THE CELLULAR REACTIONS TO LIPOID FRACTIONS FROM ACID-FAST BACILLI

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The biological tests thus far made in this laboratory (1–12) with chemical fractions isolated from or liberated by the growth of tubercle bacilli have been directed toward a clearer understanding of the tissue changes in tuberculosis and the rôle of the various chemical factors in the production of these changes. Sabin (12) and Anderson (13) have recently made critical reviews of the earlier chemical and biological investigations of tubercle bacilli and analyzed the results obtained by the various groups now working under a plan for cooperative research on tuberculosis (14).

The lipoid portion of the organisms, notably a phosphatide constituent, possesses the capacity of producing tubercular tissue; that is, epithelioid cells and giant cells (1–5, 9). The so called waxes cause a proliferation of fibroblasts (9). The acetone-soluble fat induces proliferation of all connective tissue cells and of blood vessels, and causes hemorrhage (15). The polysaccharide is chemotactic for and toxic to leucocytes (6, 9). The protein is probably responsible for fever, and in addition causes a proliferation of plasma cells (16).

By means of the recent observations it has been possible to divide the disease, so to speak, into several parts, for each of which a particular chemical fraction of the bacilli is responsible.

Sabin and Doan (2) made cultures from the phosphatide and from the tissues of animals which received the phosphatide, with consistently negative results. Nevertheless, in the present study cultures and stains for acid-fast organisms have again been made to rule out contamination of the phospholipins by living, dead, or partially defatted organisms. Furthermore, a comparison has been made between the reactions produced in the tissues by the heat-killed and partially

defatted organisms and those produced by certain of the lipoids. Comparative observations have been made on the effects produced by introducing into the tissues of animals the phosphatides from five strains of acid-fast bacilli. These were: the human, bovine, and avian tubercle bacilli, the timothy grass bacillus, and a strain of acid-fast bacilli isolated from a leprous human being. When it was found that each of the bacterial phosphatides produced similar cellular reactions, attention was directed toward the manner in which these changes were brought about. Observations were then made on the fate of the injected lipoid and the newly formed tissue produced by it. In this way the complete life cycle of the epithelioid cell has been determined and information gained regarding the physiological properties and potentialities of this strain of connective tissue cells.

Material and Methods

The bacteria for the chemical analyses have been grown in large quantities under standardized conditions by the H. K. Mulford Co. The human tubercle bacillus used was the H-37 (Saranac) strain. The bovine and avian strains were of known virulence. The timothy grass and lepra strains, avirulent for laboratory animals, were obtained from the Hygienic Laboratory, Washington, D. C. This was the name of the laboratory at the time the organisms were obtained. Since June, 1930, it has been known as the National Institute of Health.

The phosphatides isolated by Anderson from human (17), bovine (18), and avian (19) tubercle bacilli, and the timothy grass bacillus (20) are gray-white, amorphous solids. That from the leprosy bacillus (21) is pale lavender in color. These substances are soluble in alcohol and ether, insoluble in acetone, and may be readily mixed with water to form milky suspensions free from flocculi or sediment. The first preparations of phosphatides received from Dr. Anderson contained traces of acid-fast debris (2). More recently the ether solution of the lipoid has been passed twice through Chamberland candles and none of the preparations contains demonstrable acid-fast material. The phosphatide from the human tubercle bacillus has been examined under crossed Nicol prisms by Dr. R. W. G. Wyckoff of The Rockefeller Institute, who pronounced it predominantly crystalline. Twenty-two plates of Petroff's egg-gentian violet media, each seeded with 10 mg. of one of the phosphatides in aqueous suspension, have remained free of acid-fast bacteria after 4 months' incubation. Guillery (22) has likewise failed to find viable organisms in the phosphatide.

Rabbits and guinea pigs have been used in the experiments. The phosphatides were introduced by the intraperitoneal, subcutaneous, and intrapulmonary routes. The material was injected into the lungs through the chest wall and by way of the trachea. The intraperitoneal route is the one of choice for determining the type

of cell affected by the foreign material. The omentum participates most actively in the reaction and is well adapted for study either of fresh tissue or fixed material. The subcutaneous route is better for determining the quantitative reaction. The reaction in the lung offers a comparison with tuberculosis itself, and an opportunity to study the effects in a site where there is a minimal amount of connective tissue.

For the intraperitoneal route the usual dose of the phosphatide was 80 mg. in aqueous suspension. The reaction has been studied after from one to thirteen injections. In some instances a single massive dose was given and the tissues were studied about 2 weeks later. Each animal receiving the divided doses was killed by intravenous injection of air 24 hours after the last injection. Fresh tissues were studied by the supravital method. Blocks of tissue were fixed in Helly's mixture, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, and by Giemsa's method.

The Phosphatide from Human Tubercle Bacilli

A. Evidence That the Phosphatide Is Phagocytized.—If a small granule of the phosphatide be placed on a slide with a drop of water over which is a thin coverslip, the material first has a granular appearance under the microscope. However, within a few minutes the edges of the lipoid advance into the liquid and take the appearance of degenerating myelin. If this procedure be repeated, using a slide on which is a thin film of neutral red, the myelin figures take a faint pink stain. This staining reaction of the free phosphatide does not change on standing overnight.

If a single layer of the fresh omentum of a normal rabbit which has received an intraperitoneal injection of the phospholipin be studied on an unstained slide within 12 hours after this injection, the cells of the milk spots will be seen to contain these same myelin figures. They are contained in large vacuoles of irregular size and shape. Stained with neutral red, the myelin figures in many of the cells show the same staining reaction as the phosphatide alone. However, if the examination be made at a later period, or if these cells with unaltered staining of the phosphatide be studied after a few hours, the material in the cytoplasm becomes a deeper red. Fig. 1 shows one of these large cells containing the myelin figures. This photograph was made from a supravital preparation of peritoneal exudate of Rabbit R 1802,[1] which had three doses of the phosphatide and was killed 24 hours after the last dose. Note the large, irregular masses in the cytoplasm and that they all are of equal density. Fig. 2 illustrates the appearance of these cells in fixed preparations. In the tissues of the guinea pig these cells may be seen 24 hours or more after the phosphatide has been injected. Fig. 13 shows two cells from the supravital preparation of peritoneal exudate from Guinea Pig R 690. Note the appearance of degenerating myelin. The smaller of the two cells shows the phagocytized material in more finely divided form. Similar cells from fixed preparations of the omentum of the same animal are shown in Fig. 14.

[1] These are serial numbers of animals used in this laboratory during a period of years.

It is clear from these results that the cells of the milk spots phagocytize the phosphatide. It will be shown that the lipoid undergoes a definite type of degradation within the cells, and that the cells which accomplish this process are derived from monocytes or their stem cells in the connective tissues.

Everywhere in the connective tissues of the body are tiny foci of young, undifferentiated connective tissue cells and monocytes. In the omentum these foci are called the milk spots. In the interspaces between the milk spots of the omentum are the fibroblasts and clasmatocytes or macrophages—the latter probably always functioning with reference to substances passing through the omentum. It is the cells of the milk spots, however, which exhibit the specific response to tuberculo-phosphatide (see Sabin, Doan, and Forkner (9, Fig. 4)).

Rabbit R 2253 received two injections of 80 mg. each of the phosphatide from human tubercle bacilli intraperitoneally at 24 hour intervals. The animal was killed 2 days following the second injection. The peritoneal fluid was slightly cloudy, quite cellular, and showed 75 per cent of cells of the monocyte series; that is, monocytes, stimulated monocytes, and epithelioid cells. The omentum was considerably thickened and there was an increase in both the number and size of milk spots. In the supravital and unstained preparations of the omentum, peritoneal fluid, and retrosternal lymph nodes small amounts of the phosphatide could be seen free in the intercellular spaces. This lipoid looked like degenerating myelin, just as in the study of the phosphatide itself. In addition, numerous cells were seen, particularly in the retrosternal lymph nodes, which were filled with the myelin figures: apparently unchanged phosphatide. In sections, the cytoplasm of these cells was filled with clear spaces of varying size and shape. (Similar cells are shown in Figs. 1 and 2.) The nuclei contained one to three nucleoli. The cytoplasm was quite basophilic, and in many of the cells numerous mitochondria were seen. The cells of the omentum containing the myelin figures were confined almost wholly to the milk spots. However, many, if not the majority of the cells of the milk spots, and those in the retrosternal nodes contained vacuoles of reduced, uniform size, all of which stained the same color with neutral red. Cells similar in appearance but from other animals can be seen in Figs. 3 and 4. In the latter the process of intracellular dispersion of the lipoid into finer and finer particles had already begun.

Rabbit R 1802 received three daily injections of 80 mg. each of the H-37 phosphatide intraperitoneally and was killed 24 hours after the last dose. The milk spots of the omentum were markedly stimulated and there were many epithelioid cells in the interspaces which had become diffusely involved. There were moderate numbers of active granulocytes in the supravital preparations of the peritoneal

fluid and in the omentum. All transitions from the most primitive mesenchymal cell to the typical fine vacuole epithelioid cell could be seen in the omentum, although the latter were not numerous. Many cells with large vacuoles of irregular size and shape (Fig. 1), all of which took the same stain with neutral red, were present in the omentum and peritoneal fluid. Again the phagocytized material, tuberculo-phosphatide, had the appearance of degenerating myelin.

Guinea Pig R 2254 received three injections of 40 mg. each of the H-37 phosphatide intraperitoneally at 24 hour intervals and was killed 4 hours following the last dose. The omentum was enormously thickened. The milk spots were large and increased in number. Numerous small, white flakes appeared on the visceral and parietal peritoneum. The peritoneal fluid was thick. The unstained spread of the omentum showed that the milk spots were bright and refractile while the interspaces were duller. The large phagocytic cells in the milk spots showed definite myelin-like figures in the cytoplasm. (Similar cells are shown in Figs. 13 and 14.) In the supravital preparations some of these myelin-like masses were pale pink, while in other cells the vacuoles were still of the same irregular shape and size but stained more deeply. After 2 hours at room temperature many of the pale cells became more deeply stained, even though the cells were obviously still alive. These cells, derived from monocytes, which contain myelin-like masses in large vacuoles of irregular size and shape but uniform staining reaction, will henceforth be called first stage epithelioid cells.

B. Evidence That the Phosphatide Is Degraded within Cells.—24 hours after a single dose of the phosphatide from the human tubercle bacillus, the milk spots of the omentum appeared enlarged: when examined microscopically it could be seen that the young connective tissue cells and monocytes had phagocytized the phosphatide in large quantities (Rabbits R 819, R 2122, and R 2185 in Table I). The lipoid was in large vacuoles of irregular size and shape (Figs. 1 and 2). The staining reaction with neutral red was uniform, slightly toward the acid range of this dye (9, Figs. 1 and 2). At this time the increase in size of the milk spots was caused by the increased size of the cells which had phagocytized the lipoid. A few of the cells of the interspaces contained a single vacuole of the lipoid, but in general they did not participate actively in the reaction. Moderate numbers of neutrophilic leucocytes responded to the first but not to subsequent injections, and those which appeared with the first injection were soon phagocytized by clasmatocytes and removed. These leucocytes exhibited no evidence of phagocytic activity for the phosphatide.

24 hours after two daily injections of the phosphatide, the milk spots of the omentum were still larger. The young, undifferentiated connective tissue cells showed evidence of rapid maturation toward monocytes, although the cytoplasm was filled with the large, irregular shaped vacuoles. Numerous mitotic figures were present (9, Fig. 3). In sections the cells of the milk spots were as large as epithelioid cells and their cytoplasm was filled with large, clear spaces from which the lipoid had been removed during fixation (R 820, Table I).

After three daily injections of the phosphatide, the omentum was markedly

thickened, due to the increased size and number of milk spots (R 821, Table I). Many mitoses were seen, as well as cells dividing by amitosis. In spreads or scrapings of the omentum another significant change was noted. More of the cells of the milk spots had vacuoles of uniform size but considerably smaller than in the earlier stages. The staining reaction to neutral red was unchanged. The cytoplasm of these cells appeared foamy in sections.

Fig. 3 shows a second stage epithelioid cell from the supravital preparation of peritoneal exudate from Rabbit R 2186. Note that the cytoplasm is filled with vacuoles of uniform size and shape, but considerably smaller than those of the cell in Fig. 1. Fig. 4 is from the fixed section of omentum of the same animal as represented in Fig. 3. Figs. 3 and 4 then show the second stage epithelioid cell as it appears in the two techniques. It will be seen that this cell becomes the typical epithelioid cell, indistinguishable from those occurring in the disease. Two stages in the life cycle of this cell may now be recognized: the first, in which the vacuoles are very large but of irregular size and shape; and the second, in which the vacuoles are smaller and of uniform size.

During the 2nd week after a single large dose, or after repeated daily doses of the phosphatide, the omentum appeared massively thickened (R 2409 and R 2408, Table I). The identity of the individual milk spots was lost in the hyperplastic mass of newly formed tissue. There was moderate hyperemia of the vessels. Scattered here and there over the intestinal serosa and parietal peritoneum were small, white nodules, not firmly adherent. Some of the new tissue could be seen on the surface of all the abdominal viscera. By this time the omentum was usually too thick for study of spreads by the supravital method. Scrapings of the omentum and other organs showed the predominating cell to be the typical epithelioid cell with rosette of fine vacuoles staining uniformly (9, Fig. 8). In sections the cytoplasm of these cells appeared homogeneous (Figs. 5 and 6). The epithelioid cells were in many places grouped together, forming tubercles closely resembling those seen in the disease. Numerous typical rosette giant cells were present. By a comparison of Figs. 1 and 2, representing first stage epithelioid cells, with Figs, 3 and 4, representing the second stage epithelioid cells, and with Figs. 5 and 6, representing the third stage cells, the gradual process of intracellular dispersion of the lipoid into more numerous and finer particles can be visualized.

At this stage, but never in the early reaction, rather numerous lymphocytes and smaller numbers of plasma cells were present. Both the lymphocytes and plasma cells tended to occur in clumps about the tubercle-like structures, and especially about clumps of Langhans giant cells, but the plasma cells remained apart and did not become scattered between the lymphocytes.

After the lipoid had been dispersed into the finest vacuoles no further perceptible change took place. The epithelioid cells so produced were remarkably persistent (R 377 and R 379, Table I); small clumps of these cells have been found 280 days after a single intrapulmonary injection of 50 mg. of the phosphatide (R 1644, Table III). However, gradual resorption of the new tissue did take place. The areas of more diffuse reaction disappeared first, the tubercles later (R 368, R 377,

TABLE I

Protocols of Rabbits Which Received Phosphatide from Human Tubercle Bacilli Intraperitoneally

Animal No.	No. of injections	Amount of each injection	Interval between last injection and autopsy	Observations
		mg.	*days*	
R 819	1	80	1	Milk spots of omentum larger than normal. Many primitive cells, monocytes, stimulated monocytes, and phagocytic cells with large, irregular vacuoles staining uniformly. Large numbers of granulocytes, free and in clasmatocytes
R 2122	1	80	1	Same as preceding animal (R 819). In addition, many monocytes and first stage epithelioid cells in retrosternal lymph nodes
R 2185	1	80	1	Same as preceding animal (R 2122)
R 820	2	80	1	Definite accentuation of milk spots of omentum. They were made up principally of primitive cells, monocytes, and first stage epithelioid cells, with a few second stage. Numerous polymorphonuclears and clasmatocytes. Considerable cell division
R 821	3	80	1	Milk spots increased in size and number. Considerable cell division. Few polymorphonuclears and clasmatocytes. Many first, a few second, and a very few third stage epithelioid cells. An occasional rosette giant cell and a few small areas of caseation
R 2123	1 1	100 100*	6	Milk spots markedly increased in size and number. Middle lobe right lung partly consolidated. Pleural and peritoneal fluids turbid due to many monocytes and second stage epithelioid cells. In the omentum and lung the cells, chiefly second and third stage epithelioid cells, were grouped into tubercle-like masses around which were numerous lymphocytes. Epithelioid cells and caseation in retrosternal lymph nodes
R 2186	10	80	1	Entire omentum greatly thickened. Abdominal viscera covered with thin film of exudate (epithelioid cells and monocytes). Massive diffuse and nodular involvement of omentum and retrosternal nodes with principally third stage epithelioid cells. Numerous lymphocytes, a few giant cells and plasma cells. Moderate amount of caseation
R 2409	10	80	2	Same as R 2186, except perhaps greater lymphocytic response
R 664	11	80	1	Massive thickening of omentum. Many tubercle-like masses of third stage epithelioid cells. Many rosette giant cells. Many plasma cells and lymphocytes. Moderate amount of caseation
R 1016	1	1000	11	Omentum massively thickened due to tubercle-like masses and diffusely scattered epithelioid cells. Massive involvement of retrosternal nodes with typical epithelioid cells. Predominating cell in omentum was the third stage epithelioid cell. Many lymphocytes, a few plasma and giant cells. Moderate amount of caseation
R 2408	1	1000	14	Same observations as in R 1016, except few lymphocytes and many plasma cells and Russell body cells about the tubercle-like masses of epithelioid and giant cells

* This injection was made intrapleurally at the same time as the intraperitoneal injection.

TABLE I—*Concluded*

Animal No.	No. of injections	Amount of each injection	Interval between last injection and autopsy	Observations
		mg.	*days*	
R 2187	10	80	7	Reaction as in R 2408, but more caseation. Extensive involvement of retrosternal and tracheal lymph nodes
R 368	13	80	30	Omentum massively thickened and bound to intestine by adhesions. Nodular masses of third stage epithelioid cells and giant cells. Many plasma cells and lymphocytes. A little caseation
R 377	13	80	90	Omentum only moderately thickened. Microscopically all the diffuse reaction had disappeared. A few masses of third stage epithelioid and giant cells without caseation or plasma cells but with a few lymphocytes. Numerous degenerating epithelioid cells
R 379	13	80	134	Omentum slightly thickened. Reaction similar to R 377 but only about one-half as extensive. Many degenerating epithelioid cells. A few epithelioid cells remained in the retrosternal nodes

and R 379, Table I). As Sabin, Doan, and Forkner (9) have pointed out, this resorption is accomplished in part by caseation, and in part by a process of resolution similar to that by which experimental tuberculosis of bone marrow regresses (25). It is in this late phase of the cellular reaction to tuberculo-phosphatide that the end stage in the life cycle of the epithelioid cell is to be seen.

Rabbits R 377 and R 379 (Table I) showed only discrete tubercle-like masses of epithelioid cells in the omentum, with an occasional giant cell. All the diffuse reaction had disappeared. There were no lymphocytes or plasma cells. Many of the epithelioid cells in the tubercle-like structures had pyknotic nuclei. In other instances the nuclei were fragmented, yet stained densely. In still other instances the nuclei were paler than normal and presented irregular fringed margins (Fig. 5). These cells with pyknotic or pale nuclei had irregular cytoplasmic borders. Large, irregular, clear spaces could be seen in the cytoplasm. They were obviously dead and disintegrating. The life cycle of this cell has now been completed. It has been seen to arise from a primitive cell (4) in the connective tissues (reticular or mesenchymal cell), to pass through the monocyte phase to that of the epithelioid cell. In the latter phase, four distinct stages have been observed; namely, that with large, irregular vacuoles, that with vacuoles of intermediate but uniform size, that with the smallest, dust-like vacuoles, and the stage of degeneration.

In every animal receiving the phosphatide there has been some caseation (Fig. 9). No area has been designated caseous unless eipthelioid cells could be seen within, or at the margin of, the caseous mass. Less caseation has been seen after the phosphatide from the human tubercle bacillus than after the other phospholipins. Further studies of the mechanisms by which caseation is produced are in progress.

The Phosphatide from the Avian Tubercle Bacillus

This lipoid (13) represents 2.26 per cent by weight of the organisms. It has been studied in the same manner as the phosphatide from the human tubercle bacillus. In the tissues of the normal rabbit the reaction to the avian phosphatide (Fig. 7) most closely paralleled that produced by the phosphatide from the H-37 strain of bacilli.

The avian phosphatide was phagocytized by the cells of the milk spots and remained in large vacuoles (R 686 and R 687, Table II) of irregular shape and size for about 5 days. However, a few of these epithelioid cells of the first stage could be seen at the end of 1 week (R 685, Table II). During the 2nd week the greater number of cells in the omentum were of the second stage, with vacuoles of intermediate but uniform size and shape (R 2177, Table II). From the 12th day, greater numbers of the cells with the finest vacuoles were seen (R 684 and R 688, Table II); but the process of intracellular dispersion of the lipoid into the finest vacuoles was not completed until the 3rd week. It is therefore clear that a little more time was required for dispersion into the finest vacuoles than was the case with the phosphatide from the human tubercle bacillus. Also, at any given period the reaction was more mixed. For example, at 7 days (Rabbit R 685, Table II), there remained a few cells of the first stage, while the majority were in the second; and very rarely a cell with the finest vacuoles could be seen.

In addition, the reaction to the avian phosphatide was characterized by greater numbers of plasma cells than were seen after any of the other phosphatides, except that from the bovine organisms. It produced more caseation than any of the other phospholipins except that from the timothy grass bacillus.

The Phosphatide from the Bovine Tubercle Bacillus

The phosphatide represents 1.55 per cent by weight of the bovine tubercle bacillus (13). Injected intraperitoneally, it produced a reaction closely resembling those just described (Fig. 8). However, the cells which phagocytized this lipoid showed slower and more irregular intracellular dispersion of the lipoid than was seen after injections of the phosphatides from human or avian tubercle bacilli.

All three stages of the epithelioid cell could be seen at the 12th day (R 1015 and R 1014, Table III). By the end of the 3rd week all the cells reached the stage with the finest vacuoles (R 1621, Table III). This lipoid produced more rosette or Langhans giant cells than any of the other phospholipins. The epithelioid and giant cells occurred both in distinct tubercles and diffusely as in the reaction to the other phosphatides.

Greater numbers of plasma cells and lymphocytes also characterized this reaction (R 1144 and R 1145, Table III). In many areas tubercles of epithelioid or giant cells were surrounded by a broad ring of lymphocytes and plasma cells.

TABLE II

Protocols of Rabbits Which Received Phosphatide from Avian Tubercle Bacilli Intraperitoneally

Animal No.	No. of injections	Amount of each injection	Interval between last injection and autopsy	Observations
		mg.	*days*	
R 686	1	80	1	Slight stimulation of milk spots of omentum. Many polymorphonuclears and clasmatocytes. Predominating cell was a young connective tissue cell or monocyte, the cytoplasm of which was filled with large vacuoles of irregular shape but uniform staining reaction to neutral red
R 687	2	80	1	Milk spots of omentum moderately increased in size and number. Tiny white nodules composed of fibrin and monocytes over abdominal viscera. Numerous polymorphonuclears free and in clasmatocytes. Predominating cell in omentum was first stage epithelioid cell. There was a little caseation
R 685	7	80	1	Marked general thickening of omentum. Microscopically many tubercle-like masses and diffusely scattered epithelioid cells of first and second stage—the latter predominating. Occasional rosette giant cell. Many plasma cells, a few lymphocytes, and a great deal of caseation
R 2177	10	96	1	Omentum greatly thickened. Identity of milk spots almost lost. Several loosely attached small, yellow-white nodules over abdominal viscera. Many tubercle-like masses in omentum. Epithelioid cells of all stages, second and third predominating. Few polymorphonuclears. Numerous plasma cells and lymphocytes. Extensive reaction in retrosternal nodes: epithelioids and rosette as well as foreign body giant cells. Moderate amount of caseation
R 684	12	80	1	Reaction closely resembled R 2177 but there were more plasma cells and more epithelioid cells with fine vacuoles
R 688	12	80	2	Reaction very closely resembled that seen in R 2177
R 1149	1	1000	14	Massive reaction in omentum and retrosternal lymph nodes. Epithelioid cells of all stages, those of second predominating. Numerous tubercle-like structures. Moderate numbers of giant cells and plasma cells, fewer lymphocytes and a moderate amount of caseation
R 1150	1	1000	15	Resembled R 1149 in every respect except that there were more rosette giant cells in the omentum

The phosphatide from the bovine tubercle bacillus produced less caseation than that from the avian but more than that from the human strain of organisms. In all other respects the reaction was identical to that produced by the other phosphatides from mycobacteria.

TABLE III

Protocols of Rabbits Which Received Phosphatide from Bovine Tubercle Bacilli

Animal No.	Route of injection	No. of injections	Amount of each injection	Interval between last injection and autopsy	Observations
			mg.	*days*	
R 1015	Intraperitoneal	11	80	1	Massive reaction in omentum and retrosternal nodes. In omentum, all stages of epithelioid cells, first and second in largest numbers. In retrosternal nodes, second and third stage epithelioids predominated. Many giant cells. Moderate numbers lymphocytes and plasma cells. Moderate amount caseation. Many tubercle-like structures
R 1014	"	12	80	1	Resembled R 1015 in every respect except that reaction was characterized by fewer first and more third stage epithelioid cells
R 1144	Intraperitoneal and subcutaneous	1	1000	13	Reaction resembled that seen in R 1015. There were many plasma cells and lymphocytes. Epithelioid cells with smallest vacuoles most numerous. Massive reaction in subcutaneous tissues
R 1145	" "	1	1000	15	Massive reaction in omentum and retrosternal nodes. Also in subcutaneous region of right groin. A few first and second and many third stage epithelioid cells. Many giant cells, plasma cells, and lymphocytes, the latter about periphery of tubercle-like structures. Moderate amount of caseation
R 1620	Into right pleura and lung	2	20 100	13	The right lung was adherent to pleura at apex. Right upper lobe consolidated. A few first and second, and many third stage epithelioid cells, both in tubercle-like masses and diffusely scattered in alveoli. Many giant cells, plasma cells, and lymphocytes. Rather extensive caseation. Other viscera normal
R 1621	Intrapleural	1	50	23	Moderately extensive reaction on the pleura. A few friable adhesions. Only third stage epithelioid cells. Numerous giant cells. Many lymphocytes about pseudotubercles. No caseation
R 1646	Intratracheal	1	50	24	Several macroscopic areas in lungs resembling tubercles. These were composed principally of second and third stage epithelioid cells, the latter being more numerous. There were many giant cells and a few lymphocytes but no caseation. Other organs normal
R 1647	"	1	50	137	No macroscopic changes. A few tiny tubercle-like masses of third stage (and degenerating) epithelioids with an occasional giant cell and a few lymphocytes were scattered through the lungs. A few epithelioid cells in tracheal lymph node. Other viscera normal

TABLE III—*Concluded*

Animal No.	Route of injection	No. of injections	Amount of each injection	Interval between last injection and autopsy	Observations
			mg.	*days*	
R 1642	Intrapleural	1	50	139	No pleural adhesions. Small, yellow-pink nodule in upper lobe of right lung. Microscopically there were scattered clumps of third stage (and degenerating) epithelioid and giant cells, surrounded by a few lymphocytes in the right lung. A few epithelioid cells were seen in the tracheal and retrosternal lymph nodes. Other organs normal
R 1645	Intratracheal	1	75	196	Animal died. Had been kept in room with tuberculous animals and had renal tuberculosis. There were scattered small lesions in the lungs with unequivocal second stage epithelioid cells, indicating acquired pulmonary tuberculosis. Unsatisfactory for comparison of reaction or study of regression
R 1644	Intrapleural	1	50	280	All organs appeared normal at autopsy. In sections of the lungs a very few small clumps of epithelioid cells with an occasional giant cell were seen. About the periphery of these were a few lymphocytes. The epithelioid cells contained carbon particles but the giant cells were typical rosette types, interpreted as a last remnant of the reaction

The Phosphatide from the Timothy Grass Bacillus

This fraction (13) represents 0.59 per cent by weight of the organisms. It has been studied less intensively than the previously discussed phosphatides. However, it is interesting to note that this fraction, from an ordinarily avirulent strain of acid-fast organisms, produced epithelioid cells and tubercles (Fig. 9) in amounts equivalent to the other phosphatides.

This phosphatide, administered intraperitoneally, was also phagocytized by the milk spots of the omentum. On the 2nd day the predominating cell was the first stage epithelioid cell with the largest vacuoles (R 1427, Table IV). At 14 days a few of these cells remained but there were greater numbers of the cells of the second stage (R 1426, Table IV). Smaller numbers of typical epithelioid cells with rosettes of the finest vacuoles were seen at this stage, together with moderate numbers of rosette giant cells, numerous plasma cells, and a few lymphocytes. There was a great deal of caseation (Fig. 9) just as characteristic as that seen in tuberculosis.

The Phosphatide from Bacillus leprae

The organisms used (No. 370) for fractionation were obtained from the Hygienic Laboratory. The strain was isolated from a leprous human being in Honolulu by Clegg and Curry in 1909. The phosphatide (13) represents 2.2 per cent by weight of these organisms. This fraction has been tested in only four rabbits, two of which received doses smaller than usual on account of the scarcity of the available material. Later two other animals received doses of this phosphatide identical with those from the other mycobacteria.

Rabbits R 1949 and R 1950 (Table IV) received ten doses at 24 hour intervals. Each was killed for autopsy on the day following the last dose. One (R 1950, Table IV) showed a massive reaction of tubercle-like structures made up chiefly of epithelioid cells of the first stage, with smaller numbers of the second, and a few third stage cells (Fig. 10). Many giant cells of complex type were seen. Some were very large with a mass of nuclei usually forming a cap at one side of the cell, and they were possibly formed by the fusion of Langhans giant cells. Numerous plasma cells, small numbers of lymphocytes, and a minimal amount of caseation also characterized the reaction.

The second animal (R 1949, Table IV) showed a cellular reaction much less extensive and of mixed type. Epithelioid cells of the first and second stages were present but did not dominate the picture. There were many monocytes, a great deal of undifferentiated connective tissue, lymphocytes, plasma cells, polymorphonuclear leucocytes, and clasmatocytes.

Rabbit R 2414 (Table IV) received a single dose of 1 gm. of the phosphatide from *B. leprae* intraperitoneally. Supravital studies and fixed tissues revealed no cellular reaction. Rabbit R 2415 (Table IV), however, received ten daily doses of 80 mg. of the same material and showed at autopsy a massive reaction closely resembling that of Rabbit R 1950. The cause of this variation in the reaction to the phosphatide from *B. leprae* has not been determined.

Reactions to Tuberculo-Phosphatide in Guinea Pigs

As it has been indicated in the discussion of the myelin-like figures observed in the connective tissue cells after one or two injections of tuberculo-phosphatide, the reaction produced by this substance in guinea pigs was similar to that which occurred in rabbits. The principal difference was that the cells of the guinea pig required more time for the intracellular dispersion of the material into the smallest vacuoles. There was also a moderate stimulation of indifferent connective tissue cells after injections of the phospholipin in guinea pigs.

Thirteen animals received the material either intraperitoneally or intrapleurally. Autopsies were performed from 1 to 21 days after

TABLE IV

Protocols of Rabbits Which Received Phosphatides from Timothy Grass and Leprosy Bacilli Intraperitoneally

Animal No.	Source of phosphatide	No. of injections	Amount of each injection	Interval between last injection and autopsy	Observations
			mg.	*days*	
R 1427	Timothy grass bacillus	1	80	2	Moderate increase in size of milk spots of omentum. Several small nodules (monocytes and fibrin) free in peritoneal cavity. Milk spots showed many first stage epithelioid cells, monocytes, and primitive cells. There were many polymorphonuclears and clasmatocytes in interspaces and in peritoneal fluid. There was some caseation. Moderate numbers of early epithelioid cells in retrosternal nodes
R 1426	" "	10	80	4	Extensive thickening of omentum. Numerous yellow nodules of epithelioid cells and caseous material in omentum and free in peritoneal cavity. Omental reaction both nodular and diffuse. Many first and second, but few third stage epithelioid cells, a few giant cells, numerous plasma cells, and a few lymphocytes. Extensive reaction in retrosternal nodes
R 1949	Leprosy bacillus	10	80	1	Omentum only slightly stimulated. There were monocytes and epithelioid cells of the first and second stage but polymorphonuclears and clasmatocytes were more numerous. Many plasma cells and undifferentiated connective tissue cells
R 1950	" "	10	80	1	Omentum massively thickened. Marked reaction in retrosternal nodes. Many tubercle-like clumps of first and second stage epithelioid cells, rosette and complex giant cells, many plasma cells, and lymphocytes in omentum. Small amount caseation. A very mixed reaction as regards types of epithelioid cells
R 2414	" "	1	1000	15	Sections of omentum, the retrosternal and tracheal lymph nodes showed moderate numbers of polymorphonuclears and clasmatocytes. No other abnormal findings
R 2415	" "	10	80	3	Massive reaction. In all respects like that in R 1950

injection of the phosphatide. None of the animals showed complete dispersion of the lipoid into the finest vacuoles during this time. Protocols of the guinea pigs which received phosphatide are in Table V.

TABLE V

Protocols of Guinea Pigs Which Received Phosphatide from Human Tubercle Bacilli

Animal No.	Route of injection	No. of injections	Amount of each injection	Interval between last injection and autopsy	Observations
			mg.	*days*	
R 691	Intraperitoneal	1	80	1	Moderate increase in size of milk spots of omentum. In the supravital preparations and fixed tissues the predominating cells were leucocytes, free and in clasmatocytes. There were numerous cells in the omentum with large vacuoles of irregular size and shape. Numerous monocytes. Considerable necrosis
R 692	"	2	80	1	Abdominal viscera covered with a thick white exudate consisting of leucocytes, clasmatocytes, and monocytes. No macroscopic stimulation of omentum. Microscopically there were many leucocytes and clasmatocytes in the interspaces; in the milk spots, many primitive cells, monocytes, and first stage epithelioid cells. Considerable necrosis. Cultures of exudate negative
R 693	"	3	80	1	Moderate amount exudate as in R 692. Omentum markedly thickened. An occasional tubercle-like structure and extensive diffuse reaction. Fewer leucocytes and clasmatocytes than R 692. Numerous monocytes, primitive cells, and first stage epithelioid cells. Considerable amount of necrosis and some typical caseation. Cultures of exudate negative
R 690	"	7	80	1	Many white nodules free in abdominal cavity or adherent to viscera. Very marked stimulation of omentum. Predominating cell was the first stage epithelioid cell with very large vacuoles (Fig. 13), both in omentum and peritoneal fluid. A few epithelioid cells of second stage were present. Moderate amount of caseation. Numerous tubercle-like structures. Moderate increase in undifferentiated connective tissue. Culture of exudate negative
R 689	"	12	80	1	Omentum extremely thickened and adherent to liver, spleen, parietal peritoneum, and diaphragm. Numerous monocytes. Predominating cell was the first stage epithelioid cell with myelin-like figures, although a few second and an occasional third stage epithelioid cells were present. Many tubercle-like structures. Moderate amount caseation. Moderate increase in undifferentiated connective tissue. Cultures of peritoneal exudate negative
R 1953	Intraperitoneal	1	500	Died < 6 days	Postmortem changes too extensive for cellular studies
R 1956	"	1	500	" "	" "

TABLE V—*Concluded*

Animal No.	Route of injection	No. of injections	Amount of each injection	Interval between last injection and autopsy	Observations
			mg.	*days*	
R 1955	Intraperitoneal	1	500	11	Bowel adherent to abdominal wall over area 2 cm. in diameter. Moderate amount white exudate. Omentum markedly thickened. Extensive reaction in retrosternal lymph nodes. Omentum and nodes showed all stages of epithelioid cells, second stage predominating. An occasional giant cell. A few lymphocytes. A little caseation. Many tubercle-like structures. Moderate increase in undifferentiated connective tissue
R 1954	"	1	500	14	Small intestines massively adherent to parietal peritoneum. Peritoneal fluid clear. Omentum massively thickened. Many tubercle-like structures. Massive reaction in retrosternal nodes. Predominating cells were the second stage epithelioid cells with coarse vacuoles of uniform size. Numerous third stage and a few first stage epithelioid cells. A few rosette giant cells and lymphocytes. Numerous plasma cells. Small amount of caseation. Moderate increase in undifferentiated connective tissue
R 1925	"	1	20	20	Each animal showed slight thickening of the omentum. There were several small tubercle-like masses of epithelioid cells and a few giant cells in each omentum. Numerous monocytes, stimulated monocytes, and fewer epithelioid cells in the peritoneal fluid. The majority of the epithelioid cells in the omentum were of the third stage. In each animal there was enlargement of the retrosternal nodes, sections of which showed rather extensive involvement with epithelioid cells. Each showed a slight increase in undifferentiated connective tissue in the omentum. Reaction more extensive in retrosternal nodes than in the omentum. R 1927 showed a little more reaction than the others, and a little caseation
R 1923	"	1	20	20	
R 1924	"	1	20	21	
R 1927	"	1	20	21	
R 2097	Intrapleural	1	20	18	Numerous typical second and third stage epithelioid cells in the pleural fluid. On the pleura was an exudate of the same cells. The retrosternal and tracheal nodes, especially the former, show massive involvement with second and third stage epithelioid cells. Moderate numbers of lymphocytes and a few plasma cells about the epithelioid cells on the pleura
R 2098	"	1	20	18	Observations same as in R 2097

Guinea Pigs R 1925, R 1923, R 1924, and R 1927 (Table V) each received 20 mg. of the phosphatide intraperitoneally and were killed about 3 weeks later. In each instance the reaction was more extensive in the retrosternal nodes (Fig. 11)

than in the omentum, although unequivocal in the latter situation (Fig. 12). On numerous other occasions, animals which have received a single injection have shown the most extensive reaction in the lymph nodes which drain the peritoneal cavity. Quantitatively the omental response to two injections or more has been greater in proportion than that to a single injection. This is in accordance with the observations of Menkin (26). He has observed rapid drainage of foreign substances through the lymphatics from a normal area; but from an area which is the site of an inflammatory reaction, drainage through the lymphatics is inhibited or suppressed.

Comparison of Cellular Reaction to Phosphatide with That to Killed Tubercle Bacilli and Defatted Bacilli

Vigorous cellular reactions are induced by parenteral injection of all the fractions of tubercle bacilli thus far tested. It is only the lipoids, however, and particularly the phosphatides, which produce reactions characterized principally by the formation of tubercle-like masses of epithelioid cells. It seemed necessary, therefore, to determine any points of difference or similarity between reactions of the cells to tuberculo-phosphatide and those to killed tubercle bacilli and tubercle bacilli from which a portion of the fat had been removed. The latter represent the bacillary residue after prolonged treatment of the original organisms with alcohol, ether, and chloroform.

Rabbit R 2192 received 100 mg. of heat-killed tubercle bacilli suspended in 2 cc. of distilled water intraperitoneally and was killed 11 days later. Rabbit R 2193 received 100 mg. of defatted tubercle bacilli in 3 cc. of distilled water intraperitoneally and was killed 12 days later. Rabbit R 2255 received 6.5 mg. of phosphatide (the amount derived from 100 mg. of human tubercle bacilli) in 1 cc. of distilled water intraperitoneally, and was killed 10 days later. Striking differences were seen in the qualitative reactions to these three foreign substances. Each produced characteristic tubercle-like structures and scattered epithelioid cells. The reactions produced by the defatted and heat-killed tubercle bacilli were much more complex. The tissues of R 2192 and R 2193 showed many phagocytic clasmatocytes, leucocytes, and fibroblasts, all of these being more numerous in R 2192, which received the heat-killed bacilli. It is as if the reaction to the phosphatide had been produced by a relatively simple substance, while that to the defatted bacilli and heat-killed bacilli had been induced by more complex substances which have also the capacity to stimulate proliferation of other types of cells than epithelioid cells. This is, indeed, not surprising since both the heat-killed and partially defatted bacilli contain proteins and polysaccharides which cause marked changes in the connective tissues, and which are not contained in the tuberculo-phosphatide.

The significance of the element of time with regard to the complexity of cellular reactions to heat-killed and partially defatted tubercle bacilli has been demonstrated in other animals receiving these substances. Guinea Pigs R 518, R 519, and R 520 each received a single intraperitoneal injection of 1 mg. of heat-killed human tubercle bacilli. Guinea Pig R 518 was killed with ether 17 days after the injection. At autopsy the omentum was moderately thickened. There was a moderately extensive reaction limited to the omentum. There were many epithelioid cells, both in clumps resembling tubercles, and scattered diffusely. Many lymphocytes were also present. There were numerous phagocytic clasmatocytes containing debris, but this aspect of the pathological picture was much less pronounced than in Rabbit R 2192. There was moderate proliferation of fibroblasts. This reaction more closely resembled that seen in R 2255 than did the reaction observed in R 2192.

Guinea Pig R 519 was killed 22 days after introduction of 1 mg. of heat-killed human tubercle bacilli. Again the entire reaction was confined to the omentum. There were no epithelioid cells scattered diffusely but numerous tubercle-like clumps of them could be seen. The clasmatocytic response noted in R 518 had disappeared and the reaction closely resembled that seen in animals receiving the phospholipin.

Guinea Pig R 520 was killed 30 days after the introduction of 1 mg. of heat-killed tubercle bacilli. The cellular reaction was confined to the omentum and consisted of many tubercle-like clumps of epithelioid cells, an occasional giant cell, and many lymphocytes. The reaction resembled that seen in R 2255 which received tuberculo-phosphatide and was killed 10 days later.

Rabbit R 1772 received one injection of 0.1 mg. and six injections of 0.2 mg. of defatted bovine tubercle bacilli suspended in 1 cc. of normal saline subcutaneously in the right groin at 3 day intervals. The animal died 48 hours after the last injection. At autopsy a non-tuberculous pneumonia and empyema were found. No acid-fast organisms were found in the lung and in the pus in the pleural cavity. In the inguinal region at the site of the injections there was an abscess of moderate size, containing a few intact acid-fast rods and fragments of them. In sections this abscess had the appearance of a mass of epithelioid cells, the central portion of which had proceeded through caseation to liquefaction.

Rabbit R 1777 received intravenously one injection of 0.1 mg. and six injections of 0.2 mg. of defatted bovine tubercle bacilli suspended in 1 cc. of normal saline at 3 day intervals. The animal was killed 16 days after the last injection. There were numerous small tubercles in the lungs, made up for the most part of rosette giant cells, with smaller numbers of epithelioid cells. A few of the tubercle-like structures had caseous centers. Many lymphocytes were arranged about the periphery of each mass. No appreciable number of fibroblasts or clasmatocytes were present. The only other lesions were in the liver and consisted wholly of isolated rosette giant cells.

It can then be seen that the tissue changes occurring 10 or 12 days after injection of heat-killed or defatted organisms differ from those

occurring at a similar time after the injection of tuberculo-phosphatide in that the former are more complex. However, at a later time the reaction to heat-killed and defatted bacilli becomes simplified by the disappearance of granulocytes and clasmatocytes, so that the tubercular tissue so produced appears much like that induced by tuberculo-phosphatide.

Antigenic Power of Phosphatides from Mycobacteria

Pinner (27) first studied the antigenic nature of products from tubercle bacilli. Doan (28) and Doan and Moore (29) then found that the sera of some tuberculous animals and human beings contained precipitins for homologous tuberculo-phosphatide. Boissevain (30) has recently stated that a lipoid isolated from human tubercle bacilli by ether extraction produced cutaneous hypersensitivity to tuberculin. He stated, however, that this lipoid contained acid-fast bacilli. The phosphatides isolated by Anderson contain no demonstrable acid-fast organisms. However, it was deemed advisable to investigate whether the latter lipoid could produce cutaneous hypersensitiveness.

Accordingly two tuberculous animals (R 2229 and R 2230, Table VI) were given 2 mg. each of tuberculo-phosphatide intracutaneously in 0.1 cc. distilled water and were observed for evidence of reaction. Simultaneously skin tests with tuberculo-protein (Ma-100, 0.1 mg. in 0.1 cc. distilled water) were made on the same animals. In addition, normal animals which had received tuberculo-phosphatide (Anderson A-3) were, after an appropriate interval, tested for cutaneous hypersensitivity to homologous tuberculo-protein (MA-100, 0.1 mg. in 0.1 cc. distilled water).

The results of the tests are in Table VI. It can be seen that the phosphatide given intracutaneously produces a nodule which persists for some time, and that this reaction is in no way comparable to hypersensitivity to tuberculin. Moreover, animals which had received phosphatide parenterally did not exhibit cutaneous hypersensitiveness to tuberculo-protein. Boissevain has attributed tuberculin hypersensitiveness and the formation of tubercular tissue to water-insoluble proteins from the tubercle bacillus.[2] Although the water-insoluble protein isolated by Johnson and Coghill (23, 24) had been tested previously in this laboratory (10), additional tests have recently been made.

[2] At the 28th Annual Meeting of the National Tuberculosis Association, in Colorado Springs, June, 1932.

The water-insoluble protein, 10 mg. suspended in distilled water, was given intraperitoneally to each of two normal rabbits daily for 10 days. At autopsy on the 11th day there was no tubercular tissue to be found. The protein had induced a cellular reaction characterized by leucocytes, phagocytic clasmatocytes, and smaller numbers of lymphocytes and plasma cells. A water-insoluble alum precipitate of a dye-protein compound of high antigenic power and deep color (31), obtained through the courtesy of Dr. Michael Heidelberger, was injected intraperitoneally into a rabbit in 10 mg. doses daily for 4 days. The cellular reaction

TABLE VI

Results of Intracutaneous Tests for Hypersensitiveness

Animal No.	Inoculated with 0.1 mg. human tubercle bacilli	Amount of phosphatide injected and route of injection	Interval between injection and skin tests	Cutaneous reaction to tuberculo-protein	Time required to subside	Cutaneous reaction to tuberculo-phosphatide	Time required to subside	Remarks
		mg.	*days*		*days*		*days*	
R 2229	Nov. 12, 1931		36	++	3	+++	30+	Neither nodule became necrotic. The regression of the phosphatide nodule was delayed and very gradual in both animals
R 2230	" "		36	++	3	+++	30+	
R 1923		20 intraperitoneal	17	0				Tuberculo-protein MA-100 and old tuberculin used. Both tests negative in each instance
R 1924		20 "	17	0				
R 1925		20 "	17	0				
R 1927		20 "	17	0				
R 2355		40 subcutaneous	22	0				Tests made with tuberculo-protein MA-100. All entirely negative
R 2356		50 "	22	0				
R 2357		50 "	22	0				
R 2358		50 "	22	0				

which ensued was studied. The animal exhibited a response similar in all cytologic aspects to the two animals receiving the water-insoluble tuberculo-protein. The living cells were studied without neutral red or other accessory staining and the ingested protein was recognized within the cells by the dye which had been introduced into the protein molecule.

From our studies, it appears that water-insoluble protein is not associated with the formation of tubercular tissue.

DISCUSSION

It now seems certain that the changes produced in the tissues by the Anderson phosphatides are initiated by substances which are relatively

pure in the biological sense, and not by bacteria which have withstood the processes of extraction and filtration. No such bacteria or fragments are demonstrable in stained preparations of these phosphatides. In addition, it is now known that the purified phosphatide is predominantly crystalline. It is well known that dead, as well as living tubercle bacilli cause hypersensitiveness to tuberculin in guinea pigs. Cutaneous sensitization has not been elicited with tuberculin after introduction of the Anderson phosphatide. Probably more significant still is the fact that the reactions of the tissues of animals to the phosphatides are characterized chiefly by one type of cell, the epithelioid. In the light of the observation of Sabin, Doan, and Forkner (9), that lecithin (from brain) produces a similar qualitative reaction, strength is given to the hypothesis that the epithelioid cell is a connective tissue cell which has phagocytized a lipoidal substance. The appearance of the cell suggests an emulsification of the lipoid.

The bacterial residue which remains after extracting human tubercle bacilli for 4 weeks with a mixture of alcohol, ether, and water, and then with chloroform, retains to some degree the property of acid-fastness. The partially defatted organisms produced tubercular tissue when injected into animals. After the phosphatide is precipitated from the ether solution with acetone, a considerable quantity of lipoid remains in solution. This partition, designated the acetone-soluble fat, has also been observed to produce epithelioid cells when injected into animals. The phosphatide, which constitutes 6.54 per cent of the human tubercle bacillus, is but one of three substances obtainable from these organisms having the capacity to produce epithelioid cells. It cannot, therefore, be expected that the phosphatide will reproduce quantitatively the reaction caused by the same weight of the tubercle bacilli from which it is derived. Nevertheless, the reaction to the phosphatide is remarkably pure, tends principally toward one type of cell, and is qualitatively specific.

The fact that a thin film of phosphatide which has been mixed with water assumed forms which, microscopically, closely resemble degenerating myelin has made possible a determination of the fate of this material after injection. The lipoid is phagocytized by monocytes, or, if the stimulus be great enough, by the primitive cells which give rise to monocytes. After the lipoid has been phagocytized it is subjected to a process of intracellular dispersion into finer and finer droplets. After

reaching the stage of the finest droplets, no further detectable change takes place. While this process of phagocytosis and intracellular dispersion is going on, the cells exhibit evidence of proliferation and maturation, it being plain that all the functions of the cell are simultaneously set into action.

In the further analysis of the lipoids from mycobacteria, Dr. Anderson (13) has obtained by hydrolysis of the phosphatide certain fatty acids of high molecular weight. When injected into animals these fatty acids cause the formation of tubercular tissue (9). However, the epithelioid cells produced by the fatty acids pass directly to the fine vacuole stage. It therefore seems probable that the intracellular dispersion of tuberculo-phosphatide may represent a process of intracellular hydrolysis. In the one instance the process takes place *in vitro*, in the other *in vivo*.

The phospholipins from avian and bovine tubercle bacilli, timothy grass and leprosy bacilli produce reactions simulating those which occur after injecting the same fraction from human tubercle bacilli. The principal difference is that the process of intracellular dispersion of the lipoid takes place at a slower and more irregular rate. If this phenomenon of intracellular degradation represents hydrolysis, then the irregularity with which it occurs in the instances now under discussion may be but the expression of differences in chemical composition of the injected lipoid, by virtue of which it is subdivided with greater difficulty by the cells. Moreover, the fact that degradation of the phosphatide from human tubercle bacilli takes place more slowly in the cells of guinea pigs than in rabbits may indicate a greater abundance in the latter of some chemical factor necessary for the reaction.

Anderson (13) has shown that the nitrogen content of the bacterial phosphatides does not exceed 1.0 per cent (bovine) and that the value for the phosphatide from human tubercle bacilli is 0.36 per cent. He has also found the greater part of this nitrogen can be easily removed as ammonia. Since it requires a considerable amount, relatively speaking, of tuberculo-protein and a somewhat protracted series of injections to produce cutaneous hypersensitiveness to tuberculin, it is not surprising that the Anderson phosphatides do not cause cutaneous hypersensitiveness.

SUMMARY

1. A comparative study has been made of the cellular reactions induced by phosphatides from five strains of acid-fast bacilli. Each of these reactions is characterized principally by epithelioid cells and giant cells.

2. The phosphatides are first phagocytized by young connective tissue cells or monocytes. The lipoid is then dispersed into fine particles with the formation of classical epithelioid cells.

3. A comparison has been made of the reactions induced by heat-killed and defatted tubercle bacilli with those induced by tuberculophosphatide.

4. Further studies have been made to determine whether or not the phosphatide causes sensitization to tuberculin. It does not do so.

5. The life cycle of the epithelioid cell has been observed in all its stages.

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EXPLANATION OF PLATES

PLATE 44

FIG. 1. A preparation of living cells from the peritoneal fluid of Rabbit R 1802 which had received three intraperitoneal injections of 80 mg. of the phosphatide from human tubercle bacilli. Note large vacuoles of irregular size and shape in the first stage epithelioid cells. Beneath this cell is a granulocyte. The cells at the right are monocytes. Note the delicate surface films. Supravital neutral red and Janus green. ×1200.

FIG. 2. Section of the omentum of Rabbit R 821 after three intraperitoneal injections of 80 mg. of phosphatide from H-37. The two cells with highly vacuolated cytoplasm are first stage epithelioid cells or the same type shown in the living state in Fig. 1. The two smaller cells are monocytes. Hematoxylin and eosin. ×1200.

FIG. 3. A film of living omentum from Rabbit R 2186 showing an epithelioid cell of the second stage. Note that the vacuoles are of uniform size and shape, but smaller than in the cell in Fig. 1. Supravital neutral red and Janus green. ×1300.

FIG. 4. Section of the omentum from Rabbit R 2186, showing epithelioid cells of the second stage from the same animal as that of Fig. 3. Note foamy appearance of cytoplasm. Hematoxylin and eosin. ×1200.

FIG. 5. Section of the omentum of Rabbit R 377, 90 days after thirteen injections of 80 mg. of phosphatide from H-37. The cell in the center is a degenerating epithelioid cell. Note indefinite cytoplasmic outline, pale vacuolated cytoplasm, and disintegrating nucleus. The other cells are third stage epithelioid cells. Hematoxylin and eosin. ×1200.

FIG. 6. Section of the omentum of Rabbit R 2187 after ten intraperitoneal injections of 80 mg. of phosphatide from H-37. Note homogeneous appearance of cytoplasm of the third stage epithelioid cells. Hematoxylin and eosin. ×1200.

PLATE 45

FIG. 7. Section of omentum of Rabbit R 688 after twelve injections of 80 mg. of the phosphatide from avian tubercle bacilli. The epithelioid cells are of the second and third stages. A few lymphocytes are in the lower portion of the photograph. Hematoxylin and eosin. ×1000.

FIG. 8. A section of the omentum of Rabbit R 1015 after eleven intraperitoneal injections of 80 mg. of phosphatide from bovine tubercle bacilli. A large rosette giant cell is in the center, and about it epithelioid cells of the second and third stages. Hematoxylin and eosin. ×1000.

FIG. 9. A section from the wall of the cecum of Rabbit R 1426 after ten intraperitoneal injections of 80 mg. of phosphatide from timothy grass bacilli. It shows a nodule of tubercular tissue with caseous center and intact epithelioid cells about the periphery. Hematoxylin and eosin. ×240.

FIG. 10. A section from the omentum of Rabbit R 1950 after ten intraperitoneal injections of 80 mg. of phosphatide from leprosy bacilli. Note that all of the epithelioid cells are highly vacuolated. Hematoxylin and eosin. ×1300.

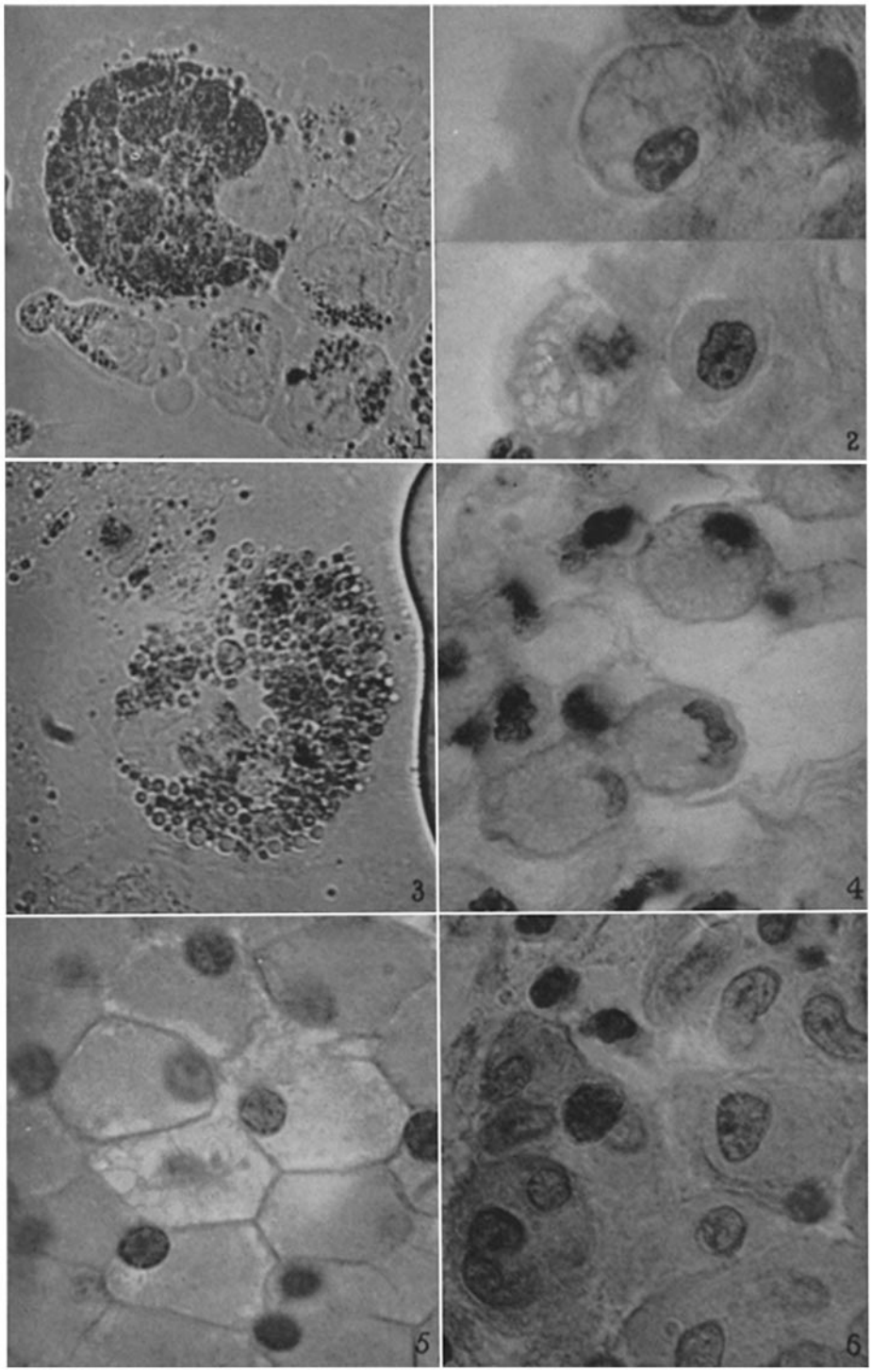
PLATE 46

FIG. 11. A section from the retrosternal lymph node of Guinea Pig R 1927, 3 weeks after a single intraperitoneal injection of 20 mg. of phosphatide from human tubercle bacilli. Note epithelioid cells surrounding a lymphoid follicle. Hematoxylin and eosin. ×250.

FIG. 12. A section from the omentum of Guinea Pig R 1927 (same animal as of Fig. 11), showing epithelioid cells and giant cells beneath the serosa. Hematoxylin and eosin. ×250.

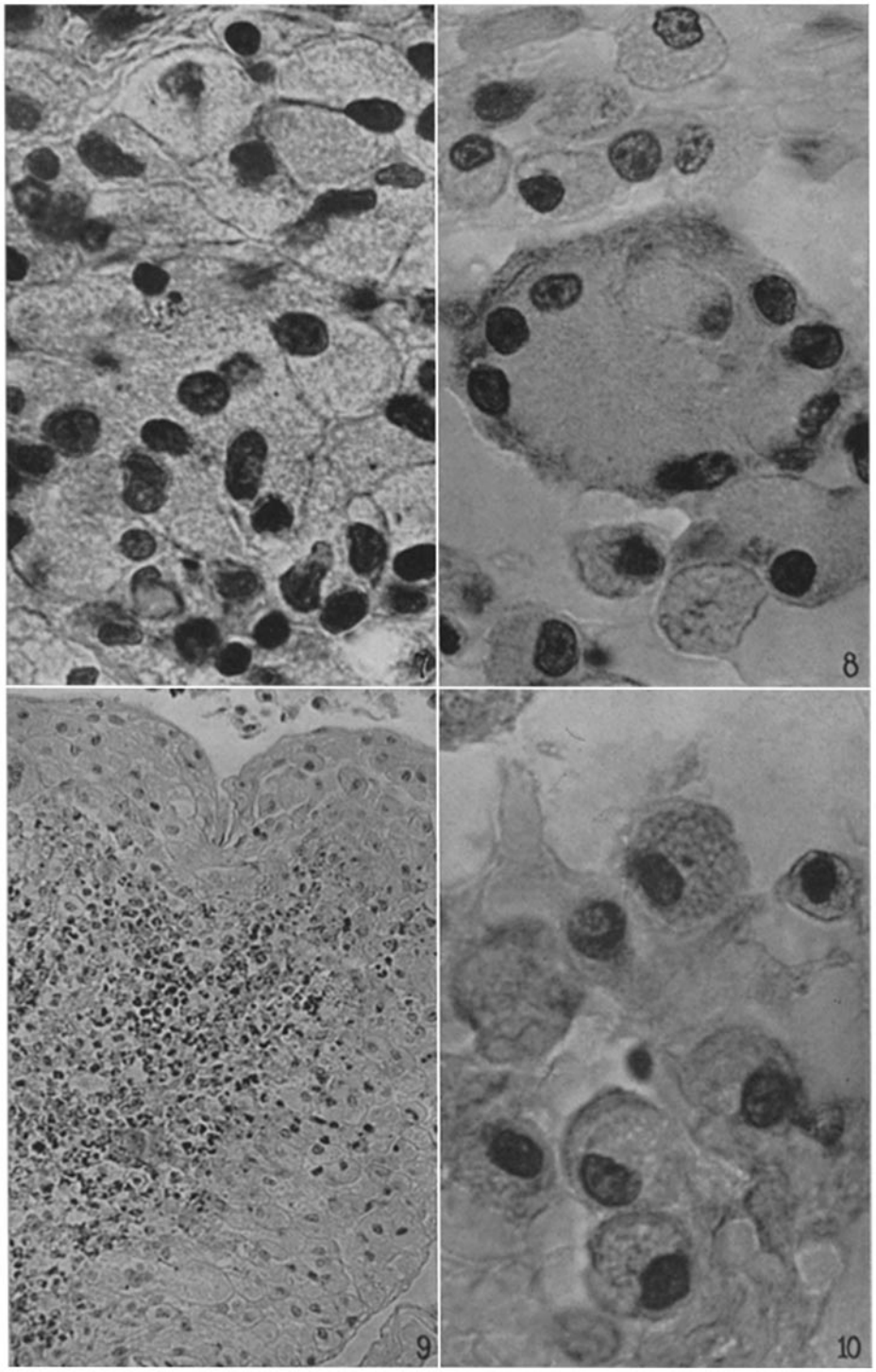
FIG. 13. A living preparation of peritoneal exudate from Guinea Pig R 690 after seven intraperitoneal injections of 80 mg. of phosphatide from human tubercle bacilli. Note myelin-like figures in the two large cells, those in the upper one being smaller than those in the lower cell. Supravital neutral red and Janus green. ×1050.

FIG. 14. A section of the omentum from the same guinea pig as that in Fig. 13. Note highly vacuolated appearance of cytoplasm in the epithelioid cells, and compare with the appearance of the same cells in the living preparation (Fig. 13). Hematoxylin and eosin. ×1000.

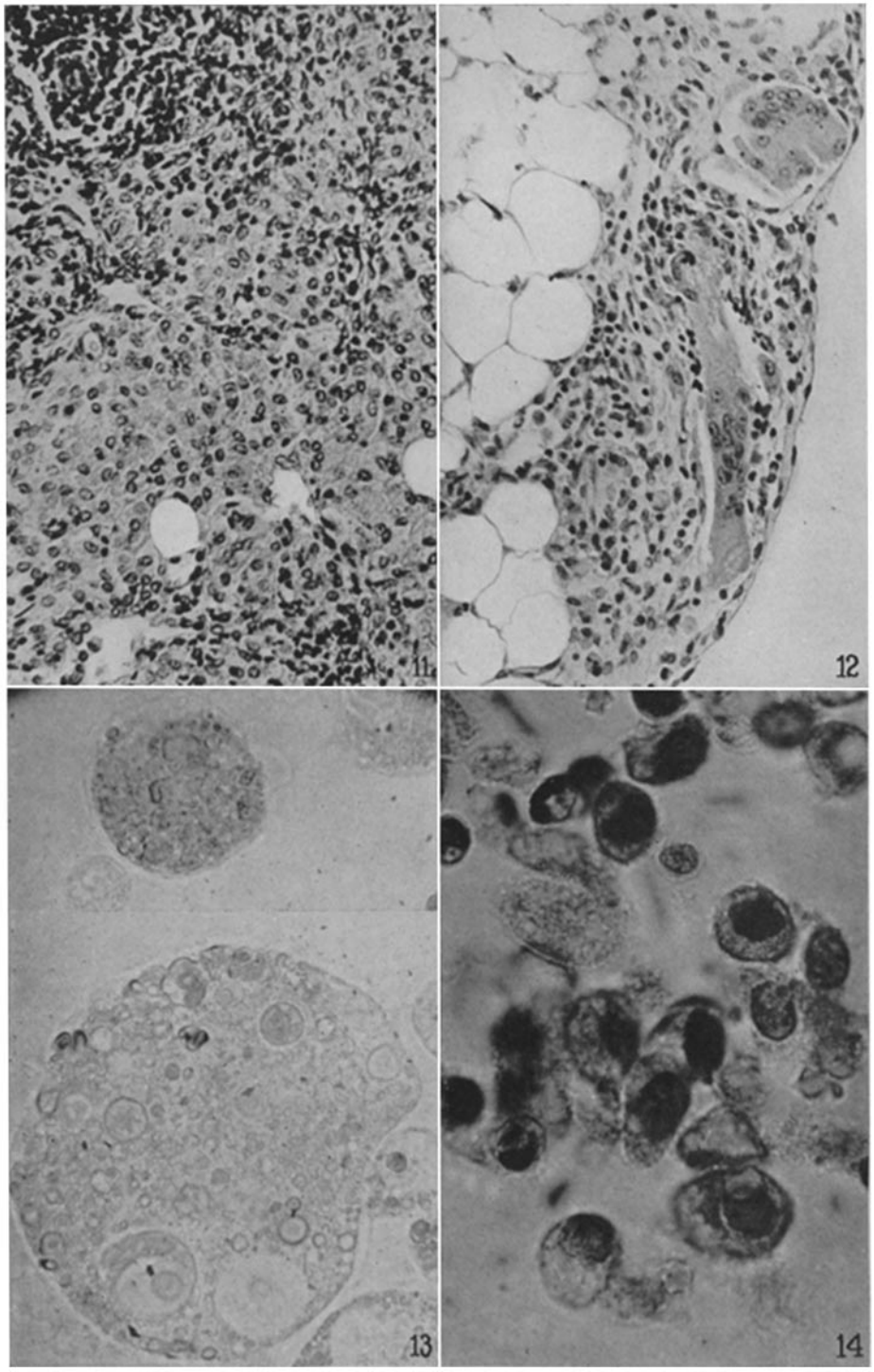


Photographed by Louis Schmidt

(Smithburn and Sabin: Lipoid fractions from acid-fast bacilli)



Photographed by Louis Schmidt



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