The Rôle of the Monocyte in Tuberculosis[1]

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INTRODUCTION

THE study of immunity may be defined as the search for the mechanism which the body itself has evolved for combating different invading organisms. It has long been known that certain organisms, such as the diphtheria bacillus, liberate a toxin by some vital activity, secretion or excretion, against which the animal reacts by producing an antitoxin. Moreover, notwithstanding the fact that neither such toxins nor anti-toxins have been analyzed chemically, some of the diseases of this type have nevertheless been controlled. It is also well known that the majority of bacteria harm the body not only by the production of exo-toxins, but by other activities as well. It has been found that certain organisms produce the so-called endo-toxins, instead of exo-toxins, and to them the reaction of the body is much more complex, consisting in the production of agglutinins, precipitins, bacteriolytic substances, etc. These different substances have likewise not yet been analyzed chemically; nevertheless, by utilizing the reactions of the animal body to such infections, certain effective immune sera and effective vaccines have already been produced. In the case of tuberculosis, it is quite clear that the human body has a marked power to produce an immunity, since pathologists have shown such a high percentage of healed tuberculosis, but we have as yet no direct control of the production of this immunity. We are thus forced to conclude that there is some factor in this particular mechanism that the body has evolved, which has so far escaped our analysis.

We are now presenting as a new factor in the study of tuberculosis the concept that it is a disease which affects primarily a single strain of cells; namely, the monocytes. Sabin, Doan and Cunningham (27) showed that the epithelioid cell, the characteristic cell of the tubercle, is a modified monocyte. We are now presenting evidence to show that the infection of tuberculosis causes an overproduction of the monocyte, including all of its stages, namely the reticular cell, the typical monocyte and its two derivatives, the epithelioid cell and the giant cell; that the tubercle bacillus so alters the cytoplasmic activity of the monocyte that the cell becomes a suitable medium in which the bacil-

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lus can live and multiply, or, in other words, that the organism of tuberculosis becomes a parasite within the cell; and that the marked overproduction of monocytes in the connective tissues is correlated, in the acute phase of the disease, with an increase in monocytes in the circulating blood.

This new concept of the essential nature of the disease and the observation that it is possible to correlate the progress of the disease with changes in the circulating blood, we believe, opens up a new experimental attack on tuberculosis, and we think it quite probable that the fact that the organism of tuberculosis can live as a parasite within the monocyte may mean that the bacillus is thereby protected to some degree from the usual reactions of the body against it. Thus, our failure to obtain an effective immune serum may be the result of the intracellular nature of the infection, and therefore may not indicate a fundamental inability of the tissues to produce antibodies of sufficient power.

In this study we propose, therefore, first to present the observations that the bacillus of tuberculosis has a remarkable power of stimulating the production of new monocytes, and then of causing the differentiation of the monocytes into a specialized form, the well known epithelioid cell; and second, to show that the analysis of these activities of the tubercle bacillus forms an essential part of the analysis of the various mechanisms involved in the biological reactions of the body in this disease.

In presenting this work we take great pleasure in expressing our deep appreciation of the assistance which we have received from Dr. William Charles White, Chairman of the Research Committee of the National Tuberculosis Association. Throughout the course of the investigation he has not only given us encouragement but has had a real share, through his critical judgment, in the interpretation of our results. In particular, it was his suggestion that the tubercle bacillus lives as a parasite within the epithelioid cell.

It is likewise a pleasure to thank Mr. James Didusch, artist of the Carnegie Institute of Embryology for the drawings of the living cells.

DISCRIMINATION OF MONOCYTES FROM CLASMATOCYTES

The theory that tuberculosis primarily affects one strain of cells, namely, monocytes, is based on two concepts; first, that the mononuclear cells of the connective tissues can be separated into two distinct strains of cells, clasmatocytes and monocytes; and second, that the monocyte becomes the epithelioid cell characteristic of the disease. These two concepts were presented by Sabin, Doan and Cunningham (27). These authors concluded that the phagocytic cells could be separated into two distinct strains, the clasmatocytes and the monocytes, on the basis of two lines of evidence, first, that they had a different embryological origin, and second, that they were both morphologically and physiologically different. They believe that the clasmatocytes are derived from endothelium; therefore, in this concept, it is wholly obvious that the clasmatocyte is the cell which has been called the "endothelial leucocyte" by Mallory and the

"endothelial phagocyte" by some of his school.

It was shown by Sabin (23) that the first white cell to circulate in the blood, as seen in the living blastoderm of the chick, was derived from the endothelial wall of the blood-vessels. These endothelial derivatives appeared on the third day of incubation and she interpreted these cells as monocytes, an interpretation which we no longer believe to be correct. Of the correctness of the observation that the first white cell to circulate in the blood-stream is a phagocytic cell, derived from endothelium, we have no doubt, but we now believe that these freed endothelial cells do not become any of the definitive white blood-cells, or in other words, that these cells are not identical with monocytes. The same observation and the same interpretation, namely, that these phagocytic cells of endothelial derivation are transient in the blood-stream, was made by Maximow (15) on mammalian embryos. The early occurrence of the endothelial phagocytes in the blood-stream of the chick embryo has now been amply confirmed by one of us (Sugiyama) working in this laboratory. These studies, in which it is possible to see a type of cell, fully identified by means of the supra-vital technique with the clasmatocyte, or endothelial phagocyte of the tissues, actually arise from the endothelial lining of a vessel, we regard as convincing proof of the original production of the clasmatocyte by endothelium in the embryo. Recently, Herzog (12) has observed the same process in the vessels of the tongue of the living adult frog.

These observations, from studies of living tissues, combined with the work of Mallory and his school, make a large body of evidence in favor of the endothelial origin of clasmatocytes. On the other hand, Cunningham, Sabin and Doan (5) have presented evidence that the monocyte does not arise from endothelium but rather from the reticular cell, a primitive embryonic rest, in common with the other white blood-cells. This reticular cell will be discussed later in connection with the effect of tuberculosis on the tissues.

The analysis of the morphological and physiological differences between clasmatocytes and monocytes has depended on modern methods for studying living cells, specifically on the use of the so-called vital and supra-vital staining. The phagocytic mononuclear cells of the connective tissues have been more generally regarded as one group, identified with the clasmatocyte, which was the first strain to be analyzed with the newer methods. The discovery of the clasmatocyte itself came from following the reactions of the cells of the connective tissues to the injection of certain dyes into the blood-stream of the living animal. This so-called vital staining was inaugurated by the work of Ribbert (21) with lithium carmine, of Bouffard (2) with isamine blue and of Goldmann (11) with pyrol blue.

In the analysis of the experiments which have been made to test the reactions of living cells, it is possible, in a very general way, to classify the substances used into five groups. First, there are certain true solutions; second, colloidal suspensions of particles too small to be visible with the highest powers of the microscope,

such as the benzidine dyes; third, insoluble substances with particles large enough to be seen with the microscope, such as carbon in suspension in India ink; fourth, bacteria; and fifth, red and white blood-cells in suspensions. In regard to true solutions, such as vital neutral red, we have had no evidence that any such dye in solution enters the nucleus of a living cell, nor are we sure that the living cytoplasm itself reacts to such dyes; but with fixed tissues, certain basic dyes, for example, do enter nuclei and react with their chromatin. If such a reaction be a chemical combination, then we might define it as "true staining." The reaction of living cells to the benzidine dyes is quite a different phenomenon from any such "true staining," because these dyes, which have been injected into the blood-stream, in colloidal suspension of ultra-microscopic particles, appear within the cells in quite large masses, readily visible even at a low magnification. We do not know in what form such dyes actually enter the cells, but the reaction of the cells, has been called "vital staining." In general the cells in which these dyes have been found in the form of particulate matter have been divided into two classes in accordance with their reactions to such dyes, first, into those that show the dye in fine particles dispersed throughout the cytoplasm, and second, those that have the power of a non-specific agglutination of the substances into very large masses. The liver cell is an example of the former and the clasmatocyte is the most conspicuous example of the latter. The reaction of cells to large particles such as those of carbon in India ink is, in general, similar to vital staining, and, in the case of the clasmatocyte, practically identical. Through this characteristic reaction of clasmatocytes of agglutinating insoluble particles of dyes, this strain of cells was separated out of the group of the cells of the connective tissues as a type with marked phagocytic power, and on account of this power it was called the "macrophage" (Metchnikoff).

One of the most significant points so far gained in the study of living cells is a further insight into the method by which cells deal with foreign material. This point is of great importance from the standpoint, first, of the separation of the monocytes, from the clasmatocytes, and second, in relation to the analysis of the special relation of the tubercle bacillus to the monocyte. When vitally stained cells, that is, cells which, while still in the animal body, have stored insoluble particles of dye, are treated with supra-vital stains, we obtain evidence regarding the mechanism which the cell uses in dealing with foreign particles. Shipley (29) took films of fresh connective tissues containing clasmatocytes, which had been chronically stained by repeated injections of trypan blue, and treated them with neutral red. He found that the agglutinated masses of trypan blue were surrounded with fluid which took up the neutral red. It is this fluid, containing the agglutinated dye, which is called the "vacuole of phagocytosis" by some authors or the "segregation apparatus" by Evans and Scott (9). This combination of the two techniques, chronic vital staining followed by the supra-

vital use of neutral red, gives the best method of analyzing these vacuoles. We have been making repeated observations in this way. In studying such bits of living tissue one often has the opportunity of watching the penetration of the neutral red into the deeper cells of a mass and in such preparations it is quite clear that the particles of the insoluble dye have been surrounded by fluid by means of some activity of the cell, and that it is this fluid which actually stains with the neutral red. The particles of blue are thus readily seen through the red-stained fluid. This clear picture of the two different colors is not retained long, for soon the entire vacuole becomes dark red or purple. It is not clear whether the masses of the insoluble dye, the trypan blue or the carmine, are merely particles of dye alone or whether the particles of dye have adhered to some substance in the cell, but the presence of the fluid around them and the staining of this fluid by neutral red can be quite convincingly demonstrated. Into this fluid the neutral red penetrates and the color of the neutral red often varies quite considerably from a salmon red, through the scarlet of the acid reaction of the dye, to a deep maroon color. As the red color becomes more dense, the particles of phagocytized dye within the vacuole gradually become more and more obscured. It is quite clear, we think, that neither the original phagocytosis of the insoluble dye, nor the subsequent staining of the fluid of the so-called vacuole in which the dye becomes segregated, is a true staining of protoplasm, for neither the phagocytized dye nor the fluid which the cell secretes around it is true protoplasm.

After such a study of clasmatocytes it becomes easy to follow the same process by staining the living cells with neutral red alone and to analyze the vacuoles that surround the debris which these cells take up during their physiological activities. Clasmatocytes supra-vitally stained with neutral red, after they had been experimentally loaded with trypan blue or with foreign blood-cells, or physiologically loaded with debris, were shown by Sabin, Doan and Cunningham in their Plate 1 (27). As will be seen in this plate, the reactions are all the same, consisting of the surrounding of a foreign body by a fluid stainable with neutral red which reacts as an indicator toward the contained substances. Clasmatocytes are characterized both by the very large size of the foreign bodies which they engulf, by their marked power of agglutinating such material, and by the fact that the engulfed material is distributed in the cytoplasm wholly without pattern.

The reaction of monocytes, on the other hand, to supra-vital staining brings out a constant and characteristic pattern. In the monocytes there are certain fine bodies that stain with neutral red which occur in a rosette around the centrosphere, and the presence of this rosette limits the zone for the storage of phagocytized material to the periphery of the cell.

We have dealt, at some length, with the vacuoles of phagocytosis and their demonstration with neutral red in cells which were known to have phagocytized foreign particles, but it must now be made clear, for the complete study of monocytes, that the

reaction of living cells to neutral red is by no means confined to the staining of the fluid secreted by cells for the purpose of dealing with phagocytized material. Not every substance stained by neutral red is to be considered as a vacuole of digestion.

The first example of a substance which reacts to neutral red, other than the fluid of the segregation apparatus of cells, is the so-called reticular substance of immature red cells. We do not regard this reaction as comparable to the other reactions toward supra-vital staining because, as Pappenheim pointed out, such a staining of red blood-cells is the result of a marked damage to the cell. Schilling (28) and Key (13), have demonstrated that the dye precipitates and clumps the basophilic substance, which, in the living cell, is uniformly distributed throughout the cytoplasm. Thus, this reaction is not true supra-vital staining because it appears as a marked distortion of the structure of the red cell, and it seems certain that no such distortion takes place in the reaction of the white cells to these dyes. The reaction of the red cell varies from the massive precipitation in the megaloblast, through the well known stages of the reticulation, to a final stage in which only two or three droplets of substance react to the supra-vital dye (see illustration given on Plate V, Doan, Cunningham and Sabin (6)).

The second reaction to supra-vital dyes is shown by the specific granulations of certain cells. These granules are probably some specific type of material produced by cytoplasmic activity and are not parts of the cytoplasm itself. Such granules are the neutrophilic, the basophilic and the eosinophilic granules of the granulocytic leucocytes. Another type of granule which reacts characteristically to neutral red is that found in the islet cells of the pancreas, as was discovered by Bensley (1). It is therefore evident that a very great range of substances within cells react to neutral red; it is, however, in no wise settled that all of these reactions are similar in their mechanism.

If we consider first the neutrophilic granules, it is clear that they are plainly visible in the living cell without any dye. With neutral red we find that there are variations in the reaction of these granules, both in comparable cells in different animals and in the same animal at different stages in its development. The early granules of the neutrophilic myelocytes stain intensely in neutral red; in the human neutrophilic leucocytes the neutrophilic granules stain throughout the life of the cell, up to the time of the non-motile phase, when the granules swell, become highly refractive and entirely unstainable. In the rabbit, on the other hand, although the neutrophilic granules stain well in the myelocytes, and in many of the leucocytes, occasionally there is but a slight reaction of these granules to the stain even during the most active phase of the leucocyte. The neutrophilic granules of the dog's leucocytes are very tiny and do not react to neutral red. All these facts show that there is some chemical evolution of the neutrophilic granules within the life of the leucocyte; nevertheless, there is a distinct substance produced by the cell which we see in the form of the neutrophilic granules and this substance is probably bound up in the

specific functions of the leucocyte. We are unable to state whether the dye actually enters the neutrophilic granules, as we think that it does enter the fluid of the so-called vacuoles, or if it occupies the interphase between the granule and the surrounding cytoplasm. It is entirely clear, however, that the neutral red is actually a chemical indicator in connection with the three types of the specific granules of the leucocytes; with the neutrophilic granule it gives an intermediate reaction, with the eosinophilic granule the reaction is toward the alkaline reaction of the dye, while with the basophilic granule the reaction is definitely the brilliant scarlet color of the acid reaction of the dye. The neutrophilic leucocyte is likewise a phagocytic cell; that is to say, it has a function which may or may not be associated with its specific granulation. This cell also reacts to phagocytized material by developing vacuoles around the debris. In case of the neutrophilic leucocyte, there are many times when the cell has no vacuoles, and again many times when they are present. These vacuoles show an entirely different color in neutral red from the neutrophilic granules, they are decidedly more scarlet, that is, more toward the acid reaction, are slightly larger than the granules, and vary markedly in size, so that they are never likely to be confused with the stained neutrophilic granules. Thus, in the neutrophilic leucocyte, there are two entirely different substances which stain with the neutral red; first, the so-called neutrophilic granules, second, the droplets of fluid which we call the vacuoles of digestion.

In the case of the supra-vitally stained monocytes, the actual analysis of the substance that reacts to neutral red has not been easy, but we think that the study of the modified monocytes of tuberculosis, the so-called epithelioid cells, has aided in this analysis. It is well known that Naegeli first analyzed the monocyte as a separate group of the white cells by showing that the cell, which Ehrlich had called the transitional cell, was entirely different from the neutrophilic leucocyte on the one hand, and from large lymphocytes on the other, in that it contains very fine azurophilic granules which are found in neither of the other two types of cells. In the supra-vital technique it was shown by Sabin ((24), see Fig. 4) that there are two types of substances in the living monocytes that react to neutral red, very tiny bodies, which in the living cell are arranged in a rosette around a clear spot, the centrosphere, and larger bodies, which are in the periphery of the rosette. The tiny bodies have a characteristic salmon-colored reaction when stained with neutral red. We have found this color both constant and characteristic. The larger bodies, on the other hand, we are quite confident, are true vacuoles of digestion, comparable of the vacuoles of the clasmatocyte; they vary markedly in size and somewhat in color; though they never show as wide a range in color, as do the corresponding vacuoles of the clasmatocyte. We are also convinced that the fine particles that stain with neutral red in the living cell are not the same as the azurophilic granules of fixed films, since the azurophilic granules are scattered without pattern in the cytoplasm. In the living, un-

stained monocyte, the fine particles of the rosette are just visible, because they have a very low index of refraction; the larger vacuoles, when present, are plainly visible, for they have a high index of refraction. We think that the fine particles of the rosette are not seen as granules in the films of blood fixed in absolute alcohol (Wright's blood stain), but they are retained in formalin; nor are the azurophilic granules of the fixed cells visible in the living state. The most difficult point in the analysis of the monocyte is that under certain conditions the entire cell may be occupied by the larger stainable bodies. Such monocytes look like clasmatocytes and the development of monocytes into this form in tissue cultures of blood has convinced Lewis, Willis and Lewis (14) that clasmatocytes and monocytes are a single strain of cells. Such a monocyte was shown by Sabin (Fig. 5, (24)) from a case of Malta fever, in which there was a very marked increase in the monocytes of the circulating blood and in which all of the monocytes became markedly vacuolated after the injection of an autovaccine. In this state the vacuoles seemed to replace the finer particles entirely. Another such monocyte was shown by Sabin, Doan and Cunningham (Fig. 19, (27)). In the former the centrosphere was still evident; in the latter the centrosphere was obscured, but the vacuoles still showed some evidence of being in a group instead of being diffusely scattered. In such a cell it is not clear what has become of the finer particles so characteristic of the cell; the question then arises as to whether they have disappeared or have enlarged into vacuoles. In the reaction of the monocytes to tuberculosis, on the other hand, it is the fine bodies of the characteristic rosette that increase in enormous proportions and it is this reaction which, as we shall now demonstrate, indicates that the epithelioid cell and the resulting giant cell are characteristically derivatives of the monocytes rather than of any other type of cell.

METHODS

General methods

The purpose of the experiments which we are reporting in this paper has been to analyze the relationship which exists between the monocytes of the blood and of the tissues, and the changes which occur, especially with regard to the monocytes, in the course of acute experimental tuberculosis. We do not feel that these experiments represent more than a very meager attempt to open up the question of the varying changes which take place in the blood cells, especially the monocytes and lymphocytes, in tuberculosis. The full exposition of this most important subject must await much more elaborate study than we have been able to carry out up to the present time.

In the course of this study we have used about 75 rabbits. The organisms which we have used were cultures obtained from the Dows laboratory of tuberculosis of the Johns Hopkins Hospital and have been numbered B1 and H37 respectively. The organism B1 was an organism of bovine tuberculosis and has been used in the majority of the experiments; while H37, an organism of human tubercu-

losis obtained from the same laboratory, has been used in only a few.

The method which we have used throughout these experiments has been to remove the bacilli to a watch crystal and weigh. The weighed bacilli were transferred to a sterile mortar and ground with a little saline, more being added as the emulsion was prepared. After about 5 to 10 minutes' grinding the suspension was filtered through sterile cotton and then centrifuged. Samples were removed from the tubes until it was shown that practically all the masses had been thrown down. A standard loop of the mixture was then spread on a slide over an area about 1 cm. square and the average number of bacilli per oil-immersion field determined. We are well aware that this method is not even approximately exact, but it at least ensures that the eventual suspension contains no large clumps or masses of the bacilli. We have generally used an emulsion containing from 15 to 50 organisms to the oil-immersion field and the suspension was, in most instances, given intravenously, although a few animals were inoculated intraperitoneally.

Sabin (24) found that, in a case of Malta fever, there was a large increase in the number of the circulating monocytes, and an additional increase in their phagocytic activity as indicated by their staining with neutral red. With this concept in mind it occurred to us that perhaps B. abortus, an organism closely related to the bacillus melitensis, might bring about a stimulation of the monocytes in our experimental animals and thus supply us with a mechanism for analyzing the results of infection with tuberculosis in the case of previously stimulated animals.

Throughout these experiments we have taken blood counts at as close intervals as was possible, many of the experimental animals having been counted daily for periods of six to seven weeks. This factor of making daily supra-vital differential counts, as well as counts of the total white blood-cells, has rendered the utilization of a larger series of animals technically impossible so that, while we recognize that our series must appear small to those workers studying allergic, serological and immunological reactions, nevertheless, it was as large as it was possible for a small group of workers to carry through. And furthermore, our results have been so striking and so easy to classify into specific groups, with regard to the monocytic reactions, that it has seemed fully justifiable to consider the series quite large enough to make reliable conclusions possible.

We have counted the blood of all experimental animals several times before injections and, whenever possible, this period of preliminary counting has been extended to several weeks' duration. It is a customary opinion that the blood counts in rabbits vary much more widely than in the other animals and we were inclined in our earlier experiments to concur in this opinion, but we found, when care was taken to have such a dilatation of the vessels of the ear that the blood flowed freely, that the variations in the total counts of the blood-cells of the rabbit were reduced to within limits not greatly in excess of those which we have demonstrated to be normal in the human blood (Sabin, Cunningham, Doan and Kindwall (25)). Such a dilatation of the ear veins can be easily obtained if the ear is stroked

or gently tapped with the back of a knife.

Throughout the study on the blood, we have been careful to take the blood for the total count at the same time at which we took the specimens for the supra-vital differentials. All of the differential counts have been made with the supra-vital technique; smears fixed in Wright's stain and also in Ziehl-Nielsen for tubercle bacilli have been made in special instances when specific observations were desired.

The autopsies have been controlled by careful studies of lungs, spleen, bone-marrow, omentum and other tissue, in special cases, made upon supra-vital preparations, according to the methods described by Sabin, Doan and Cunningham (27). Sections were also prepared from tissues fixed in the routine manner and stained both by the ordinary histological stains and for tubercle bacilli.

The supra-vital technique

The method we have used was developed by Sabin (24). The essential point in the technique is to obtain a perfectly even, thin film of a vital dye or combination of dyes on a slide, which is to be used for a preparation of fresh blood. In this way the dyes, to which the living cells react, dissolve in the normal plasma as the film is made, so that the cells are not subjected to any accessory fluids. For the technique it is first essential to remove all traces of grease from the slides and covers. This is done by the usual technique. They are kept in concentrated sulphuric acid to which a few crystals of potassium bichromate have been added for 3 to 4 days; then they are rinsed thoroughly in running tap water, preferably hot, and transferred to distilled water and then 80 per cent alcohol. They are wiped from the alcohol with cheesecloth and flamed thoroughly to remove the last traces of grease. The slides are then ready to be flooded with the stain.

We have found vital neutral red and a combination of vital neutral red and vital Janus green the most useful stains. The neutral red alone does not inhibit motility and all of the normal blood-cells react to it characteristically. The addition of Janus green, which stains the mitochondria, does check motility, but is of especial value in discriminating immature blood-cells, the cells of organs and the cells of the connective tissues. Therefore, for the routine blood-counts with relatively normal cells, we use the neutral red alone, but for all of the studies of abnormal blood and of the fresh tissues from the autopsies we have used the double stains.

The films of stain are made as follows: we keep a saturated stock solution of vital neutral red in absolute alcohol; from this a dilute solution is made by adding from 20 to 30 drops of the saturated solution to 10 cc. of absolute alcohol; the strength of the stain is best judged by the color, which is a rose red; the exact strength must be tested with the material to be stained, in fact, the amount of stain must vary with the number of cells that take the dye in a given preparation. Any staining of the nuclei is a sign that the stain is too strong. The double stain is made by taking 1 cc. of the dilute neutral red and

adding from 3 to 6 drops of a saturated solution of vital Janus green in absolute alcohol. We have found that 3 drops of Janus green per 1 cc. of dilute neutral red is the correct strength for the cells of normal blood, but for preparations from tissues more Janus green should be used, up to 6 drops. Beyond this strength the cells are killed.

The slides are prepared with the dyes as follows; after they have cooled from the flaming, they are held in a horizontal position and flooded with the stain, which is quickly drained back into the bottle; the stain must neither be allowed to stand long on the slide, since the alcohol will evaporate, nor to touch the fingers in this process, lest a little grease be added to the solution. The slides are then placed upright until they are dry. If the film of stain is uneven, some of the cells will be killed, and the technique is in no sense differential for dead cells. The stain can be used over and over unless it becomes greasy or full of dust.

The preparations of fresh blood are made by the usual technique of obtaining the drop on a coverslip and inverting it on the slide as soon as the blood has spread, the coverslip must be rimmed with vaseline of a high melting point; we have used salvoline. The preparation is then placed and studied in a warm box, kept at 37°C. For preparations of the tissues the technique varies according to the organ to be studied. For the lung, liver and kidneys we scrape a freshly cut surface of the organ gently and mount the material as if it were a blood film. It is important to have an amount of tissue so small that it will spread out in a film practically as thin as a blood film; this is important for two reasons, first, because the cells are then reached by the dye, and second, because the necessity of using accessory fluids is avoided. Such preparations must also be sealed with vaseline. For the free cells of lymph glands, spleen and bone-marrow we have found that better preparations can be made by drawing the material up into capillary pipettes from the anaesthetized animal in which the circulation is intact. In studying the cells of the diffuse connective tissues, such as subcutaneous tissue we have seldom found enough fluid present for our preparations and in this case it has proved to be better to make an artificial oedema by the injection of neutral red (1 to 10,000 in Ringer's solution) and to mount bits of the resulting gelatinous tissue. We have found that it is the cells of the circulating blood especially which are the most sensitive to accessory fluids and consider that motility of cells can never be correctly judged when they are studied in artificial solutions. From the autopsies of our animals we have made the supra-vital studies of the tissues of the lungs, liver, kidneys, spleen, lymph glands, bone-marrow and omentum and of any other tissues that have shown signs of tuberculosis in the gross material. We have found these studies of the utmost value and consider that they permit a much better diagnosis in certain particulars than can be obtained from fixed sections; in the first place, the supra-vital technique is differential for cells that cannot be discriminated in sections, and secondly, certain structural points such as the

relative independence of cells, for example, whether monocytes are structurally bound together in tubercles or actually free in the tissues, are more easily determined by this technique than in sections of fixed tissues.

EXPERIMENTAL DATA

Effect of tuberculosis on the monocyte in the circulating blood and in the tissues

The immediate effect on the monocyte of the ingestion of the tubercle bacillus is an inhibition of the motility of the cell. We judged this because we have found, in following the blood of rabbits which had been infected with tuberculosis, that a short time after the infection there appeared in the blood monocytes which had apparently lost their power of motility. These monocytes were quite different from the normal cells and we have called them "modified monocytes," since we are unable to say exactly in what way they have been changed. These cells were large, usually round and had apparently lost their power of motility; they stained intensely in neutral red and had the stained vacuoles scattered throughout the peripheral zone around the rosette. Such a cell is shown in Fig. 1, from Rabbit TB 49. This cell was somewhat irregular but showed no locomotion on the slide. We have demonstrated the tubercle bacilli within such monocytes of the circulating blood by means of the Ziehl-Nielsen technique. We consider that the cessation of motility is a sign that the cell has been damaged. The interpretation of the increase in the stainable vacuoles as the very first effect of the bacillus within the cell is an important point. It is possible to explain this change in three different ways, as evidence of increased activity on the part of the cell, as evidence of cellular injury, or as an indication that the cell is attempting to compensate by increased cytoplasmic activity for an actual damage of its structure.

In the case of the vacuoles of the clasmatocyte and of the neutrophilic leucocyte, we are confident that they are functional structures. Every reaction of the monocyte in the development of the large vacuoles may not be quite so clearly functional; it is frequently true that these vacuoles take longer to stain in our preparations than the vacuoles of the clasmatocyte, but we do not believe that this change is evidence of immediate or extreme injury or that the monocyte is quickly killed by harboring the tubercle bacillus within its cytoplasm. It is, however, quite clear that both by its actual presence in the cell and possibly by substances which it produces, which reach the cell through the circulation, the tubercle bacillus can profoundly modify the morphological appearance and the physiological activities of the monocyte.

The next stage in the effect of tuberculosis on the monocyte we have also seen in a cell of the circulating blood, namely, the very beginning of the formation of the epithelioid cell. Such a cell is shown in Fig. 2. This cell was drawn from the blood of Rabbit TB 43, 26 days after the infection of the animal. We have found that the effect of the tubercle bacillus in producing the epithelioid cell is very characteristic and consists in two things; first, the suppression of the

vacuoles which are normally present in the periphery of the cell, and second, a most characteristic and enormous multiplication of the fine particles of the rosette. A cell comparable to the one of Fig. 2, but taken from the tissues, is shown in Fig. 3, This cell was from the liver of a tuberculous rabbit (TB 36). By this multiplication of the fine bodies of the rosette of the monocyte, the rosette becomes the essential characteristic of the so-called epithlioid cell. It must be brought out very clearly that the presence of granules arranged around the centrosphere is not found in monocytes alone. All young granulocytes, of course, have a centrosphere, and at a certain stage, the stage in which the cytoplasm is well filled with the specific granules, these granules are arranged in radiating lines, thus accentuating the centrosphere; this is true of the neutrophilic, the basophilic, and the eosinophilic myelocytes; in the monocyte there is likewise a specific substance, in the form of fine granules, that makes the rosette in radiating lines around the centrosphere and, in this type of cell, in contrast to the granulocytes, there is a marked permanence of the pattern of the rosette. The cell of Fig. 3 was small as compared with the more developed epithelioid cells. It happened to have two nuclei, thus showing the same tendency toward amitosis exhibited by the normal monocyte. We have not seen division in monocytes except by amitosis. The mitochondria characteristic of the peripheral zone of monocytes were obvious in the case of Fig. 3. The most striking and characteristic change in monocytes infected with the tubercle bacillus consists, then, in the multiplication of the fine bodies of the rosette. If the monocytes in Plate 11 of Sabin, Doan, Cunningham (27) are compared, it will be seen that there is some variation in size in the fine bodies of the rosette; for example, in their Figs. 14, 15, 16, and 18, the fine bodies of the rosette are all small, whereas in the cell of Fig. 17 they are markedly larger. All of the cells on this plate were drawn at the same magnification, so that the size of the granules can be compared. It is interesting to note that the fine bodies of the rosette in Fig. 18 are small, and this was a cell which had been stimulated to marked phagocytic activity. The cell in question had engulfed a red blood-cell and several white blood-cells. In the young epithelioid cell shown in Fig. 3, which was taken from Rabbit TB 36, there had been an increase in the number of the fine bodies of the rosette; they were at the same time slightly larger than the fine bodies of the rosette of the average normal monocyte, such as the ones already referred to and as the monocyte from normal human blood shown by Sabin (Fig. 4, (24)).

In the cell of Fig. 3, rabbit TB 36, the centrosphere was obvious in the center of the rosette. As will be seen in the drawing, there is a slight tone of the dye between the granules; in some instances in the epithelioid cells we have found it difficult to tell whether this tone was due to a true staining of some substance between the granules or simply to an optical effect on account of the great number of the granules. It may also be true that this staining between the granules is a diffusion of the dye from the

granules due to the gradual damage to the cell. The rest of the cytoplasm of this cell was practically clear, except for the mitochondria. The rabbit from which this cell was drawn showed very many of these young epithelioid cells from the liver, together with many large epithelioid cells and giant cells; in the lung of this animal we found comparatively few of the younger epithelioid cells, but, on the other hand, we found many that were much further differentiated, together with a considerable number of clumps of the undifferentiated reticular cells.

As the monocyte becomes more and more affected by the tubercle bacillus, the rosette becomes larger and the bodies which form it become smaller Such a cell is shown in Fig. 4 (Rabbit TB 33). This cell was found in a scraping from the cut surface of the lung. In this cell the rosette was so large that it almost completely filled the cytoplasm, leaving only a small peripheral zone. Such epithelioid cells occur, but a wider peripheral zone is more frequent. In the edge of this rosette was one vacuole which stained in neutral red and a single refractive body shown in white which we interpreted as fat. These bodies stain with Sudan III. Most of the epithelioid cells from the lung of this animal showed a marked development of these droplets of fat. The most striking thing about this cell, shown in Fig. 4, was the enormous multiplication of the fine bodies of the rosette. These bodies were slightly smaller than those shown in Fig. 3, but still were not as fine as those shown in Figs. 6 and 8. This great increase in the actual number of the fine bodies of the rosette is the characteristic morphological change, as seen in supravitally stained films, which is brought about in the monocyte by the tubercle bacillus or its products. At the same time there is a very considerable suppression of the larger vacuoles occupying the periphery of the rosette. The cytoplasm around the rosette in this cell was like ground glass and contained no mitochondria.

The next cell of the series, shown in Fig. 5 (Rabbit TB 16) was also a typical epithelioid cell. It was also taken from the lung. This cell showed the very characteristic division of the cytoplasm into two zones, the rosette and the peripheral zone. There was a greater variation in the size of the small bodies of the rosette than of the other cells, and in the lower border there were a few bodies which were decidedly larger than the rest. The wide peripheral zone of this cell was markedly granular and very characteristic of many of the epithelioid cells. Nothing in this granular peripheral zone stained with either the neutral red or the Janus green, but in this area there were two bacilli which were very characteristic. It is in this peripheral zone that cells and other particulate material that a monocyte has phagocytized are always seen, and when such cells or debris have been phagocytized, they are always to be found within stained vacuoles of digestion. On this account we stress the fact that there were no stained vacuoles in the peripheral area of this cell (Fig. 5); the bacilli showed not the slightest staining reaction around them and they were seen moreover to shift their position slightly in the cytoplasm, possibly through some slight movement of the latter. We have now seen bacilli

several times in the living cells and are convinced that the monocyte does not show any of the signs toward engulfed tubercle bacilli which ordinarily indicate that the material taken in is being digested. So that it seems to us as most reasonable to assume that the bacilli remain alive and capable of multiplication. This forms one factor in the evidence that leads us to suggest that the bacilli are harbored by these modified monocytes instead of being destroyed by them.

A most marked rosette with the finest division of the granules is shown in the cell of Fig. 6, which was obtained from the lung of Rabbit TB 39. The rosette in this cell was very sharply defined and was made up almost wholly of fine bodies; furthermore, in this cell the bodies reached the maximum fineness in the cells we have seen. In the cell shown in Fig. 6, there were around the rosette a few small refractive droplets which we interpreted as fat and which we think probably indicate a beginning degeneration of the cell. In the peripheral zone of this epithelioid cell there was a small red blood-cell, which we presume had just been taken in, because its color was exactly like that of the surrounding red cells. There was not a trace of neutral red about this red cell. On the other hand, many of the epithelioid cells of this rabbit showed a small amount of debris in the peripheral zone, as evidenced by stainable vacuoles. Such cells indicate that the power of phagocytosis is not entirely suppressed in the epithelioid cells. In the edge of the rosette of the cell shown in Fig. 6, there were a few highly refractive bodies; they did not stain at all in neutral red. A specimen studied in Nile Blue Sulphate did not show any staining of these droplets. In many of the epithelioid cells of this animal the entire periphery of the cytoplasm was packed with these refractive droplets, but, in frozen sections these refractive bodies stained heavily with Sudan III and hence we have concluded that they are lipoids of some type or other. We have not, as yet, studied these refractive bodies more thoroughly, although this should be done, as they represent a most obvious and important change in the cell and one which we think is probably degenerative in character.

A very large epithelioid cell is shown in Fig. 7, from the lung of Rabbit TB 38. This cell showed several vacuoles in the edge of the rosette, which clearly indicated that the cell had phagocytized some debris; in this cell there was only one of the lipoid bodies, shown in white in the edge of the rosette, but in the lung of this animal the majority of the epithelioid cells showed the fat droplets either filling the entire peripheral zone, leaving the rosette intact, or else filling the entire cell, as is shown in Fig. 9.

The last phase of the epithelioid cell tends either toward a fatty degeneration or toward the formation of a giant cell, which may also pass into the same terminal phase of fatty degeneration. The cell of Fig. 7, Rabbit TB 38, shows the very beginning of the fatty degeneration. The next cell of the series (Fig. 8) was taken from the lung of Rabbit TB 36 and shows that fat droplets first fill the periphery of the cytoplasm of the epithelioid cell. These droplets of fat increase in number until they

occupy the entire peripheral zone of the cytoplasm. They then go on increasing in number until they entirely obscure both the rosette and the nucleus of the living cell. The same stages of the development of refractive droplets can be followed in the giant cell. Just why some epithelioid cells undergo this extreme degeneration before there is any nuclear division, while others go on to various stages of the giant cells, we have not been able to determine, but this condition must be associated with the extent of the injury inflicted upon the cell by the bacillus or with the general physiological condition of the cell.

Fig. 9 is of a cell in which the entire cytoplasm has become filled with the refractive droplets referred to above; this cell, while still alive, as shown by the fact that the nucleus did not stain with neutral red, nevertheless, was probably in an advanced stage of degeneration. There was only a single nucleus visible in this cell, which indicated that there had been no progression toward the giant cell type.

The cell of Fig. 8 had two nuclei. This division of the nucleus without a resulting division of the cell is the method by which we believe the giant cell of tuberculosis is formed from the monocyte. Again, it seems to us that this is further evidence that there is a marked change in the cytoplasmic activities of the monocyte in animals infected with tuberculosis. Sabin, Doan and Cunningham (27) have shown first, that the monocyte has a marked tendency to divide by amitosis, and secondly, that amitosis is to be defined as a condition in which nuclear division precedes the division of the centrosome; complete amitosis involves three processes in definite sequence, nuclear division, division of the centrosome, and subsequent division of the cell. The division of the cell seems to be dependent on the previous division of the centrosome; if this be true, we have an adequate concept of the sequence of events that give rise to the giant cell, namely, repeated nuclear division with inhibition of the division of the centrosome. We, therefore, suggest that, in general, giant cells of the Langhans type are derived from monocytes.

It is thus clear that the effect of the infection of tuberculosis on the monocytes causes them to increase in size and to develop a very marked differentiation of the cytoplasm into two distinct zones, the zone of the rosette and the peripheral zone. When films made by scraping the freshly cut surface of a tuberculous lung are treated as blood films and stained with the Wright's blood stain, the division of the cytoplasm into these two zones is very marked. The central zone of the rosette stains a diffuse pink in eosin; thus the fine granules of the living cell that make the rosette seem to have been dissolved in the alcohol so that they no longer appear as discrete particles. The peripheral zone of the cell is markedly basophilic and has the same muddy blue color as the monocyte of the circulating blood. In these cells one can count about 50 to 60 of the azurophilic granules. Thus the relationship of the epithelioid cell to the monocytes is again brought out in the presence of the azurophilic bodies characteristic of that cell. These observations lead us to conclude that the fine bodies

of the rosette of the monocyte are substances visible in the living cell; that they are not the same as the azurophilic granules of the fixed films which appear in the monocyte after fixation in alcohol; that the fine bodies of the rosette are retained in formalin; that when they are very markedly increased in number, as they are in the epithelioid cells, they give to the cytoplasm an acidophilic reaction in Wright's blood stain. This reaction is not seen in the normal monocyte of the peripheral blood, which we interpret as due to the smallness in amount of the substance in the normal cell as compared with the epithelioid cell. It may be, however, that the presence of this substance in the monocyte is the factor which makes the sharp differentiation between the very clear blue of the cytoplasm of the lymphocyte in Wright's blood stain, and the muddy or smoky blue of the cytoplasm of the monocyte.

One of the points by which the discrimination between clasmatocytes and monocytes was made by Sabin, Doan and Cunningham (27) was a difference in origin of the two types. They obtained evidence which indicated that the clasmatocyte originally comes from endothelium, while the monocyte arises throughout the life of the animal from an undifferentiated, embryonic type of cell, the so-called reticular cell. Thus the monocyte is derived by a process of maturation just as are all other types of white blood-cells. The above named authors (5) have been able to identify this primitive, embryonic rest, the so-called reticular cell, in the living connective tissues, so that the type is no longer a hypothetical progenitor for the white blood-cells, but a cell which can be readily found and identified. The name "reticular cell" is not very specific but the cell itself can be quite clearly defined both as to its appearance and in its location. This reticular cell was identified by Doan, Cunningham and Sabin (6) in fresh films of bone-marrow stained with supra-vital dyes. In bone-marrow so simplified by experimental procedures that there were no cells in the marrow except fat, endothelium and these reticular cells, this discrimination was easy. The reticular cell in the living state shows a complete absence of differentiated structures, both in the nuclei and in the cytoplasm. A small clump of such cells as seen in the living state could be drawn only as a mass with a definite but common outline and with a uniform gray tone; the nucleus may not show at all in the living cell; but if such a cell or group of cells be watched, the nuclei gradually appear, probably as the cells die. There are no discrete granules whatever to be seen in the cytoplasm which has the uniform appearance of ground glass. The cytoplasm of the reticular cell appears to have a much more definite tone, however, than the clear part of the cytoplasm of a squamous epithelial cell, for example. In Wright's blood stain the cytoplasm and nucleus of the reticular cells show a practically uniform but very faint basophilic reaction. These cells can be found in great numbers in every film made from scrapings obtained from the cut surface of a lymph gland and studied either supra-vitally or stained with any methylene blue-azure mixture. The lack of a striking structure is what

has made the reticular cell remain a hypothetical type of cell for so long. The nucleus has little chromatin, the nuclear border is never as sharp as in a lymphocyte or in an epithelial cell; the cytoplasm has no specific structure by which it can be discriminated from other cells. There are no mitochondria whatever; this absence of mitochondria sharply discriminates this cell both from the primitive white blood-cells and from the lymphocyte (see Cunningham, Sabin and Doan (5)). This reticular cell is the progenitor of the primitive white blood-cell which it becomes as soon as mitochondria develop in the cytoplasm. There is no other cell in the body with such a lack of discriminating features; that is to say, it is the most undifferentiated cell of the adult organism.

The reticular cell is most easily found in normal tissues in a scraping from the freshly cut surface of any lymph gland, because there are more of them in lymph glands than anywhere else; it is also readily obtained from the spleen. It is much more difficult to find these cells in normal bone-marrow because the marrow is so crowded with myelocytes. The reticular cell can be found in very small numbers in a fresh preparation made by scraping the freshly cut surface of any normal lung. We have found it more easily from the septa of the lung than in subcutaneous tissue; however, if very tiny bits of fresh connective tissues are mounted on a film of neutral red and Janus green, of sufficient strength, so that all of the more differentiated cells, the fibroblasts, the clasmatocytes and the various types of the white blood-cells are well stained, these very primitive reticular cells, which do not react at all to either of the dyes, can be found.

From the supra-vital studies of the material of tuberculous animals at autopsy, we have found that the condition of the lungs has varied markedly in the relative proportions of the different stages of the monocytes. For example, in the lung of Rabbit TB 39 (Protocol on page 255, Chart 3) the cells in the fresh scraping seemed to have come wholly from the septa because there was none of the characteristic elastic tissue from around the air sacs and also no epithelium. The most striking thing about this tissue was the large masses of the primitive reticular cells which were present in the scraping; some of these masses contained only three or four cells, but others filled the whole field under the oil-immersion lens. Most of them had no granules whatever; a few had some granules, like those of Fig. 5, of Cunningham, Sabin and Doan (5), which did not react to Janus green. These granules may well have been the precursors of mitochondria. Even when we increased the strength of the Janus green until the cells were killed, we were unable to demonstrate any mitochondria. Besides these very large masses of the reticular cells, there were enormous numbers of epithelioid cells, some of them having rosettes that practically filled the cells, while others, like the one shown in Fig. 6, which was taken from this rabbit, had a wide peripheral zone. Only a few of the epithelioid cells of this rabbit had mitochondria, and these were very tiny and were in the extreme periphery of the cells. Very large numbers of the epithelioid cells of this rabbit had a

few fat droplets in the periphery. Thus, in this specimen, there were cells in two different phases; first, there was a very marked production of new reticular cells, and second, the epithelioid cells were of the fully developed type and many of them showed the beginning of degeneration in the diminution of the mitochondria and in the development of fat in their cytoplasm.

In the scrapings from the lung of Rabbit TB 16 (Protocol on page 264; Chart 11A) there were, on the other hand, no reticular cells to be found, but the tissue likewise seemed to have come from the septa, for no elastic tissue was present. The scraping was practically a pure culture of modified monocytes of the type shown in Fig. 5. Films from the lung of this animal were stained for tubercle bacilli and as many as ten were found in the peripheral zone of the epithelioid cells. There were very large giant cells present, some of which contained enormous numbers of fat droplets. From these two records, it can be seen that there is probably a tendency toward the development of the cells in cycles; thus, the first rabbit showed a marked wave of the production of new reticular cells, with the epithelioid cells of a preceding generation just beginning to degenerate, while the second rabbit was killed when there was comparatively little development of new reticular cells, but when there were vast numbers of the matured epithelioid cells.

From the observations described in the preceding pages it seems legitimate to conclude that the effect of infection with tuberculosis is to cause an increase in the reticular cells of any organ which becomes infected. This local effect on the reticular cells we consider is probably a chemical one and not a direct effect due to the presence of bacilli in the cells, because so far we have no evidence that the reticular cell can phagocytize the bacilli. Furthermore, the infection brings about a rapid maturation of these reticular cells into the typical monocytes and the further change of the monocytes into the epithelioid cells. We are quite sure that the monocytes and epithelioid cells take in the tubercle bacilli, but we are also sure that this multiplication of the reticular cells and their transformation into monocytes and epithelioid cells can be brought about without the immediate presence of the bacilli themselves. In this report of our series of rabbits we shall show that some of the monocytes of the circulating blood become modified, and we have demonstrated the presence of tubercle bacilli within them. The fully matured epithelioid cell contains the bacilli, as can be proved by seeing them in the living cell and by staining them with carbol fuchsin. Moreover, it can be demonstrated that the epithelioid cell does not show a reaction toward these engulfed bacilli as demonstrated by neutral red, a reaction which we believe indicates that the cell destroys and digests the material taken up.

The giant cells are produced by the multiplication of the nuclei of the epithelioid cell which always takes place in the peripheral zone, leaving an undivided and central centrosome surrounded by an enormously developed rosette. In one of the fresh specimens, a very large double giant cell was seen, that is to say, a single cell with two

rosettes, each of which had a partial rim of nuclei. Both the epithelioid cells and the giant cells finally show the development of fat droplets, which begins near the periphery of the rosette, after which they gradually increase in number until they fill the entire peripheral zone of the cell and ultimately seem to replace the entire rosette. In some of the cells this increase in the amount of lipoid granules is so great that there is no staining to be seen at all (Fig. 9). The specific effect of the tubercle bacillus on the monocyte is the enormous increase in the numbers and the decrease in the size of the fine bodies staining with neutral red that characterize the living monocyte. This development divides the cell into two zones, the central zone of the rosette and a peripheral zone which usually contains no stainable substance, that is, has no reaction to vital dyes but does contain the tubercle bacilli. The rosette more rarely may entirely fill the cell. The fine granules of the rosette give the zone of the rosette a pink reaction in Wright's stain, in which the typical azure granules of the monocyte can be seen. The periphery of the cell retains the same muddy blue reaction in Wright's blood stain as that which characterizes the cytoplasm of the normal monocyte. The giant cell of tuberculosis has all of the signs of having come from a monocyte; it is a type of giant cell in which the rosette, characteristic of the monocyte, has become enormously enlarged and is centrally placed in the cell so that the multiple nuclei are confined to a peripheral zone. In this characteristic, the giant cell of tuberculosis is an entirely different type of cell from the so-called foreign-body giant cell and the osteoclast.

Thus the effect of tuberculosis is on one strain of cells, and consists in the increase of reticular cells and their maturation into monocytes and the characteristic derivatives of monocytes —the epithelioid cells, and a special type of the giant cell.

Ratio of the monocytes to the lymphocytes in the blood and the correlation of this ratio with the cells of the tissues.

In our series of rabbits we used ten animals as controls and made repeated counts of the peripheral blood on them to obtain the normal number of the white blood-cells and the normal percentage of the different types of these cells. For the data concerning the normal blood of rabbits, we have also included the counts made on the rest of the animals before they were infected. From these data we have found that the average number of the white blood-cells in the normal rabbit is 11,281, taken from counts on 54 rabbits. The average normal percentage of the monocytes proved to be 8 per cent, the actual number per cubic millimeter being 943; the corresponding data for the total lymphocytes is 25 per cent and 2805 cells per cubic millimeter. We are showing in Charts 1 and 2 the relative frequency of the total number of the white blood-cells and the average percentages of the monocytes in the normal rabbit to demonstrate that the range of variation is not great. In Chart 2 we are giving comparative data for the monocytes of the normal and after infection with tuberculosis.

In following the blood of rabbits which have been infected with such massive doses of tubercle bacilli as to give a comparatively acute reaction, we have found that there is a marked correlation between the progress of the infection in the tissues and the condition of the blood. There are two striking effects to be seen in the blood; first, there is an actual and marked increase in the percentage of the monocytes which may go as high as 53 per cent; and second, the normal ratio of monocytes to lymphocytes is reversed. Thus, in a rabbit with active, acute tuberculosis the monocytes of the circulating blood surpass the lymphocytes in number. So important is this ratio between monocytes and lymphocytes in the circulating blood that we have been able to make a correct judgment concerning the condition of the animal in the majority of experiments by following the ratio between these two types of cells. We have found that, as is well known, there is a general lowering of the production of both white and red blood-cells in tuberculo-

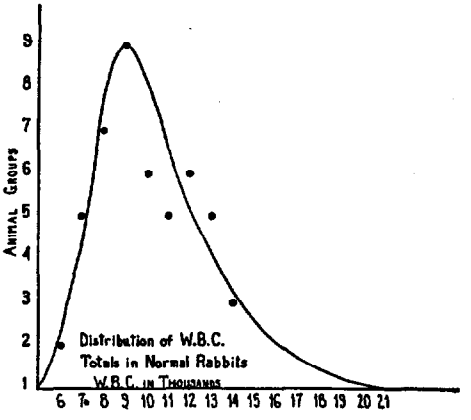

Chart 1. Chart Showing the Distribution of the Average Counts of the Total White Blood-cells in Normal Rabbits

On the abscissae are given in thousands the average number of white blood-cells per cubic millimeter. On the ordinates are given the number of animals in each group corresponding to a given number of thousands on the abscissae. It will be seen that from 54 rabbits, the largest group, namely 9, had a count between 9 and 10,000; and that the extremes were represented by very few animals.

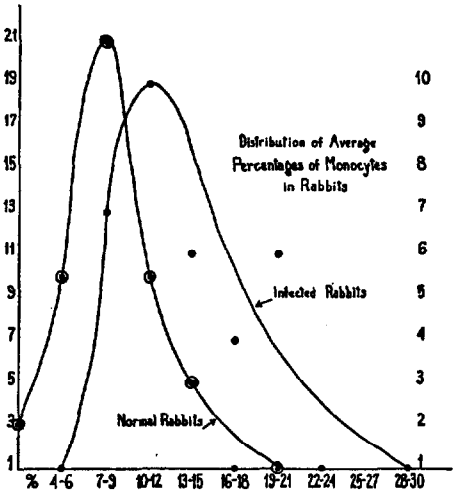

Chart 2. Chart Showing the Distribution of the Average Percentages of Monocytes in the Blood of Normal Rabbits, and in Rabbits Which Have Been Infected with Tuberculosis

On the abscissae are given the percentages in groups; on the ordinates are given the number of animals corresponding to a given range of percentages on the abscissae. The figures on the left margin correspond to the curve from the normal rabbits, while those in the right margin correspond to that of the tubercular rabbits. It will be seen that the most frequent range in percentages in normal rabbits is from 7 to 9 per cent, for 21 out of a total of 54 animals were in this group, while the corresponding most frequent range in percentages for infected animals is from 10 to 12 per cent; ten out of 34 animals were in that range.

TABLE I

Studies on the blood of rabbits

ANIMAL	BEFORE TUBERCULOSIS				AFTER TUBERCULOSIS				WHITE BLOOD CELLS		REMARKS
	Percentage of lymphocytes	Percentage of monocytes	Total lymphocytes	Total monocytes	Percentage of lymphocytes	Percentage of monocytes	Total lymphocytes	Total monocytes	Before tuberculosis	After tuberculosis	
TB 1	15	6	998	422	20	19	1,402	1,425	6,620	5,687	Bacillus abortus, arrested tuberculosis
3	24	13	2,373	1,288	26	10	2,703	1,211	9,590	10,884	Miliary tuberculosis
6	30	14	2,995	1,423	34	12	3,647	1,208	10,200	10,052	Arrested tuberculosis
7	29	10	3,545	1,251	33	14	4,237	1,797	12,520	12,840	Found dead fifth day after inoculation. Slight pneumonia
8	27	6	2,635	740	23	20	2,443	2,048	11,680	10,150	Moderate tuberculosis
10	21	7	1,836	654	21	11	1,947	949	8,600	8,331	Arrested tuberculosis
11	21	5	2,010	433	16	20	1,449	1,812	9,080	9,060	Moderate tuberculosis
13	32	9	3,717	1,034	13	19	2,112	3,374	11,500	18,368	Miliary tuberculosis
15	39	4	4,599	467					10,455		Control
16	27	8	2,584	1,144	18	20	2,799	3,469	13,455	9,720	Extreme tuberculosis
17	25	11	4,335	1,734	27	9	3,683	2,599	17,540	16,632	Bacillus abortus only
18	52	9	4,118	712	33	7	4,312	947	7,920	15,066	Bacillus abortus only
19	21	9	2,268	870					11,125		Control
20	25	7	4,708	1,235					16,700		Control
21	19	8	2,419	1,124					14,150		Control
22	30	13	3,740	1,655					19,250		Control
23	34	5	6,781	1,156					21,100		Control
24	22	8	3,453	1,354					15,700		Control
25	25	8	3,034	949					12,300		Control
26	26	6	4,171	987					14,222		Control
27	20	5	1,935	497					9,244		Control
29	23	6	2,300	536	42	7	2,700	506	8,900	6,338	Arrested tuberculosis
30	29	7	2,860	637	43	8	3,895	752	9,800	9,042	Arrested tuberculosis
31	21	6	1,659	485					7,364		Control
32	27	7	2,488	655					9,102		Control
33	21	8	1,489	589	26	14	1,687	1,071	7,140	6,588	Moderate tuberculosis
34	26	10	1,893	771	24	12	1,811	1,006	7,348	7,563	Moderate tuberculosis
35	27	12	3,865	1,754	41	11	2,652	841	13,943	7,000	Moderate tuberculosis
36	41	8	5,576	1,088	27	17	2,876	1,491	13,600	10,038	Moderate tuberculosis
37	17	13	3,128	2,392	33	14	2,810	1,030	18,400	7,945	Moderate tuberculosis
38	40	20	3,760	1,880	33	20	2,763	1,621	9,400	8,197	Extreme tuberculosis
39	33	2	2,838	172	22	29	2,275	3,217	8,600	10,722	Extreme tuberculosis
S 59	21	7	1,922	844	17	15	1,743	1,597	9,045	9,506	Moderate tuberculosis Bacillus abortus
60	13	9	1,191	867	21	19	2,171	2,903	8,696	14,140	Arrested tuberculosis

TABLE I—*Continued*

ANIMAL	BEFORE TUBERCULOSIS				AFTER TUBERCULOSIS				WHITE BLOOD CELLS		REMARKS
	Percentage of lymphocytes	Percentage of monocytes	Total lymphocytes	Total monocytes	Percentage of lymphocytes	Percentage of monocytes	Total lymphocytes	Total monocytes	Before tuberculosis	After tuberculosis	
73	22	11	2,547	1,273	16	17	1,501	1,811	11,580	8,309	Moderate tuberculosis Bacillus abortus
76	19	11	1,679	972	13	14	1,223	1,346	8,840	10,888	Arrested tuberculosis
79	32	12	3,247	1,180	19	10	2,059	1,084	9,840	10,840	Arrested tuberculosis
65	31	8	3,902	979	18	12	2,264	1,612	12,293	13,668	Moderate tuberculosis
P 1	19	3	1,996	358	22	15	1,813	1,365	10,240	9,551	Moderate tuberculosis
2	15	8	1,693	865	20	16	2,053	1,814	11,360	12,747	Extreme tuberculosis
3	18	9	2,332	1,231	14	14	1,600	1,673	12,960	11,606	Miliary tuberculosis
5	31	7	1,971	445	41	8	4,435	952	6,360	9,936	Extreme tuberculosis
6	6	4	917	564	20	17	2,347	1,945	14,120	11,180	Moderate tuberculosis
7	24	3	2,289	381	30	7	4,503	1,128	12,720	14,952	Extreme tuberculosis
8	19	4	2,126	422	27	20	3,287	2,476	10,560	12,245	Miliary tuberculosis
9	22	10	1,739	875	27	11	2,472	1,016	8,000	9,141	Arrested tuberculosis
10	35	10	4,382	1,309	34	7	4,162	826	13,000	11,582	Arrested tuberculosis
11	24	7	2,475	778	28	8	2,339	650	10,360	8,307	Arrested tuberculosis
12	21	6	2,006	600	26	8	2,465	810	9,760	9,421	Arrested tuberculosis
13	21	6	3,001	843	21	4	1,492	307	13,940	7,284	Arrested tuberculosis
14	24	10	2,667	1,184	24	10	1,878	800	10,360	7,886	Arrested tuberculosis
15	22	8	2,916	987	26	8	2,065	745	12,240	8,593	Miliary tuberculosis
16	20	13	1,545	938	23	12	2,076	1,075	7,680	8,839	Miliary tuberculosis
Average..	25	8	2,805	943	25	14	2,465	1,455	11,281	9,978	

sis as the disease becomes chronic, unless there is a secondary infection; but in the leucopenia which develops, there is an alternation in the proportions of the different types of white cells.

We are giving in Table I the records of 54 of our rabbits, including the controls, the animals infected with B. abortus and with tuberculosis, with the relative percentages and the actual numbers of monocytes and lymphocytes before and after infection, correlated with the condition found at autopsy. From these data, it becomes clear that there is an increase in the percentage and in the actual number of monocytes after infection with tuberculosis; that the average percentage of lymphocytes remains the same, with, however, a slight decrease in their number; while there is a decrease in the actual number of the white blood-cells, *i.e.*, a slight general leucopenia. The effect of tuberculosis on the peripheral blood becomes more striking when the cases are analyzed with regard to the grade of tuberculosis found at autopsy, as is shown in Table 2, in which it will be seen that

in severe infection the monocytes increase from an average of 8 per cent up to an average of 15 per cent. In this table it is interesting to note that the leucopenia is greater in the groups marked "moderate" and "arrested" than in the group marked "severe," which we interpret as due to the longer duration of the former experiments.

When the records of our experiments were analyzed, it was found that on the basis of our studies of the blood the animals fell into three groups,

TABLE II

Percentage and number of monocytes and lymphocytes in the peripheral blood of rabbits

PERCENTAGE OF LYMPHOCYTES	PERCENTAGE OF MONOCYTES	TOTAL LYMPHOCYTES	TOTAL MONOCYTES	WHITE BLOOD CELLS	GROUPS
25	8	2,805	943	11,281	Normal before infection
24	15	2,723	1,896	11,401	Extreme and miliary tuberculosis
25	15	2,253	1,414	9,200	Moderate tuberculosis
25	11	2,304	1,064	9,223	Arrested tuberculosis

somewhat correlated with the clinical groups of Table II. In the first group we have included those animals in which, shortly after the infection, the monocytes increased so that the normal ratio of monocytes to lymphocytes was reversed and in which this unfavorable ratio was maintained throughout the experiment. All of these rabbits showed either miliary tuberculosis or a grade of infection even more extreme, which will be described later. These animals showed consistently a low resistance to tuberculosis. In the second group were the animals in which the lymphocytes remained consistently above the monocytes, even though there was some rise in monocytes. These animals at autopsy either showed no microscopic evidence of tuberculosis at all or a condition which we interpret as characteristic of arrested tuberculosis, that is, they showed a consistently high resistance. In the third group, which represents the largest of the three, the blood showed a repeatedly shifting ratio between monocytes and lymphocytes and the result at autopsy correlated with the condition of the blood which was obtained when the animal was killed. It will be noted that this grouping does not bring together all the animals that had the same grade of the disease at the time of the autopsy, for, while in Group 1 all of the cases were severe at autopsy and in Group 2 all were arrested, in Group 3, on the other hand, the results at autopsy were mixed, being extreme, moderate or arrested. The basis of our classification rather has been the nature of the reaction of the animal throughout the experiment; thus in Group 1, the entire reaction of the animal was unfavorable; in Group 2, the entire reaction was favorable, while in Group 3 the animals showed attempts to build up a resistance alternating with periods of low resistance; that is to say, there were alternating periods of active and arrested phases of the disease. Through this type of classification we have been able to make a better study of the ratio of monocytes to lymphocytes in tuberculosis. High monocytes and low lymphocytes have been found associated with active tuberculosis, while low

monocytes and high lymphocytes have been found associated with arrested tuberculosis.

For Group 1, representing animals with a consistently low resistance, we are giving protocols and charts (Nos. 3 to 6) of four experiments, Rabbits TB 39, P 2, P 3 and TB 13.

Protocol, Rabbit TB 39

4/24/25. Weight 3050 grams. W.B.C. 8600. For the records of the blood see Table I and Chart 3.

4/25/25. Injection of 2 cc. saline emulsion of tubercle bacilli, B1, 50 bacilli per oil-immersion field, intravenously.

4/25/25 up to 5/22/25. Weight decreased to 2830 grams, W.B.C. as shown on Chart 3. Monocytes and lymphocytes before and after the inoculation with tuberculosis on Table I. There was no leucopenia, the average of the last four counts being 11,575.

5/22/25. Animal killed on account of the high monocytes. *Autopsy:* Extreme, diffuse tuberculosis of the lungs; supravital studies showed large masses of reticular cells from the septa and enormous numbers of free epithelioid cells of the type shown in Fig. 6. Very few of the epithelioid cells had mitochondria, but they had numerous refractive droplets of fat in the peripheral zone. Spleen surrounded by an enormous clot, which suggested an organised rupture. Mesenteric lymph glands enormously enlarged.

As will be seen on Chart 3 (Rabbit, TB 39), there was a marked increase in monocytes up to 3000 per cubic millimeter in this animal 5 days after the intravenous injection of the tubercle bacilli, when, of the total number of the white blood-cells, 29 per cent were monocytes. From this time on, the monocytes were consistently high, reaching a maximum of 52 per cent on May 21st. On May 5th, it was first noted that some of the monocytes of the circulating blood were strikingly changed and from that time on there were marked variations in the monocytes. Certain of them were found to be very young forms, obviously resulting from an increase in cell division by amitosis. That cell division was increased was also shown by the observation that, on May 21st, 4 of the 52 monocytes per 100 cells were found in division. In the blood of this animal occasional degenerating monocytes were found.

The phenomenon of the modified monocytes in the circulating blood is a most interesting one. These modified monocytes were characterized by the fact that they were round, appeared to be larger than normal and showed no motility. As was described in the preceding section, these modified monocytes of the circulating blood contained tubercle bacilli. We believe that these monocytes in the circulating blood, infected with the bacilli, give the best chance to study the very first effects of the organism on the cells. The most important of these effects are a cessation of motility and an increase in the substances stainable with neutral red. At this stage, as is shown in Fig. 1, from Rabbit TB 49, the line of demarcation between the fine bodies of the rosette and the vacuoles of the periphery is not sharp, since there is such a marked scattering of the vacuoles. We are unable to say whether or not the presence of these scattered vacuoles, in the monocytes which are just beginning to show the effects of damage by the infection or more specifically by having taken in the bacilli, indicates that the immediate and normal reaction of the monocyte is an attempt to kill the invading bacillus. We consider, however, that the

cessation of motility represents an immediate damage of the monocytes.

At autopsy, Rabbit TB 39 showed a diffuse, generalized tuberculosis of the lungs. No tubercles were seen in the gross specimen; in supra-vital preparations the septa of the lungs showed great masses of reticular cells and enormous numbers of scattered typical epithelioid cells like the one of Fig. 6. Many of these epithelioid cells were beginning to show fatty degeneration. This lung was an example of a tuberculous lesion which we regard as even more severe than the diffuse miliary tuberculosis, namely, a diffuse and invasive mononucleosis in which the process is so extensive, or, as it were, so malignant that there is not yet any reaction of the body in clumping the epithelioid cells into even tiny tubercles. Supra-vital studies from a scraping of such a lung show scattered epithelioid cells everywhere with no indication whatever that they had been held together by any of the usual framework of the tubercle; most of them are single, some may be in small clumps. Sections of such tissues show small clumps of monocytes, it is true, but these clumps are not confined by any framework of connective-tissue fibres, nor are they surrounded by lymphocytes, so that the principal and overwhelming characteristic of this grade of infection is an extensive invasion of the tissues with single epithelioid cells. We have other examples of

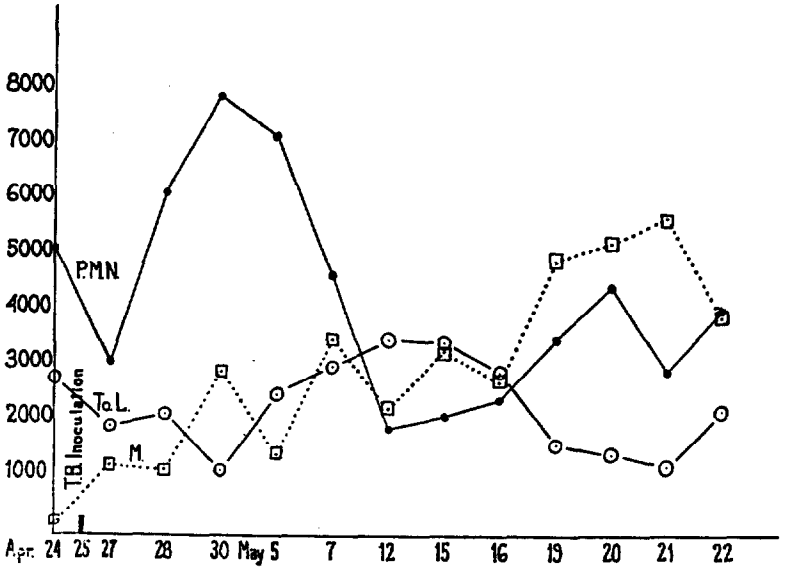

CHART 3. CURVES SHOWING THE MONOCYTES, LYMPHOCYTES AND POLYMORPHONUCLEAR NEUTROPHILIC LEUCOCYTES OF THE PERIPHERAL BLOOD FROM RABBIT TB 39

The dates are given on the abscissae and the numbers of cells on the ordinates. The curve of the total white count has not been plotted, but, in a general way, it can be judged from the other lines on the chart. The date of the inoculation of the animal with tubercle bacilli, strain B 1, is indicated on the chart. The animal belonged to our Group 1, of a severe infection without remissions. Result at autopsy, extreme tuberculosis.

this type of reaction in even more extreme form than in Rabbit TB 39.

In Charts 4 and 5 are shown the blood counts from Rabbits P 2 and P 3, and these experiments were likewise on animals in which the infection was

Protocol, Rabbit P 2

1/12/25. Weight 1930 grams. Temp. 102.5. W.B.C. 11,360. For types of white blood-cells see Table I, Chart 4.

1/16/25. Injection of 1 cc. saline suspension of tubercle bacilli, Bl, 15 organisms per oil-immersion field, intravenously.

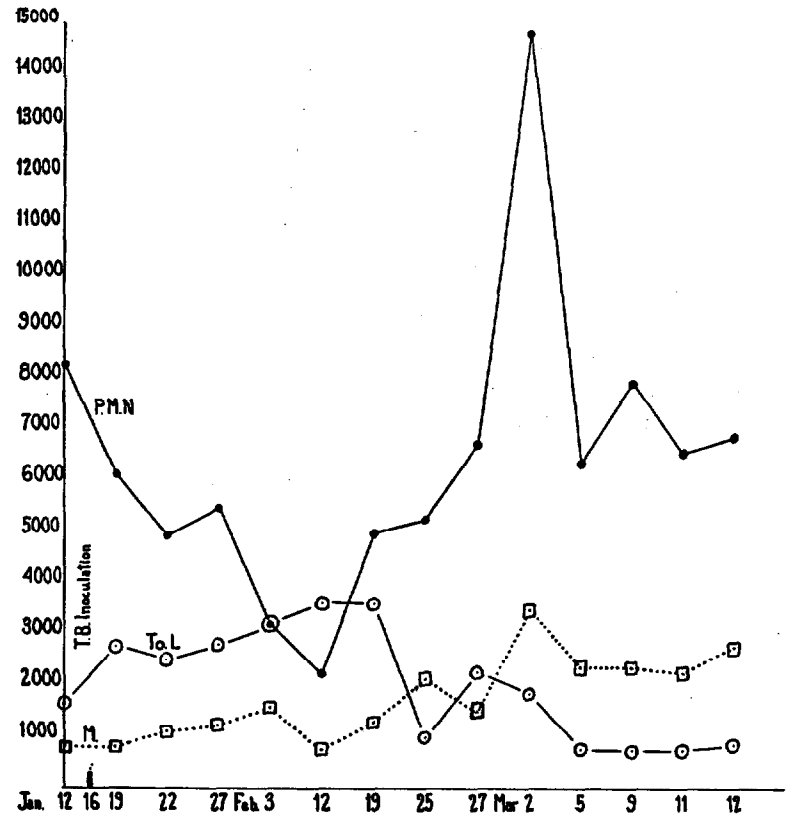

CHART 4. CURVES SHOWING DATA FROM THE BLOOD OF RABBIT P 3, SIMILAR TO THOSE ON CHART 1

The animal belonged to our Group 1, of a severe infection without remissions. Result at autopsy, extreme tuberculosis.

severe from the start; in neither of them did the increase in the monocytes follow the injection of the bacilli as quickly as in TB 39, but in both they remained consistently high after they had once surpassed the lymphocytes.

For data on monocytes and lymphocytes see Table I.

3/ 2/25. The W.B.C. were consistently normal in number except on this date when they rose to 20,960.

2/28/25. Lowest weight, 1695 grams.

3/12/25. Weight 2000 grams. W.B.C.

10,800. Animal killed. *Autopsy:* Extreme tuberculosis of the lungs, kidneys, pericardium and spleen. Supra-vital studies of the lung showed large tubercles together with great masses of free epithelioid cells; a few typical clasmatocytes, almost no lymphocytes. Visceral pericardium showed miliary tuberculosis; in the kidney many tubercles were found but no scattered epithelioid cells. The spleen showed greater numbers of scattered epithelioid cells than in any other animal of the series. A few scattered epithelioid cells in the liver.

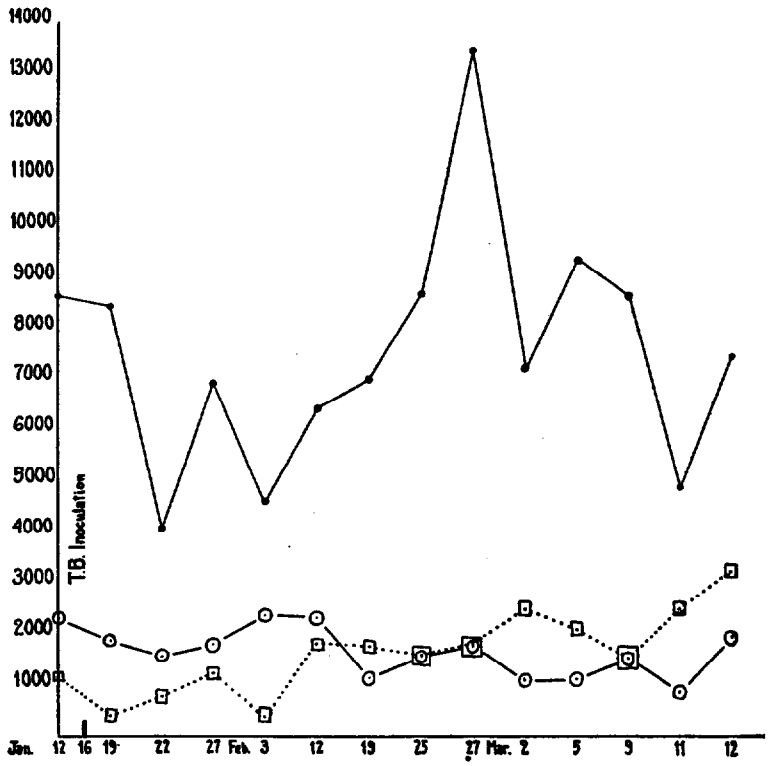

CHART 5. CURVES SHOWING DATA SIMILAR TO THOSE ON CHART 3, FROM THE BLOOD OF RABBIT P 3

The animal belonged to our Group 1, of a severe infection without remissions. Result at autopsy, miliary tuberculosis.

In Rabbit P 2, the organisms were given intravenously and at autopsy there was most extensive miliary tuberculosis of the abdominal viscera, miliary tuberculosis of the pericardium and extensive tuberculosis of the lungs. The septa of the lungs showed extensive mononucleosis. We have not seen such extensive masses of epithelioid cells in the spleen of any other animal of our series. There were very few lymphocytes in these tissues.

In Rabbit P 3, there was marked tuberculosis of the lungs and of the kidneys.

Protocol, Rabbit P 3

1/12/25. Weight 2210 grams. Temp. 102.5. W.B.C. 12,960. For types of white blood-cells see Chart 5.

1/16/25. Injection of tubercle bacilli same as for Rabbit P 2. For data on monocytes and lymphocytes see Table I.

2/27/25. Highest count of the W.B.C., 17,800, with P.M.N. at 13,439.

2/24/25. Lowest weight, 1615 grams.

3/12/24. Weight 1765 grams. W.B.C. 14,000. Killed. *Autopsy:* Lung showed very marked involvement but on the left side there were normal areas in the gross and there were zones which suggested healing. The kidneys showed numerous small tubercles. Supra-vital studies of the lung showed tubercles and many clumps of three or four epithelioid cells; it was noted that they were surrounded by clumps of lymphocytes, indicating the beginning of a reaction favorable to the animal. Many free lymphocytes. The kidney showed tubercles and an occasional free epithelioid cell.

It was interesting in this animal, that, though the involvement was marked, there were nevertheless signs of the beginning of a reaction of increased resistance on the part of the animal, since we interpret an increase in lymphocytes in the tissues involved in the tuberculosis as indicative of such a reaction. The lymphocytes of the blood had, however, not yet responded to this increase in the tissues and the ratio of monocytes to lymphocytes was still unfavorable for the prognosis. Rabbits P 2 and P 3 both showed rises in leucocytes at about the same time, which involved primarily the neutrophilic leucocytes, and were thus probably due to a super-added infection.

The fourth example of this group is shown on Chart 6 (Rabbit TB 13).

Protocol, Rabbit TB 13

11/28/24. Weight 2470. Condition good.

12/ 9/24. W.B.C. 11,320. Types of W.B.C. shown on Chart 6.

12/17/24. Injection of 2 cc. saline suspension of tubercle bacilli having 50 organisms per oil-immersion field.

1/12/25. Weight 2000. Found with paralyzed hind legs; marked leucocytosis, W.B.C. 21,360 a.m. and 33,600 p.m., due to an increase in the P.M.N. of 14,524 and 23,184, respectively, and in monocytes, 4272 and 5880. Killed. *Autopsy:* Marked miliary tuberculosis of the entire peritoneum, including the mesentery and the abdominal viscera. Supra-vital studies showed the tubercles to be made up of typical epithelioid cells with massive rosettes. Very few young monocytes; only very slight fatty degeneration of the epithelioid cells.

We did not make as many counts of the blood of this rabbit as would have been desirable, but all that were made showed that the monocytes were consistently higher than the lymphocytes with a marked increase in monocytes just before the animal was killed. The autopsy showed the most extreme miliary tuberculosis of the peritoneal wall and of the abdominal viscera, together with some involvement of the lung and a zone of red hepatization in one lung which probably accounted for the rise in the leucocytes. The injection of the bacilli into this rabbit was given intraperitoneally.

It will be seen that all of the animals of this group had severe tuberculosis at the time of autopsy; two cases we have classified as extreme and two as miliary. All of them, with the exception of Rabbit TB 13, (the one found paralyzed) might have lived longer, and in the case of Rabbit P 3 it is possible that a resistance might have been built up. The records of these four experiments show that a continued severe infection was indicated in the circulating blood by an unfavorable ratio of monocytes to lymphocytes.

The second group of our series represents animals that were markedly resistant to the infection throughout the

period of the experiment. We are giving the protocols of four animals from this group, Rabbits TB 6, TB 10, TB 29 and TB 30, as representative of markedly resistant animals.

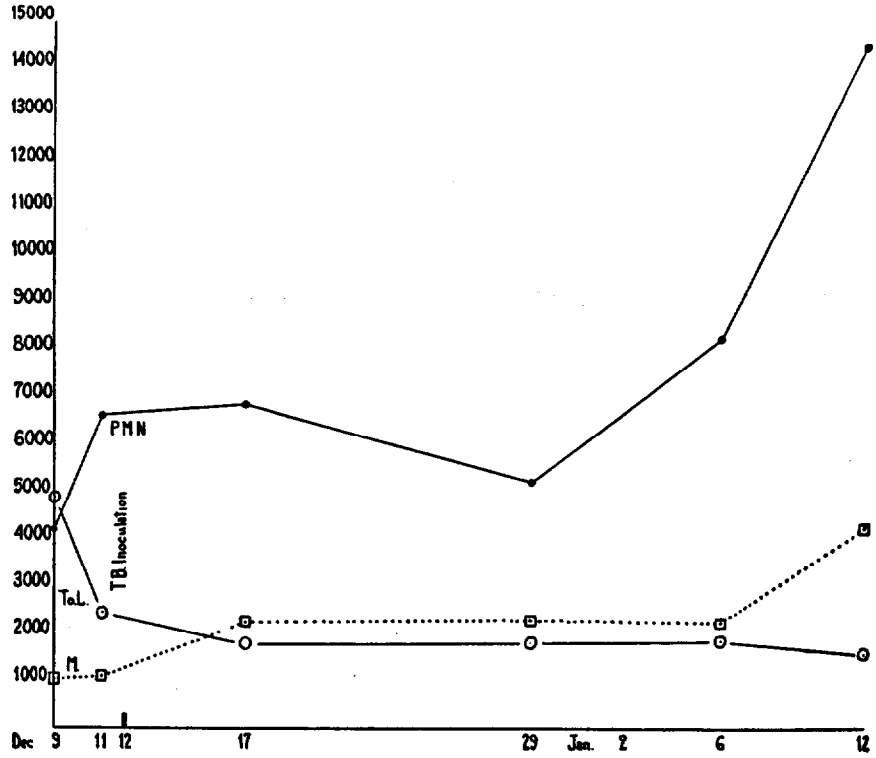

CHART 6. CURVES SHOWING DATA FROM THE BLOOD OF RABBIT TB 13, SIMILAR TO THOSE ON CHART 3

The animal belonged to our Group 1, of a severe infection without remissions. Result at autopsy, miliary tuberculosis.

Protocol, Rabbit TB 6

12/ 9/24. Weight 1860 grams. W.B.C. 9600. Chart 7.
12/12/24. Injection of 2 cc. saline suspension of 50 organisms to the oil-immersion field, intraperitoneally.
1/12/25. Weight 2080. Condition good.
2/19/25. W.B.C. 10,000.
2/24/25. W.B.C. 5600.
2/27/25. W.B.C. 7400.
3/ 2/25. W.B.C. 8400.
3/ 4/25. W.B.C. 7120. Killed. *Autopsy:* The lungs showed a few old scars. There was an enlargement of the lymph nodules of the blind pouch of the intestine, and supra-vital studies showed one or two stimulated monocytes from them. Otherwise no signs of tuberculosis whatever. In the films from the spleen there were unusually large masses of yellow pigment in some of the clasmatocytes, while others were filled with red blood-cells.

Protocol, Rabbit TB 10

12/ 9/24. Weight 1800. W.B.C. 9200.
12/17/24. Injection of 2 cc. of saline suspension of tubercle bacilli, 50 organisms to oil-immersion field, intraperitoneally. For the types of white blood-cells see Table I and chart 8.

1/22/25. W.B.C. 6880. Time of the leucopenia.

1/27/25. W.B.C. 5480. Time of the leucopenia.

2/ 9/25. W.B.C. 8400.

2/16/25. W.B.C. 9400.

Weight 1880 grams. Animal in excellent condition. Killed. *Autopsy:* A few healed calcified lesions in the omentum surrounded by lymphocytes. Lungs had a few healed lesions; no epithelioid cells found.

pale, contained an average amount of air; no tubercles seen on gross inspection. Supra-vital studies disclosed a few tubercles in the lung made of monocytes which were full of droplets of fat. Around these tubercles were great numbers of small lymphocytes. No calcified or caseated lesions.

Protocol, Rabbit TB 30

4/14/25. W.B.C. 10,200. For the records of the blood see Table I and Chart 10.

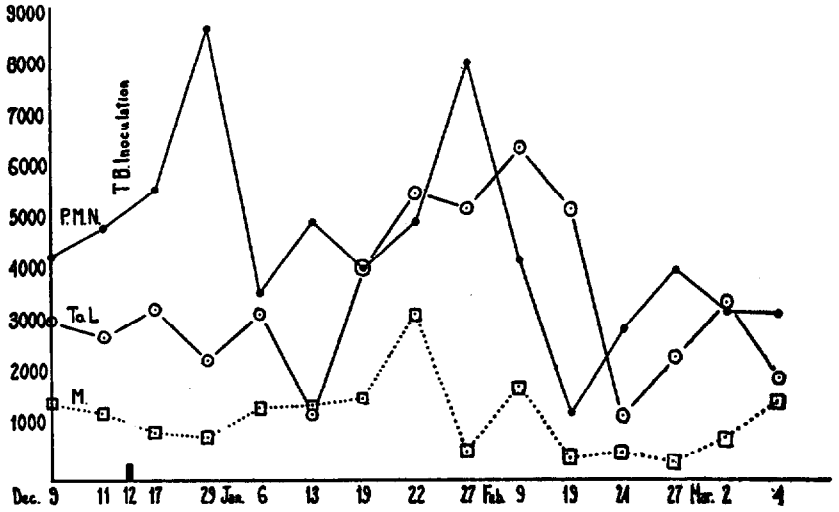

CHART 7. CURVES SHOWING DATA FROM THE BLOOD OF RABBIT TB 6, SIMILAR TO THOSE ON CHART 3

The animal belonged to our Group 2, in which a high resistance to the infection was maintained. Result at autopsy, arrested tuberculosis.

Protocol, Rabbit TB 29

4/14/25. W.B.C. 10,800. For the records of the blood see Table I and Chart 9.

4/20/25. Weight 1660 grams.

4/25/25. Injected 2 cc. of a saline suspension of tubercle bacilli B1, having 50 bacilli to an oil-immersion field, intravenously.

5/ 5/25. W.B.C. 5600. Beginning leucopenia.

5/ 7/25. W.B.C. 6400.

5/25/25. W.B.C. 3800. Low blood count.

5/27/25. W.B.C. 2800. Lowest blood count. Weight 1750 grams. Animal in excellent condition. Killed. *Autopsy:* Lungs

4/20/25. Weight 1700 grams.

4/25/25. Injected 2 cc. of a saline suspension of tubercle bacilli B1, having 50 bacilli to an oil-immersion field, intravenously.

4/27/25. W.B.C. 14,600. Highest blood count, correlated with the highest number of P.M.N., 9198.

4/28/25. Weight 1420 grams. Looks sick.

5/ 8/25. Weight 1570 grams. Gaining.

5/12/25. Weight 1720 grams.

5/25/25. W.B.C. 6200, P.M.N. 1550—lowest blood count up to this time. Weight 1380 grams. Animal looks sick.

5/27/25. W.B.C. 4800. P.M.N. 1968. Weight 1220 grams.

5/29/25. W.B.C. 4000. P.M.N. 1960.
6/ 1/25. Animal found dead. *Autopsy:* Lungs markedly red and inflamed; leathery in consistence with marked bleeding, though the animal had been dead a long time. Blood watery in consistence. Apparently a generalized acute reaction with a healed tuberculosis in the background. Rest of tissues normal.

The charts (7 to 10) of all of these animals are similar; all of them record a time after the infection when the monocytes rose, showing a reaction to the tuberculosis, but in none of them

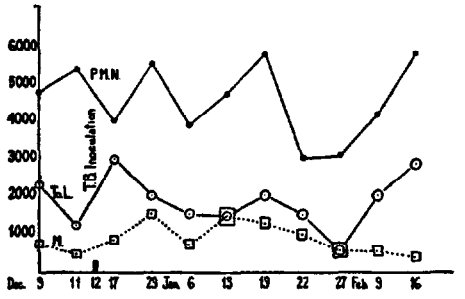

CHART 8. CURVES SHOWING DATA FROM THE BLOOD OF RABBIT TB 10, SIMILAR TO THOSE ON CHART 3

The animal belonged to our Group 2, in which a high resistance to the infection was maintained. Result at autopsy, arrested tuberculosis.

did the monocytes ever go above the lymphocytes. All of them likewise showed that the period of the leucopenia followed the period of the rise in monocytes rather than occurring synchronously with it. Thus, the leucopenia is a residual effect after the animal has already controlled the infection. It will be noted that the greatest rise in lymphocytes was in Rabbit TB 6, shown in Chart 7, and our records show that there was an increase not only in their numbers but also in their motility. The records of the autopsies all indicate that these animals had no demonstrable infection or were in a state in which the disease was markedly arrested. There were in some fibrosed and calcified tubercles; there was also a marked increase in the lymphocytes in the tissues which had been involved in the tuberculosis and this increase was accompanied by an increase in lymphocytes in the circulating blood. It will be seen in Table 1 that all of the animals in this group showed either no increase or only a slight increase in the monocytes after the infection, but, on the other hand, there was a marked rise in lymphocytes. In animals TB 6 and TB 10, the increase in percentage of the lymphocytes was not marked; but in TB 29 and TB 30 the percentages of lymphocytes rose to 42 and 43 respectively.

The third group was by far the largest of our series and includes the animals which neither succumbed at once to the infection nor showed a consistent resistance. We have selected 5 animals (TB 16, TB 1, TB 38, S 65 and P 1) from this group whose blood is shown on Charts 11 to 15. In general, two tendencies are to be noted in the curves on these charts; either the ratio of monocytes to lymphocytes fluctuated repeatedly, first one form preponderating and then the other; or there were periods in which both lymphocytes and monocytes were increased but tended to run along more or less parallel to each other. Some of the curves show combinations of these two types. In this group, the reaction of the animal to the disease was obviously much more complex than with the animals of our Groups 1 and 2. In general, it was possible to

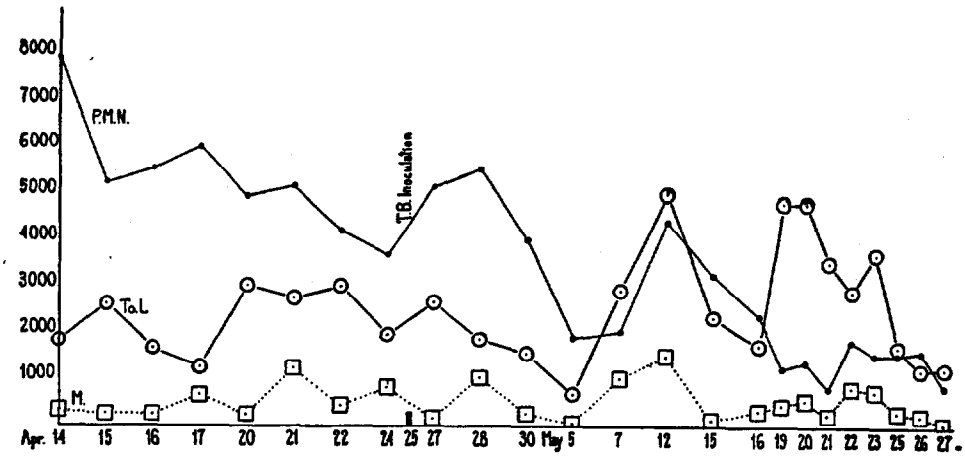

CHART 9. CURVES SHOWING DATA FROM THE BLOOD OF RABBIT TB 29, SIMILAR TO THOSE ON CHART 3

The animal belonged to our Group 2, in which a high resistance to the infection was maintained. Result at autopsy, arrested tuberculosis.

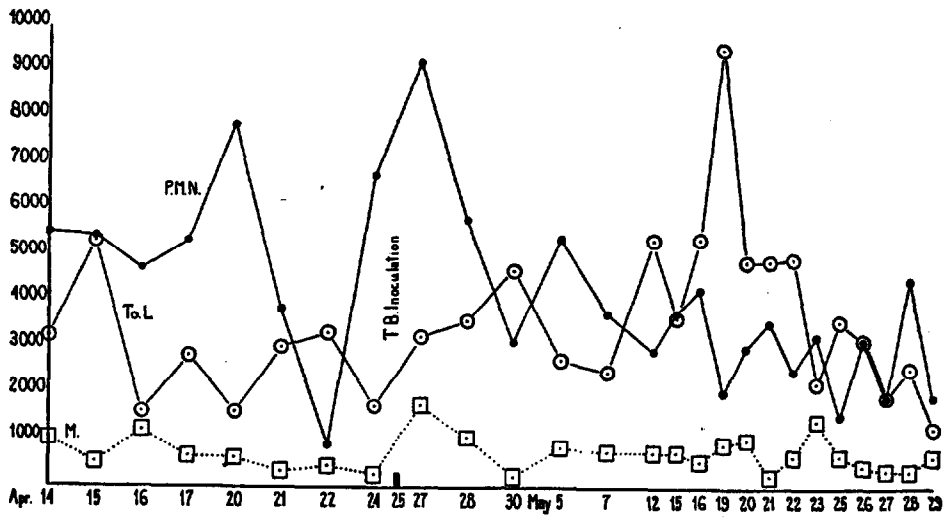

CHART 10. CURVES SHOWING DATA FROM THE BLOOD OF RABBIT TB 30, SIMILAR TO THOSE ON CHART 3

The animal belonged to our Group 2, in which a high resistance to the infection was maintained. Result at autopsy; arrested tuberculosis with a superimposed acute infection.

correlate the condition at autopsy in these animals with the ratio of monocytes to lymphocytes at the time of the autopsy, provided the reaction had obtained for a sufficient time. In this group when the monocytes were markedly above the lymphocytes at the time of autopsy, marked tuberculosis was found; those in which the monocytes had been at the normal level for some time showed arrested tuberculosis, while the intermediate and the mixed types of curves corresponded to a moderate grade of the disease.

The first example of this group, from Rabbit TB 16, (Charts 11 A and 11 B) was the most extreme example of malignant, invasive tubercular mononucleosis of our entire series.

Protocol, Rabbit TB 16

1/21/25. W.B.C. 12,160. For records of the blood see Table I and Chart 11.

1/26/25. Weight 1650 grams.

1/27/25. W.B.C. 17,440. W.B.C. and P.M.N. rather high, as shown on Chart 11, until the time of the injection of tubercle bacilli.

2/ 5/25. W.B.C. 11,840.

2/ 7/25. Injection of 5 cc. of saline suspension of tubercle bacilli, B1 50 organisms to an oil-immersion field, intravenously.

2/ 9/25. W.B.C. 8400.

2/19/25. W.B.C. 6200. Lowest count. P.M.N. 2108

4/ 1/25. Animal showed marked loss in weight. Killed at the time of a rising count of monocytes. *Autopsy:* Lungs showed massive gelatinous pneumonia, looking as if they were a solid mass of cells. Supra-vital studies of scrapings from the freshly cut surface of the lung showed an almost pure culture of epithelioid cells, such as the one in Fig. 5. No elastic tissue at all in the scrapings, as if the entire tissue had come from the septa, and no epithelium from the air sacs. Epithelioid cells contained tubercle bacilli, demonstrated with Ziehl-Nielsen technique. Some enormous giant cells loaded with fat. Result: an extreme grade of invading monocytosis with little clumping of the epithelioid cells into circumscribed tubercles.

It is obvious that by the term mononucleosis we mean the increase of the modified monocytes or epithelioid cells. It is interesting that the average number of the monocytes in this animal was high before the injection of the bacilli, being 1144 as against a normal average of 943 (see Table 1). After the injection of the bacilli, the average of the monocytes was 3469. We are giving two charts of this animal, the first one showing the total number of the cells and the second the corresponding percentages. In this animal the lines of monocytes and lymphocytes crossed repeatedly until the last few days, when the blood showed the following astonishing numbers of monocytes; 4189, 4864, and 6364. At *autopsy*, the lungs in the gross showed a massive, diffuse gelatinous pneumonia; they were pale and, on section, little blood was seen; they looked as if the entire pulmonary tissue was a mass of cells. The fresh scraping from the cut surface was practically a pure culture of infected monocytes, *i.e.*, of epithelioid cells of the type shown in Fig. 5. Bacilli were demonstrated in these epithelioid cells. The specimens looked as if they had come from the septa entirely, for there was no elastic tissue whatever. There were some enormous giant cells full of fat droplets. Sections of the lung confirmed the supra-vital studies, for the septa showed great masses of epithelioid cells. Blood-vessels were seen full of these cells and some were present in

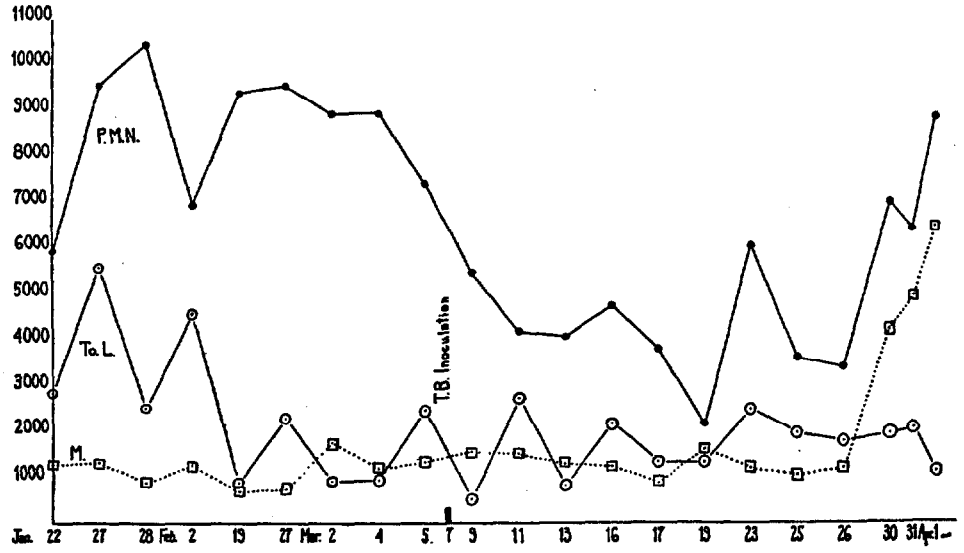

CHART 11A. CURVES SHOWING DATA FROM THE BLOOD OF RABBIT TB 16, SIMILAR TO THOSE ON CHART 3

The animal belonged to our Group 3, in which there were alternating periods of active and inactive tuberculosis. Result at autopsy, severe tuberculosis.

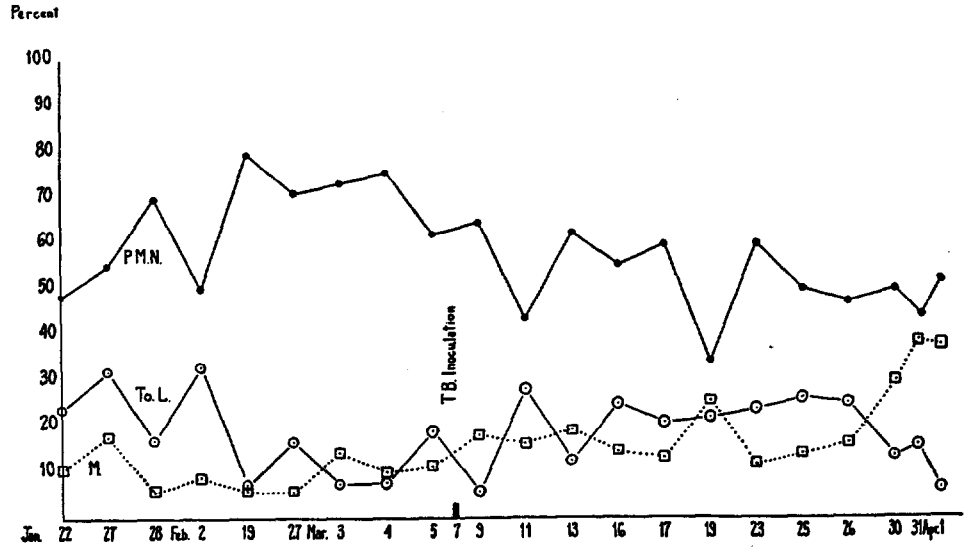

CHART 11B. CURVES SHOWING THE DATA FROM THE BLOOD OF THE SAME RABBIT (TB 16) AS ON CHART 11A, BUT PLOTTED IN PERCENTAGES

the exudate of the alveoli. It is obvious that this animal was killed in the most active phase of the disease.

The next experiment Rabbit TB 1 (Chart 12) in this group was a long one, extending from December 9th to April 7th.

Protocol, Rabbit TB 1

11/28/24. Weight 2350 grams. For records of the blood see Table I and Chart 12.

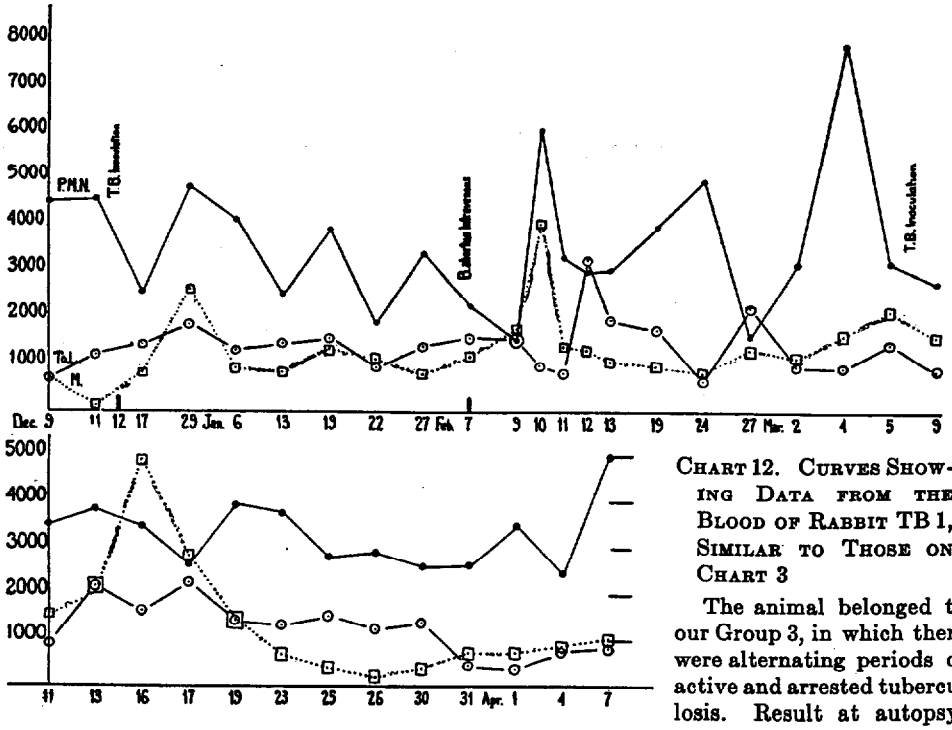

CHART 12. CURVES SHOWING DATA FROM THE BLOOD OF RABBIT TB 1, SIMILAR TO THOSE ON CHART 3

The animal belonged to our Group 3, in which there were alternating periods of active and arrested tuberculosis. Result at autopsy, arrested tuberculosis.

12/ 9/24. W.B.C. 6440.

12/12/24. Injection of 2 cc. of saline suspension of tubercle bacilli B1, intraperitoneally. 50 bacilli to an oil-immersion field.

12/17/24. W.B.C. 5800.

12/29/24. W.B.C. 11,560.

1/22/25. W.B.C. 4560. Lowest count.

2/ 7/25. Injected 1/5 of a 24-hour culture of living B. abortus in 1 cc. of saline intravenously.

2/10/25. W.B.C. 12,200.

3/ 7/25. Second injection of TB—5 cc. of saline suspension of B1—intravenously. 50 bacilli to an oil-immersion field.

3/31/25. W.B.C. 4200.

1/12/25. Weight 2330.

1/26/25. Weight 2370. Condition good with actual gain in weight.

4/ 7/25. Weight 2130. Condition good.

4/ 8/25. W.B.C. 7400. Killed. *Autopsy:* Peritoneal walls and viscera normal. Lungs showed a few small white masses surrounded by yellow lines suggesting healed tuberculosis. Supra-vital studies of the lung showed an occasional monocyte with a marked rosette, suggesting young epithelioid cells. A few epithelioid cells were filled with fat. The omentum showed great masses of lymphocytes and cells suggesting plasma cells. Result: healing tuberculosis.

This picture represents the reverse of the condition found in Rabbit TB 16, for Rabbit TB 1 was killed when the

animal was in excellent condition and the monocytes of the blood were low. In following the chart it will be noted that the lines of the monocytes and lymphocytes crossed repeatedly and that on four occasions the monocytes were markedly increased. We have no doubt that if this animal had been killed on March 16th when the monocyets were 4929, the condition at autopsy would have been entirely different. It may be that the rise of the monocytes on April 10th, was correlated with the chondria, while others showed fatty degeneration. Thus the tubercular process was still present but markedly in abeyance. The tissues from the lung showed many lymphocytes. This experiment represents an animal with relatively high resistance.

The next animal in this series, TB 38, Chart 13, is mentioned on account of an interesting correlation between the supra-vital studies of the lungs at autopsy and the chart of the peripheral blood.

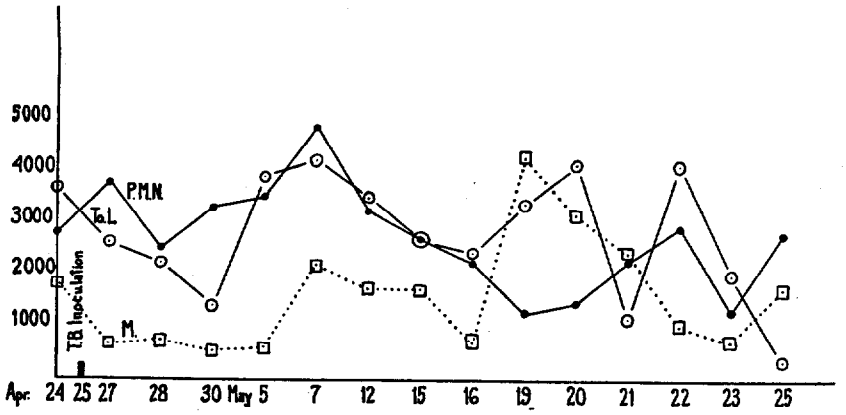

CHART 13. CURVES SHOWING DATA FROM THE BLOOD OF RABBIT TB 38, SIMILAR TO THOSE ON CHART 3

The animal belonged to our Group 3, in which there were alternating periods of active and inactive tuberculosis. Result at autopsy, extreme tuberculosis.

injection of B. abortus rather than with an exacerbation of the tuberculosis. When this animal was killed, the monocytes had been normal in numbers for some days. The only abnormalities in this animal were in the lungs. In the gross specimen the lungs showed some small white masses surrounded by yellowish borders, suggesting healing tubercles. A few rather small epithelioid cells, typical of tuberculosis, were found; some of them were young with many mito-

Protocol, Rabbit TB 38

4/24/25. Weight 1700 grams. W.B.C. 9400. For the records of the blood see Table I and Chart 13.

4/25/25. Injection of 2 cc. of a saline suspension of tubercle bacilli B1, having 50 bacilli to an oil-immersion field.

5/ 8/25. Weight 1720 grams. Condition good.

5/21/25. Weight 1650 grams.

5/23/25. W.B.C. 4200. Lowest count.

5/25/25. Weight 1400 grams. Animal sick. Killed. *Autopsy:* Lungs showed intense gelatinous pneumonia; spleen markedly enlarged. Supra-vital studies of the lung

showed great masses of epithelioid cells like the one of Fig. 7. There were large numbers of epithelioid cells in the lung in which the rosette entirely filled the cell and again very large numbers of them in which the entire periphery of the cell was filled with fat droplets. Result: extreme tuberculosis with beginning degeneration of the epithelioid cells.

The chart is quite complex. The first rise in monocytes was preceded by a marked rise in lymphocytes, so that there was a period between May 5th and May 16th when both monocytes and lymphocytes were above normal. After this period there was one sharp rise in monocytes followed by a gradual fall associated with marked fluctuations in the lymphocytes. It is obvious that such a chart indicates complex reactions on the part of the animal, on the basis of our concept that the condition of the blood is actually representative of specific changes in the reaction of the animal to the infection. The condition at autopsy was interesting. There was a marked gelatinous pneumonia and the scraping from the lungs showed very large masses of epithelioid cells, one of which is shown in Fig. 7. The most striking thing about this lung was the large proportion of epithelioid cells which showed the beginning of fatty degeneration. This observation leads us to suggest that there was a cycle of young infected monocytes, that is, young epithelioid cells between the 19th and 21st, which were beginning to degenerate at the time of the autopsy. The first modified monocytes in the blood of this rabbit were recorded on May 7th, the time of the first rise in their number. In the interval between the 19th and the 21st the monocytes were recorded as dividing, modified and degenerating. On the last day of the experiment, out of 33 monocytes, 15 were modified, while the 18 unmodified were noted as in part very young forms and in part actively motile mature monocytes. On the 23rd, degenerating monocytes were recorded in the circulating blood. We may therefore consider that the monocytes of the period from the 19th to the 25th of May represent the cycle in one generation of monocytes from the time of the full maturity of the epithelioid cells of one exacerbation of the disease to the time of their beginning degeneration. We regard our observations on such cycles as only suggestive, but we give them considerable weight because our entire experience indicates that there is a correlation between the condition of the monocytes of the circulating blood and the condition of the monocytes in the general tubercular process. It is, therefore, of great significance in following the blood of these tuberculous animals to record minutely all of the appearances of the monocytes.

The other two animals of this group Rabbits S 65 and P 1, showed similar curves (Charts 14 and 15). In both, the lymphocytes were, for a time, above the monocytes, followed by a period of repeated crossing of the two curves; the monocytes were above the lymphocytes at the time of autopsy.

Protocol, Rabbit S 65

10/14/24. Weight 1605 grams. For records of the blood see Table I and Chart 14.

10/20/24. W.B.C. 11,920.

11/ 5/24. From this time until 12/8/24 the animal received intravenous injections of gelatin in doses of from 10 to 20 cc. of a 2 per cent solution. This was given for a possible effect on the clasmatocytes and monocytes, but without results.

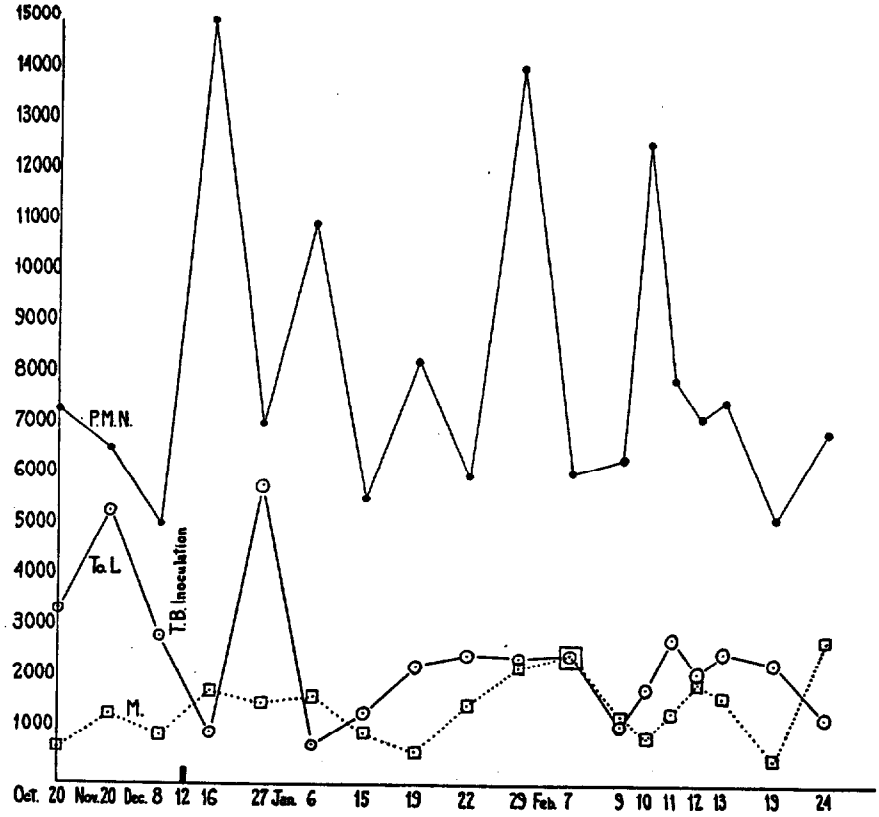

CHART 14. CURVES SHOWING DATA FROM THE BLOOD OF RABBIT P 1, SIMILAR TO THOSE ON CHART 3

The animal belonged to our Group 3, in which there were alternating periods of active and inactive tuberculosis. Result at autopsy, moderate tuberculosis.

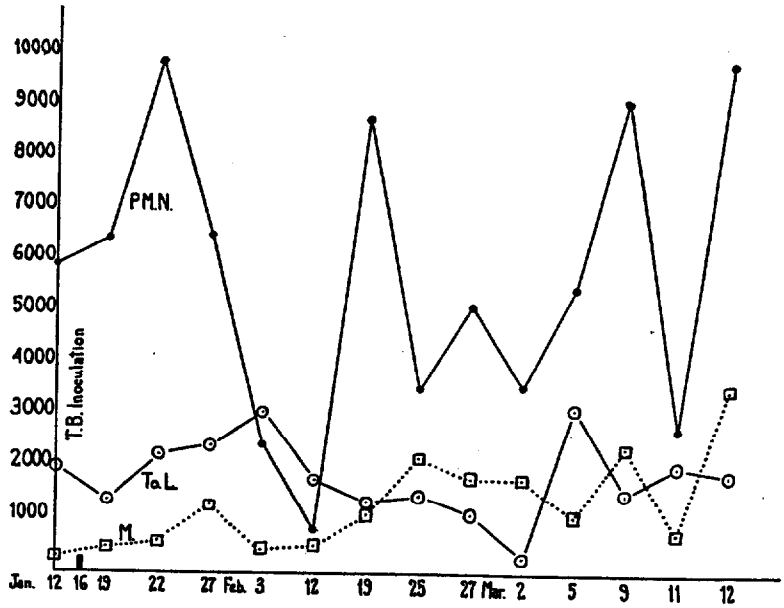

CHART 15. CURVES SHOWING DATA FROM THE BLOOD OF RABBIT S 65, SIMILAR TO THOSE ON CHART 3

The animal belonged to our Group 3, in which there were alternating periods of active and inactive tuberculosis. Result at autopsy, moderate tuberculosis.

12/12/24. Injection of 2 cc. of a suspension of tubercle bacilli, H 37, intraperitoneally. 50 bacilli to an oil-immersion field.
12/16/24. W.B.C. 20,360. White blood cells remained high for the month of January.
1/26/25. Weight 1970 grams. Condition good.
1/29/25. W.B.C. 20,840.
2/ 7/25. W.B.C. 14,600. Injection of 1/5 of a 24-hour culture of living B. abortus, intravenously.
2/10/25. W.B.C. 16,000. Highest of the last period of the experiment.
2/24/25. W.B.C. 11,520. Killed. *Autopsy:* Lungs showed scars in which no epithelioid cells were found, but considerable numbers of lymphocytes. Liver showed a moderate number of epithelioid cells.
Result: Moderate tuberculosis.

Protocol, Rabbit P 1

1/12/25. W.B.C. 10,240. For records of the blood see Table I and Chart 15.
1/16/25. Injection of 1 cc. of a saline suspension of tubercle bacilli Bl, 15 bacilli to an oil-immersion field, intravenously. Dose about 1.7 mgm.
1/17/25. Weight 2,090 grams.
1/22/25. W.B.C. 13,200.
1/27/25. W.B.C. 13,680.
2/11/25. Weight 2,000 grams.
2/12/25. W.B.C. 3,680. Lowest count.
2/28/25. Weight 1,700 grams. Lowest weight.
3/12/25. W.B.C. 16,000. Highest count. Weight 1885. Animal in good condition. Killed. *Autopsy:* Lungs showed healing tuberculosis. Kidneys showed a few encapsulated tubercles. Supra-vital studies showed clumps of epithelioid cells in the omentum; in the kidneys there were some epithelioid cells both free and in small clumps. No free epithelioid cells in the lung and no reaction of lymphocytes around the epithelioid cells. *Result:* Moderate tuberculosis.

On Chart 14 (Rabbit S 65) the monocytes were above the lymphocytes on the last count, but when the chart as a whole is studied, it will be seen that the lymphocytes were above the monocytes throughout most of the experiment. In this animal there were some infected monocytes, typical epithelioid cells, seen in the scrapings from the liver, but not any marked tuberculosis to be made out on gross inspection. The animal whose blood is shown on Chart 15 (Rabbit P 1) showed the monocytes above the lymphocytes more than in the preceding example. At autopsy, some tubercles were found in the omentum and in the kidneys but the process was not severe.

When the records of these three groups of reactions are compared, it will be seen that there are four different types of curves. The first two groups of animals represent experiments in which the reactions of the animals were so consistent that the records can be shown as composite curves. Chart 16 is a composite curve of Group 1, in which the number of different blood-counts is shown on the abscissae and the number of cells on the ordinates. This is the type of curve of a marked infection in animals with low resistance to tuberculosis, in which it will be seen that the normal position of monocytes and lymphocytes has been reversed. The monocytes are finally quite steady at the level of about 3000 per cubic millimeter while the lymphocytes are at about the level of 1000 per cubic millimeter. That is to say, in this type of curve the monocytes are above normal while the lymphocytes are below normal after the infection had been established.

On Chart 17, we are giving a curve of the counts of the animals in Group 2 in which there was a consistent state

of marked resistance to the infection. On this chart there are two slight rises of monocytes, not in any degree, however, comparable to the marked elevation of the level of the lymphocytes, which were for a considerable time about 4000 cells per cubic millimeter with a single extreme rise over 5000 cells. The period of the very marked rise in lymphocytes between the 8th and the 14th counts represents the time of the very favorable reaction on the part of the animals, with a subsequent return to more usual numbers of lymphocytes and monocytes. This later period, from the 14th to the 18th counts, represents the period of leucopenia, so that the blood charts still show that the animals had been infected. Thus, this type of curve is the exact reverse of the one of Chart 16, for in this one the monocytes were below normal while the lymphocytes were markedly above normal. These two charts may be considered as representative of the two groups of animals having a low and a high resistance and may be used as typical curves for corresponding cases.

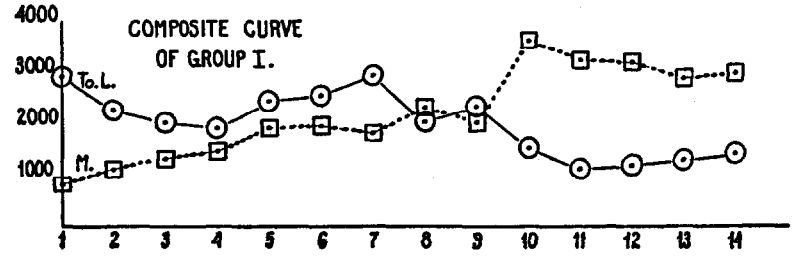

CHART 16. COMPOSITE CURVE OF THE BLOOD OF GROUP 1 (CHARTS 3–6 INCLUSIVE), SHOWING THE TYPE OF THE CURVES OF THE MONOCYTES AND LYMPHOCYTES IN TUBERCULOSIS IN RABBITS THAT HAD NO REMISSIONS OF THE DISEASE

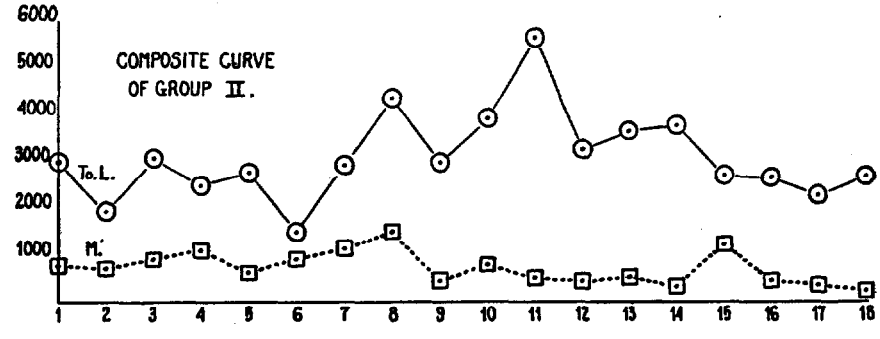

CHART 17. COMPOSITE CURVE OF THE BLOOD OF GROUP 2 (CHARTS 7–10 INCLUSIVE), SHOWING THE TYPE OF THE CURVES OF THE MONOCYTES AND LYMPHOCYTES IN TUBERCULOSIS IN RABBITS WITH A CONSISTENTLY HIGH RESISTANCE TO THE DISEASE

For the last group we could not plot any composite type of curve because the reactions of the animals were so varied. In general in this group there were two types of curves; first, a condition in which the lines of lymphocytes cross and recross each other re-

peatedly, and second, a condition in which both lines are elevated above the normal levels, more or less parallel to each other. These two different tendencies in the ratio of the monocytes to lymphocytes in the blood can be found at different times in the same animal. The final outcome in this group was exceedingly varied, one of the animals showing the most extreme reaction of our series, a second showing marked signs that the infection was arrested, while the rest showed moderate tuberculosis.

From these studies we think that the autopsies of all of our animals demonstrate that in relatively acute tuberculosis the peripheral blood can give definite information concerning the progress of the disease, which correlates quite accurately with the records of loss in weight and general loss of strength. In our series we have found that extremely large numbers of monocytes in the blood accompanied extensive, malignant mononucleosis in the infected tissues; moderately high monocytes have accompanied miliary tuberculosis and tissue mononucleosis of a less marked intensity, while monocytes within normal limits and below normal, together with lymphocytes markedly above normal, have been correlated with a marked arresting of the disease. Such a relation between a marked increase in two of the types of blood-cells, namely monocytes and lymphocytes, in infected tissues and an increase of these types of cells in the circulating blood we consider a significant point both in relation to tuberculosis and in connection with the relationship of the cells of the connective tissues and the cells of the blood. We have found that widely disseminated infected monocytes represent the most severe lesion of tuberculosis; on the other hand, we have found that the surrounding of tubercles by masses of lymphocytes is evidence that some healing is taking place.

The experiments with B. abortus we did not find as helpful in relation to the study of tuberculosis as we had hoped. We used the organism alone in two animals and in connection with an infection with tuberculosis in several others. We found that, in the animals infected with B. abortus alone (see Rabbits TB 17 and TB 18, on Table 1), although there was an increase in the actual numbers of the monocytes, there was nevertheless a decrease in their percentages. This shows that the increase in monocytes was due to an increase in the total number of the white blood-cells rather than a specific effect on the monocytes themselves. We did not find that an infection with B. abortus increased the intensity of the infection with tuberculosis, in spite of the added increase in monocytes, as will be seen on Chart 12, Rabbit TB 1, which showed arrested tuberculosis in spite of the secondary infection with B. abortus.

DISCUSSION

In these studies on blood we are offering as a new concept for an experimental attack on the problem of tuberculosis the theory that the tubercle bacillus attacks one specific type of cell—the monocyte—and that the complete analysis of the effect of tuberculosis on monocytes is a necessary factor for laying a new foundation for progress in the study of this disease. We have found that the effect of tu-

berculosis on the whole system of the monocytes is a profound one, and consists primarily in a marked over-production of the diffusely scattered reticular cells which are the parent cells of monocytes. This reticular cell is also the parent form of all of the other white blood-cells, so that the effect of the tubercle bacillus on this cell is definitely to force it toward the production of monocytes. In normal connective tissues the markedly undifferentiated reticular cell exists only in small numbers, so that, for example, in a scraping from the freshly cut surface of a normal lung, only an occasional cell of this type will be found. At a certain stage in tuberculosis, representing some cycle in the reaction of the body to the disease, these reticular cells may be found in an infected area, such as the septa of a tuberculous lung, in such large sheets as to cover an entire field or more, under an oil-immersion lens. The effect of the production of such an increase in reticular cells and their maturation into monocytes must be a chemical one affecting the cells through the surrounding medium, since neither reticular cells nor very young monocytes have been found containing tubercle bacilli. There is further evidence of this fact in some experiments which we are just beginning on the effect of one of the protein fractions obtained from the tubercle bacilli with which we have injected several animals.

The next stage in tuberculosis is the differentiation of vast numbers of monocytes into epithelioid cells; this effect certainly takes place in monocytes which have ingested the bacilli, though we have not yet analyzed the phenomenon in terms of cause and effect. It is, of course, known that tubercle bacilli are engulfed by many other cells; for example, they have been found in the Kupffer cells of the liver (Evans, Bowman and Winternitz (7)). Maximow (16) has shown tubercle bacilli within a wide range of cells in tissue cultures, in lymphocytes as well as in monocytes and in some highly branched cells of the connective tissues. But it seems to us that the monocytes can be sharply discriminated from all other cells by this fact that with the monocytes the major effect is on the monocyte itself rather than on the bacillus. We are unable to go so far as to state that the monocyte has no power whatever to destroy tubercle bacilli, but we do think the evidence points to the concept that such power, if present, is limited. Thus, histological appearance of living monocytes, in which bacilli have been seen, leads us to the conclusion that the bacilli are alive within the cell; and the numbers which can be seen within an individual monocyte point also to the idea that the bacilli multiply within the cell. The figures of cells shown by Maximow from tissue cultures infected with tubercle bacilli bear out this concept, for it will be noted that those cells which are definitely monocytes, or the giant cells derived from monocytes, have consistently the greatest number of bacilli within them; a maximum is reached in the cells of his Fig. 21, Plate 5, which have bacilli in uncountable numbers. Such numbers of bacilli are not seen in the monocytes in the body of the animal but it may be that the monocytes of the tissue cultures have very much less resistance to the bacilli than ever occurs in the cells of an infected animal. We

suggest that the tubercle bacillus lives as a parasite within monocytes; moreover, it seems to us that the concept that certain bacilli, of which the tubercle bacillus is one example, live as parasites within specific types of cells gives a new key for the study of immunity.

The effect of the tubercle bacillus on the monocyte is the accentuation of one of its normal structures and the suppression of another. Under the influence of this infection there is an enormous increase in the number, accompanied by a decrease in the size, of the fine particles that make the rosette of the normal cell. This rosette then becomes the positive characteristic of the epithelioid cell; the particles that make the rosette are visible in the living cell, they are stainable in neutral red, soluble in alcohol but fixed by formalin. In Wright's blood stain the zone of the rosette stains a diffuse pink in which the azure granules, typical of the monocyte, can be seen. The peripheral zone of the epithelioid cell is the area occupied by the bacilli; it shows a marked decrease in the stainable vacuoles; nevertheless, an occasional epithelioid cell can take foreign bodies such as a red blood-cell into this zone. The cytoplasm of this peripheral zone shows the first signs of degeneration in the development of droplets of fat which may ultimately fill the cell completely. The type of the giant cell which comes from the monocytes shows also the characteristics of the parent cell or monocyte, for the giant cell arises by an abortive amitosis when the rosette has become so enormous as to inhibit completely the division of the centrosome. Therefore, the giant cell of the Langhams type which has the nuclei in the peripheral zone around the centrally placed rosette, is a derivative of a monocyte. The giant cell shows the same type of fatty degeneration as the epithelioid cell.

It is clear that the tubercle bacillus is not the only stimulus through which the monocyte may become an epithelioid cell, because typical epithelioid cells develop in small numbers, for example, in Hodgkin's disease. We have also studied these epithelioid cells from lymph glands in Hodgkin's disease with the supra-vital technique and have found that they stain like those of tuberculosis, but in our limited experience this reaction is not so intense.

In this connection, it is very significant that Lewis, Willis and Lewis (14) have demonstrated the production of epithelioid cells from monocytes by following drops of sterile blood in tissue cultures. They have also considered these epithelioid cells as like those of tuberculous infections. It may be that the development of the epithelioid cells in the tissue cultures is an effect of a decreased nutrition on the part of the cell, due to an unfavorable environment, which may be interpreted as indicating that the tubercle bacillus interferes with the nutrition or some other physiological property of the normal monocyte.

The effect of tuberculosis on the monocyte is so specific that it seems to us to contribute additional evidence in favor of the concept that the monocyte is a different strain of cells from the clasmatocyte. The monocyte is originally a cell in which cytoplasmic pattern is an especial characteristic as contrasted with the clasmatocyte and

it is this pattern which is accentuated in the infected monocyte or epithelioid cell. In the septa of an infected lung, such as the specimen described from Rabbit TB 16, it is true that we saw no clasmatocytes, for the tissues had such an enormous over-production of the epithelioid cells that the films looked like pure cultures of these modified monocytes. We believe that additional proof of the concept that the clasmatocytes and monocytes are two different physiological strains of cells could be obtained by taking an animal, in which such a stimulation of monocytes had been produced by tuberculosis, and giving it repeated doses of carmine or trypan blue until all of the clasmatocytes had been stained. This test we have not yet applied.

In our studies on tuberculosis we have found that the effects of tuberculosis on the monocytes are so profound as to be seen in the monocytes of the peripheral blood; in our animals in which we have induced a practically acute reaction, the monocytes of the peripheral blood became infected. In these monocytes, in which we have demonstrated bacilli, we can follow to some extent the first reaction of the cell to the disease. We were led to record the changes in these monocytes before we had found that they were infected with bacilli. These monocytes have frequently made up about half of the monocytes in the differential counts; they have occurred when both the percentages and the actual numbers of the monocytes had been markedly increased.

In following the blood of the infected rabbits we have proved that the ratio of monocytes to total lymphocytes is an important law in connection with the disease. We have found that the normal ratio of monocytes to lymphocytes is 943 to 2805, as shown in Table 2. In the first group of animals which we are showing in Charts 3 to 6, summarized on Chart 16, in which the reaction to the disease was consistently unfavorable to the animal, the ratio was 2129 to 1694. This is a higher ratio of monocytes to lymphocytes than is obtained when all of the severe cases, some of which had remissions of the disease, are averaged, as shown in Table 2. In Rabbit TB 16 (Chart 11A) the entire ratio after the infection with tuberculosis was 3469 to 2799, while the ratio during the unfavorable reaction at the end was 5139 to 1694, being the average of the last three counts. On the other hand, the ratio for the group of animals shown in Charts 7 to 10, summarized in Chart 17, in which the reaction was consistently favorable to the animal, was 854 to 3047. When these figures are reduced to a ratio with the monocytes shown as one, the normal ratio of monocytes to lymphocytes is 1 to 2.97; the unfavorable ratio of Group 1 is 1 to 0.79, while the favorable ratio of Group 2 is 1 to 3.56. These same data are shown graphically in Charts 16 and 17.

Thus a marked and continued reversal of the normal ratio, provided that the monocytes are markedly increased in actual numbers is, in our experience, a consistent indication of a malignant stage in the reaction of the animal to the disease. The reverse condition is likewise true, for when the lymphocytes are markedly and consistently higher than the monocytes, even though the actual numbers of the monocytes are somewhat above

normal, we have found arrested tuberculosis without exception.

Our experience corroborated the well known fact that a leucopenia develops in tuberculosis. In the stage of the leucopenia we have found, in some instances, an increase in the percentage of monocytes or lymphocytes even with an actual decrease in their numbers, showing that the disease affects primarily monocytes and lymphocytes with a final consistent decrease in the granulocytes. We have found the leucopenia persisting in animals in which the infection was healed or quiescent (see Charts 9 and 10). We are thus led to suggest that the effects of tuberculosis are primarily on the monocytes, with secondary effects such as a depression of the entire blood-forming tissues and a loss in weight of the animal.

The majority of the rabbits of our experiments have not shown the two extremes of a consistently favorable or consistently unfavorable reaction, but rather have shown a varying reaction in which there have been repeated crossings of the lines of the monocytes and lymphocytes in our curves, with intervals in which the lymphocytes were rather steadily above the monocytes but in which both were decidedly above normal. We think that such studies give a new key for following the development of an immunity in an animal, but we are well aware that one count a day cannot be relied upon to give more than a general indication of the condition of the blood of the animal, because Sabin, Cunningham, Doan and Kindwall (25) have shown a marked daily rhythm of the white blood-cells. A daily count is, however, able, as the records herein recorded demonstrate, to bring out marked variations. We therefore conclude that the law of the ratio of monocytes to lymphocytes, in the circulating blood, in acute tuberculosis is of fundamental importance. In these studies we have found that there is a more severe type of infection than miliary tuberculosis, namely, a condition of diffuse, spreading mononucleosis in which infected monocytes increase in enormous numbers in the tissues, with no reaction on the part of the tissues to wall them off into tubercles. This type of infection, which we have found most markedly in the lungs and in the spleen, has been correlated with the most marked increase in the percentages and in the actual numbers of the monocytes of the circulating blood. For example, our maximum figures in this regard are the ratio of monocytes to lymphocytes in the last count of Rabbit TB 16, as 6348 to 1118, the percentages being 37 to 13 per cent. In Rabbit TB 39 we had the ratio of monocytes to lymphocytes on 5/20/25 as 5293 to 1449, with percentages 52 to 11 per cent; and on the next day a slightly greater disproportion, namely 5720 to 1210, with the percentages approximately the same. These figures are extreme examples of an increase in monocytes above the total number of the lymphocytes.

We have so far stressed the point of the increase in the monocytes and the reversal of the ratio of monocytes to lymphocytes because we regard that as the distinctively new point, but it is no less significant that an increase in lymphocytes has a relation to the healing of tuberculosis. We have consistently found, as has been well

known, that lymphocytes surround healing tuberculous lesions, and that a ratio indicating healing tuberculosis is one in which the lymphocytes are above the monocytes and are increased in actual numbers above the average normal limit. We therefore consider that the study of the development of immunity to tuberculosis must include an intensive analysis of the lymphocytes. For example, it might prove that measures for the stimulation of lymphocytes, which we have already at our disposal, as for instance, certain doses of X-rays, might be used to much greater advantage when the ratio of lymphocytes to monocytes was already favorable.

We think it a significant point, with reference to the close connection between the diffuse connective tissues and two of the strains of the white blood-cells namely, monocytes and lymphocytes, that there should be such a marked change in their numbers in the blood corresponding to an increase in their numbers in the connective tissues of an infected area. In the case of the monocytes we have given evidence to show that the increase in these cells in the connective tissues is due to a local differentiation of new monocytes, from the local overproduction of the reticular cells.

From these studies we think that it is clear that the immediate steps for progress in the study of tuberculosis involve the attempt to find the substances by which the change of the monocytes into the epithelioid cells is brought about. We believe that there is such a substance and that its discovery is of major importance. We think it is clear that the infection with tuberculosis brings about an increase in the number of reticular cells; it is not yet clear whether the change of the monocyte into the epithelioid cell is initiated by some chemical substance produced by the infection, which affects the cell structurally and permits bacilli to enter it and live in it more easily, or whether these changes are initiated by the presence of bacilli within the cell, or perhaps by both methods. We are, however, quite sure that the major effect of tuberculosis in the body is on one strain of cells, namely, the monocytes; we think that there is every indication that the monocyte is the cell primarily damaged by the infection and that it subsequently becomes the host to the invading organism. In a word, tuberculosis is a disease of the monocytes. The discovery of the substances which bring about the increased production of monocytes and effect their transformation into epithelioid cells, with the idea in mind that the discovery of the source of such a substance or substances is the first step toward producing an antibody for them, would be not only rational but a promising program. With a substance by which the overproduction of monocytes might be checked, then the problem of immunity in tuberculosis might be more analogous to the problem in those diseases which have already been controlled.

That this concept is hopeful is shown by an experiment which we have started with one of the fractions, obtained by chemical analysis of tubercle bacilli, given to us by the Committee for Research of the National Tuberculosis Association from the work of Prof. Treat B. Johnson. We took a normal rabbit (TB 40) in our series, in which

we had a long series of counts of the blood-cells, as a control and gave, daily, 10 mgm. of the fraction intraperitoneally in 20 cc. of distilled water. After the third dose, there was an increase in the percentage of the monocytes, from an average before the experiment of 13 to 20 per cent. Twice before the injection of the fraction the percentage of monocytes had been high in this animal but the cells on these two occasions were small and not changed in type. On the fourth day after the first injection, when three doses had been given, we found several monocytes such as the one shown in Fig. 10, in which there had been a very marked increase in the fine bodies of the rosette. This is the exact change which we have shown is characteristic of the transformation of the monocyte into the epithelioid cell.

The major problem in connection with the study of the blood at the present time is the analysis of the normal stimuli which have to do with the continued maturation of the cells of the blood throughout the life of the animal, because the discovery of any factors involved in this normal mechanism may lead toward the establishing of methods by which the production or activity of a given type of cell can be controlled. It is clear that all of the experiments involving the study of the effects of various tissue extracts bear on this problem, and that the newer technique of studying living cells both supra-vitally, in cells taken directly from the animal, and in tissue culture, offers a much improved method of analyzing the results of such experiments.

The next step in the study of tuberculosis seems to us to involve an intensive study of methods for controlling monocytes; these methods might possibly change the rate of production of these cells or modify their specific activities. Inasmuch as, in the disease of tuberculosis, there is both an overproduction of monocytes and a marked change in them as well, which we interpret as a lessened power toward the destruction of the bacilli, methods for counteracting this overproduction and for stimulating their cytoplasmic activities seem to be especially indicated. Since our evidence indicates that the monocytes phagocytize the tubercle bacilli but then fail to destroy them, it seems particularly important to seek for methods of decreasing the phagocytic activity of these cells or else of increasing their cytoplasmic activities so that the ingested bacilli will be destroyed instead of being allowed to live and multiply. The approaches that seem to us feasible are further studies with extracts made from tissue cultures of blood in which there had been a great overproduction of monocytes, according to the methods of Lewis and Lewis and of Carrel; of tissue extracts made from the lungs of such an animal as our Rabbit TB 38 in which the tuberculous infection had given rise to a very great overproduction of the immature forms of the monocytes; the study of the effect of the various chemical fractions from the bacilli such as we have referred to above, and the study of the effect of various extraneous chemical compounds which may be found to affect monocytes. Thus through a cooperation between the modern chemical studies on the nature of immunity and the modern experimental analysis of the physiology of the cells of the

blood and the connective tissues there has developed a new method of attack on the problem of tuberculosis. The next steps in tuberculosis involve studying the various chemical factors in the disease in their effect on monocytes.

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PLATE 1

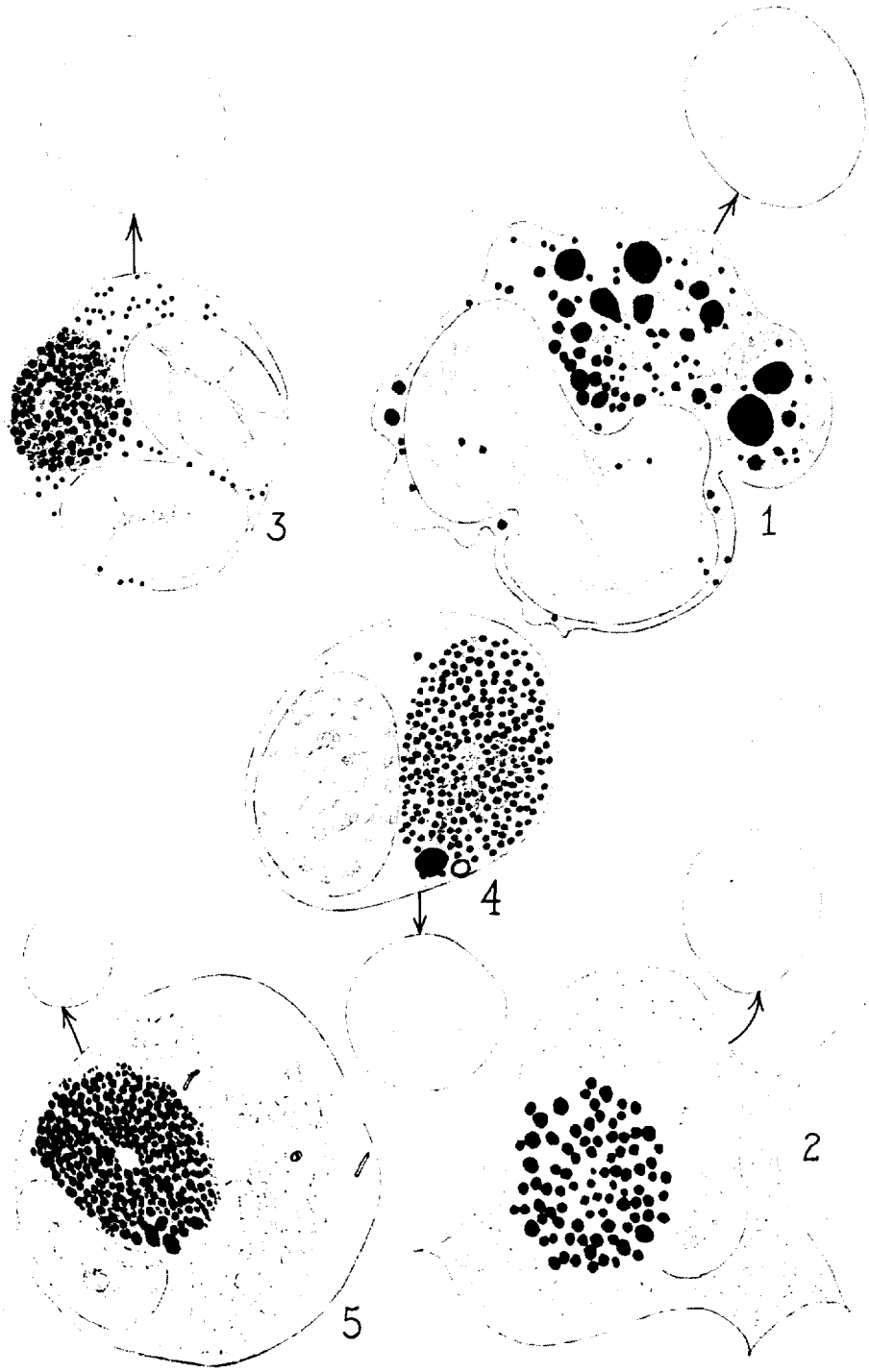
FIG. 1. Modified monocyte from the circulating blood of Rabbit TB 49. Intraperitoneal injection of tubercle bacilli on 5/29/25; cell drawn 6/23/25. Cell was supra-vitally stained with neutral red. Mitochondria are shown in black. The magnification of all the cells is indicated by the accompanying red blood-cell.

FIG. 2. Monocyte of the circulating blood of Rabbit TB 43, showing the earliest signs of the change into an epithelioid cell. Intravenous injection of tubercle bacilli on 5/29/25; cell drawn on 6/19/25. Cell was supra-vitally stained with neutral red.

FIG. 3. Epithelioid cell from a scraping of the liver of Rabbit TB 36, drawn immediately after the animal had been killed on 6/4/25. Data on the blood on Table 1. The peripheral blood on that day had 40 per cent monocytes. The tissues from the liver showed many of these very young epithelioid cells. This cell had two nuclei and many mitochondria in the cytoplasm. Cell was supra-vitally stained with neutral red and Janus green.

FIG. 4. Epithelioid cell from a scraping of the lung of Rabbit TB 33, drawn on 6/2/25. Data on the blood on Table 1. The peripheral blood on that day showed 17 per cent monocytes. This was a very young epithelioid cell, with a single vacuole stained red in the periphery of the rosette and one refractive lipoid body, shown in white, outlined in black. Supra-vitally stained with neutral red and Janus green.

FIG. 5. Epithelioid cell from a scraping of the lung of Rabbit TB 16, drawn 4/1/25. Data on the blood in Table 1. The peripheral blood on that day showed 37 per cent monocytes (see Charts 11A and 11B). This was a typical mature epithelioid cell with marked rosette of fine bodies and wide peripheral zone in which two bacilli were seen. Films from this lung stained for tubercle bacilli showed that most of them were within the epithelioid cells. Supra-vitally stained with neutral red and Janus green.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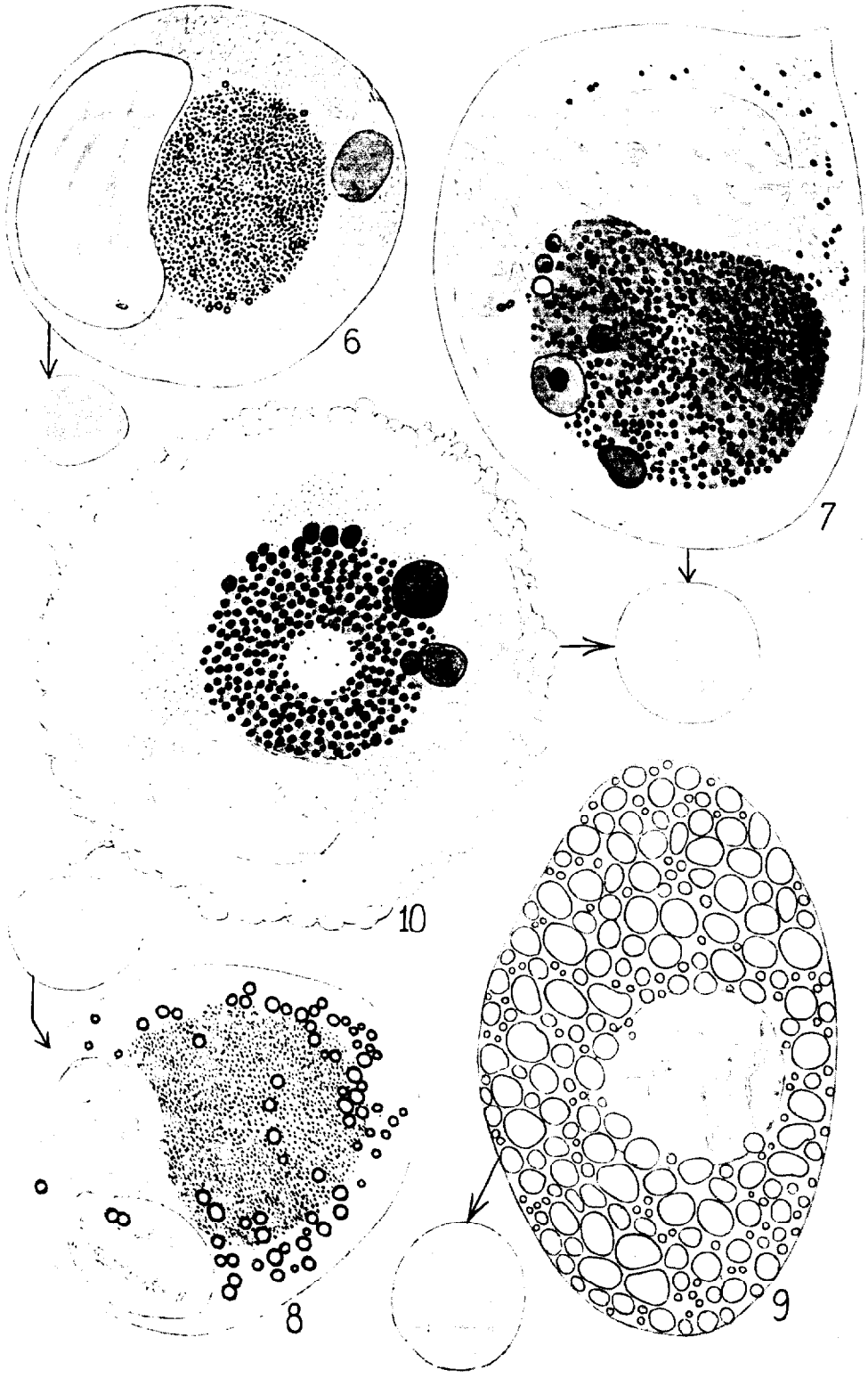
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