

THE HISTOLOGICAL EXPRESSION OF THE NATURAL
RESISTANCE OF RABBITS TO INFECTION
WITH HUMAN AND BOVINE TYPE
TUBERCLE BACILLI.

BY PAUL A. LEWIS, M.D., AND EVERETT S. SANDERSON, PH.D.

*(From the Department of Animal Pathology of The Rockefeller Institute for Medical
Research, Princeton, N. J.)*

(Received for publication, September 25, 1926.)

The separation of mammalian tubercle bacilli into two types, designated respectively the human and the bovine in accordance with their usual derivation, as first made by Theobald Smith depended primarily on a constant difference in virulence. The recently isolated cultures of bovine type in minimal doses cause a progressive, usually fatal tuberculosis when reinoculated on young cattle or on rabbits. In equivalent doses cultures of the human type cause only local, non-progressing lesions in these animals, lesions which frequently heal entirely. Smith later described differences in the progressive changes in the reaction curve when the type cultures are grown on glycerol broth of appropriate constitution, and also other less definitely measurable cultural distinctions were clearly drawn.

The foregoing statements of fact may be paraphrased with the reaction of the animal as a primary consideration. We would then say that cattle and rabbits have a high degree of natural immunity or resistance against infection with tubercle bacilli of the human type and are very susceptible to infection with those of bovine type. This natural immunity toward bacilli of the human type is by no means absolute. In the case of the rabbit the kidneys frequently become the seat of a slowly progressive local disease which eventually destroys their capacity to function and leads to the death of the animal. At times also quite extensive loci of disease develop in the lungs. The restraint is, however, sufficient to prevent a generalized spread of the infection, and death when it occurs is caused by interference with some local function.

The nature of this natural immunity is quite unknown and is a subject of considerable interest. It is known that the injection of human tubercle bacilli intravenously does at times lead to the formation of miliary tubercles in the lungs and that these must subsequently be reabsorbed to account for the generally normal condition of these organs when the animals are killed 2 or 3 months after infection. The resistance does not therefore depend on a total failure of the tissues to react to the presence of the bacillus. Nor can the natural resistance have all the qualities of the acquired immunity possessed against reinfection in greater or less degree by all tuberculous animals which have been infected for several weeks. It is generally considered that hypersensitiveness as manifested by a hyperactivity of exudative and destructive processes is in considerable measure responsible for this induced partially immune condition. The hypersensitiveness is lacking, of course, in the normal animal and takes as a matter of fact 2 or 3 weeks to develop its effectiveness. It must be assumed that this feature is lacking in the early days of a primary infection and can be at most a reinforcing factor in any expression of natural resistance. Beyond these two points, the certainty of some positive reaction to the human bacilli and the certain absence of the hypersensitive factors in the earlier stages at least of this reaction, present knowledge fails us.

As a preliminary to a further study of this matter we have carried out a comparison of the reaction of the lung of the rabbit to the two types of bacilli using histological methods. The purpose of the present paper is to report these observations.

Krause¹ outlines a somewhat similar study as being then in progress in his laboratory. His comments, directed wholly to problems related to the localization of the infection, make no reference to the comparative aspects of the study and do not suggest any particular reference to the question of immunity.

EXPERIMENTAL PART.

Fifteen rabbits of approximately the same weight and age were injected intravenously with 1 mg. of a culture of living human tubercle bacilli, and the same

¹ Krause, A. K., *Am. Rev. Tuberc.*, 1922-23, vi, 1.

number with 1 mg. of a living bovine strain. At 2 day intervals an animal of each series was killed, tissues being preserved in formalin and Zenker's fluid. Sections were cut in paraffin. Staining was done with the eosin-methylene blue method after Mallory and Wright and for tubercle bacilli by the Ziehl-Neelsen method. A description of the conditions found in representative individuals of each series on microscopic examination follows. The gross appearances were not informing.

The human type culture (No. 7156) used was a typical one isolated from sputum at the Henry Phipps Institute of the University of Pennsylvania in the fall of 1922. It has been cultivated on glycerol agar with monthly transfers. The virulence for guinea pigs is normal for the type.

The bovine type culture (Bovine xiv) was isolated from a cervical abscess occurring in a 9 months old calf in May of 1919. The location and character of the lesions were rather atypical. The material was injected into guinea pigs and cultures isolated from them on egg medium in the usual way.

After four monthly transplantations on the egg medium the culture was placed on glycerol agar and has since been maintained on this medium. The growth characteristics and virulence for guinea pigs and rabbits are characteristic for the type.

Rabbit 1.—Human bacilli injected. 2 days. There is much thickening of the interalveolar septa very irregularly distributed. This is partly due to a very intense capillary congestion and stasis, in part to an exudation of mononuclear and polynuclear leucocytes. The mononuclear cells are of varying size and conformation, chiefly large cells of "endothelial" type. Small lymphocytes are rare, and definite phagocytic macrophages are not seen. The polynuclear cells are chiefly of eosinophilic type, showing large granules rather indistinct in outline with a tendency to fusion. These cells are found especially in foci about the smaller arterioles, sometimes in small collections in capillaries and venules. The alveoli are generally empty of either cellular or serous exudation. Occasionally one is found to be filled with an exudate chiefly made up of large mononuclear cells. The smaller bronchial branches are frequently filled with similar cells showing that while, as measured by the alveoli affected, the exudation to the surface is slight, yet "*in toto*" it is significant.

The peribronchial lymphoid tissue is not large in amount. About the smaller divisions it shows some congestion in the peripheral zone and a slight increase of large mononuclear cells toward the center of the mass. A small amount of cellular debris is found in this region also. In connection with the larger bronchial branches lymphoid nodules are found between the cartilaginous plates and the mucosa, which are swollen, have broken into and in one instance through the mucosa.

A particular search for mitotic figures in those places where large mononuclear cells are collected in foci, both in the interalveolar septa and in the lymphoid tissue, failed to reveal any.

Tubercle bacilli were found in small numbers in the peribronchial lymphoid tissue. They appeared to be extracellular. None were found in other places.

Rabbit 4.—Human tubercle bacilli injected. 8 days. The interalveolar septa are much thickened and folded decreasing appreciably and generally the area of the alveoli. The thickening is due chiefly to an exudation of large mononuclear leucocytes. A moderate number of polymorphonuclear leucocytes are found. There is no congestion.

At widely scattered points the thickening of the septa is more massive and one or more alveoli are obliterated. In a few instances at these points there is organization into definite tubercles. In these cases the central cells are mononuclears, more uniform in character than in the exudate generally. In some cases they are of moderate size with basophilic cytoplasm; in others they are larger and both cytoplasm and nuclei stain rather faintly. None show any intense staining with eosin or other evidence of necrosis.

Eosinophil leucocytes are found occasionally in the exudate generally. They are very numerous in the lymphoid tissue about the arteries and arterioles. In one instance an arteriole is thrombosed, the cells of the thrombus being large mononuclears and eosinophils. The eosinophils are found in the muscular coat of this vessel as well as massed in its neighborhood.

Mitotic figures occur occasionally in the large mononuclear cells of the exudate generally. They are found in moderate numbers in the lymphocytes of the peribronchial lymphatic tissue.

Tubercle bacilli are found in moderate numbers in those portions of the interalveolar septa where the formation of tubercles is evident or suggested, but not elsewhere.

Rabbit 7.—Human bacilli injected. 14 days. The general description given of Rabbit 4, the 8th day animal, might well be applied to this animal. The following points of difference are to be noted. The tubercles are on the whole somewhat larger and some of them have a definite peripheral zone of lymphocytes. Tubercle bacilli are found in smaller numbers than in the 8th day animal, and like that one, only in the more central portions of the tubercles. The proliferative process, as evidenced by mitotic figures in large mononuclear cells in the interalveolar septa and in the tubercles, is still active. Eosinophil leucocytes are found but in much smaller numbers than previously.

Rabbit 11.—Human bacilli injected. 22 days. In the gross, there are minute, translucent, non-caseous tubercles scattered over the surface of the lungs. The organ is not definitely more firm to the touch than normal. In general the microscopic picture presented by this animal closely resembles that of the 8th and 14th day ones in this series. The interalveolar septa are markedly thickened, perhaps slightly less so than in the preceding animals. Mitotic figures are not found in them.

The tubercles are slightly more numerous in this animal. They are always single. The centers of the larger ones are almost unstained when viewed with

the low power and have a glassy appearance in some places. The high powers show the nuclei faintly outlined, the cytoplasm hyaline, the whole having taken very little stain. The outlines of individual cells can seldom be made out, small groups being fused and the mass apparently cracked and channeled (perhaps due to fixation shrinkage). The hyalinization process is found to extend in streaks along columns of cells to the periphery of the tubercle in numerous places. This is not accompanied by any crowding of the remaining cells, the consequence being that the whole tubercle is appreciably less in its content of well preserved cells than in the earlier animals. The well preserved cells are mostly large mononuclears. Occasional large phagocytes of the macrophage type are seen. There is a small amount of free cellular debris.

The lymphoid tissue is not prominent. One peribronchial lymphoid mass contains a definite tubercle which is hyalinized as just described. In other places the lymphoid tissue shows aggregates of large mononuclear cells.

Mitotic figures are found in large mononuclear cells in the peripheral portions of the tubercles in small numbers.

Eosinophils and amphophil polynuclear leucocytes are found scattered singly in the septa, in the peripheral zones of the tubercles, and in the lymphoid aggregates. Masses or clumps of these cells are not found.

Tubercle bacilli are found in very small numbers in the centers of the tubercles.

Rabbit 15.—Human bacilli injected. 30 days. The interalveolar septa in the subpleural regions are definitely thickened but in a very irregular way. In small patches this thickening is sufficient to obliterate alveolar spaces, in others it amounts to but the presence of an extra layer of mononuclear cells in the septum. The patches are rather definitely most marked beneath the pleura and in the regions about the vascular trunks fading through the less thickened septa to normal lung tissue in the midzone. Definite tubercles are found both in the subpleural region and in the region about the main vascular trunks. In the less thickened septa there are found small collections of large mononuclear cells more or less interspersed with lymphocytes. Occasional giant cells quite isolated from other cell collections are also found in this midregion.

The peribronchial and perivascular lymphoid tissue is scanty.

The tubercles, some of them large, consist almost entirely of large mononuclear cells. There is no definite demarcation into zones except for the suggestion of an imperfect capsular layer one or two cells thick. The cells are chiefly large mononuclears. Some of them are hyaline both as to nucleus and cytoplasm, staining very faintly. Others, perhaps the majority, are of normal appearance. The normal and abnormal cells are intermixed with no regular pattern. Occasional lymphocytes and a minimal amount of cellular debris are found among the mononuclear cells.

No mitotic figures were found and no tubercle bacilli.

Rabbit 16.—Bovine bacilli injected. 2 days. There is a very marked general thickening of the alveolar walls by an exudation consisting of large and small

mononuclear and polymorphonuclear cells. Many of the latter are of eosinophil type. The thickening of the septa is sufficient in many places to almost obliterate the alveoli by compression although the alveolar wall remains clearly defined and the alveoli generally are quite free of exudate. There is no marked congestion either generally or focally. The mononuclear cells of the exudate are on the whole rather large and faintly staining. The nuclei tend to take a violet tinge with eosin-methylene blue. There are scattered, rather large aggregations of mononuclear cells, with a moderate amount of cytoplasm, which retain a rather intense, clear blue with the stain. An occasional mitotic figure is found in the interalveolar septa, pertaining to cells of the large mononuclear series.

The lymphoid tissue about the bronchial vessels is greatly increased in amount chiefly by an increase in the size of the aggregates. Lymphoid cells undergoing mitosis are found in very large numbers in the peripheral zone of the nodules. The lymphoid tissue shows a scattering infiltration with large mononuclear and polymorphonuclear leucocytes.

Tubercle bacilli, taking the sections generally, are found in but small numbers and then in the interalveolar septa; none were found in the lymphoid tissue. In a few focal points—those previously described where the septa were most swollen—collections of large numbers of bacilli, single and in clusters were found. The arrangement of the clusters was such as to suggest the intracellular position of the bacilli.

Rabbit 19.—Bovine bacilli injected. 8 days. The interalveolar septa are generally much thickened and folded due to the excessive cellular content consisting of large mononuclear cells chiefly. Many polymorphonuclear cells are also found, eosinophils being more numerous than amphophils. Over considerable areas the thickening of the septa is of such extent as to nearly or quite obliterate the alveoli, the wall of the latter often remaining intact. In many places there is a very definite organization of the cellular content of the septa into tubercles and these are in various stages. Some are little more than collections of large mononuclear cells with a tendency to basophilic staining associated with a few amphophilic and eosinophilic polymorphonuclear leucocytes. In others similar groups of cells are partly surrounded by and merged in the periphery with lymphocytes. In a few places the mononuclear cells are massed closely and show decreased staining intensity. There is no definite necrosis. The tubercles are more numerous and somewhat further advanced than in the "human" animal of the same date.

The periarterial lymphoid tissue shows masses of eosinophils.

The peribronchial lymphoidal aggregates are of large size. In some instances their central portions almost to the periphery are occupied by large mononuclear cells. In these instances the central portions also contain small foci of lymphocytes, some polymorphonuclear leucocytes, and a moderate amount of cellular debris. In some instances also the submucosa and mucosa of the adjacent bronchial vessel are infiltrated with lymphocytes even to the surface.

Mitotic figures are abundant in large mononuclear cells in the interalveolar septa; in large mononuclear cells in the tubercles; in large mononuclear cells in the central portions of the lymphoid aggregates in those instances in which these cells are prominent there; and in the lymphocytes in the lymphoid tissue generally.

Tubercle bacilli, single and in clusters, are abundant in the central portions of the more advanced tubercles. They are found single and in small numbers in the peripheral portions of the tubercles among the lymphoid cells and in the peribronchial lymphoid tissue.

Rabbit 22.—Bovine bacilli injected. 14 days. In the gross, the pleural surfaces of all lobes are marked with minute, glistening, translucent, raised tubercles, giving the lungs a granular appearance. Some of these tubercles have coalesced. Organ is firm.

The interalveolar septa generally are somewhat less thickened than in the previous rabbits of the bovine series. Mitotic figures are no longer to be found in any number in the septa when these are definitely removed from well defined tubercles.

The tubercles are much more numerous than in the 8th day animal, the addition in numbers being apparently due in large part to the organization of new, small tubercles in contiguity with the older ones. The lines of demarcation while generally rather distinct are not always so. The larger tubercles have undergone a very definite central necrosis.

Eosinophilia is pronounced in the peripheral regions of the tubercles, in the interalveolar septa, and particularly in the periarterial lymphoid tissue.

Proliferation as evidenced by mitotic figures is still in evidence in the large mononuclear cells in the peripheral portions of the smaller tubercles and in similar cells in some of the lymphoid aggregates. No mitoses are found among the lymphocytes.

The lymphoid tissue shows some well defined tubercles. The lymphocytes are quite generally replaced by large mononuclear cells.

Tubercle bacilli are very abundant in the tubercles particularly in the central portions of the older ones. They are also abundant in those areas of the lymphoid tissue where the large mononuclear type of cell predominates.

Rabbit 26.—Bovine bacilli injected. 22 days. In the gross, the lungs are much enlarged, very firm to touch, and granular. The surface is heavily marked with translucent miliary tubercles most of which have gray centers.

Microscopically, the prevailing picture is that of conglomerate miliary tubercles, the majority of which show central necrosis. These occupy fully two-thirds of the lung. Between some of the tubercles there are large alveolar spaces containing a little exudate and cellular debris, while similar ones are entirely empty. The interalveolar septa where not in contact with tubercles are but slightly thickened.

The peribronchial and periarterial lymphoid tissue is involved in the tuberculous process which here is continuous with that in the adjacent lung structure so

that the lymphoid structure is no longer demarcated or clearly recognizable. Small collections of lymphocytes are scattered here and there in the tuberculous masses. These are largest in the peribronchial and periarterial regions. In these foci mitotic figures are readily found. Occasional mitoses are found in large mononuclear cells in the peripheral zones of the tubercles. Clusters of polymorphonuclear cells both amphophils and eosinophils are recognizable but are always fragmented and irregular in staining reaction.

The blood vessels and bronchial vessels contain much cellular debris.

Wherever in the section tubercle formation is definite or where groups of cells have undergone hyaline transformation tubercle bacilli are abundant. In the definitely necrotic centers of the tubercles they are present in enormous numbers. In the remaining, more normal interalveolar septa they are scanty for the most part or absent. They are also difficult to find in bronchial and blood vessels even where the contents of these are obviously necrotic cellular remnants.

Rabbit 29.—Bovine bacilli injected. 27 days. Microscopically, discrete and conglomerate tubercles dominate the picture occupying approximately four-fifths of the area of the section. Most of the recognizable cells in the tubercles are large mononuclears. The nuclei stain faintly and the cytoplasm staining also faintly has a hyaline appearance. Many of the tubercles show complete necrosis at the centers. Only in the peripheral zones of some of the tubercles are there small areas where the mononuclear cells stain normally. Even in these areas mitotic figures are not found.

The interalveolar septa adjacent to the tubercles are thickened by mononuclear cell increase. In the small areas which are distinctly removed from tubercles the septa are quite normal in appearance. The alveoli contain very little exudate of any kind.

The lymphoid tissue of the peribronchial regions is almost obliterated by the formation of discrete and conglomerate tubercles. Such remains as are seen show no mitotic figures or other evidence of activity.

The bronchial vessels contain a small amount of cellular debris.

Throughout the section, usually in the tissue between contiguous tubercles, there are found small collections of polymorphonuclear leucocytes. These are almost always in some stage of degeneration.

Tubercle bacilli are readily found within the tubercles and wherever these are necrotic, bacilli are found in large numbers. Outside tubercles bacilli are found with difficulty.

Comparative Description.

A comparison of the foregoing protocols, as well as those not printed, reveals a close similarity in the effects of the two types of bacilli up to the 8th day. The lesions during this period are primarily an increasing interstitial exudation of mononuclear and polymorphonuclear leuco-

cytes affecting the walls of the alveolar spaces. While more or less irregular, the distribution of this lesion may properly be said to be general and diffuse. It is remarkable that the rather extensive changes as revealed by the microscope have caused no definite macroscopic change. The alveoli are very free from an exudation either serous or cellular. Considering the extent or the quality of this interstitial inflammation by itself, and it is the dominant feature of the picture, there is no difference to be made out between the human and bovine series.

In the human series the interstitial inflammation persists with little change until about the 20th day and then gradually is reabsorbed. By the 30th day only traces of it remain in the midzonal portions of the lung, while in the subpleural regions and about the central main vascular trunks the thickened interstitial tissue, more irregular than before, is still plainly evident. In the bovine series the interstitial diffuse inflammation is apparently persistent, but as the tubercles develop gradually ceases to be the dominant feature in the picture. In the last animals of the series the alveolar walls where adjacent to tubercles are thickened, while those more distant are quite normal. This suggests that a great deal of reabsorption of the exudate has taken place in this series also.

Tubercle formation is evident in both series from the 2nd to the 4th day and is equally marked in every respect till the 8th or 10th day. From this point the two series diverge in an extreme degree. In the human series the tubercles enlarge somewhat up to the 18th day, but do not become more numerous, do not become conglomerate, and show no necrosis. After the 18th day the texture of the tubercles becomes looser, individual cells become somewhat hyaline, and the appearances generally suggest that they may be undergoing a reabsorption. By the 30th day they seem definitely less numerous. Those that remain are in some instances quite large and present ill defined connective tissue formation in the peripheral zone. In the bovine series after the 8th day the tubercles increase progressively in number, they coalesce to form conglomerate masses which undergo a process of fusion, and they become necrotic at the centers. Necrosis is evident at 14 days. These changes continue to develop to the end of the series when three-fourths to four-fifths of the area of the sections is occupied by clearly defined tubercles.

Tubercle bacilli are not abundant in either series up to the 8th day, and the two series show about equal numbers during this period. They are found by locating in the diffuse exudate those more definite clusters of mononuclear cells which seem precedent to the formation of well defined tubercles. Associated with those cells, sometimes within them, sometimes apparently between them, the bacilli are found.

After the 8th day bacilli become very difficult to find in the human series. In the bovine series they become increasingly numerous and very rapidly so. By the 14th day in this series, when tubercle formation is well advanced and necrosis has begun, the bacilli are abundant in the tubercle, and after this time are frequently found there in enormous numbers. Even in this series it is very difficult and usually impossible to demonstrate bacilli in the interalveolar tissue apart from all collections which are definitely segregated in such a way as to suggest tubercle formation.

The lymphoid tissue shows a marked hyperplasia in the bovine series from the 2nd day to about the 20th day. After this the lymphoid tissue becomes increasingly occupied by tuberculous tissue and finally almost disappears as such. Throughout this series frank development of tubercles in the lymphoid tissue is later than in the interalveolar exudate. In the human series the lymphoid tissue undergoes no definite hyperplasia, as estimated by the amount of tissue present, and does not become tuberculous to any considerable extent. Mitotic figures are frequently found in cells of lymphocytic type showing that there is here also an increased lymphoidal activity. Occasional large mononuclear cells associated with a few tubercle bacilli were found in lymphoid tissue early in the human series.

Experiments with Killed Bovine Bacilli.

As a check on the series of observations just described a shorter series was carried out in which 1 mg. of the bovine culture previously used was injected into two series of rabbits; in the one series living bacilli were used; in the second series the same suspension was used after heating to 60°C. for 1 hour, a procedure known to be lethal to the bacillus. Animals in each series were killed at 4 day intervals and examined in the same manner as that described.

The series injected with living bacilli developed in all respects in a comparable manner to that of the previous bovine series.

In the series injected with the killed bacilli the diffuse interstitial exudation developed with much the same intensity as that described for the living cultures either bovine or human. This exudate persisted in a way comparable to that previously observed in the human series, being still well defined at the 20th day which was as far as the series was carried. Well defined tubercles were not formed, but in the earlier members of the series small clusters of large mononuclear cells were segregated and with these were sometimes found a few tubercle bacilli. Polymorphonuclear cells were much less abundant in the exudate with killed bacilli than in the case of the living, either human or bovine. They did occur as collections of eosinophils chiefly and for the most part localized in foci of considerable mass in the perivascular tissues, particularly in the peribronchial submucosa. The lymphoid tissue shows an indefinite hyperplasia in this series marked by an increase in mitotic figures in the small lymphocytes within the nodules, but with no definite increase in the size and number of the nodules.

DISCUSSION AND SUMMARY.

From the foregoing description it is evident that when rabbits are inoculated intravenously with equal amounts of tubercle bacilli of bovine and human type respectively, they are subject to an immediate reaction in the form of an interstitial pulmonary exudation, which, being of equal severity and character does not serve to distinguish the type.

There is an hyperplasia of the lymphoid tissue which is much more pronounced in the bovine series and which may distinguish this type. Opinion on this point may well be reserved until other typical cultures are examined for this response.

The two types are sharply distinguished by the behavior of the tubercle bacillus and by the progression of tubercle formation. Tubercles are formed by both types and for about 1 week after inoculation they are not distinguishable. Progressively thereafter those formed as a response to the bovine bacillus become more numerous; they caseate and become conglomerate, finally coming to occupy the major part of the pulmonary tissue and its associated lymph nodes.

With the human type the tubercles do not progress to caseation, do not become more numerous after their first well defined formation, and finally tend to disappear. The human type bacillus does not multiply considerably, if at all, and disappears early. The bovine bacillus suffers little or no restraint in growth and finally multiplies enormously. It seems clear that so far as histologic evidence goes the fundamental difference in the reaction of the rabbit to the two types of tubercle bacilli is referable to the ability of the animal to restrain the growth of the human type or to the prevalence of conditions which permit a most vigorous multiplication of the bovine type. The initial cellular responses seem to be qualitatively of the same order and their quantitative distinctions are for the most part developed coincidentally with the manifest growth of the bovine type bacillus.

If we undertake to state the observed results in the terminology of immunity we can say only that the histologic picture discloses a difference in the rate of bacillary multiplication which suggests that a difference in the physiologic requirements for growth of the two types of bacilli is satisfied or unsatisfied, in the respective cases, by the rabbit as host; or on the other hand, that there is a positive growth-restraining action exerted with efficiency against bacilli of the human type. It is evident that the present observations furnish no points of discrimination between these alternatives. There is, however, an occasional result of the injection of human type bacilli into rabbits (not seen in this series) which offers a suggestion.

When animals so injected are allowed to live for 2 or 3 months, the lungs at autopsy not infrequently present a few nodules of large size, often 1 cm. in diameter, which are found to be well encapsulated, soft, caseous masses. These often contain large numbers of tubercle bacilli. Since we know nothing of the particular conditions which give rise to these rather exceptional formations it is impossible to draw general conclusions from them, but they do suggest that the rabbit is not lacking in the food materials required by the human type bacillus; and that if the more usual suppression of this type is due to failure of its essential nutritives, it is rather a question of the distribution within the animal than an absence which is responsible. The usual result would then appear to be due to a positive growth-restraining action exerted against the human type bacillus.

Certain other points of interest in the histologic picture described are worthy of comment.

The lymphocytes do not appear as active cells in any preponderant way in either series and they are much less in evidence in the immune case (human type) than in the non-immune (bovine type). This might suggest that the activity of this cell type is a response to infection rather than that it furnished an effective preexisting barrier against infection in this particular case. If the lymphocytes were the most important agents in the immune reaction, it might be expected that they would show an immediate sharp response in the human series.

The large mononuclear type of cell is clearly most closely related physically to the tubercle bacillus within the body of the rabbit and this without distinction as to bacillary type. Foci of these cells are the loci of the disappearing bacilli of human type, and in either the active or necrotic state similar cell collections are the site of the most vigorous multiplication of the bovine bacilli. These cells undoubtedly stand in a central position in any picture which can be drawn of experimental tuberculosis in the rabbit and deserve as a consequence all of the very considerable attention they have received at the hands of numerous observers in recent years.

It has been quite usual of late to consider that the whole of the essential reaction of the animal against tubercle bacilli is carried by the cells of the mononuclear series, either lymphocytes or large mononuclears according to the predilections of the observer. We cannot, however, entirely overlook the presence in very large numbers of polymorphonuclear leucocytes, both amphophilic and eosinophilic, in this experimental series. They are much less prominent in the animals injected with the killed culture and hence can hardly be neglected on the assumption that they are merely a part of a reaction to an indifferent foreign body. They are in large measure a reaction to the living organism: whether a primary and direct or a secondary, indirect consequence of its presence we are unable to decide.

These cells are not massed in any regular relationship to the well formed tubercles or to the clusters of mononuclear cells initiating tubercle formation. They are also very much less abundant in the very severe late lesions of the bovine type where enormous numbers of

bacilli are enclosed in the tubercles. It seems possible that the polymorphonuclears are a response to the living free tubercle bacilli as contrasted with either the dead bacilli or the living bacilli segregated in mononuclear cell clusters or in tubercles. They would appear also to be related to something apart from the bacillus itself, either a diffusion or disintegration product, since phagocytosis of bacilli, or the presence of bacilli in close physical relation to polymorphonuclear leucocytes, is so infrequent in general as not to have been observed in this series of experiments.

CONCLUSIONS.

The natural resistance of the rabbit to infection with the tubercle bacillus of human type is apparently referable to a failure of this type of bacillus to multiply in the body of this species to any considerable extent. Exceptionally there are localized lesions (kidneys and lung nodules) which are associated with abundant growth and which show that this failure to multiply may be due to some positive growth-restraining factor rather than to a failure of suitable nutritive materials. There is no qualitative difference in the cellular response to the bacillus of human type contrasted with that to the bovine type, which seems adequate to account for the difference in susceptibility. The eventually more abundant tubercle formation and the degenerative changes occurring in the tubercles formed with the bovine type bacillus seem to be a consequence of the abundant growth of this type; the initial response to equal quantities of the two when injected intravenously being approximately the same both quantitatively and qualitatively.