CCLIV. THE EFFECT OF CHOLESTEROL FEEDING OF RABBITS ON THE TISSUE LIPOIDS

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In spite of much research work, our knowledge of the absorption, transport and utilization of cholesterol in the animal body is still vague. Cholesterol is known to be absorbed from the intestine in relatively small amounts, depending on the intensity of fat absorption and possibly on the nature of the fatty acids themselves. Spanner & Baumann [1932] have suggested that the solubility of cholesterol in bile may also be an important factor.

The path of absorption appears to be through the lacteals and, as cholesteryl esters have been demonstrated in the chyle following the administration of cholesterol, it is possible that absorption is accompanied by esterification [Mueller, 1915; Frolicher & Sullman, 1934; Gardner & Gainsborough, 1930]. Because of the presence of large amounts of cholesteryl esters in normal blood, Bloor [1923] has suggested that these esters may play an important part in the transport of fatty acids in the animal body.

One of the methods of approach to the problems of cholesterol metabolism has been to feed animals on diets containing various amounts of cholesterol and to study the effect on the tissues. Much of the early work on these lines was histological [Anitschow & Chalatow, 1913] and although many changes were observed, little attempt was made to investigate them quantitatively. In recent years, however, the problem has received renewed attention as part of the investigation of the effects of choline and other factors in the production of fatty livers. It has been shown that when rats are fed on diets containing cholesterol, deposition of cholesterol (mainly in the esterified form) occurs in the livers [Okey, 1933, 1, 2; Chanutin & Ludewig, 1933; Sperry & Stoyanoff, 1935; Beeston *et al.* 1935; 1936; Cook, 1937; Best *et al.* 1934]. The last-named authors were able to demonstrate that choline had a marked effect on the prevention of the "cholesterol" fatty liver, the glyceride fraction being reduced almost to normal (from 9·5 to 1·5%), while the percentage of cholesteryl ester fell appreciably (from 4·35 to 1·77%). Later Aylward *et al.* [1935] showed that when rats were fed on a diet containing 2% cholesterol in addition to 40% fat, the accumulation of cholesterol as ester began in the liver within a very few hours of feeding and increased progressively with time.

The purpose of the present work was to continue the investigation of the production of fatty livers by cholesterol feeding, with special reference to the comparative rates of change of the individual lipoids, in order to gain more complete information regarding the factors influencing the "cholesterol" fatty liver. Further, because of the paucity of knowledge regarding the quantitative aspects of the effect of cholesterol feeding on organs other than the liver, it was decided to examine also the kidney, heart, lungs, brain, spleen and adrenals. Most of such previous investigations as have been carried out on these tissues

have been restricted to histological study only, and even when chemical analyses have been carried out they have usually been confined to cholesterol determinations. Consequently there are few records of the effects of cholesterol diets on the phosphatide and glyceride fractions of the tissues. Furthermore the majority of workers have confined their attention to a very limited number of tissues and thus it is impossible from their data to obtain a clear picture of the changes throughout the body.

Experimental

Three groups of rabbits were used: (*a*) the first group ("normal") of six animals was fed for 6 weeks on a mixed grain diet with the addition of cabbage leaf. The analytical figures obtained from this control group were compared with those of two further groups (*b*) the "high fat" group of ten animals which received a diet of sunflower-seed meal and (*c*) the "cholesterol" group of fourteen animals which received sunflower-seed meal with the addition of 2% cholesterol. The last two groups also received cabbage leaf on alternate days. They were fed for periods up to 9 weeks.

Table I. *Composition of sunflower-seed meal*

	g. per 100 g. meal
Protein (N × 6·25)	19·42
Water	7·39
Ash	5·61
Choline	0·113
Total ether extract	36·97
Unsaponifiable matter	0·74
Total fatty acids	34·26

Sunflower-seed meal was used because it is a plant food of sufficient fat content to promote cholesterol absorption. The commercial sunflower-seed meal was received crushed and ground and it was sieved to remove as much of the husk as possible. In the preparation of the cholesterol diet, powdered cholesterol was mixed and sieved with the meal. It was then kneaded to a dough with water and dried by an electric fan to give a hard, dry mass, which was readily consumed. About 100 g. of this dried material were supplied to each rabbit per day and on an average 70 g. were ingested. From the data of Table I (obtained by direct analysis) it was calculated that each rabbit consumed per day 24 g. fatty acids, 13·6 g. protein and 79 mg. of choline, while the cholesterol group ate in addition 1·4 g. cholesterol.

The control group of six animals were killed together after 6 weeks' feeding. From the second and third groups usually two animals were killed at varying intervals after the fifth day, and the liver, kidney, heart, lung, brain, adrenals and spleen were removed for analysis. The fat extractions and the subsequent analyses for the various lipoids were carried out by the methods given by Best *et al.* [1934], except that the method of extraction of the livers was modified so that the moisture content could be determined [Channon & Wilkinson, 1934]. In the cases of the spleen and adrenals, the lipoid analyses were carried out on the pooled extracts from several organs. From the determinations of total fatty acids, lipoid phosphorus, free and total cholesterol, the amounts of the various lipoids present were calculated on the assumption that the phosphatide was dioleyl-lecithin, the glyceride triolein and the cholesteryl ester, the oleate.

Results

Table II summarizes the extensive analytical data obtained from the "normal", "high fat" and "cholesterol" groups.

The data for the individual animals of the "normal" group will not be considered and the mean values only are recorded ("N."). Further, except in the liver, the variations due to the "high fat" diet were relatively slight and for this group also the mean values only are therefore given ("H.F."). In the "cholesterol" group the changes were much more striking and the mean values over three periods are given:

C. 1	0–2 weeks	3 rabbits
C. 2	3–5 „	5 „
C. 3	6–9 „	6 „

The "normal" group

The more noticeable features shown by the data for the "normal" group may now be summarized. The phosphatide fraction is highest in the brain (4·71 %) and lowest in the spleen (1 %). The amounts of glyceride show rather wide variations, being very low in the brain (0·69 %) and very high in the adrenals (9·49 %). The percentage of free cholesterol in all the tissues except the brain is between 0·16 and 0·5 %, but it increases in the latter tissue to 2 %. The amount of cholesteryl ester is negligible in all the tissues except the spleen (0·56 %) and the adrenals (15 %).

Although many authors have carried out cholesterol, lecithin or other lipoid analyses on the tissues under consideration, few have made any attempt to estimate all the main lipoids in the tissues of the same animal, so that the figures given in Table II must be compared with those collected from a number of sources. Many of the lipoid analyses performed are recorded in papers (to be referred to later) on the effects of cholesterol diets on the tissues but some of the data are collected by Ellis & Gardner [1912]; Hess-Thaysen [1914]; Chamberlain [1928]; Thierfelder & Klenk [1930] and Magistris [1931].

The reason for the great differences observed in the various tissues is not known but there are a number of possible explanations. It is probable that some of the lipoids are concerned with cellular equilibria and that different types of cells may require different proportions of the lipoids. Alternatively some of the tissues may act as depots for particular lipoids or as centres for active metabolism.

The high fat content of the adrenals, due mainly to the presence of large amounts of glyceride and cholesteryl esters, is a matter of great interest. Histological work has shown that the accumulation of lipoids is mainly in the cortex and it has been suggested that the normal cortex is concerned with the storage of cholesteryl esters or alternatively with their metabolism [Rosenheim & Tebb, 1909; Whitehead, 1934; Grollman, 1936].

It should be mentioned that the individual variations found in this tissue are greater than in any of the others examined and this has also been observed by previous workers. Kay & Whitehead [1935] examined the adrenals of groups of six male and six female rabbits and found that the amounts of cholesteryl ester were 7·6 and 15 % (mean values) respectively, but even within the groups there were considerable variations. Chamberlain [1928] obtained similar results. In view of the possible function of the adrenals in storage of cholesteryl esters, it seems likely that the amount present in the "normal" animal will depend to a great extent on the animal's previous dietary history.

Table II. *The lipoids of the tissues of rabbits receiving various diets*

Group	Tissue as % body wt.	% fresh tissue					mg. per kg. body wt.				
		Phosphatide	Free cholesterol	Cholesteryl oleate	Glyceride	Total	Phosphatide	Free cholesterol	Cholesteryl oleate	Glyceride	Total
Liver:											
N.	3·30	3·35	0·28	0·09	0·55	4·27	1106	92	31	180	1410
H.F.	2·86	3·34	0·30	0·22	2·37	6·23	908	82	60	650	1700
C. 1	3·50	3·11	0·58	3·34	5·74	12·77	1089	203	1170	2010	4470
C. 2	4·19	2·60	0·90	8·02	4·04	15·56	1090	377	3360	1690	6520
C. 3	6·45	2·42	1·04	13·86	2·21	19·53	1561	671	8940	1430	12600
Kidney:											
N.	0·57	2·43	0·37	0·010	2·06	4·87	139	21	1	117	278
H.F.	0·65	2·61	0·44	0·022	3·33	6·40	170	29	1	216	416
C. 1	0·61	2·08	0·42	0·011	1·56	4·08	127	26	1	95	249
C. 2	0·76	2·21	0·51	0·655	1·32	4·70	168	39	50	100	357
C. 3	1·02	2·08	0·64	1·199	2·62	6·54	212	65	123	267	667
Heart:											
N.	0·26	1·42	0·16	0·020	3·17	4·78	37	4	1	82	124
H.F.	0·26	1·64	0·22	0·015	5·21	7·10	43	6	1	135	185
C. 1	0·30	1·25	0·18	0·13	4·58	6·14	38	5	4	137	184
C. 2	0·25	1·76	0·28	0·22	3·89	6·15	44	7	6	97	154
C. 3	0·41	1·41	0·52	0·58	3·97	6·48	58	21	24	163	266
Brain:											
N.	0·41	4·71	2·12	0·017	0·69	7·53	193	87	1	28	309
H.F.	0·35	4·07	2·13	0·046	1·23	7·48	142	75	2	43	262
C. 1	0·38	4·20	1·98	0·011	0·83	7·02	160	75	1	31	267
C. 2	0·40	4·61	2·08	0·388	0·90	7·99	184	83	16	36	319
C. 3	0·35	3·88	2·02	0·550	0·93	7·38	136	71	19	33	259
Lung:											
N.	0·40	2·54	0·50	0·019	3·14	6·20	102	20	1	126	249
H.F.	0·41	2·53	0·54	0·010	3·93	7·01	104	22	1	161	288
C. 1	0·38	2·19	0·56	0·156	2·50	5·41	83	21	6	95	205
C. 2	0·48	2·79	0·78	1·260	1·49	6·32	134	37	60	72	303
C. 3	0·61	2·88	0·95	2·717	3·88	10·43	176	58	166	237	637
Spleen:											
N.	0·046	1·00	0·34	0·56	1·57	3·47	5	2	3	7	17
H.F.	0·031	1·24	0·36	0·37	1·95	3·92	4	1	1	6	12
C. 1	0·041	1·52	0·83	2·93	1·95	7·23	6	3	12	8	29
C. 2	0·183	1·97	1·37	8·98	2·60	14·92	36	25	164	48	273
C. 3	0·240	1·93	1·57	10·39	3·57	17·46	46	38	249	86	419
Adrenals:											
N.	0·029	2·00	0·48	14·96	9·49	26·93	6	1	43	28	78
H.F.	0·017	2·34	0·67	11·55	8·77	23·33	4	1	20	15	40
C. 1	0·033	2·52	0·68	15·29	14·59	33·08	8	2	51	48	109
C. 2	0·033	2·99	0·94	33·36	1·89	39·18	10	3	110	6	129
C. 3	0·094	2·00	1·40	36·82	5·78	46·00	19	13	346	54	432

The effect of the experimental diets on the tissues

Before discussing the changes in the lipoids of the tissues, some remarks must be made about the extensive changes which occurred in the actual weights of the tissues. Table II shows that the mean value of the liver weight as a percentage of body weight is 3·30 for the control animals, and no great change occurred in the "high fat" group or during the early period of cholesterol feeding. However, after the fifth week of this latter diet there is a sharp increase in the proportionate liver weight, the average figure in this period being 6·45 %. This increase is partly due to the decrease in body weight which occurred at the same time in this group.

The variations in liver weight are accompanied by changes in the moisture content of the livers, and while the "high fat" group (69 %) shows little change from the normal (67 %) wide variations occur in the "cholesterol" group. During the first 6 weeks of cholesterol feeding there is a definite decrease in the moisture percentage (C. 1, 61 %; C. 2, 57 %—the minimum individual figure being 46·4 %), but towards the end of the experiment the values rise again (C. 3, 63 %). The explanation of these changes probably lies in the interplay of a number of factors such as the increase in the total lipoid and in the increase in weight of the liver itself. The increase in fat content of the liver does not account for more than a part of the increase in liver weight and it appears certain that fat deposition causes imbibition of water, presumably to maintain cellular equilibrium. It is also quite possible that both cholesterol and fat infiltration may cause a true physiological enlargement of the liver, by stimulating the growth of new cells.

The change in weight of the tissue due to the cholesterol diet is not confined to the liver, but is shown by all the tissues investigated except the brain. The spleen and adrenals in particular were grossly enlarged during the later stages of cholesterol feeding and in the extreme case the spleen was eight times and the adrenals five times their normal weights.

These changes in tissue weight and moisture content are of great importance in the interpretation of the effects of diets on the lipoids and the significance of variations due to diets can only be fully assessed by expressing the amounts of tissue lipoids first as a percentage of the tissue weight (fresh or dry) and secondly as a percentage of the body weight. In Table II the results are therefore recorded also in terms of the weights of the different lipoids present in the tissues of a 1 kg. rabbit, and these figures will be referred to as "absolute weights". If a pronounced change in body weight occurs data calculated by this method must be interpreted with caution.

(*a*) *The livers.* As the changes in the percentages of the liver lipoids are of particular interest they are shown graphically in Figs. 1 and 2. Each point represents the mean value for two animals. Fig. 1 shows that in the "high fat" group the percentages of phosphatide, free cholesterol and cholesteryl ester do not vary to any great extent from the normal values. The glyceride fraction, however, undergoes relatively large changes. Early in the experiment there is a marked rise followed by a decrease to a value which always remains above normal. This sequence of changes may be due to the circumstances that the rabbit is not accustomed to such a high fat diet (25 g. fat per day). Consequently, in the early stages of the experiment the absorbed fat may not be utilized, whereas later, more complete metabolism may occur.

None of the livers of this group is excessively fatty in spite of the fact that the diet contains nearly 40 % of fat, an amount sufficient in the early experi-

ments of Best *et al.* [1932] to produce a high degree of glyceride infiltration in the livers of rats. Any or all of four factors may explain this finding—the difference in the species, the chemical nature of the fat fed, the protein content of the diet and the presence of choline, betaine and allied substances in the sunflower-seed.

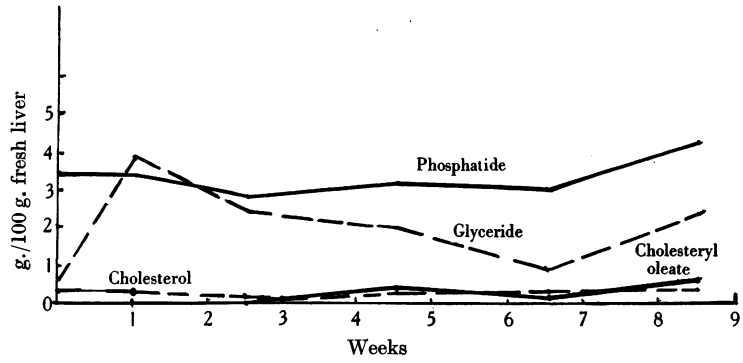

Fig. 1. Liver lipoids of "high fat" group.

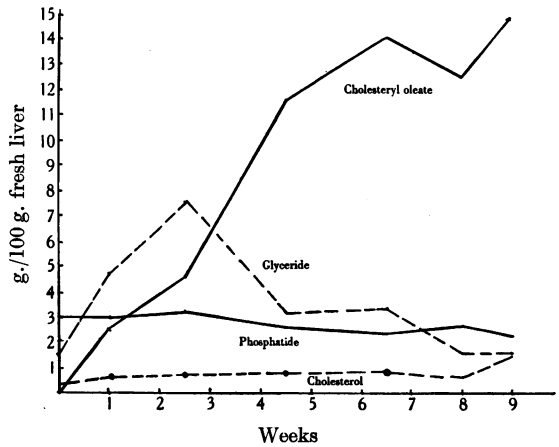

Fig. 2. Liver lipoids of "cholesterol" group.

It is impossible to say which of these effects is of major importance in the present experiment. The sunflower-seed fat has a high I.V. (130) and it has been shown by Channon & Wilkinson [1936] that a diet containing unsaturated glyceride is far less effective in producing fatty livers than a diet containing a relatively saturated fatty acid mixture (e.g. beef fat). So far as the protein is concerned, Beeston *et al.* [1935; 1936] have shown convincingly that caseinogen exercises some control of glyceride infiltration in the liver of the rat, and that a plant protein, edestin, has a similar action. Although few other proteins have yet been investigated, it is quite possible that the high percentage of sunflower-seed protein (19·4 %) in the diet used is responsible in part for the absence of notably fatty livers. The daily intake of choline (80 mg.) must clearly be of importance also.

Considerable contrast with both the "normal" and "high fat" groups is presented by the results of the "cholesterol" group. Thus Fig. 2 shows that the most striking change is the pronounced increase in the cholesteryl ester fraction,

which is noticeable when the first animals are killed (the fifth day) and is progressive throughout the period of the experiment. After 9 weeks the liver contains 16% of cholesteryl ester equivalent to 8·9 g. per kg. body weight, whereas the normal liver contains but traces. During the course of 8 weeks each rabbit ingested about 80 g. cholesterol and of this over 10 g. were deposited in the liver (2 kg. rabbit).

This deposition of cholesteryl ester in the liver is in agreement with the histological and chemical findings of the authors already referred to, but the degree of infiltration in this experiment is very much greater than that recorded previously. The progressive increase during the period of the experiment bears out the observations of Aylward *et al.* [1935], to which reference has already been made.

In contrast with the cholesteryl ester fraction, the free cholesterol of this group shows a relatively small but progressive increase ("normal" 0·28%, maximum individual value in "cholesterol" group 1·67%). Expressed in another way, there is 0·67 g. free cholesterol per kg. body weight in the later period of cholesterol feeding.

The graph shows a slight decrease in liver phosphatide during the course of the cholesterol feeding, but this apparent decrease in amount of lipoid is entirely due to the increase in liver weight. The absolute weight of liver phosphatide is approximately constant during the early period (mean value 1·1 g.), but after 5 weeks it increases almost 50% (to 1·56 g.). This increase is probably due in part to the decrease in body weight which took place in this period.

The changes in the glyceride fractions are of particular interest in that during the first 5 weeks of the experiment there is a definite increase (0·55–7·72%) after which a progressive decrease occurs. The same series of changes is seen if one examines the amounts of glyceride expressed in terms of body weights, and a similar though less marked sequence of changes occurs in the "high fat" group.

In all the experiments carried out on the production of the "cholesterol" fatty liver in rats, an accumulation of both glyceride and of cholesteryl ester has been observed, but there is not any constant ratio between the amounts of these lipoids; in some cases the cholesteryl ester is in excess, in others the glyceride. The "cholesterol" group of the present experiment shows a constant change (over the period of 9 weeks) in the relative amounts of the glyceride and the cholesteryl ester, but the results differ from those of other workers in that they show a very definite decrease in the glyceride fraction during the later stages of the cholesterol diet, in spite of a continuous accumulation of cholesteryl ester.

These results seem therefore to dissociate the two phenomena of glyceride and cholesteryl ester infiltration and to suggest that they are in some measure independent. It has already been pointed out that cholesterol is not absorbed from the intestine unless a considerable amount of fat is present. In previous experiments on the "cholesterol" fatty liver, the cholesterol was administered with a high fat diet which of itself would produce a definite degree of glyceride infiltration, but in this experiment the diet of sunflower-seed, while readily promoting the absorption of cholesterol, does not cause a deposition of glyceride. This is shown by the absence of excessively fatty livers from the "high fat" group, in spite of the high percentage of fat in the diet. It seems probable therefore that the glyceride infiltration is determined largely by the protein and choline contents of the diet and by the amount and nature of the fat fed, whereas the cholesterol infiltration is determined mainly by the amount absorbed and the period of feeding and only to a less extent by the other factors.

(*b*) *The kidney, heart, brain and lungs.* The changes in the kidney, heart, brain and lungs are relatively slight compared with those occurring in the livers and they will therefore be discussed together. The glyceride fraction usually shows an increase on both the experimental diets, although anomalies occur in the kidney and lung during the early part of cholesterol feeding. While none of the increases are of the order found in the livers, they nevertheless appear significant. It seems therefore that a sunflower-seed meal diet with or without added cholesterol, causes some degree of glyceride infiltration. The phosphatide fraction on the other hand shows no appreciable change in any of the groups. The free cholesterol is quite constant in the brain during the course of the experiments, but in the other three organs it increases both in percentage and in absolute amount during the later period of cholesterol feeding. These changes run parallel with those observed in the livers.

As in the case of the liver, the cholesteryl ester of these tissues is the lipoid most affected by the cholesterol diets, being progressively deposited throughout the period of the experiment. Increases occur in all four organs, but in the cases of the brain (0·55 %) and the heart (0·58 %) they are slight and even in the lung not more than 2·7 %. That these changes are significant is clearly brought out by the actual weights of lipoids in these tissues.

Weltman & Black [1913]; McMeans [1915]; Bailey [1916] and Sperry & Stoyanoff [1935], all recorded deposition of cholesteryl ester in the kidney as a result of cholesterol feeding and the data obtained in the present experiments are in agreement with their results. Chanutin & Ludewig [1933], however, reported that they could find no change in the kidney, heart and brain and Page & Menschick [1932] reported that cholesterol was not deposited in the brain. These results differ from those presented here. Since in the present experiments a considerably greater amount of cholesteryl esters appeared in the livers than in the experiments of these authors, it is not surprising perhaps that cholesterol deposition has occurred in these other tissues.

(*c*) *The spleen and adrenals.* The distribution of the lipoids in the normal spleen and adrenals differs considerably from the distribution elsewhere. The large increases in the weights of these tissues due to cholesterol feeding have already been mentioned and the considerable changes in the lipoids will now be discussed. The percentage of phosphatide in both organs is increased by the "high fat" and "cholesterol" diets, except in the case of the adrenals of the final "cholesterol" group. When, however, the amounts of tissue phosphatide per kg. body weight are examined, a different picture is seen; in both tissues the "high fat" group has normal amounts of phosphatide but there is a progressive increase due to the cholesterol diet.

The glyceride content of the spleen, whether measured as a percentage of the tissue or of the body weight, increased in both the "high fat" and "cholesterol" groups. In the adrenals, however, a different sequence of changes takes place. The "high fat" group shows decreases in both the percentage and absolute amount of glyceride. The percentage in the "cholesterol" group rises in the first 2 weeks from the "normal" figure of 9·49 to 14·59 % and then in the following 2 weeks it decreases to the exceptionally low value of 1·89 %, only to increase later to 5·78 %. A similar series of changes is seen when the absolute amounts of glyceride in the tissue are examined. It is possible that the anomalies in the case of the adrenals are partly due to the excessive individual variations found in these organs as shown by the analytical figures for normal adrenals. On the other hand it seems that the decline in glyceride may be related to the large increase in cholesteryl ester which occurs about the same time.

In both organs there is a large increase in the amount of free cholesterol present after cholesterol feeding, but the "high fat" diet has no effect. The free cholesterol content of the spleen rises from the normal figure of 0·34 to 1·57 % after 8 weeks of the "cholesterol" diet and the corresponding change in the adrenals is from 0·48 to 1·4 %. These increases are even more striking when the absolute amounts of the free cholesterol are considered.

The "high fat" diet has no effect on the cholesteryl ester content of the organs, but the "cholesterol" diet causes changes comparable with those taking place in the liver. In the spleen there is an increase from the normal figure of 0·56 to 10·39 % and in the adrenals from 14·96 to 36·82 % and these changes are progressive with time. In the final period the "cholesterol" group has 0·25 g. of cholesteryl esters in the spleen and 0·35 g. in the adrenals, amounts very much above normal.

Evidence that the spleen may play some part in cholesterol metabolism has been put forward by a number of authors [Leites *et al.* 1933; Peretti *et al.* 1933; Goebel, 1934; McMeans, 1915], and although the present results would agree with this hypothesis, they could be interpreted equally well by saying that the spleen in certain circumstances can act as a depot for cholesteryl esters.

The data for the adrenals which show a large increase in cholesteryl ester and a smaller increase in free cholesterol in the "cholesterol" group are in agreement with previous work [Wacker & Hueck, 1913; Weltman & Black, 1913; McMeans, 1915; Bailey, 1916; Kay & Whitehead, 1935]. The changes observed by the last-named authors were not so pronounced as those found in the present work, owing probably to the low fat content of the diets they used. It is quite evident from the present work that the adrenals like the liver and spleen possess the power of taking up large amounts of cholesteryl esters, but whether this is for storage or metabolism cannot be decided.

In all the tissues investigated the cholesterol is deposited mainly in the form of its ester, in spite of the fact that the normal tissues (except the spleen and adrenals) contain little or no combined cholesterol. The free cholesterol which is normally present in the tissues may be part of the cell structure and as such would not be concerned with metabolic processes. Possibly, therefore, reserves of cholesterol can only be stored within the cells when esterified, in which condition it would be readily available for transport in the blood stream.

The presence of large amounts of cholesteryl ester in the tissues of the rabbits on the "cholesterol" diet indicates that there must be in the animal body an efficient mechanism for the esterification of cholesterol, but where this process takes place is not clear. Possibly all the tissues (and especially the liver, spleen and adrenals) contain a cholesterol esterase. On the other hand, the esterase may be mainly in the mucosa of the small intestine, where it would cause a synthesis of cholesteryl esters during the course of cholesterol absorption. If this were so then the cholesterol fed would enter the circulation as ester and as such would be taken out of the blood stream by the tissues. The presence in blood plasma of large amounts of cholesteryl esters is in agreement with this hypothesis.

Summary

1. A study has been made of the variations in the lipoids of the liver, kidney, heart, lungs, brain, spleen and adrenals of groups of rabbits receiving either a normal diet or a diet high in fat with or without added cholesterol.

2. All the organs except the brain increased in size on the "cholesterol" diet and this was particularly noticeable in the cases of the liver, spleen and adrenals.

3. Striking changes in the distribution of the lipoids took place as a result of the diet containing cholesterol and smaller changes due to the "high fat" diet. With the "cholesterol" diet deposition of cholesteryl ester took place in all the organs studied but particularly in the liver, spleen and adrenals. In the livers the percentage of cholesteryl esters reached the value of 16%, representing an accumulation of 10 g. cholesterol in the liver of a 2 kg. rabbit.

4. No evidence is available from the present experiments to show whether these organs act merely as depots for excess cholesterol or whether they are concerned with its metabolism.

5. The reason why cholesterol is deposited in the tissues mainly in the form of an ester is discussed.

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REFERENCES

Anitschow & Chalatow (1913). *Zbl. allg. Path. path. Anat.* **24**, 1.
Aylward, Channon & Wilkinson (1935). *Biochem. J.* **29**, 169.
Bailey (1916). *J. exp. Med.* **23**, 69.
Beeston, Channon & Wilkinson (1935). *Biochem. J.* **29**, 2659.
—— —— Loach & Wilkinson (1936). *Biochem. J.* **30**, 1040.
Best, Channon & Ridout (1934). *J. Physiol.* **81**, 409.
—— Hershey & Huntsman (1932). *J. Physiol.* **75**, 56.
Bloor (1923). *J. metab. Res.* **4**, 549.
Chamberlain (1928). *J. Physiol.* **66**, 249.
Channon & Wilkinson (1934). *Biochem. J.* **28**, 2026.
—— —— (1936). *Biochem. J.* **30**, 1033.
Chanutin & Ludewig (1933). *J. biol. Chem.* **102**, 57.
Cook (1937). *Biochem. J.* **31**, 410.
Ellis & Gardner (1912). *Proc. roy. Soc.* B, **85**, 385.
Frolicher & Sullman (1934). *Biochem. Z.* **274**, 21.
Gardner & Gainsborough (1930). *Quart. J. Med.* **23**, 465.
Goebel (1934). *J. Physiol. Path. gen.* **32**, 59, 408.
Grollman (1936). The Adrenals, p. 228. (London: Baillière, Tindall and Cox.)
Hess-Thaysen (1914). *Biochem. Z.* **62**, 89.
Kay & Whitehead (1935). *J. Path. Bact.* **41**, 293.
Leites, Koslowa & Jussin (1933). *Dtsch. med. Wschr.* **59**, 214.
McMeans (1915). *J. med. Res.* **33**, 475, 481.
Magistris (1931). *Ergebn. Physiol.* **31**, 165.
Mueller (1915). *J. biol. Chem.* **22**, 1.
Okey (1933, 1). *J. biol. Chem.* **100**, lxxv.
—— (1933, 2). *Proc. Soc. exp. Biol. N.Y.* **30**, 1003.
Page & Menschick (1932). *J. biol. Chem.* **97**, 359.
Peretti, Reale & Cioglia (1933). *Riv. Patol. sper.* **11**, 73.
Rosenheim & Tebb (1909). *J. Physiol.* **38**, liv.
Spanner & Baumann (1932). *J. biol. Chem.* **98**, 181.
Sperry & Stoyanoff (1935). *J. Nutrit.* **9**, 131.
Thierfelder & Klenk (1930). Die Chemie der Cerebroside u. Phosphatide. (Berlin: Springer.)
Wacker & Hueck (1913). *Arch. exp. Path. Pharmak.* **74**, 450.
Weltman & Black (1913). *Z. exp. Path. Ther.* **14**, 213.
Whitehead (1934). *Brit. J. exp. Path.* **15**, 279.