LIV. ON THE CHOLESTEROL CONTENT OF THE TISSUES OF CATS UNDER VARIOUS DIETETIC CONDITIONS AND DURING INANITION.

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Since the discovery of cholesterol by Conradi in 1775, and its analysis by Chevreul in 1815, it has been found to be very widely distributed in the animal, and in its isomeric forms in the vegetable, kingdom, and it is now generally recognised as an integral constituent of all cells in the animal body [Dorée, 1909]. The constant presence of such a substance clearly suggests its importance from a vital standpoint, and necessitates its recognition as a primary constituent of all protoplasm. Though a considerable amount of work has been done on the subject, however, we have as yet little definite knowledge of its physiological functions.

As a result of experiments on herbivorous animals published by one of the present writers, and various colleagues and the researches of Pribram [1906], Kosumoto [1908, 1 and 2], Harley and Barratt [1903] the working hypothesis was put forward that in these animals cholesterol is a substance which is strictly conserved in the animal organism; that when the destruction of the red blood corpuscles and possibly other cells takes place in the liver, their cholesterol is excreted in the bile, and that the cholesterol of the bile is reabsorbed in the intestine along with the bile salts and finds its way into the blood stream to be used in cell anabolism [Dorée, Ellis, Fraser and Gardner, 1908-1912]. In the case of carnivorous animals there would not appear to be the same necessity for such economy, as cholesterol is a normal constituent of their food, whereas the food of herbivora contains no cholesterol, but instead the isomeric phytosterols, and these only in minute quantity. The absorption of the food cholesterol would, however, be limited by the reduction of cholesterol to coprosterol by the bacteria of the gut. In the

human subject cholesterol is excreted in the faeces entirely in the form of coprosterol under ordinary conditions [Bondzynski, 1896; Bondzynski and v. Humnicki, 1896], though it has been shown by Müller [1900] that a prolonged milk diet leads eventually to the excretion of cholesterol as such. In the case of dogs and cats the change into coprosterol is generally only partial, though under some conditions it may be complete, and takes place only on meat diets [Dorée and Gardner, 1908, 2]. Feeding experiments [Dorée and Gardner, 1908, 2; Kosumoto, 1908, 2; Ellis and Gardner, 1909, 2] on dogs and cats have shown that the total cholesterol found in the faeces, either as cholesterol or coprosterol can be more than accounted for by the cholesterol taken in with the food, provided that the animal remains in health and constant in weight. This is also probably the case in man [Ellis and Gardner, 1912, 3]. Feeding cats, however, on artificial diets free from cholesterol led to inconclusive results from the point of view of deciding whether in the case of carnivora the cholesterol of the bile is normally reabsorbed along with the bile salts in the intestine [Ellis and Gardner, 1909, 2]. Earlier experiments made by Dorée and Gardner [1909] to trace a connexion between the percentage of cholesterol in the blood of dogs and the cholesterol content of the food taken, also led to inconclusive results, partly owing to the defects in the methods then available for estimating cholesterol in the tissues. Recent improvements in the methods of estimating cholesterol—notably the digitonine process of Windaus [1910]—rendered it desirable to repeat and extend the experiments referred to. In the present paper we give an account of analyses of blood and the tissues of cats fed on diets containing different amounts of cholesterol and also of cats kept in a state of inanition.

It is much more difficult in the case of carnivorous animals to maintain constant conditions during experiment than in the case of herbivorous animals such as the rabbit. In the latter animals a natural standard diet, free from cholesterol and similar bodies, to which measured portions of cholesterol could be added was available; and as these animals are practically continuous feeders the bile flow and consequently the cholesterol content of the blood due to this source would remain practically constant. In the case of cats we had to make use of different foods, though the difference was not so marked as in the earlier experiments. Little is known concerning the influence of food on the secretion of the bile, but from experiments that have been made by various observers, there is good reason to suppose that the nature of the diet would not be without influence [*vide* Goodman, 1907]. Furthermore the cat and the dog are discontinuous feeders and the flow

of bile into the intestine would be intermittent. Under these circumstances the portion of the floating as distinguished from the constituent cholesterol in the blood and the tissues due to the reabsorption of the cholesterol of the bile, would not necessarily be strictly comparable in different cases. What would be the limits of such variation, if any, we have no data at present upon which to form an opinion, but a variation of the kind suggested might wholly or partially mask any variation due to the cholesterol absorbed from the food, which at best would not be great in absolute magnitude. We did not therefore expect to obtain results comparable in precision with those obtained in the case of herbivora, but we attempted to render the conditions as comparable as possible by careful selection of diets, and by always killing the animals during a period of active digestion.

DETAILS OF THE DIETS USED.

Diet A, cholesterol free. We had some difficulty in finding a cholesterol-free diet which cats would take freely, but ultimately the following proved suitable. The whites of four eggs were taken raw and mixed with an equal weight of mashed boiled potatoes. The well-mixed material was flavoured with a small quantity of Liebig's extract of meat, and heated until the white of egg coagulated. The animals appeared to relish this diet, not the slightest trouble being experienced in getting them to eat it.

Diet B. Diet A, with the addition to each daily ration of a weighed amount of free cholesterol.

Diet C. Lean cooked beef.

DETAILS OF THE ANIMALS USED.

Diet A, cholesterol free.

Cat No. 1. The animal was fed for eight days and consumed 1003 grams white of egg and 1003 g. potatoes, the total weight when cooked being 1932 g. During this period its weight remained quite constant—2·7 kilos. It was killed one hour after the last meal. The stomach was full, and all the organs appeared normal.

Cat No. 2. This cat ate the same amount of food as No. 1, and during the eight days of the diet period its weight was as follows:—2·67, 2·58, 2·6, 2·55, 2·5, 2·5 and 2·55 kilos. It was killed two hours after the last meal. The stomach was full, and all the organs seemed normal.

Cat No. 3. This cat was pregnant, and during 11 days consumed

728 g. of egg white, 728 g. of potatoes, weighing when cooked 1396 g. It lost weight gradually during the diet period—3, 2·7, 2·7, 2·5, 2·4, 2·3 and 2·3 kilos. It was killed one hour after the last meal.

Cat No. 4. This animal ate in the course of 11 days 1138 g. egg white and 1210 g. of potatoes, weighing when cooked 2188 g. It remained approximately constant in weight—3·2, 3·2, 3·2, 3·1, 3·0, 3·1, 3·1, 3·1 kilos. It was killed *three* hours after the last meal. The stomach was half full, and the organs seemed normal.

Diet B.

Cat No. 5. During 11 days this cat consumed 693 g. of egg white and 693 g. of potatoes, weighing when cooked 1256 g., and 2·75 g. of cholesterol, i.e. about twice the amount it would have got from the same weight of meat. It weighed 2·3 kilos. at the beginning of the experiment and 2·2 kilos. at the end. It was killed one hour after the last meal, and all its organs were quite normal.

Cat No. 6. This cat consumed during 11 days 728 g. of egg white and 728 g. of potatoes, weighing when cooked 1303 g., and 2·75 g. of cholesterol. It weighed 2·4 kilos. at the beginning and gradually decreased in weight to 2·1 kilos. It was killed 1·5 hour after the last meal. Its organs seemed normal.

Diet C.

Cat No. 7. This animal was fed for ten days on lean cooked beef as free as possible from fat and consumed 1563 g. of meat weighing in the raw state 2165 g. This would correspond to an intake of about 1·7 g. of cholesterol. The weights taken from time to time were 3·37, 3·3, 3·4, 3·33, 3·42, 3·45 and finally 3·43 kilos. It was killed four hours after the last meal. The stomach was full and all the organs normal.

Cat No. 8. This cat consumed during 11 days 1910 g. of cooked lean beef, weighing in raw state 2433 g. This would correspond to an intake of about 1·9 g. of cholesterol during the period. The animal remained constant in weight—3·3 kilos. It was killed four hours after the last meal. The stomach was full and the organs normal.

ANIMALS IN A STATE OF INANITION.

In these experiments well-fed animals were kept without food for seven days, but were allowed as much water as they wished. At the end of this period they all appeared to be in good health. No faeces were passed.

Cat No. 9. The weights taken daily, except on Sunday, were 3.1, 2.9, 2.8, 2.6, 2.55 and 2.5 kilos. The loss of weight was therefore 19.4%. After the animal was killed the stomach was found to be completely empty. There was a little fluid in the small intestine, and some faecal matter in the rectum.

Cat No. 10. The weights recorded were 3.1, 2.95, 2.8, 2.75, 2.7, 2.6 and 2.5 kilos., the loss in weight being as before 19.4%. The stomach was quite empty; a small amount of fluid was found in the small intestine and some faeces in the rectum.

Cat No. 11. This animal similarly treated only lost 10% of its body weight, the weights recorded from time to time being 2.8, 2.7, 2.6, 2.5 and finally 2.5 kilos. The stomach was empty, but there was some fluid in the intestine and faeces in the rectum. The weight of the contents was 92 g., and this was found to contain 0.24 g. of cholesterol.

Immediately the animals were killed, they were cut up and the various tissues submitted with the least possible delay to the treatment described below. The following were examined—blood, liver, muscle taken from various parts of the body, kidney, suprarenal glands, heart, brain, lung and contents of the gall-bladder.

METHOD OF EXTRACTION AND ESTIMATION OF CHOLESTEROL.

The tissues were finely minced and ground up with sand and enough plaster of Paris to make the whole mass fairly dry and friable. It was allowed to stand until quite dry, powdered and extracted in a Soxhlet's apparatus with ether for about 14 days. The ethereal extract was made up to known volume, and divided into two portions, or in some cases into suitable aliquot portions. In one portion the free cholesterol was estimated and in the other the total free and ester cholesterol after saponification in ethereal solution by means of sodium ethylate. The cholesterol was estimated by the modification of Windaus' digitonine process described by Fraser and Gardner [1910]. The ester cholesterol was thus determined by difference, and it was assumed that no hydrolysis of the esters took place in the time occupied in dissecting out the tissue, mincing up and drying with plaster of Paris. Though we think this assumption is probably justifiable in the case of most of the tissues, it is perhaps open to some question in the case of liver. This tissue is known to contain ferments, which hydrolyse cholesterol esters, but the hydrolysis does not appear to take place rapidly even under most favourable conditions. Thus a sample of liver which on treatment as

described above was found to contain 0.28% of total cholesterol and 0.13% of free cholesterol and therefore 0.15% of ester cholesterol, was still found to contain 0.05% of ester after incubating for four days at 37° in saline suspension in the presence of a little toluene, though another sample allowed to putrify in saline suspension at 37° yielded no ester. We endeavoured however to reduce any such error to a minimum by carrying out the operations as rapidly as possible. •

DISCUSSION OF ANALYTICAL RESULTS.

Blood. The results of the analysis of the blood of the eleven animals are collected together in Table I.

TABLE I.

No. of cat	Diet	Weight of blood taken for analysis, in grams	Total free and combined cholesterol in g. per 100 g. of blood	Free cholesterol in g. per 100 g. of blood	Cholesterol in form of esters per 100 g. of blood
1	A (non chol.)	68.2	0.044	0.044	Nil
2	A	87.9	0.060	0.046	0.014
3	A	84.1	0.058	0.056	0.002
4	A	83.2	0.044	0.040	0.004
		Mean of 4 cats	0.052	0.047	0.005
5	B (cholest.)	74.2	0.102	0.098	0.004
6	B	59.8	0.178	0.049	0.129
		Mean of 2 cats	0.140	0.074	0.066
7	C (meat)	126.2	0.083	0.066	0.017
8	C	74.2	0.078	0.054	0.024
		Mean of 2 cats	0.081	0.060	0.021
9	Inanition	65.8	0.163	0.074	0.089
10	Inanition	76.5	0.196	0.069	0.127
11	Inanition	75.6	0.151	0.126	0.025
		Mean of 3 cats	0.170	0.090	0.080

On comparing the figures in column four of Table I it will be seen that the total cholesterol of the blood increases with the cholesterol taken in with the food. In the case of the animals in a state of inanition, which were living on their own tissue, the increase is very marked. This increase is in all cases chiefly due to an increased quantity of ester cholesterol, though the free cholesterol itself also increases. The high ester content is very marked in the case of animals in a state of inanition. This may very likely have

something to do with the transference of the fat from the depots during starvation, and in this connexion it may be noted that in the case of cats 9 and 10 which lost 19% of their body weight their blood contains 0.089 and 0.127% of ester cholesterol, while that of cat 11, which only lost 10% of body weight, is 0.025. In the case of cats 2 and 3, both of which lost weight during the diet period, the total cholesterol of the blood is somewhat higher than in the case of cats 1 and 4 which had the same diet but remained constant in weight. The same thing will be noticed in the case of cat 6 compared with cat 5 on the same diet.

The variations observed are similar to those found in the case of rabbits fed on diets containing varying amounts of cholesterol, and the figures are of much the same order as will be seen from Table II in which only average values are given.

TABLE II.

Number of animals of which the figures are the average	Animal	Diet	Total free and combined cholesterol per cent.	Free cholesterol per cent.	Ester cholesterol per cent.
4	Cat	Cholesterol-free A ...	0.052	0.047	0.005
4	Rabbit ¹	Bran extracted by ether ...	0.058	0.044	0.014
2	Cat	B (A + cholesterol) ...	0.140	0.074	0.066
4	Rabbit ¹	Extracted bran + cholesterol	0.081	0.059	0.022
2	Cat	C (lean beef) ...	0.081	0.060	0.021
2	Rabbit ¹	Ordinary bran ...	0.082	0.069	0.013
2	Rabbit ¹	Cabbage leaves and stalk ...	0.068	0.063	0.005
3	Cat	Inanition ...	0.170	0.090	0.080
2	Rabbit ¹	Inanition ...	0.154	0.113	0.040

¹ [Ellis and Gardner, 1912, 2.]

Liver. In Table III we give the total cholesterol, free and combined, actually found in the livers of the animals, also the weights of cholesterol per cent. of liver and the weights of liver cholesterol per kilo. of body weight. On comparing the figures for animals fed on diet A (cholesterol free) it will be seen that both the total percentages of cholesterol in the liver and the total liver cholesterol per kilo. of body weight are fairly uniform, though not so constant as was found to be the case in rabbits fed on cholesterol-free diet. On comparing these figures with those of cats 5 and 6 which were fed for a period on the same diet, but with the addition of free cholesterol, a marked increase in the total cholesterol is obvious. This increase is partly due to free cholesterol, but mainly to ester cholesterol. On the other hand in the case of cats 7 and 8, fed on lean meat, i.e. a diet containing

TABLE III.

No. of cat	Diet	Wt. of animal when killed, in kilos.	Wt. of liver, in g.	Wt. of total cholesterol in liver, in g.	Wt. of free cholesterol in liver, in g.	Wt. of ester cholesterol in liver, in g.	Wt. of cholesterol per cent. of liver			Wt. of liver cholesterol per kilo. of body wt.		
							Total	Free	Ester	Total	Free	Ester
1	A	2.70	59.8	0.128	0.092	0.036	0.214	0.154	0.060	0.047	0.034	0.013
2	A	2.55	54.6	0.136	0.041	0.095	0.249	0.076	0.173	0.053	0.016	0.037
3	A	2.30	63.0	0.102	0.102	Nil	0.162	0.162	Nil	0.044	0.044	Nil
4	A	3.10	70.8	0.192	0.048	0.144	0.254	0.067	0.187	0.062	0.015	0.047
Mean values							0.220	0.115	0.105	0.052	0.027	0.025
5	B	2.20	48.4	0.194	0.089	0.105	0.401	0.185	0.216	0.088	0.041	0.047
6	B	2.10	43.7	0.228	0.049	0.179	0.466	0.117	0.349	0.108	0.023	0.085
Mean values							0.434	0.151	0.283	0.098	0.032	0.066
7	C	3.43	77.5	0.243	0.221	0.022	0.316	0.285	0.031	0.071	0.064	0.007
8	C	3.30	107.7	0.362	0.362	Nil	0.346	0.346	Nil	0.109	0.109	Nil
Mean values							0.331	0.316	0.015	0.090	0.087	0.003
9	Inanition	2.50	50.3	0.345	0.063	0.282	0.550	0.125	0.425	0.138	0.025	0.113
10	Inanition	2.50	49.8	0.295	0.069	0.226	0.637	0.143	0.494	0.118	0.027	0.091
11	Inanition	2.50	48.2	0.245	0.059	0.186	0.509	0.122	0.387	0.098	0.024	0.074
Mean values							0.565	0.130	0.435	0.118	0.025	0.093

a considerable proportion of cholesterol but no added carbohydrate, there is a similar increase in total cholesterol, but the increase is almost entirely free cholesterol, the ester being nil in one case and considerably below the average of diet A in the other. In the case of cats 9, 10 and 11, in which the animals were living on their own tissues, we find a similar storing up of the cholesterol in the liver. It is stored almost entirely in the form of esters and there is only a slight increase if any of free cholesterol. This may also have something to do with the transference of the fat from the depots to the liver during starvation. In the case of cat 11, which lost only 10% of body weight, this storing up is not quite so great; in this cat the percentage in the blood was also somewhat lower than in the case of cats 9 and 10. Those changes are generally similar to those observed by Ellis and Gardner [1912, 1] in the case of rabbits during starvation. Assuming that the loss in weight of the cats during inanition is due to muscle tissue, then, if the cycle postulated for herbivora be true for carnivora, cats 9 and 10 both of which lost 600 g. of body weight should have accumulated each about 0.5 g. of cholesterol in the blood, liver and the contents of the intestine. Taking the average figures for diet A as representing the normal cholesterol content of the blood and liver under conditions in which body weight is kept constant, but no cholesterol is absorbed with the food, 0.42 g. of cholesterol was accumulated in the blood and liver of cat 9 and 0.41 in cat 10. The

contents of their intestines were not analysed, but in cat 11 about 0.2 g. was found.

Suprarenal Glands. This organ was only examined in five of the eleven cats, and the results are given in Table IV.

TABLE IV.

No. of cat	Diet	Weight of organ, in g.	Total cholesterol, free and combined, actually found	Free cholesterol actually found	% of total cholest.	% of free cholest.	% of ester cholest.
3	A	0.6569	0.0116	0.0060	1.77	0.91	0.86
4	A	0.5886	0.0155	0.0034	2.63	0.58	2.05
5	B	0.6310	0.0373	0.0373	5.92	5.92	Nil
6	B	0.4408	0.0228	0.0070	5.13	1.59	3.58
11	Inanition	0.5208	0.0048	0.0035	0.92	0.67	0.25

Here the addition of cholesterol to the diet produces a marked increase in the total cholesterol content of the organ, and the change appears to run parallel to that in the blood. In cat 5, for instance, the increase in the blood is all free cholesterol, the actual amount of ester cholesterol being slightly below the average of the cats on diet A. In the suprarenal the increase is also all free cholesterol, the ester being nil or very small. In cat 6 on the other hand, where the increase is almost entirely in ester cholesterol, the ester cholesterol of the blood is very high, while the free cholesterol is almost exactly equal to the average on diet A. In inanition however this parallelism no longer appears to hold, for while the cholesterol accumulates in the blood it decreases almost to the vanishing point in the suprarenals. More experiments will however be required before this interesting point can be settled. The inanition result appears, however, to be in agreement with the observations of Landau [1913]. In this connexion it may also be noted that Albrecht and Weltmann [1911] and Hueck [1911] have shown that in diseases such as Carcinoma, Tuberculosis, etc., both the free and ester cholesterin of the suprarenals sink and sometimes are absent altogether.

Muscle. The muscle analysed was a mixed sample cut from various parts of the body. The results are given in Table V.

TABLE V.

No. of cat	Diet	Percentage total cholesterol	Percentage free cholesterol	Percentage ester cholesterol
1	A	0.056	0.031	0.025
2	A	0.047	0.032	0.015
7	C	0.087	0.083	0.004
8	C	0.081	0.073	0.008
9	Inanition	0.059	0.053	0.006
10	Inanition	0.065	0.051	0.014

It will be noticed that on diet A, which contained no cholesterol, but was rich in carbohydrate, there is a marked increase in the ester cholesterol compared with the cats on meat diet C. The free cholesterol is however much lower. In the case of rabbits, Ellis and Gardner were unable to trace any influence of food cholesterol on the cholesterol content of muscle.

Heart. The results are given in Table VI.

TABLE VI.

No. of cat	Diet	Wt. of organ, in g.	Total cholest. per cent.	Free cholest. per cent.	Ester cholest. per cent.
1	A	7.9	0.080	0.073	0.007
2	A	8.8	0.068	0.068	Nil
7	C	16.3	0.073	0.073	Nil
8	C	12.5	0.072	0.063	0.009
9	Inanition	10.5	0.071	0.063	0.008
10	Inanition	9.4	0.091	0.039	0.052

The cholesterol of this organ is very constant, and nearly all present in the free state. The percentage is much the same as that given for heart muscle in the ox, 0.066 to 0.071 by Ellis and Gardner [1908].

Kidney. The results on this organ are given in Table VII.

TABLE VII.

No. of cat	Diet	Wt. of animal when killed, in kilos.	Wt. of kidney, in g.	Total cholesterol, in g.	Free cholesterol, in g.	Ester cholesterol, in g.	Wt. of cholesterol per cent. of kidney			Wt. of kidney cholesterol per kilo. of body wt.		
							Total	Free	Ester	Total	Free	Ester
1	A	2.7	21.6	0.064	0.064	Nil	0.298	0.296	0.002	0.024	0.024	Nil
2	A	2.6	17.8	0.056	0.056	Nil	0.326	0.326	Nil	0.022	0.022	Nil
7	C	3.43	27.5	0.073	0.069	0.004	0.265	0.252	0.013	0.021	0.020	0.001
8	C	3.3	24.8	0.077	0.070	0.007	0.313	0.284	0.029	0.023	0.021	0.002
Mean values							0.301	0.290	0.011			
9	Inanition	2.5	20.8	0.070	0.053	0.017	0.335	0.212	0.123	0.028	0.021	0.007
10	Inanition	2.5	23.8	0.066	0.054	0.012	0.275	0.226	0.049	0.026	0.022	0.004

The diet as in the case of rabbits appears to have no influence on the kidney cholesterol. The average values for normal animals are not very dissimilar from the mean of those given by Windaus [1910] for normal human kidneys, viz. 0.24 free and 0.02 ester per cent. In the case of the starved animals the ester cholesterol shows a marked increase, which Ellis and Gardner also showed to be the case in the rabbit. These high ester values recall the high ester value found by Windaus in human pathological kidneys.

Lung. The results are given in Table VIII.

TABLE VIII.

No. of cat	Diet	Wt. of organ taken for analysis, in g.	Percentages of cholesterol		
			Total	Free	Ester
1	A	17.1	0.416	0.295	0.121
2	A	15.6	0.361	0.296	0.065
7	C	17.5	0.435	0.247	0.188
8	C	11.4	0.453	0.270	0.183
9	Inanition	13.4	0.445	0.413	0.032
10	Inanition	16.5	0.376	0.376	Nil

The cholesterol content, as one would expect, appears to have nothing to do with diet. The values are of much the same order as those given for rabbits by Ellis and Gardner, viz. average of six animals, free 0.442 and ester 0.053. The ester cholesterol, as in the case of rabbits, is very variable.

Brain. The brains of cats 1 and 2 on diet A, 7 and 8 on diet C and 9 and 10 in a state of inanition, were examined with the following results:—3.05, 2.65, 2.94, 2.91, 2.33 and 2.06 per cent. In no case was any ester found. The average is 2.66. For rabbits, Ellis and Gardner give as an average of eight experiments 2.32, with a variation of 2.02–2.88, and in no case was there any evidence of the presence of esters.

Bile. Attempts were made to estimate the cholesterol content of the bile of the cats under different diets and during starvation, but the contents of the gall-bladder were so small that it was difficult to estimate the cholesterol with any degree of accuracy. We give however the results for what they are worth in the following Table IX.

TABLE IX.

No. of cat	Diet	Wt. of bile in gall-bladder, in g.	Percentage of cholesterol		
			Total	Free	Ester
1	A	0.96	0.56	0.42	0.14
2	A	1.48	0.28	0.28	Nil
7	C	0.61	0.36	0.34	0.02
8	C	1.30	0.61	0.61	Nil
9	Inanition	2.53	0.25	0.03	0.22
10	Inanition	1.62	3.01	3.01	Nil

The results of the experiments on diets A and C do not indicate any connexion between either the actual amount of cholesterol in the bile or the percentage content and the diet. In the case of cat 10 there was a relatively high percentage of cholesterol in the bile, and this appeared to be all or mainly in the free state. This is in agreement with the observations of Ellis and Gardner on starving rabbits. On the other hand in the case of cat 9, also in a state of starvation, both the actual amount found and the percentage are of much the same order as in the fed animals, but it was nearly all found in the ester condition.

GENERAL CONCLUSIONS.

The results obtained in these experiments are strikingly similar to those obtained by Ellis and Gardner on the rabbit, and are in agreement with the hypothesis advanced at the beginning of this paper, viz. that cholesterol is a constituent constantly present in all cells, and that when these cells are broken down in the life process the cholesterol is not excreted as a waste product, but is utilised in the formation of new cells. A function of the liver is to break down dead cells, e.g. blood corpuscles, and eliminate their cholesterol in the bile. After the bile has been passed into the intestine in the process of digestion, the cholesterol is reabsorbed, possibly in the form of esters, along with the bile salts and is carried in the blood stream to the various centres and tissues for reincorporation into the constitution of new cells. Waste of cholesterol is made up from that taken in with the food.

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