The Liver-Lipid Constituents of Male and Female Rats

2. EFFECTS OF THE FAT-DEFICIENCY SYNDROME, AGGRAVATED BY DIETARY CHOLESTEROL

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Feeding cholesterol to rats depresses liver vitamin A concentrations in male rats but not in females (Green, Horner, Lowe & Morton, 1957). The difference between the sexes in this effect of cholesterol suggested that there might be a metabolic link with the essential fatty acids. It has been shown, however, that deficiency of these essential fatty acids did not itself affect liver vitamin A levels in either sex (Morton & Horner, 1961). The experiments reported below were begun at the same time as those just referred to and differed only in that all the animals had 1% of cholesterol added to their basal fat-free diets. Previous work (Peifer & Holman, 1955; Holman & Peifer, 1960) had shown that dietary cholesterol accelerated the onset of fat deficiency.

Our first aim was to find how the cholesterol-supplemented diets influenced liver vitamin A levels. During this investigation interrelationships between essential fatty acids and either endogenous cholesterol (Morton & Horner, 1961) or exogenous cholesterol were examined.

EXPERIMENTAL

Animals and diets. Hooded rats were fed on a basal fat-free diet from weaning. This differed from the diet described by Morton & Horner (1961) in that 1% of crystalline cholesterol (Croda Ltd.) was included at the expense of an equal weight of sucrose.

Procedure. The experimental plan resembled that described for rats that did not receive cholesterol (Morton & Horner, 1961). Ten males and twelve females from six litters delivered within a period of six days were given the basal diet at weaning. The experimental groups were formed when weaning was completed. In group 2 five males received the basal diet. In group 1 five males received the basal diet plus 100 mg. of linoleic acid per day. In group 4 six females received the basal diet. In group 3 six females received the basal diet plus 25 mg. of linoleic acid per day. The diets were fed *ad lib.* and water was freely provided. Linoleic acid was given as cottonseed oil.

The males were killed after 78–81 days. The females were killed between the 138th and 146th days.

Vitamin A dosage. Each rat received eight equal doses of 800 i.u. of vitamin A given by mouth at weekly intervals from the start of the experiment.

Analysis of livers. The methods described by Morton & Horner (1961) were used. The cholesteryl esters from group 4 were accidentally lost during saponification. The value given in Table 1 for the total fatty acids from the fraction was calculated by assuming that the yield of fatty acids from the esters was 36.7% by weight. This was the mean recovery of fatty acids from twenty other samples of cholesteryl esters.

RESULTS

Body weights and liver weights with the liver-lipid analyses are given in Table 1.

Growth and deficiency symptoms. The deficient males had all ceased to grow (i.e. became stationary in weight) by the 72nd day at an average weight of 107 g. Their controls were still growing and then averaged 175 g. Deficient females ceased to grow by the 110th day, averaging 124 g. in weight. Their controls then weighed 173 g. on average.

All the deficient animals had scaly tails but other lesions were relatively mild. In particular, only the hind paws were scaly. All symptoms were less marked in the females and control animals of both sexes were perfectly healthy in appearance.

Liver vitamin A. The mean liver vitamin A of the deficient males was 15% lower than the value for the controls. This difference was not statistically significant at an acceptable level of probability ($t = 1.483$, $P < 0.2 > 0.1$). On the other hand, the average liver vitamin A content of deficient females was 48% lower than the control value and the difference was significant ($t = 3.340$, $P < 0.01$).

Liver lipids. The results can be compared with those of earlier experiments (Morton & Horner, 1961) in which rats received no cholesterol. In doing so the terms 'normal' males and 'normal' females refer to control groups A and C respectively in the preceding paper.

Concentrations of lipid in the livers of groups 1 and 2 exceeded the 'normal' value by factors of 2.96 and 2.77 respectively. In females also the 'normal' value was exceeded, by factors of 1.11 in group 3 and 1.24 in group 4. These increases over

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Table 1. *Body weights and liver weights, liver vitamin A and the weights of the various lipid fractions and their constituent fatty acids in the livers of fat-deficient male rats (group 2) and their controls (group 1) and in fat-deficient female rats (group 4) and their controls (group 3)*

All animals received a dietary supplement of cholesterol (1% by weight). Control males were given 100 mg., and control females 25 mg. of linoleate per day. N.D. indicates not determined.

Group ...	1	2	3	4
Mean final body wt. (g.)	184	107	198	124
Mean liver wt. (g.)	6.75	5.33	7.78	6.37
Mean total liver vitamin A (i.u.)	1781	1509	975	507
	(± 113)*	(± 145)	(± 134)	(± 43)
Mean total liver lipid (mg.)	495	364	254	233
Mean wt. (mg.)/liver of:				
Phospholipids	64.1	63.4	106.2	63.2
Triglycerides	96.0	78.1	88.0	69.5
Cholesteryl esters	253.7	196.0	23.8	70.1
Free cholesterol	23.3	20.8	21.4	18.2
Hydrocarbons	5.8	5.9	4.4	3.4
Mean wt. (mg.)/liver of:	Phospholipid fatty acids			
Dienoic	2.30	0.95	2.17	1.19
Trienoic	0.86	3.99	2.53	5.30
Tetraenoic	1.97	1.00	2.99	1.28
Pentaenoic	0.46	0.21	1.30	0.25
Hexaenoic	0.49	0.48	0.47	0.13
Total polyunsaturated acids	6.08	6.63	9.46	8.15
Monoenoic and saturated	19.61	28.74	33.45	29.61
Mean wt. (mg.)/liver of:	Triglyceride fatty acids			
Dienoic	4.35	0.75	1.31	0.64
Trienoic	0.75	0.79	0.57	0.61
Tetraenoic	0.72	0.13	0.38	0.10
Pentaenoic	0.18	0.05	0.12	0.04
Hexaenoic	0.26	0.21	0.20	0.07
Total polyunsaturated acids	6.26	1.93	2.58	1.46
Monoenoic and saturated	71.37	70.34	74.21	58.39
Mean wt. (mg.)/liver of:	Cholesteryl ester fatty acids			
Dienoic	3.79	0.87	0.23	N.D.
Trienoic	0.47	1.21	0.10	N.D.
Tetraenoic	0.90	0.20	0.13	N.D.
Pentaenoic	0.10	0.05	0.02	N.D.
Hexaenoic	0.16	0.06	0.04	N.D.
Total polyunsaturated acids	5.42	2.39	0.52	25.72†
Monoenoic and saturated	70.19	77.21	7.09	

* Standard deviation of the mean.

† Assumed value for total fatty acids from cholesteryl esters (see text).

'normal' liver-lipid concentrations were due to accumulations of cholesteryl esters and triglycerides, whereas phospholipid levels fell slightly.

The concentrations of cholesteryl esters in control and deficient males exceeded the 'normal' value by 46.4 and 45.6 times respectively. The corresponding values for control and deficient females were 4.94 times and 17.7 times greater respectively than the 'normal' female level.

Triglyceride concentrations in control and deficient males were 2.95 and 3.05 times the 'normal' value, whereas in females they exceeded the 'normal' value by factors of only 1.15 in controls and 1.11 in the deficient animals.

In groups 1 and A the combined weights of

dienoic and tetraenoic acids in the livers were 12.76 and 14.03 mg. respectively. Values for the concentrations of di-, tri- and tetra-enoic acids for the experimental groups in the present work and in the preceding paper are given in Table 2.

The distribution of polyunsaturated acids between the three classes of liver ester differed markedly in groups 1 and A. The combined weight of dienoic and tetraenoic acids in the cholesteryl esters of the cholesterol-fed group was 15.1 times as high as in the 'normal' group. (However, the concentrations of these acids as percentages of the total cholesteryl ester acids did not increase. The values for groups 1 and A were 6.2 and 6.9% respectively.)

Table 2. *Concentrations of dienoic, trienoic and tetraenoic acids in liver cholesteryl ester, triglyceride and phospholipid fractions and in the total liver lipid*

Concentrations are expressed as percentages by weight of the total acids recovered from each fraction. The data are for the experimental groups in the present work (groups 1, 2, 3 and 4) and in the experiment described in the preceding paper (Morton & Horner, 1961) (groups A, B, C and D). Lipid class: C.E., cholesteryl ester; T.G., triglyceride; Ph., phospholipid.

Group	Lipid class	Diene	Triene	Tetraene	Triene	Diene
					Tetraene	Tetraene
1	C.E.	5.01	0.62	1.19	0.52	4.21
	T.G.	5.60	0.97	0.93	1.04	6.04
	Ph.	8.95	3.35	7.67	0.44	1.17
	Total	5.83	1.16	2.01	0.58	2.91
2	C.E.	1.09	1.52	0.25	5.05	4.35
	T.G.	1.04	1.09	0.18	6.08	5.77
	Ph.	2.69	11.28	2.83	3.99	0.95
	Total	1.37	3.20	0.71	4.50	1.93
3	C.E.	3.02	1.31	1.71	0.77	1.77
	T.G.	1.71	0.74	0.49	1.50	3.45
	Ph.	5.06	5.90	6.97	0.85	0.73
	Total	2.91	2.51	2.75	0.91	1.06
4	C.E.	* —	—	—	—	—
	T.G.	1.07	1.02	0.17	6.10	6.40
	Ph.	3.15	14.04	3.39	4.14	0.93
	Total	4.149	4.79	1.12	—	—
A	C.E.	5.13	2.46	1.79	1.38	2.88
	T.G.	4.02	0.77	0.50	1.52	8.00
	Ph.	7.82	3.91	10.95	0.36	0.71
	Total	6.15	2.56	6.29	0.41	0.98
B	C.E.	2.52	1.92	0.72	2.67	3.50
	T.G.	0.86	0.67	0.14	4.73	6.07
	Ph.	2.54	12.89	3.82	3.37	0.66
	Total	1.41	4.11	1.18	3.49	1.20
C	C.E.	4.79	2.87	5.26	0.55	0.91
	T.G.	1.61	0.60	0.35	1.70	4.57
	Ph.	5.38	5.40	8.71	0.62	0.62
	Total	3.39	2.83	4.23	0.67	0.80
D	C.E.	2.45	2.45	0.61	4.00	4.00
	T.G.	1.44	0.90	0.15	6.00	9.56
	Ph.	2.75	17.17	4.24	4.04	0.65
	Total	1.91	6.35	1.53	4.17	1.25

* This fraction was lost during saponification.

The weight of dienoic and tetraenoic acids in group 1 triglycerides exceeded that in group A 2.7 times. In this case the concentration was higher, being 6.5% in the cholesterol-fed group compared with 4.5% in the 'normal' group.

Increased weights of polyunsaturated acids in the cholesteryl esters and triglycerides accumulated 'at the expense of' the phospholipids. The weights of phospholipid dienoic and tetraenoic acids were 0.52 and 0.32 respectively of group A values.

In cholesterol-fed and 'normal' females, groups 3 and C, the combined weights of dienoic and tetraenoic acids in the livers were 7.21 and 9.46 mg. respectively. The distribution of these acids among the different classes of liver ester differed less strikingly than in the corresponding groups of males, because liver-lipid levels in females were much less influenced by cholesterol feeding.

The weight of dienoic plus tetraenoic acids in the cholesteryl esters of group 3 was 1.71 times the value for group C. The concentrations of these acids as fractions of the total acids esterified with cholesterol were 0.05 in group 3 and 0.1 in group C.

The weight of triglyceride-bound dienoic and tetraenoic acids in group 3 exceeded that of group C by a factor of 1.32, but the fractional concentrations of these acids were almost the same for the two groups.

In group 3 the weight of phospholipid-bound tetraene was 0.65 of that in group C. Phospholipid diene in group 3 was 0.94 of the group C value.

Comparisons may also be drawn solely between groups in the present experiment. In group 2, the total weights of liver dienoic and tetraenoic acids were smaller (0.25 and 0.37 respectively) than the values for the control, group 1. Trienoic acid was

2.88 times higher in the deficient group. The falls in dienoic and tetraenoic acid levels were most marked in the triglycerides and least pronounced in the phospholipids. Conversely, the rise in trienoic acid was greatest in the triglycerides and least in the triglycerides.

The accidental loss of the cholesteryl esters from group 4 prevents a full comparison being made between the groups of females. Changes from the control weights of dienoic and tetraenoic acids were greater in the triglycerides; the rise in triene was more marked in the phospholipids.

DISCUSSION

In males, liver vitamin A stores were not significantly affected by fat deficiency. As in the previous experiment (Morton & Horner, 1961) it was not possible to differentiate between impaired absorption and storage of vitamin A and its subsequent rate of utilization. Experiments to determine vitamin A utilization specifically have been done with fat-deficient animals, and will be reported elsewhere. In females, however, absence of cottonseed oil from a diet containing cholesterol caused a marked and significant decrease in liver vitamin A.

This sex difference differs from that described by Green *et al.* (1957), which was that the addition of cholesterol to a diet containing adequate fat caused a lowering of liver vitamin A in males, but not in females.

Differences emerged in the percentages of administered vitamin A found in the livers. Morton & Horner (1961) gave the animals 8800 i.u. of vitamin A and at the end of the experiment the livers of males contained 8% of the intake and those of females 11%. In the present work the animals were given 6400 i.u. and 25% of this was found in the livers of males, whereas in female controls the livers contained 16% of the intake as against 8% for deficient females.

These results might appear to be in conflict with an earlier finding (Horner & Morton, 1960) that vitamin A absorption or storage is depressed in male rats by dietary cholesterol. In that investigation the diets contained 10% of arachis oil. Control males given 10 000 i.u. before being placed for 42 days on a vitamin A-free diet had 40% of the dose in their livers. Another group of male controls, which received 3360 i.u. as daily doses of 60 i.u. for 56 days and were then killed, retained 37% of the dose in their livers. Thus vitamin A absorption or storage by rats given adequate dietary fat was more efficient than in any of our fat-deficient animals, or in their controls which received very small amounts of cottonseed oil.

The body weights given in Table 1 support the

work of Holman & Peifer (1960), showing that cholesterol accelerates the onset of fat deficiency, by causing earlier cessation of growth. The Table shows how the female rat is less responsive than the male to these effects of dietary cholesterol. Morton & Horner (1961) found that without dietary cholesterol females ceased to grow after 170 days at a mean weight of 133 g.; males after 160 days at 167 g. In the present work females grew for 110 days, to a mean weight of 124 g. and in fact attained a higher final body weight than males, which ceased to grow after 72 days and averaged 107 g. in weight.

In the experiments of Bromer (1953) cholesterol had no significant effect upon the growth of male or female rats fed diets free from fat or containing hydrogenated arachis oil (2.9%) for 14 weeks. These findings are at variance with the present work and that of Holman & Peifer (1960). Bromer did, however, find that dermal symptoms were aggravated by cholesterol in the sense that they appeared appreciably sooner in males than in females and were more severe.

Liver lipids. Exogenous cholesterol which was deposited in the livers in these experiments was all esterified, as was the extra endogenous cholesterol which accumulated in fat-deficient animals (Morton & Horner, 1961). Increases in the concentrations of cholesteryl esters in the livers of fat-deficient animals given cholesterol were much more marked in males than in females. Bromer (1953) made a similar observation. The cottonseed oil which we gave to control animals did not alter this effect in males, but reduced it in females. Okey & Lyman (1957), under very different experimental conditions, observed that cholesterol feeding caused a greater accumulation of total lipid and cholesterol in the livers of males than in females, irrespective of the nature of the dietary fat.

The accumulation of exogenous cholesterol in the livers was much greater than the increase of endogenous cholesterol caused by fat deficiency alone.

Liver-triglyceride concentrations were very similar in control and deficient males. In both cases the values were lower than in deficient males which received no cholesterol (group B). The triglyceride levels of control and deficient females were both very similar to the group D value. Therefore dietary cholesterol did not increase the liver-triglyceride levels of fat-deficient animals. However, when fed together with cottonseed oil it caused an increase in liver triglycerides about equal to that caused by fat deficiency.

Accumulation of cholesteryl esters was caused by dietary cholesterol, by lack of linoleate, or by a combination of these factors. In no case was there any tendency for cholesteryl dienoate and tetraenoate to maintain or increase their normal con-

centrations, indeed the concentrations of these esters invariably fell as total cholesteryl ester levels rose.

Similarly, triglycerides which accumulated in fat-deficient animals were predominantly esterified with monoenoic and saturated acids. However, in males dietary cholesterol caused an increase in the percentages of dienoic, trienoic and tetraenoic acids bound to glycerol, whether the diet contained fat or not (compare group A with group 1 and group B with group 2). In females these effects were less marked and triglyceride dienoate concentration was actually lower in group 4 than in D.

Dietary cholesterol decreased the concentrations of dienoic and tetraenoic acids, particularly the latter, in the phospholipids of control animals. Both these effects were much more marked in males in spite of the fact that they received the larger dosage of linoleate. Popják (1946) reported a similar effect on rabbit plasma, wherein feeding a low-fat diet containing cholesterol caused a decrease in the unsaturation of plasma phospholipids.

There is noticeable a relative constancy of liver-phospholipid concentrations. In this experiment and the earlier work of Morton & Horner (1961) the liver-phospholipid levels ranged from 9.5 to 14.5 mg./g. of liver, with six of the values between 12.0 and 14.5 mg./g. In the present work each group had a lower liver-phospholipid concentration than the corresponding group in the previous experiments (Morton & Horner, 1961), which did not receive cholesterol. This effect of cholesterol was most marked in control males (group A, 13.7 mg. of phospholipids/g. of liver; group 1, 9.5 mg./g.), and least evident in control females (group C, 14.5 mg./g.; group 3, 13.7 mg./g.).

Similarly, Patil & Magar (1960) observed that dietary cholesterol lowered liver-phospholipid levels in male rats fed with various fats at the 5% level for 10 months (e.g. animals fed with arachis oil: 37.5 mg. of phospholipids/g. of liver; arachis oil plus cholesterol: 30.4 mg./g.). The example cited was less dramatic than the effect of adding cholesterol to diets containing coconut oil, ghee or dalda but we wish to compare it with an experiment of our own. Horner & Morton (1960) fed young male rats with a diet containing arachis oil (10%) for 6 weeks; half the animals also received cholesterol (2%). The control and cholesterol-fed groups had liver-phospholipid levels of 15.1 and 14.6 mg./g. respectively (Horner, 1958). Cholesterol did not affect liver-phospholipid concentrations appreciably under these conditions, and the phospholipid levels were very similar to those of fat-deficient animals and their controls. Possibly liver-phospholipid levels are less responsive to dietary changes in young rats than in adults.

Trienoic:tetraenoic acid ratios. Dietary cholesterol accelerated the onset of essential fatty acid deficiency as judged by earlier cessation of growth, appearance of dermal symptoms and lower final body weight.

It is difficult to find a 'chemical' criterion of fat deficiency which shows that the syndrome is more severe in cholesterol-fed animals. Holman (1960) has used the trienoic:tetraenoic ratio as an index of the degree of fat deficiency. High values of this ratio are indicative of fat deficiency because the rise in trienoic acid characteristic of this condition is always accompanied by a fall in tetraenoic acid. Table 2 shows that the value of this ratio for total liver fatty acids is always higher in fat-deficient animals.

Comparison of each group in the previous experiment with corresponding groups in the present work indicates that dietary cholesterol consistently raised the ratio for total liver fatty acids. This second effect, which was smaller than that due to fat deficiency, was observed whether or not the diet contained cottonseed oil.

In every experimental group the highest trienoate:tetraenoate ratio was that of the triglycerides. Deficiency of fat invariably raised the ratio above the control value in all three classes of liver lipid. Dietary cholesterol always raised the corresponding value in phospholipid fractions, but did not exert this effect consistently in cholesteryl esters or triglycerides.

Trienoate:tetraenoate ratios in phospholipid fractions always approximated to the corresponding values for total liver lipids. This fact allows group 4 to be included in a discussion of these values as an index of degree of fat deficiency.

By the standard of phospholipid trienoate:tetraenoate ratios, deficient females (group D) were more severely deficient than males (group B), the value for females being higher by a factor of 1.20. The addition of cholesterol to the diet increased the ratio by a factor of 1.22 for deficient males but had no significant effect on the value for females. Thus a direct comparison of the triene:tetraene values for the phospholipids of deficient rats fed with cholesterol shows no notable difference between the sexes.

The phospholipid trienoate:tetraenoate ratios of female control groups C and 3 were higher than those of the corresponding groups of male controls by factors of 1.72 and 1.93 respectively. These differences may be due to the smaller weight of cottonseed oil given to female controls. The chosen dosage levels were based on the data of Greenberg, Calbert, Savage & Deuel (1950).

Holman (1960) has suggested that linoleic acid should provide at least the equivalent of 1% of the total calorie value of the food eaten by male rats if

fat deficiency, as judged by the heart trienoate:tetraenoate ratio, is to be prevented. Our male controls received linoleic acid equivalent to 1.5 % of total calories (assuming a daily intake of 15 g. of the basal diet). Female controls obtained the equivalent of 0.5 % of their calorie intake as linoleic acid (food consumption 12 g./day).

Holman (1960) has also shown that a triene:tetraene ratio in plasma, erythrocytes or heart tissue less than approximately 0.4 indicates that the minimum requirement of linoleate has been met. This criterion cannot be applied to other tissues. Ratios based on the data of Rieckehoff, Holman & Burr (1949) [the data were recalculated and amplified with correction formulae derived from the spectral constants given by Holman (1957) for polyunsaturated acids isomerized by the method of Holman & Burr (1948)] indicate that in male rats given 100 mg. of corn oil per day, the value for heart (0.27) was considerably lower than that of liver (1.16) or any other tissue examined (i.e. kidney, brain, skin, liver, muscle and depot fat). In fat-deficient animals the value for heart was 4.30, higher than in any other tissue. The liver value in fat deficiency was 2.03. The increase in the heart trienoate:tetraenoate ratio was a much more sensitive indicator of fat deficiency than the corresponding change in the liver. (The second most responsive tissue 'indicator' was skin with ratios of 0.79 in controls and 3.86 in deficient animals.)

Similarly in an experiment carried out under very different conditions (Peifer, Janssen, Ahn, Cox & Lundberg, 1960) the trienoic:tetraenoic ratios given for heart lipids were consistently lower than the values which can be calculated for total liver lipids (e.g. heart, 0.33, liver, 1.49 in a group of hypercholesterolaemic male rats fed ethyl oleate).

Dienoic:tetraenoic ratios. Dietary cholesterol invariably raised the dienoic:tetraenoic ratios in total liver lipids and in the phospholipids and cholesteryl esters. The opposite effect was consistently observed in triglycerides. The changes did not depend on whether the diets contained cottonseed oil or not. They suggest that dietary cholesterol impairs the conversion of linoleate into arachidonate, an inference made by Klein (1959).

If group B is compared with group 2 and group D with group 4 the degree of this effect is seen to be virtually identical in deficient males and females; cholesterol increased the diene:tetraene ratio for phospholipid fatty acids by a factor of 1.4. There is a sex difference in the degree of this effect of cholesterol in control animals, but interpretation of its significance is complicated by the extra variable of linoleate dosage. Diene:tetraene ratios can be calculated from the data of Patil & Magar (1960). The addition of cholesterol to four diets each containing 5 % of a fat consistently raised the diene:

tetraene ratio in cholesteryl esters and lowered it in the triglycerides of livers of male rats. Our results obtained under very different experimental conditions confirm these findings. In the Indian experiment, however, cholesterol did not significantly change the diene:tetraene ratio in the phospholipids.

Females were more resistant to fat deficiency than males by several criteria. They continued longer to put on weight on a fat-free diet, and showed milder dermal symptoms. Dienoic and tetraenoic acids comprised a higher percentage of their total liver fatty acids (3.44 % in group D; 2.59 % in group B). Nevertheless by another criterion females were more deficient than males in that their liver triene:tetraene ratio was higher. Mead, Slaton & Decker (1956) have shown that in male mice linoleate oxidation is increased by fat deficiency, but the sexes were not compared.

Fat-deficient female rats were more resistant than males to the additional stress of dietary cholesterol. They grew for a longer period and became heavier than deficient males. Again dienoic and tetraenoic acids accounted for a greater percentage of the total liver fatty acids in females (> 2.61 %) than in males (2.08 %). By the tests of liver triene:tetraene and diene:tetraene ratios there was no sex difference in cholesterol-fed deficient rats. According to Lynn & Brown (1959) feeding cholesterol to rabbits, but not rats, stimulates the oxidation of all fatty acids by mitochondria, especially the unsaturated fatty acids. In rats cholesterol did not speed up the oxidation of linoleate by liver.

Feeding cholesterol caused a much greater deposition of cholesteryl esters in the livers of deficient males than of deficient females. The weight of dienoic and tetraenoic acids esterified to cholesterol in group 2 was 1.07 mg., equivalent to 27.4 % of the total dienoic and tetraenoic acids in the liver. In group B the corresponding values were 0.27 mg. and 6.6 %. If the essential fatty acids esterified to exogenously derived cholesterol were partially or wholly metabolically inert, i.e. unavailable to other classes of lipids or to other tissues, their deposition would accelerate fat deficiency. Further, their greater accumulation in males would explain why females are more resistant to a diet containing cholesterol and lacking linoleic acid.

Our experiments have revealed sex differences in the fat-deficiency syndrome and the effects of cholesterol, but do not lead to full explanations. The rates of turnover and the interconversions of the individual fatty acids in the various classes of lipids must be determined under differing dietary conditions. A beginning has been made by Dittmer & Hanahan (1959), who have studied the liver lipids

of normal male rats in this way, and Fulco & Mead (1959) have shown how eicosa-5:8:11-trienoic acid arises in fat-deficient rats.

SUMMARY

1. The liver vitamin A stores of fat-deficient male rats fed on a diet containing cholesterol (1%) did not differ significantly from those of controls which received linoleic acid.

2. The liver vitamin A stores of fat-deficient female rats fed on a 1% cholesterol diet were 48% lower than those of their controls which had been given linoleic acid.

3. The addition of cholesterol to a fat-free diet aggravated fat deficiency as judged by more rapid cessation of growth at a lower body weight. Both effects were appreciably more pronounced in males than in females.

4. Cholesterol-supplementation of the diet caused much larger accumulations of cholesteryl esters in livers than are caused by fat deficiency alone. The effect was much greater in males. Linoleic acid (100 mg./day) did not alter the severity of this phenomenon in males; a smaller dose (25 mg./day) reduced the effect considerably in females.

5. The rise in liver triglyceride concentration caused by fat deficiency was not altered by adding cholesterol to the diet. Cholesterol caused an increase in the concentrations of polyunsaturated acids in triglycerides, both in deficient animals and their controls. This effect was more pronounced in males.

6. Dietary cholesterol decreased the concentrations of dienoic and tetraenoic acids, particularly the latter, in the liver phospholipids of control animals. Both effects were appreciably greater in males than in females.

7. Dietary cholesterol consistently raised the triene:tetraene ratio of liver phospholipids.

8. Dietary cholesterol invariably raised the diene:tetraene ratio in total liver lipids, phospholipids and triglycerides, and lowered this value in the triglycerides. These effects occurred both in deficient animals and in controls.

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Ester-Linked Long-Chain Fatty Acids of Nervous Tissue

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It has long been recognized that nervous tissue contains a high proportion of lipid which on hydrolysis gives long-chain fatty acids in substantial amounts (Thudichum, 1901). Extracts of this lipid comprise a number of compounds that are now well characterized; the quantities in which these are present, and their chemical structures, have in many cases been elucidated (see LeBaron &

Folch, 1957). Although the results of early qualitative work and of later quantitative studies on the acids from isolated fractions of different lipids extracted from nervous tissue have appeared in the literature, no survey of the nature and distribution of these acids in total lipid extracts of nervous tissues had been reported before the account by Johnston & Kummerow (1960) of the acids in two