CELLULAR REACTIONS TO WAXES FROM MYCOBACTERIUM LEPRAE

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PLATE 34

(Received for publication, July 9, 1935)

The plan for chemical analysis of the Mycobacteria sponsored by the National Tuberculosis Association included an acid-fast strain isolated from a case of leprosy, as well as selected strains of human, bovine, and avian tubercle bacilli, and this study deals with the waxes extracted from *Mycobacterium leprae*.[1] The chemical analyses have not yet been completely reported (1, 2). The materials used have been in different stages of purification and have therefore provided the opportunity to compare the complex cellular reactions to mixtures of substances with the simple response to highly purified crystalline alcohols.

Materials and Methods

The strain of organisms used for the analysis was obtained from a case of leprosy (Apa case) in Honolulu, about 1909. It has been kept at the Hygienic Laboratory, Washington, D. C., as Strain 370; on Feb. 4, 1926, a subculture was given to the Mulford Biological Laboratories, Glenolden, Pennsylvania. It was there grown on Long's synthetic medium in quantities adequate for chemical analysis.

Five preparations of wax fractions from the *Bacillus leprae* have been studied.

1. A Crude Chloroform-Soluble Wax.—This was obtained in the same manner as the corresponding fraction from tubercle bacilli (3). This fraction has not yet been analyzed completely, but Dr. Anderson reports that it is almost free from phosphorus and nitrogen and hence is not a phosphatide (1). It is a red, wax-like substance, looking like beeswax except for the color.

2. A Crude Wax Obtained from the Purification of the Lepra Phosphatide.—This material could be removed from the crude phosphatide because it is more

[1] We are indebted for this material to Dr. R. J. Anderson and his associates at Yale University, who extracted the waxes from *Mycobacterium leprae*.

soluble in a mixture of ether and acetone than the phosphatide, and it came out on cooling the acetone solution (1). Concerning this product, Dr. Anderson has written us, "This product consists mainly of a solid glyceride which I call 'Leprosin.' It contains a number of fatty acids and the alcohols $C_{20}H_{42}O$ and $C_{18}H_{38}O$, identical with the alcohols isolated from the wax of the timothy grass bacillus." This material is a slightly pink, amorphous powder; that is, it carries some of the pigment so characteristic of the *B. leprae*.

3. Leprosin.—This was obtained from the purification of the preceding material. Though a glyceride instead of an alcohol, it corresponds in a general way in its physical properties to the unsaponifiable material from tubercle bacilli. It is a snow white powder, with a melting point of 54°C. It is optically active.

$$[\alpha]_D^{23} \text{ in chloroform } + 4.2°.$$

4. Leprosinic Acid.—This was obtained from the analysis of the leprosin and in physical properties is like it. It has a melting point of 61–62°C. and is dextrorotatory:

$$[\alpha]_D^{23} \text{ in chloroform } + 4.7°.$$

All four of these materials from the *B. leprae* are acid-fast.

5. Alcohol II from the Unsaponifiable Matter of the B. leprae.—This is a pure white material consisting of feather-like crystals. It has a melting point of 84°C. and a formula of $C_{25}H_{44}O_2$. It is not acid-fast.

Besides this alcohol from the *B. leprae* we have also received from Dr. Anderson a similar material as follows:

Alcohol Isolated from the Wax of the Timothy Grass Bacillus.—This is a pure white material made up of fine, needle-like crystals. Its melting point is 63°C. It is dextrorotatory:

$$[\alpha]_D^{21} \text{ in ether } + 6.93°.$$

Its formula is $C_{20}H_{42}O$. It is not acid-fast.

None of these preparations has been given in nujol, the method used in our first study of wax fractions from tubercle bacilli (4). Rather it has been found better to introduce these materials in the form of a dry powder through an incision under ether anesthesia. The crude waxes can be ground into a fine powder if they are first chilled with dry ice until brittle. The more purified substances, the leprosin, the leprosinic acid, and the alcohols, do not need more than simple grinding to separate the clumps of crystals.

The method of preparing these waxes from the *B. leprae* in colloidal suspension, which was developed by one of us with the unsaponifiable material from the tubercle bacillus (5), was tried with the lepra fractions but with less success. It consisted of dissolving the wax in chloroform and adding an equal amount of hot alcohol. To this solution the same amount of distilled water was added drop by

drop, which procedure threw down the wax in the form of a precipitate. The suspension obtained from the crude chloroform-soluble wax was not as fine as that from the unsaponifiable material from the tubercle bacillus but rather consisted of particles about 7μ in diameter which settled out into quite large aggregates. This material, therefore, had to be freshly prepared for each injection.

When the crude wax obtained from the purification of the lepra phosphatide was prepared for colloidal suspension, the precipitate was of more or less regular pentagonal particles, highly refractive and suggesting a crystalline form. There was no agglutination of these particles.

In attempting to prepare similar suspensions of leprosin and leprosinic acid, the addition of distilled water resulted in the separation of minute droplets of an oily nature which formed an unstable emulsion. This emulsion had a tendency to curdle and adhere to the surface of the flask. This was in marked contrast to the stability of the suspension prepared from the unsaponifiable material from H-37.

RESULTS

Reactions to the Crude Chloroform-Soluble Wax from B. leprae.—The crude chloroform-soluble wax from the *B. leprae* was given intraperitoneally to six rabbits, as shown in Table I.

Rabbits R 4268 and R 4269 both received the material after it had been powdered with dry ice. The material did not give a simple reaction; where the wax had lodged, the area became infiltrated with leucocytes making an abscess. Around these abscesses were monocytes, fibroblasts, and clasmatocytes, filled with leucocytes. In the omentum the reaction was not uniform; there were abscesses, bands of fibroblasts, and foci of monocytes, some showing partial fusion into giant cells. There was a marked dilatation of the vessels. The retrosternal lymph nodes had their sinuses filled with monocytes and in some places they were present also in the follicles.

The same tendency toward the formation of abscesses followed the introduction of the material in colloidal suspension.

This preparation showed a marked tendency to a clumping of the particles, with the result that the dose varied; Rabbit R 4189 received a small dose, R 4187 an average dose, and R 4188 a massive dose. The abscesses were smaller because the masses of the wax had been smaller. They were filled with clasmatocytes containing leucocytes. Around the abscesses were monocytes and giant cells, many of them vacuolated, indicating a phagocytosis of the wax. The edge of one of the abscesses with a narrow border of giant cells is shown in Fig. 1, from Rabbit R 4188. Bands of fibroblasts were extensive; in one animal of the group, R 4188, there was an extreme involvement of the retrosternal lymph nodes with

TABLE I

Protocols of Rabbits Which Received the Crude Chloroform-Soluble Wax from B. leprae

Animal No.	No. and amount of injections	Time	Method of preparing material	Peritoneal exudate			Tissues
				Percentage of			
				PMN	Lymph.	Mono.	
		days					
R 2795	1 20 mg.	4 K	Dry powder through cannula	—	—	—	Most of the wax in one bolus. Coccidiosis present
R 4268	1 50 mg.	6 K	Material powdered with dry ice and introduced through incision under anesthesia	15.0	14.0	70.80	Many abscesses, 2 to 3 cm. in diameter in omentum, on cecum, in the peritoneal wall, and in incision. Abscesses surrounded by monocytes and fibroblasts, with clasmatocytes containing leucocytes in the border. Omentum almost too massive to study as film and very complex: zones of fibroblasts; many small foci of monocytes showing partial fusion into giant cells, the center of which contain leucocytes. Marked vascular dilatation. Retrosternal nodes have sinuses filled with monocytes
R 4269	1 50 mg.	6 K	" "	63.68	0	36.31	Many abscesses 2 to 3 cm. in diameter in omentum surrounded by monocytes, fibroblasts, and clasmatocytes containing leucocytes. Many small foreign body giant cells with vacuolated cytoplasm. Diaphragm shows tubercles of giant cells in which there is only partial fusion of the monocytes. Leucocytes infiltrating the tissues. Bands of fibroblasts; marked dilatation of the vessels. Extensive reactions of the same type in retrosternal nodes replacing part of the follicles

R 4187	3 20 mg.	4 4 K 5 days after third injection	Colloidal suspension	1.78	22.19	76.02	No symptoms. No rise in temperature. Small abscesses in body wall surrounded by fibroblasts and clasmatocytes containing leucocytes. Omentum has no large abscesses but many small tubercles of foreign body giant cells and monocytes, some vacuolated. In the center of the tubercles were leucocytes. Dilatation of the vessels and marked bands of fibroblasts. Few giant cells in retrosternal lymph node. One small hemorrhage in one lung, infiltrated with monocytes
R 4188	3 20 mg.	4 4 K 5 days after third injection	" "	8.88	24.36	66.75	No symptoms. No rise in temperature. Abscesses 4 to 10 mm. in diameter on surface of cecum, in capsule of liver and spleen, on diaphragm, in body wall, and in the omentum. Abscesses contain clasmatocytes filled with leucocytes and are surrounded by a narrow band of monocytes. Omentum has many small tubercles of foreign body giant cells and monocytes, some of them vacuolated. Dilatation of vessels and bands of fibroblasts. Lungs had several small, translucent nodules which were made up of monocytes. Retrosternal nodes show extreme involvement with monocytes and giant cells both in the sinuses and in the follicles. In the fresh tissue, typical epithelioid cells were seen, confirmed in sections
R 4189	3 20 mg.	4 4 K 5 days after third injection	" "	0.25	42.45	57.28	Very slight reaction; a few abscesses in the body wall and a few foreign body giant cells in the omentum

TABLE II

Protocols of Rabbits Which Received the Wax Obtained in the Purification of the Phosphatide of B. leprae

Animal No.	No. and amount of injections	Time	Method of preparing material	Peritoneal exudate			Tissues
				Percentage of			
				PMN	Lymph.	Mono.	
		days					
R 2715	1 20 mg.	3 K	Dry powder through cannula blown in with air	1.02	5.10	93.87	Omentum showed increased size of milk spots with particles of wax on them. Increase in monocytes; occasional fusion into giant cells, some having as many as 50 nuclei. Few epithelioid cells. Infiltration with neutrophilic leucocytes and a few myelocytes. No increase in fibroblasts. Retrosternal node has many monocytes in the sinuses
R 2800	1 20 mg.	4 K	Dry powder through incision under anesthesia	1.13	1.13	97.72	Omentum showed increased size of milk spots with particles of wax on them. Around the particles were young monocytes showing no signs of phagocytosis; that is, no vacuoles staining with neutral red. Few neutrophilic and eosinophilic leucocytes. No increase in fibroblasts. Eosinophilic myelocytes prominent in the bone marrow
R 2798	2 20 mg.	4 D	Dry powder through cannula. Died after second injection which punctured the liver	—	—	—	Wax in single bolus and reaction slight. Few foreign body giant cells in omentum. Blood in retrosternal lymph nodes 5 min. after puncture of the liver

R 2763	2 20 mg.	4 K 3½ hrs. after second injection	Dry powder through cannula blown in with air	Cells all dead			3 hrs. after the second injection the animal had a convulsion, was extremely sensitive on being touched, became cyanotic, pulse was about 250 and respiration was slow. Temperature was 111.5° and the animal was killed. Small abscesses in mesentery, diaphragm, and omentum. Omentum showed increased density of the milk spots with monocytes and giant cells, dilatation of the vessels, neutrophilic leucocytes, both free and in clasmatocytes, and a few epithelioid cells. Marked damage of the lymphocytes in the nodes, probably due to the temperature. Congestion of the lungs and liver. Hemorrhage in the bone marrow
R 4184	3 20 mg.	4 4 K 6 days after the third injection	Colloidal suspension in 4 cc. water	5.59	45.29	49.10	No symptoms; no rise in temperature. Small abscesses on peritoneal wall near places of injections, on the large intestine, on the diaphragm, and in the omentum. Milk spots of omentum increased in size and number. Abscesses of omentum contain many clasmatocytes filled with leucocytes and have a wide border of giant cells. Many giant cells in tubercles and diffusely scattered. Marked vascularization, bands of fibroblasts, and increase in fibrous tissue. Acid-fast stain negative. Retrosternal nodes show extreme numbers of monocytes in the sinuses and the follicles are almost replaced by them. Small, translucent nodules in lungs made up of monocytes

TABLE II—*Concluded*

Animal No.	No. and amount of injections	Time	Method of preparing material	Peritoneal exudate			Tissues
				Percentage of			
				PMN	Lymph.	Mono.	
		days					
R 4185	3 20 mg.	4 4 K 6 days after the third injection	Colloidal suspension in 4 cc. water	27.62	18.31	54.05	No symptoms. No rise in temperature. Lesions like those of R 4184, except less formation of fibers in the omentum and less reaction in the retrosternal nodes. Nodules of monocytes in the lungs
R 2771	3 20 mg.	4 4 K 5 days after the third injection	Dry powder through cannula	5.20	13.54	81.25	No symptoms after the first injection. After the second, rapid respiration and temperature fell 2.1° and then rose 3.3°. After the third the temperature fell 2.5° and then rose 4.8°. One of the injections had lodged in the sheath of the external oblique muscle, where there was a nodule of giant cells, and monocytes, many of them vacuolated; very few neutrophilic leucocytes but many eosinophilic. Omentum showed increased density of the milk spots due to monocytes and giant cells, whose cytoplasm was filled with vacuoles. Some wax still to be seen on the milk spots. Some typical epithelioid cells. Moderate increase in fibroblasts

monocytes and giant cells, as shown in Fig. 3 which is a section through the sinuses. The follicles of this node were also extensively replaced by similar cells. Besides these reactions, typical epithelioid cells were found in the omenta and the retrosternal lymph nodes of these animals, both with the supravital technique and in sections.

Reactions to Wax from the Purification of the Phosphatide of B. leprae.—The wax obtained from the purification of the lepra phosphatide was given to seven rabbits, as shown in Table II. This was the only material given which was followed by any symptoms and they proved to be inconstant.

The early reaction was shown by two rabbits, R 2715 and R 2800, one of which received the material through a cannula and the other through an incision. In neither animal were there symptoms after the injection. The wax had lodged on the milk spots of the omentum, and around the particles of the wax there had been a multiplication of monocytes; in places these monocytes had started to fuse into giant cells. There were occasional epithelioid cells but there was no increase in fibroblasts.

Rabbit R 2763 showed extreme disturbance after the second injection, 3 hours later it had a convulsion, the temperature rose to 111.5°, and the animal was therefore killed. A second rabbit, R 2771, had a rise in temperature of 3.3° after a third injection but survived. We did not determine the cause of the fever in Rabbit R 2763. The material injected showed no bacteria. The brain and cord were normal; there were small abscesses in the omentum, in the mesentery, and on the diaphragm, but not as many as in the rabbits of the preceding series. There was the same reaction of monocytes and giant cells around the wax which had lodged on the milk spots of the omentum.

Rabbit R 2771 showed similar cellular reactions, complex in type; there were foreign body giant cells around the particles of wax, a few neutrophilic leucocytes, and many eosinophilic leucocytes. There were epithelioid cells and an increase in fibroblasts.

The material was then given to two rabbits, R 4184 and R 4185, in three doses in colloidal suspension. None of these injections caused symptoms and there was no rise in temperature. In making the suspension, the material had been heated. There were small abscesses wherever the wax had lodged. The abscesses had wide borders of monocytes and giant cells, as is shown in Fig. 2. The width of the border of giant cells can be seen to be greater than in Fig. 1, which was taken from an animal which had received the crude chloroform-soluble wax. Indeed, almost the entire section shown in Fig. 2 is of monocytes except the abscess on the lower border. Besides this, the material from Rabbit R 4184 showed many large masses of giant cells, some in tubercles and some diffusely scattered, so that when the total reaction was compared with that in the animals

TABLE III

Protocols of Rabbits Receiving Leprosin

Animal No.	No. and amount of injections	Time	Method of preparing material	Peritoneal exudate			Tissues
				Percentage of			
				PMN	Lymph.	Mono.	
		days					
R 2801	2 20 mg.	4 K 4 days after second injection	Dry powder through incision under anesthesia	6.5	5.0	88.0	No symptoms after either injection. Omentum shows marked increase in size of milk spots with foreign body giant cells enclosing the wax. Marked bands of fibroblasts growing as in tissue culture. No epithelioid cells. Eosinophilic leucocytes in the retrosternal lymph nodes
R 4264	1 50 mg.	7 K	Colloidal suspension	5.0	13.50	80.75	In omentum the predominant reaction is an extensive formation of tubercles of foreign body giant cells. Some have hollow centers and dense cytoplasm; others have a vacuolated cytoplasm. A few abscesses in the omentum, with walls dense with giant cells. Except for these abscesses very slight infiltration with neutrophilic leucocytes. Increase in fibrous tissue. Abscess in body wall near site of injection with monocytes and giant cells in its border. Retrosternal nodes show an extensive infiltration of sinuses and follicles with foreign body giant cells and many eosinophilic leucocytes
R 4265	1 50 mg.	7 K	" "	0	3.20	96.66	Reaction slight; a few milk spots of the omentum showed wax with foreign body giant cells. One giant cell with four nuclei found in the peritoneal exudate

R 2812	2 20 mg.	4 K 14 days after the second injection	Dry powder through incision under anesthesia	—	—	—	No symptoms. Omentum shows foreign body giant cells singly and in tubercles. The giant cells have enclosed the wax and a few leucocytes. Very little vacuolization of the cytoplasm of the giant cells. Increase in fibroblasts and in bands of fibers. A few small abscesses, but almost no neutrophilic leucocytes elsewhere. No eosinophilic leucocytes
R 2807	2 20 mg.	4 K 24 days after the second injection	" "	0	9.64	90.35	Every milk spot of omentum has foreign body giant cells, some extremely large. Their cytoplasm is markedly vacuolated. Tubercles of giant cells seen in section. Many bands of fibroblasts and many eosinophilic leucocytes in the omentum. Many eosinophiles in the retrosternal nodes

that received the crude chloroform-soluble wax, it was clear that this preparation had a much greater proportion of the factor, that is the wax, that produces the foreign body giant cells. Besides the giant cells there was a marked dilatation of the blood vessels, seen also in Fig. 2, as well as signs of the new formation of fibroblasts and of fibrous tissue.

Reactions to Leprosin.—Five rabbits have received the leprosin and their protocols are given in Table III. The formation of abscesses, so marked a feature of the reaction to the two preceding materials, was much reduced, appearing only in Rabbit R 4264.

There were two constant reactions to the material, an extensive formation of foreign body giant cells and a marked development of bands of fibroblasts. These bands are shown in Fig. 4, from the omentum of Rabbit R 2801. The photograph was taken from a fresh film, stained with neutral red, and was made while the cells were living. It will be noted that the bands of fibroblasts are growing in a manner to simulate a tissue culture. The giant cells occurred singly and in tubercles and had eosinophilic leucocytes around them. No epithelioid cells were seen after the injection of the leprosin.

Reactions to Leprosinic Acid.—The cellular reactions to the intraperitoneal injections of leprosinic acid are shown in five rabbits, the protocols of which are given in Table IV. In every instance the reaction to this material has been of a single cell type; namely, there has been a multiplication of monocytes around the particles of the wax and their fusion into giant cells.

In the omentum it was clear that this material also lodged only on the milk spots which became tubercles of giant cells. The differential counts of the cells of the peritoneal exudates of these animals show how small a part of the reaction is made by neutrophilic leucocytes, for only in the first animal of the series were there any of these cells and in that instance, only 2.5 per cent. Instead the tissues were infiltrated with eosinophilic leucocytes.

Reactions to Alcohols from the Waxes of the B. leprae and B. phlei.—These two highly purified, crystalline alcohols, $C_{25}H_{44}O_2$, from the unsaponifiable wax material of the *B. leprae*, and $C_{20}H_{42}O$, from the *B. phlei*, or timothy grass bacillus, were each given intraperitoneally to two rabbits in doses of 15 mg. They were given in each instance as the dry crystals through an incision under ether anesthesia.

Rabbits R 4272 and R 4241 each received the alcohol from the *B. leprae* and were killed in 5 days. Rabbit R 4236 and R 4238 received the alcohol from the

TABLE IV

Protocols of Rabbits Receiving Leprosinic Acid

Animal No.	No. and amount of injections	Time	Method of preparing material	Peritoneal exudate			Tissues
				Percentage of			
				PMN	Lymph.	Mono.	
		days					
R 2769	1 20 mg.	5 K	Dry powder through cannula	2.52	7.07	90.40	Omentum showed accentuated milk spots with wax enclosed in giant cells. Tubercles of foreign body giant cells on diaphragm infiltrated with eosinophilic leucocytes. Many eosinophiles in the retrosternal lymph nodes
R 4271	1 30 mg.	22 K	Dry powder through incision under anesthesia	0	14.81	85.18	Omentum showed flecks of wax on the milk spots surrounded by foreign body giant cells. These giant cells were not as large as in R 4270 but there were as many as 30 to 40 on some milk spots. Foci of monocytes in septa of lungs and some giant cells in the retrosternal lymph nodes
R 4270	1 30 mg.	23 K	" "	0	13.40	86.59	Omentum showed flecks of wax on the milk spots surrounded by foreign body giant cells. Some of them had only three or four nuclei; others were so large that they completely filled a low power field. Many monocytes; no leucocytes seen. No foci of monocytes found in the lungs
R 4266	1 40 mg.	37 K	Imperfect colloidal suspension	0.51	15.97	83.50	Omentum showed many giant cells on the milk spots, some with 30 to 40 nuclei. There were 2.5% eosinophilic leucocytes in the peritoneal exudate and there was one giant cell about 100μ in diameter. A focus of giant cells and monocytes near the point of injection was infiltrated with eosinophilic leucocytes. A few foci of monocytes in the lungs and extensive involvement of both sinuses and follicles of retrosternal lymph nodes with monocytes
R 4267	1 40 mg.	38 K	" "	0	14.79	85.20	Less reaction than in R 4266 but of same type. Monocytes and foreign body giant cells containing wax on the milk spots of the omentum

timothy grass bacillus and were killed in 4 and 6 days respectively. The reaction was of giant cells around the crystals of alcohol; these crystals lodged on the milk spots of the omentum. The giant cells were proportional to the size of the crystals, whether they occurred singly or in a clump. There were more neutrophilic leucocytes in the peritoneal fluid than when the time interval had been longer, ranging from 5 to 11 per cent, except in the case of Rabbit R 4238, in which there was an adhesion of a part of the omentum to the peritoneal wall and to the liver, and the leucocytes were 39.08 per cent.

These experiments have not been completed; they involve a study of the reactions to these materials in the tuberculous rabbits, as well as in the normal ones.

DISCUSSION

The materials from the lepra bacillus have given an interesting opportunity to follow the chemical separation of complex mixtures by cells instead of in the test tube. It is clear that all of these materials contain some substance which, like the solid alcohol $C_{25}H_{44}O_2$, gives the formation of the simple foreign body giant cell. The crude chloroform-soluble wax and the wax obtained from the purification of the lepra phosphatide contain, however, many more substances that give cellular reactions. With both of them the most striking phenomenon is the formation of large abscesses wherever the masses of the wax lodge. Thus there is some substance chemotaxic to leucocytes in or on the wax which acts like the tuberculo-polysaccharide. That is, this material calls leucocytes from the blood stream and so damages them that they are readily engulfed by clasmatocytes. The leucocytes are found in clasmatocytes both in the local lesions and in the spleen. These abscesses are not at all like caseation for there is no basis of dead cells, but rather it is the wax itself which becomes infiltrated with leucocytes.

Besides this reaction, there is the formation of the foreign body giant cells with the fused monocytes enclosing both the wax and the leucocytes. In the case of the wax obtained from the analysis of the lepra phosphatide, this reaction is much greater in amount than with the crude chloroform-soluble wax, indicating that the wax-like material is in much greater proportion in this fraction. The tissues also become infiltrated with eosinophilic leucocytes and lymphocytes.

These materials also are irritants, causing a marked dilatation of the blood vessels and probably a new growth of vessels. They also contain substances that are remarkable stimulants for the formation of fibroblasts and of new fibers. Besides all of these properties, they contain some material that gives rise to typical epithelioid cells, exactly like the reaction to the tuberculo-phosphatide. Thus these two materials give reactions as complex as those aroused by the acetone-soluble material from the tubercle bacillus (6). The cellular reactions to them are similar to those aroused by the crude wax which can be separated from tubercle bacilli. These complex cellular reactions reflect the fact that some of all the different types of lipoids come out with the first use of lipoidal solvents on mixtures.

The leprosin is an entirely different type of material; it is a pure white powder in crystalline form which does not give the cellular reactions characteristic of complex mixtures of lipoids, such as have just been described. Rather its reactions are reduced to two simple properties: It causes a remarkable new growth of fibroblasts, as is shown in Fig. 4, making them grow as they do in tissue cultures. Then it produces the formation of foreign body giant cells around the particles of the wax, and subsequently gives an infiltration of the tissue with the eosinophilic leucocytes. With the leprosinic acid the material which causes the new growth of the fibroblasts has been split off from the molecule of the leprosin, and the resulting fraction causes only the formation of the foreign body giant cells. This property is seen in its purest state with the crystalline solid alcohol, in which each giant cell is proportional in size and shape to a single crystal or a clump of crystals which may happen to lodge on a milk spot.

CONCLUSIONS

1. The waxes from the *B. leprae*, like those from tubercle bacilli, are remarkable stimulants of cells.

2. The crude wax separated from the *B. leprae* is a mixture of lipoids and other materials, and gives reactions that include the types of cells characteristic of the response to the tuberculo-polysaccharide, phosphatide, and wax.

3. The wax obtained from the purification of the lepra phosphatide shows similar cellular reactions but with a greater proportion of foreign body giant cells.

4. Leprosin, though a glyceride, corresponds in its physical properties to the unsaponifiable material from the tubercle bacillus. It stimulates two strains of cells, fibroblasts and monocytes. The monocytes fuse into foreign body giant cells to engulf the wax.

5. The cellular reaction to the leprosinic acid and to the crystalline alcohols is of one type only, represented by the foreign body giant cell.

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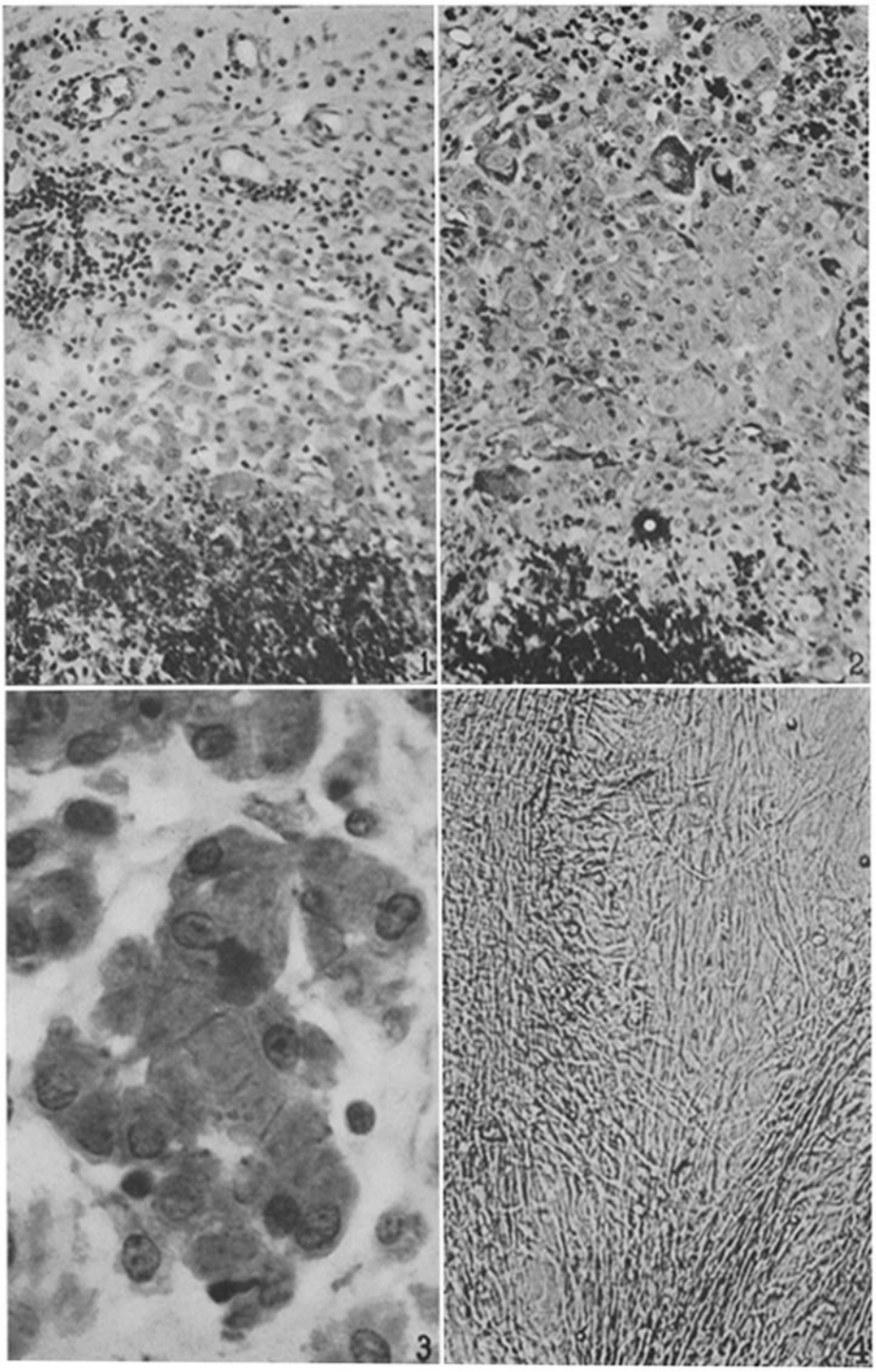
EXPLANATION OF PLATE 34

FIG. 1. Wall of an abscess in the omentum of Rabbit R 4188, which had received three intraperitoneal injections of 20 mg. each of the crude chloroform-soluble wax from the *B. leprae*. The wax was in the form of a colloidal suspension. The animal was killed 5 days after the third injection. There is a narrow band of monocytes and giant cells along the edge of the abscess. Masson stain to show the deep basophilia of the cytoplasm of the monocytes. × 210.

FIG. 2. Wall of an abscess in the omentum of Rabbit R 4184, which had received three intraperitoneal injections of 20 mg. each of the wax obtained from the purification of the lepra phosphatide. The animal was killed 6 days after the third injection. There is a wide band of monocytes and giant cells along the edge of the abscess. Masson stain. × 250.

FIG. 3. Sinuses of one of the retrosternal lymph nodes of the same animal as Fig. 1. It shows a pure and extensive reaction of monocytes and giant cells. Masson stain. × 1,000.

FIG. 4. Film of the omentum of Rabbit R 2801, which had received two intraperitoneal injections of 20 mg. of leprosin in the form of a dry powder. The animal was killed 4 days after the second injection. The preparation was stained with neutral red and the photograph was taken while the cells were still living, and shows bands of fibroblasts. × 150.



Photographed by Louis Schmidt

(Sabin *et al.*: Waxes from *Mycobacteriuml eprae*)