A CHROMATOGRAPHIC STUDY OF SKIN LIPIDS IN LIPOID PROTEINOSIS*

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Lipoid proteinosis is a rare dermatosis of considerable interest, especially in South Africa where about 30% of all known cases are found (1).

Not only is this disease of interest because of its rarity and wide range of clinical manifestations, but also for the vexed and very controversial questions of etiology and pathogenesis. Histochemical evidence suggests a lipoidosis (2) but widely differing results have been obtained by various workers. Direct chemical analyses of diseased tissue have been attempted by only a few investigators with equally contradictory findings (3, 4, 5).

Consequently, when we had the opportunity of obtaining tissue specimens from several cases of lipoid proteinosis† it was decided to investigate the lipid composition by thin layer chromatography in an attempt to see whether any tissue-foreign lipid could be demonstrated.

MATERIAL AND METHODS

Lipid Extraction

Specimens of skin were obtained from eight patients (10 to 50 years of age) and from normal controls by means of an 8 mm biopsy punch after cleansing the skin with alcohol. Each piece of tissue was then cut into several smaller sections and left for at least 12 hours (usually overnight) in a solution of chloroform-methanol (4:1, v/v); the ratio of the solvent to tissue being approximately 50:1. Subsequently the mixture was heated until the volume of fat solvent had evaporated to half the original. These extracts were then decanted and stored until just before use when all the samples were concentrated to approximately 0.1 ml (chloroform-methanol extract).

Isolation of Lipid Component of Compound Lipids

In order to isolate remaining lipid and/or the alcoholic component of these lipids, the remaining tissue was hydrolyzed by refluxing with 0.1 N alcoholic KOH for 10 minutes. The hydrolysate was added to distilled water in a separating funnel and exhaustively extracted with ether. The aqueous layer was discarded. After washing the ether extract several times with distilled water to remove all traces of alkali, the mixture was finally dried over anhydrous sodium sulfate. Upon filtration of the sodium sulfate and evaporation of the ether, an oily residue was obtained which was redissolved in chloroform and chromatographed (hydrolysis product).

Chromatography

Thin layer chromatography was performed according to 2 methods. The first, as described by Barbier *et al* (6) utilizes Silicagel G (Stahl, E. Merch A. G.) as adsorbent and cyclohexane/ethylacetate (70:30 v/v) as the solvent system. Chromatograms are developed by spraying with a 20% solution of antimony trichloride in chloroform, and subsequent heating at 120° C. They are then examined in daylight and under ultraviolet light (method 1). This procedure is suitable for the separation and detection of steroids, steroid esters and other alcoholic compounds which may be formed by the hydrolysis of lipids. Both extraction products were examined by this method.

The method of Wagner *et al* (7) which is specific for neutral fats and phospholipids was used to investigate the chloroform-methanol extract.

The same adsorbent is used as in the first method, but chloroform/methanol/water (65:25:4) is employed as the solvent system. Chromatograms are sprayed with a 10% solution of phosphomolybdic acid in alcohol and heated for 20 minutes at 120° C. to develop the color (method 2).

In all the chromatograms a test sample was run with a control sample on the same plate for comparative purposes.

RESULTS

No qualitative differences have been detected between the chloroform-methanol extract of test and control samples (method 1). In all samples cholesterol and its esters were the major components.

Chromatography of these extracts by method 2, showed no qualitative differences either, as in both test and control samples neutral fat, cephalin, lecithin and sphingomyelin were detected. However, it appeared that the phospholipid concentration was very much increased in relation

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to the neutral fats in the test samples as compared to the control samples (see fig. 1).

The hydrolysis product investigated by method 1 showed no qualitative differences between test and control samples. Both had the same seven fractions in roughly equal proportions when examined under ultraviolet light.

Because of differences in the quantity of subcutaneous fat obtained when taking biopsy specimens, it was considered necessary to confirm our results of an apparent clearcut increase in the phospholipid content of the diseased tissue. To do this, chloroform-methanol extracts of normal skin containing subcutaneous fat and extracts of normal skin not containing subcutaneous fat, were chromatographically compared with diseased skin according to method 2. This time a similar distribution of lipids was found in the diseased skin and normal skin not containing subcutaneous fat (Fig. 2).

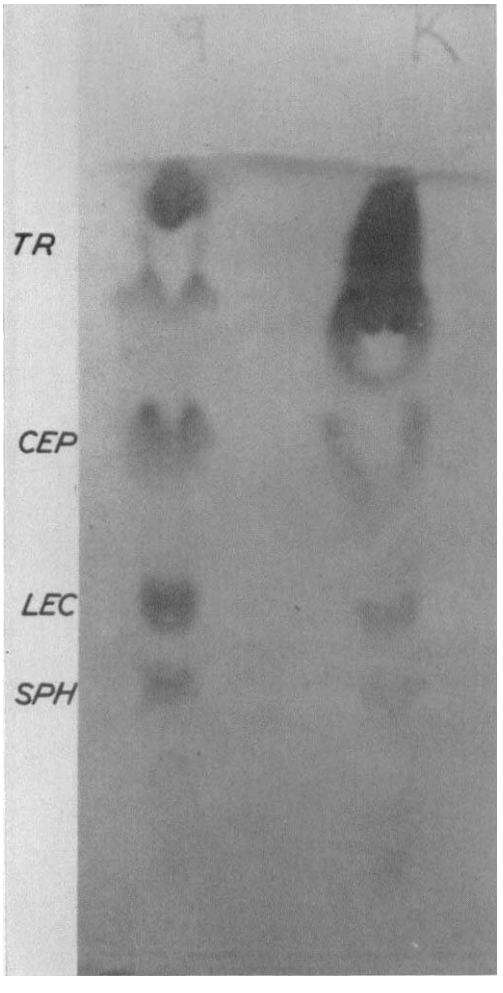

FIG. 1. Chromatogram of the chloroform-methanol extract of diseased skin (9) and normal skin (K). Tr = Triglyceride; Sph = Sphingomyelin; Lec = Lecithin; Cep = Cephalin.

DISCUSSION

Most of what is known about the nature of lipoid proteinosis has been derived from histochemical investigation. Original results suggested a lipoidosis, and the disease was classified as such by Thannhauser (8). Some investigators have found abnormalities in the serum lipids and occasional association with diabetes mellitus, which supported this concept. However, as more and more cases became known, it appeared that only a minority demonstrated these findings. In addition, as histochemical reports accumulated, it became increasingly evident that conflicting results were being obtained. Whereas Urbach and Wiethe found the deposits in the skin to contain largely phospholipids (a result with which several other authors concurred), others like McCusker and Caplan (9) considered them to consist of a glycoprotein with associated free or loosely bound lipid in the form of cholesterol, a small amount of neutral fat and only a trace of phospholipid. Wood, Urbach and Beerman (10) thought the deposit most likely to be a glycolipid. Ungar and Katzenellenbogen (11), reinvestigating one of Urbach and Wiethe's original cases 28 years later, could not identify any lipids except for a small amount of neutral fat. Weyhbrecht and Korting (12) concluded that the deposits contained large amounts of saturated triglycerides.

A second approach to the problem was that of lipid-extraction from tissue slices before staining. This also led to conflicting results, *e.g.* Urbach and Wiethe found the deposits only soluble in hot acetone, while Ungar and Katzenellenbogen were able to extract all sudanophil substances with cold alcohol.

The most complete results of chemical analysis of diseased tissue were published by Price *et al* (5), and are reproduced in Fig. 3. It is interesting to see that the total lipid per wet weight of tissue is *less* than that of the normal control, thus not supporting the concept of a lipoidosis. It is also noteworthy that in the diseased skin cholesterol constitutes 18.2% and phospholipids 3.6% of the

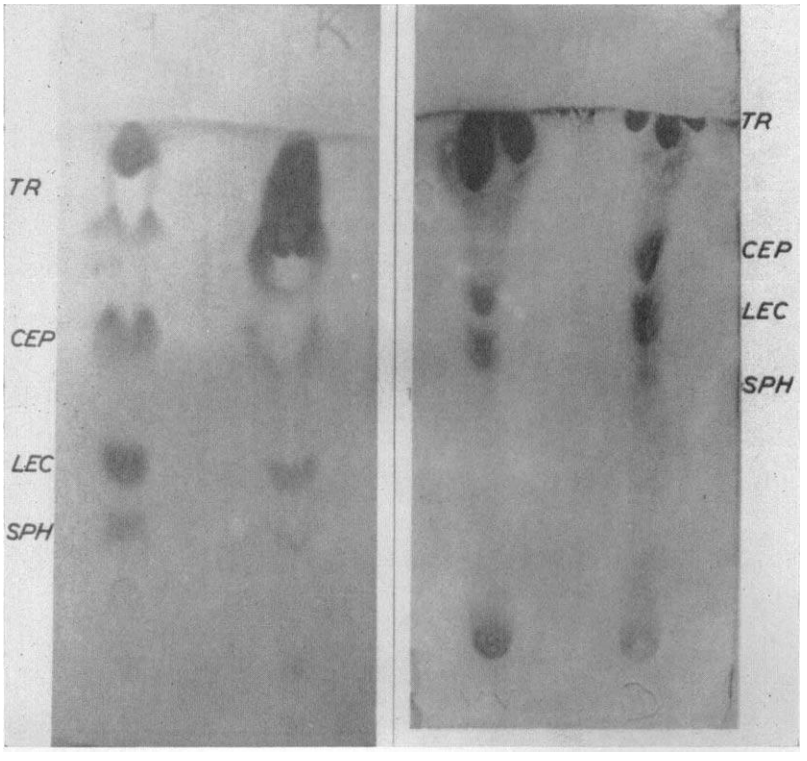

FIG. 2. Combined chromatograms of diseased skin (9), normal skin (VV), and dermis of normal skin (D). Tr = Triglyceride; Sph = Sphingomyelin; Lec = Lecithin; Cep = Cephalin.

	Diseased Tissue		Normal Skin	
Wet weight	436	512	20.50	0.956
Weight of:				
Total lipids	64	33	601	44
Cholesterol	12	6	21	1
Phospholipids	4	1.2	24	1.54
% of wet weight:				
Total lipids	1.47	0.64	2.92	4.60
Cholesterol	0.27	0.12	0.10	0.10
Phospholipids	0.09	0.02	0.12	0.16
% of total lipids:				
Cholesterol	18.7	18.2	3.5	2.28
Phospholipids	6.25	3.6	4.0	3.5

FIG. 3. Results of chemical analyses of skin, taken from Price et al.

total lipid as against 2.28% and 3.5% respectively in a control sample taken from a comparable area in a normal subject.* This does not agree with histochemical results which suggest an increase of phospholipids but do not reveal significant amounts of cholesterol in the majority of cases.

In our study uniform results were obtained in all eight cases, indicating that there is no qualitative difference in lipid content between the diseased skin and normal controls. Although no quantitative conclusions can be drawn, the same fractions were obtained in both control and sample specimens in roughly the same proportions, and only minor variations in quantity can be expected.

CONCLUSIONS

When these results and the more recent reports in the literature are considered together, it seems reasonable to conclude that lipoid proteinosis is no lipoidosis in the real sense of the word.

If the specificity of positive lipid stains is accepted, then it seems clear that the deposition of fat in the skin occurs as an incidental phenomenon during the course of the disease. Only normally occurring lipids are found in these deposits, but they may vary their composition from time to

* See original text.

time. This may conceivably occur either through actual change (re-absorption with deposition of a different lipid) or, more probably, by unmasking (release) of lipids during the process of tissue degeneration.

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