purified caseinogen	20 parts	20 parts	20 parts
„ starch	70 „	50 „	50 „
Salt mixture	5 „	5 „	5 „
Yeast extract	5 „	5 „	5 „
Hardened vegetable oil	—	20 „	—
Butter fat	—	—	20 „

All three batches were kept under the closest observation for a period of three months.

Lot C nourished upon the butter fat ration showed throughout the whole period a normal standard of nutrition. At the conclusion of the experiment they were killed and subjected to post mortem examination. With the exception of one rat which showed a small worm encysted in the liver, no abnormalities were noticed in any case.

Lot B received a diet which contained an adequate supply of fat, but no detectable traces of fat-soluble *A*. These animals maintained their weight well and showed no apparent symptoms of ill-health until towards the end of the second month when they one by one began to develop the characteristic condition of sore eyes.

Two deaths occurred in the early part of the third month from broncho-pneumonia and at the conclusion of the experiment the surviving animals were losing weight and all suffering from the external eye disease.

These survivors were killed and carefully examined. In every case there was a large amount of body fat present and there was no indication of in-

anition. The organs all presented a healthy appearance as far as the eye could detect. The histological examination has not been completed.

The body temperature of the rats in this group remained normal, except in the two cases which contracted an acute infection of the lung. This observation has been confirmed by the records obtained from a large number of rats fed upon diets deficient in fat-soluble *A*. There is therefore no indication that lack of fat-soluble *A* brings about a disturbance of the heat regulating mechanism, as does a deficiency of water-soluble *B* [Drummond, 1918, 2; Dutcher, 1918].

The batch of animals fed upon ration A showed very similar behaviour to those nourished upon ration B. Their food consumption was throughout higher owing to the fact that the calorific value of their diet was lower than that of diet B. They all maintained a good appearance until towards the end of the second month when sore eyes began to appear. Body weights then showed declines and there was one death from lung trouble. The remaining five at the end of the third month had all lost weight and exhibited more or less severe external eye trouble.

Post mortem examination of these animals failed to reveal any gross lesion, except in the lungs which were congested in some cases. The amount of body fat was apparently normal. The histological examination of the organs has not yet been completed.

These experiments indicate that the appearance of the symptoms associated with a deficiency of fat-soluble *A* is not postponed by withholding fat from the diet.

It has recently been shown that rats may live in perfect health for several months upon a diet containing relatively large amounts of free fatty acids [Halliburton, Paton, Drummond and others, 1919]. The classical researches of Munk [1880, 1884] showed that free fatty acids are absorbed from the intestinal tract and combined with glycerol derived from the body to form neutral fat.

The possibility of fat-soluble *A* playing a rôle in the resynthesis of fats suggested itself and an attempt to obtain information on this point was made. Two batches of rats were fed upon the following rations:

				Diet B	Diet D
Purified caseinogen	...	...	...	20 parts	20 parts
„ starch	...	...	...	50 „	50 „
Salt mixture	...	...	...	5 „	5 „
Yeast extract	...	...	...	5 „	5 „
Hardened vegetable oil	...	...	...	20 „	—
Fatty acids derived from hardened oil				—	15 „

The period of observation was as in the last experiment three months. The batch receiving fat but no fat-soluble *A* showed a behaviour closely similar to that described in the previous case. At about the end of two months symptoms of ill-health and external eye disease appeared and the animals gradually declined.

The batch which received the fatty acid ration (diet D) ate their diet with avidity throughout the experiment. After the lapse of some seven weeks ill-health began to appear accompanied as in the other cases by the characteristic condition of sore eyes.

Analyses of the faeces made on several occasions during the three months indicated that the absorption of the fatty acids was excellent, the highest value being 92 % and the lowest 86 %. At the expiration of the three months the surviving animals were killed—two had died during this period, one from bronchopneumonia and the other as a result of an accident.

Post mortem examination showed no obvious abnormality. Body fat was plentiful and all organs appeared normal as far as the naked eye could detect.

These animals had, therefore, been capable of absorbing large amounts of free fatty acids and of effecting the synthesis of these into neutral fats over a considerable period of time in the absence of any detectable traces of fat-soluble *A* in the diet. It is therefore justifiable to assume that this factor plays no important part in the synthesis of fatty acids into fats following absorption, for the power to absorb fatty acids and effect fat synthesis is apparently retained after the animal has passed the period during which it was able to maintain health in spite of the deficiency, and during which it is surmised that it may have been utilising reserve supplies of the essential factor.

The appearance of the characteristic symptoms associated with a deficiency of fat-soluble *A* in approximately the same time whether the ration is deficient in fat, contains an ample supply of fat or merely a supply of fatty acids, is an interesting observation. It has been confirmed in two out of the three cases in an experiment with young rats.

Two batches of twelve young rats were fed upon diets A and B; that is one lot received fat and the other none, whilst neither obtained fat-soluble *A*. The two groups were as far as possible comparable, and the following data were obtained as a result of close observation.

	12 young rats fed on diet A con- taining neither fat nor fat- soluble <i>A</i>	12 young rats fed on diet B con- taining fat but no fat-soluble <i>A</i>
Average weight at commencement of experiment	80.2	82.8
Average weight at 10 days 	89.0	92.1
Average weight at 20 days 	93.4	97.1
Average weight at 30 days 	86.4	88.2
Average weight at 40 days 	80.1	80.6
First appearance of case of sore eyes 	28th day	26th day
Number of cases of sore eyes at 40th day ...	6	9

These observations appear to have an indirect bearing upon the question of the dispensability of fat in the diet of the animal organism. If an animal is deprived of fat upon an artificial ration of the type employed in this work it eventually succumbs to certain disturbances of nutrition which it is believed result from a deficiency of fat-soluble *A*. If it receives a pure fat it succumbs

in an exactly similar manner and after an interval of the same order. It therefore appears that no decision upon the question of the dispensability of fat can be arrived at until the fat-soluble factor has been obtained in a form free from fat. Then and then only can the nutritive effect of feeding diets deficient solely in fat be studied. But the onset of the symptoms alluded to above after the same interval, no matter whether the diet contains fat or not, does suggest that fat itself is a dispensable component of food.

The experiments recorded by Osborne and Mendel [1912] are of interest at this juncture. The diets they employed were practically fat-free, but as the components had not been extracted with fat solvents it is very probable that traces of the fat-soluble factor were present. Considerable growth over long periods was shown by young rats fed upon such rations.

INFLUENCE OF A DEFICIENCY OF FAT-SOLUBLE *A* ON FAT METABOLISM.

Two pairs of healthy rats weighing about 150 g. each were selected from stock and one pair was placed upon rations B and C respectively.

The pair which received ration C thrived for the whole three months during which they were under observation and were in excellent health at the close of the experiment. During this time their absorption of fat was studied for five periods of seven days. As will be seen from Table II, the absorption of fat was excellent in all three periods, averaging 97·3 %.

Table II.

Diet	Period of experiment	Condition of the animals	Total consumption of dry food in g.	% fat in food	Total intake of fat in g.	Total weight of dry faeces in g.	% total fat in dry faeces	Total excretion of fat in faeces in g.	% utilization	M. P. of fat in diet
C	1st week	Excellent	122	16·7	20·4	8·14	6·18	0·50	97·5	30·2°
C	3rd "	"	134	16·3	21·8	10·06	6·10	0·62	97·2	
C	6th "	"	141	16·3	23·0	6·02	13·30	0·80	96·5	
C	9th "	"	136	15·9	21·6	9·20	5·99	0·55	97·5	
C	12th "	"	142	16·1	22·8	9·42	5·72	0·54	97·6	
Average									97·3	
B	1st "	Excellent	126	15·2	19·2	9·09	14·2	1·29	93·4	38·5°
B	3rd "	Good	135	15·8	21·3	8·02	15·5	1·24	94·2	
B	6th "	"	132	16·4	21·6	9·11	14·5	1·32	89·2	
B	9th "	Ruffled appearance, one rat shows slight sore eyes	136	16·0	21·8	10·10	25·7	2·69	87·6	
B	12th "	Both losing weight and eyes very bad	126	15·7	19·8	7·12	30·4	2·16	89·1	
Average									90·7	

The two animals receiving diet B, which contained a fat known to be deficient in fat-soluble *A*, were studied in a similar manner. They showed good health for about six weeks but during the latter half of the period of observation they were failing and both contracted sore eyes during the third month.

Fat absorption was, however, good in all five periods, even in the final one when the physical condition of the animals showed that they suffered from a serious deficiency of fat-soluble *A*.

The coefficient of absorption shows a slight decrease in the later periods, but it is thought that this may represent a slightly lower efficiency of the digestive system consequent on the failing health. The reduction of the percentage absorbed is too small to be interpreted as evidence that fat-soluble *A* is directly concerned in fat absorption.

Estimations of fat in the faeces were carried out by the wet extraction method described by Saxon [1914].

SUMMARY.

1. Fat-soluble *A* is necessary for maintenance of health in the adult rat.
2. Further confirmation of the importance of supplying the female with an ample supply of fat-soluble *A* during pregnancy and lactation is given.
3. A deficiency of fat-soluble *A* in the diet of adult rats apparently brings about a serious lowering of the resistance against diseases of bacterial origin.
4. No obvious characteristic pathological lesion has been detected in adult rats which have been deprived of fat-soluble *A* for long periods. Such animals usually show normal reserves of body fat.
5. The symptoms associated with a deficiency of fat-soluble *A* appear in comparable groups of rats after the same period of time whether the diet contains neutral fat or not.
6. Rats are able to absorb large amounts of fatty acids, and presumably synthesise these into fats, in the absence of fat-soluble *A* in the diet.
7. A deficiency of fat-soluble *A* in the diet does not exert any direct influence on the absorption of fat.
8. Indirect evidence deduced from these experiments with rats suggests that pure neutral fats may be dispensable components of the diet.

REFERENCES.

- Drummond (1918, 1). *Lancet*, Oct. 12th.
— (1918, 2). *Biochem. J.* **12**, 25.
Dutcher (1918). *J. Biol. Chem.* **36**, 63.
Halliburton, Paton, Drummond and others (1919). *J. Physiol.* **52**, 328.
McCollum, Simmonds and Pitz (1916, 1). *J. Biol. Chem.* **27**, 33.
— (1916, 2). *Amer. J. Physiol.* **41**, 361.
Munk (1880). *Virch. Arch.* **80**, 10.
— (1884). *Virch. Arch.* **95**, 407.
Osborne and Mendel (1912). *J. Biol. Chem.* **12**, 81.
Saxon (1914). *J. Biol. Chem.* **17**, 99.