the iodine number from 126 to 101. The larvae are no doubt consuming a higher proportion of saturated fat than is indicated by analysis of the medium alone.

SUMMARY

1. A study of the changes in the moisture and lipid content of the housefly, *Musca vicina*, during growth and metamorphosis is recorded.

2. The apparent drop in water content at the time of pupation is due to the formation of the puparium with its low moisture content rather than an actual loss in water.

3. The lipid content of *M. vicina* rises in 7 days from 2·5 μg. in the egg to 1225 μg. in the late third-stage larva. Within 24 hr. of the onset of pupation, the fat content drops to 880 μg. The 48 hr. old mature pupa contains 714 μg. fat of which, 2 days later, 294 μg. can be recovered from the emergent housefly and 22 μg. from the puparium. It appears that 400 μg. of lipid is used in the first part of the pupation period and about 400 μg. in the latter part.

4. No appreciable change in the saponification number is observed during larval growth and pupation. The fat of the emergent housefly, however, appears to contain a significantly higher proportion of shorter-chain fatty acids.

5. The amount of sterol and unsaponifiable lipid remains relatively constant throughout growth and metamorphosis.

6. During larval growth, the fatty acids of the wheat-bran medium are laid down in their unsaturated state. With the onset of pupation they are saturated or broken down into shorter-chain fatty acids.

7. 78 % of the rearing media lipid is consumed by the larvae during growth.

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Changes in Chemical Composition During the Development of 'Cholesterol Fatty Livers'

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Many investigations have been made of the effects of feeding cholesterol to rats. Even a brief review of the work from the laboratories of Schoenheimer, Okey, Channon, Sperry, Cook, and others, would be too voluminous for presentation here. In spite of the many publications in this field, relatively few data are available showing the rate at which the different lipid components accumulate in the liver when different amounts of cholesterol are fed.

The data to be presented show that in female rats consuming a cholesterol-rich, hypolipotropic diet, the percentages of total lipids and of cholesteryl esters in the liver reach more or less limiting values within about 3 weeks and then remain relatively constant (throughout the period of observation, 7 weeks). The livers continue to increase abnormally in size at a constant rate and in this experiment were double the normal weight at the termination of the study.

The impression obtained from the usual method of expressing the results, as percentage of fresh liver weight, is that the excessive deposition of lipids has

come to a stop after about 3 weeks and that a condition of equilibrium has been reached. The present results indicate, however, that actually both glycerides and cholesteryl esters increase in absolute amount throughout the experimental period. The concomitant increase in liver weight occurs at such a rate as to produce the impression of a dynamic equilibrium after the rats have been consuming the rations for about 3 weeks. The material accumulating during these first 3 weeks consisted mainly of glycerides. As time went on, the proportion of glycerides in the material being deposited decreased and the percentage of water increased. Deposition of cholesteryl esters was fairly rapid during the first 3 weeks in both males and females. Males continued to accumulate esters at a considerable rate but in females the rate of deposition appeared to decline. There are not sufficient data, however, to establish this as a real sex difference.

EXPERIMENTAL

The care of the animals, preparation of the diets and analytical procedures have been described by Ridout, Lucas, Patterson & Best (1952). The basal diet, which was essentially free from choline, had the following percentage composition: casein (fat-free, vitamin-free) 8, gelatin 6, soya protein ('Alpha protein', Soya Products Division, Glidden Co., Chicago, Ill.) 6, salt mixture 3, celluflour 2, 'sucrose–vitamin mixture' 1, sucrose 62·0, beef fat 10, corn oil 2, cod liver oil concentrate (Ayerst, McKenna and Harrison, Ltd., Montreal) 0·015 and α-tocopheryl acetate 0·010. The following salt mixture (devised by Lucas & Patterson) is one used in this laboratory, made from salts commercially available in finely powdered form and supplying amounts of mineral believed optimum for growth of rats: 1 kg. contains $CaCO_3$, 110 g.; $CaHPO_4$, 325 g.; K_2HPO_4, 275 g.; $MgSO_4,3{\cdot}5H_2O$, 100 g.; NaCl, 150 g.; ferric citrate, 30 g.; 'trace-element mixture', 10 g. The latter contains (g./100 g.): $CaCO_3$, 70; $MnSO_4,4H_2O$, 19·9; $ZnSO_4,7H_2O$, 3·5; $CuSO_4,5H_2O$, 4·0; KI, 0·05; NaF, 0·05; $Al_2(SO_4)_3,K_2SO_4,24H_2O$, 0·4; $CoCl_2,6H_2O$, 0·05; $Na_2SiO_3,9H_2O$, 2·0 and $NaAsO_2$, 0·1. Celluflour is a non-nutritive material to supply bulk, obtainable from Chicago Dietetic Supply House, Chicago, Ill. The 'sucrose–vitamin mixture' consisted of thiamine hydrochloride, 500 mg.; riboflavin, 250 mg.; pyridoxine hydrochloride, 200 mg.; calcium pantothenate, 1·00 g.; nicotinic acid, 1·00 g.; folic acid, 50 mg.; biotin, 30 mg.; 2-methyl-1:4-naphthoquinone, 100 mg.; *p*-aminobenzoic acid, 10 g.; inositol, 50 g. and finely powdered sucrose (100-mesh) to 1000 g.

Female white rats of the Wistar strain (120–150 g. in weight) were kept in individual cages and fed the cholesterol-containing diet *ad lib.* for varying periods of time. In the first experiment a supplement of 0·5% crystalline cholesterol (British Drug Houses Ltd., London) was added to the basal diet at the expense of sucrose. Groups of ten rats chosen at random were killed after 3, 7, 14, 21, 35 and 49 days, respectively; liver lipids were extracted with hot ethanol and analysed as described previously (Best, Lucas, Patterson & Ridout, 1946).

The effect on liver lipids of feeding diets containing different amounts of cholesterol was investigated in four groups of male rats (100–120 g.). Supplements of crystalline cholesterol (0, 0·2, 0·8, and 1·6%, respectively) were added to the basal diet. The animals were fed *ad lib.* for 3 weeks. Ten rats from each group were killed and ten rats each were continued (for a total of 90 days) on the diets containing 0·2 and 0·8% cholesterol, respectively.

RESULTS

The development of the cholesterol fatty liver in female rats is shown graphically in Fig. 1. Deposition of lipids appears to reach an equilibrium after about 3 weeks when the results are expressed as percentage of fresh liver (Fig. 1*a*). Actually, there is a continued deposition of glycerides and cholesteryl esters as shown in Fig. 1*b*. The average

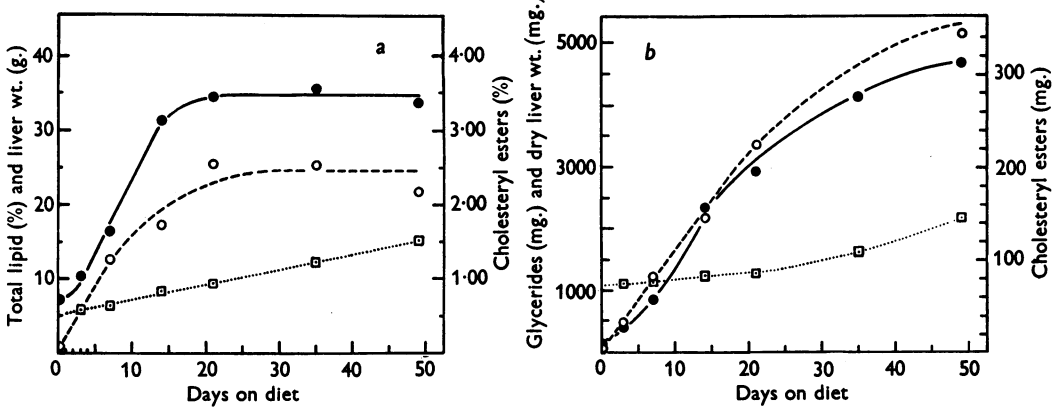

Fig. 1. Rate of accumulation of lipids in livers of rats. Groups of ten rats fed an hypolipotropic diet containing 0·5% cholesterol for periods shown. Data in Fig. 1*a* as % of fresh liver, in Fig. 1*b* as mg. per liver. In Fig. 1*a* left-hand scale gives values for % total lipids (●) and fresh weight of liver (⊡) in g.; right-hand scale % cholesteryl esters (○). In Fig. 1*b* left-hand scale gives values for mg. glycerides (●) and mg. dry, fat-free liver (⊡); right-hand scale gives mg. cholesteryl esters (○).

Table 1. *Changes in composition during the development of 'cholesterol fatty livers'*

Groups of ten female rats (120–140 g.); average food consumption 9–10 g./day; dietary cholesterol 0·5%.

	Days on ration				Material deposited in periods shown					
	0	21	35	49	0–21		21–35		35–49	
	Mean weight in g. ±S.E.				(g.)	(%)	(g.)	(%)	(g.)	(%)
Component										
Fresh liver	5·57±0·17	9·27±0·91	12·27±1·61	15·93±0·77	3·70	—	3·00	—	3·66	—
Dry, fat-free tissue	1·12±0·02	1·31±0·05	1·47±0·10	2·20±0·12	0·19	5·1	0·16	5·3	0·73	20·0
Total lipids	0·38±0·008	3·40±0·59	4·74±0·87	5·33±0·37	3·02	—	1·34	—	0·59	—
Water (by difference)	4·07±0·14	4·56±0·30	6·06±0·76	8·40±0·61	0·49	13·2	1·50	50·0	2·34	64·0
Glycerides (by difference)	0·17±0·007	2·94±0·58	4·15±0·81	4·68±0·34	2·77	74·9	1·21	40·3	0·53	14·5
Cholesterol plus ester*	0·018±0·0004	0·244±0·028	0·345±0·054	0·376±0·030	0·226	6·1	0·101	3·4	0·031	0·8
Phospholipids	0·191±0·003	0·213±0·010	0·249±0·018	0·275±0·017	0·022	0·6	0·036	1·2	0·026	0·7
						99·9		100·2		100·0

* Cholesteryl esters were calculated as oleate by multiplying bound cholesterol by 1·68.

Table 2. *Composition of material found in livers after feeding cholesterol-containing diets for the periods shown*

Groups of ten male rats (100–120 g.); average food consumption 9–10 g./day.

	Cholesterol in diet (%)						
	0	0·2		0·8		1·6	
	At start	21 days	21 days	90 days	21 days	90 days	21 days
	Mean weights in g. ±S.E.						
Component							
Fresh liver	5·15±0·27	9·19±0·57	9·91±0·59	16·28±1·25	10·68±0·92	21·69±0·82	11·11±0·79
Dry, fat-free tissue	1·08±0·05	1·58±0·10	1·47±0·17	2·17±0·13	1·70±0·10	2·60±0·07	1·80±0·08
Total lipids	0·30±0·014	2·20±0·26	3·01±0·21	5·87±0·54	3·32±0·57	7·94±0·47	3·54±0·43
Water (by difference)	3·77±0·21	5·41±0·29	5·43±0·42	8·24±0·68	5·66±0·30	11·15±0·45	5·77±0·30
Glycerides (by difference)	0·12±0·009	1·98±0·25	2·71±0·20	5·23±0·49	2·91±0·53	6·23±0·42	3·01±0·39
Cholesterol plus ester	0·013±0·0007	0·047±0·006	0·104±0·010	0·359±0·060	0·229±0·027	1·344±0·093	0·338±0·037
Phospholipids	0·169±0·006	0·176±0·006	0·197±0·008	0·281±0·018	0·180±0·014	0·369±0·011	0·188±0·012

weight of the fatty livers at the end of the experiment was 15·9 g. which is just about double that from a normal rat of the same weight. The liver weight of a normal rat on a stock diet may be calculated with reasonable accuracy, being about 4–5 % of body weight. After 49 days on the basal diet the average weight of the rats was 185 g., thus the expected weight of the liver would be about 8 g.

It may readily be shown that the abnormal increase in size of the liver was due mainly to deposition of glycerides and water. At the beginning, the average weight of the rats was 130 g. with liver weight of 5·57 g. Normal female rats of our colony have average total liver lipids of about 6–7 % and an average dry, fat-free liver residue of 20–22 %. The absolute weights of these fractions at the beginning of the experiment were 0·38 and 1·12 g., respectively. By difference, the amount of water in these livers would be 4·07 g. and by calculation the glyceride portion of the lipid was 0·17 g. Values found by actual determination on the livers of rats fed the diet containing 0·5 % cholesterol for 21, 35 and 49 days are included with these data in Table 1. The absolute weights of free cholesterol in the livers did increase slightly but these increments are so small in comparison with the much larger changes in cholesteryl esters that the two have been combined in the table.

During the first 3 weeks most of the increase in weight of the liver was due to deposition of glycerides (74·9 %); the next largest increase was in water (13·2 %). These two components accounted for about 90 % of the materials deposited during the first 21 days, which is the period adopted in most laboratories for studying fatty livers. In the period following the establishment of the apparent equilibrium (Fig. 1*a*) the material being deposited is of a rather different composition although glycerides and water still constitute 80 to 90 % of the increment in weight. The ratio in which they are laid down, however, is greatly altered; about twice as much water as fat is deposited during the interval 21 to 49 days. The ratio in which glycerides, water and other components accompany the deposition of cholesteryl esters after 3 weeks are such as to leave the percentage composition of the liver relatively constant. The material deposited during the period 21–49 days contained about 30 % of total lipids which is essentially the percentage of total lipids in the liver at 21 days. Thus the constancy of the total lipids as percentage of wet weight is explained.

The amount of dry, fat-free tissue increased progressively with the growth of the rat (Table 1). Several interesting trends may be seen in these data. The rate at which total lipids increased fell off and the percentage of water in the material deposited increased. The sum of water plus glycerides remained relatively constant (90 and 79 %) during the periods 21–35 and 35–49 days. The percentage of cholesterol plus cholesteryl esters in the material deposited diminished, as did the absolute amount.

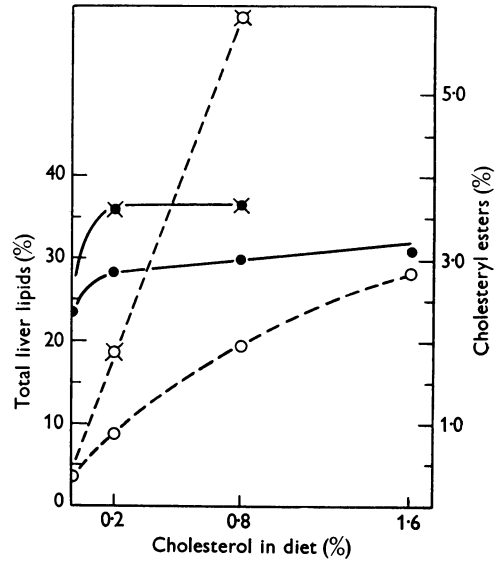

Fig. 2. Influence of dietary cholesterol on liver lipids. Left-hand scale shows total lipids as % fresh liver after rats had consumed rations for 3 weeks (●) and 3 months (✖); right-hand scale gives cholesteryl esters as % fresh liver after 3 weeks (○) and 3 months (□).

Table 3. *Percentage composition of material deposited in livers of male rats under dietary conditions shown*

For experimental details see Table 2 and text.

	Cholesterol content of diet (%)					
	0	0·2		0·8		1·6
Component	0–21 days	0–21 days	21–90 days	0–21 days	21–90 days	0–21 days
Dry, fat-free tissue	12·4	8·2	11·0	11·2	8·2	12·1
Water	40·6	34·9	44·1	34·2	49·9	33·6
Glycerides	46·0	54·4	39·6	50·5	30·2	48·5
Cholesterol plus ester	0·8	1·9	4·0	3·9	10·1	5·5
Phospholipids	0·2	0·6	1·3	0·2	1·7	0·3
Total	100·0	100·0	100·0	100·0	100·1	100·0

Phospholipid was the only fraction which appeared to be relatively constant in its rate of deposition.

The effect of increasing the concentration of cholesterol in the diet of male rats may be seen in Fig. 2, where data for total liver lipids and for cholesteryl esters are presented as percentage of fresh liver weight. More complete data about the composition of these livers are available in Table 2. The increases which occurred in each component were calculated (as in Table 1) and the percentage composition of the material deposited in the livers under each of the dietary regimens was calculated. These data are shown in Table 3. Again it may be seen that regardless of the amount of cholesterol in the diet, the material deposited in the liver consists principally of glycerides and of water. As might be anticipated, the deposition of cholesteryl esters increases with increasing amounts of dietary cholesterol. In these male animals the cholesteryl esters continued to rise throughout the period of observation. At the start of the experiment, the absolute amount of free cholesterol was about 10 mg. and that of cholesteryl esters about 3 mg., making a total of 13 mg. (cf. Table 2). In the case of rats consuming the diet containing 0·8 % cholesterol, the absolute amounts of free cholesterol at 21 and 90 days were 20 and 66 mg., respectively, and the corresponding values for cholesteryl esters were 209 and 1278 mg. Since the liver gained 5·53 g. in weight in the first 21 days and cholesterol plus esters account for 216 mg. of this, they represent 3·9 % of the material deposited in this interval (cf. Table 3). When rats were maintained on the same diet for a total of 90 days the cholesterol plus ester portion increased to 10·1 % of the material deposited during the period of 21–90 days. The female rats used in the first experiment did not exhibit the same increase. Actually, in female rats, the rate at which cholesteryl esters were accumulating decreased with time (after 21 days).

SUMMARY

1. Analyses have been made of the lipids accumulating in the livers of rats fed a hypolipotropic diet containing different amounts of crystalline cholesterol (0, 0·2, 0·5, 0·8 and 1·6 %, respectively) and the rates at which the different components accumulate in the liver have been determined.

2. The material deposited during the first 3 weeks consisted mainly of glycerides and of water, these two accounting for between 80 and 90 % of the increase in weight of the liver. When the cholesterol-containing diet was fed for longer periods, the nature of the material being deposited changed: the amount of glyceride decreased and that of water increased, but the sum of these two continued to account for about 80 % of the gain in liver weight.

3. Although the percentage of total lipids in the livers reached a more or less limiting value after feeding the diets for about 3 weeks, the absolute amount of glycerides and of cholesteryl esters continued to increase throughout the period of observation.

4. In the livers of rats consuming diets containing cholesterol, cholesteryl esters increased about 200-fold in absolute amount, but since the normal concentration is low, the cholesteryl esters never accounted for more than a small percentage of the total lipid material deposited.

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Preventive and Curative Studies on the 'Cholesterol Fatty Liver' of Rats

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Some preventive and curative studies of the so-called 'cholesterol fatty liver' were made almost twenty years ago. This early work has been reviewed by Best & Ridout (1939) and by McHenry & Patterson (1944). Increased knowledge of dietary requirements indicated the desirability of re-investigating this type of fatty liver in rats fed more adequate diets. The preventive effect of choline chloride and of inositol in improved rations containing graded doses of cholesterol was reported recently from this laboratory (Ridout, Lucas, Patterson & Best, 1952). These experiments revealed two points upon which further data seemed desirable: (1) the effect of a change in the nature of the dietary protein, and (2) the effect of somewhat larger doses of choline chloride. Preventive and