CCXXXI. CHOLESTEROL FEEDING AND FAT METABOLISM.

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THE experiments to be described were undertaken as part of a study of the chemical changes produced in artificially induced fatty infiltrations and degenerations. Recent work has shown that feeding of cholesterol to animals brings about well marked pathological changes. The subject of arteriosclerosis and its possible relation to dietary cholesterol is dealt with in Cowdry's [1933] book and the later work is reviewed by Brody [1935]. The cholesterol "fatty liver" has also been the subject of much investigation and it is mainly with this aspect that the writer is concerned.

Cholesterol and growth.

(a) *Experiments with rats.* The animals used were drawn from the piebald laboratory stock. Litter-mates of approximately the same weight were taken soon after weaning and were 4-6 weeks old. The room in which the animals were confined was kept at a constant temperature. The diets fed to the animals were made up as shown in Table I.

Table I. *Rats' diets.*

	All figures in g.			
	Fat-free	Fat-free plus cholesterol	Normal synthetic	Normal synthetic plus cholesterol
Caseinogen	23	23	23	23
Rice starch	50	50	40	40
Cane sugar	22	22	17	17
Arachis oil	0	0	15	15
Salt mixture	5	5	5	5
Yeast	7.5	7.5	7.5	7.5
Cod liver oil	0	0	2.5	2.5
Radiostoleum	One drop per diem		0	0
Cholesterol (B.D.H.)	0	2	0	2
Calories per 100 g. food	354	347	434	426

NOTES. The caseinogen used for the growth of rats shown in Fig. 1 was "Glaxo physiological caseinogen AB". This is heat-treated ashless caseinogen. In all subsequent experiments, Series B "Glaxo ashless casein E." was used. This is ashless caseinogen exhaustively extracted with alcohol to the maximum possible removal of ether-soluble matter. The salt mixture is No. 185 of McCollum & Davis [1915]. The yeast is a dried yeast preparation of trade name "Ceramealo".

The cholesterol was the normal commercial preparation (B.D.H.) and was not further purified. It was carefully mixed with the other constituents of the diet. No calorigenic value is given to the cholesterol but the cod liver oil is counted as dietary fat. The cholesterol intake of the rats is approximately 0.25 g. per rat per day.

Series A (Fig. 1) shows the growth curves obtained when the dietary source of caseinogen was the normal physiological caseinogen. Series B (Fig. 2) shows the result of using alcohol-extracted caseinogen. This type of caseinogen was substituted in the diets to ensure that the extraneous fats were minimum.

Fig. 1. Growth curves of animals fed on various synthetic diets plus 2% cholesterol. The weights are the average of two males in each series. Series A using physiological caseinogen.

—x— Fat-free diet; ○— fat-free diet + cholesterol; - - - x - normal synthetic;
●— normal synthetic + cholesterol.

Fig. 2. Series B using alcohol-extracted caseinogen. The weights are the average of four males in each series. Markings as for Fig. 1.

The males of series A alone are shown, but the female litter-mates were also fed and gave the same type of growth curves. The results for the liver and body fats of the females are included in Table III.

Table II gives the calorie intake of the animals and shows that the calorie intake of food on the diets is well matched except that in series B there is a significantly decreased intake for the animals on the normal synthetic diet plus cholesterol. It will be seen from the figures that the presence of cholesterol affects the growth rate in both cases but particularly so in series B. This point will be discussed later. The earlier part of the growth curves only is shown. Some of the animals in series A were actually fed over 130 days. The difference in the food intakes during the later periods between the control animals and those fed with cholesterol was not significant. It averaged about 60 calories per rat per day. In addition to the animals fed on the synthetic diets a series was put

Table II.

Food intake expressed as calories per rat per day on the various diets. The figures are average intakes over the period stated in the first column. For series A the diet of 2 male rats is averaged and for series B that of 4 males.

Period of feeding days	Series A				Series B			
	Fat-free plus cholesterol		Normal synthetic plus cholesterol		Fat-free plus cholesterol		Normal synthetic plus cholesterol	
	Fat-free	Normal synthetic	Fat-free	Normal synthetic	Fat-free	Normal synthetic	Fat-free	Normal synthetic
0-15	32.9	32.0	37.2	36.7	25.0	25.4	29.7	26.5
15-28	46.9	45.1	50.3	52.5	35.2	32.7	41.6	30.8
28-40	55.6	45.8	47.4	50.5	43.8	45.8	51.3	34.4
40-58	60.0	60.3	60.1	60.0	49.1	52.3	56.0	42.3
58-82	62.1	59.2	62.6	60.0	—	—	—	—

up on the normal stock laboratory diet. This consists of bread and milk with corn and greenstuff added. The animals grew well on this diet and the rate of growth of normal animals and those taking 2% cholesterol in their diet is shown in Fig. 3. The difference in the curves is due only to the initial difference in the weights of the animals. The added cholesterol has little effect on the growth rate. Mature animals were also given the stock diet but there was no noticeable effect. Thus 4 mature females of average weight 230 g. fed for 64 days with a diet containing 2% cholesterol showed an average increase in weight of 27 g. as compared with an increase in the weight of the controls of 20 g.

At the end of a feeding period the animals were killed by cutting their throats under light anaesthesia and collecting the blood which was estimated with the carcass. The liver was always weighed as far as possible free of blood. Autopsy of the animals revealed that the fatty liver was only to be found in those animals which were given the normal synthetic diet, i.e. that containing added fat. Those on the stock diet showed no obvious change, a fact that was borne out on analysis. It was also observed that the animals showing the "fatty liver" appeared to be generally in a poor state of nutrition. There was very evidently less body fat. The carcass was therefore analysed for its fat content by the method of Leathes & Raper [1925]. This method does not give any idea of the distribution of the lipin material but it serves as an admirable and fairly rapid method of estimating total fat. The fats are estimated as fatty acids and the cholesterol as the free alcohol together with any other unsaponifiable matter. The question of the distribution of the lipins is at present in hand but it is not intended to deal with it in this communication. The carcass was normally saponified directly, the fur being included. The animals were not gutted. About one-sixth the weight of the carcass of KOH added as 50% solution was sufficient to saponify the carcass after heating on a water-bath for 2 hours, alcohol being added after about 1½ hours. The mixture was made up to volume and the fat estimated in an aliquot portion. The results obtained are shown in Table III. The figures for fat determinations after the food had been taken for varying times are shown for the stock diet. It will be seen that there is no very marked effect. Normally the animals on the synthetic diets had the remains of the previous day's food in the dishes. It is somewhat more difficult with synthetic diets than with the stock diet to ensure the animals feeding at definite times, the tendency being for frequent feeding. Table III shows that the cholesterol effect is not very marked until after about 60 days' feeding. The

Table III. *The fat contents of the livers and carcasses of control and cholesterol-fed rats.*

a is the control animal and *b* the cholesterol-fed. In some experiments two animals were pooled for analysis.

Diet	No.	Sex		Days on diet	Wt. of animals g.		Av. wt. of livers g.	Liver as % body wt.	"Fat" in liver %	Carcass	
										g. fat	As % of body wt.
Fat-free Series A	1	♀	<i>a</i>	85	158	170	7.5	4.5	0.32	37.0	11.85
			<i>b</i>		175	153	8.1	4.9	0.78	37.0	12.0
	2	♂	<i>a</i>	131	298	270	12.0	4.2	1.41	43.4	8.35
			<i>b</i>		267	260	11.3	4.3	1.33	38.8	7.40
Series B	1	♂	<i>a</i>	59	180	210	9.8	5.0	0.23	47.6	12.85
			<i>b</i>		186	197	9.6	5.0	0.54	36.8	10.25
	2	♂	<i>a</i>	87	180	158	7.2	4.2	2.93	42.2	13.2
			<i>b</i>		186	194	9.2	4.8	3.48	41.8	11.45
Normal synthetic Series A	1	♀	<i>a</i>	89	152	188	8.2	4.8	0.21	43.2	13.4
			<i>b</i>		139	220	11.4	6.4	13.8	34.9	10.5
	2	♂	<i>a</i>	107	302		12.4	4.1	1.11	—	—
			<i>b</i>		354		12.8	3.6	12.5	—	—
Series B	3	♂	<i>a</i>	117	274		11.5	4.2	3.6	20.0	7.6
			<i>b</i>		237		12.6	5.3	12.7	6.3	2.7
	1	♂	<i>a</i>	41	190		9.7	5.1	4.43	14.2	7.9
			<i>b</i>		132		7.8	5.9	8.23	4.9	4.0
Full diet (laboratory stock diet)	2	♂	<i>a</i>	63	230	216	10.2	4.6	1.13	64.6	15.4
			<i>b</i>		162	133	7.3	4.9	10.0	32.0	11.6
	3	♂	<i>a</i>	73	190		8.7	4.6	3.45	27.8	15.7
			<i>b</i>		123		6.0	4.9	5.25	9.1	8.7
Full diet (laboratory stock diet)	1	♂	<i>a</i>	64	378		17.6	4.7	2.5	54.2	15.35
			<i>b</i>		382		18.3	4.8	2.63	61.5	17.2
	2	♀	<i>b</i>	92	187	187	16.7	4.5	1.83	42.6	11.4
			<i>a</i>		167		7.3	4.4	2.98	—	—
Full diet (laboratory stock diet)	3*	♀	<i>a</i>	66	185		8.0	4.3	3.78	—	—
			<i>b</i>		192		6.9	3.6	3.52	—	—
	4*	♀	<i>a</i>	66	209		7.7	3.7	3.20	—	—
			<i>b</i>								

* No. 3, full diet. The animals were killed one hour after feeding. No. 4, six hours after feeding.

Fig. 3. Growth curves of rats fed on the laboratory normal stock diet alone and plus 2% cholesterol. The weights are the average of four females in each series.

Fig. 4. Growth curve of guinea-pigs fed on stock diet and on stock diet plus 2% cholesterol. The weights are the average of two animals in each set.

action on the depot stores of fat estimated as carcass fat is noteworthy. An interesting observation with the animals fed on the fat-free diet is the low fat content of the liver in the earlier periods of growth and this notwithstanding the fact that the depot stores of fat are high. In general it may be said that livers with a fat percentage greater than 5% show the typical "fatty" appearance.

As the cholesterol effect is so marked with a fat-containing diet the possibility suggested itself that the cholesterol was only absorbed in the presence of fat. The faeces of some of the animals were therefore collected, the animals being placed in metabolism cages. For the estimation of lipins in the faeces, the latter were dried at 100° to constant weight. They were then powdered and extracted in a Soxhlet apparatus for 2-3 days with light petroleum, B.P. 50-60°. The results obtained are given in Table IV.

Table IV. *The lipin content and unsaponifiable matter in the faeces of rats fed on various synthetic diets.*

Diet and no. of rats	Days	Dry wt. g.	Total lipin g.	Unsaponi- fiable matter g.	Cholesterol in faeces as unsa- ponifiable matter in faeces minus that in control g.	Chole- sterol in food over collection period g.	Chole- sterol excreted %
Fat-free 2	7	7.7	0.49	0.36	—	—	—
Plus cholesterol 2	7	11.6	4.13	4.01	3.65	3.8	96
Normal synthetic 1	4	1.5	0.21	0.14	—	—	—
Plus cholesterol 1	4	2.0	0.69	0.66	0.52	0.6	87
Normal synthetic 2	7	9.0	1.54	0.34	—	—	—
Plus cholesterol 2	7	11.5	4.15	2.28	1.94	3.4	57

It will be seen from Table IV that there is practically complete excretion on a fat-free diet. The absorption is far from complete even on the fat-containing diet. Individual differences in the excretion of cholesterol in the case of the rats on this diet will be observed, and these are reflected to some extent in differences in fat in the liver. Experiments are at present in progress to study the question of absorption on diets containing varying amounts of fat.

(b) *Experiments with guinea-pigs.* As is well known these animals cannot be easily reared on a synthetic diet. The animals were therefore fed on a stock diet. This consisted of bran oats and egg yolk (A), and mangold roots or swedes or cabbages (B) were also given. The food intakes were on the average per animal per day 35 g. A and 40 g. B. The animals used in these experiments were about 6 months old. The cholesterol was mixed with the foodstuff A so that it was in a concentration of 2%. Thus approximately 0.8 g. cholesterol per animal per day was eaten. The growth curves of the control animals and those given cholesterol are shown in Fig. 4.

Table V gives the results of the fat estimations on the animals.

The cholesterol feeding has a marked effect on these animals. A point of interest here is that the liver size is greatly increased. The liver of a cholesterol-fed animal may be as much as 33% larger than of the control.

The question of the absorbability of the cholesterol was also investigated. The results are shown in Table VI.

The figure for unsaponifiable matter in the normal diet has been brought to the amount present in the same weight of faeces as in the cholesterol-fed animals in making the correction for the amount of cholesterol in the faeces.

Table V. *The fat contents of the livers and carcasses of control and cholesterol-fed guinea-pigs.*

The second animal in each set has been fed on the cholesterol-containing diet.

No.	Days on diet	Wt. of animals g.	Wt. of liver g.	Liver as % of body wt.	"Fat" in liver %	Carcass	
						g. fat	As % of body wt.
1	40	445	16.5	3.7	2.46	20.8	4.9
		409	20.6	5.0	12.6	12.5	3.2
2	45	351	24.6	7.0	1.86	—	—
		360	36.8	10.4	6.57	—	—
3	57	367	14.7	4.0	2.92	16.9	4.9
		262	20.7	7.9	6.7	8.3	3.6

Table VI. *Cholesterol content of guinea-pig faeces.*

Two animals were on the normal diet and three on the same diet plus cholesterol. The faeces were collected over 4 days.

	Dry wt. g.	Total lipin g.	Unsaponifiable matter g.	Cholesterol in faeces as unsaponifiable matter in faeces minus that in control	Cholesterol in diet g.	Cholesterol excreted %
				g.		
Normal diet	95	1.10	0.57	—	—	—
Plus cholesterol	113.5	6.35	4.90	4.22	8.0	53

It will be seen that there has been very definite absorption of the cholesterol in these animals and this notwithstanding the fact that the diet does not contain fat in large amounts as does the rats' diet.

DISCUSSION.

This communication must be regarded as preliminary in nature. The purpose aimed at is a study of the action of cholesterol in producing such marked disorders of fat metabolism. The growth curves show that cholesterol feeding of rats maintained on synthetic diets has only one effect and that deleterious. This action is found only when the diet contains fat. On fat-free diets there is no effect, either beneficial or otherwise, and this has been shown to be due to the absence of absorption. On the fat-containing diet the growth rate is decreased and the fatty liver develops. On a normal stock diet cholesterol is without action. There appears to be an influence of some dietary factor, possibly choline [Best, Channon & co-workers], in the difference observed between normal dietary caseinogen and caseinogen that has been extracted to remove ether-soluble matter. Thus over a period of 60 days while the control animals in both series A and B gained 156 g. in weight those given cholesterol in series A gained 139 g., those in series B only gained 100 g. With series A there was no significant difference in the food intakes of controls and cholesterol-fed animals. A similar result was found for the females on the same diet. In both series however fatty livers were found. As no experiments were made on the absorption of cholesterol in the series A animals or in those given the stock diet it is impossible to state how much of this action is due to the choline effect. In studies with cholesterol it is perhaps pertinent to include a study of cholesterol excretion. In the case of the rat the action of the cholesterol appears to depend definitely on the absorption taking place in the presence of free fat in the diet. Thus on transferring a rat

which had been reared on the stock diet to the normal synthetic diet plus cholesterol the typical fatty liver developed. With regard to the question of absorbability it is of interest that the guinea-pig is able to absorb relatively large amounts of cholesterol and this on a diet which contains little fat. The experiments of Page & Menschick [1932] on another herbivore, the rabbit, show that as much as 69% of the ingested cholesterol was either destroyed or (to a small extent) stored in the animal.

Some of the organs were subjected to a histological examination. I am indebted to Dr T. Day of the Pathology Department for his help. With the rat livers although the liver cells were full of fat there did not appear to be any marked degenerative or necrotic changes. In the case of the rats the relatively small increase in liver weight as compared with that in the controls is to be remarked. Examination of the aorta revealed little evidence of atheroma, although there were some signs in animals which had been fed for an extended period. On the other hand in the guinea-pigs' livers there was evidence of degenerative changes. The liver weight was increased considerably over that of the controls. Sections of the liver showed marked degeneration and necrosis, principally central. Sections of the aorta showed atheromatous changes. A more extended study of the pathological changes is in progress with Dr G. P. McCullagh.

In comparing the rat and the guinea-pig the species difference and difference in diet are to be considered. In general it may be said, however, that the changes induced by cholesterol feeding are of the same nature, being different only in degree.

SUMMARY.

1. The growth of rats and guinea-pigs fed on various diets containing cholesterol has been investigated.
2. Cholesterol affects the growth curve of rats on a synthetic diet only when fat is present.
3. Guinea-pigs are very susceptible to the action of cholesterol in their stock diet, which contains but little fat.
4. Cholesterol is absorbed by rats only when fat is present in the diet.
5. Fatty livers are produced in the rats on the fat-containing diet only, but in the guinea-pigs on the stock diet also.
6. There is a marked decrease in the depot fats. The action of the cholesterol appears in part to be in mobilizing the fat in the liver.

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