THE DIPHASIC NATURE OF TUBERCULOSIS IN RABBITS AFTER INTRAVENOUS INOCULATION WITH BOVINE TUBERCLE BACILLI

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Studies with reference to the correlation existing between the cellular reactions in the tissues in experimental tuberculosis and the changes in the circulating blood cells have been carried on in this laboratory since 1925. Observations on the connective tissue cells have been made, both with the supravital technique and with fixed tissues prepared by the standard methods (1). General studies of the changes in the blood cells associated with changes in the lesions during the course of the disease have been reported (2, 3) and certain changes in the blood cells directly related to the pathological findings in the bone marrow have been described. Since these observations were reported, a considerable number of animals have been inoculated with tuberculosis as specific controls for groups of animals under various treatments. The accumulated mass of data relevant to the course of tuberculosis in rabbits is reviewed and analyzed in the present paper.

Material and Methods

The changes in the blood cells following inoculation with tuberculosis have been followed in a group of 175 animals. 140 of these received no treatment and were allowed to die of tuberculosis. The remaining 35 were either killed for comparative studies or were given some treatment, in which latter instance no further studies of their blood were included in this analysis. From the group of 140 animals, sections were available for study in 109 instances.

The animals used have been of various breeds and weighed 2,000 gm. on the average. Male and female rabbits have been employed in approximately equal numbers. The season of the year in which inoculations were made was not limited, groups of animals having been inoculated in every month except July and August. An analysis of the records showed that the course of the disease in

animals inoculated in June or September was similar to that observed in animals inoculated in December or January. They were kept under uniform housing and dietary conditions, and during the period that they were in our animal house there were no acute infectious diseases of rabbits which assumed epidemic proportions. There were sporadic cases of severe snuffles, and some cases of pneumonia of undetermined origin. An occasional animal showed evidence of spontaneous encephalitis. The effect of these isolated instances of deaths due to accessory diseases, on the general study of mortality rates from tuberculosis is slight.

The organism used was a strain of bovine type tubercle bacillus designated B-1 which has been cultivated on artificial media since its isolation from a bovine lesion by Dr. Theobald Smith in 1893. It has been virulent for rabbits during the 6 years that it has been in use in this laboratory. The organisms were harvested from colonies grown on Petroff's egg media and weighed directly. They were then triturated in 0.9 per cent sodium chloride solution and suspended so that the infecting dose was contained in a suitable quantity of fluid, 0.5 or 1.0 cc. All animals were inoculated in the marginal ear vein, the dose varying from 0.001 mg. in the case of four animals up to 2.0 mg. In the past 2 years 0.1 mg. has been used as the infecting dose.

During the course of the disease the animals were weighed and the temperature taken on the day the blood counts were made. The intervals between counts varied in the different groups but in general the animals were counted at weekly intervals for the first 2 months after inoculation and at 2 to 4 week intervals thereafter. At death the animals were autopsied and blocks were saved from each organ for sectioning.

The Rate of Mortality in Tuberculous Rabbits

The mortality rate in a population subject to an acute infectious disease usually follows a course somewhat as follows:—

There is at first a comparatively low rate of mortality which precedes a period during which the mortality increases rapidly and reaches a peak; following this there is a decline in the rate of mortality which is less rapid than the increase prior to the peak. When the total mortality of such a group is plotted against time, the curve assumes a sigmoid shape, skewed more or less toward the later time periods. This distribution of mortality is interpreted as reflecting the distribution of susceptibility in the whole group. The early deaths are attributed to the greater susceptibility of a portion of the population, the larger portion succumbing at or near the point of peak mortality representing the members of average resistance.

In Fig. 1, the actual number of deaths per 15 day period is shown for the group of 140 animals mentioned above. It is seen that there

were two definite peaks in the mortality rate, one occurring at the end of the 1st month, and the other occurring at the 5th month, with an intervening period at the end of the 3rd month, when the mortality rate was much lower than the general rate for the entire period. In

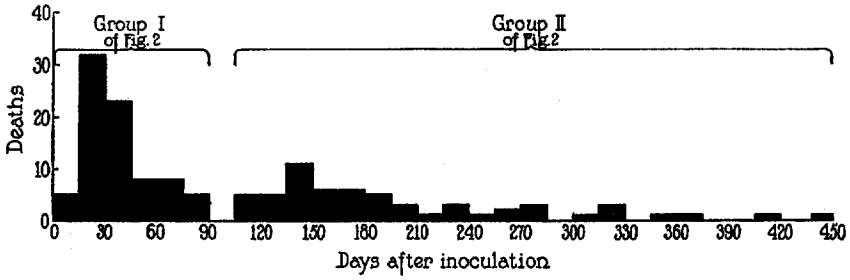

FIG. 1. Death rates during 15 day periods after inoculation in 140 tuberculous rabbits.

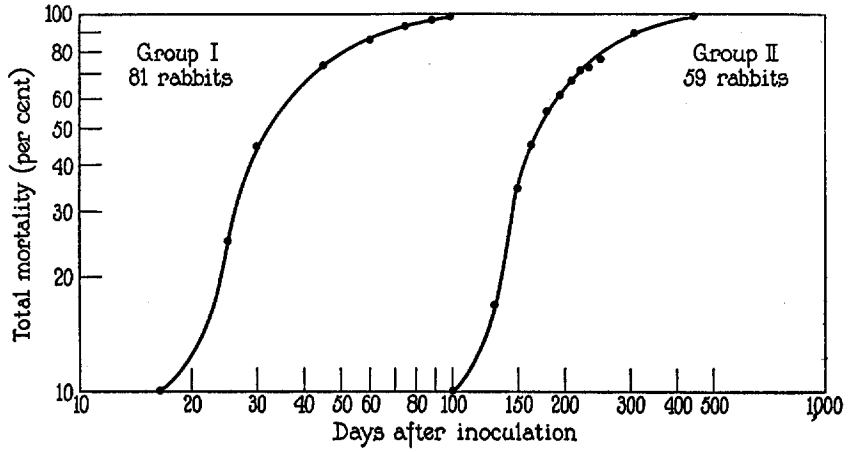

FIG. 2. Total mortality of 81 rabbits which died under 100 days (Group I) and 59 rabbits which died between 100 and 437 days (Group II).

this series no deaths occurred in the period from 85 to 100 days. In Fig. 2, these data are rearranged so that the total mortality of each of the two groups of animals, *i.e.* those dying before 85 days and those dying after 100 days, is shown. The per cent of animals dead is plotted on the ordinate and the time after inoculation along the

abscissa. Both sets of values are plotted as logarithms. It is obvious that the mortality for the whole group of 140 animals is in reality the summation of the mortalities occurring during two separate phases of the disease, and that in each instance the distribution of deaths is similar to that described above as typical of an acute infectious disease. The effect of these two separate mortality pressures has been evident in groups varying in size from 5 animals to 40. It has been seen also with all doses used save the 0.01 mg. and 0.001 mg., that a large dose resulted in a greater number of animals succumbing in the first phase of the disease, while a small dose allowed a greater number to survive the first phase. In the case of the animals which received 0.01 and 0.001 mg., four in each case, all survived the first phase of the disease.

It would seem therefore that if the resistance of rabbits to tuberculosis initiated through intravenous inoculation is to be measured by the longevity of the animals inoculated, it must be expressed in terms of resistance to two distinct phases. The use of an average obtained by dividing the sum of all the days lived by the number of rabbits in the group, may be quite misleading, since the distribution of deaths in two groups might be significantly different and yet result in the same average.

Differences in Lesions in the Two Phases

The sections of 109 animals were found to be suitable for study. The extent and type of the lesions were noted and the findings were then grouped according to the longevity of the animal. The pathological picture during successive 15 day periods was then summarized.

A. Lesions in the First Phase

The Lungs.—A tuberculous pneumonia was found in almost every animal that died during the 1st month after inoculation. The alveoli were filled with an exudate made up of monocytic and epithelioid cells. The alveolar walls were thickened and showed monocytic cells in and adherent to them. There were some polymorphonuclear leucocytes and clasmatocytes present. Exudation of red cells and fibrin was rarely noted. In the gross the lungs were of normal color, but did not collapse completely when the thorax was opened. The surface frequently showed a number of small, pearly gray tubercles, with translucent borders, 1 to 2 mm. in diameter. On gentle pressure of the lungs a small amount of exudate

could be expressed from the trachea. Caseation of portions of the pneumonic tissue was seen at this time. In those animals which died between 13 and 20 days, these lesions were quite pronounced; however, at the end of the 1st month many animals showed practically the whole lung consolidated with pneumonia. During the 2nd and 3rd months the extent of the pneumonic involvement decreased steadily, and in those animals which died at the end of the 3 months only small scattered collections of epithelioid cells could be found. The classic, fully developed tubercle, formed of epithelioid cells and giant cells, enveloped by lymphocytes and fibrous tissue, was rarely present in the 1st month; however, in the 2nd and 3rd months they were present in increasing number together with Langhans giant cells free in the alveoli. In Fig. 3, the occurrence of these various lesions has been plotted. The occurrence of tuberculous pneumonia, and of fully

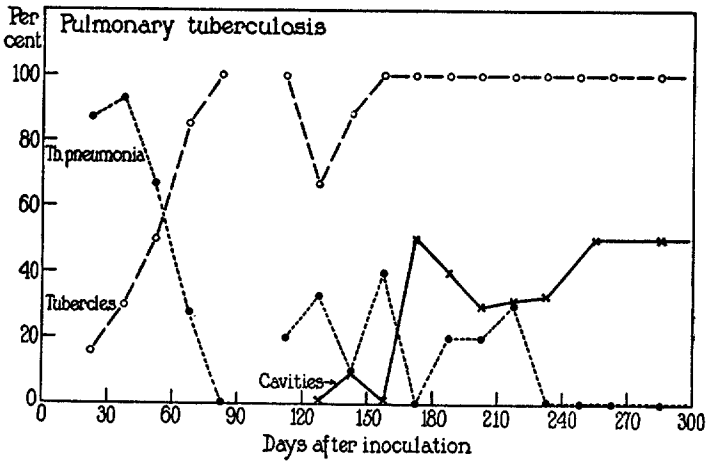

FIG. 3. The distribution in relation to time of various pulmonary lesions.

developed tubercles, is plotted as per cent for successive 15 day periods. No attempt was made to estimate the total extent of all types of lesions, as sections taken one from each lobe do not give sufficient data for such a comparison. It is seen that the incidence of tuberculous pneumonia decreases rapidly after the 1st month, while the incidence of fully developed tubercles increases steadily from the 1st month onward. It seems highly probable that the small amount of exudate found at the end of the 3rd month is in part residual, since the injection of dead tubercle bacilli intravenously is followed by the appearance of a caseous pneumonia in a very short time, and small collections of epithelioid cells entirely similar to those mentioned above may persist in the lungs for as long as 60 days. It is probable that the infection with living, virulent organisms results in the production of large amounts of exudate in which bacilli are either killed or fail entirely to multiply and which is removed from the lung slowly. Any attempts to

estimate the state of activity of a particular lesion by study of the histologic sections alone must take this factor into consideration.

The Spleen.—In the first 4 weeks the spleen was involved in almost every instance. The extent of the monocytic and epithelioid cell reaction was greater in the spleen at this time than in any other organ. In many cases the normal histologic appearance of the organ was completely obliterated, both pulp and follicles being invaded. The weight of the organ was increased; the average weight of the spleen at this time was 3.2 gm. as against a normal average of 0.9 gm. In one instance the weight of the spleen was 17 gm., and section showed the entire organ to be filled with monocytes and epithelioid cells. Congestion of the spleen with blood was not seen. During the 2nd and 3rd months, however, the spleens examined showed a marked diminution in the extent of the lesions, going on to com-

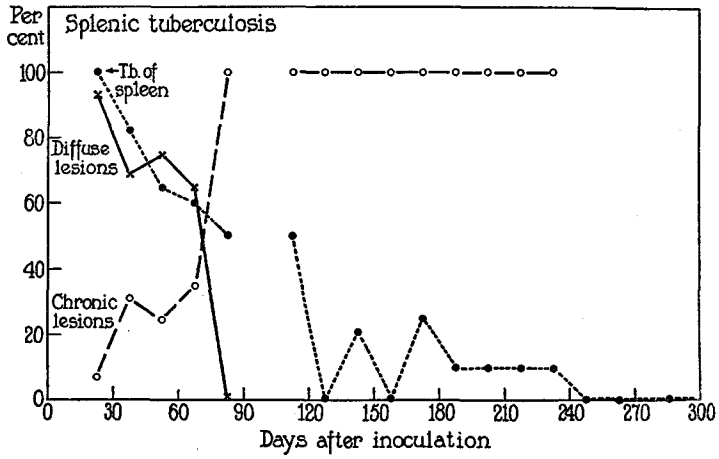

FIG. 4. The distribution in relation to time of lesions of the spleen.

plete healing in many instances. This process of healing did not seem to have taken place by the caseation of a large mass of the tuberculous tissue with fibrosis of the cavity formed, but by the elimination of the cells by absorption and possibly by phagocytosis by clasmatocytes. The epithelioid cells in these regressing lesions lost their staining reaction and their normal morphology; the nuclei and cytoplasm both appeared degenerated and in many instances only faintly stained cell outlines could be made out. Lymphocytes and polymorphonuclear leucocytes were seen in and around such areas, possibly aiding in the complete removal of such cells. In but a few instances localized tubercles were seen at the end of the 3rd month. In Fig. 4 the number of spleens in which tuberculous lesions were found is plotted and shows a steady decline after the 1st month. The number in which diffuse lesions were found is expressed in per cent and is seen to decline rapidly after the 1st month. Small, localized tubercles consisting most often of a few epithelioid

cells or an occasional Langhans giant cell were classed as lesions of a more chronic nature and their increase is shown in Fig. 4.

The Bone Marrow.—The course of the tuberculous infection in the bone marrows of a large group of rabbits has been studied and reported by Doan and Sabin (2). They demonstrated that there were no significant changes visible in the marrow before the 8th day, when a definite infiltration with monocytes and epithelioids was seen. At this time the fat was seen to be breaking up and disappearing, and from the 8th day on up to the end of the 4th week, the marrow showed an increasing amount of the diffuse epithelioid cell reaction. In a few animals that survived longer than 30 days, they reported a steady regression of the lesions to complete healing at the end of 3 months.

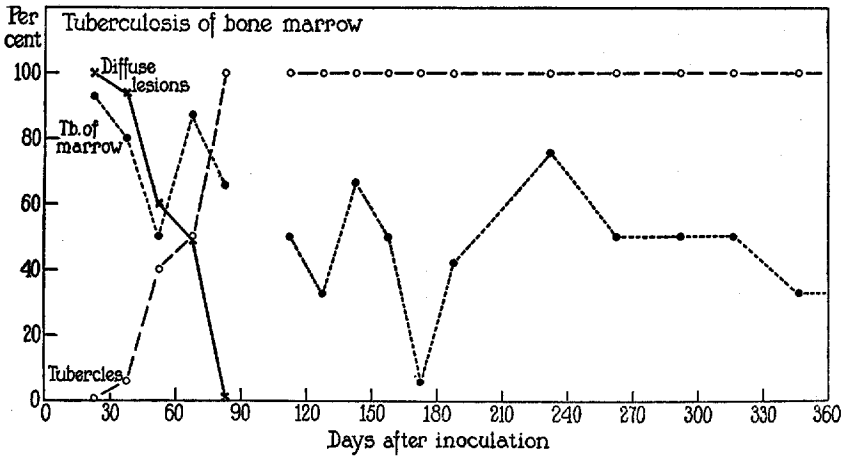

FIG. 5. The distribution in relation to time of lesions of the bone marrow.

In the present series of animals similar findings were noted in the marrows of those succumbing in the 1st and 2nd months. At the end of the 3rd month about half of the animals showed no tuberculous involvement of their marrows whatever, while the remainder revealed varying numbers of small, definitely localized tubercles scattered throughout the tissue. These relations are shown in Fig. 5.

The Lymph Glands.—In the 1st month after inoculation, almost every lymph node showed tuberculous involvement. The lesions consisted of a diffuse infiltration of the entire node with monocytes and epithelioid cells. Caseation was found in a considerable number. During the 2nd and 3rd months, however, the incidence of tuberculous lymph nodes decreased and the lesions were definitely less extensive. The phenomenon of regression was noted in a considerable number, similar in appearance to the regression of the lesions in the spleen and bone marrow. Caseation of tuberculous nodes was much less frequent in the 2nd and 3rd months than in the 1st. Definitely localized tubercles did not make their

appearance in the 2nd and 3rd months, although giant cells were more frequent. The lesions at the end of the 3rd month resembled those seen in the spleen, where small collections of epithelioids and giant cells remained with no evidence of fibrous tissue forming about them.

The Liver.—In the liver, tuberculous lesions apparently mature more rapidly than in the other organs, as in this series of animals the early reaction of monocytes and young epithelioid cells was not observed. The lesions found were constant in type, namely, a scattering of Langhans giant cells throughout the organ, occurring most often singly, but occasionally in groups of two or more. Had animals been studied during the first 2 weeks after inoculation a much more active type of reaction would probably have been observed. The number of animals showing any involvement of the liver during the 1st month was 60 per cent, and this figure decreased rapidly in the ensuing months.

The Kidneys.—Renal tuberculosis was found to exist in about 70 per cent of the animals that died in the 1st, 2nd, and 3rd months. This rate of incidence did not lessen as time went on, a fact which would suggest that healing of the renal lesions once under way is uncommon. The lesions in the kidneys were for the most part macroscopic tubercles, located both in the cortex and in the medulla. They tended to extend between the tubules, and frequently made their way into the pelvis of the kidney, where tuberculous pyelitis was set up. When the pelvis was so involved, there was evidence of fresh invasions of the medulla from the pelvis. It was common to find the pelvis completely filled with caseous debris. Epithelioid cells were occasionally seen in the glomeruli, and some small tubercles which evidently had their origin from such a lesion. Histologic sections of the ureters and bladder were not made, but no gross tuberculous involvement of these portions was observed.

The Reproductive System.—In but two cases were tubercles found in the testicles of animals that died in the acute phase; these two were small, pin-point tubercles without caseation. No ovarian tuberculosis has been recorded.

B. Lesions Found in Animals Surviving beyond 3 Months

The Lung.—In the second or chronic phase of the disease all of the changes associated with long standing tuberculosis were found in the sections. Tubercles of varying size and in varying stages of development were found. Large confluent tubercles which had apparently extended into adjacent lung tissue were in some cases walled off and in others surrounded by a zone of tuberculous pneumonia. Many small tubercles were seen to be well enveloped by lymphocytes and fibrous tissue. It was noteworthy that in general the greater the longevity of the animal the more sharply localized were the lesions; and the rest of the lung showed less and less evidence of any inflammatory reaction, in sharp distinction to the findings in the acute phase where the whole lung from apex to base was frequently involved. In those animals which survived a year or more, the extent of the lesions was markedly reduced, and in some instances confined to the presence of a few small cavities with dense fibrous walls.

The Spleen.—The spleen was seldom found to be tuberculous in the chronic phase of the disease. Occasionally a few scattered epithelioid cells or Langhans giant cells were observed. No large confluent tubercles were found. In a number of spleens, particularly from animals that died after 5 or 6 months, there was a marked change in the histologic appearance, involving a necrosis of the cells of the pulp and much fibrosis; the lymphoid follicles were remarkably reduced in size. The average weight of the spleen was approximately normal, but in a considerable number of cases the organ was very small and shrunken.

The Peripheral Lymph Nodes.—The incidence of tuberculous lymph nodes during the chronic phase of the disease decreased steadily. The nodes found in animals surviving over a year were in general very small and difficult in many cases to locate at autopsy, but apparently normal in other respects. The popliteal lymph node was more often involved than any other peripheral node, and an analysis of the records showed this to be associated with tuberculous infection of the bone marrow of the tibia and femur. The incidence of the two lesions together was frequent, while the occurrence of either lesion alone was not common.

The Bone Marrow.—Of the animals dying in the chronic phase of the disease, about half had tubercles in the marrow. These tubercles were discrete, usually small, and made up of from 5 to 25 epithelioid cells in section. The tubercles often had a lymphocytic reaction about them, but none showed any evidence of an increase in fibrous tissue. Caseation was not seen, and there was no evidence of any inflammatory reaction in the adjacent marrow.

The Kidneys.—The kidneys were tuberculous in about 65 per cent of the animals that died in the chronic phase of the disease. The extent of the lesions was perhaps greater in some of the animals which had survived for a long time, but the nature of the lesions was essentially the same as that described in the acute phase of the disease. There was in no instance any evidence of regression or repair of the renal lesions.

The Liver.—In the chronic phase of the disease the incidence of hepatic tuberculosis was very low. Only about 15 per cent of the animals showed any lesions at all and these consisted of an occasional Langhans giant cell.

The Reproductive System.—Tuberculosis of the testicle was seen in a number of instances in animals that died in the chronic phase of the disease, and in these the whole organ was caseous in each case, frequently involving the epididymis.

C. Comparison of the Lesions in the Two Phases

The two phases of the disease are characterized by different pathological findings. In the first 3 months the spleen, bone marrow, lymph nodes, and the liver were extensively involved, as well as the lung and kidney. In animals dying after 3 months the lesions in the spleen, bone marrow, lymph glands, and liver were minimal, whereas the lesions in the lung and kidney were extensive. In addition, the lesions

in the first 3 months were most frequently a diffuse infiltration of the organs with a mass of monocytes and epithelioid cells, which showed a tendency to regress without caseation, while in the later phase the formation of typical tubercles together with cavitation and fibrosis went on to the exclusion of any other type of lesion.

In connection with these studies the work of Lurie is most valuable and interesting. In 1928 (4) he made an extensive study, following the course of tuberculosis in rabbits after intravenous inoculation with bovine and human type bacilli, with especial reference to the number of organisms which could be recovered from the various organs. He sacrificed animals at varying intervals after inoculation, making cultures from small weighed portions of spleen, liver, bone marrow, and lung. The number of colonies that grew out during 10 weeks' observation of the cultures was determined, and the colony counts from the various organs compared. The data as presented showed that there was apparently a lag period of approximately 10 days, as cultures made from animals sacrificed 8 days after inoculation showed either no growth or a very few colonies, while cultures obtained from animals sacrificed after 2, 3, and 4 weeks showed increasing numbers of positive growths as well as colonies. Cultures made from the spleen revealed growth earlier and in greater profusion than those from the other organs. After the 4th week the number of colonies obtained from the spleen, liver, and bone marrow declined, while the number of colonies obtained from the lung and kidney increased with time, the lung showing a greater increase and the kidney but a slight increase.

The number of bacilli which can be grown from an organ is dependent on the rate of multiplication and destruction of organisms in that organ and on the number of organisms originally seeded into it; the estimation of the number of viable organisms throws no light on the ratio of living to dead bacilli. The fact that dead organisms produce lesions similar to those produced by living virulent organisms serves to complicate the pathological studies. However, lesions produced by dead organisms do differ from the active lesions in that the former show regression, a phenomenon which is clearly recognizable in sections and which may be easily followed in rabbits inoculated with heat-killed tubercle bacilli. It is probable that the regressing lesions seen in the 2nd and 3rd months in the spleen and bone marrow and lymph

glands were due to the large number of dead organisms present in those tissues after the 1st month. The rise and fall of the number of organisms recovered by Lurie from the different organs occurs coincidentally with the period of increasing and decreasing lesions in our series, and parallels the rise and fall of the death rate during the first 2 months.

More recently Lurie (5) has made studies similar to those referred to above but in which sections of tissue adjacent to the block of tissue taken for culture were fixed and sectioned, and a correlation between the bacteriological and the histological findings determined. The sequence of pathological changes reported in this recent study is substantially in agreement with the course of the changes reported in our series for the first 3 months. Lurie made a more intensive study of the events occurring up to 2 months after inoculation, recording but a few instances of studies on animals allowed to live longer.

All of the factors brought out so far in the first phase of the disease, including the mortality rate and the nature and extent of the lesions, are significantly related to the widespread multiplication of the tubercle bacilli in the various organs and their subsequent partial destruction. While the trend of total mortality in the second phase sets this portion of the disease apart as an entity, it is not so apparent that the rise and fall of the death rate in this phase is related to or dependent upon the same factors.

The Changes in the Blood Cells in the Two Phases

During the first 3 months after inoculation, the lesions were widespread and constantly changing in extent in the various organs. The clinical condition of the animals during this period was apparently normal in almost every instance, and those animals which died showed little evidence of wasting or malaise. The younger and lighter animals, in fact, frequently gained weight steadily during this period. The changes in the blood cells, however, reflected faithfully the changes in the lesions in each animal as well as for the whole group. The striking correlation between lesions in the bone marrow and the blood cell changes is evident in Fig. 6, where the total number per c.mm. of the various cells is plotted by 10 day periods after inoculation. 175 animals were included in this summation, which was extended to the 190th day. The number of counts available from ani-

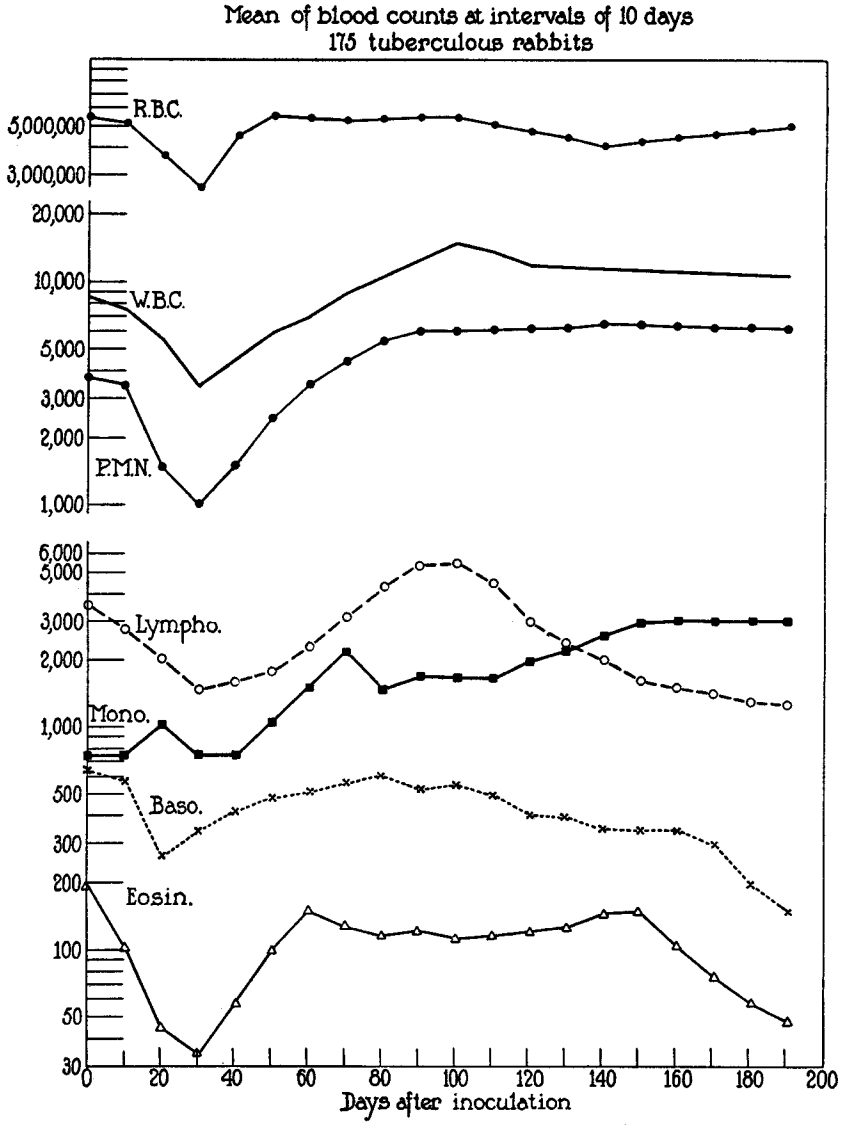

FIG. 6. The total number of the various types of blood cells per c.mm. during successive 10 day periods after inoculation, in a group of 175 tuberculous rabbits.

mals surviving beyond this time was too small to allow a significant average to be taken. It is seen that at the end of the 1st month there was an anemia and a leucopenia of all cells except the monocytes. This fall in total numbers of these cells coincided with the increase of lesions in the bone marrows, shown in Fig. 5.

Following this period of anemia and leucopenia, the cells tended to return to their previous levels, during the 2nd and 3rd months. Again this change coincided with the period in which the bone marrows were recovering from the extensive lesions present in the 1st month.

The lymphocytes fell in total numbers during the 1st month and recovered during the 2nd and 3rd months. This fall in total numbers is apparently due more to a drop in the total number of intermediate and large lymphocytes than to a proportionate decrease in all three types of lymphocytes. The lesions in the lymph glands and spleen, extensive in the 1st month, regressed in the 2nd and 3rd months, and undoubtedly these changes are related directly to the variations in the number of lymphocytes in the circulating blood. The difficulty in estimating the extent of the lesions in all of the possible sources of lymphocytes makes a close correlation between the qualitative changes in the cells and the pathological changes in the lymphoid tissues difficult to obtain.

The monocytes showed a small rise in total numbers during the 1st month. The most significant change in this cell type was a qualitative one and it is brought out sharply when the monocytes are differentiated into normal and "stimulated" forms.

The stimulated form of the monocyte seen in tuberculosis differs from the normal monocyte in the following characteristics. The only nuclear change is a greater tendency on the part of the stimulated cells toward amitotic division, so that more of the stimulated forms show two nuclei. The essential changes are cytoplasmic and consist in an increase in the size of the rosette of vacuoles staining with neutral red, correlated with an increase in the size of the whole cell. In the stimulated forms the individual vacuoles of the rosette are smaller but much more numerous. The normal monocyte has a small rosette of vacuoles of moderate size, while the epithelioid cell has a very large rosette of tiny vacuoles. The stimulated form of monocyte is intermediary between the two, and the transition from monocyte to

stimulated monocyte ending with the epithelioid cell has been demonstrated by Sabin (6). It is significant that, during the period when stimulated monocytes are found in great numbers in the circulating blood, epithelioid cells, identical with those found in the lesions, are occasionally found in the blood (7, Plate 26,

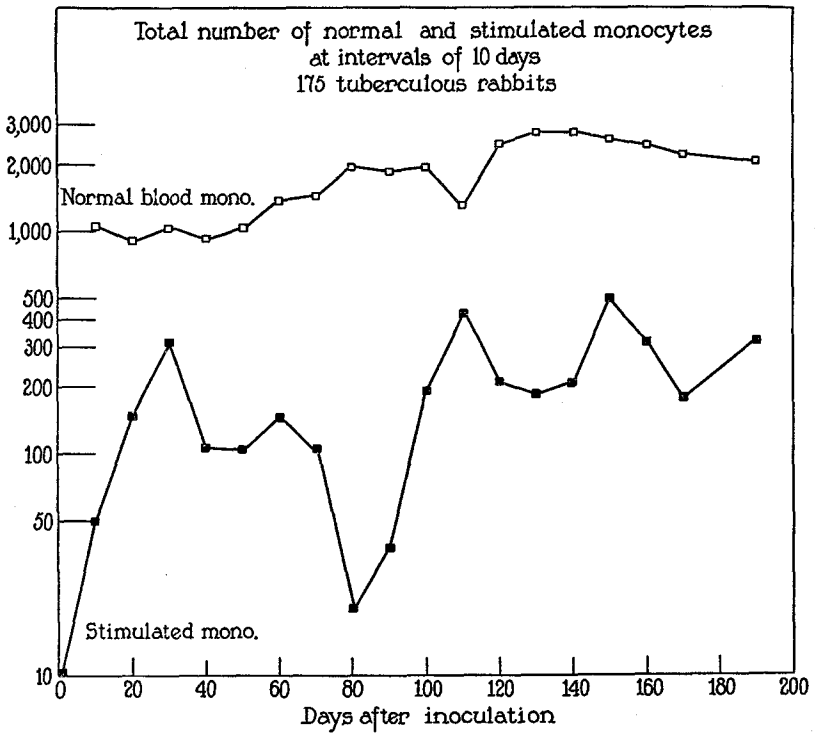

FIG. 7. The relative number of normal and stimulated monocytes in the blood during successive 10 day periods after inoculation, in the same group of 175 tuberculous rabbits.

Fig. 3), whereas at other times when there are no stimulated forms present epithelioids are not seen. The surface film and the mitochondria of the two types of monocyte are similar. These qualitative differences between cells of the monocytic strain are only to be seen with the supravital technique. Fig. 7 shows the total number of normal monocytes and stimulated monocytes plotted separately,

instead of together as in Fig. 6. It is clear that the small rise in the total number of monocytes which occurred in the 1st month was due to the accession of several hundred of the stimulated forms per c.mm. The normal monocytes remained at their initial level until the 2nd month, when they rose steadily. The appearance of stimulated monocytes in the peripheral blood is a constant phenomenon following inoculation with tuberculosis, particularly in the 1st month (8). After this time these stimulated cells decrease somewhat in number and frequency, reappearing during periods of great extension of lesions.

The basophilic leucocytes and the eosinophils reacted as did the neutrophils, showing a leucopenia at the end of the 1st month. Eosinophils became exceedingly scarce, the average number per c.mm. at the end of the 1st month being but 35. Both eosinophils and basophils returned to approximately normal levels after the 1st month.

It is seen in Fig. 6 that the neutrophils after the period of leucopenia returned to their former level and went on to a definite leucocytosis, which was maintained throughout the course of the disease in most instances. This leucocytosis was probably a reaction to the presence of caseous lesions. Caseation occurred so constantly that it is difficult to determine what other factors, if any, contributed to the increase in neutrophils.

Thus the complex of lesions during the first phase, at first extending and then regressing in some organs, while steadily progressing in others, can be viewed not only with the aid of the autopsy findings, but followed from day to day and from week to week in the living animals, by means of repeated studies of the blood cells.

In the chronic phase of the disease, the lesions were for the most part confined to the lungs and to the kidneys. The other organs showed minimal lesions. It was also evident that the rate of extension of the lesions was not so great in the chronic phase, since many animals showed no evidence of any change in their clinical condition for long periods of time and died with relatively few lesions. During this period of relative inactivity, studies of the blood cells showed few significant changes. However, in each case in which the studies were made at frequent intervals up to the time of death, it was found that for a period of 1 to 2 months before death there was a change in the blood picture, of a constant nature. The lymphocytes, which had

been maintained at or near the normal level, about 3,500, during the long period of inactivity, started to fall; this fall was steady and continued until the total numbers of lymphocytes just before death were but 600 to 1,200 cells. During this period of decline the monocytes frequently increased in total number and stimulated forms became more frequent. The animals' clinical condition went steadily downward, the weight falling at about the same rate as the lymphocytes. This decrease in the number of lymphocytes and increase in the number of monocytes at a time when the animals' resistance to the disease has obviously failed is a change that occurs so constantly that it is extremely useful as an index or basis for prognosis.

It will be noted that no mention has been made of a relation between the extent of the lesions and the longevity in the chronic phase. The number of animals that have been studied is not large enough to draw conclusions from, and it is not clear that such a relation exists, at least as measured by the extent of the cellular changes. The fact that many animals of extraordinary resistance, surviving for a year or more, show at autopsy only a few lesions, most of which are very well walled in with fibrous tissue, would suggest that the extent of the lesions in the chronic phase rose and fell somewhat parallel to the death rate, as in the acute phase. However, when the group of animals in the chronic phase which showed the most extensive lesions were compared with those which showed the least, it was found on analyzing the blood studies and the weight records that the two groups showed the same prolonged period of decline and wasting before death. It would appear that the factors responsible for the loss in weight and malnutrition are in part independent of the extent of the lesions. The close correlation between the maintenance of weight and the maintenance of the lymphocytes, and the simultaneous decline in both suggests that the lymphocytes are either directly or indirectly related to the state of resistance to the disease.

The Relation of the Preinoculation Blood Counts in Rabbits to Their Resistance to Tuberculosis

The records of 66 animals, all of which were inoculated with 0.1 mg. of tubercle bacilli and which had an average of 5.4 counts each before inoculation, were examined in an effort to find what relationships

could be discovered between the blood counts before infection and the resistance of the animal. The counts on each animal were summated and the mean obtained. The means for the group of 66 animals were then arranged in a frequency distribution and the mean, mode, and standard deviation calculated. It was found that the separate values distributed themselves in the unimodal fashion of a normal frequency distribution. A correlation table was then made for each of the various cell types, plotting the longevity of the animal as the ordinate and fractional units of standard deviation on either side of the mode along the abscissa. It was found that a positive correlation existed between longevity and blood counts of or near to the modal value. Those animals of exceptional longevity had counts which deviated but slightly from the mode, while conversely those animals whose counts varied significantly from the mode were found to have survived but a very limited time. Such relationships have been shown by Casey and Pearce (9) to hold in regard to the susceptibility of rabbits to a malignant neoplasm. This correlation interestingly enough was evident when the whole group of animals was considered. However, when the animals were grouped according to whether or not they survived less than or longer than 90 days, it was seen that the correlation was slight in the first group and very definite in the group surviving beyond 90 days. This indicates apparently that some factors other than those expressed in the circulating blood cells are responsible for the division of any group into those which will survive and those which will not survive the first phase of the disease, since there was no significant difference in the blood counts of the first group and the second. The total number of animals used was not large enough, however, for such an analysis, and it may well be that with additional data the conclusions will be modified or amplified.

The relation between longevity and the value of the deviation of the count was most evident in the red blood cells; among the white cells the lymphocytes and basophils showed a closer correlation than did the neutrophils and monocytes. No direct relationship between longevity and the preinfection ratio of lymphocytes to monocytes could be demonstrated, a very high ratio apparently being as frequently associated with low longevity as a low ratio.

SUMMARY

It has been shown that in a large group of rabbits inoculated intravenously with bovine tubercle bacilli the disease which follows resolves itself into two distinct phases. The first phase manifests itself in widespread diffuse lesions which subsequently regress. The mortality rate shows a rise and fall during this period, which have been correlated with the extent of lesions and with the changes in the blood cells. The duration of this phase is approximately 80 to 90 days. Following this period the mortality rate again rises and falls, during a period when all animals show lesions of a chronic nature. In an infected group the number of fatalities in the first phase is a function of the size of the dose. When the size of the dose and other conditions are held constant, a definite basis upon which to compare the reactions of treated animals is established.

Studies of the blood cells during the course of the disease show that the changes in these cells reflect the course of the lesions in the first phase when other signs are lacking and offer a valuable means of making an estimate of the state of resistance of the animal from time to time.

Studies of blood counts made on rabbits before their inoculation with tuberculosis were analyzed with regard to the relative longevity of the animals. It was found that those animals whose blood cells of various sorts deviated least from the modal value for the entire group survived longer than those animals whose counts were significantly high or low.

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