THE BIOLOGICAL REACTIONS IN RABBITS TO THE PROTEIN AND PHOSPHATIDE FRACTIONS FROM THE CHEMICAL ANALYSIS OF HUMAN TUBERCLE BACILLI.

By FLORENCE R. SABIN, M.D., and CHARLES A. DOAN, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

Plates 26 to 29.

(Received for publication, June 20, 1927.)

Through the organization of the Research Committee of the National Tuberculosis Association, for cooperative research, we have had available for biological testing certain protein and phosphatide fractions isolated in the chemical analysis of tubercle bacilli. Two specimens of water-soluble proteins, designated 304 and 903 respectively, were received from Professor Treat B. Johnson of the Sterling Chemistry Laboratory of Yale University, and two phosphatide fractions, A-3 and A-4, from Dr. R. J. Anderson, of the same laboratory (1, 2). The material was obtained from the analysis of human tubercle bacilli, strain H 37.

In another paper in this *Journal* (3) evidence has been given to show that clasmatocytes and monoytes react differently toward tubercle bacilli. By some investigators, particularly those using the technic of tissue culture, it is believed that this phagocytic mononuclear group represent essentially a single strain of cells, variations in the physiological environment being thought sufficient to account for differences in the histological structure within the limits observed. However, an increasing weight of evidence accumulating as a result of the direct analysis of the response of the cells of the connective tissues *in situ* to pathological conditions, is necessitating the subdivision of this group on functional, as well as genetical and structural (4, 5) grounds. For example, while both clasmatocytes and monocytes phagocytize tubercle bacilli, the clasmatocyte on the one hand fragments the bacteria readily and rapidly as it does all debris with

which it has to deal; the monocyte, on the other hand, retains the specific bacillus intact so long that the relationship may be considered one of symbiosis.

And now, again, in analyzing the responses of tissue to the protein and phosphatide fractions from the tubercle bacilli, it has been found necessary to express the differential reactions in terms of the same two types of cells within this mononuclear phagocytic group: the response to the protein fractions has been predominantly clasmatocytic, in sharp contrast to the development of monocytes, epithelioid cells, and Langhans giant cells, making the typical lesions of tuberculosis, which is the overwhelming reaction to the phosphatide fractions.

The amount of material available limited the administration of the protein fractions to seven rabbits, the phosphatide fractions to five rabbits, with a control group consisting of six rabbits.

The Protein Fractions.

Both the proteins, 304 and 903, were given intravenously from saturated solutions made up each day in water freshly distilled from glass. From a preliminary experiment (6) it was known that rabbits could readily withstand a dosage of 10 cc. of the saturated aqueous solution of 304. With this experience as a basis a similar dosage of 903 was given to Rabbit R 138, with an immediately fatal result simulating anaphylactic shock. A second rabbit, R 132, survived the initial dose of 10 cc. of 903, but died immediately after a second dose 24 hours later. In the latter animal the white count before the first injection was 9350; on the next day, just before the second inoculation, the white cells had risen to 25,000. At autopsy the lungs showed hemorrhagic foci and the free cells were predominantly clasmatocytes and leucocytes. The protein fraction 903 was, thus, found to be far more toxic than 304, since in the present experiment two rabbits survived ten and eleven doses, respectively, of the latter in the dosage that proved fatal with 903.

To determine the upper limit of sublethal dosage for 304, Rabbit R 152 was given two injections of 20 cc. each at an interval of 24 hours. The studies of the peripheral blood of this animal are shown on Chart 1, the data concerning the white cells being given on a scale one-half the magnitude of the other graphs to cover the rise in leucocytes. The dosage of 20 cc., while not lethal, was too toxic to study other than the acute reaction; the day after the first injection the animal appeared ill, ate but little, and showed a rectal temperature of 107.6°F. (normal 102–104°F.); following the second dose the condition did not warrant further injections. From the chart it is obvious that the animal was developing an anemia, the red cells

falling from 6,800,000 to 4,100,000 and the hemoglobin from 65 to 53 per cent; while the white cells mounted from 10,000 to 27,000. On the two counts made before the injection, the neutrophilic leucocytes were low, 38 and 29 per cent, as can be seen on the chart by a comparison of their number and the total number of the white cells. However, the total myeloid cells were about half the white cells, 54 and 57 per cent, due to the relatively large percentage of basophilic cells, which were 13 and 22 per cent in respective counts. 24 hours after the first injection a marked rise in the total number of white cells may be seen to have been due wholly to an increase in neutrophilic leucocytes and this continued to be true throughout the experiment. The basophilic and eosinophilic leucocytes, on the other hand, remained unaffected at their original levels indicating a specific stimulating effect on one group of granulocytes only, namely the neutrophils. There were interesting qualitative as well as quantitative changes in the neutrophilic leucocytes: on Jan. 4, of the 67 per cent neutrophils, 57 per cent were of the non-motile (7) type. These are the forms that appear as ruptured cells with scattered granules in fixed films, showing that they are fragile. 24 hours after a second dose of 304 was given, the white cells showed some further increase but now there were found two types of neutrophils, old, degenerating types with fragmenting nuclei and a new group of peculiar young forms. These young leucocytes had indented nuclei, not yet two lobed; they were markedly deficient in the specific granulation and rich in mitochondria; and they were not actively ameboid. In fixed films these cells had the other characteristic of incomplete maturation, namely a strongly basophilic cytoplasm. At this examination the leucocytes were about equally divided between the old forms and the young. A second count, in the afternoon, showed a fall in total number of white cells to within normal limits with only 6 per cent of the degenerating leucocytes remaining. On the next day the total white count had risen again to 27,000 and the young leucocytes as described were the predominating type. As will be seen on the chart, both monocytes and clasmatocytes rose slightly while the lymphocytes remained at the low level to which they had fallen after the first dose.

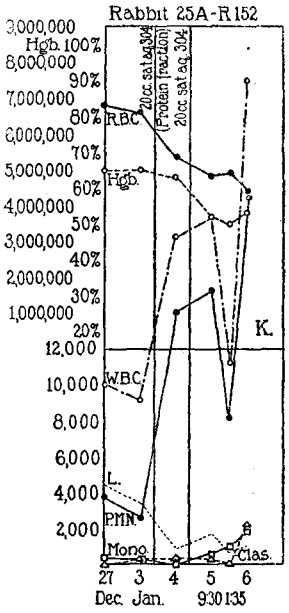


CHART 1.

The studies of bone marrow following the autopsy on the 4th day revealed something of the nature of the processes already indicated in the studies of the peripheral blood. The bone marrow had been depleted rapidly of its store of mature myelocytes, Type C (8): this was evident in the supravital preparations of the fresh marrow and in sections. The sections showed that the interspaces

between the fat cells had been denuded of cells so that they were like the sections of marrow shown by Doan *et al.* (9) both from experimental animals treated with repeated doses of dead typhoid bacilli and from a human case of typhoid fever. In the supravital preparations of this marrow the immature myelocytes, Type B, with fewer of the specific ganulations, showed a premature indication of polymorphism of the nuclei and the beginning of motility. Thus the leucocytes of the blood stream had had certain of the characteristics of the B myelocytes, namely the basophilic cytoplasm, the large content of mitochondria, and the low content of specific granules; while their nuclei were, on the other hand, of the type of the metamyelocyte, that is indented but not yet lobed. The nuclei showed that the cells were being transformed into functionally active leucocytes without complete maturation relative to their full quota of granules. These cells therefore were entirely different from the immature and unchanged B myelocytes that occur in the blood stream in leucemia (8). This type of cell, the immature but motile leucocyte, is especially interesting because it occurs in the blood in typhoid fever (10), probably for the same reason as in this rabbit with the massive doses of protein. It must be discriminated with care from the monocyte both in the fixed films and in the supravital technic. The discrimination is to be made by the nature of the granulations. In this rabbit, it is interesting to note that the effect of the protein was limited to the neutrophilic (pseudo-eosinophilic) leucocytes, both the basophilic and the eosinophilic cells being unchanged in the blood and in the bone marrow; the reaction thus represents a response to a protein in one strain of granulocytes. This type of reaction is of course not specific of a protein from tubercle bacilli only.

The autopsy showed a general increase in clasmatocytes in the tissues in the lung; as determined in supravital studies, many of these clasmatocytes contained fragments of red cells. In sections the interalveolar septa were thickened and a few foreign body giant cells were found. As in all the animals with the protein fractions, there were hemorrhages. In this animal they were especially marked in the bone marrow as shown by sections. There were gross hemorrhages in the retroperitoneal tissues extending into the psoas muscle on either side.

The liver showed changes. There were certain chronic periportal lesions entirely independent of the experiment. The acute lesions of the liver were irregular, small zones of necrosis, involving 20 to 30 liver cells, sometimes centrally placed, but more often in the middle of the lobule part way between a portal space and a central vein. In these zones the liver cells were extremely vacuolated with no evidence of normal granulation and the nuclei showed extensive fragmentation. Frozen sections stained with Sudan III showed these cells loaded with fat. Leucocytes and highly phagocytic clasmatocytes infiltrated these areas, with destruction so great that the vessels could not be analyzed. There were also degenerations in the convoluted tubules of the kidney, some of which contained cellular debris and some fragments of red cells. Thus the effects of the protein in this animal were a specific leucocytosis, fever, an increase in clasmatocytes in the lung, multiple hemorrhages, focal fatty necrosis of the liver cells, and some damage to the cells of the kidney tubules.

TABLE I.

Protocols.

No. of rabbit	Dose	Length of life
	All protein fractions given intravenously	
R 138	1 dose of 10 cc. of 903	Died immediately after first dose
R 132	2 doses of 10 cc. of 903	Died after second dose
R 152	2 doses of 20 cc. of 304	Killed 48 hrs. after second dose. Maximum temperature 107.6°
R 131	26 doses of 2 to 5 cc. of 903. Total of 72 cc. in 33 days	Killed 3 days after last injection. Maximum temperature 107°
R 137	25 doses of 1 to 3 cc. of 903. Total of 32 cc. in 31 days	Died immediately after last injection; complicating peritonitis found, due to *B. lepisepticum*. Maximum temperature 108.2°
R 129	13 doses of 6 to 10 cc. of 304. Total of 126 cc. in 15 days	Killed 24 hrs. after last dose. Maximum temperature 108.5°
R 130	12 doses of 6 to 10 cc. of 304. Total of 116 cc. in 15 days	Died night after last dose. Maximum temperature 108.3°
	Phosphatide fractions	
R 156	2 doses of 80 mg. of A-3 intravenously	Acute death suddenly after second dose, from emboli
R 153	2 intravenous and 12 intraperitoneal doses of from 80 to 160 mg. of A-3. Total of 1280 mg. in 15 days	Killed 24 hrs. after last dose
R 160	12 intraperitoneal doses of from 80 to 160 mg. of A-3. Total of 1120 mg. in 13 days	Killed 48 hrs. after the last dose
R 158	1 intravenous and 12 intraperitoneal doses of from 40 to 160 mg. of A-4. Total of 1000 mg. in 14 days	Killed 6 days after the last dose
R 159	1 intravenous and 12 intraperitoneal doses of from 40 to 160 mg. of A-4. Total of 1120 mg. in 14 days	Killed 4 days after the last dose
	Controls	
R 243	15 intraperitoneal doses of from 80 to 122 mg. of lecithin. Total of 1242 mg. in 17 days	Killed in excellent condition 24 hrs. following the last injection
R 244	15 intraperitoneal doses of from 80 to 122 mg. of lecithin. Total of 1242 mg. in 17 days	Killed in excellent condition 24 hrs. following the last injection

TABLE I—*Concluded.*

No. of rabbit	Dose	Length of life
	Controls—*Concluded*	
R 245	15 intraperitoneal doses of lecithin plus tubercle bacilli, H 37, inactivated at 60° for 1 hr. Total of 1242 mg. lecithin and 3.75 mg. bacilli in 17 days	Killed in excellent condition 24 hrs. following last dose
R 246	15 intraperitoneal doses of lecithin plus tubercle bacilli, H 37, inactivated at 60° for 1 hr. Total of 1242 mg. lecithin and 4.00 mg. bacilli in 17 days	Killed in excellent condition 48 hrs. following last injection
R 247	12 intraperitoneal doses of tubercle bacilli, H 37, inactivated at 60° for 1 hr. Total of 3 mg. in 14 days	Killed in excellent condition immediately after last injection
R 248	16 intraperitoneal doses of from ¼ to 3 mg. of tubercle bacilli, H 37, inactivated at 60° for 1 hr. Total of 7 mg. in 19 days	Killed in excellent condition 24 hrs. after last injection

With the studies on toxicity just cited as a basis for dosage the remaining material was given to a small number of animals, rather than less amounts to a larger series in the hope of securing if possible a striking and characteristic effect. The results have seemed to justify this plan. Two rabbits (R 131 and R 137) received the protein 903 in repeated daily doses of 1 to 2 cc. of a saturated solution, with final doses of from 3 to 5 cc.; while two others (R 129, R 130) were given daily intravenous injections of 304 in the original amounts of 10 cc. per dose. With these dosages in total amounts as shown in Table I, the effects noted in the four animals were in general comparable.

Chart 2 is given as representative of the two animals with 903, Chart 3 for the two with 304. These charts are given for the sake of comparison of the effects on the peripheral blood of the chemical fractions from tubercle bacilli with the known effects in the rabbit of the living tubercle bacilli in large doses. In an earlier paper in this *Journal* (11) it has been shown that the changes in the blood after an intravenous injection of 1 or 2 mg. of bovine tubercle bacilli reflect directly, and may be interpreted in terms of, two different processes. First, an anemia combined with the fall in platelets and granulocytes indicates a direct effect on the bone marrow; second, an increase in monocytes and at times in clasmatocytes combined with a fall in lymphocytes is correlated with the general progress of the tubercular process in the tissues (6).

It has been demonstrated that the effect revealed in the blood in red cells, plate-

lets, and granulocytes is due to an extensive involvement of the marrow with tuberculosis, a constant finding at a certain stage with the doses mentioned; that the local tubercular lesions are sufficient to displace the fat and to reduce the marrow to the level of the early erythroblasts for the red cells and the early myelocytes for the white cells. Rabbit R 131 received 903, in doses as seen in Chart 2. It will be seen in this chart that there is a sharp fall of the red cells, correlated with a drop in hemoglobin beginning after the 8th dose of protein; the lowest point was reached Dec. 30 with 3,760,000 red cells. There was later a partial recovery of the red cells, but to an average of 5,000,000 rather than to the original average of 6,000,000. This fall in red cells was not correlated with a fall in granulocytes,

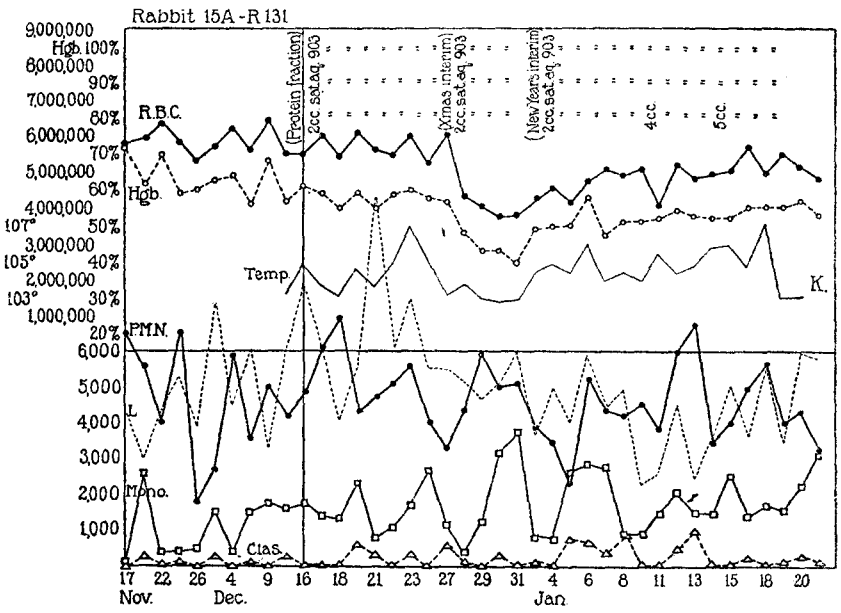


CHART 2.

the latter remaining practicllly at their original level. The anemia was found to be due to multiple hemorrhages and was not therefore a local effect on bone marrow except in so far as there were, as in every case, hemorrhages in the bone marrow itself as well as multiple ones outside the marrow. The anemia was thus secondary to the hemorrhage.

In regard to the white cells, the total numbers are not shown on the chart but their range before the injections was from 7250 to 15,600; while after the injections there were four moderately high counts: Dec. 21, 17,000; Dec. 23, 15,600; Dec. 31, 15,400; and Jan, 6, 15,900. These numbers are not excessive for the rabbit and the experiments already recorded with R 132 and R 152 indicate that a marked

effect of the protein on the leucocytes is obtained only with larger doses. The chart also differs from a typical tubercular chart in a negative effect on lymphocytes. On the other hand, there was a somewhat rhythmic rise in monocytes and clasmatocytes which is like tuberculosis. At the times of the increase in monocytes in the peripheral blood there were some qualitative changes, namely some decreased motility, an increase in small scattered neutral red bodies in the cells, and some cells with accentuated rosettes; but no true epithelioid cells were found such as had been seen in the preliminary tests with 304 (6).

Rabbit R 137 received smaller doses of 903, namely, twenty of 1 cc., three of 2 cc., and two of 3 cc., making a total of 32 cc. as contrasted with a total of 72 cc. for R 131. R 137 had the only complication of the series, namely a terminal peritonitis due to the *B. lepisepticum*, identified by Dr. Ida Pritchett. The only difference between this chart and that of R 131, as far as the white cells were concerned, was a fall in both leucocytes and lymphocytes during the last 4 days. In this animal there was no anemia. The autopsy showed only small fresh hemorrhages so the interpretation is that the animal did not survive the hemorrhages long enough to show their effect in the peripheral blood. The curves of monocytes and clasmatocytes were the same as in the corresponding rabbit, R 131.

The peripheral blood in the two animals, R 129 and R 130 (Chart 3), that received the protein fraction 304, showed the same effects as 903. R 129 received eleven doses of 10 cc. and one of 6 cc. Rabbit R 130 had ten doses of 10 cc. and one of 6 cc. Both animals showed a gradually progressive anemia; the lowest count on R 129 was 3,680,000 from an original base line of 5,500,000; while the lowest count on R 130 was 2,970,000 from a level of 5,000,000 to 6,000,000 before the injections. Of all the animals with the protein fractions, R 130 showed the most marked anemia and had the most extensive hemorrhages. In both R 129 and R 130 there was a neutrophilia and a tendency toward lower lymphocytes. In R 130, though not in R 129, there was the same somewhat rhythmic rise in monocytes as is shown on Chart 2.

In summary,the protein fractions 903 and 304 were both toxic; both produced anemia with neutrophilia of greater or less degree; four of the rabbits died, two (R 138 and R 132) in the acute reaction to large doses of 903, one (R 130) after repeated doses of 304, and the other (R 137) after 903 but with a complicating infection so that death could not be ascribed to the protein alone. There were no signs of secondary infection in the other animals. One rabbit (R 152) was killed when in a critical condition; the other two (R 129, R 131) were killed while still in good condition All the rabbits appeared more or less ill with temperatures reaching between 107° and 108.5°.

At autopsy the findings in the series were quite uniform. The only signs of abnormality obvious in the gross inspection were multiple hemorrhages. In every animal, on opening the abdominal cavity, there was found edema of the prevertebral connective tissues, with small hemorrhagic foci scattered throughout from diaphragm to pelvis. These hemorrhages extended characteristically into the septa of the psoas muscles on either side and in one animal were found to have

dissected into the prevertebral tissues of the thoracic cavity and were found also in the axillæ. The question arose as to whether the psoas hemorrhages, so uniform in distribution in the gross, could be traumatic in origin, but they were present in only these five out of more than seventy rabbits handled in the same way by the same people; moreover, all the rabbits in this group had also hemorrhages in the bone marrow as well as in some of the other organs. The animal with the infection (R 137) had a peritonitis with a thick, purulent exudate covering the entire peritoneal surfaces, but typical hemorrhages were found in sections of the subperitoneal prevertebral tissues.

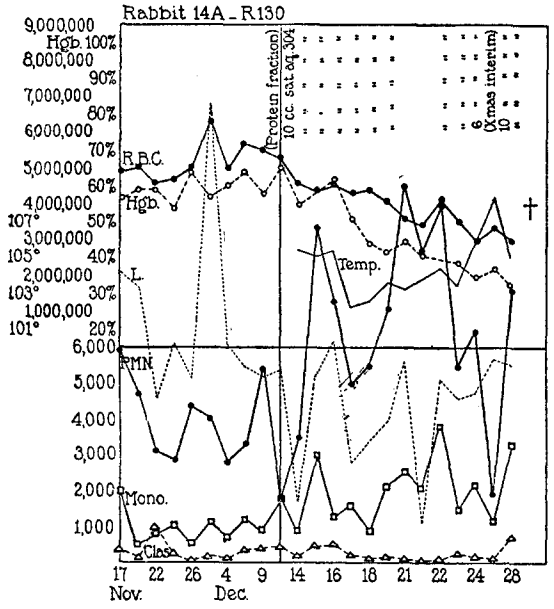


CHART 3.

In Fig. 6, is shown a drawing from the central part of the mesenteric lymph gland of Rabbit R 131, which drained a hemorrhagic area. The lymph cords are covered, or, in other words, the lymphatic sinuses are lined by endothelial cells which are engorged with debris from red cells. In this lymph gland the peripheral sinuses were entirely denuded of endothelium leaving quite bare the scanty framework of reticular cells which showed no phagocytic activity whatever. All the central sinuses of the gland show a complete phagocytic endothelium except for an occasional endothelial cell which has dropped off as is indicated by the arrow *b* (Fig. 6). At the point marked with the arrow *a* on the drawing there is a reduplication of the endothelium, with the outer cell rounding up as if to des-

quamate. At the point marked *c*, there is an endothelial cell stretching along the reticular framework. Within the lymph cords there are a few phagocytic cells exactly like the lymphatic endothelium; some of these are obviously adventitial cells to the blood vessels of the lymph cords; others appear to be free of any relation to the adventitia of a blood vessel. There was no involvement of the blood vascular endothelium *per se* in the phagocytic activity. In some of the central sinuses there were great numbers of free round phagocytes loaded with the debris, which cells probably account for the endothelium of the denuded peripheral sinuses. This lymph gland offers as good evidence as can be had that certainly some of the cells of the free phagocytic type, the typical clasmatocyte, may originate from endothelium, in this case from lymphatic endothelium. It also gives quite conclusive evidence that there are two types of cells in the lymphatic sinuses, the nonphagocytic reticulum and the potentially active endothelium; and it shows as well that the endothelium is complete or incomplete according to the functional state of the gland. It is probably true that the extensive response of the endothelium in this lymph gland was not a primary effect of the protein injected but was rather a response secondary to the presence of a massive amount of the debris of red cells. In this rabbit there was one rather large hemorrhage in the edge of the psoas muscle near the kidney and there are clumps of clasmatocytes loaded with yellow debris and others with brown pigment from the red cells in the surrounding tissues. This is the only rabbit in the series in which there has been any breaking down of the hemoglobin in any of the hemorrhagic areas sectioned. On Chart 2 from this animal (R 131), it will be seen that there was a sudden marked fall in the red cells, 24 days before the date of the autopsy; whereas on Chart 3, the fall in the red cells was a very gradual one. All the other hemorrhagic areas studied in the other animals show the condition illustrated in Fig. 9, in which there are intact free red cells in the tissues exactly like those in the vessels without any appreciable phagocytosis. Obviously there has been a marked increase in connective tissue cells, with many mitoses present. In the supravital studies of the tissues adjacent to that of Fig. 9, the predominating cell was identified as the clasmatocyte in its characteristic reaction to neutral red but no phagocytosis of the red cells was found. The interpretation is that these areas show a preliminary increase in the clasmatocytes by division before the red cells are engorged. In the fixed tissue, as shown in Fig. 9, it is clearly not possible to discriminate between fibroblasts and clasmatocytes; and it is well known that the inactive clasmatocyte has never been discriminated in fixed tissues. With the supravital technic as applied to the connective tissues, however, it is possible to distinguish readily the cell types morphologically differentiated and known as the fibroblast, the resting clasmatocyte, the stimulated clasmatocyte, the epithelioid cell, and the serosal lining cell (see plates, etc., in preceding papers (4, 6)).

Beside the hemorrhages, all the rabbits with the protein fractions showed a thickening of the interalveolar septa in the lungs. The maximum grade of this lesion found in any of the series is shown in Fig. 7. In the gross specimens the lungs did not look abnormal, but there was an increased resistance to the knife on cutting

and the supravital preparations all showed an increase in free cells which were of the clasmatocytic type. In all the lungs the areas of thickened septa were in patches and varied in amount from a lesion just perceptible up to the grade shown in Fig. 7. This section is from a rabbit, R 130, which was found dead. The lungs were mottled, which was postmortem, but supravital preparations were recorded as showing a marked increase in free cells, entirely due to clasmatocytes which were still living as shown in their characteristic reaction to neutral red. It is interesting to note that this type of reaction has already been found in response to the injection of both living and dead tubercle bacilli. It is this reaction which Lewis and Sanderson (30) found in the lungs 48 hours after the injection of massive doses of living bacilli, demonstrated to be an increase in clasmatocytes in another paper in this *Journal* (3). The reaction in the lung to the injection of dead bacilli or of chloroform extracts has been described in the literature (14, 18, 31) as an interstitial pneumonia, which is the lesion illustrated in Fig. 7.

In describing the autopsy results in Rabbit R 152, with massive doses of 304, it was noted that there were small necrotic areas in the liver. There was another rabbit in the series, R 130, with extensive acute necrosis of the liver cells. At autopsy, the hepatic lesions were easily visible in the gross, giving a marked mottling to the surface; frozen sections showed areas of cells loaded with fat and in fixed sections these zones showed extreme necrosis of the liver cells with destruction of their nuclei. The lesions are the same as in Rabbit R 152 but much more extensive. These are acute lesions, having no relation to the chronic periportal cirrhosis found in all the rabbits of the series. Inasmuch as the acute necrosis of liver cells was obvious in only two of the five animals, it cannot be regarded as a constant effect of the protein; it must, however, be kept in mind in the further biological testing of the material.

The lesions found in all the animals that received the protein fractions were multiple hemorrhages from small vessels and an increase in clasmatocytes, especially in the so called interstitial pneumonia of the lungs. These proteins were thus toxic to endothelium. In general, the proteins were toxic to the animal, lethal in large doses, and gave high temperatures.

Phosphatide Fractions.

Known weights of the phosphatide fractions were rubbed into a fine emulsion in water freshly distilled from glass. The dose determined with reference to the proportion of this material to the total weight of the dry bacilli was 80 mg. The first injections were made intravenously but since one of the animals dropped dead instantly, probably from an embolus, the intraperitoneal route was substituted.

Four rabbits were given repeated intraperitoneal doses of the two fractions, as shown in Charts 4 and 5. Two rabbits, R 153 and R 160, received A-3, while two rabbits, R 158 and R 159, had A-4. These two fractions were entirely nontoxic and there was no rise in temperature in any animal. The two rabbits that received A-3, of which Chart 4 is representative, both showed an anemia with a

fall of about 2 million red cells and a corresponding drop in the hemoglobin. At the same time both of them showed a rise in monocytes reaching the level of 4000 cells per c.mm. The two rabbits that received the fraction A-4, of which Chart 5 is representative, showed the same response in less degree. In these animals, R 158 and R 159, the fall in red corpuscles was a little over a million cells while the rise in monocytes was to the level of 2000 cells. The changes in leucocytes and lymphocytes were inconstant and perhaps negligible; Rabbits R 153 (A-3) and R 159 (A-4) both showed the slight drop in leucocytes and lymphocytes indicated on Chart 4, while the other two had no changes, as shown on Chart 5. Thus all

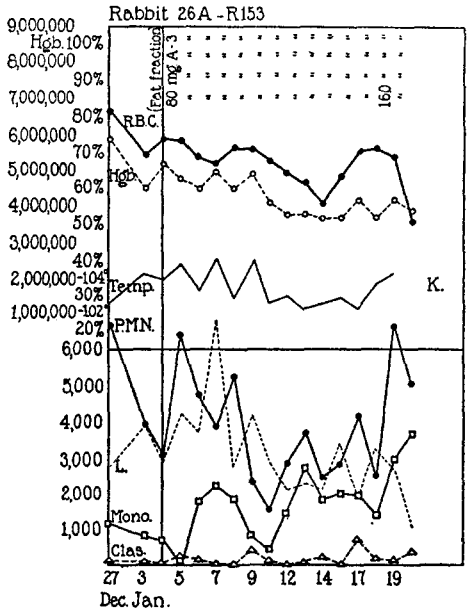


CHART 4.

four animals showed some anemia, which was possibly due to the distilled water introduced, since there were no hemorrhages and no changes were detected in the bone marrow. In all four animals there was a rise in monocytes, after A-4 to 2000 cells, after A-3 to 4000 cells. Thus the greater anemia and the more marked monocytosis were with the fraction A-3.

The four animals with the phosphatide fractions, in striking contrast to those receiving the protein, showed no clinical symptoms whatever during the course of the injections. The rabbits, therefore, were autopsied while all were yet in excellent condition when all the available material had been used. The extent of the pathological lesions was thus entirely unexpected. The findings in general

were identical in type in the four animals and the pathology in general was confined essentially to the peritoneal cavity. The local lesions were in every case so massive that it was difficult to differentiate quantitatively their relative extent in the individual cases. Rabbit R 128, which received A-4, had perhaps the greatest amount of involvement though it was one of the rabbits with the lesser reaction in the peripheral blood.

On opening the peritoneal cavity there were no ascites and no adhesions but all the animals showed an extensive involvement of the parietal and visceral peritoneum with what proved to be typical tubercular tissue. The omentum in every case

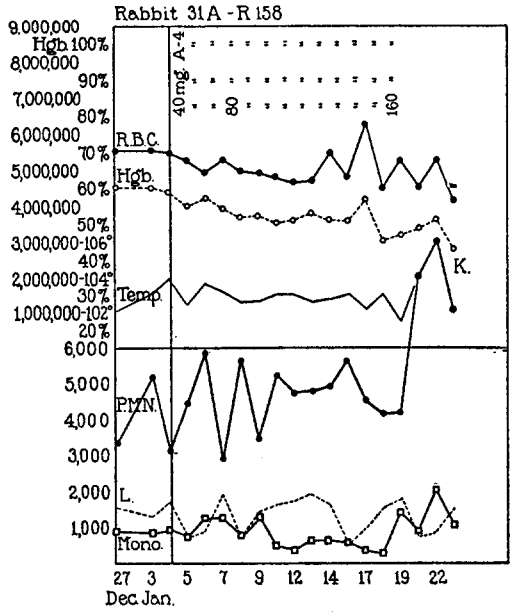


CHART 5.

was a dense thickened, nodular, dull reddish mass, markedly different from the delicate membrane of the normal state. The characteristic color was probably due to the increased vascularization. Some idea of the thickness of these omenta may be obtained from Fig. 11, in which the original thickness of the omentum illustrated can be seen at the upper right hand corner; the rest of the photograph gives about one-third of the actual thickness of the tissue at that point. The proliferation is specifically of epithelioid cells with a great excess of giant cells of the Langhans type in this area. The small darker nuclei indicate clusters of lymphocytes. Fig. 10 illustrates another area in the same animal (R 153) with fewer giant cells and a marked proliferation of typical epithelioid cells. The

predominating cell from the omentum of this animal is shown in the supravital technic as Fig. 5. This epithelioid cell is to be compared with a similar one, Fig. 3, which was found in the peripheral blood of a rabbit, R 19, 16 days after the injection of 1 mg. of living bovine tubercle bacilli. The graph showing the peripheral blood of this animal is given as Chart 1 in another paper in this *Journal* (11), and the cell was drawn on Dec. 2 when there were 6000 monocytes per c.mm. in the peripheral blood. In both of these epithelioid cells is shown the typical rosette of tiny bodies, with the carmine reaction to supravital neutral red, which is the characteristic of the cell that makes the tubercle. In contrast to these epithelioid cells (Figs. 3 and 5) which far outnumbered any other type of cell in the lesion, are three other cells illustrated from the same omentum as the cell of Fig. 5. Fig. 1 was an unstimulated fibroblast, entirely typical of the type. The cells of Figs. 2 and 4 were in our interpretation somewhat stimulated, rounded up clasmatocytes. They are to be compared with the unstimulated clasmatocyte of the omentum shown in Fig. 3 from an accompanying paper in this *Journal* (3). This cell was from the omentum of another of the rabbits, R 160, that received the phosphatide fraction A-3 , and was identical with many found in R 153. But beside these long forms there were also found cells, such as the ones shown in Figs. 2 and 4. In our interpretation these latter cells were types to be classified as clasmatocytes. The cell of Fig. 2 is the more typical; the cell of Fig. 4 showed the tendency of the smaller vacuoles to fading toward yellow; the vacuoles when originally seen were a deeper red than is shown in the drawing, but they were ultimately orange and yellow in color while all the epithelioid cells in the preparations still showed an unchanged carmine reaction. Reference to the photograph, Fig. 10 (R 153), from the connnective tissues near the bladder, shows the predominating cell to be the epithelioid type, with eccentric nucleus, but there is an occasional atypical cell with the nucleus in the center as in Figs. 2 and 4. Proof will be given in the discussion of the controls that the clasmatocyte as well as the monocyte takes in lipoids. However, the contrast in the reaction of the cells under discussion in an environment as identical as there would be within the area of omentum included in one oil immersion field of the microscope is strikingly shown in Figs. 2, 4, and 5, or in Figs. 2 and 3 in the accompanying paper in this *Journal* (3).

These are the observations. Concerning them, two different interpretations are possible; either the same type of cell is responding in two different ways, thereby making it necessary to postulate differences in the environment; or, two potentially different strains of phagocytes are reacting differently in the same environment in their disposal of phagocytized material. Our interpretation at the moment favors the latter explanation as the more probable.

In all four of the phosphatide animals there was marked involvement of the parietal peritoneum, especially near the site of injections. The visceral peritoneum covering the pelvic organs and the mesentery were extensively involved while the surface of the intestine had everywhere patches of the same tubercular tissue that characterized the omentum. Most of the lesions were nodular, white

or of the same dull reddish color as the omentum. In Fig. 8 is shown a section of the duodenum at the site of involvement in Rabbit R 159. It shows that the lesions are here limited to the serosal coat. The magnification is low to show the extent of the new growth. The mass of the tissue is of epithelioid cells. The dark patches in the center are lymphocytes; the two dark areas on the left show necrosis with infiltration of leucocytes. The zone in the upper left corner is the more markedly necrotic. In one of the animals there was some involvement of the submucosa, probably by extension. In this same animal (R 159) there was an occasional small tubercle in the liver and a few epithelioid cells in the lung, but in general the reaction was almost entirely localized to the peritoneum. In one animal only, R 158, was there an involvement of the mesenteric lymph glands, as shown in Fig. 12. Here the peripheral follicles are seen extensively involved with typical epithelioid cells, giving a picture that would be described as early tuberculous involvement, though no acid-fast bacilli could be found.

Complete parallel surveys of all tissues from all animals receiving either the protein or phosphatide fractions have yielded uniformly negative results with the Ziehl-Neelsen technic. The phosphatide material itself gave a diffuse reddish reaction with the carbolfuchsin and some of the large giant cells, such as are shown in Fig. 11, stained a faint, diffuse pink. In the case of the lymph gland of Fig. 12 there were certain areas within the follicles and in the reticulum of the lymphatic sinuses in which groups of cells were found containing acid-fast debris. There were, however, no intact, acid-fast bacilli found in any instance.

The contrast between the reaction of the connective tissues to the protein fractions on the one hand and to the phosphatide material on the other may be seen at a glance in comparing the tissues of the lung, as shown in Fig. 7, with the omentum of Fig. 11. In Fig. 7, the reaction is not at all that of tuberculosis. The contrast is, however, much clearer in Figs. 9 and 10, where the tissues involved are entirely comparable. Fig. 9 is from the prevertebral tissues near the kidney after the protein, and Fig. 10 from the connective tissue near the bladder after the phosphatide fraction. The character of the round cells with eccentric nuclei, together with the typical giant cells of a tubercular lesion in the latter, contrast markedly with the long and the branched cells with no trace of a tubercular reaction in the former. In both of these photographs there is shown a little perivascular reaction. In none of the tissues in the entire series was any tendency towrad fibrosis seen. In the tubercular lesions after the phosphatide fractions there was a considerable new growth of vessels.

These are the preliminary tests of substances from the chemical analysis of the tubercle bacillus. Under the plans of the Research Committee of the National Tuberculosis Association, Dr. William Charles White, chairman, these tests are to be extended, both to the products from the further analysis of these protein and phosphatide fractions and to comparable substances from other organisms.

As far as these studies have progressed, it is clear that certain of the responses to the proteins are like those to other proteins; for example, the anaphylactoid response to large doses, and the direct effect on the neutrophilic leucocytes from an excessive dose, and possibly the temperature. The acute effect on the liver cells in two animals can only be evaluated with a larger series of animals. With the proteins, all the rabbits showed fever, multiple hemorrhages, and a tendency toward an increase in the cellular response of clasmatocytes. How far the toxic effect on endothelium and the pressor effect on clasmatocytes are specific awaits further tests. The phosphatide fractions showed only one response, namely the local production of the typical lesion of tuberculosis. There was a massive local increase in monocytes, epithelioid cells, and Langhans giant cells; there was some infiltration of this tubercular tissue with lymphocytes and there was some necrosis. Thus, with these two types of substances from the analysis of the bacilli many of the effects of the actual disease have been reproduced. The typical distribution of the lesions of the infection with living bacilli was not reproduced. If the observations that in the disease itself clasmatocytes fragment the living bacilli, that the clasmatocytes are in turn disintegrated, and the resulting debris rephagocytized, actually mean that large numbers of bacilli are killed and broken down by the cells of the animal, then the actual progress of a tubercular infection involves the effects of both living and dead bacilli on the body.

Controls.

Before venturing any interpretation as to the specificity of the above reactions of the fractions from tubercle bacilli, adequate controls are, of course, essential. It will be possible to secure soon various bacterial proteins with which to compare and contrast the reactions already noted with the protein fraction from the tubercle bacilli. In some respects the acute reactions with this material have approximated anaphylactoid phenomena; in other respects the responses have been unlike the non-specific protein reactions with which the literature deals. Therefore, the real significance of these observations awaits further studies.

As a partial control for the findings with the specific phospholipins of the tubercle bacilli, certain appropriate, if inadequate, experiments were carried out. When stained by the Ziehl-Neelsen technic, it was found that this material, as received, contained a limited number of intact, acid-fast bacilli, the protein fractions being free of such findings. However, when the protein and phosphatide fractions were injected into guinea pigs directly, or inoculated on Petroff's media,

or when the tissue suspensions from rabbits, treated as described above with these materials, were inoculated into guinea pigs, there was no evidence either from repeated tuberculin tests or at autopsy, that any of the bacilli were viable. Ten guinea pigs were used; five were autopsied 2 months after inoculation with completely negative findings, and five are still living and in perfect health 7 months after. Therefore, the control series of rabbits was divided into three groups: one to receive lecithin (12)[1] alone in doses comparable to that given in the case of the phospholipin; another to receive the same dosage of lecithin plus a known added weight of dead human tubercle bacilli, Strain H 37; the third to receive dead bacilli alone, all conditions being identical with those existing during the phosphatide series.

It has long been known that the injection of dead tubercle bacilli can produce lesions when injected into the living animal (13, 14). Koch (15) himself found that they caused aseptic pus upon subcutaneous inoculation. That the lesions produced with the dead organisms may be tubercles with typical epithelioid and giant cells is also well recognized (14, 16–20). Typical of this early work is that of Prudden and Hodenpyl (21) who in 1891 injected rather large amounts of dead bacilli, 2 to 3 cc. of a milky suspension, by various routes, subcutaneous, intrapleural, intraperitoneal, and intravenous. With the intrapleural and the intraperitoneal routes, two animals, one in each series, developed a few small nodules of epithelioid cells in the serosa. With the intravenous route, the tubercular tissue was confined to the lungs. Prudden (22) then studied the lungs after intratracheal injections, in which he also found some reaction in the pulmonary tissues.

In the attempts to explain the mechanism of the reactions noted, the nonspecific foreign body stimulus of the bacteria as such is mentioned (18, 23), but it is evident that the preponderant opinion of investigators has been that the histological lesion caused by the tubercle bacillus is due to a poison liberated from the body of the bacillus by the action of the tissue cells (13, 14, 17, 23, 24, 25). Among the first to attempt an analysis of the effects of chemically separate constituents of the dead tubercle bacilli was Weyl (26). He obtained two substances from the bacillus, one of indifferent nature with the tinctorial properties of the bacteria, and the other a toxomucin which, upon subcutaneous injection, caused dry necrosis of the skin. Auclair (27) reported the production of caseation necrosis in guinea pigs by subcutaneous and intratracheal injections of lipoidal materials from human tubercle bacilli extracted by ether, chloroform, xylene, and benzene. In 1903 he studied the tissue reactions following ether extracts of typhoid, Friedländer's, and Loeffler's bacilli, streptococcus, *Staphylococcus aureus*, gonococcus, and actinomyces, finding in contrast to the effects of extracts of tubercle bacilli no typical epithelioid proliferations but rather in each instance only inflammatory lesions comparable to those resulting from the original organism in its usual manifestations. Morse and Stott (23) produced typical cellular tubercles, without caseation, in rabbits and white rats, by subcutaneous intraperitoneal, and intravenous injections of the alcohol extract, but found the

[1] We are indebted to Dr. P. A. Levene for the lecithin used in the control animals.

ether extract to be inert. They attributed the characteristic microscopic lesion to waxy substances acting as a peculiar type of foreign body. Gaehlinger and Tilmant (28) found non-specific "liver lipoids," when injected subcutaneously, capable of producing tubercles with caseation, though Ray and Shipman (29) could not confirm this observation. The latter investigators found chloroform-soluble lipins extracted from tubercle, grass, and colon bacilli to produce "entirely similar epithelioid tubercles" when injected *via* the subcutaneous and intrapulmonary routes in guinea pigs. The differences in the degree of the involvement were not considered significant, the conclusion being drawn that the characteristic histological features of tubercles are "merely a foreign body reaction." However, their controls of sodium stearate emulsion, with and without 0.5 per cent chloroform, and olive oil, with and without 0.5 per cent chloroform, gave no lesions. True caseation was never seen by them and they question previous findings of this nature because of the close macroscopic resemblance of the solid pus of rabbit and guinea pig to caseous material. Thus it will be seen that the literature provides to some degree controls for the work presented in this investigation. However, the chemistry of bacteria is being placed upon a new foundation today with the improved methods of quantity production of known strains of organisms on synthetic media of known composition, and with the development of chemical procedures yielding material in amounts sufficient both for analysis chemically and biologically. Also the newer criteria for cell identifications are making the study of the biological reactions more specific and exact than was possible in the earlier investigations.

Charts 6, 7, and 8 are representative of the peripheral blood, respectively, in the three control groups here cited. No changes whatever are revealed in any of the graphs representative of temperature, weight, red cells, hemoglobin, or white cells of the various strains, in the periods of experimentation following the control periods. The fluctuations noted are quite within the range of normal for each determination. Thus, the clinical and peripheral blood findings indicate even less of a disturbance in the normal mechanisms in these control animals than was the case with the phosphatide fractions from the tubercle bacilli.

Because of the intraperitoneal route for the injections and the striking findings with the phosphatide fractions limited essentially to the local manifestations in the peritoneal cavity, the greatest interest attaches to the survey of this region, particularly the peritoneum and omentum. Both parietal and visceral peritoneum in five of the animals were glistening, smooth, and uninvolved throughout; only in Rabbit R 245, of the second group, were there found several strong, fibrous adhesions extending between the site of injection in the left abdominal wall and the serosa of the descending colon; the latter were probably secondary to some trauma incident to one of the injections.

In the two animals receiving lecithin alone (R 243, R 244), the omentum in the gross showed a patchy, dusky, red color, with an increase in milk bodies, but lacked the extensive nodular thickening present in the animals receiving the phosphatide fractions. Supravital studies of films of omentum spread on neutral

red-Janus green slides showed a dilatation of the omental capillary bed accounting for the gross appearance. The reaction of the adventitial cells along the small veins was particularly prominent. The milk spots were focal accumulations of highly phagocytic clasmatocytes, their vacuoles reacting brilliantly with the neutral red. The phagocytized debris was globular but clearly not red cells. No monocytes or epithelioid cells could be identified as such. These identical preparations were subsequently fixed *in situ* in formalin, which faded the neutral red of the clasmatocytes, and were then subjected to staining with Sudan III. On reexamination of the same areas previously studied in the supravital, it was

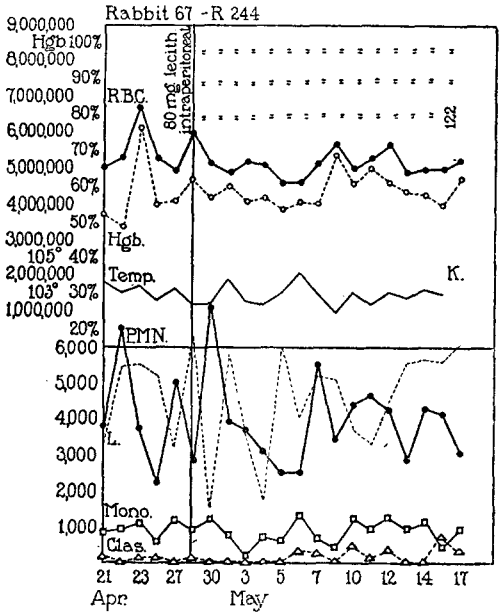


CHART 6.

found that the vacuoles formerly reacting to the neutral red also reacted to the fat stain, quantitatively, indicating a specific response to the foreign phospholipin of the strain of cells of the connective tissues chiefly responsible for the handling of non-specific debris.

The human tubercle bacilli, Strain H 37, used in the last four animals of this control group, were grown under the same conditions as those used for the chemical analyses yielding the proteins and phosphatides of this experiment, and were, indeed, dried weights from identical lots used by Dr. Johnson and Dr. Anderson. Known weights were inactivated at 60° for 1 hour and the individual dosage in all four animals was the same though the total dosage varied as indicated in Table I.

It was the desire to compare the relative reactions of known weights of dead bacilli alone *via* the peritoneal route with comparable dosages in the presence of a non-specific phospholipin.

The two animals receiving lecithin in dosages comparable to those of the phosphatide fractions, plus known quantities of inactivated human tubercle bacilli (R 245, R 246), also showed the evidence of dilated vessels and increased milk bodies in the omentum. However, supravital studies of omental spreads from these rabbits showed the predominating reaction in R 245 to be the typical epithelioid cells, with clasmatocytes, as in the preceding group, stimulated though

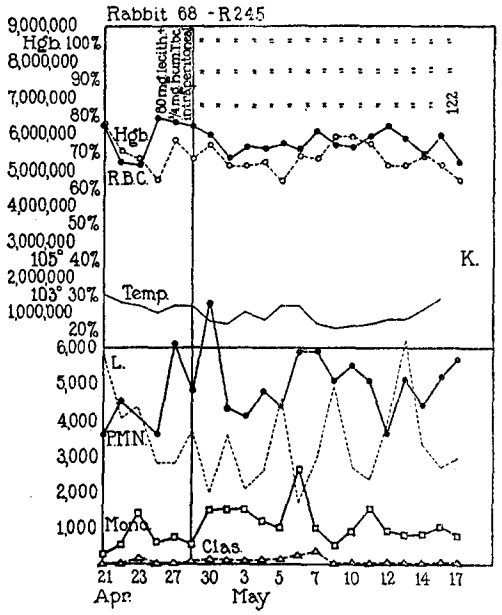


CHART 7.

less in number; while in R 246, the predominant reaction was clasmatocytic with a less pronounced response of epithelioid and giant cells.

In the two animals receiving dead bacilli only (R 247, R 248), the omentum had the gross appearance already noted. Supravital studies showed the more densely cellular areas to be predominantly clasmatocytic, though without the excessive phagocytosis of fat globules so prominently a part of the picture seen in the preceding four rabbits. The Sudan III preparations showed many of the cells characteristic of the clasmatocyte group with fine orange globules and an occasional larger fat body, but the majority of the cells did not react. This was in particularly striking contrast quantitatively to the studies in rabbits receiving the

lecithin. Typical epithelioid cells and giant cells of the Langhans type while present were a minority finding in these omenta subjected to dead bacilli.

Thus it will be seen that the intraperitoneal injections *per se* caused more or less of a vascular dilatation of the omentum, giving rise to a characteristic dull pink coloration, with a local cellular stimulation predominantly clasmatocytic in all the animals with the exception of R 245. Those rabbits receiving only lecithin showed no reaction of the monocytic strain of cells which could be identified with the usual criteria of the supravital technic, but rather there was a profound phagocytic response on the part of clasmatocytes as shown by surveys with

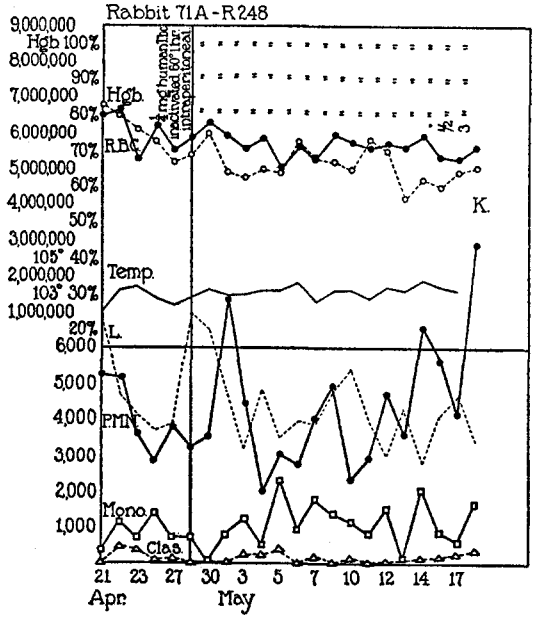


CHART 8.

Sudan III. The response of epithelioid cells was minimal except in the case of Rabbit R 245, in which both lecithin and bacilli had been given, and here there was nothing comparable in the extent and specificity of the reaction to that seen in any of the animals receiving the special phosphatide fractions from the tubercle bacilli.

These reactions of peritoneum and omentum are an adequate index of the general findings in the other tissues of the several groups. The mesentery showed some increase of cellular bodies comparable histologically to those described in the omentum. The mesenteric lymph glands in all animals were swollen, tense, and on section a copious flow of opaque fluid was released, which coagulated quickly

on standing. There was in every instance a great excess, four to five per oil immersion field, of typical clasmatocytes loaded with debris, but in all supravital studies only two typical epithelioid cells were seen (R 245) and no giant cells. The bone marrows in every animal were normal in their hemopoietic activity, and no epithelioid or giant cells were found in any case. There was only one instance in the control group of six animals in which there was an acute splenic tumor. The spleen of R 244 weighed 2.4 gm., but on supravital study showed only the normal cellular differential except for numerous red blood cells. Only one spleen (R 245) showed epithelioid cells, three in number.

Pleural cavity, lungs and heart, liver, intestinal tract, kidneys, from the animals receiving lecithin were negative in the gross and microscopically.

Both animals receiving lecithin plus bacilli showed some increase of monocytes in the lung with occasional typical epithelioid cells, but with the predominant cell being the clasmatocyte. The large intestine in R 245 was involved, possibly at the site of an injury incident to the injection, the small intestine being unaffected throughout.

Rabbit R 247, receiving dead bacilli only, showed a few epithelioid cells in the lung preparations, but in R 248 none were found, the usual clasmatocyte predominance being the obvious finding in both cases.

Certainly it may be accepted that dead bacilli alone in repeated doses and in the amounts cited, when injected intraperitoneally, can excite in the peritoneal cavity only a very limited development of the cellular pathology pathognomonic of tuberculosis; that lecithin is quite incapable of inducing other than a non-specific phagocytic response; and that the combination of lecithin and dead bacilli together reacts essentially independently with only a minimal response of epithelioid cells.

From the literature it is clear that the intravenous injection of dead tubercle bacilli produces in the lungs two distinct reactions: first, the so called interstitial pneumonia, primarily a proliferation of clasmatocytes; and second, small foci of typical epithelioid and giant cells. The protein and phosphatide fractions from tubercle bacilli here used sharply differentiate and separate these two reactions.

It may not be surprising to find the occasional epithelioid cell in other pathological cellular responses, for in the limited surveys with the supravital technic already made, they have been found in Hodgkin's disease and in the septa of certain tumors. The exact nature and distribution of the specific stimulant, possibly a lipoid, remain to be determined. However, the very special relationship

which this reaction bears to tubercular infection, and the apparent symbiosis of tubercle bacillus and epithelioid cell, present an approach to this disease which is thought at present to be significant.

In the further studies with the chemical products from the tubercle bacilli it will be important to analyze the biological reactions in terms of the phagocytic mononuclear cells of the connective tissues, with the possible differentiation functionally into monocytes, epithelioid cells, and clasmatocytes.

SUMMARY.

1. The clasmatocyte, the cell with the power of fragmenting tubercle bacilli, the cell making the lesion of the so called interstitial pneumonia, has been shown to be the overwhelming response to the special protein fractions, 304 and 903. Multiple hemorrhages, high fever, and toxicity have marked the use of these fractions in every instance.

2. The epithelioid and the giant cell of the Langhans type, making typical tubercular tissue, have been the massive and specific response of the peritoneal cavity to intraperitoneal injections of the phosphatide fractions, A-3 and A-4. These fractions have been entirely non-toxic in the dosages used.

BIBLIOGRAPHY.

1. Johnson, T. B., and Coghill, R. D., *Tr. 22nd Ann. Meeting, Nat. Tuberc. Assn.*, 1926, 277.
2. Anderson, R. J., The separation of lipoid fractions from tubercle bacilli, *J. Biol. Chem.*, 1927, lxxiv, 525; A study of the phosphatide fraction of tubercle bacilli, *J. Biol. Chem.*, 1927, lxxiv, 537.
3. Sabin, F. R., and Doan, C. A., *J. Exp. Med.*, 1927, xlvi, 627.
4. Sabin, F. R., Doan, C. A., and Cunningham, R. S., *Carnegie Institution of Washington, Pub. No. 361, Contributions to Embryology*, 1925, xvi, 125.
5. Cunningham, R. S., Sabin, F. R., and Doan, C. A., *Carnegie Institution of Washington, Pub. No. 361, Contributions to Embryology*, 1925, xvi, 227.
6. Cunningham, R. S., Sabin, F. R., Sugiyama, S., and Kindwall, J. A., *Bull. Johns Hopkins Hosp.*, 1925, xxxvii, 231.
7. Sabin, F. R., *Bull. Johns Hopkins Hosp.*, 1923, xxxiv, 277.
8. Sabin, F. R., Austrian, C. R., Cunningham, R. S., and Doan, C. A., *J. Exp. Med.*, 1924, xl, 845.
9. Doan, C. A., Cunningham, R. S., and Sabin, F. R., *Carnegie Institution of Washington, Pub. No. 361, Contributions to Embryology*, 1925, xvi, 163.

10. Sabin, F. R., unpublished observations.
11. Doan, C. A., and Sabin, F. R., *J. Exp. Med.*, 1927, xlvi, 315.
12. Levene, P. A., and Rolf, I. P., *J. Biol. Chem.*, 1927, lxxii, 587.
13. Maffucci, A., *Centr. allg. Path. u. path. Anat.*, 1890, i, 825.
14. Masur, A., *Beitr. path. Anat. u. allg. Path.*, 1894, xvi, 256.
15. Koch, R., *Deutsch. med. Woch.*, 1891, xvii, 101.
16. Wyssokowitsch, W., *Z. Hyg. u. Infectionskrankh.*, 1886, i, 3.
17. Straus, I., and Gamaleia, N., *Arch. méd. exp. et anat. path.*, 1891, iii, 705.
18. Vissman, W., *Virchows Arch. path. Anat.*, 1892, cxxix, 163.
19. Grancher, J., and Ledoux-Lebard, *Arch. méd. exp. et anat. path.*, 1892, iv, 1.
20. Kostenitsch, J., *Arch. méd. exp. et anat. path.*, 1893, v, 1.
21. Prudden, T. M., and Hodenpyl, E., *New York Med. J.*, 1891, liii, 637, 697.
22. Prudden, T. M., *New York Med. J.*, 1891, liv, 617.
23. Morse, P. F., and Stott, E., *J. Lab. and Clin. Med.*, 1916–17, ii, 159.
24. Abel, *Deutsch. med. Woch.*, 1892, xviii, 482.
25. Grancher, J., and Martin, H., *Centr. Bakt.*, 1891, x, 298.
26. Weyl, Th., *Deutsch. med. Woch.*, 1891, xvii, 256.
27. Auclair, J., Thesis, Paris, 1897; *Rev. tuberc.*, 1898, 97; *Arch. méd. exp. et anat. path.*, 1899, xi, 363; 1900, xii, 189; 1903, xv, 725.
28. Gaehlinger, H., and Tilmant, A., *Compt. rend. Soc. biol.*, 1911, lxxi, 345.
29. Ray, L. W., and Shipman, J. S., *Am. Rev. Tuberc.*, 1923, vii, 88.
30. Lewis, P. A., and Sanderson, E. S., *J. Exp. Med.*, 1927, xlv, 291.
31. Jaffé, R., *Frankf. Z. Path.*, 1915, xvii, 34.

EXPLANATION OF PLATES.

PLATE 26.

FIG. 1. Typical resting fibroblast from the omentum of a rabbit, R 158, which had received 13 intraperitoneal doses of the phosphatide fraction A-4 on successive days, and was killed 6 days after the last injection. Stained in supravital neutral red. Magnification about 1940, as indicated by the red cell near Fig. 5.

FIG. 2. Stimulated, round clasmatocyte from the same preparation as the cell of Fig. 1.

FIG. 3. Epithelioid cell, stained supravitally with neutral red, taken from the peripheral blood of a rabbit, R 19, 16 days after the injection of 1 mg. of living bovine tubercle bacilli into the ear vein. The round, unstained bodies are fat. The chart of the blood of this animal is shown in another paper in this *Journal* (11) as Chart 5; there were 6000 monocytes in a c.mm. of the blood at the time, Dec. 2, when this cell was drawn. Magnification shown by the red cell.

FIG. 4. Slightly stimulated clasmatocyte from the same preparation as the cell of Fig. 1.

FIG. 5. Typical epithelioid cell, from the same preparation as the cell of Fig. 1, showing the rosette, with the carmine tone in supravital neutral red, characteristic of the type.

FIG. 6. Drawing of lymph cords and sinuses from the center of a mesenteric lymph gland of a rabbit, R 131, after 26 injections of the protein fraction 903. Tissue fixed in Zenker-formol. Stained with hematoxylin and eosin. Drawn with the camera lucida, magnification about 870. The lymphatic endothelium and the clasmatocytes which have become free from it are marked by their content of the debris of red cells from a hemorrhagic area which was being drained. *a*—reduplication of endothelium with the outer cell becoming rounded up; *b*—a denuded area where the endothelium has desquamated; *c*—an endothelial cell stretching across the sinus. In the center is a tiny lymph cord with a perivascular clasmatocyte; to the left are free clasmatocytes in the lymph cord.

PLATE 27.

FIG. 7. Photograph of a section of the lung from Rabbit R 130, which had received 12 intravenous injections of the protein fraction 304. Animal found dead the morning after the last dose. Shows the maximum thickening of the septa with clasmatocytes, the type proved by supravital studies since the cells were still living. All the tissues were fixed in Zenker-formol and stained in hematoxylin and eosin. × about 90.

FIG. 8. Photograph of the duodenum of a rabbit, R 159, which had received 13 intraperitoneal doses of the phosphatide fraction A-4, and was killed 4 days after the last dose. To show the extent of the serosal lesion. The mass of the tissue is of epithelioid cells, with the dark spots in the center of lymphocytes and two large masses of necrosis to the left. × about 9.

PLATE 28.

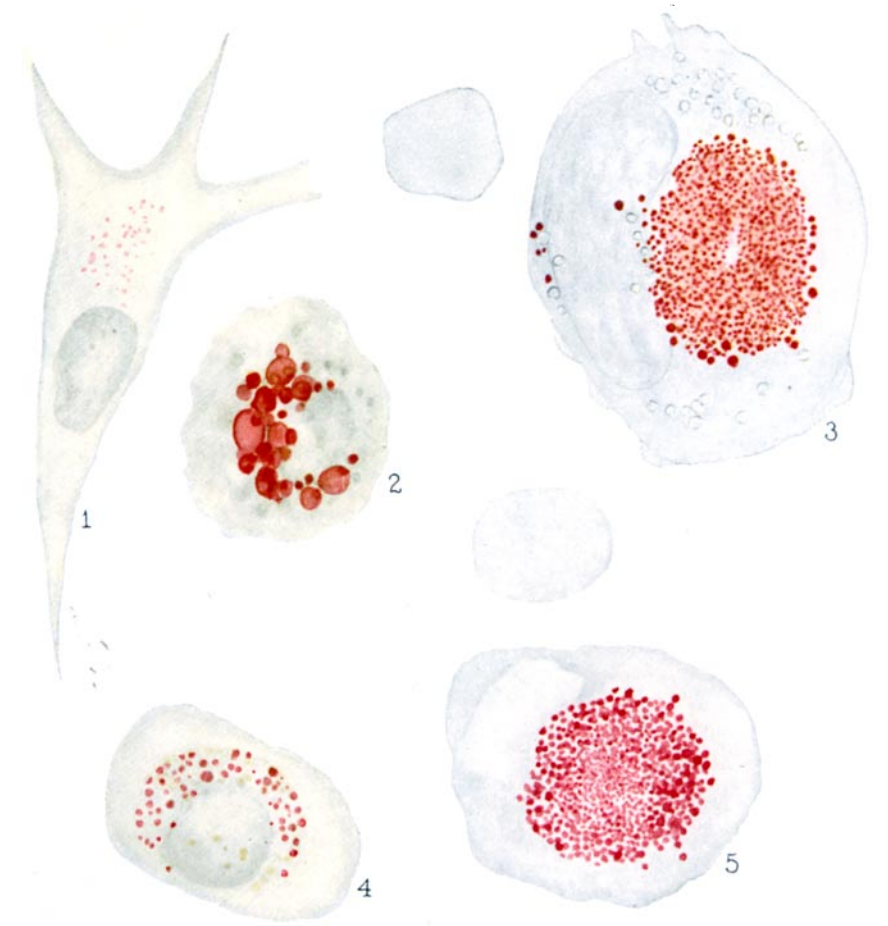
FIG. 9. Photograph of the retroperitoneal connective tissue, in the edge of the psoas muscle near one kidney, of a rabbit, R 129, which had 13 doses of the protein fraction 304. Killed 24 hours after the last dose. The tissue is hemorrhagic and edematous, and shows a stimulation of the connective tissue cells which were predominately clasmatocytic in the supravital studies. × about 455.

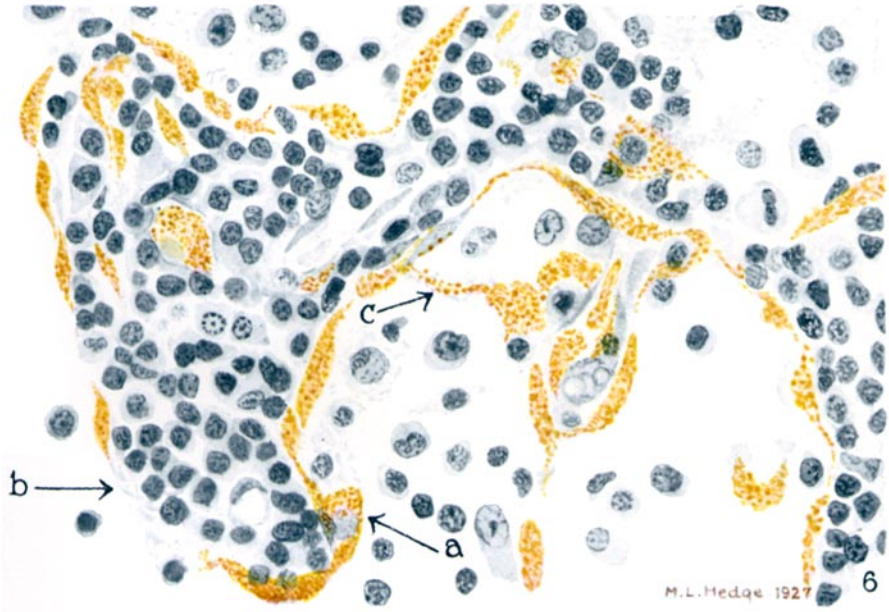
FIG. 10. Photograph of the connective tissue near the bladder from a rabbit, R 153, which received 14 intraperitoneal doses of the phosphatide fraction A-3, and was killed 24 hours after the last dose. It shows a reaction predominately of epithelioid cells and giant cells of the Langhans type. × about 455.

PLATE 29.

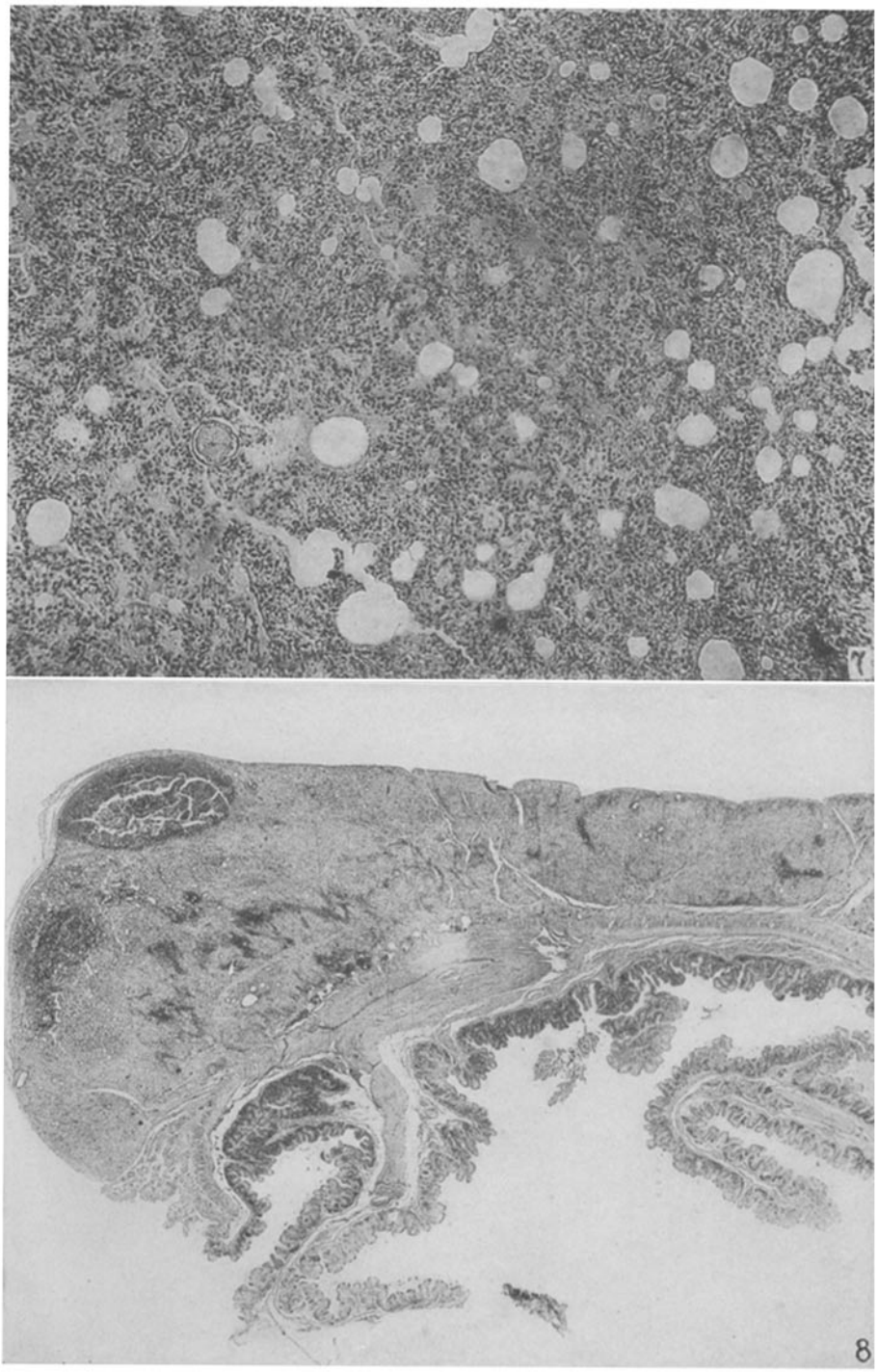
FIG. 11. Photograph of the omentum of a rabbit, R 153, which had received 14 intraperitoneal doses of the phosphatide fraction A-3, and was killed 24 hours after the last dose. It shows a reaction predominately of the giant cells of the Langhans type. There are many blood vessels and some clumps of lymphocytes. × 195.

FIG. 12. Photograph of the mesenteric lymph gland of a rabbit, R 158, which had received 13 intraperitoneal doses of the phosphatide fraction A-4, and was killed 6 days after the last dose. The peripheral follicles with pale centers show extensive involvement with typical epithelioid cells. × about 90.

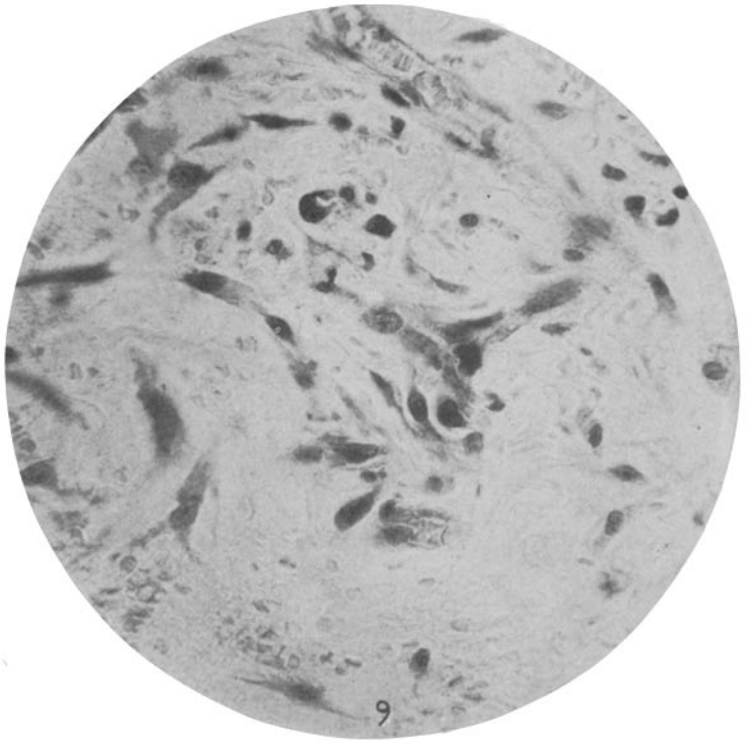


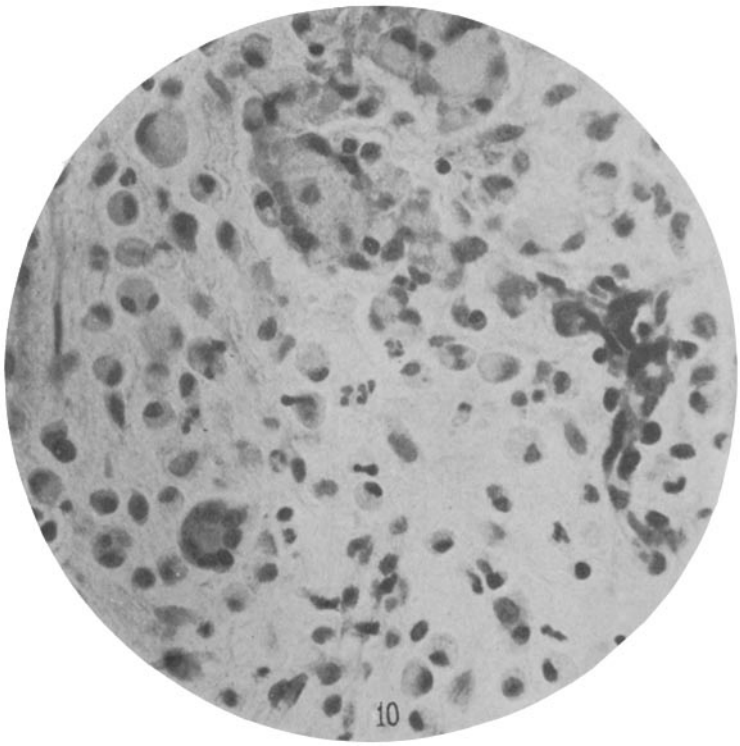


(Sabin and Doan: Chemical analysis of human tubercle bacilli.)

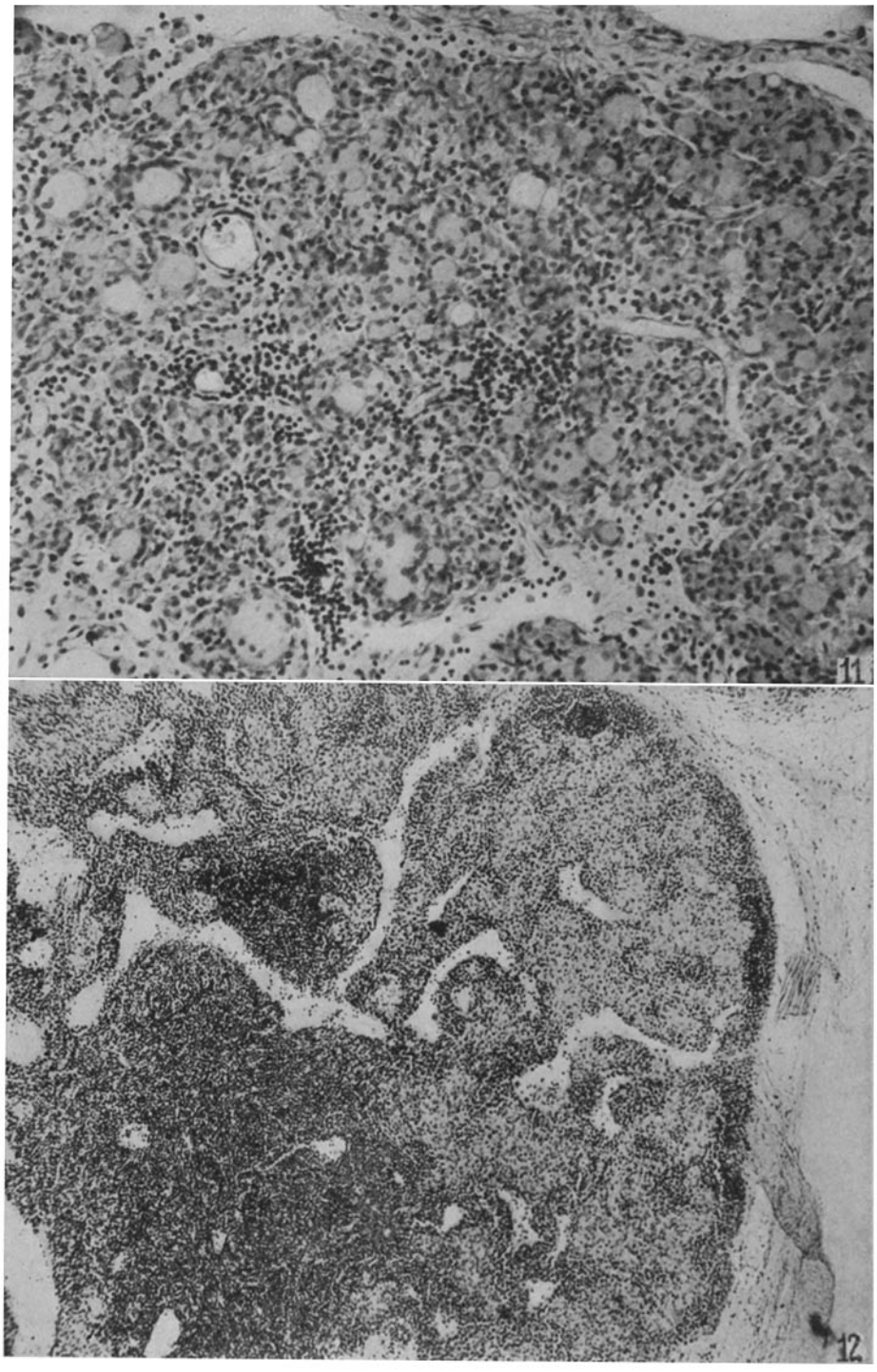


(Sabin and Doan: Chemical analysis of human tubercle bacilli.)





(Sabin and Doan: Chemical analysis of human tubercle bacilli.)



(Sabin and Doan: Chemical analysis of human tubercle bacilli.)