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The Lipids in Xanthomata*

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Since the original description (1) in 1850 of "vitiligoidea" in association with chronic jaundice and diabetes, it has become apparent that lipid containing fibrous masses, now called xanthomata, may appear in the skin and occasionally other tissues in many disorders of lipid metabolism (2). The commonest form, xanthoma of the eyelids (xanthelasma), and some of the rarer disseminated types may occur in patients with normal serum lipids (2, 3). However, the larger xanthomata (tuberous on pressure areas such as elbows, knees, and buttocks, and tendinous in the tendons) are almost always associated with an increase in the patient's total serum lipids.

Xanthomata from different sites are similar histologically (2). There is much fibrous and connective tissue with conglomerations of sudanophilic material mostly in foam cells; giant cells may be present. In older lesions nests of cholesterol crystals may be seen.

Xanthomata may wax and wane with rise and fall in the serum lipids (4), and it has long been supposed that the lipids in the lesions may be derived from the serum (5). Credence would be given to this hypothesis if the lipids in the xanthomata were the same as in the serum lipids, but published data are sparse and were mostly accumulated before satisfactory methods of separating small amounts of the lipid classes and fatty acids became available. Because of the continuing interest in the interrelationships of tissue and serum lipids, we thought the problem worth re-examining.

Methods

Seven patients with elevated serum lipids and xanthomata or xanthelasmata were studied (Table I). In ad-

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dition, A.T. was obese, mildly hypertensive, and complained of bilateral intermittent claudication. Arterial pulses were absent in both legs below the femorals. G.W. had gout, and his deceased mother was said to have had lumps on her hands like G.W.'s xanthomata. T.E. had suffered a cerebrovascular accident, and one leg had been amputated for arterial occlusion; atheroma of the femoral artery had been found. T.H. had femoral artery atheroma confirmed at operation. His serum lipid level was lower than those usually associated with xanthomata, but there was no doubt as to the nature of the lesion removed. Patient D.L. had developed nephrosis 7 years before, and xanthelasmata had appeared at about that time. Despite excellent control of the nephrosis with prednisolone and a normal serum albumin for several years past, the xanthelasmata had enlarged and the serum lipids remained elevated. We believe that the abnormality of lipid metabolism was probably unrelated to the nephrosis. In all the patients' liver function tests, serum albumin and protein-bound iodine were normal, and the urine was free from sugar. Except for G.W. none of the patients knew of any relative with xanthomata or elevated serum lipids, but the patient's relatives were not examined.

The xanthomata were excised under local anesthesia either at the patient's request or with his clear understanding that it was for experimental purposes. The specimens were immediately stored at -20° C until processed a few days later. All except one xanthoma (A.T.) were examined histologically, and the typical appearances were demonstrated. As far as possible, skin and adipose and connective tissue were trimmed off the fibrous masses, which were then sliced into fragments <1 mm thick and extracted in at least 40 times their volume of chloroform:methanol, 2:1, at 5° C for 3 to 4 days. In one instance the effectiveness of the extraction was examined by alkaline digestion of the residue and estimation of the fatty acids thereby liberated. Extraction of fatty acids by the chloroform:methanol had been $>99\%$ complete.

In four patients a sample of subcutaneous fat was obtained from the buttock by a technique similar to that of Hirsch and co-workers (6), and the fatty acids were analyzed as described below.

While all patients were fasting 20 or 30 ml of venous serum was extracted in 20 vol chloroform:methanol, 2:1. After filtration all lipid extracts were equilibrated overnight at room temperature with 0.2 vol 0.5 M phosphate buffer (7). Samples of the chloroform phase were taken for gravimetric and total phosphorus (8) analyses. Appropriate portions of the concentrated

TABLE I
Personal details of the patients with xanthomata

Patient	Sex	Age	Other conditions present	Lesions examined	Duration	Comment
A.T.	M	42	Obesity, femoral artery atheroma	Tuberous xanthomata of elbows	12	Low fat diet plus unsaturated fats discontinued 2 years before
G.W.	M	36	Gout	Tendinous xanthomata of hand and elbow	18	Low fat diet plus unsaturated fats discontinued 2 years before
T.E.	M	54	Femoral artery atheroma, cerebrovascular disease	Pedunculated tuberous xanthomata of trunk	26	Treatment with sunflower seed oil discontinued 2 years before
T.H.	M	48	Femoral artery atheroma	Tuberous xanthomata of elbow	"Several"	No previous treatment
J.O.	M	34	None	Tuberous xanthomata of elbow	10	On low fat diet plus corn oil, also on Atromid for 8 months; xanthomata much reduced in size
F.E.	M	51	None	Tuberous xanthomata of elbow	12	On Atromid for 9 months, previously having corn oil; xanthomata unchanged
D.L.	F	62	Nephrosis	Xanthelasmata	7	See text

crude extracts to give loadings of less than 15 mg per g were applied to 20-g columns of prepared silicic acid (9) activated overnight at 110° C and atmospheric pressure and packed in 1.0-cm i.d. columns. Neutral lipids (NL) were eluted with 200 ml chloroform and then the phospholipid (PL) classes by rising concentrations of methanol in chloroform according to the proportions recommended by Philips (10) but with appropriately larger volumes.

Fifteen-ml cuts were collected, and the phosphorus content of alternate tubes was estimated. Tubes were pooled in five fractions, A to E, and the total phosphorus content of each fraction was determined. Compared to the phosphorus content of the crude extracts, recoveries were 70 to 80%. Similar columns run with standards give recoveries of > 80%.

The identity of the phospholipid in each peak was examined by thin-layer chromatography (TLC) against standards. Glass plates 8 × 8 cm were coated with a 0.25-mm layer of silica gel G¹ slurried in water. They were dried in air and then at 110° C for 30 minutes before storage in a desiccator. The plates were developed in unlined glass tanks for 10 to 15 minutes with methanol:chloroform:water, 62:25:4, as the solvent system. Phosphatidyl ethanolamine and serine are not separated in this system.

Lipid spots were detected by warming the plates and spraying with 10% (wt/vol) phosphomolybdic acid in absolute ethanol. A standard mixture of phosphatidyl ethanolamine, lecithin, and sphingomyelin was run at both sides of each plate. The phosphatidyl ethanolamine and lecithin were a mixture prepared in this laboratory from hens' eggs (11); the sphingomyelin was obtained commercially.²

Fraction A usually contained two or three unidentified components with R_f's greater than cephalin, fraction B was almost pure cephalin, and C almost pure lecithin.

Sphingomyelin was present in fractions D and E and comprised the major portion of the latter.

From the extracts of two xanthomata the cephalin fraction was hydrolyzed by heating at 100° C for 4 hours in 5 N methanolic HCl.

The reaction mixture was taken to dryness; the residue was dissolved in distilled water and spotted onto TLC plates prepared as above. Ethanolamine and serine (obtained commercially) were applied to the same plates, which were developed in methanol:water:7 N NH₄OH, 6:3:1. The spots were detected by warming the plates after spraying with 0.5% Ninhydrin in butanol. Over 90% of the base liberated by the hydrolysis was ethanolamine. In the cephalin fraction from sera examined similarly, serine was present only in trace amounts.

The NL fraction was passed through a 5.0-g column of Amberlite IR 45 (OH) ion exchange resin from which the free fatty acids (FFA) were subsequently recovered (7). The FFA was estimated, after methylation with diazomethane, by a hydroxamic acid method (12).

The NL's were separated at room temperature on a 10-g column of prepared silicic acid (9) with a diethyl ether:petroleum ether (boiling point, 40 to 60° C) solvent pair (13). Only small traces of lower glycerides were found. The identity of the cholesterol ester (CE), triglyceride (TG), and cholesterol peaks was checked by TLC as above except that the solvent system was petroleum ether (boiling point, 40 to 60°):diethyl ether:glacial acetic acid, 85:15:2. The peaks were then estimated gravimetrically and by their cholesterol (14) or esterified fatty acid content (12). When the lipid samples were small, the size of the columns and solvent volumes was scaled down.

Over-all recoveries of lipids were estimated by comparing the sum of all the individual classes identified to the total solid content of the chloroform phase of the crude extract. Recoveries were 50 to 95% for the xanthomata and 70 to 90% for serum. With standard mixtures recoveries with these techniques are usually better

¹ E. Merck, Rahway, N. J.

² L. Light & Co., Colnbrook, England.

TABLE II
*Lipid classes of xanthomata and serum**

Patient	Wet wt	% lipid (wet wt)	Xanthomata % of total identified lipids				% of cholesterol unesterified	Serum					% of cholesterol unesterified
			TG	PL	CE	C		TL	TG	PL	CE	C	
	<i>mg</i>	<i>%</i>						<i>mg/100 ml</i>					<i>%</i>
A.T.	160	15.5	16†		54†	30†	50	2,033	598	259	414	118	33
G.W.	4,030	11.7	7	15	72	6	12	1,649	228	480	588	152	31
T.E.	1,990	27.5	0.2	7	14	79	91	1,857	620	436	440	168	40
T.H.	960	7.5	6	14	58	22	40	1,159	297	263	290	70	30
J.O.	400	3.1	41	22	24	13	50	1,180	260	310	260	61	29
F.E.	5,960	6.1	2	31	12	55	89	971	169	299	188	38	26
D.L.	410	9.3	31	22	36	11	31	1,175	164	334	336	52	21

* TG = triglyceride, PL = phospholipids, CE = cholesterol esters, C = free cholesterol, and TL = total lipids.

Note that "cholesterol esters" refers to the total weight isolated in this fraction, i.e., cholesterol and fatty acids. The "percentage of cholesterol unesterified" was calculated by comparing the amount of free cholesterol with the total amount present, i.e., free cholesterol plus the cholesterol contained in the cholesterol esters.

† Of neutral lipids only.

than 90% for all components, and we suppose that much of the missing material from the crude extracts was either unidentified lipids or nonlipid material held in the chloroform phase, although some loss on the columns and in handling seems inevitable.

Methyl esters of fatty acids from all the lipid classes except FFA (which were treated with diazomethane) were formed by treatment with 5% concentrated H₂SO₄ in methanol at 85° C for 4 to 6 hours under a reflux condenser. Later, 0.1 ml of 0.1% hydroquinone was added to each tube. The completeness of methylation was confirmed by TLC. Methyl esters were separated using a Pye argon gas chromatograph with Celite 100- to 120-mesh packing coated with 10% polyethylene glycol adipate at 175° C and sometimes also with 5% Apiezon L coating at 200° C. Gas flow was 50 ml per minute with inlet pressures of 8 to 12 pounds per square inch.

When the National Heart Institute fatty acid standards A, B, and F were analyzed, the results agreed with the stated composition with a relative error less than 10% for major components and less than 15% for minor components except that methyl myristate was underestimated, when in low concentration, by about 40% (15). Esters were identified by their retention times compared to NHI standards. Peak areas were meas-

ured by triangulation on analyses using the adipate liquid phase and results expressed as percentage of total by weight. All solvents were reagent grade, purified (16) and redistilled before use.

Results

The weights of the trimmed specimens ranged from 160 mg to 4.0 g, the total lipid content being 3.1 to 27.5% of wet weight (Table II). The lowest value was in J.O., whose xanthomata had become much smaller with treatment and contained loose connective tissue. The highest value was in the old xanthomata of T.E., which contained many cholesterol crystals. The percentage of the individual lipid classes present is also shown. Cholesterol predominated, and the percentage unesterified ranged from 12 to 91%, the low figure being in the tendinous xanthomata, and the high ones in old xanthomata from T.E. and F.E. In F.E. there were cavities up to 0.5 cm in diameter containing brown cheesy material

TABLE III
Proportions of phospholipid fractions in xanthomata and serum

Patient	Xanthomata*					Serum*				
	A	Cephalin	Lecithin	D	E	A	Cephalin	Lecithin	D	E
			<i>%</i>					<i>%</i>		
G.W.	4	36	41	8	11	1	4	68	16	11
T.E.	7	32	39	5	17	2	5	66	9	18
T.H.	8	33	31	22	6	3	8	60	6	23
J.O.	5	19	(42)		34	1	5	69	15	10
F.E.	4	20	41	16	19	1	6	71	9	13
D.L.	7	7	(77)		9	2	5	82	6	5

* For composition of fractions A, D, and E, see text.

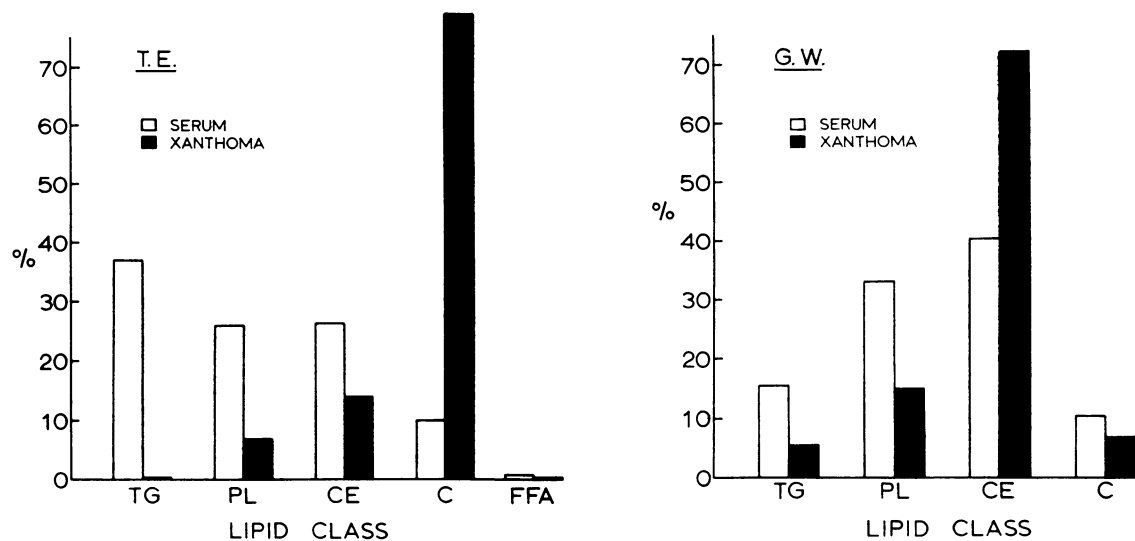


FIG. 1. PROPORTIONS OF LIPID CLASSES BY WEIGHT IN SERA AND XANTHOMATA FROM PATIENTS G.W. AND T.E. Abscissa: triglyceride (TG), phospholipid (PL), cholesterol esters (CE), free cholesterol (C), and free fatty acids (FFA).

which when extracted and examined by TLC was found to be almost pure cholesterol. High relative amounts of TG were recorded in J.O. and in the xanthelasmata of D.L.

The analytical results for fasting venous serum are given also (Table II) in terms of weight per 100 ml. Patients A.T., T.E., and T.H. had an increased level of triglyceride and G.W. a pre-

dominantly hypercholesterolemia; J.O. and F.E. had been modified by treatment.

The proportions of the lipid classes were different in the xanthomata and the serum. This is illustrated in Figure 1, where data from patients G.W. and T.E. are shown with the results from xanthomata and serum expressed in the same way, as percentage of recovered lipid by weight.

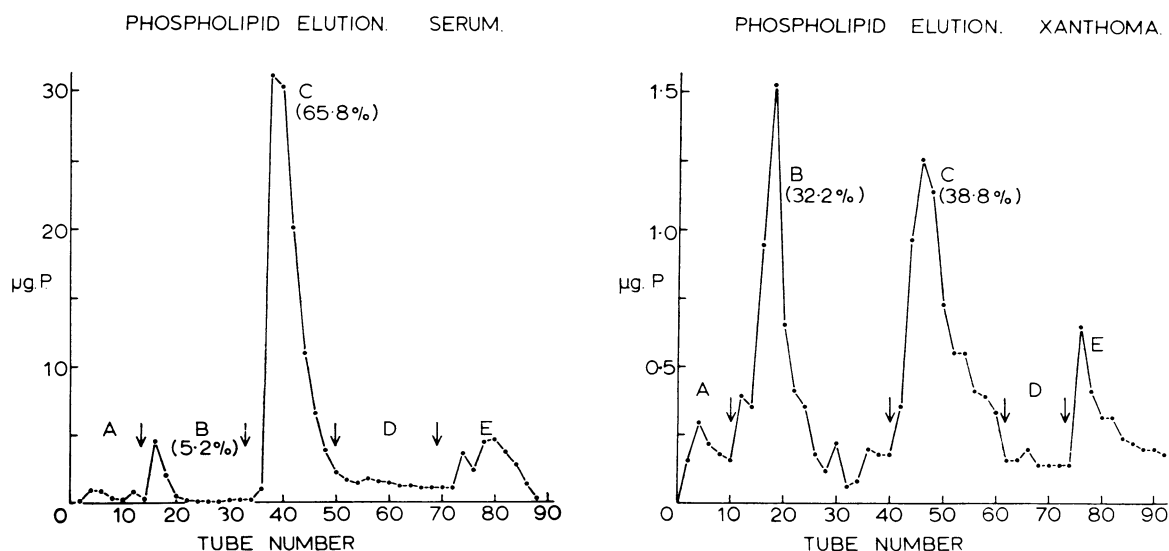


FIG. 2. PHOSPHOLIPID ELUTION PATTERNS FROM SERUM AND XANTHOMATA OF PATIENT T.E. Arrows indicate the points of division of the fractions A to E into which the tubes were pooled.

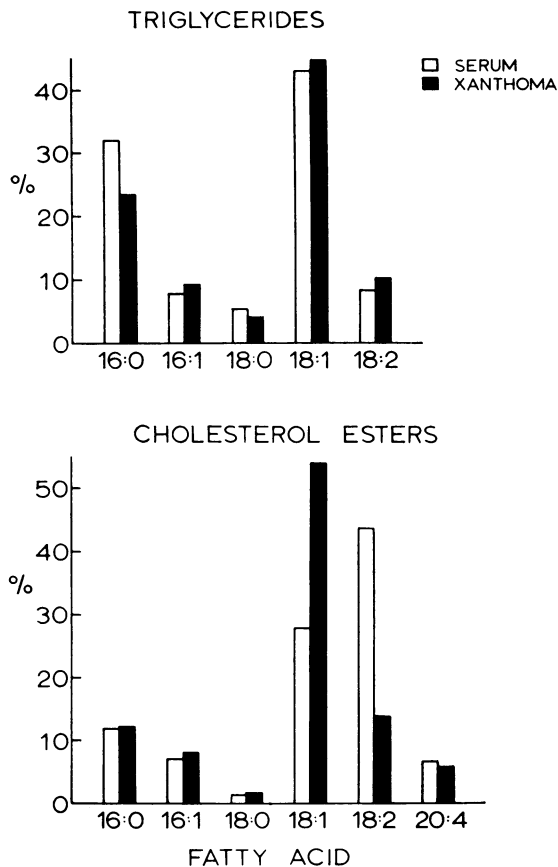


FIG. 3. PROPORTIONS OF FATTY ACIDS PRESENT IN TRIGLYCERIDES AND CHOLESTEROL ESTERS FROM SERA AND XANTHOMATA.

In Table III PL analyses, where available, are shown. Compared with serum, the most marked difference in the xanthomata PL was the reduction in lecithin and the increase in the cephalin fraction until, in three instances, they were approximately equal. This is illustrated in Figure 2, where the elution patterns of the PL's from the xanthoma and serum of T.E. are shown. If we assume that all the sphingomyelin was in fractions D and E, the proportion of this lipid (except perhaps in J.O.) was similar to that in the serum. An FFA fraction was isolated from three xanthomata. There were 2.3 and 1.3 μ Eq per g wet tissue in patients T.H. and F.E., respectively, and 14.2 μ Eq per g wet tissue in patient T.E.

The differences between the xanthomata and serum in the fatty acid content of the lipid classes (Table IV) were more consistent than the differences in the total amounts of the classes present.

The use of mean values must be suspect considering the variable nature of the lesions analyzed, but for convenience they have been calculated and are shown in Figures 3 and 4, where they are compared with the mean values from the sera of the five patients who were not being treated at the time of examination.

The TG's (Figure 3) contained less palmitic acid in the xanthomata, and arachidonic acid was sometimes present, but the general pattern, particularly in the C-18 acids, was similar to that in the serum TG. The CE's, however, were quite different. The percentage of oleic acid was about twice as great in the xanthomata as in the serum, and this increase was all at the expense of linoleic acid. Arachidonic acid was the same, but eicosatrienoic acid (20:3) was increased from < 1% in the serum CE's to 3.5%, on average, in the

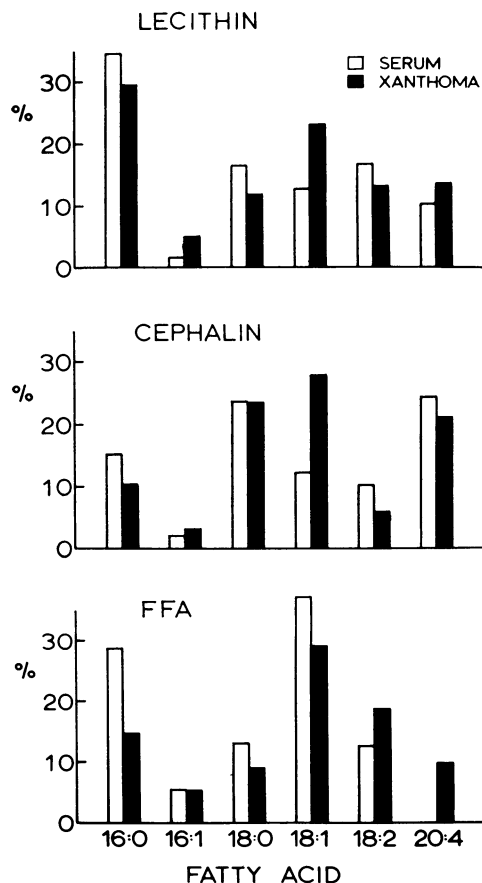


FIG. 4. PROPORTIONS OF FATTY ACIDS PRESENT IN LECITHIN, CEPHALIN, AND FREE FATTY ACID FROM SERA AND XANTHOMATA.

TABLE IV
*Per cent of fatty acids in the lipid classes in the xanthomata**

Acid	Cholesterol esters							Triglyceride						
	A.T.	G.W.	T.E.	T.H.	J.O.	F.E.	D.L.	A.T.	G.W.	T.E.	T.H.	J.O.	F.E.	D.L.
16:0	12.7	5.8	9.1	10.2	14.5	11.6	20.6	18.3	27.3	20.6	27.9	20.6	24.3	26.6
16:1	8.1	9.7	8.0	6.1	10.5	9.3	4.9	8.5	9.4	11.0	8.0	8.4	11.2	9.4
18:0	1.2	0	1.1	1.4	3.6	1.3	2.6	4.9	3.1	4.6	2.9	7.8	3.8	2.8
18:1	60.8	63.0	52.5	56.2	32.7	47.7	63.6	44.0	44.2	41.4	44.6	40.6	46.9	52.6
18:2	11.4	12.2	15.2	11.7	22.4	17.8	6.0	6.0	13.3	9.2	9.4	17.7	11.9	5.3
20:4	4.1	3.0	7.8	6.2	7.7	5.7	?	1.9	0	5.1	0.7	0	0	0

Acid	Cephalin						Lecithin						Free fatty acids			
	G.W.	T.E.	T.H.	J.O.	F.E.	D.L.	G.W.	T.E.	T.H.	J.O.	F.E.	D.L.	G.W.	T.E.	T.H.	F.E.
16:0	10.7	8.3	4.0	10.7	6.8	21.9	30.3	25.3	27.4	27.3	36.5	29.6	21.8	12.9	18.2	15.7
16:1	1.9	2.2	1.7	2.6	1.2	9.4	6.7	5.1	3.8	5.4	4.0	5.3	5.0	5.4	4.5	6.4
18:0	30.5	26.9	22.9	18.8	29.6	12.8	7.9	11.5	12.7	11.8	11.0	15.7	10.7	7.9	9.5	8.1
18:1	24.6	23.3	24.7	27.1	24.7	43.0	25.6	22.2	21.2	21.3	19.8	28.7	34.0	27.5	22.9	31.4
18:2	4.9	7.0	5.2	8.3	5.1	4.8	12.5	14.1	12.3	12.3	14.8	13.5	15.9	18.8	21.0	18.8
20:4	24.8	27.0	27.0	21.8	21.6	4.4	13.1	15.4	15.9	17.7	13.3	7.1	?	9.3	6.6	13.3

* Total percentages listed for each subject usually do not reach 100 because of the presence of small amounts (usually < 5%) of unidentified material.

xanthomata. Linolenic acid (18:3) was not detected.

In lecithin from the xanthomata (Figure 4) oleic acid was also twice that in the serum, but the corresponding fall was in palmitic and stearic acids, as well as linoleic. Palmitoleic acid was more than twice that in the serum, and arachidonic acid was also increased. The changes in the cephalins were similar except that arachidonic acid was less in the xanthomata than in the serum and the increase in palmitoleic acid in the xanthomata was less marked. Eicosatrienoic acid was present in small amounts (0.5 to 2.0%) in the PL's of serum and xanthomata. Linolenic acid was not detected.

The adipose tissue from patients A.T., G.W., T.E., and T.H. contained, on the average, the following fatty acids: myristic, 4.2%; palmitic, 23.6%; palmitoleic, 10.3%; stearic, 2.2%; oleic, 48.7%; and linoleic, 10.5%. Arachidonic acid was virtually absent. These values are similar to published data in normals (6) and also to those found in normals in this laboratory.

Discussion

The presence of lipid is the most striking histologic feature of xanthomata, and it accounted, on the average, for about 10% of the masses with wide individual variations. Whatever the pat-

tern of elevation of the serum lipids, cholesterol predominated in the xanthomata. In the only example of tendinous xanthomata examined (G.W.), the cholesterol was nearly all esterified, but in all the other specimens there was a higher proportion of free cholesterol than in the serum, particularly when deposits of cholesterol were present.

The proportion of PL in the xanthomata lipids was reduced on the average to half that in the serum. The proportion of TG was reduced usually even more, and only in the xanthomata of J.O., which had become smaller and softer with treatment, and in the xanthelasmata was TG present in substantial proportions. In the latter, histology showed some adipose tissue in the material analyzed. A high neutral lipid (which we take in this context to mean TG) content of 4.4% of wet weight has been reported previously in a xanthelasma (17).

An extensive study in 1938 (18) of various kinds of xanthomata reported similar total lipid contents, i.e., 5 to 15% of wet weight. Cholesterol was 10 to 40% in the free form, the latter figure being unexpectedly low, as some of the xanthomata contained many cholesterol crystals. Because of the way in which the results were expressed other comparisons are difficult, but in general similar proportions to those reported here were found. Thannhauser (2) gives one com-

parable analysis in a hypercholesterolemic tuberous xanthoma. The total lipid was 11% of wet weight, about 20% of the lipid was PL, there was little TG, and 50% of the cholesterol was free.

The proportion of the PL classes in the xanthomata differed from those in the serum in that cephalin was increased up to sixfold and lecithin about halved. The proportion of sphingomyelin was usually about the same. The xanthomata PL's therefore resemble in some respects those in human red cells (19), where cephalin and lecithin are 39 and 36%, respectively. Muscle also contains relatively more cephalin; in human psoas muscle unpublished analyses in this laboratory showed an average PL composition of 24% cephalin and 51.5% lecithin.

The fatty acid in the CE's of the xanthomata contained greatly increased amounts of oleic acid, at the expense of linoleic acid, compared to the CE's of the serum. The usual amount of arachidonic acid was present, however. Less marked differences were present in the PL's; therefore all the xanthomata lipids except FFA tended to have, in respect of oleic and linoleic acids, the fatty acid pattern characteristic of TG and adipose tissue. A similar finding is implied by the report (17) that the fatty acid pattern in a xanthomatous infiltration of the skin in a patient with diabetes mellitus was the same as that in normal adipose tissue. Compared to serum a higher oleic acid content of CE's has been found also in the tonsillar deposits of lipid in Tangier disease (20).

The possible effects of treatment must be considered. Most of the patients had previously eaten diets high in unsaturated fat, but usually these had been discontinued years before, and there was no evident effect of them in the serum lipids and adipose tissue. Furthermore, the acid patterns in the tissues of T.H., who had always taken a normal diet, and D.L., whose diet had been normal in respect of fats, were similar to those in the other patients. Two patients had been treated with Atromid,³ a mixture of androsterone and ethyl-(p-chlorphenoxy) isobutyrate, which in most persons is an effective serum cho-

lesterol-lowering agent. In one (J.O.) the lesions had regressed, and the TG content was high. In both, the cephalin content of the PL's and the oleic acid content of the C.E.'s were lower than in the other xanthomata. The only other relationship noted between the analytical results and the clinical condition of the patients was the presence of tendinous xanthomata containing little free cholesterol in the patient with predominantly hypercholesterolemia.

Presumably some of the lipids analyzed were structural lipids of the fibrous tissue and perhaps also to some extent of coexistent adipose tissue, but most of the lipids present in substantial quantities must have been in the foam cells or deposits. Apparently they are far from being inert deposits of the serum lipids.

Xanthoma cells are reported to have intricate pseudopods (21) that bear some resemblance to the brush border of the small intestinal mucosa. Perhaps the pseudopods aid the entry into the cells of serum lipids in particulate form, after which the particles coalesce (21). It seems that during this process lipids other than cholesterol are removed faster so that cholesterol accumulates. By synthesis, transesterification, or selective catabolism the cells appear to impose on the lipids accumulating within them a pattern that tends in some respects towards the PL's of normal tissues and the fatty acids of adipose tissue. These suppositions receive some support from isotope incorporation studies (22) which showed that slices of rabbit and human xanthomata had little capacity to synthesize cholesterol but made PL's and fatty acids readily. The metabolism of lipids in xanthomata is evidently complex, and the means whereby the new lipids are synthesized remain obscure, but it seems unlikely that the FFA isolated were acting as a precursor pool. Indeed, their composition suggests that they were produced in part by a liberation of fatty acids from CE's in the serum.

Summary

1. Xanthomata were removed from seven patients with idiopathic hyperlipidemia. Lipids from venous serum of the fasting patients and the xanthomata were separated into the major neu-

³ Imperial Chemical Industries, Ltd., Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, U. K.

tral and phospholipid classes, and the fatty acid spectrum of each class was determined.

2. Cholesterol always predominated in the xanthomata, although some of the patients had predominantly hypercholesterolemia and some hypertriglyceridemia. The degree of esterification of the cholesterol in the xanthomata was, with the exception of the only example of tendinous xanthomata examined, less than in the serum particularly when deposits of cholesterol were present.

3. In the phospholipids of the xanthomata the proportion of cephalin was greater and that of lecithin smaller than in the serum.

4. The fatty acid spectra of the triglycerides were similar in the xanthomata and serum, but in the cholesterol esters of the xanthomata oleic acid was greater (nearly 60%) and linoleic acid was less than in the serum. There was a similar but less marked difference in the phospholipids. The free fatty acids may have contained acids derived from cholesterol esters in the serum.

5. We suggest that these results and published evidence are consistent with the hypothesis that serum lipids enter xanthomata, cholesterol is retained, and the other lipids are modified or replaced by newly synthesized material so as to have a pattern in some respects similar to the lipids in other tissues.

Acknowledgments

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