Lipids in Leprosy

2. Histochemistry of Lipids in Human Leprosy[1, 2]

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It is well known that lepra cells contain large amounts of lipid substances, especially phospholipids and fatty acids. Opinions as to the origin of these lipids have, however, been varied ([1, 8, 14, 16, 17, 28–32]).

This progressive accumulation of fatty substances in lepromatous macrophages and the absence of such accumulation in tuberculoid leprosy warrants continuing investigation with respect to its relationship to the apparent defect in cellular immunity that is characteristic of lepromatous leprosy. The following observations are a continuation of preliminary investigations initiated in this area.

MATERIALS AND METHODS

Skin biopsies from 21 Hong Kong and Taiwan Chinese patients with leprosy were divided into three groups: (1) 14 cases of histologically lepromatous leprosy, either untreated or treated with diaminodiphenyl sulfone or Ciba-1906, (2) two cases of histologically tuberculoid leprosy, and (3) five instances of leprosy treated with B.663 for from two months to over a year. The latter group included two cases of lepromatous and three cases of intermediate (dimorphous) leprosy. The B.663-treated cases were regarded as a distinct group because reflections of cellular lipid metabolism in these patients have characteristics differing from that in the other groups.

The specimens were immediately fixed in three different fixatives: Ridley's modified Zenker-formol solution for Triff stain ([34]), 10 per cent neutralized formalin for various stains useful in the identification of lipids, and formol-calcium solution for Baker's and Klüver-Barrera's methods for phospholipids.

Each of the tissues fixed in Ridley's solution was transferred to 70 per cent ethyl alcohol, dehydrated in graded alcohols, embedded in paraffin and cut at four microns. Tissues fixed in either 10 per cent neutralized formalin or formol-calcium solutions were embedded in carbowax (polyethylene glycol, Union Carbide Corp.), which does not interfere with the various lipid stains ([22]). They were cut at 4 to 8 microns in a low humidity, air-conditioned room at a temperature of approximately 22°C. The sections were then placed in a flotation bath made of 40 volumes of diethylene glycol, 50 volumes of distilled water and 10 volumes of 40 per cent formalin to dissolve the embedding medium and to spread the sections. They were then picked up on glass slides precoated with a thin film of gelatin consisting of a mixture of 10 gm. gelatin, 60 ml. distilled water, 50 ml. glycerine and 1 ml. phenol. The mixture had been preheated to dissolve the gelatin, and filtered.

Histochemical procedures were performed mainly in accord with the methods detailed by Pearse ([19]) and Okamoto *et al.* ([18]). The paraffin-embedded tissues were stained with hematoxylin and eosin, periodic acid-Schiff, Ziehl-Neelsen's stain, Triff stain ([34]), Sudan black B stain combined with lipid extraction by ethanol, pyridine, or chloroform-methanol (2:1) mixture for firmly bound lipids and ceroid. Sudan black B stain together with extraction by acidified chloroform-methanol mixture (1% conc. hydrochloric acid in chloroform-methanol, 2:1, mixture) was used as a negative control for the latter procedure. The carbowax-embedded tissues were subjected to the following battery of pro-

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	Sudan III & Fat Red		Nile Blue Sulfate		Neutral Fat
	No. of cases		No. of cases		
Lepromatous	14	orange-yellow	10	pink-blue or blue	— ~ +
Tuberculoid	2	trace	2	blue	—
B.663-treated	5	mostly red	5	mostly red	+ ~ +++

FIG. 1. Histochemistry of neutral lipid in human leprosy.

cedures: Sudan III stain (Daddi's method) for lipid in general; fett rot stain for lipid in general; Nile blue sulfate method for neutral fat and lipoid; Fischler's method for fatty acids together with lipid extraction by pyridine as a negative control; Okamoto, Shimamoto and Sonoda's methods for cholesterin and its esters; Okamoto, Ueda, Kusumoto and Hashimoto's method (modified Molisch reaction) for glycolipids; Okamoto, Shimamoto, Ueda, Kusumoto and Shibata's mercury diphenylcarbazone method for phospholipids and glycolipids; Baker's acid hematein method for phospholipids, together with Baker's pyridine extraction technic as a negative control; Klüver and Barrera's phthalocyanin method for phospholids excluding sphingomyelin, and probably glycolipids.

RESULTS

Neutral fat (Fig. 1). Three methods were employed for the identification of neutral fat as well as lipids in general. These consisted of the Sudan III, fett rot and Nile blue sulfate stains. There are no specific histochemical methods available for neutral fat. The Sudan and fett rot dyes stain not only neutral fat but also other lipids. The presence of neutral fat in tissues, however, may be suggested by a red color reaction with Sudan III and fett rot as well as by metachromasia (pink-red) in the Nile blue sulfate method. Other lipids than neutral fat such as cholesterol esters, phospholipids and fatty acids may be stained orange-yellow, and glycolipids are not generally stained with Sudan III. Neutral fat should stain red, cholesterols are pale red and other lipoid substances such as phospholipids are blue by means of Nile blue sulfate method ([18, 19]). As shown in Figure 1, lepra cells in lepromatous leprosy are stained orange-yellow by Sudan III and fett rot stains, and blue or pink-blue by Nile blue sulfate, suggesting that some lepra cells contain a small amount of neutral fat, but the main components are probably lipoid substances as first pointed out by Mitsuda ([16]). In contrast to lepromatous leprosy epithelioid cells in lesions of tuberculoid leprosy present only trace of color in both Sudan III and fett rot stains, and are stained light blue by Nile blue sulfate. Tuberculoid leprosy lesions contain only small amounts of lipids, which can scarcely be demonstrated by histochemical procedures.

Four of five cases treated with B.663 showed red reaction in lepra cells by the Nile blue sulfate method, and were stained red by Sudan III and fett rot stains, instead of orange-yellow as seen in untreated lepromatous leprosy. One of these five cases yielded almost similar results to that found in untreated lepromatous leprosy. This case had been treated for the shortest period among five cases. Most macrophages

	Number of cases	Fischler's method	Leprosy bacillus
Lepromatous	14	+++	many
Tuberculoid	2	— ~ +	none or few
B.663-treated	5	— ~ +	few

FIG. 2. Histochemistry of fatty acid in human leprosy.

(lepra cells) in B.663-treated cases have larger vacuoles or globules in their cytoplasm which probably contain larger amounts of neutral fat than in untreated lepromatous tissues.

One exception was found in the untreated lepromatous group. These sections revealed nonspecific panniculitis in reactional phase. The color reaction in lipid staining was peculiar in this case, differing from that in the other cases of lepromatous leprosy. Some macrophages had a great deal of neutral fat, demonstrating as bright red in Sudan III and fett rot stains, and as pink-red in the Nile blue sulfate method. Such neutral fat-laden cells are commonly seen in the deep corium and subcutis, or around the dermal appendages. Besides lepromatous changes, this case showed lepra reaction manifested as nonspecific panniculitis, vasculitis and less bacilli. Neutral fat deposition in macrophages in this case may have resulted from phagocytosis of necrotic fat cells in the subcutaneous tissue.

Free cholesterol and cholesterol ester. Sections were strained with the Okamoto *et al.* sulfuric acid-acetic acid and sulfuric acid methods ([18, 19]). All the cases studied are negative to both methods.

Fatty acid (Fig. 2). Fischler's method was employed for fatty acids. As shown in Figure 2, all of the 14 cases of lepromatous leprosy were positive for fatty acid staining (Fig. 3), whereas most cells in tuberculoid lesions were negative. Leprosy bacilli are themselves stained with this method and the bacilli had been destroyed and digested in the tuberculoid lesions. Large globi in lepromatous lesions stained dark blue. B.663-treated cases varied in reaction to fatty acid staining. Two of five cases were slightly positive and the other three were negative. Positive cases presented granular forms of bacilli which are stainable with this method. The positive results were reconfirmed by pyridine-extraction.

Glycolipid. Okamoto, Ueda, Kusumoto and Hashimoto's modified Molisch reaction was employed for the identification of glycolipids. All cases in the three groups studied were negative by this method.

Phospholipid (Fig. 4). Phospholipids were evaluated by three different methods consisting of Okamoto *et al.* mercury diphenylcarbazone method, Baker's acid hematein method, and Klüver and Barrera's copper phthalocyanin method. The results are shown in Figure 4.

Baker's method seemed to be the most sensitive. Positive reaction was clearly shown as dark blue against a yellowish brown background (Fig. 5), whereas in the other methods a positive reaction was presented as darker tone in a lighter background of the same color so that evaluation was relatively difficult.

The results of phospholipid-histochemistry indicate that lepromatous leprosy macrophages yield a strong reaction in most instances, whereas tuberculoid lesions are

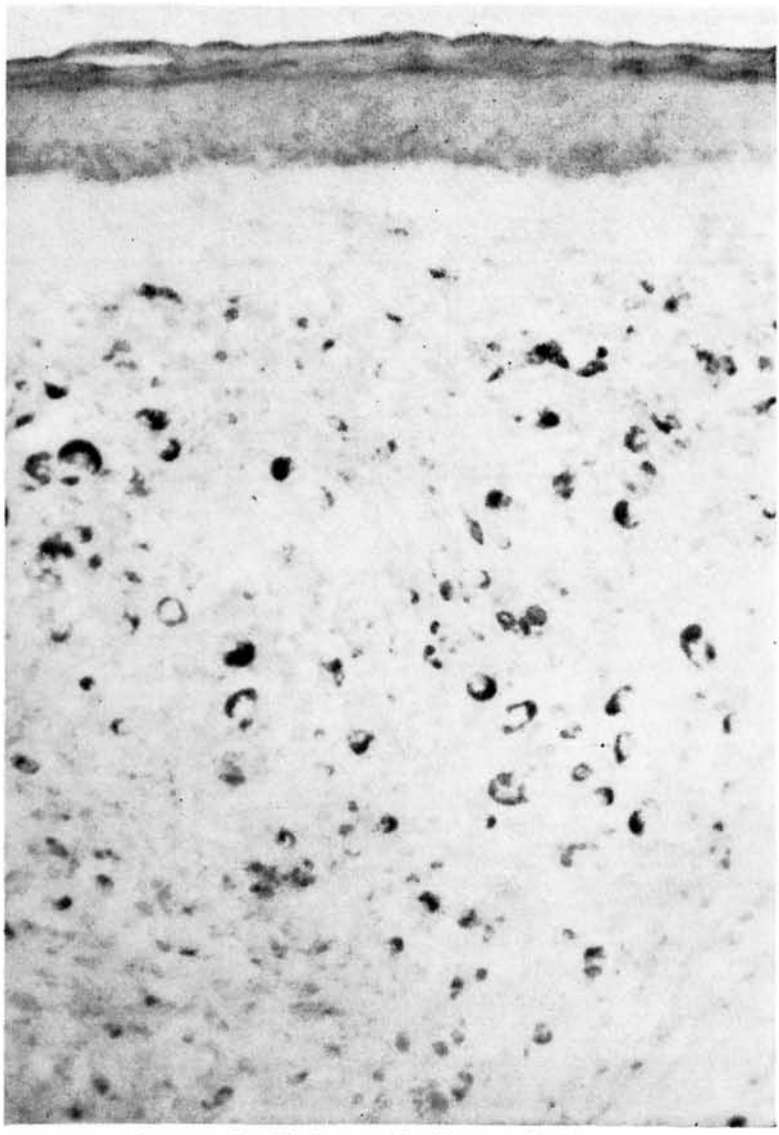


FIG. 3. Lepromatous leprosy. Fischler's stain for fatty acid. Original magnification X100.

negative. It is interesting that B.663-treated leprosy varied between negative and weakly positive.

Firmly bound lipids (Fig. 6). It is well known that certain kinds of lipid are not dissolved in ordinary fat solvents such as ethanol, methanol, chloroform, acetone or pyridine, because they are firmly bound to proteins or polysaccharides. These can be dissolved in acidified or alkalized solvents. Such lipids have been termed "firmly bound lipids." On the basis of such chemical characteristics of firmly bound lipids, a histochemical method for their identification has been designed in this laboratory. After extraction of paraffin-embedded tissues by a chloroform-methanol (2:1) mixture, most lipids should be removed from the tissues but firmly bound lipids should remain. They can be visualized by Sudan black B staining. If positive by this method, the firmly bound lipids should be removed when extracted with acidified organic solvents. This can be used as a negative control. Accordingly after extraction of paraffin-embedded tissues with a chloroform-methanol (2:1) mixture, the sections were stained with Sudan black B and coun-

	Okamoto et al method*				Baker's method		Kluver-Barrera		
	No. of cases	common	A	B	No. of cases		No. of cases	method	Phospholipid
Lepromatous	12	±~+	±~+	±~+	14	+++	14	+++	+++
Tuberculoid	2	−~±	−~±	−~±	2	—	2	—	—
B.663-treated	1	±	±	±	5	−~+	1	±	− ~ +

* Okamoto et al method;
Common method; positive for all phospholipids
"A" method ; positive for sphingomyelin
"B" method ; positive for sphingomyelin and lecithin
negative for cephalin

FIG. 4. Histochemistry of phospholipids in human leprosy.

terstained by neutral red. The negative control was provided by extraction with acidified chloroform-methanol (1% conc. hydrochloric acid in chloroform-methanol, 2:1, mixture).

The results obtained by this method are shown in Figure 6. Firmly bound lipids were absent or present in only small quantities in both lepromatous and tuberculoid leprosy, whereas B.663-treated leprosy presented strongly stained substances in macrophages. It is of interest that acid-fast substances, which are obviously different from mycobacteria morphologically, are shown in the same location within lepra cells even after lipid extraction in B.663-treated cases, (Fig. 7). Thus, the firmly bound lipids seen in B.663-treated cases presumably have another histochemical characteristic which is acid-fastness. The acid-fast, sudanophilic substances are shown around the rim of vacuoles in lepra cells. These histochemical characteristics of the substances in macrophages in B.663-treated leprosy are consistent with those of ceroid pigment, which is also a lipid derivative, acid-fast, sudanophilic and insoluble in ordinary organic solvents. Clinically the leprous lesions in B.663-treated patients are well known to be pigmented ([4]). Such pigmentation is probably due to accumulation of ceroid-like substances, in addition to the deposition of the drug itself in macrophages ([23]).

Summary of results (Fig. 8). In summary, Figure 8 notes that lepromatous leprosy presents strong lipid staining and has large quantities of fatty acids and phospholipids, and small amounts of neutral fat and firmly bound lipids, whereas tuberculoid leprosy reveals only a trace of lipid staining. B.663-treated leprosy, which shows a brown pigmentation intensively concentrated in leprous lesions, discloses peculiar characteristics in lipid histochemistry, and is remarkably different from other groups. B.663-treated lepromatous leprosy has more neutral fat and firmly bound lipids, and less fatty acids and phospholipids in the lesions than untreated lepromatous leprosy. Brown pigment in the leprous lesions of B.663-treated patients can be shown in lepra cells in the dermis on thick sections at 10 to 20 microns even after extraction with organic fat solvents, which are able to remove the drug from tissues ([23]). The pigmentation also remains in the paraffin-embedded tissues processed by alcohols and chloroform (Fig. 9). Lepra cells in the lesions of B.663-treated patients present acid-fast and sudanophilic substances, which are morphologically different from mycobacteria, around the rims of vacuoles even after most lipids and the drug have been removed by extraction with organic solvents. These characteristics are similar to those of ceroid pigment, which is a lipid derivative, sudanophilic, acid-fast and insoluble in ordinary fat solvents, and appears around the rim of fat globules in phagocytes ([23]).

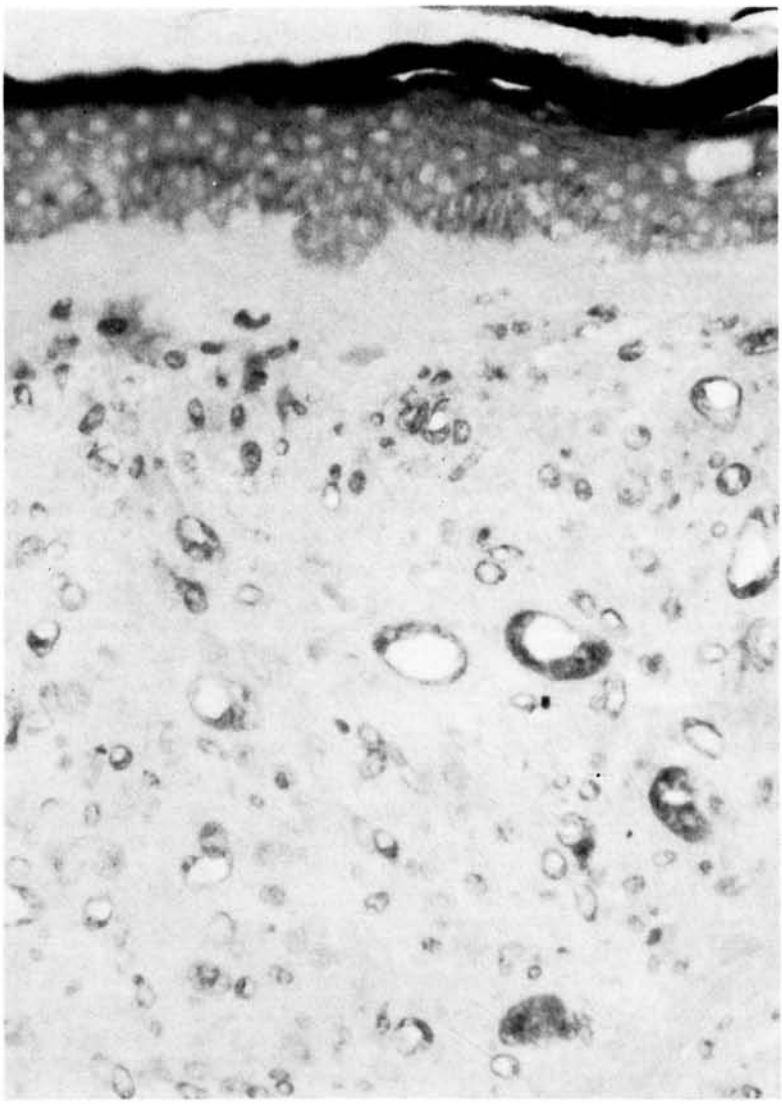


FIG. 5. Lepromatous leprosy. Baker's acid hematein method for phospholipid. Original magnification X100.

DISCUSSION

Lipid composition of lepra cells and its origin. The lepra cells were first named by Virchow, in 1863, ([33]) who noticed that these cells characteristically had a tendency to show vaculoes. These he thought to be the result of hydropic degeneration. Unna in 1898 ([32]) suggested on the basis of osmic acid stained tissues that the vacuoles contained lipid substances, and Mitsuda in 1918 ([16]) reported the presence of a certain kind of lipoid (phospholipid or glycolipid) and fatty acids, and absence of neutral fat and cholesterols in lepra cells. According to several subsequent studies phospholipids and fatty acids are regarded as major lipid components of lepra cells (Fig. 10). There are, however, some differences in concepts regarding the nature and origin of stored lipids. Some investigators have held that neutral fat is one of the important components of the cell lipids ([10, 13, 17]). Some report that cholesterol is contained in the stored lipids in advanced stages of lepromas ([29,30, 31]), while others deny the presence of lepra cell cholesterol ([9, 11, 12]). There seem to be three different views regarding

	Number of cases	Sudan black B stain after extraction by chloroform-methanol	Negative Control after extraction by acidified chloroform-methanol	Firmly bound lipid
Lepromatous	14	— ~ +	—	— ~ +
Tuberculoid	2	— ~ +	—	— ~ +
B.663-treated	5	+ ~ +++ (acid-fastness)	— ~ ±	+++

FIG. 6. Histochemistry of firmly bound lipid in human leprosy.

the origin of the stored lipids ([28]). One holds that the lipids are derived from bacilli phagocytosed by the macrophages ([11, 12, 16]). Another maintains that these lipids originate from fatty degeneration (lipophanerosis) occurring in the cytoplasm of lepra cells ([1]). A third view is that the lipid storage results from phagocytosis of fat droplets by macrophages. These fat droplets are believed to result from destruction of the subcutaneous fat by leprous infiltration ([10, 17]). Most observers, however, seem to hold that both factors, bacilli and lipophanerosis, may play a role in the origin of lipid storage in lepra cells ([13, 29, 30, 31]). Davison *et al.* ([8]) and Ghosh *et al.* ([11, 12]), pointed out that tuberculoid macrophages present only minimal lipid staining whereas lepromatous macrophages have a great deal of accumulated lipid substance. It is evident that lepromatous macrophages readily phagocy-

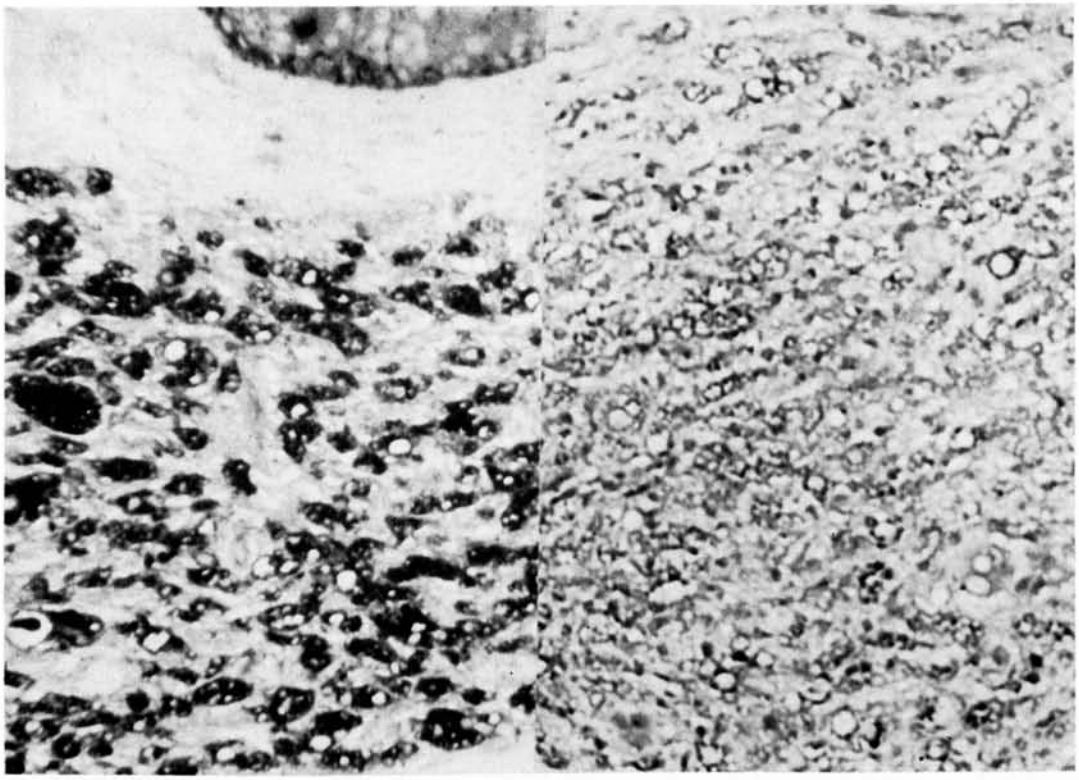


FIG. 7. B.663-treated leprosy. Sudan black B stain (left) and Ziehl-Neelsen stain (right) after lipid extraction. There is a sudanophilic acid-fast substance around the rim of vacuoles in lepra cells, which is insoluble in fat solvents. Original magnification X100.

	Lepromatous	Tuberculoid	B.663-treated
All Lipids	positive	trace	positive
Neutral fat	−~+	−	+++
Fatty acid	+++	−~+	−~+
Cholesterol & its ester	−	−	−
Glycolipid	−	−	−
Phospholipid	+++	−	−~+
Firmly bound lipid	−~+	−~+	+++
Leprosy bacillus	+++	−~+	+

FIG. 8. Lipid histochemistry in human leprosy.

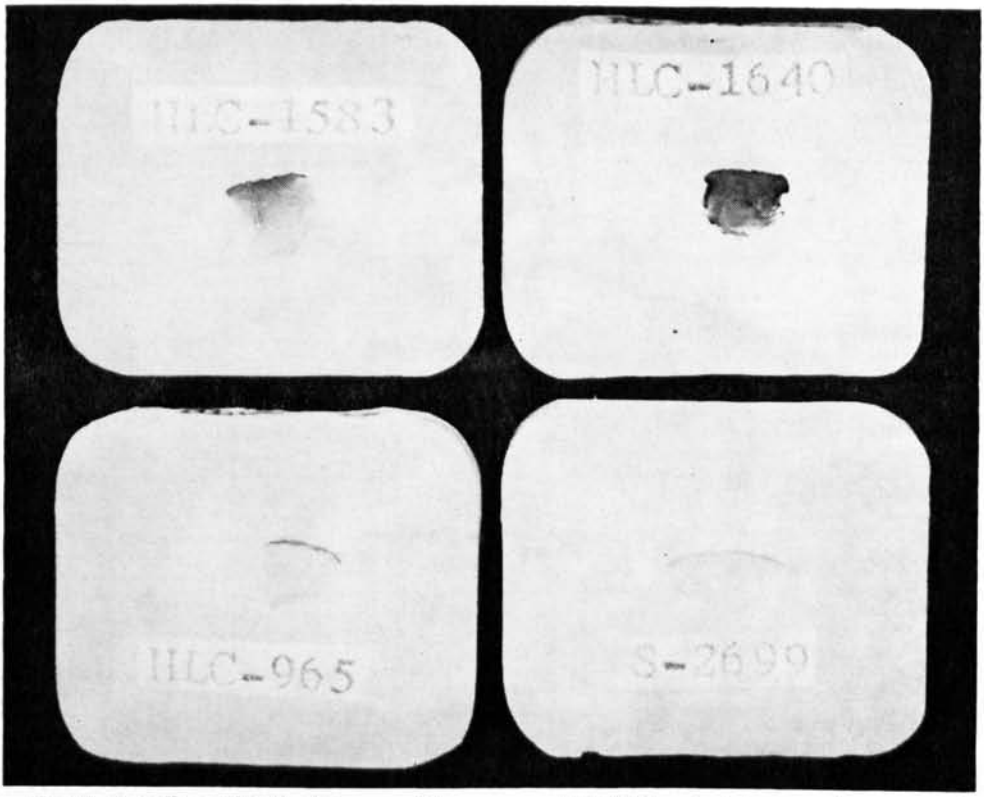


FIG. 9. Paraffin-embedded tissues of two cases of B.663-treated leprosy (upper row), compared with DDS-treated (left lower) and untreated (right lower) lepromatous leprosy. B.663-treated leprosy presents a pigmentation throughout the dermis even after lipids and lipophilic B.663 have been removed from tissues by fat solvents.

	NEUTRAL FAT	FATTY ACID	CHOLESTEROL FREE	CHOLESTEROL ESTER	GLYCO-LIPID	PHOSPHO-LIPID	LIPO-PROTEIN	FIRMLY BOUND LIPID	LIPID ORIGIN
MITSUDA, 1918	—	+ bacilli	—		+ lipoid				BACILLI
UEDA, 1948	±	—	—	−~+	—	+ lecithin			BACILLI & CELL DEGENERATION
HARADA, 1955	−~+ early late	+				+	+ early		BACILLI & CELL DEGENERATION
SUGAI, 1958	−~+	+ bacilli	− ~ +		—	+ lecithin			BACILLI & CELL DEGENERATION
ARAKAWA, 1958									CELL DEGENERATION
IMAEDA, 1960		+ saturated			+	+	+		LIPOPROTEIN FROM BLOOD
FUKUSHI, 1960	+								LIPOPHAGIA
GHOSH, 1962	±	+ bacilli	−	−	−	+			BACILLI
SAKURAI & SKINSNES	−~+	+ bacilli	−	−	−	+		−~+	BACILLI & CELL DEGENERATION

FIG. 10. Histochemistry of lipids in lepra cells and their origin.

tose *M. leprae* and many of the phagocytosed bacilli have undigested, lipid-rich cell walls. Figure 10 compares the present study with those of others. The findings are similar to those previously reported by Ueda ([31]), Harada ([13]), Sugai ([29]) and Ghosh *et al.* ([11, 12]), except for cholesterol, which was shown by Ueda and Sugai but not identified in this study. Fite ([9]) indicated that cholesterol was not present in early stages of lepromas, and Harada ([13]) stressed that neutral fat tends to be present in lepra cells with large fat globules or globi in advanced stages of lepromatous leprosy. Also from electron-microscopic studies it has been suggested by Yamamoto *et al.* ([36]) and Imaeda ([14]) that the foamy structure of human lepra cells is a terminal phase of intracellular structural changes. Histochemical methods for fatty acids and phospholipids stain the mycobacterial cell walls on bacterial smears ([5, 24]). Dharmendra also demonstrated that phospholipids were contained in *M. leprae* ([7]). Thus fatty acids and phospholipids are major lipid components of *M. leprae*. It seems reasonable to think that the stored lipids, especially fatty acids and phospholipids, are mainly of bacillary origin, and that neutral fat and cholesterol, both of which begin to appear in advanced stages, may possibly result from lipophanerosis of macrophages.

Comparison with lipid staining in tuberculosis and suppurative lesions is contributive to understanding. Kusumoto ([15]) stated that early tuberculous granulomas, without caseation, contain only minimal fat substances, mainly phospholipids. At this stage fairly large numbers of *M. tuberculosis* are usually present. As lesions progress and caseation necrosis develops, lipid staining becomes more intense and the bacilli in the lesions decrease in number. In advanced tuberculous granulomas having caseation necrosis, neutral fat and cholesterol, and phospholipids are found mainly in peripheral areas of caseation necrosis adjacent to the surrounding granulomatous zone. Lipids in nonspecific suppurative lesions are mainly composed of neutral fat and cholesterol fat whereas those in tuberculosis are mainly phospholipids (Fig. 11). Thus it seems reasonable to think that phospholipids are probably the main exogenous lipid component of reticuloendothelial cells in lesions caused by mycobacterial infections. The findings reported

			NEUTRAL FAT	FATTY ACID	CHOLESTEROL FREE	CHOLESTEROL ESTER	GLYCO-LIPID	PHOSPHO-LIPID	TUBERCULOSIS BACILLUS
TUBERCULOSIS	MILIARY	EARLY TUBERCLE WITHOUT CASEATION	±	−	−	−	−	+	+++
		TUBERCLE WITH CASEATION	++	−	−	++	−	++	+
	PRODUCTIVE	EARLY TUBERCLE	++	−	−	±	−	+	++
		TUBERCLE WITH MARKED CASEATION	++	−	+	+++	−	+++	±
SUPPURATIVE LESION: ABSCESS & GANGRENE			++	−	−	+	−	+	

FIG. 11. Lipid histochemistry of tuberculosis and suppuration.

by Kusumoto for other inflammatory conditions also seem to support the concept that neutral fat and cholesterol in macrophages may result from cell degeneration, and phospholipids may be derived from mycobacterial cell walls.

Chromatographic analyses by a thin-layer technic done recently in this laboratory ([26]) have shown that all major lipid fractions such as phospho- and glycolipid fractions, free cholesterol, free fatty acid, triglyceride, methyl ester of fatty acid and cholesterol ester, are identifiable in purified *M. lepraemurium* suspensions as well as in both human and murine leprous tissues, as shown in Figure 14. Murine leprosy bacilli contain large amounts of lipoid substances (phospho- and glycolipid) and free fatty acid, whereas murine leprosy tissues have more free cholesterol than do *M. lepraemurium* and normal skin. This indicates also that accumulation of free cholesterol in the tissues is probably caused by tissue lipophanerosis. Murine lepromas and human lepromatous tissues contain more free fatty acids and lipoid substances (phospho- and glycolipid) than control tissues or tuberculoid leprosy lesions. Both free fatty acids and lipoid substances are also richly contained in the bacilli. These results also support the concept concerning the origin of stored lipids in lepra cells outlined above. Further studies on lipids in leprosy by chromatographic analyses are in preparation ([26]).

B.663 pigmentation. Leprous lesions having the peculiar pigmentation caused by B.663 treatment show yellowish-brown pigment in the cytoplasm of macrophages, which can be identified in thick unstained or methylene blue stained paraffin-sections of tissue at 10 to 20 microns, even after B.663 and most lipids have been removed by organic solvents ([23]). Since B.663 is a red dye, abnormal coloration of the skin can be produced by a deposition of the drug itself. In addition, however, there must be other pigment than the drug in the leprous macrophages which are not dissolved in organic solvents.

We have noted ([25]) that a rabbit fed B.663 (50 mg./kg. for 5 days) in vegetable oil, at autopsy showed that its fat tissues had an usual yellow tint which turns to orange-red shortly after exposure to the air. These fat tissues, however, retain their normal appearance if they are kept in a nitrogen gas atmosphere with exposure to sun light but without exposure to the air. B.663 is dissolved in oil or organic fat solvents, but is not water-soluble. This suggests that B.663, a red dye, becomes colorless after absorption through the digestive canal, is deposited in fat cells and may again be-

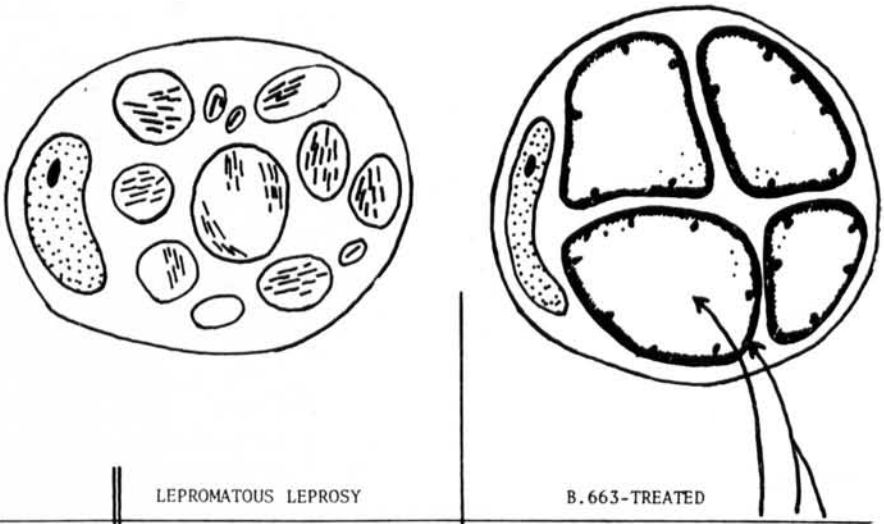
	CEROID	PIGMENT IN B.663 TREATED
NATURAL COLOR	yellow brown	yellow brown
LOCATION	rim of fat globules of phagocytes	rim of fat globules of lepra cells
WATER	insoluble	insoluble
FAT SOLVENTS	insoluble	insoluble
BLEACHING	— ~ +	— 2hrs + 24hrs
IRON REACTION	— ~ +	— ~ +
GMELIN R.	—	—
STEIGER R.	—	—
ACID FASTNESS	+	+
HUECK METHOD	— ~ +	+
SCHMORL R.	— ~ +	+
CHROME ALUM HEM.	— ~ +	+
P A S	— ~ +	+

FIG. 12. Characteristics of pigment in B.663-treated leprosy.

come red on oxidation. Intraperitoneal injection of B.663 dissolved in vegetable oil (25 mg. in 0.5 ml. of oil) into mice infected with *M. lepraemurium* resulted in a reddish-brown or reddish-black pigmentation limited to nodular granulomas in the peritoneum by eight weeks after injection ([25]). Peritoneal spread preparations and frozen sections of various organs such as liver, kidney, lung and spleen showed dark red, elongated cylindric crystals. These crystals were completely dissolved and removed from the tissues by alcohols, and the pigmentation disappeared after extraction with fat solvents. On the basis of these results and other reports ([2, 3, 6, 27, 35]), it seems evident that the early stage of red pigmentation is due to a deposition of B.663 itself in tissues, mainly in fat cells, and that the deposited drug can be removed by fat solvents. However, in human lesions of patients treated with B.663 for several months or for over a year, there appear to be other pigments than the B.663 present in macrophages which are not removed by organic solvents. This is yellowish-brown and usually located around the rim of the fat globules in macrophages. As shown in Figure 9, pigmentation still remains after tissues have been processed in alcohols and chloroform which extract the drug from the tissues ([23]).

In various histochemical stainings, B.663-treated leprosy is significantly different from untreated lepromatous leprosy. The B.663 treated lesions contain more neutral fat and firmly bound lipids, and lesser amounts of fatty acids and phospholipids (Fig. 8). Leprosy bacilli are far fewer in the lepra cells in the B.663 treated and are granular. Histochemical characteristics of the pigment in macrophages of the B.663-treated lesions are quite similar to those of ceroid pigment, as shown in Figure 12 ([23]). Most macrophages in the lesions of B.663-treated leprosy have large fat vacuoles in the cytoplasm, which are filled with neutral fat and the rim of which is made up of acid-fast, sudanophilic ceroid-like substance that is insoluble in ordinary fat solvents (Figs. 7 & 13). Some investigators have proposed a melanin-theory for late B.663 pigmentation ([4]). Late B.663 pigmentation, however, is usually seen also deep in the corium, where melanin is not generally present except in certain abnormal conditions such as blue nevi or Ota's nevus.

Since ceroid is a lipid derivative ([20]), it may be that this pigment is produced from



	LEPROMATOUS LEPROSY	B.663-TREATED
LEPROSY BACILLUS	NUMEROUS, SOLID	A FEW, GRANULAR
MAIN LIPIDS	PHOSPHOLIPID, FATTY ACID	NEUTRAL FAT, FIRMLY BOUND LIPID
PIGMENT	NONE	YELLOW BROWN, ACID FAST, SUDANOPHILIC INSOLUBLE IN FAT SOLVENTS

FIG. 13. Diagram of histochemical characteristics of lepra cells in B.663-treated leprosy as compared with untreated leprosy.

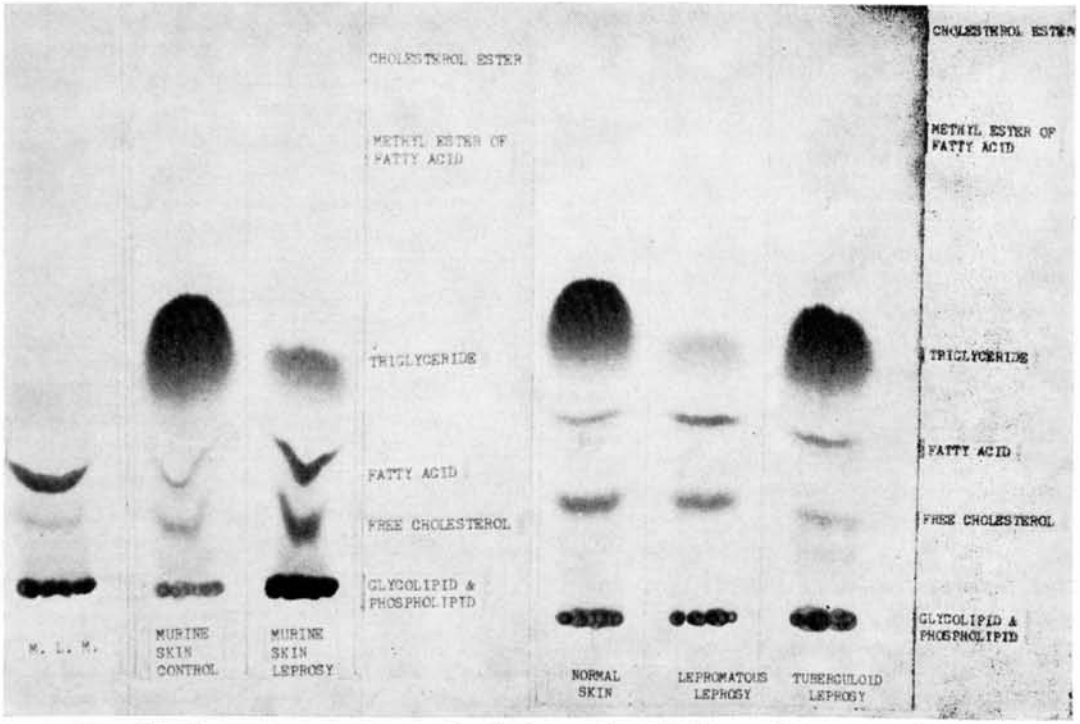


FIG. 14. Separation into major lipid classes by thin-layer chromotography in *M. lepraemurium*, and murine and human leprosy tissues. *Thin-layer plate*: Silica gel H, 250 microns thick (Analtech). *Solvent*: Petroleum ether-diethyl ether-acetic acid (90:10:1). *Detection*: 100% sulfuric acid with charring.

acid-fast, lipid components of bacilli which have been altered by action of the drug. The released acid-fast components of mycobacteria may be bound with substances which are insoluble in fat solvents, as suggested by Reeves and Anderson ([21]).

Problems in the field of lipid histochemistry. As shown in Figure 14, our chromatographic studies indicate that all major lipid fractions are present in the bacilli as well as in leprous tissues, although cholesterols are not identified by histochemical methods. Histochemical methods for certain lipids are not sensitive enough to identify small amounts of lipids. However, histochemistry has some advantages over biochemical analysis. In identifying chemical substances by color reactions within cells or tissues there is no sacrifice of morphologic observations, and the locations of such chemical substances within cells or tissues is indicated. Biochemical analyses of lesions often include normal cellular or tissue components in addition to the lesion material under analysis. Combination of both methods of study, as here attempted, seem more valuable and helpful to an understanding of the problems posed in the area of the lipid metabolism of lepra cells.

SUMMARY

Histochemical studies on lipids were performed on human biopsy materials which included lepromatous (14 cases), tuberculoid (2 cases), and B.663-treated (5 cases) lepromatous leprosy. Lepromatous leprosy presents strong lipid staining whereas tuberculoid reveals only minimal staining. Lepra cells in lepromatous leprosy contain much phospholipids and fatty acids, and only small amounts of neutral fat. Cholesterols were not found in this study. Stored phospholipids and fatty acids probably originate from phagocytosed *M. leprae*. Neutral fat may result from lipophanerosis. B.663-treated leprosy presents yellowish-brown pigment in macrophages of leprous lesions, which is similar to ceroid pigment in histochemical characteristics, being insoluble in fat solvents, acid-fast and being sudanophilic. Compared with results from chromatographic analyses of lipid in leprosy, histochemical technics are not sensitive enough to identify small amounts of lipids.

RESUMEN

Utilizando métodos histoquímicos, se efectuaron estudios de los lípidos de material de biopsia de pacientes con lepra lepromatosa (14 casos), tuberculoide (2 casos) y lepra lepromatosa tratada con B.663 (5 casos). La lepra lepromatosa presenta tinción intensa de lípidos mientras que la lepra tuberculoide revela solamente una tinción mínima. Las células de lepra en la lepra lepromatosa contienen muchos fosfolípidos y ácidos grasos y sólo pequeñas cantidades de grasa neutra. En este estudio no se encontraron colesteroles. Los fosfolípidos y ácidos grasos almacenados probablemente se originan de los *M. leprae* fagocitados. La grasa neutra puede ser consecuencia de lipofanerosis. Los casos de lepra tratados con B.663 presentan un pigmento amarillo-marrón en los macrófagos de las lesiones de lepra, que es similar en sus características histoquímicas al pigmento ceroide, ya que es insoluble en solventes de lípides, es ácido resistente y sudanofílico. En comparación con los resultados obtenidos de los análisis cromatográficos de lípidos, las técnicas histoquímicas no son lo suficientemente sensibles como para identificar pequeñas cantidades de lípidos.

RÉSUMÉ

Des études histochimiques des lipides ont été menées sur du matériel provenant de biopsies humaines, qui comprenaient 14 cas lépromateux, 2 cas tuberculoïdes, et 5 cas de lèpre lépromateuse traités par le B.663. Dans la lèpre lépromateuse, on a noté une forte coloration des lipides, alors que dans la lèpre tuberculoïde, la coloration était très minime. Dans la lèpre lépromateuse, les cellules lépreuses contiennent beaucoup de phospholipides et d'acides gras, mais seulement de petites quantités de graisse neutre. On n'a pas relevé de cholestérol au cours de cette étude. Les phospholipides et les acides entreposés ont probablement leur origine dans les *M. leprae* phagocytés. La graisse neutre peut provenir de la lipophanérose. Dans les cas de lèpre traités par le B.663, on a observé un pigment brun-jaunâtre dans les macrophages des lésions lépreuses, semblable au pigment céroïde au point de vue des caractéristiques histochimiques, insoluble dans les solvants de graisse, acido-résistant et soudanophile. Quand on compare ces résultats à ceux des analyses chromatographiques des lipides dans la lèpre, on conclut que les techniques histochimiques ne sont pas assez sensibles pour identifier de petites quantités de lipides.

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