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Experimental Tuberculous Meningitis in Rabbits*

III. Sequence of Histological Changes Following Intracisternal Infection in Sensitized and Non-sensitized Animals

By

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With 5 Figures in the Text

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Although tuberculosis has been the subject of many and extensive studies, there still exists a paucity of knowledge concerning certain manifestations of the tuberculous process. Thus for example, there is little available information on the chronological sequence of early histological lesions in brain and leptomeninges following tuberculous infection of the subarachnoid space. This was already noted by RICH and MCCORDOCK (1933) who wrote that "histological descriptions of the lesions have been either very sketchy or, more usually, completely lacking in reports". In the thirty years which have followed this comment, the attention of investigators, including the above-mentioned ones, has not been particularly set at describing the detailed histopathology of the primary complex in tuberculous meningitis and at following at close intervals its developments as compared to that in previously sensitized subjects.

The present work was done in an endeavor to fill this gap in information.

Material and methods

Thirty-two rabbits weighing approximately 2 kg each were used. Animals underwent sensitization with killed tubercle bacilli of the bovine strain. The bacilli were grown on Dubos medium for 14 days and then killed by heating for one hour at 80 degrees centigrade. Various procedures for sensitization were tried, since rabbits do not sensitize well to tuberculin as judged from skin reactions (TAYLOR and HUNTER 1957). The best results were obtained with two fortnightly intraperitoneal injections of one billion killed tubercle bacilli suspended in 1 cm^3 of normal saline. Ten out of fourteen rabbits thus treated showed a positive skin reaction when tested with 0.2 cm^3 of a 1:10 dilution of O. T. injected intracutaneously 3 weeks following sensitization. Reactions were read at 24, 48 and 72 hours.

The 10 animals sensitized successfully formed the experimental group referred to hereafter as "sensitized rabbits"; 22 non-sensitized rabbits formed the experimental group referred to hereafter as "non-sensitized rabbits".

All animals of both experimental groups were inoculated with 100,000 live tubercle. The suspension was injected into the cisterna magna following withdrawal of the same amount of clear cerebro-spinal fluid.

Animals of both experimental groups died, or were killed, with in one tofour weeks following the intracisternal inoculation. The whole brains were removed and fixed in 10% formalin.

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Seven standard coronal sections including brain with leptomeninges, parts of the optic nerves as well as roots of the III, V and VII cranial nerves, were embedded in paraffin. Histological sections were stained with hematoxylin and eosin. The Ziehl-Neelson method, as modified by VERHOEFF, was used together with controls for identifying acid-fast bacilli in histological sections.

Histological findings

1. Non-sensitized animals

At 3 and 4 days following the intracisternal inoculation with live tubercle bacilli no reactive changes were observed within leptomeninges, brain, reticular tissue around blood vessels of brain, choroid plexuses, optic nerves and chiasma, roots of cranial nerves. No acid-fast bacilli, either free-lying or intracellular, could be demonstrated.

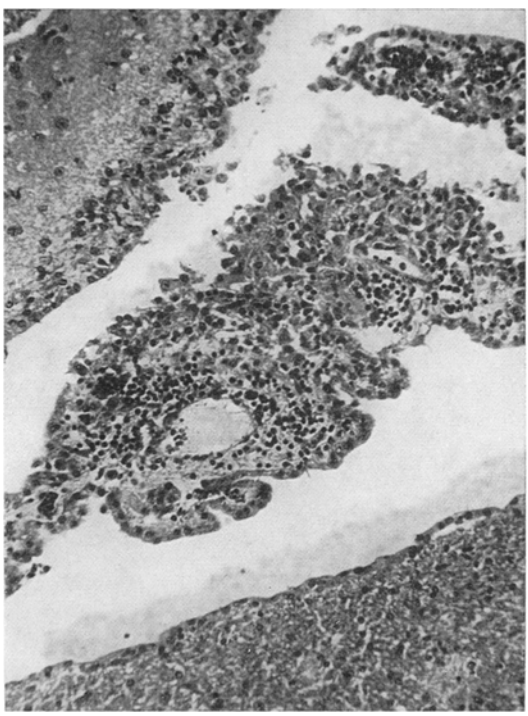

Fig. 1. Non-sensitized rabbit, 7 days following intracisternal inoculation with live tubercle bacilli. Choroid plexus of lateral ventricle is infiltrated by lymphoid cells, and by larger mononuclear cells interpreted as activated local histiocytes (Hematoxylin-eosin ×134)

At five days a small number of lymphoid cells appeared within the pia-arachnoid membrane alone. No signs of phagocytic activity were present, no acid-fast bacilli were identified in the sections.

At seven days the lymphoid cells were still scarce within the pia-arachnoid; however, there were now present swollen and rounded mononuclear cells with an eosinophilic cytoplasm and a pale nucleus, accumulated around leptomeningeal blood vessels. Such cells, which were interpreted as activated local elements of the reticuloendothelial system, were also present in the reticular tissues around intracerebral blood vessels, as well as around vessels within the optic chiasma, optic nerves, and choroid plexuses (Fig. 1). Signs of an exudative process were not present and acid-fast bacilli could not be demonstrated.

At 8, 9, 10 and 12 days, there was a gradual increase in number of the activated reticuloendothelial cells, many of which were functioning as macrophages, having engulfed acid-fast

bacilli. Acid-fast bacilli were also found outside macrophages, among the activated and proliferated reticuloendothelial cells. Actually, it was impossible to demonstrate the origin of these cells, although one could assume their R. E. S. origin. The above changes, including the presence of intra- and extracellular acid-fast bacilli, were observed in the leptomeninges and also in the perivascular reticular tissue within brain, cranial nerves and choroid plexuses.

The appearance of noticeable signs of an acute exudative inflammatory reaction was first observed at 15 days following the intracisternal infection of the animals. It was represented by a fine network of fibrin in the leptomeninges and the perivascular reticular tissues of the brain, optic chiasma, optic nerves and roots of III, V and VII cranial nerves, infiltrated by many polymorphonuclear leucocytes and lymphoid cells. Leptomeningeal and intracerebral

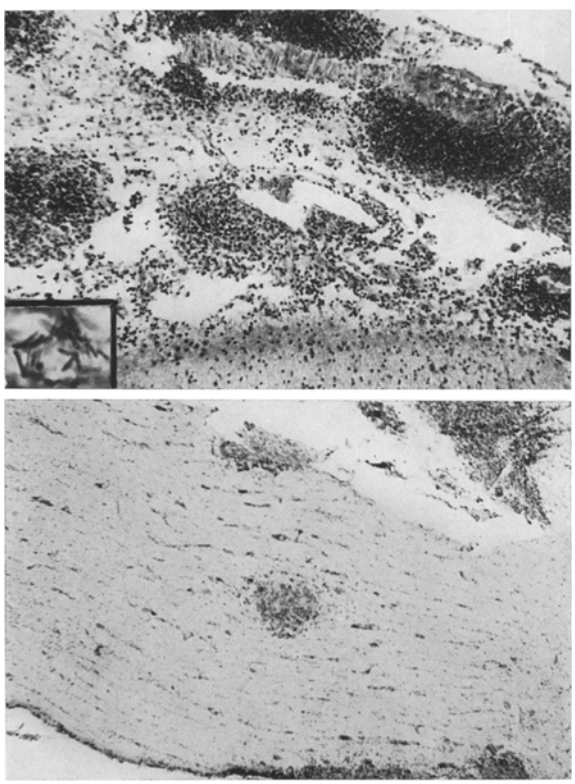

Fig. 2a and b. Non-sensitized rabbit, 15 days following intracisternal inoculation with live tubercle bacilli. a Acute necrotising hyperergic angiitis of leptomeningeal blood vessels (Hematoxylin-eosin ×100). Small square at left bottom shows acid-fast bacilli in necrotising exudate (Ziehl-Neelsen ×1300). b Tuberculous foci in optic nerve (Hematoxylin-eosin ×33)

blood vessels, as well as blood vessels within the optic chiasma, optic nerves and choroid plexuses were simultaneously affected by an acute necrotising inflammatory process of the type commonly referred to as hypersensitivity angiitis, with acid-fast bacilli in the exudate (Fig. 2a). From this location the vasculitis and accompanying perivasculitis had, here and there, broken through pial-glial membranes into the adjacent brain or nerve tissue and had thus caused an acute focal tuberculous encephalitis and cranial neuritis (Fig. 2b). Many acid-fast bacilli were present in these lesions.

Besides the inflammatory cellular exudate, a large number of epithelioid granulomata, some of them caseated, all containing large amounts of acid-fast bacilli, were present in the pia-arachnoid membrane and in the perivascular reticular tissue of the brain, cranial nerves and choroid plexuses.

At 17 days the necrotising hypersensitivity angiitis had subsided. Some of the epithelioid granulomata had now acquired a sharper outline due to the appearance of peripheral lymphocytic rims typical of classical tubercles (Fig. 3). Caseation, with or without multinucleated Langhans cells, was present in some tubercles. Many acid-fast bacilli were identified within the latter.

The leptomeningeal tuberculous process had spread contiguously along penetrating vessels (Fig. 4a), some of which could be followed down to the subependymal plate of the lateral ventricles. From here the process had broken into the cerebral parenchyma and through the ependymal ventricular lining, causing ulceration of the ventricular wall (Fig. 4b). Thus, a pathway was established for the tubercular infection, connecting the subarachnoid space with the ventricular lumen and feeding tubercle bacilli into the latter.

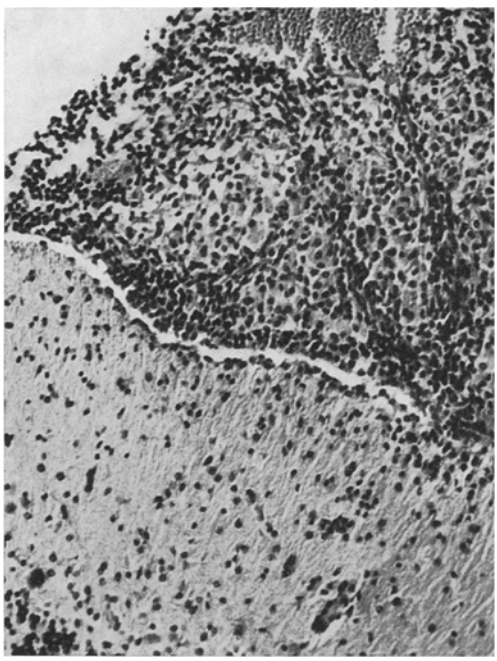

Fig. 3. Non-sensitized rabbit, 17 days following intracisternal inoculation with live tubercle bacilli. Epithelioid cell granulomata with peripheral lymphocytic rims, in leptomeninges of cerebrum (Hematoxylin-eosin ×150)

At 25, 26 and 27 days the histological appearances of the brains were identical with one another. Many leptomeningeal tubercles had coalesced into tuberculomata, distending and obliterating the subarachnoid space, pressing upon and indenting the cerebral cortex, and leptomeningeal arteries had their walls segmentally involved in a productive tuberculous process which caused stenosis, to complete obstruction, of the lumen (obliterative panarteriitis) with small, anemic infarctions in corresponding areas of the brain. There was a multifocal tuberculous encephalitis, cranial neuritis, choroiditis and ulcerative ependymitis. Many acid-fast bacilli were present in the lesions.

II. Sensitized animals

The shortest interval, following intracisternal inoculation, at which animals of this group were killed was 2 days.

At 2, 3 and 5 days following the intracisternal inoculation with live tubercle bacilli, there was an acute exudative leptomeningitis with polymorphonuclear leucocytes, lymphoid cells, large mononuclear cells with pale nuclei and substantial cytoplasm, and many acid-fast bacilli. A similar inflammatory reaction was observed in the perivascular tissue of blood vessels

within the brain, roots of the cranial nerves included in the sections, and the choroid plexuses. Pia-arachnoid blood vessels were involved in an acute, but non necrotizing, hypersensitivity angiitis.

At 9, 12 and 15 days, the acute inflammation had subsided. Only a rare focus of acute angiitis was seen. A considerable number of histiocytes, some engaged in phagocytosis of acid-fast bacilli, others arranged in compact collections having the appearance of epithelioid granulomata, were present in the leptomeninges covering the brain (Fig. 5a), the optic nerves and the roots of other cranial nerves, as well as in the perivascular reticular tissue of these structures, and in the choroid plexuses. Some of the granulomata had peripheral lymphocytic rims and, though caseation centres were not present, they could be promptly identified as tubercles containing large numbers of acid-fast bacilli. The walls of pia-arachnoid blood vessels were involved, too, in this productive tuberculous process which caused stenosis or obliteration of the vessels' lumens, and spread along the perivascular tissue of perforating blood vessels and therefrom, across pial-glial membrane, into the parenchyma of the brain.

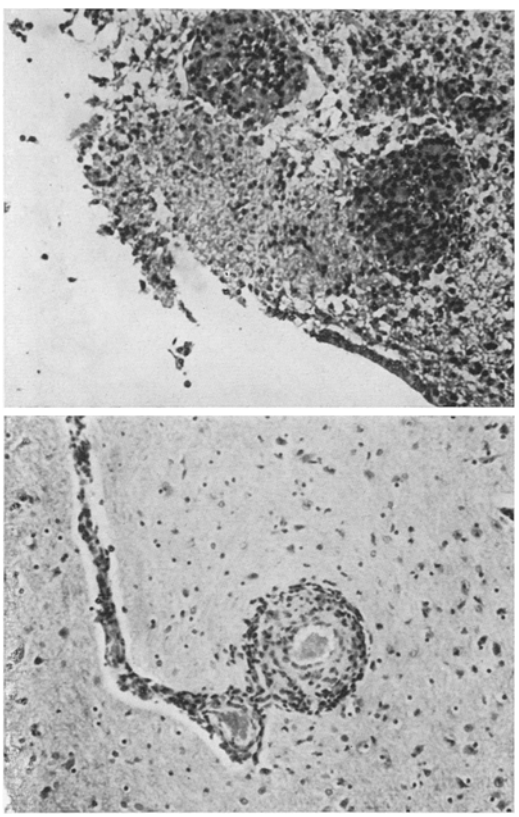

Fig. 4a and b. Non-sensitized rabbit, 17 days following intracisternal inoculation with live tubercle bacilli. a Tuberculous inflammation spreading contiguously along penetrating blood-vessels deep into the brain substance (Hematxoylin-eosin × 134); b Ulceration of wall of lateral ventricle by tuberculous process spreading along blood vessels (Hematoxylin-eosin × 150)

At 18, 23 and 27 days the histological pictures in leptomeninges, brain substance, optic nerves, roots of III, V and VII cranial nerves, and choroid plexuses were identical with those at 25, 26 and 27 days in non-sensitized animals.

Discussion

In our experiments the major reactions of the rabbit brain to the intracisternal injection of live tubercle bacilli were histologically similar in both non-sensitized and sensitized animals, but differed in the time of their appearance and in intensity, being more severe, and appearing, on the whole, later, in the non-sensitized animals (Table). These reactions took place not only in the leptomeninges, but also in the reticular tissue accompanying the blood vessels which perforate the brain, the roots of the cranial nerves and the choroid plexuses. In other words, what predominantly appeared as a tuberculous leptomeningitis was, in fact, a simultaneous meningo-encephalo-neuro-choroiditis. It follows from this observation, that tubercles in the choroid plexuses, in cases of spontaneous tuberculous meningitis in humans, must not of necessity be a precursor of the leptomeningeal infection, as claimed for a proportion of cases by KMENT (1924) HUEBSCHMANN (1928) and ENGEL (1944), "the bacilli passing through the natural channel of

communication to the base of the brain and thus producing the basal type of tuberculous meningitis" (ENGEL). Our findings show that the tuberculous involvement of the choroid plexuses may be a common result of the infection, which was elicited *simultaneously* in the various tissues and structures which had been brought in contact with the tuberculoprotein circulating with the cerebrospinal fluid. It has already been shown that tuberculous involvement of the substance of the optic nerves and chiasma is part and parcel of the usual course of experimental tuberculous meningitis in rabbits (BEHAR et al. 1960).

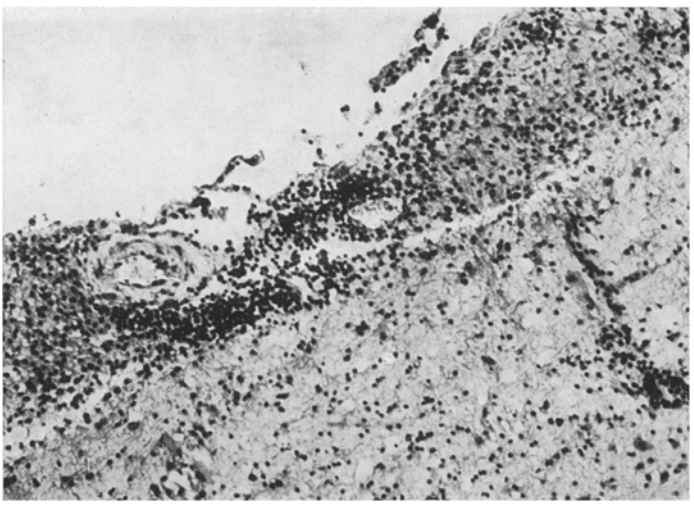

Fig. 5. Sensitized rabbit, 9 days following intracisternal inoculation with live tubercle bacilli. Epithelioid-cell granulomata in leptomeninges of cerebrum (Hematoxylin-eosin ×150)

It appears from our findings that direct infection of the cerebral leptomeninges of a non-sensitized rabbit with live tubercle bacilli will not cause first a focal primary lesion (Ghone lesion), as in the cases of primary pulmonary and intestinal tuberculosis, but will, after a quiescent period of two weeks, suddenly manifest

Table

	Sensitized group	Non-sensitized group
First signs of phagocytosis of tubercle bacilli	2nd day	8th day
Appearance of an acute nonspecific inflammatory exudate	2nd day	15th day
Appearance of local signs of a hypersensitivity reaction	2nd day	15th day
Appearance of epithelioid-cell tubercles	9th day	15th day
Appearance of caseation	18th day	15th day

itself as a diffuse acute specific inflammation with components of an allergic reaction (acute angiitis). This reaction is evoked, in the sensitized rabbits, much more rapidly (see Table) and does not bear the signs of hyperergy with massive caseation as it does in the non-sensitized group. A swift response to the intracisternal injection of tuberculoprotein has already been observed by HUNTER and TAYLOR (1957) who described cell and protein changes in the cerebrospinal fluid of sensitized rabbits 24 hours after they were injected intrathecally with P.P.D.

On the other hand, the productive component of the inflammation, i.e. tubercle formation, although it appears, on the whole, earlier in the sensitized than in the non-sensitized animals, does not do so as suddenly in the former group as it does in the latter (see Table).

It also appears, from our observations, that the aforementioned reactions in both sensitized and non-sensitized animals are preceded by a transformation of local histiocytes into active Koch-bacilli—engulfing macrophages—a process which, perhaps, is necessary for the bacilli to discharge their sensitizing breakdown products, which are then circulated with the cerebrospinal fluid.

The conspicuous absence of acid-fast bacilli from the histological sections of the brains of non-sensitized rabbits during the first week following intracisternal inoculation could, we believe, be explained in the following manner: During the stage preceding phagocytosis the bacilli apparently circulate with the cerebrospinal fluid as free-lying, unattached particles which could be easily washed out of the subarachnoid space during the various technical procedures of the histological sections.

It may be of interest now to consider, briefly, the extent to which the clinical course of human tuberculous meningitis would be, by parallelism, compatible with such histological findings as observed in our animals.

In the vast majority of patients with tuberculous meningitis the process is secondary to a lesion in another organ. One may, therefore, assume that the patients' leptomeninges are already sensitized to tuberculoprotein at the time they are infected with tubercle bacilli, as suggested strongly by the work of SWITHBANK et al. (1953). If so, one should expect, in all these cases, a rapid inflammatory response of the leptomeninges, parallel to that observed in our sensitized animals. Indeed, the existence of tuberculous meningitis of acute onset (TAYLOR et al. 1955) is well known and the fulminant initial reaction is presumably caused by the entry of massive quantities of tubercle bacilli into the subarachnoid space and by their coming in contact with hypersensitive leptomeninges. However, these are the rarer cases of human tuberculous meningitis, the usual clinical course of the disease being one of insiduous onset. One may assume, as suggested by our experiments, that in these cases, too, as in the rarer, fulminant ones, the response of the leptomeninges, provided they are sensitized, is prompt; but that differences in clinical course will reflect quantitative differences in the initial inflammatory reaction, this being dependent upon the various extrinsic and intrinsic factors which are known to determine the degree of tissue allergy in tuberculosis.

Summary

Rabbits sensitized by intraperitoneal injections of tubercle bacilli and later showing positive skin reactions to tuberculin, as well as non-sensitized rabbits, were given a single inoculation with live tubercle bacilli into the cisterna magna. The animals' brains were examined histologically, 2 to 27 days following the inoculation, for a comparative study of local reactions and their temporal sequence. It was found that in both sensitized and non-sensitized rabbits the major reactions of the brain were histologically similar in type but differed in intensity, in the time of their appearance and in the intervals between them. These reactions were more severe and, on the whole, slower in appearance in the non-sensitized animals

and consisted of phagocytosis of tubercle bacilli by activated local histiocytes, the appearance of acute, non-specific inflammation with components of tissue hypersensitivity, tubercle formation by epithelioid cells and caseation, in that order of sequence. These reactions were evoked not only in the cerebral leptomeninges, but also, and simultaneously, in the perivascular reticular tissue of intracerebral, intraneural and choroid-plexus blood vessels. It thus appears that tuberculous involvement of the brain parenchyma, cranial nerves, ependymal lining and choroid plexuses in tuberculous meningitis may not be a complication but a common manifestation of that process.

Zusammenfassung

Kaninchen, die mit intraperitonealen Injektionen von Tuberkelbacillen sensibilisiert waren und daraufhin positive Hautreaktionen auf Tuberkulin zeigten, und nicht sensibilisierten Kaninchen wurde eine einmalige Injektion von lebenden Tuberkelbacillen in die Cisterna magna verabreicht. Die Tiergehirne wurden (2—27 Tage nach der Beimpfung) zum Zwecke der vergleichenden Untersuchung der Lokalreaktionen und deren zeitlicher Folge histologisch untersucht. Es wurde gefunden, daß in beiden Fällen, also sowohl bei den sensibilisierten als auch bei den nichtsensibilisierten Kaninchen die Hauptreaktionen des Gehirns in der Zeit ihres Auftretens und in den Intervallen histologisch ähnlich, jedoch dem Grade nach verschieden waren. Diese Reaktionen waren schwerer und vor allem von langsamerer Manifestation bei den nichtsensibilisierten Tieren und bestanden in Phagocytose der Tuberkelbacillen durch aktivierte ortständige Histiocyten. Auftreten von akuter, unspezifischer Entzündung mit Komponenten der Gewebsüberempfindlichkeit, Tuberkelbildung aus epithelioiden Zellen und Verkäsung in dieser Reihenfolge. Diese Reaktionen wurden nicht allein in den Gehirnmeningen hervorgerufen, sondern auch — gleichzeitig — im perivasculären retikulären Gewebe der intracerebralen, intraneuralen und der Plexus-Blutgefäße. Es scheint also, daß der tuberkulöse Befall des Gehirnparenchyms, der Hirnnerven, des Ependyms und des Plexus bei tuberkulöser Meningitis nicht eine Komplikation, sondern eine übliche Manifestation im Rahmen des Grundprozesses darstellt.

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