LVII. FAT FEEDING AND CHOLESTEROL ABSORPTION

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In a previous paper [Cook, 1936] it was shown that with growing rats cholesterol is absorbed only in the presence of free fat in the diet, the cholesterol "fatty" liver being induced solely under these conditions. The following experiments were carried out to test whether the amount of cholesterol absorbed could be increased by raising the quantity of fat in the diet.

DIET AND ANIMALS

The diets fed to the animals were made up of the same basic constituents as described previously [Cook, 1936, Table I]. The fat (arachis oil) was given in percentages of the basic diet as 15 % or "normal synthetic", 20 and 30 %. The fat displaced some of the carbohydrate in the higher concentrations. These percentages are reckoned on the basic constituents. Actually the 30 % fat diet for example contained 30 g. of fat in 110 g. of food, the extra 10 g. being made up of the vitamin supplements. Alcohol-extracted caseinogen was used. The cholesterol (B.D.H.), 2 g. per 110 g. of food, was mixed with the oil and heated on a water-bath for some minutes and then mixed while still hot with the other dietary constituents. There was too much cholesterol for complete solution in the oil at room temperatures but the sterol is intimately mixed with the oil by the above procedure. The calorie value of the diets per 100 g. is for the 15 % control diet 434 Cal., 20 % 457 Cal. and 30 % 502 Cal. For the diets plus cholesterol 15 % 426 Cal., 20 % 448 Cal. and 30 % 494 Cal. No calorific value is given to the cholesterol.

The animals used were of the piebald strain and were litter-mates about 4 weeks old taken immediately after weaning. A male and a female were used in each set of experiments. The average weights were males 56 (± 6) g. and females 50 (± 5) g. The animals were placed in Hopkins metabolism cages, the sexes being segregated. The faeces were collected free from urine. The food was weighed out daily and wetted in the dish. After the first few days there was little spilling of food and any spilt was returned to the dish. The supply of water was unlimited. Table I gives the food intakes of the animals expressed in calories and the weight increases over successive 3-weekly periods during their growth. The first period is immediately after weaning, i.e. about 4 weeks after birth.

It will be seen that during the first period there is a significantly decreased intake on the cholesterol-containing diet and this is reflected in the smaller increase in body weight as compared with the control animals. This effect is most marked with the males. Later periods show that the food intakes and weight increases are approximately the same in the various sets. There is some evidence that this is due to adaptation to the diets. The similar calorie consumptions on the varying fat diets are to be noticed as is also the fact that the animals appear to grow best on the high fat diet. These points will be returned to later.

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Table I. *Food intake in calories per day and increase in weight for period stated of control (a) and cholesterol-fed (b) rats.*

Period of days	15% fat				20% fat				30% fat			
	Food intake		Increase in wt. g.		Food intake		Increase in wt. g.		Food intake		Increase in wt. g.	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0-20 <i>a</i>	46	42	71	51	46	43	72	53	50	44	86	44
<i>b</i>	35	36	38	54	38	38	56	55	41	38	56	37
20-41 <i>a</i>	66	43	59	31	58	48	48	34	67	55	59	45
<i>b</i>	49	48	62	27	52	46	54	26	60	45	51	30
41-62 <i>a</i>	56	43	27	21	59	45	43	24	56	43	28	8
<i>b</i>	58	46	36	13	54	46	27	10	54	36	30	16
62-83 <i>a</i>	58	48	7	6	67	52	12	7	56	45	7	10
<i>b</i>	56	43	11	2	55	47	14	9	62	41	14	10
Increase in wt. over total period	<i>a</i>		164	109			175	118			180	107
	<i>b</i>		147	96			151	100			161	93

EXCRETION OF CHOLESTEROL

The faeces were collected at 2-day intervals and air-dried until the requisite amount had been gathered. They were then dried at 100° to constant weight, powdered and an aliquot amount extracted in a Soxhlet apparatus with light petroleum, B.P. 50-60°, for not less than 40 hours. The total lipoid or "fat" was then weighed and the unsaponifiable matter (U.M.) determined. The saponification was done with 10% alcoholic KOH for 3-4 hours; four extractions with petroleum were normally made of the saponified material, enough alcohol to make a concentration of about 25% being added to break the emulsion. At first some difficulty with emulsification was encountered in washing the petroleum extracts with water but by considerably increasing the volume of petroleum and adding alcohol this was easily overcome. The method of estimating cholesterol (as U.M.) has been tested by the recovery of known amounts of cholesterol similar in quantity to that found in the faeces. Series A was set up as shown in Table II.

Table II. *Cholesterol recovered*

	Amount added	3 extractions	%	4 extractions	%	5 extractions	%
	g.	g.		g.		g.	
Series A, cholesterol alone	2.808	—	—	2.763	98.4	—	—
	1.249	—	—	1.245	100	—	—
	2.334	—	—	2.320	99.8	—	—
				Mean	99.4	—	—
Series B, cholesterol	1.618	1.432	88.4	1.543	95.1	1.597	98.7
	1.848	1.687	91.3	1.791	96.7	1.831	99.4
plus 2 g. arachis oil	2.671	2.422	90.4	2.545	95.3	2.604	97.5
	2.881	—	—	2.784	96.5	—	—
				Mean	95.9 (±0.82)	—	—

These were saponified for 3 hours with 50 ml. of 10% alcoholic KOH and then washed into the funnel with about 100 ml. water and extracted 4 times with petroleum. The combined extracts were washed twice with water. In series B arachis oil was added and 75 ml. of KOH were used for saponification. A point of some practical importance is to be observed in the extractions. The volume of material to be extracted is about 200 ml., to this for the first extraction are added

100 ml. of petroleum. This is well shaken and allowed to stand. With series B and in the case of the faecal lipoids separation into 3 layers normally occurs. The middle layer is dark coloured. For the second extraction this middle layer is removed with the aqueous layer and the whole re-extracted. In the second extraction using 50 ml. of petroleum this phenomenon does not occur and the aqueous layer and the petroleum show a clear-cut separation.

As far as possible all the estimations were done in duplicate. The results are shown in Table III. A criticism of the method is that the U.M. may not be regarded as being entirely cholesterol but its crystalline form and M.P. (145°) showed that it was mainly unchanged cholesterol. From Table II it will be seen

Table III. *Details of the analyses of the lipoid and unsaponifiable matter (U.M.) in the faeces of the rats fed on varying fat concentrations. Series A are the controls and Series B the cholesterol-fed animals, 2 animals in each series*

Diet	No.	Period of days	Dry wt. faeces g.	Aliquot extracted g.	Lipoid U.M.		Totals in faeces over period†		Lipoid as % of faeces	U.M. as % of lipoid
					g.	g.	Lipoid g.	U.M. g.		
A 15% fat	1	4-15*	8.5	8.5	1.30	0.72	1.30	0.72	15.3	55.4
	2	15-57	39.8	10	0.74	0.51	2.94	2.03	7.4	69
	3	57-78	22.4	10	1.26	0.34	2.82	0.76	12.6	27
B plus 2% cholesterol	1	4-15*	9.75	9.75	3.41	2.13	3.41	2.13	34.9	62.4
	2	15-57	54.2	10	3.34	—	20.30	12.70	37.4	62.5
				10	4.02	2.49				
				20	7.56	4.55				
	3	57-78	28.2	10	3.50	2.45	8.95	6.66	31.8	74.3
				10	2.85	2.28				
A 20% fat	1	1-15	10.3	10.3	1.31	0.57	1.31	0.57	12.7	43.5
	2	15-57	40.9	10	1.04	0.58	4.25	2.37	10.4	55.7
	3	57-78	22.5	10	1.43	0.51	3.22	1.14	14.3	35.4
B plus 2% cholesterol	1	1-15	16.25	16.25	5.75	3.60	5.75	3.60	35.3	62.6
	2	15-57	57.3	10	3.75	—	22.10	13.55	38.6	61.2
				10	4.06	2.49				
				10	3.78	2.24				
	3	57-78	31.4	10	3.48	2.20	10.10	6.35	32.1	62.9
				10	2.99	1.85				
A 30% fat	1	1-15	12.4	10	1.81	0.41	2.24	0.51	18.0	22.8
	2	15-57	44.1	10	1.15	0.54	6.94	2.86	15.8	41.3
				10	1.78	0.76				
	3	57-78	24.2	10	1.12	0.41	2.67	0.99	11.0	37.0
				10	1.09	0.41				
B plus 2% cholesterol	1	1-15	16.8	10	3.78	—	6.71	3.61	40.0	53.7
	2	15-57	60.0	6.7	2.93	1.44				
				10	3.64	—	21.75	12.46	36.2	57.3
				10	3.69	2.25				
	3	57-78	31.2	10	7.20	3.98	10.40	6.13	33.3	59.0
				10	3.46	1.95				
				10	3.22	1.98				

* The first 4 days' collections of these series are not included.

† With reference to the duplicate determinations of lipoid and U.M. in the faeces of the control animals on the 15 and 20% fat diets, second and third periods, the materials left over after the initial estimations had been made were pooled and estimations carried out by Mr G. N. Jenkins. The results obtained were; 84.2 g. of faeces gave 9.81 g. lipoid and 4.27 g. U.M. This corresponds to estimated values of 9.43 and 4.08 g. respectively which would be expected by comparison with the writer's figures.

that there is a 5 % loss in recovery with 4 extractions. In the results no correction is made for this. It is obvious that the errors in an experiment of this nature must be relatively high and in a negative direction.

Table IV shows the cholesterol excretion.

Table IV. *The excretion of cholesterol in the faeces as measured by the amount of U.M. excreted. The figures in the third column have been obtained by subtracting the U.M. in series A (see Table III), i.e. the controls from the appropriate set in series B*

Diet	Period	Excess U.M. excreted in faeces of cholesterol- fed animals g.	Food g.	Dietary cholesterol g.	% cholesterol- fed excreted in faeces	Cholesterol absorbed per rat per day g.
15 % fat	1	1.41	214	3.82	36.9	0.11
	2	10.67	986	17.60	60.4	0.08
	3	5.90	491	8.77	67.3	0.07
	Total	17.98	—	30.19	59.6	—
20 % fat	1	3.03	277	4.95	61.2	0.07
	2	11.18	923	16.50	67.9	0.06
	3	5.21	466	8.32	62.5	0.07
	Total	19.42	—	29.77	65.1	—
30 % fat	1	3.10	262	4.67	66.4	0.06
	2	9.60	841	15.00	63.9	0.06
	3	5.14	410	7.32	70.2	0.05
	Total	17.84	—	26.99	66	—

A possible criticism of these results is that the deduction of the amount of U.M. in the faeces of the control animals from the U.M. of the cholesterol-fed animals may not be justified. It will be seen that the weight of the faeces in the case of the latter animals is much greater than that of the controls and that this difference appears to be due to the cholesterol excreted. It has been assumed, however, that this difference does really represent the true cholesterol excretion and it will be seen later that on making a cholesterol balance sheet this assumption is apparently justified. Part of the U.M. of the controls is probably derived from the U.M. of the arachis oil fed. The content of U.M. in the oil has been estimated by Mr G. N. Jenkins to be 0.16 %. If this was excreted unchanged the quantities present for example during the second period on the 15, 20 and 30 % fat diets would be 0.22, 0.21 and 0.20 g. respectively. It will be seen, however, from Table III that the quantity of U.M. excreted is much greater than this. A complete study of the faecal lipoids is in progress. In the final column of Table IV is given the absorption of cholesterol per rat per day. It will be seen that this is highest on the 15 % fat diet and particularly high for the first period. No allowance is made for absorption followed by excretion. For the final period of feeding the absorption per kg. rat per day is 0.3–0.4 g. cholesterol.

THE UNSAPONIFIABLE MATTER OF THE ANIMALS

In an attempt to find the fate of the cholesterol the animals used in the experiments were analysed. They were not fed for the final 24 hours, being left with the remains of their food. The animals were bled under anaesthesia, the blood being analysed with the carcasses. The methods used for the analysis of lipid are the same as described previously with the exception that the saponified

material was first extracted at least 4 times for the U.M. It was then acidified and the fatty acids were determined. The results are shown in Table V. The typical "fatty" liver is to be observed in the cholesterol-fed animals. It will be seen that there are some indications that the high fat diet increases the fat in the liver but how far this is significant is not known.

A balance sheet of the cholesterol may now be constructed. This is shown in Table VI.

Table V. *The analyses of the animals for "fat" or total lipid and unsaponifiable matter (U.M.) at the conclusion of the experiment*

Diet	Sex	Wt. of animal	Liver						Carcasses (pooled samples)						Gut (pooled samples)		
			Wt.	as % of body wt.	Pooled samples				Wt.	"Fat" g. (a)	U.M. g. (b)	a as % of body wt.	b as % of body wt.	Wt. g.	"Fat" g.	U.M. g.	
					"Fat" g.	U.M. g.	% "Fat"	% U.M.									
15% control	♂	215	7.3	3.4	—	0.02	3.1	—	320	48.3	0.36	15.1	0.11	20.5	2.17	0.07	
	♀	144	6.1	4.2													
Plus cholesterol	♂	188	9.4	5.0	2.49	0.72	14.4	4.15	282	57.0	0.54	20.2	0.19	16.2	2.17	0.10	
	♀	137	7.9	5.8													
20% control	♂	216	6.7	3.1	—	0.02	3.9	—	344	61.5	0.76	17.9	0.22	24.6	2.90	0.09	
	♀	171	6.8	4.0													
Plus cholesterol	♂	196	10.0	5.1	2.75	0.63	14.2	3.27	294	49.0	0.69	16.7	0.23	21.5	2.05	0.23	
	♀	147	9.4	6.4													
30% control	♂	238	8.5	3.6	—	0.02	2.7	—	352	72.2	0.29	20.5	0.08	17.7	2.52	0.08	
	♀	153	6.7	4.3													
Plus cholesterol	♂	208	10.3	4.9	3.80	0.89	18.35	4.28	307	67	0.39	21.8	0.13	18.5	2.64	0.22	
	♀	148	10.4	7.0													

Table VI. *Cholesterol balance*

Cholesterol in	Fat in diet		
	15% g.	20% g.	30% g.
Faeces	17.98	19.42	17.84
Livers	0.70	0.61	0.87
Carcasses	0.18	0.0	0.10
Gut	0.04	0.14	0.14
Total	18.90	20.17	18.95
Fed	30.19	29.77	26.99
% accounted for	62.5	67.7	70.1

The values for cholesterol in Table V have been obtained by subtracting from the cholesterol-fed animals the amount of unsaponifiable matter present in the control animals.

DISCUSSION

Perhaps the most striking result is the relatively large amount of cholesterol which the rat is capable of absorbing. The dynamics of cholesterol absorption have never been exhaustively studied. Dorée & Gardner [1908] studied the absorption of cholesterol in the rabbit and cat but did not make a balance sheet of their experiments. Page & Menschick [1932] have studied the cholesterol absorption in the rabbit but as their experiments are on a herbivore which does not normally eat a diet containing much fat their figures can hardly be compared with the results obtained for the rat. That cholesterol is absorbed only in the presence of fat seems to have been tacitly assumed; the only experimental observation that the writer can find is that of Thannhauser [1923] whose published experiments on this point do not seem to be very extensive. The deleterious

effect on the growth of the animals appears to be most marked on the 15 % fat diet. It may be observed that this series in this communication did not show the lowering of body fat seen in the previous paper [Cook, 1936]. Further experiments are in progress and indicate that this effect varies to some extent with the sex of the animals, the males being apparently more susceptible to the action of cholesterol. With the higher fat concentrations, whilst the growth rate is less than that of the controls the deleterious effect is less marked.

The analyses of the animals show, in agreement with other workers, that cholesterol is largely concentrated in the liver. There appears to be a considerable metabolism of cholesterol by this organ.

It is not intended to deal with the fate of the cholesterol in the present communication. It may be mentioned, however, that the discrepancy can be accounted for in part, at least, by an increase in the acid fraction of the faecal lipoids.

SUMMARY

1. Growing rats were fed on diets containing 15, 20 and 30 % fat with and without 2 % cholesterol. The food intakes, growth of the animals and excretion of cholesterol in the faeces over various periods of the growth curve have been measured.

2. Cholesterol has a deleterious effect on the growth rate, which is most marked with the 15 % fat diet. This effect is probably due partly to a lessened food intake during the first 3 weeks; thereafter the rats appear to adapt themselves to the diet.

3. The absorption is not increased by raising the fat concentration in the diet although this effect is offset to some extent by the fact that the animals maintain the same calorie consumption, i.e. they eat less of the high-fat diet. But as the cholesterol is present in greater concentration than would allow for complete absorption this does not affect the conclusions. The quantity absorbed is greatest for the first 3-weekly period on the 15 % fat diet but eventually falls to the same value as for the other diets, viz. 0.3–0.4 g. per kg. rat per day.

4. The animals were analysed; the cholesterol appears to be concentrated in the livers. The "fatty" liver appears to be slightly more marked on the high-fat diet.

5. On constructing a balance sheet approximately 30 % of the cholesterol fed remains unaccounted for by the unsaponifiable fractions found in the faeces and in the animals' bodies. This discrepancy is greatest on the 15 % fat diet.

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