STUDIES OF THE BLOOD IN EXPERIMENTAL TUBERCULOSIS: THE MONOCYTE-LYMPHOCYTE RATIO; THE ANEMIA-LEUCOPENIA PHASE

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The work of our group is, as you know, a part of the plan for co-operative research which is being carried out under the supervision of a committee of the National Tuberculosis Association. Our studies on blood and connective tissues have been made on living cells and our conclusions are based on the newer criteria that are being developed from their reactions to normal and abnormal stimuli. This method, which involves the use of the so-called vital dyes, is an offshoot of the method of tissue culture, but, in contrast with tissue culture, involves the study of the cells immediately after they are taken from an animal; for this reason it has been called the supravital technique.

Our work with tuberculosis began with the study of fresh human, tubercular lymph glands, removed for diagnosis, in which we found that the epithelioid cells reacted in a characteristic manner to supravital staining. This observation reopened the question of the origin of the epithelioid cell.

In the study of early stages of experimental tuberculosis in rabbits, we found, in fresh scrapings, tiny tubercles, so small that every cell in them could be seen and so young that there was no reaction of the fibrous tissue around them; according to our interpretation these tubercles were made of young monocytes, younger than had been seen in the circulating blood of the animal, and of transitional types between these young monocytes and the epithelioid cells. The changes by which

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a monocyte becomes an epithelioid cell consist in an increase in the number and a decrease in the size of certain tiny bodies that react to neutral red and make a rosette around the centrosphere.

From these studies, there developed the concept that the epithelioid cell arises in the tissues from a tissue cell which is of the same strain that gives rise to the monocytes of the blood, and this idea led to the study of the blood in experimental tuberculosis; the first results of these studies were presented to this Association last year. The work was done with rabbits, infected with massive doses of bovine tubercle bacilli introduced intravenously.

In these experiments it was found that it was possible to study the progress of the tuberculosis in the tissues by following the white blood cells, because there were marked changes, both quantitative and qualitative, in the monocytes. The plan of the work was to study the blood by means of supravital counts taken every day or, once in 2, 3 or 4 days and kill the animal for the study of the tissues when the changes in the blood cells had become striking. It was found advisable to compare the actual numbers of the white cells rather than their percentages. Within two or three days after an injection of tubercle bacilli into the rabbit, there is a transient rise in the number of monocytes in the blood. We have not followed the reactions in the tissues at this stage. Subsequently, two or three weeks after the infection, there is a second more sustained rise in monocytes, with marked alterations in their type. The changes consist in a lessening of motility and involve all the transitions between monocytes and epithelioid cells. In severe reactions typical epithelioid cells appear in the blood.

In the normal rabbit monocytes occur in an average of 8 per cent, making a total of 900 cells per cubic millimeter. In severe tuberculosis the average rose to 15 per cent, with 52 per cent as the maximum in our series. Whenever an animal was killed at the time of an extreme rise in abnormal monocytes in the blood, there was always an extreme grade of tuberculosis of the lungs. When all the records were analyzed, however, it proved that the correlation of the state of the tuberculosis in the tissues could not be made from monocytes alone in the blood but rather from the ratio of lymphocytes to monocytes. The normal ratio of lymphocytes to monocytes in the rabbit is about 3 to 1. It was found that when the tubercular process in the tissues was quiescent or receding, and the lesions in the tissues were surrounded by lymphocytes, the lymphocytes had been increased in the blood both in actual numbers and in proportion to monocytes; on the other hand, an advancing tuberculosis of the tissues was indicated by a rise in monocytes in the blood with a reversal of the normal ratio to lymphocytes. Thus a high lymphocyte count with a low monocyte count in the blood was

correlated with healing lesions, and the reverse of high numbers of monocytes with low numbers of lymphocytes indicated advancing lesions in the tissues. We therefore think that the lymphocyte-monocyte ratio in the peripheral blood is a significant factor in tuberculosis.

These studies, with the blood of rabbits infected with a bovine strain of tubercle bacilli, have been repeated during the past year by Cunningham, Camp and Luton at Vanderbilt University on guinea pigs infected with a human strain (H 37). They have found the same type of reaction in the blood, but, in accordance with the greater susceptibility of the animal, the reaction has been of greater severity and beginning at a shorter interval after infection.

In our studies of fresh blood we found organisms which we interpreted as living tubercle bacilli in the modified monocytes; likewise, in both the tissues and blood we found bacilli in the living epithelioid cells; these bacilli almost never gave the reaction to the supravital staining which we have found to be characteristic of dead bacilli within cells. From these observations came the concept that the epithelioid cell is a type so damaged by the infection that the tubercle bacilli can survive in it. In any preparation stained for tubercle bacilli, it is obvious that in the rabbit vastly more epithelioid cells are formed than actually harbor tubercle bacilli. Under the Research Committee of this Association, we had the opportunity to make a biological test of some of the fractions isolated in the analysis of tubercle bacilli by Johnson and Coghill, and we found that the intravenous injection of one of the proteid fractions into rabbits was followed by an increase in the monocytes and the appearance of typical epithelioid cells in the blood stream. This demonstration that the epithelioid cell can be produced by a definite chemical substance without the presence of any bacilli we deem of real significance; in that it suggests that the typical lesion of tuberculosis, the epithelioid cell, is produced by a chemical factor yielded by the dead bacilli. If this should prove to be true, then the study of tuberculosis involves the reaction of the body both to dead and to living bacilli. The hypothesis may be formulated as follows: The bacilli killed within the body give rise to a substance that transforms monocytes into epithelioid cells and between these epithelioid cells and the tubercle bacilli which they phagocytize there develops a symbiosis, through which bacilli both survive over long periods of time and multiply within the cell.

It has now been found by Sabin and Doan, that in addition to this definite chemical factor operative in tuberculosis, a second chemical factor operative in the infection also exists. This second substance acts directly on the bone marrow. In following the blood of tubercular rabbits, we have found that there is a characteristic anemia combined with

a leucopenia. The leucopenia involves specifically the white cells that arise normally in the bone marrow, namely the granulocytic series.

These studies have been made on 68 rabbits, infected with either one or two milligrams of bovine tubercle bacilli injected intravenously. In these animals, the fall in the red cells has been from an average of 5,425,697 before infection to 2,741,764; the hemoglobin from 62 to 35 per cent: while the neutrophilic (pseudoeosinophilic in the rabbit) leucocytes have fallen from 44 per cent, with a total number of 3,883, to 17 per cent and a total number of 940. The lowest red count in the series was 1,410,000, and the lowest hemoglobin was 17 per cent. There were several instances of an extreme fall in neutrophilic leucocytes under 10 per cent, with total numbers between two and three hundred cells. The fall in red cells, in hemoglobin and in neutrophilic leucocytes comes about the same time, averaging 11 days after infection for the red cells and 9 days for the white cells.

The animals of this series fall into three groups in respect to the anemia and leucopenia. The first group consists of 33 animals that died during the first month, with an average survival of 23 days after infection; the second group, of 14 animals that died during the second month with an average of 35 days; and a third, of 21 animals that lived into the chronic phase of the disease for an average of 119+ days. None of the days are exact averages because we killed a few of the animals in each group at times when the peripheral blood indicated that they were near death in order to follow the tissues with the supravital technique. When the animals die during the night this opportunity is lost. In each instance the autopsy demonstrated that the tubercular process was comparable with that in the others of the group that had died spontaneously. Of the last group, 8 of the animals are still living, and certain experimental procedures have been introduced that may have influenced the length of life but not the course of the anemia and leucopenia.

The first group of 33 animals all died (or were killed) as the red cells, the hemoglobin and the leucocytes were either decreasing or at the lowest level. The second group, the animals that lived into the second month, all show the beginning of a recovery of the bone marrow. The rise in hemoglobin usually precedes the rise in the red cells by one or two days; with this exception, red cells, hemoglobin and granulocytes rise together. In this group we see the astonishing results of animals dying of an advancing tuberculosis in the tissues while the bone marrow is recovering from the anemia and the leucopenia. In other words, all rabbits that live into the second month after infection become resistant to the factor that depresses the bone marrow quite independently of the tuberculosis in the tissues. Supravital studies of the bone mar-

row of each animal have shown that the depression of the marrow is not due to local tuberculosis, for though some of the animals showed a few epithelioid cells in the bone marrow there was no correlation of the lesion with the course of the anemia and the leucopenia.

From the study of the third group, we find that the average duration of the anemia and the leucopenia is 33 days. Then the marrow passes into a phase of hyperactivity which involves all its cellular elements, the red cells and the three strains of granulocytes. This period of stimulation of the marrow is likewise independent of whether the tuberculosis is quiescent or advancing. In the stage of hyperplasia, the red cells rise above their normal level, reaching six, seven and even nine million cells; the hemoglobin rises to levels that give a color index of plus one when reduced to a unit comparable with the human index. The neutrophilic leucocytes fluctuate but rise to a base level above the normal. In regard to the basophilic leucocytes, the normal percentage varies markedly in different rabbits; they are not markedly reduced in the phase of the leucopenia but they are increased in the stage of the stimulation of the marrow. Eosinophiles are normally low in the rabbit, running from 2 to 3 per cent: they disappear entirely from the blood of rabbits during the leucopenic stage and reappear during the phase of the hyperplasia of the bone marrow. As far as our records go, the period of stimulation is temporary and the cells from the bone marrow return to their normal levels.

From these studies we conclude that there must be a definite chemical factor in tuberculosis that acts on the bone marrow entirely independently of the specific protein factor that produces the tubercular lesion in the tissues. All rabbits with primary tuberculosis show an anemia together with a leucopenia which involves the granulocytes; all that survive into the second month become resistant to this factor; all that survive into the chronic phase show a period in which the bone marrow becomes hyperactive in all its elements. During this phase, the red cells become increased above the normal both in number and in content of hemoglobin, and all the granulocytic strains are produced in higher percentages and numbers than normally. As far as our records go this period of hyperplasia of the marrow is transient. For the anemia and the leucopenia the sole factor is the length of survival of the animal. Through further studies with the fractions which have been isolated from the tubercle bacilli by Johnson and Coghill, it may be possible to identify this depressant factor on the bone marrow, to which rabbits have the power of becoming resistant.