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HISTOPATHOLOGY AND HISTOCHEMISTRY OF YERSIN TYPE TUBERCULOSIS IN RABBITS

Development of the Disease after intravenous Infection with Mycobacterium avium

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Abstract

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After intravenous aplication to rabbits of *Mycobacterium avium* the first morphological changes appeared 48 hours post infection. The most characteristic morphological feature of the infection was a strong proliferation of the cells of mononuclear macrophage system.

Epitheloid cells were formed 7 days after infection and multinucleated giant cells appeared after the 10th day.

In following intervals the process grew enormously above all in the liver and spleen. In this period the number of mycobacteria increased and this process was evident also in the cytoplasm of epitheloid cells.

Starting from the 14th day after infection epitheloid cells in the center of larger foci underwent necrobiotic changes.

Tuberculosis, Mycobacterium avium, Yersin type, macrophages, epitheloid cells, giant cells.

In rabbits, two types of development of tuberculous infection are known and they differ among other features by morphological changes as well. Villemin type tuberculosis is usually caused by *Mycobacterium bovis* or fairly low doses of *Mycobacterium avium* (Yamamoto et al. 1961a, b). Morphological features of this type of tuberculosis appear in form of tuberculous nodules. The other type is the Yersin type tuberculosis described by Yersin and the course of this disease is usually fairly acute (Yamamoto et al. 1961a, b, c, 1962; Černý 1965; Mohelská et al. 1975 etc.). The description of this type of the disease gave Yamamoto et al. (1961a), Černý (1965) and an electron microscopic study of the Yersin type tuberculosis conducted Mohelská et al. (1975). In the morphological picture dominant affection of the liver and spleen is prominent with very clearly increasing number of mycobacteria and reaction of the cells of mononuclear macrophage system.

In this paper which describes a preliminary experiment the main task is the study of development of microscopic changes in various organs and study of the growth of *Mycobacteria* in this type of tuberculosis.

Materials and Methods

In the experiment 15 rabbits were used weighing about 1,200 g. Experimental animals were infected intravenously with a suspension of virulent culture of *Mycobacterium avium*, the dosis being 0.02 mg for each rabbit. The animals were gradually sacrificed at intervals of 2, 4, 6, 12 and 24 hours and of 2, 5, 7, 9, 10, 12 and 14 days. Two remaining rabbits died 16 and 18 days after infection.

Sacrificed animals were necropsied and smears from various organs were prepared and stained by Ziehl-Neelsen method. At the same time the organs from rabbits sacrificed up to 5 days after infection were cultivated for mycobacteria. Tissue samples from the lungs, liver, spleen and kidneys were fixed in neutral formol and paraffin sections were stained by hematoxylin-eosin and Ziehl-Neelsen method for mycobacteria.

Results

In none of the rabbits sacrificed 2, 4, 6, 12 and 24 hours after infection microscopic changes were present. Histopathological examination of the lungs, liver, spleen and kidney of these animals did not reveal any changes which could be explained as caused by the infection. In some of the liver sinusoids and in the spleen tissue, mononuclear cells were present. In the smears stained by Ziehl-Neelsen method finding of very few mycobacteria was only exceptional although by culturing the mycobacteria were present in all organs and in the blood of experimental animals.

Two days after infection locally thickened alveolar septa were seen in lungs. Very rare alveolar macrophages were found and close to the walls of some blood vessels neutrophil leucocytes and monocytes were scattered. In the liver small groups of few mononuclear cells were seen. The spleen and kidneys were microscopically intact. In the sections from spleen and liver single mycobacteria were present. Cultures from all organs revealed mycobacteria.

Five days after infection the interalveolar septa were clearly thickened. The thickness of the septa was caused by proliferation of mononuclear cells. Alveolar macrophages in alveoli were present. The changes of liver were clear and consisted

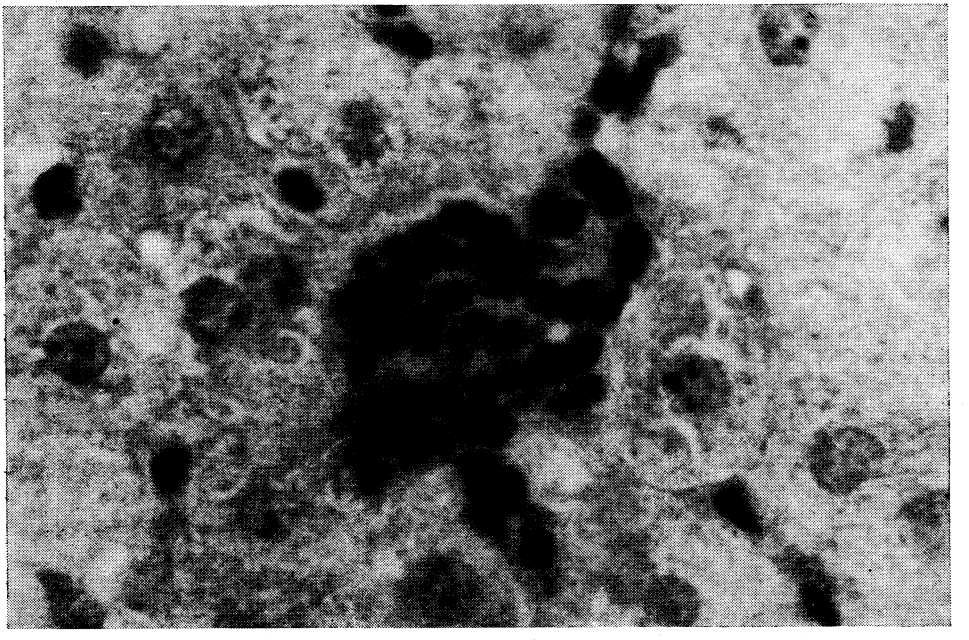

Fig. 1.
Small accumulation of macrophages in liver 5 days after infection. H. E., 1.600×.

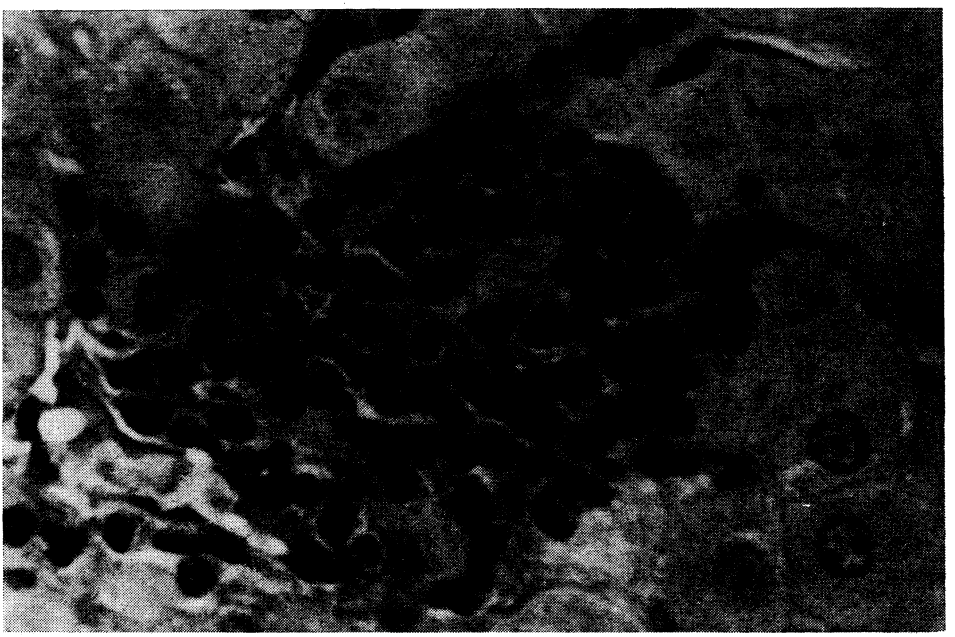

Fig. 2.
Enlargement of macrophages foci and formation of epitheloid cells 9 days after infection. H. E., 1.600×.

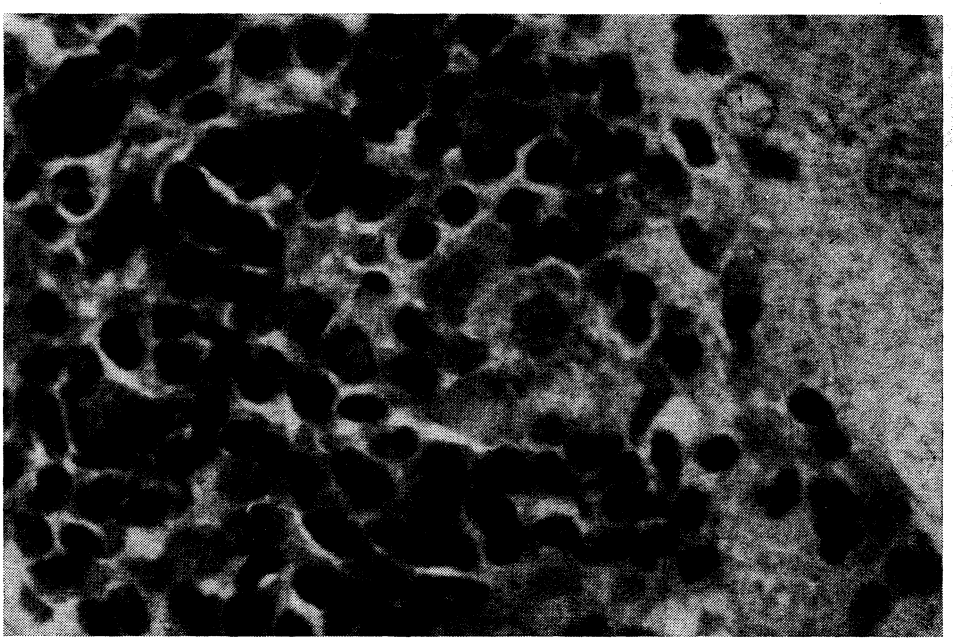

Fig. 3.
Formed epitheloid cells 10 days after infection. H. E., 1.600×.

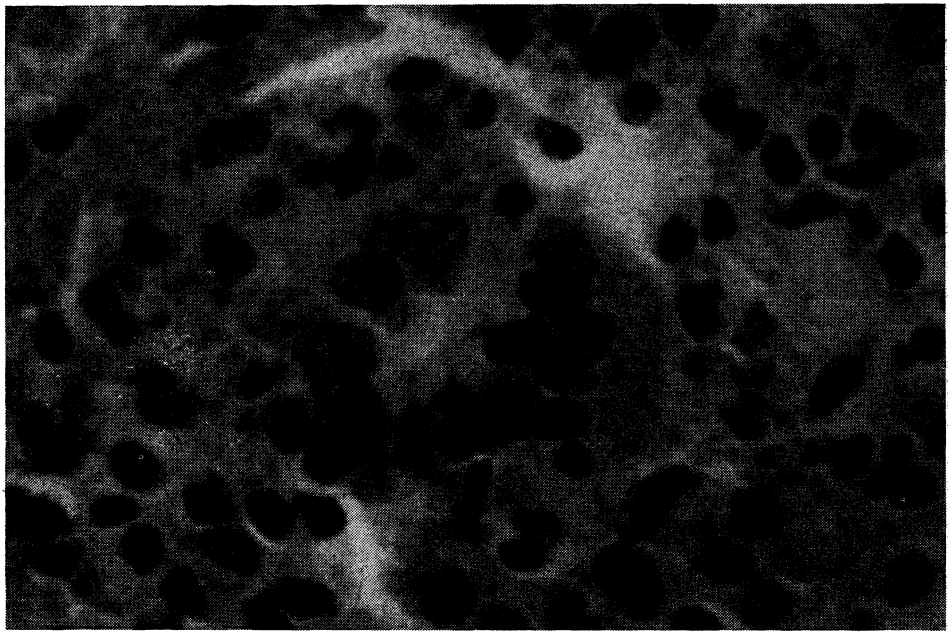

Fig. 4.
Formation of multinucleated giant cells by fusion of epitheloid cells, 12 days after infection H. E., 1600×.

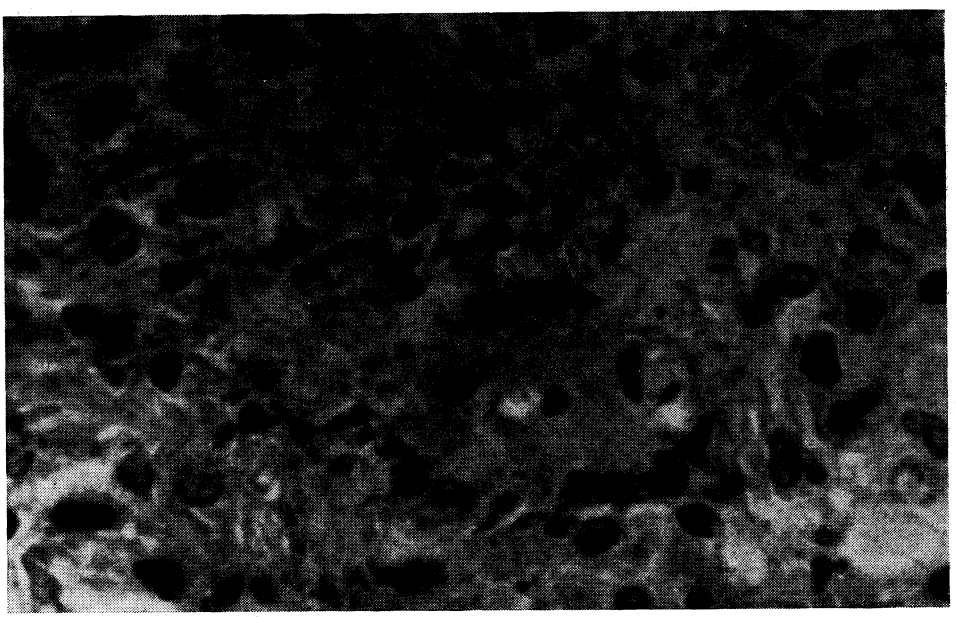

Fig. 5.
Beginning of necrobiotic changes with pycnotic nuclei of epitheloid cells in the middle of focus 16 days after infection. H. E., 1600×.

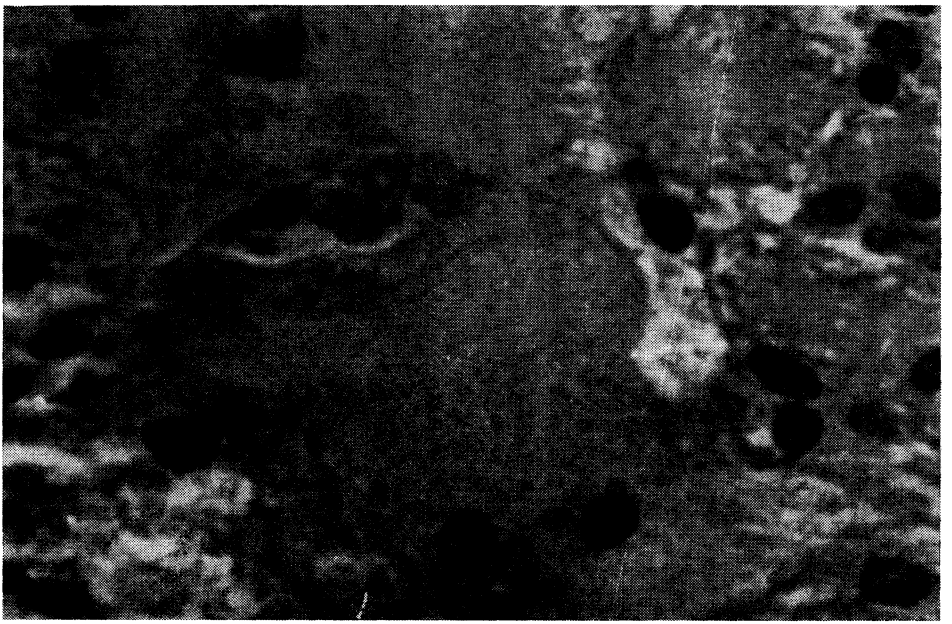

Fig. 6.
Necrotic center of a tuberculous nodule 18 days after infection. H. E., 1600×.

of proliferating macrophages and increased number of Kupffer's cells in the liver sinusoids. In the spleen, proliferation of macrophages was fairly prominent. Mycobacteria were present in the sections of the liver and spleen and they were mostly phagocyted in cytoplasm of macrophages. Cultures of all organs revealed mycobacteria.

Seven days after infection the dominant feature in all organs was the formation of epitheloid cells. In lungs small granulomas composed of macrophages and few epitheloid cells in the center were found. The sections of liver revealed increased number of small foci, scattered throughout entire parenchyma. These foci were formed in liver lobules and in portal areas. Very few similar foci were found in the kidney and they were mostly localized around the glomeruli. Proliferation of macrophages in the spleen was diffuse. In cytoplasm of macrophages and epitheloid cells in liver and lungs were present phagocyted mycobacteria.

Microscopic changes in the organs of rabbits sacrificed 9 and 10 days after infection revealed that the progress of tuberculous process in lung was very limited and there was no solidification of the lung tissue. But the number of small foci in the liver increased considerably and the main feature was formation of epitheloid cells. In the spleen proliferation of macrophages and small foci of epitheloid cells were found. These cells were present in small nodules in the kidneys as well. Some of the epitheloid cells contained several mycobacteria.

welve days after infection the changes in the lung did not increase in size and free alveolar macrophages were often vacuolised and in some of them nuclei revealed pycnotic changes. Tuberculous lesions in the liver increased considerably, the cellular foci were bigger and among epitheloid cells giant multinucleated cells

were formed. Similar granulomas were present in the spleen and they contained also forming giant cells. Foci in the kidneys were not considerably enlarged and the main type of cells present were epitheloid cells, similar to other organs. Mycobacteria were found practically in all foci, mostly in the liver and spleen and they were localized, for the most part, in the middle of tuberculous lesions.

On 14 day after infection the spleen was macroscopically enlarged. The microscopic picture was not different from that in the lungs, but the alveolar macrophages mostly revealed necrobiotic changes. At this stage the liver showed profound microscopical changes. Tuberculous lesions developed further and their main constituents were epitheloid and giant cells. The structure of liver tissue was considerably destroyed. Similar development of the process was seen in the spleen. In both these organs the number of mycobacteria increased very strongly.

Rabbits which died 16 and 18 days after infection showed again macroscopical enlargement of the spleen and microscopically, the main changes were again present in the liver and spleen. Structure of the liver was completely destroyed, growing tuberculous tissue forming irregular shapes connected with each other. Epitheloid cells in the centers of such foci revealed pycnotic and necrobiotic changes. Microscopic picture was characterised by very strong proliferation of the cells of mononuclear macrophages system and their change into epitheloid and giant cells. Similar features revealed the spleen with growing and connecting tuberculous foci. Necrotization of epitheloid and giant cells in the middle of foci was similar to that in liver. Cytoplasm of epitheloid and giant cells contained numerous mycobacteria and few of them were present in necrotic tissue as well.

Discussion

In the development of tuberculous lesions caused in rabbits with big doses of *Mycobacterium avium* was evident that the first and very minute lesions formed during 48 hours after infection. Small nodules of macrophages were present 5 days after infection and after the 7th day epitheloid cells formed. Multinucleated giant cells were present after ten days. The course of the disease was typical for the Yersin type tuberculosis and the longest interval to death of experimental animal was 18 days.

Dominant feature of the morphological picture was destruction of the liver and spleen while the lesions in the lungs were not very grave. Another morphological characteristic was enormous reaction of the cells of the mononuclear macrophages system. This was very clearly seen in the liver and spleen where the macrophages proliferation was in the latest stages almost diffuse. This feature of Yersin type tuberculosis described Yamamoto et al. (1961 a, b, c, 1962), Černý (1965), Mohelská et al. (1975). This finding was connected with the development of the cells of the macrophage system. Spector and Lykke (1966) found that during 5 days after infection macrophages changed into epitheloid cells. In our cases this interval was 7 days, that was the time, when several epitheloid cells formed. Epitheloid cells were formed from alveolar macrophages, macrophages of the spleen, Kupffer's cells and blood monocytes. The origin of epitheloid cells seems to be clear (Hess et al. — 1971, Erochin — 1978, Turk — 1978), but Volkmann (1976) expressed the opinion that macrophages of the liver and peritoneal cavity formed a special population.

Considering the macrophages it is necessary to mention the dynamics of the growth of mycobacteria. It was evident that the number of mycobacteria

present in the liver and spleen increased during the whole course of the disease. In this connection it would be pertinent to consider data of Tonaki et al. (1976) who found that after application of test emulsion with I^{131} 90—95 % of activity was found in the liver. It showed the very high phagocytic activity of Kupffer's cells. One could consider that by intravenous propagation of mycobacteria the Kupffer's cells and the macrophages of spleen would phagocytose these mycobacteria very intensively and the end result of this process would be high grade of activation of these cells. This process is also connected with immunological processes. Nezelof and Vildé (1976) characterized granulomas as cellular societies which eliminated or surrounded foreign material or agent. In this process, in its first stage there was a non-specific phagocytosis which was a most simple defense mechanism. The very high phagocytosis present in the Yersin type of tuberculosis shows that the immunological defense in this disease is incompetent. With this fact were connected the findings of Mariano et al. (1976) who found that macrophages and epitheloid cells gradually lost their phagocytic activity. This was connected with the loss of specific receptors and their ability to destroy mycobacteria diminished. In granulomas mycobacteria were found mainly in the center of foci i. e. there, where older epitheloid cells, with diminished phagocytic and destructive activity were present. It means that the high phagocytic activity of these cells is transient and the older epitheloid and multinucleated giant cells are not able to destroy phagocytosed mycobacteria. Similar localization of the mycobacteria in the middle of tuberculous foci described also Otto and Bertram (1969).

In Yersin type tuberculosis mycobacteria act evidently as intracellular parasites (Černý 1965; Mohelská et al. 1975). This is known to apply to tuberculosis in other species as well, e. g. in birds (Černý 1965; Hejlíček 1977). Therefore it is evident, that in Yersin type tuberculosis mycobacteria are not destroyed in the process of phagocytosis and after necrotisation of epitheloid and giant cells they may liberate themselves to invade other cells.

Histopatologie a histochemie Yersinova typu tuberkulózy u králíků. Vývoj onemocnění po intravenózní infekci Mycobacterium avium

Po intravenózní aplikaci *Mycobacterium avium* králíkům se první morfologické změny projevily mikroskopicky za 48 hodin po infekci. Nejvýraznějším morfologickým projevem onemocnění je silná proliferace buněk systému mononukleárních makrofágů.

Epiteloidní buňky se tvořily od 7. dne po infekci a obrovské mnohojaderné buňky po 10. dni.

V následujících dnech se proces šířil mohutně hlavně v játrech a ve slezině. Při tom se výrazně pomnožovaly mykobakterie, a to i v cytoplazmě epiteloidních buněk.

Od 14. dne po infekci podléhají epiteloidní buňky v centru větších ložisek nekrobiotickým změnám.

Гистопатология и гистохимия типа Иерсена туберкулеза кроликов. Развитие заболевания после внутривенной инфекции *Mycobacterium avium*

После внутривенного применения *Mycobacterium avium* кроликам первые морфологические изменения проявились в течение 48 часов микроскопически. Самым выразительным морфологическим проявлением заболевания является интенсивная пролиферация клеток системы мононуклеарных макрофагов.

Эпителиоидные клетки возникали с 7 дня после инфекции и огромные многоядерные клетки — после 10 дней.

В последующие дни процесс расширился весьма интенсивно главным образом в печени и селезенке. При этом существенным образом увеличивалось число микобактерий даже в цитоплазме эпителоидных клеток.

С 14 дня после инфекции эпителиоидные клетки в центре более крупных очагов подвергаются некробиотическим изменениям.

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