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Studies on the Pathogenesis of Brucellosis.*

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The mechanism by which Brucella produces infection and continues to grow in the tissues of immune individuals has been suspected but not clearly demonstrated. One of the most interesting features of the disease in man is the prolonged sequence of periods of illness and apparent recovery for which it has been given the name of undulant fever. The finding of Brucella for months and even years after the beginning of the infection places this disease in a class with tuberculosis and syphilis, in which the infecting organism continues to multiply in spite of the considerable degree of immunity displayed by the host. However, brucellae are not quite resistant to cellular and humoral defences as is the case in tuberculosis. Therefore, its ability to remain active within the organs and eventually to be found in the blood stream must depend on factors peculiar to the microorganism.

One of the most interesting observations on the relationship of Brucella to the infected tissues was made early in the history of the disease, when Theobald Smith demonstrated that a considerable number of *Brucella abortus* crowded the cytoplasm of the cells of the chorium in the placenta of aborting animals. This finding did not seem to have been fully appreciated as a possible guide in the explanation of the remarkable persistence of Brucella in the organism in spite of the coexistence of high degree of immunity.

Corroborating Smith's findings, Goodpasture and co-workers[1] in a study of bacterial infection in the developing chick embryo found that Brucella multiplied within the cytoplasm of certain cells more intensely than could be observed in extracellular positions. More recently Buddingh and Womack[2] following the method of Goodpasture found that *Br. abortus* and *suis* had a tendency to select cells of mesodermal origin while the melitensis variety was only found in ectodermal cells. The 3 brucellae could be found in considerable numbers within macrophages not merely phagocytized but actually multiplying within the cytoplasm of such cells. These authors advanced the idea that the monocytes acting as a natural means of defense against the infecting agent supplied a favorable refuge where multiplication took place and therefore, constituted the source of continual infection from cell to cell. The intracellular position of Brucella in tissues of infected persons was first reported by Meyer[3] in a case of acute infection with *Br. suis*. The brucellae were easily seen within parenchyma cells of the kidney. After Meyer's report we decided to investigate intracellular brucellae in fatal cases of human brucellosis. It was discouraging to find so few visible organisms while cultures from the organs showed considerable numbers of colonies. Therefore, it was considered of interest to become familiar with the microscopic aspect of the disease in experimental animals. The following is a presentation of some findings after inoculation of guinea pigs, rabbits and mice with the 3 varieties of Brucella. Guinea pigs and rabbits were inoculated intravenously and also directly into the testicle. Mice were inoculated by intravenous and intranasal routes. The strains used as inoculum were *Br. abortus* and *suis* sent to us by Dr. Huddleson and a recently isolated

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1 Goodpasture, E. W., and Anderson, K., *Am. J. Pathology*, 1937, **13**, 149.

2 Buddingh, G. J., and Womack, F. C., *J. Exp. Medicine*, 1941, **74**, 213.

3 Meyer, K. F., *Essays in Biology*, University of California Press, 1943, pp. 439-459.

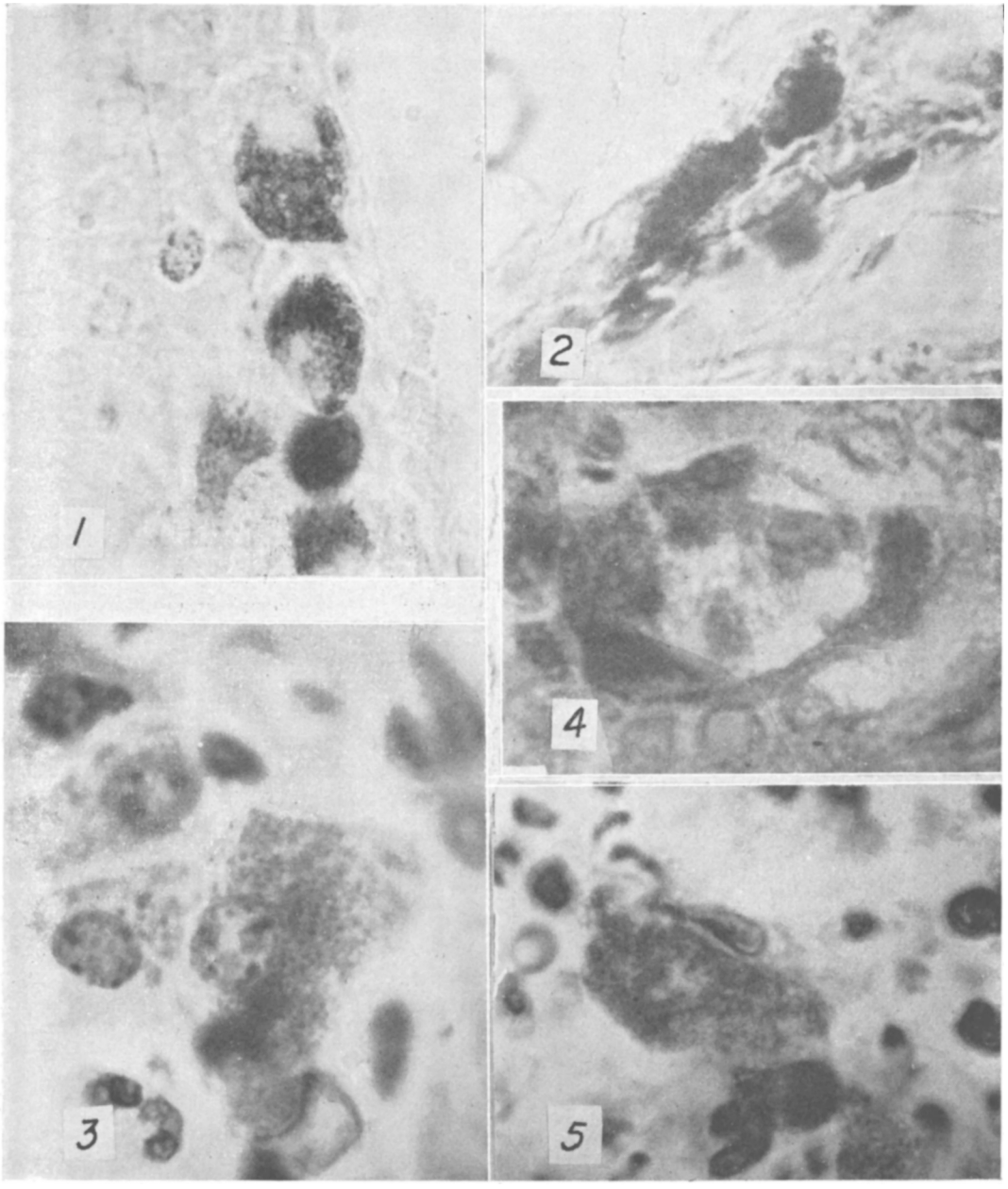

FIG. 1.—Testis of a guinea pig inoculated locally with *Br. melitensis*. Parasitized macrophages. No extracellular brucellæ are detected. Formalinized Giemsa. × 1000.

FIG. 2.—Fibroblasts parasitized with *Br. abortus*. Guinea pig testis. Same stain. × 900.

FIG. 3.—Testis of guinea pig. Interstitial cell heavily parasitized with *Br. abortus*. Nyka's stain. × 1000.

FIG. 4.—Same testis. Endothelial cells of a capillary stuffed with *Br. abortus*. Formalinized Giemsa. × 1000.

FIG. 5.—Lung of a mouse inoculated intranasally with *Br. melitensis*. Alveolar cell packed with brucellæ. Nyka's stain. × 1600.

strain. After being inoculated, the animals were killed at various intervals in order to follow the infecting organism through the various stages of the process. Smears and

prints made from the organs were stained with our methylene-blue-safranin stain for rickettsiae. The sections were stained with formalinized Giemsa or by Nyka's stain.[4] The latter producing delicately stained cellular elements in which violet stained brucellae could be easily found.

It is unnecessary to include here the observations concerning the cytological response to the infecting organism which will be reported by one of our associates.

The search of brucellae in smears or prints from animals inoculated intracardially showed relatively few extracellular organisms in the spleen and liver, and even less in other organs. Often one could detect polymorphonuclear leucocytes containing few phagocytized organisms and also macrophages containing brucellae in the cytoplasm. The optimum time for the finding of parasitized macrophages was about the 5th day after inoculation. Some of the mononuclear cells contained rather large numbers of brucellae but the microscopic aspect of the parasitized cells was far from being suggestive of intracellular multiplication as reported by Buddingh and Womack. On the other hand, the observation of smears from the testicle of animals inoculated locally showed cells with the cytoplasm crowded with brucellae. In smears from the locally infected testicle there were found a considerable number of polymorphonuclear leucocytes phagocytizing brucellae and also many macrophages stuffed with organisms. Although in sections of the liver and spleen it was not difficult to find infected cells, the testicle afforded better material for the study. Fig. 1 shows cells from the testicle of a guinea pig infected with *Br. melitensis* which leave little doubt of the intracellular growth in cells which are likely to be macrophages. Fig. 2 shows the growth of *Br. abortus* in the cytoplasm of a fibroblast and in Fig. 3 there is no doubt about the multiplication of *Br. abortus* within a cell, probably an interstitial cell, if compared with neighboring cells of similar morphology. In Fig. 4 it seems that cells from a capillary wall have been parasitized by *Br. abortus*. Most of these preparations were made from animals killed from the 3rd to the 5th day, and it has been our impression that the parasites within the cells have increased not because of phagocytosis but by the actual development of Brucella colonies after some of the organisms gained entrance into the cytoplasm. It is interesting to find so few or even no extracellular organisms close to the parasitized cells. However, in certain portions of the testicle one could see large numbers of extracellular organisms and it was usually in zones in which considerable numbers of polymorphonuclear leucocytes were present.

We have not yet sufficient information concerning the type of cells which allow brucellae to develop in the cytoplasm, nor the possible selectivity of the 3 varieties of organisms for a special group of cells. We can only indicate that we found macrophages, fibroblasts and, particularly in the spleen, endothelial and reticular cells which contained brucellae in the cytoplasm. To this we may add parenchymatous cells from the kidney and interstitial cells of the testis.

The inoculation of mice by intravenous or intranasal route has afforded a practical method for the study of the relationship of brucellae to the cells of the infected animal. Even 48 hours after the inoculation it has been possible to find parasitized cells in various organs. In the lung the alveolar cells are found to be parasitized in a similar manner to that which has been observed in typhus. Fig. 5 shows the aspect of an infected alveolar cell which does not look unlike similar cells parasitized with typhus rickettsiae. We have also seen macrophages stuffed with brucellae and polymorphonuclear leucocytes containing phagocytized organisms, usually in small numbers. It is interesting to find that, contrary to what has been observed in typhus-infected animals the bronchial epithelium seems to have no tendency to favor multiplication of brucellae.

Comment. There seems no doubt that most workers experienced in the clinical aspect of brucellosis consider that some of the

[4] Nyka, W., *J. Pathology and Bacteriology*, 1945, **57**, 317.

symptoms shown by the patients are due to a state of hypersensitivity to brucellar antigens. Since 1938 we have advocated this view based on the observation of typical manifestations of allergy and the favorable effect of desensitization of the patients. To illustrate our discussion we shall give a brief description of the general aspect of the caprine type of brucellosis. The disease most frequently begins like typhoid or typhus infection or less frequently as a mild febrile influenza-like infection. The first attack, of variable duration, represents the cyclical phase of the infection ending when the individual has developed sufficient immunity to prevent further injury by the infecting agent. Then, a state of convalescence closes the incident. But in a great number of cases Brucella continues to grow as indicated. The cell to cell infection as suggested by Buddingh and Womack insures the preservation of the organism in spite of the state of immunity of the patient. The constant discharge of brucellar products into the system increases this state of immunity and at a certain moment the reactivity of the tissues and the general reactions acquire sufficient intensity to produce considerable discomfort and fever. This constant irritation may become very harmful, overcome the body defenses and even allow considerable multiplication of brucellae either focally or again as a general infection. An important factor in the failure to destroy Brucella in the immune individual seems to be the reduction in numbers of the polymorphonuclear leucocytes.

The demonstration of brucellae within cells of various types is a clear indication of the mechanism of preservation of the organisms in the immune individual but most important, for the understanding of the pathogenesis of the numerous clinical manifestations of the infection, seems to be the facility with which Brucella are phagocytized by macrophages which may be transported to all parts of the body and become the source of new colonies since the cytoplasm supplies suitable material for the multiplication of the organisms.

Up to the present time we have studied relatively few animals from which only a limited number of preparations have been suitable for the investigation of intracellular brucellae, therefore, we have not been able to gather sufficient information concerning special affinity of the 3 varieties of the organism for certain cells as observed in the chick embryo by Buddingh and Womack.

According to the observations made by various authors, in experimental brucellosis and in human material, the histological lesions are, fundamentally, the nodules described by Fabyan. The nodular formation could be explained as a reaction around wandering macrophages carrying brucellae or local parasitized cells, which are surrounded first by monocytes and perhaps polymorphonuclear leucocytes and walled off by lymphocytes. It is obvious that in brucellosis there is a considerable call for the latter cells and that the infected individual endeavors to supply them. It seems likely that this mechanism of focal growth of Brucella followed by the process of nodule formation explains many of the complications of brucellosis including nervous manifestations, abscesses, focal necrosis, etc. The tendency of brucellae to select the spleen as a refuge during the course of the disease is probably due to exceedingly rich material suitable for the intracellular growth and the hyperplasia observed in this organ must be accounted for the manifestations of hypersplenism often observed in the patients.

Conclusions. The studies referred to, and our own observations in experimental brucellosis are in our opinion, good evidence of the ability of Brucella to use the cytoplasm of a variety of cells as a source of material for its growth. The fact that even in the early stages of the infection, when the host has not developed immunity against brucellae, important groups of this organism are found only within the cytoplasm of certain cells, seems to indicate that the intracellular growth is the most convenient for the preservation of the infecting agent. When the intracellular colony has reached its maximum, the cells are destroyed and the brucellae exposed to unfavorable conditions, namely to phagocytosis by polymorphonuclear leucocytes. From the scarcity of extracellular organisms

one may think that the intracellular fluids are not suitable for an active multiplication of Brucella, exception is made of zones where the tissues have been subject to considerable damage. In these zones one may find extracellular Brucella in large numbers. One would consider that the organism could be placed together with true intracellular parasites, but this is not the case since Brucella have its own enzyme systems with which it is able to use rather ordinary material for its metabolism.

Considering as likely that in man Brucella grow in a manner similar to that which has been observed in the experimental infection, the pathogenesis of the disease seems to us better understood.

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Thromboplastic Action of Plasma Protease (*Tryptase*).*

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Indirect evidence, based on experimental analogy with trypsin, papain, etc. was reviewed[1] in support of the theory that blood coagulation normally involves natural plasma protease (*tryptase*[1] or *plasmin*[2] or *fibrinolytic enzyme*[3]) apparently identical with the agent responsible for fibrinogenolysis, fibrinolysis, and other proteolytic phenomena. Plasma tryptase resembles pancreatic trypsin in many respects, especially relevant to these problems, but does differ in 1. origin,[4] 2. kinase-specificity,[3] and 3. other important ways.[2] The following experiments confirm some similarities and lead to *direct* evidence that plasma tryptase can participate as a "thromboplastic" factor in the blood-clotting system.

Reagents. In the first paper[5] of this series, on the activation and inhibition of the fibrin(ogen)olytic protease (tryptase) in certain plasma fractions, are described: 1. Borate buffer, pH = 7.7, *viz.* 45 vol. 2.5% H_3BO_3, 45 vol. 0.5% NaCl, 10 vol. 4% $Na_2B_4O_7$, $12H_2O$, used as solvent and diluent (to constant volume) throughout; 2. Harvard (courtesy Drs. E. J. Cohn, J. T. Edsall, and colleagues) human plasma Fraction-I (H.F.), which contains about 60% fibrinogen, a trace of active protease (tryptase) and considerable enzyme-precursor (tryptogen) that can be activated by a suitable kinase (Table I, A); 3. Armour's (courtesy Dr. J. B. Lesh) enzyme-free[5] bovine plasma Fraction-I (B.F.), of about the same fibrinogen content as H.F.; 4. Thrombin (THR.), a 1% soln. of lyophilized rabbit "hemostatic globulin," Lederle's (courtesy Dr. I. A. Parfentjev); 5. Streptokinase (STREP.), the streptococcal material (Garner and Tillett, 1934) of which a 1% extract is an excellent kinase-type activator of the plasma protease; 6. Crystalline trypsin-inhibitors, protein (or polypeptide) in nature, from pancreas (T.I.)

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