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The Early Events of Experimental *Brucella* Infection in the Mouse

Relationships of Bacteria With Phagocytic Cells and Lymphoid Tissue Responses

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Summary. The local lymph node and the spleen of the mouse were examined with combined techniques during the 10 days following a subcutaneous inoculation of living *Brucella suis*. Light and electron microscopy on epoxy-embedded tissues provided information on the modifications in histological architecture and on the differentiation of various cell series. Incorporation of tritiated thymidine, observed by autoradiography and scintillation counting indicated the degree of cellular activation. Finally, the use of purified, specific, peroxidase-conjugated antibodies allowed a more precise observation of the dissemination of inoculated bacteria, their relationship to phagocytic cells, and the degree of their degradation. It was observed that early activation was mainly seen in macrophages and reticular cells. The participation of the latter cells was apparently more important than in comparative studies concerning other bacteria inducing delayed hypersensitivity (DHS). In contrast, activation of lymphoïd cells was delayed and moderate; the slow response of plasma cells was noticeable, especially in the lymph node. Dissemination from the site of inoculation of bacteria was probably continuous during the entire observation period. Free, non-altered bacteria were encountered in the sinuses of both organs. Intracellular bacteria showing very different degrees of degradation were seen in macrophages and reticular cells; here again, the participation of reticular cells seemed a special feature of Brucella infection. These data give to Brucella infection of the mouse the features of a T-cell immunological response, but delayed as compared to other immunogens of the same type, and accompanied by peculiar cell reactions. These results are discussed in the light of comparative studies it is suggested that the timing of the induction of antibody production, could play a critical role in the establishment of DHS.

Key words: *Brucella* – Mouse experimental infection – Phagocytic cells – Immunological response – High resolution autoradiography.

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Introduction

Some infections caused by bacteria are characterized by a marked degree of delayed hypersensitivity (DHS) which plays a prominent role in their physiopathology. The best known example is that of *Mycobacterium tuberculosis*. Sharing this property with Mycobacteria are some Gram-negative species, such as *Brucella*, *Pseudomonas pseudomallei*, *Salmonella typhi*, *Hemophilus ducreyi* and *Treponema pallidum*, an a few Gram-positive bacteria such as *Listeria monocytogenes*, Group A streptococci and the pneumococcus.

The importance of cellular participation in DHS has been emphasized for a long time, and most studies deal with the cellular events in established DHS. It is possible, however, that the afferent immunogenic arc may also have peculiar features in these cases. The early processes of these infections have some aspects in common: the phagocytic cells involved are mononuclear, a granulomatous reaction develops, and bacteria multiply within tissues and phagocytic cells. Later, the infection frequently tends to form a subacute or chronic pattern. From this point of view, *Brucella* seems to be a promising tool for experimental studies, since, besides the features mentioned above, it also elicits the synthesis of specific antibodies. Therefore, we investigated the early stages of lymphoïd stimulation after a primary infection by *Brucella*, using a number of complementary techniques, in order to fully document the changes at the cellular level.

Materials and Methods

Infection of Animals

Specific pathogen-free, white albino mice, weighing 16–18 g, were used. During experiments they were kept in a controlled environment. Mice were housed to a maximum of six in sterile boxes protected with filter caps ("Isocaps", Iffa-Credo, 69210 St Germain/l'Arbresle, France). The "straw" was sawdust sterilized by dry heat (1 h at 160° C). Animals were fed with pellets (U.A.R., 91360 Villemoisson, France) and supplied with acidified water (0.8 ml of concentrated HCl per liter), both sterilized by autoclaving. *Brucella suis* strain 1330 (Brinlay Morgan, biotype), was used for this study. Bacteria were grown during 16 h at 37° C on a rotary shaker in Albimi's Brucella broth (Chas-Pfizer C°, N.Y., 10036, USA).

The culture was centrifuged, washed 3 times in sterile saline and suspended in saline to an approximate concentration of 10^9 bacteria per ml; this was monitored, according to previous standardization, by adjusting the optical density of the suspension to 0.8 at 700 nm in a Coleman H 14 photometer. Animals were inoculated subcutaneously by injecting 0.2 ml of the standardized suspension into the right posterior leg near the groin.

Histological Techniques

Sixty animals inoculated as described were killed by cervical disruption after 6 and 24 h, and 2, 3, 4, 5, 6, 8 and 10 days (6 mice per group). One group of 6 uninoculated mice was used as control. The inguinal lymph nodes of the infected side and several thin slices (approx. 0.1 mm) of various parts of the spleen were taken from each animal. The specimens were pre-fixed in 2.5% glutaraldehyde (Ladd Res. Inc., Burlington, VE, 05401) in 0.05 M cacodylate buffer, pH 7.1, for 2 h at room temperature, then washed in the same buffer supplemented with 0.5% of sucrose, and finally post-fixed in 1% OsO^4 in distilled water, for 1 h, at room temperature. Fixed specimens

were washed 3 times in Sörensen's phosphate buffer, pH 7.2, and embedded in Araldite (Ladd Res. Inc.). Thin sections (0.7 to 1 μ) were performed in a Reichert OMU2 ultratome. Sections were stained for 30 min in 1:50 Giemsa stain dilution Gurr Ltd (London, NW9, Great Britain). Photographs were taken with an Orthomat Leitz camera using a Kodak plus X-pan film (125 ASA).

Electron Microscopy Techniques

Specimens of the same materials were embedded in Epon (Ladd Res. Inc.) according to Luft (1961), ultra-thin sectionned, and stained with lead citrate and uranyl acetate according to Reynolds (1963). Observations were performed with a Philips EM 200 electron microscope.

Radio-Isotopic Techniques

Mice were pulsed, 2 h before sacrifice, by an intravenous injection of 0.1 ml (containing 100 μCi) of tritiated Thymidine, specific activity 7.2 mCi/mM (Commissariat à l'Energie Atomique, 91190 Gif/Yvette, France). Measurements of labelled compound incorporation were made by sampling a part of the specimen, which was dissolved in a scintillation vial with Soluene (Packard Inst. C°, Downers-Grove, Ill., 60515), 1 ml/100 mg of tissue. Samples were dissolved at 60° C for 1 h. The coloration due to the presence of blood cells in spleen samples was cleared by 0.1 ml of a 0.1% solution of sodium hypochlorite. Ten ml of Instagel (Packard) were added to each vial and the emission was counted in a Packard Tricarb 300 spectrometer, using an external standard for quenching correction. For autoradiography, grids of material embedded and treated as described in the preceding paragraph were dipped in Ilford L3 emulsion (Ilford Ltd, Sussex, Great Britain) and exposed for 25 to 30 days, at 4° C.

Preparation of Labelled Specific IgG

Antisera to *Br. suis* were raised in rabbits by a single injection of 0.2 ml of a 16 h, living culture. Rabbits were bled after 15–20 days. Sera were pooled and antibodies were evaluated by Wright's agglutination (titer, 1:1280) and indirect immunofluorescence (titer, 1:1280). Globulins were isolated by continous flow electrophoresis in a model CP cell (Beckman Inst., Fullerton, CA, 92634) using a veronal buffer, pH 8.6, ionic strength 0.02. The Ig peak was concentrated, dialyzed against 0.2 M Tris-HCl buffer, pH 7.2, and purified by chromatography on a Whatman DE 23 cellulose Column (Reeve Angel & C° Ltd, London, Great-Britain). Specific antibodies were then absorbed on the centrifugation pellet of a rich culture of *Br. suis*, previously washed 3 times with Dulbecco's phosphate buffered saline (PBS). After 30 min at 37° C, bacteria were centrifuged, washed several times with PBS, and specific IgG eluted with 0.1 M acetate-acetic acid buffer, pH 3.9, during 1.5 h at 37° C. Elution was repeated until the absorbing strain no longer showed a noticeable fluorescence with fluorescein-conjugated anti-rabbit globulins (Sylvana C°, Milburn, N.J., 07041). Conversely, the absorption of IgG was repeated until it no longer displayed a noticeable fluorescence with *Br. suis* by the same technique. Purified specific IgG were concentrated in a dialysis bag until the concentration reached approximately 5 mg of proteins per ml, dialysed against PBS and conjugated with horseradish peroxidase (Boehringer Gmbh, Mannheim, West Germany) according to the technique of Avrameas (1969). These are referred to hereafter as peroxidase-conjugated purified antibodies (PCPA).

In situ Labelling of Bacteria

Forty eight mice were inoculated as described before and divided into 8 groups (Table 1). Two groups of six were not inoculated and kept as controls. Inguinal lymph nodes and spleen samples were removed and cut in small pieces (approximately 2 mm) in a 2.5% solution of glutaraldehyde in distilled water. After 2 h at 4° C, the pieces were cut again into fragments as thin as possible with sharp razor blades, and washed with 5% glucose in PBS.

Table 1. Repartition of animals for labelling of bacteria in situ

Group reference	N° of animals	N° of inoculated animals	Examination after	Treatment of samples[a]	
				PCPA+DAB	DAB
A	6	6	12 h	4	2
B	6	6	1 day	4	2
C	6	6	2 day	4	2
D	6	6	3 day	4	2
E	6	6	4 day	4	2
F	6	6	6 day	4	2
G	6	6	8 day	4	2
H	6	6	10 day	4	2
C_1	6	0	10 day	6	–
C_2	6	0	10 day	–	6

[a] See material and methods for abbreviations

Fragments were then dipped in a 1:10 dilution of PCPA (in the same glucose - PBS) and kept at 4° C for 16 h. After 3 washings in glucose-PBS, peroxydase activity was revealed by 3-3' diamino benzidin (DAB-Merck France, 75009, Paris, France), 75 mg/100 ml of 0.01 M phosphate buffered saline, pH 7.2, with hydrogen peroxide at a final concentration of 0.001% for 1 h at room temperature (Kuhlmann and Miller, 1971). Fragments were then abundantly washed in glucose-PBS and post-fixed in 2.5% glutaraldehyde for 2 h at room temperature. Finally, they were post-fixed with OsO^4 and embedded as described in electron microscopy techniques. Some of the sections were not stained with lead and uranyl acetate in order to observe the structures labelled by PCPA with a sharper contrast.

Results

No deaths were observed among inoculated animals during the period of observation (10 days).

I. Morphological Changes in Lymphoïd Organs

Macroscopically, inguinal *lymph nodes* of inoculated animals were enlarged; the maximum extent of this swelling was observed on the 5th day. Histological changes took place very early. By the 6th hour, the *lymph node cortex* showed hyperaemia and oedema with enlargement of the sinuses and of the intercellular spaces. Cellular changes were also seen. There were numerous large cells with extended cytoplasm and an indented nucleus, showing a well-defined nucleolus, that we considered "macrophage-like". Lymphoïd cells were rather dispersed (Fig. 1). There was also a moderate increase in the number of slender, elongated cells with tapered nuclei (fibroblasts). These changes increased up to the 5th day. At this time, the cortex showed marked hyperaemia. Macrophage-like cells were numerous and sometimes contained clusters of nuclear debris (Flem-

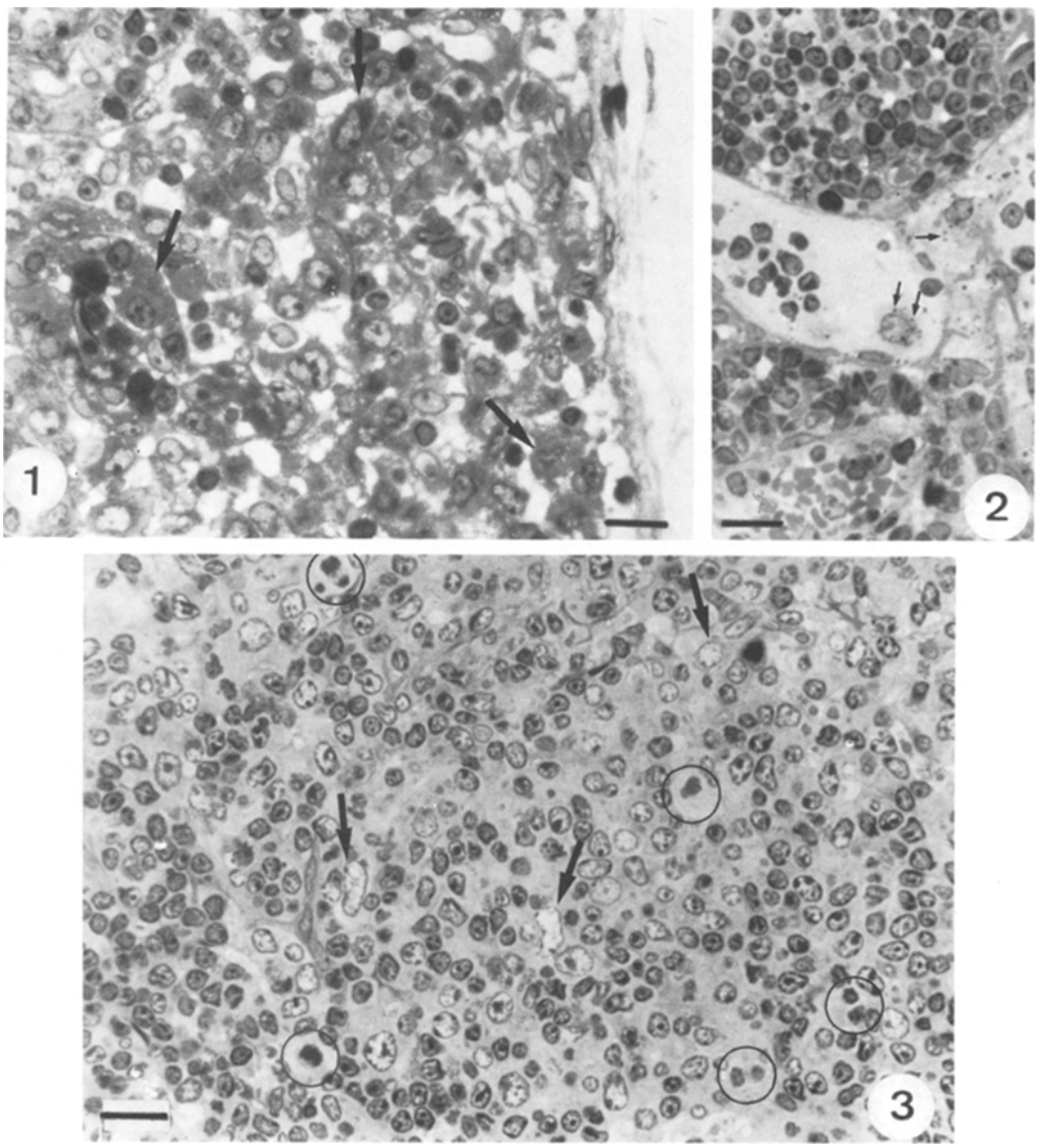


Fig. 1. Lymph node cortex, 6th h after inoculation. Oedema with distension of the connective network. "Macrophage-like" cells are frequent (*arrows*). Light microscopy (*LM*); bar: 20. Original magnification (*OM*): ×400. (Dimensions of the bars are given in μ)

Fig. 2. Lymph node cortex, 5th day. Enlargement of capillaries (bottom of the picture) and of sinuses (middle). Densely packed cells at the top are a part of a germinal center. Bacteria are seen in "macrophage-like" cells (*small arrows*). LM; bar: 30. OM: ×240

Fig. 3. Spleen cortex, 5th day. Mitoses (*circles*). Arrows point to "reticular-like" cells. LM; bar: 30. OM: ×240

ing's tingible bodies – Fig. 2). Otherwise, lymphoïd cells were organized in dense secondary centers. After the 5th day, oedema and hyperaemia decreased and lymphoïd and phagocytic cells underwent important changes which will be described in the electron microscopy section. The *lymph-node medulla* also showed early hyperaemia and oedema, which decreased from the 5th day onwards. Lymphocytes and plasmocytes were seen, but the latter dis not seem to increase noticeably in number until the 10th day. Between days 5 and 10 we observed an increasing number of large, clear cells with an extended, interdigitating cytoplasm, and a clear nucleus with peripheral chromatin, which we refer to as "reticular-like" cells. Some negative features are worthy of mention; there was no participation of polymorphonuclear leukocytes, proliferation of blood vessels was not observed, and finally no fibrosis developed during the 10 days of the experiment.

In the spleen, changes were slower. During the first two days, the increase in volume was only moderate. Splenomegaly was marked on the 5th day and thereafter. Microscopically, inflammatory aspects were less marked than in the lymph node during the first four days. Important changes were seen in the *white pulp* on the 5th day. Oedema appeared; germinal centers showed large numbers of densely packed cells. Mitoses were seen, mostly in large cells resembling lymphoblasts. Reticular-like cells were numerous (Figs. 3 and 4). These appearances persisted until the 10th day without much modification. In the *red pulp*, the most noticeable change was an increase in the number of plasmocytes after the 5th day.

II. Cytological Changes Observed by Electron Microscopy

In the lymph node, *lymphoïd cells* did not show marked changes during the first days, even in the cortex. After 24 h, one could observe a slight modification in the organization of the chromatin, and certain degree of cytoplasmic oedema (Fig. 5). Important changes began on the 5th day. In the follicular zone, cellular activation was apparent with the development of centrioles (sometimes divided), the extension of the Golgi complex and the organization of ribosomes into rosettes (Fig. 6). This activation went on until the 10th day, even though the cellular oedema decreased after the 5th day. *Plasma cells* were infrequently seen before the 5th day; between the 5th and 10th day their number increased in the interfollicular and medullary zones, but to a moderate extent.

In contrast, there was an unmistakable activation of cells in the interfollicular area of the cortex which probably corresponded to the "macrophage-like" cells seen in light microscopy, and which bore features regarded as distinctive for lymph node macrophages (Hoefsmit, 1975): irregular surfaces with vacuolar progresses, indented or bilobar nucleus, well-developed Golgi complex close to the cytocentre and peripheral lysosomes and rough endoplasmic reticulum. Figure 7 shows this type of macrophage, whose activation was proved by the dividing centriole (see also Fig. 8). These cells were frequently encountered in the follicular area of the cortex, from the 2nd day onwards. In the same area, we observed, among inoculated animals, an increase in the number of

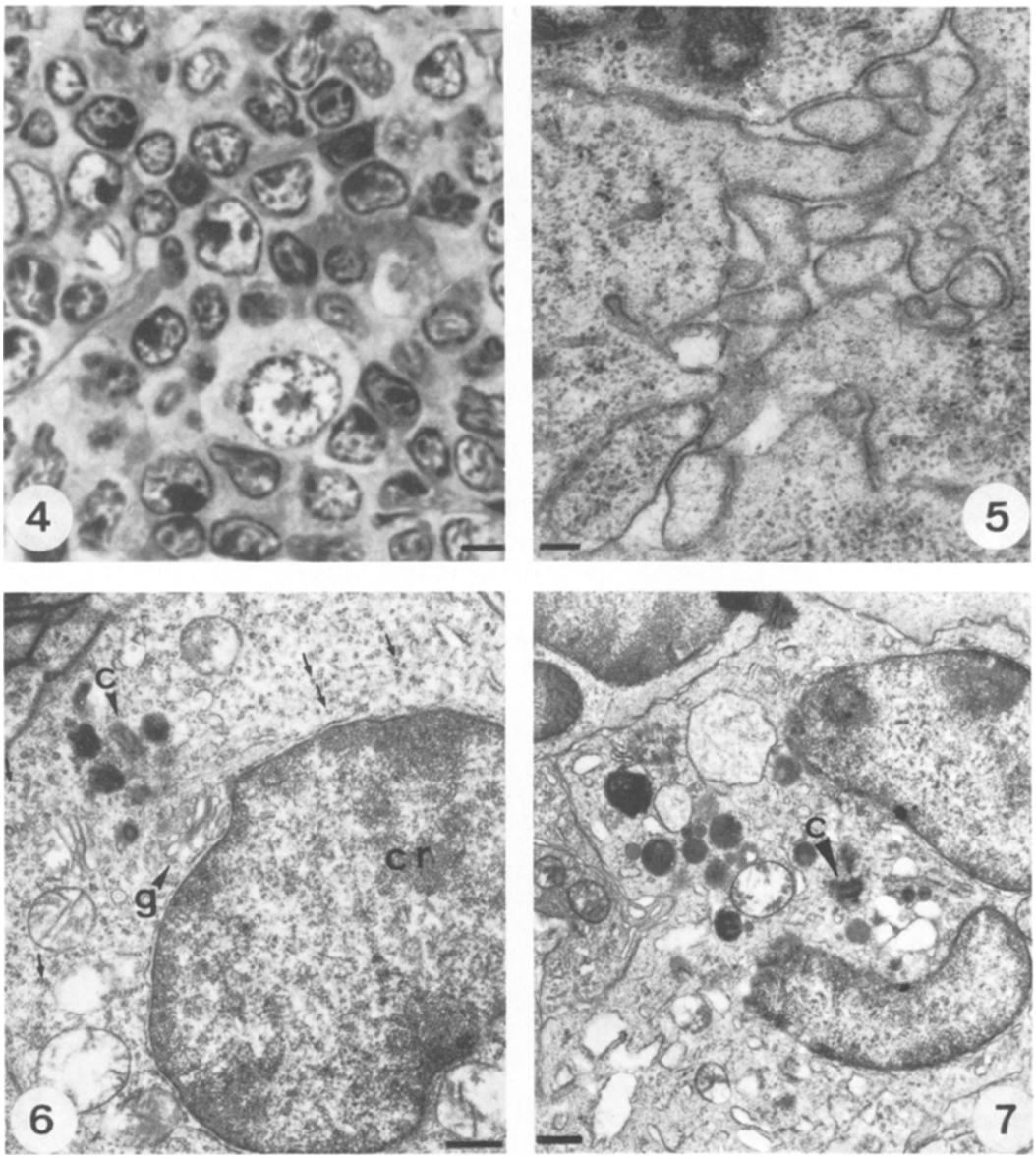


Fig. 4. Spleen cortex, 5th day. Most of the cells are quiescent lymphocytes: there is a lymphoblastic cell with modified chromatin. LM; bar: 10. OM: ×260

Fig. 5. Lymph node cortex, 24th h. Aspect of cytoplasmic oedema with complex folding of membranes. Electron microscopy (*EM*); bar: 1. OM: ×5,400

Fig. 6. Lymph node cortex, 5th day. A lymphoïd cell with modified chromatin (*cr*) and a divided centriole (*c*). *g*: Golgi complex. *Small arrows*: polyribosomes. EM; bar: 1. OM: ×4,000

Fig. 7. Lymph node cortex, 6th h. An activated macrophage, with a dividing centriole (*c*), extended Golgi complex, lysosomes and a vacuolated cytoplasm. EM; bar: 2. OM: ×4,000

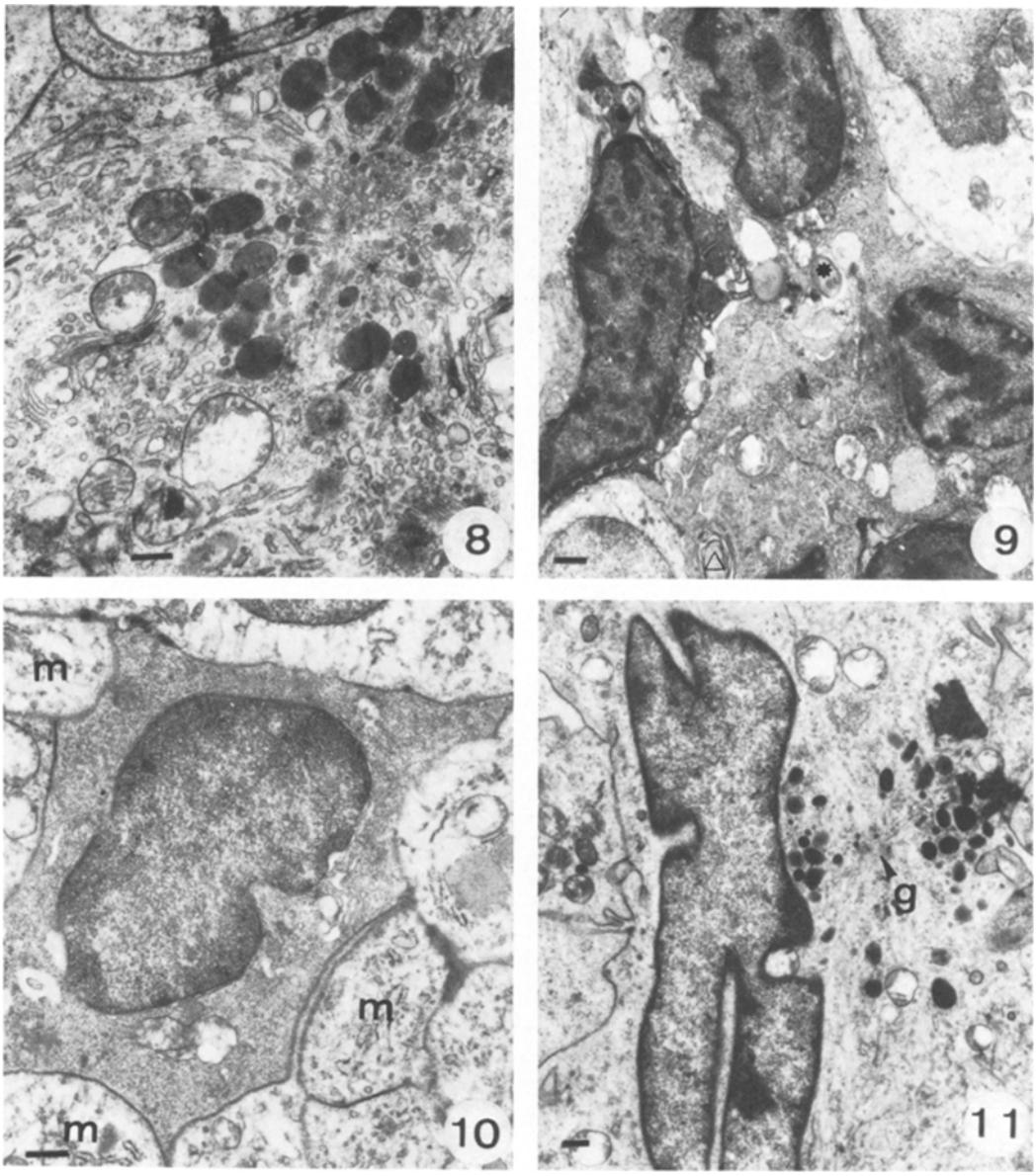


Fig. 8. Lymph node cortex, 6th h. Part of the paranuclear zone (cytocentre) of a macrophage showing marked activation. EM; bar: 2. OM: ×2,900

Fig. 9. Lymph node follicle, 24th h. A cluster of electron dense cells described as "reticular cells". The nucleus is large and shows quadrangular or triangular sections. The relationships of cytoplasms are extremely complicated; in this section it is difficult to see if brucellae (*) and myelinic debris (△) are included within vacuoles. EM; bar: 2. OM: 2,400

Fig. 10. Spleen cortex, 4th day. Numerous macrophage pseudopodes surrounding a fairly dense cell showing little differenciation. Ribosomes are numerous but not organized in rosettes; other cytoplasmic organelles are scarce. The aspect of the nucleus is not in favour of a reticular cell. EM; bar: 2. OM: ×2,900

Fig. 11. Spleen cortex, 4th day. A macrophage showing a rather indented nucleus and a clear cytoplasm with an activated Golgi complex (*g*). EM: bar: 2. OM: 2,300

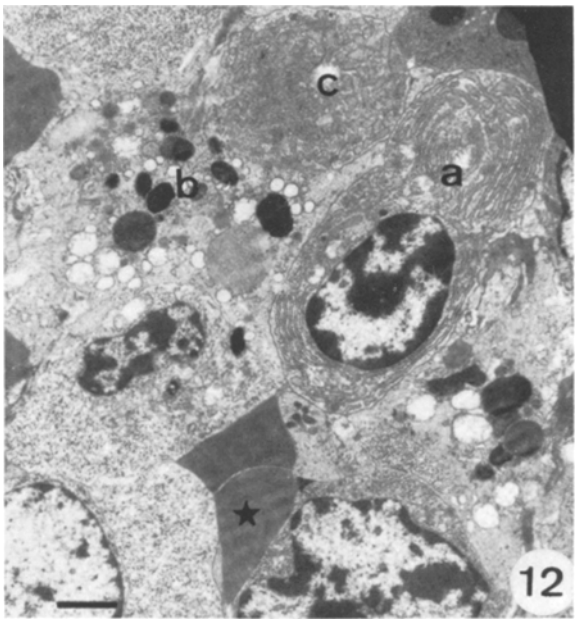


Fig. 12. Spleen, white pulp, 6th day. Adjacent to a typical proplasmocyte (*a*), there is a cell showing the cytoplasmic features of a proplasmocyte (*c*) associated to those of a macrophage (*b*); no membrane can be clearly observed between these two part. *: red blood cells. EM; bar: 5. OM: ×1,500

cells showing interdigitating contacts with adjacent cells; their cytoplasm was electron dense and contained few organelles: a reduced Golgi complex, some vacuoles (sometimes containing bacteria) and a few mitochondria (Fig. 9). The features of these cells were akin to those of the "fixed reticular cells" of Galindo and Imaeda (1962), the "primitive reticular cells" of Moe (1964) or the "dendritic reticular cells" of Nossal et al. (1968), and we shall refer to them hereafter with the generic name of reticular cells.

There was little difference in the appearance of the spleen. The cellular oedema was less marked, but lasted longer. In the follicular zone of the cortex, changes were identical to those of the lymph node, but only after a two-day interval. After the 5th day, unlike the changes seen in the lymph node, the density of plasma cells increased in the red pulp. Stimulated macrophages and reticular cells were also observed (Figs. 10 and 11).

Besides the quantitative and structural changes in pre-existing cells, the cytology of lymphoïd organs underwent changes which led to cellular appearances not seen in controls (Fig. 12). These were evident after the 5th day and seemed more frequent in the spleen.

III. 3H-Thymidine Incorporation

Figure 13 shows the increase in the incorporation within the spleen and lymph nodes of inoculated animals, when compared with controls. A significant peak appeared on the 3rd-4th day in the lymph node, and on the 4th day in the spleen. In the latter, a moderate peak was also seen on day 8.

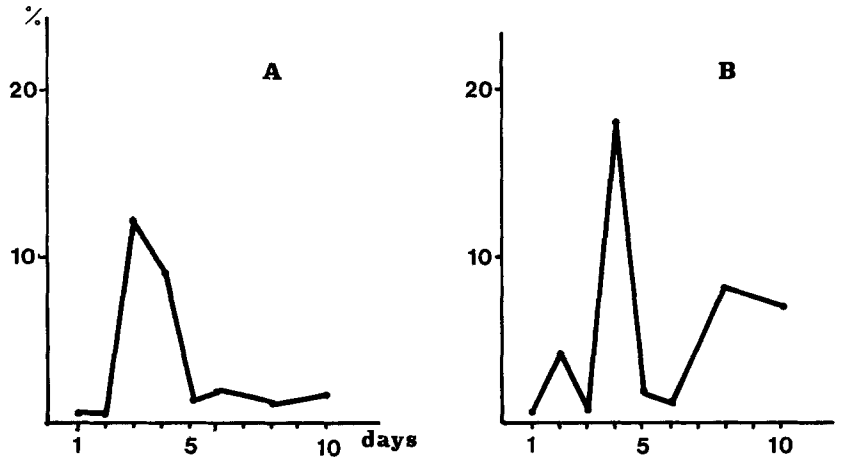


Fig. 13 A and B. Diagram of the 3H-Thymidin incorporation (liquid scintillation countings) in: **A**, lymph-node; **B**, spleen. Abscissa: days after inoculation; ordinate: percentage of increase as compared to controls

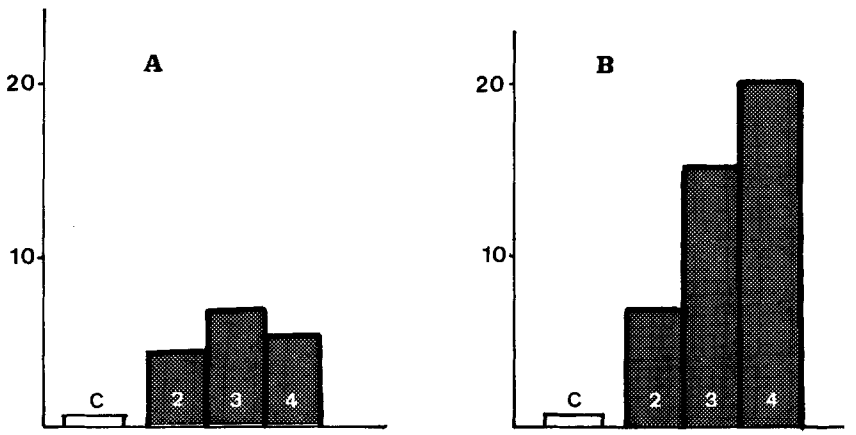
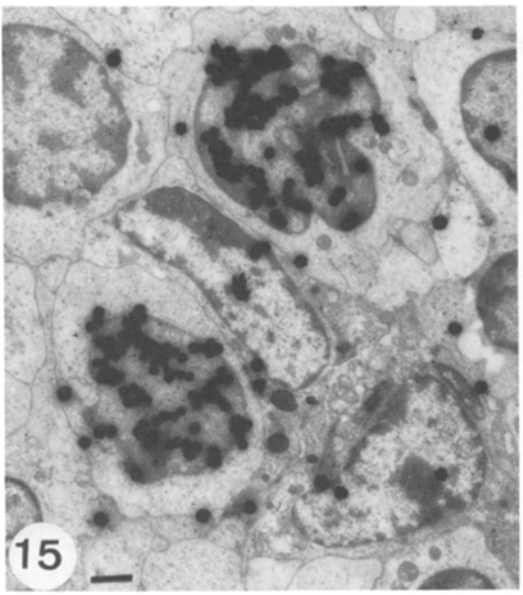
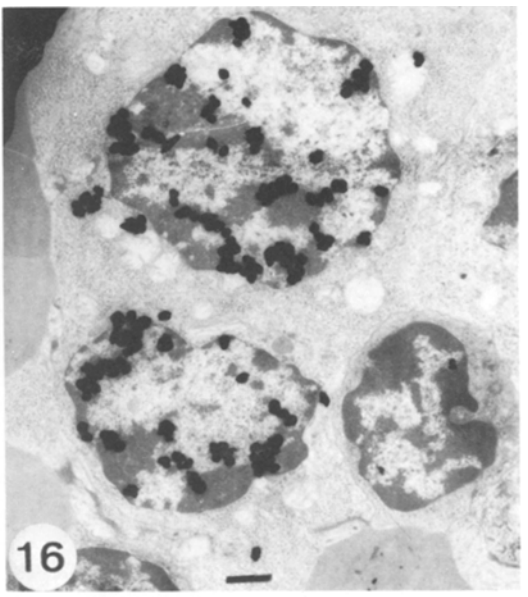
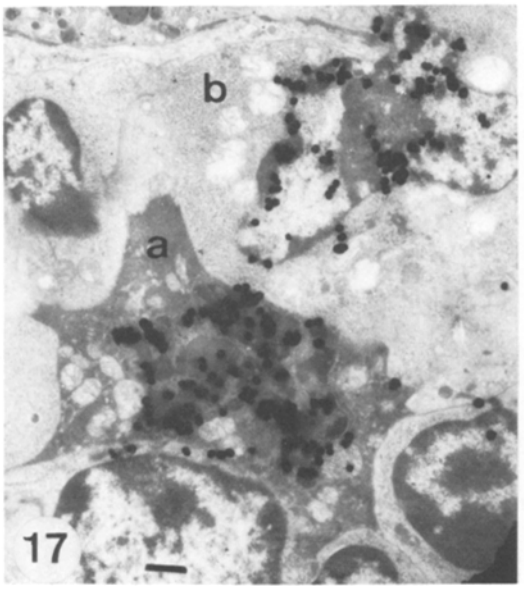


Fig. 14 A and B. Diagram of 3H-Thymidin incorporation (autoradiographic evaluation) in: **A**, lymph-node; **B**, spleen. Abscissa: days after inoculation (*c*: control); ordinate: percentage of cells showing a density at least 10 times that of the background for a comparable surface

Figure 14 gives a semi-quantitative evaluation of labelled cells observed in electron microscopic sections. In controls, the number of labelled cells was very low in the lymph node; there were a few lymphocytes, and even fewer reticular cells. The density of labelling was weak; this incorporation reflected the cell turnover. In the spleen, the labelling was identical in lymphoïd and reticular cells and labelled erythroblasts were numerous in the red pulp. Among inoculated animals, a significant rise in cell labelling was observed only on the 3rd and 5th days, and was less marked in the lymph node than in the spleen. In the lymph node cortex, the incorporation of 3H-T into lymphocytes was occasionally observed earlier than morphological appearances of activation (Figs. 15 and 16). The incorporation of 3H-T was also marked in reticular







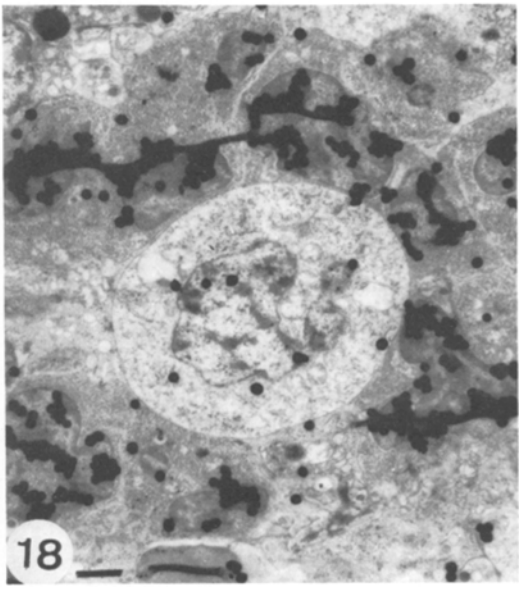


Fig. 15. Lymph node cortex, 2nd day. Two lymphocytes with incorporation of 3 H-T in the nucleus, but no aspects of nuclear or cytoplasmic activation. EM; bar: 5. OM: ×1,250

Fig. 16. Spleen, 4th day. Two activated lymphocytes with a modified chromatine and the appearance of polyribosomes in the cytoplasm. EM; bar: 2. OM: ×5,000

Fig. 17. Lymph node cortex, 4th day. *a*: an electron-dense cell showing strong incorporation. The aspect of the cytoplasm with the start of numerous intercellular expansions, is that of a reticular cell; mitochondries, however, are rather abundant. *b*: a lymphoïd cell showing nuclear and cytoplasmic activation

Fig. 18. Spleen, red pulp, 8th day. An electron-lucent cell (macrophage?) showing very little incorporation, surrounded by actively incorporating erythroblasts ("peripolesis"). EM; bar: 5. OM: ×1,250

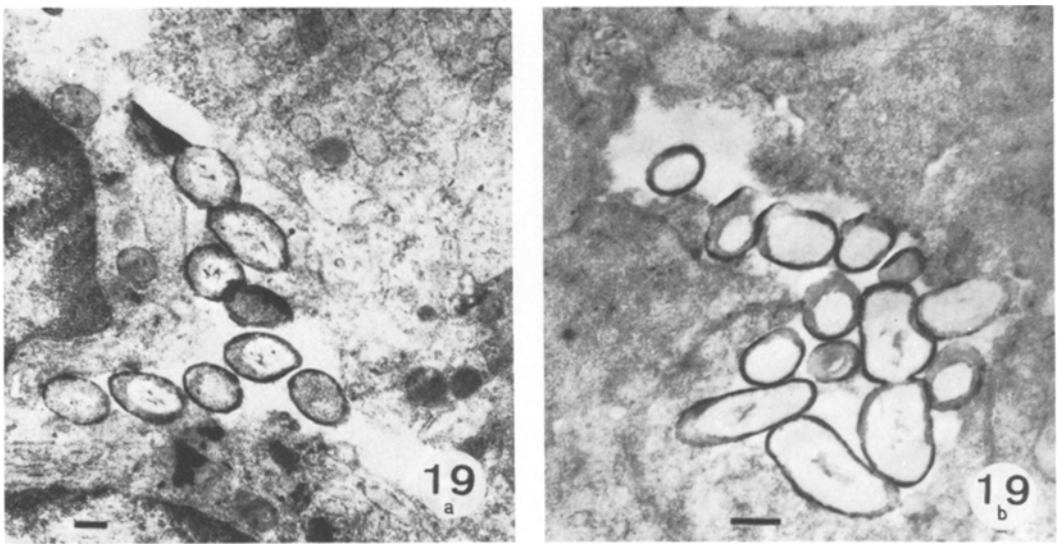


Fig. 19. a Brucellae in a sinus of the spleen cortex, 8th day. Bacteria are labelled with PCPA. EM; bar: 1. OM: ×4,500. **b** PCPA-labelled Brucellae within a deteriorated cell (macrophage?), spleen, 8th day. Bacteria show no changes as compared to those of the preceeding figure. EM; bar: 1. OM: ×6,500

cells (Fig. 17). In the spleen, the intensity of labelling on days 3 to 5 was higher, and the percentage of labelled cells greatest. Besides similar changes in lymphoïd and reticular cells, we found that the erythroblasts also showed a burst of activation (Fig. 18).

IV. Observations of Brucellae within Lymphoid Organs

As a general rule, a tiny abscess was observed at the site of inoculation. On histological sections, brucellae were seen on the connective capsule of the lymph node and within cortical sinuses; eventually, they were enclosed within macrophages in the lymph node cortex (see Fig. 2). However, the small size of these bacteria does not allow a precise evaluation of their relationships with cells of lymphoid organs, especially in the spleen. This was better observed by electron microscopy, with the help of peroxidase-labelled antibodies. Labelled brucellae were seen free in the sinuses of the lymph node and of the spleen. They were usually grouped, and did not seem to have undergone morphological degradation (Fig. 19a). Intracellular bacteria were observed within macrophages and reticular cells, and showed variable degrees of degradation (Figs. 19b and 20). Several changes observed among infected animals only on PCPA-DAB treated non-stained sections, suggest that the mobilization of particulate antigen(s) within phagocytic cells, after the degradation of bacteria could be visualized by this technique (Fig. 21).

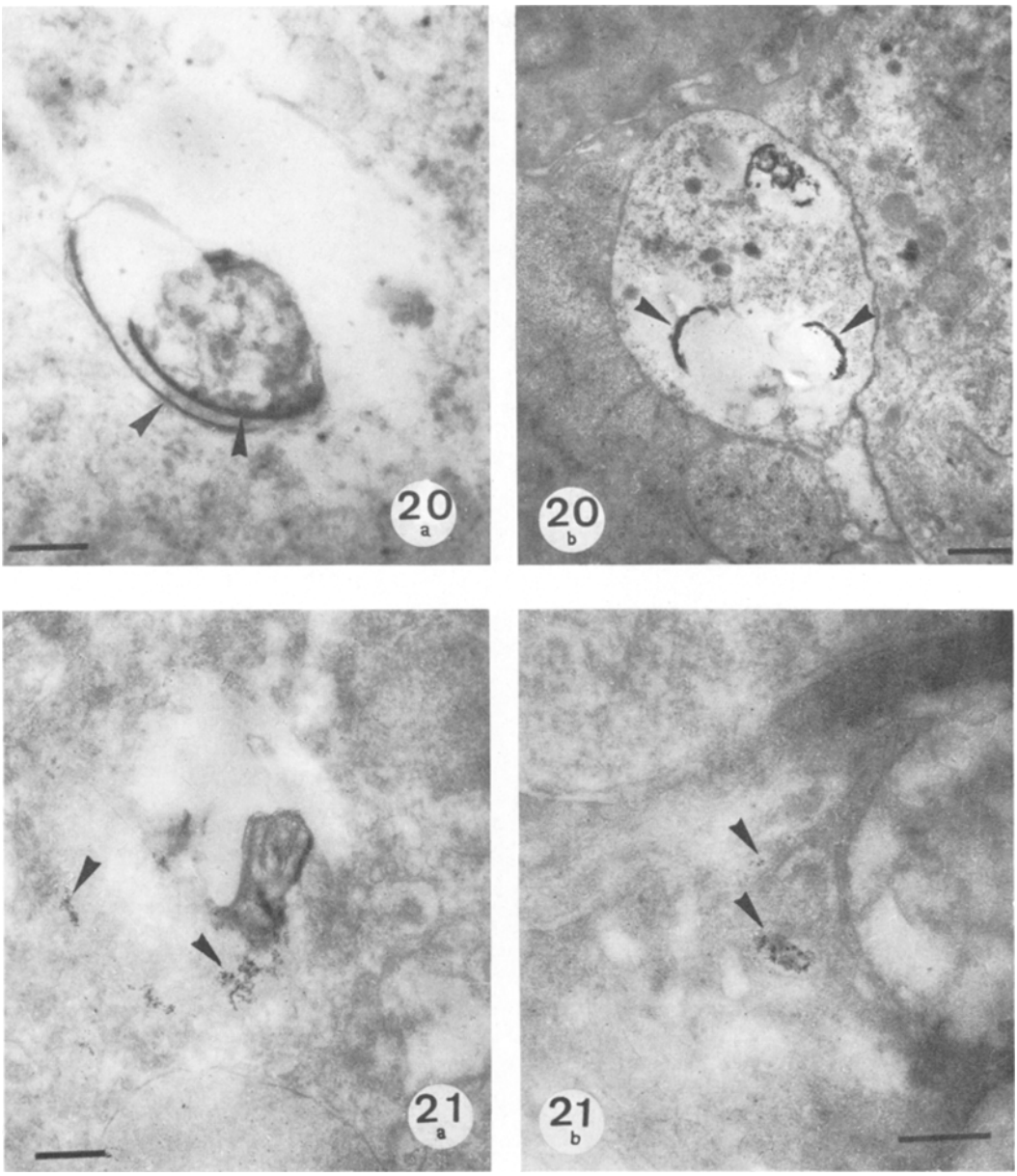


Fig. 20a and b. Aspects of bacterial degradation within vacuoles, spleen, 8th day. **a** Degradation of partially stained Brucella. Note the apparent breaking of the densely labelled wall (arrowheads). The section is not counterstained. EM; bar: 0.5. OM: 22,500. **b** Other aspect in a macrophage vacuole; note the density of labelling of cell wall remnants (arrowheads). Counter stained section. EM; bar: 1. OM: $\times 6,500$

Fig. 21a and b. Aspects of fine particular deposits within the cytoplasm of some phagocytic cells. These aspects were generally seen in the vicinity of vacuoles. Although some structures (e.g.: peroxysomes) were seen in cells after the DAB revelation these fine, pulverulent and dense particles were never observed in control animals. Sections are not counterstained. EM; bar: 0.5. OM, **a**: $\times 22,500$; **b**: $\times 26,500$

Discussion

The observations made on mice after primary stimulation by living *Br. suis* may be summarized as follows. In the lymph node an early reaction in the cortical region was observed, with a major influx of macrophages, and an increase in the number of reticular cells; these two cell groups showed activation from the 2nd day onwards. Changes in lymphoid series began after the 4th day with activation of a limited number of lymphocytes and the development of secondary germinal centers. Development of plasma cells was not observed before the 5th day, and was limited between the 5th and the 10th days. This change was also observed in the lymph node medulla. In comparison, the splenic cortex showed the following features: changes were a little delayed when compared with those of the lymph node, there were more activated cells, and plasma cells were more numerous. Bacteria were observed within the connective tissue of the lymph node capsule by the 24th h. In the spleen, free brucellae were encountered within sinuses, whilst phagocytozed bacteria were more often seen in the follicular area, enclosed within macrophages and reticular cells. The degree of bacterial degradation did not seem related to the time elapsed after inoculation, and phagocytozed bacterial showed unequal degradation.

Experimental brucellosis in laboratory animals is generally a very mild infection, unless huge quantities of bacteria are used (Margolis et al., 1945). Mice, however, develop antibodies and cellular resistance (Feldman et al., 1935; Olitzki et al., 1953). We choose a subcutaneous inoculation in order to be able to observe more precisely the local lymph node involved in the initial response to infection, and to provide a more reasonable model of spontaneous infection than intravenous injection (Janbon and Bertrand, 1955).

The early response of mouse lymphoïd tissues to a primary injection of *B. suis* bore the features of a T cell-mediated reaction (Mackaness, 1972). Some differences, however, may be found between the changes observed here and those seen with other T-stimulating antigens. Xenogenic red blood cells, for instance, induced very rapid changes in the spleens of mice: loss of germinal centers and numerous divisions of pyroninophilic cells by the 24th h; appearance of plasma cells (with circulating antibodies) and invasion of the red pulp by lymphoïd cells on the 3rd day; and finally a beginning of recovery by the 4th-5th day (Congdon et al., 1961). Similar rapid changes were described after injection of xenogenic bone marrow, or heterologous albumin into mice (Congdon, 1964).

In these examples, the differentiation of antibody-producing cells, although induced by a cooperation between T and B cells, was conspicuously quicker than in mouse brucellosis. A comparison with the response of other species of laboratory animals cannot be easily made, studies on experimental brucellosis being rather scarce. Observations made on dogs (Margolis et al., 1945) cannot be compared to ours, since the purpose of this study was quite different, and the doses of Brucella used beyond all comparison. In the guinea pig (Moulton and Meyer, 1958), a peculiarity of the response was the importance of polymorphonuclear leucocytes participation; in the spleen, an hyperplasia of "reticular

cells" was observed by the 7th day, plasma cells were seen in large number by the 7th–10th days. As observed in earlier studies concerning guinea pigs, numerous abcesses were observed in the parenchyma after the 20th day.

It is interesting to compare the response of the mouse to brucella with that to *Mycobacterium tuberculosis*; in the spleen the lymphocyte activation was conspicuous by the 2nd day, germinal center did not develop, and by the 6th day the cellular response was much more marked in the red pulp than in the white; no plasma cells were observed (North, 1972). After *Listeria monocytogenes* inoculation, a marked activation of lymphocytes, starting at the 12th h, was observed; the paracortical area was the site of granulomas, more evident on the 6th–8th days (Raez et al., 1974), this period corresponding to the maximal protective activity of spleen cells (Mackaness, 1969) or thoracic duct cells (Kostiala et al., 1975). Compared with these descriptions, the main characteristics of our observations are the slow activation of lymphocytes, the absence of granulomas, the development of germinal centers and an increased participation of reticular cells.

Since invading bacteria reached the lymph node via the cortical sinuses, the first cells they encountered were probably the macrophages occluding the perforations of the inner wall of subcortical sinuses, and the reticular cells (Clark, 1962). Later, when bacteria reached the spleen, they either came into contact with the cells of the walls of limiting sinuses, which are identical to fixed reticular cells (Galindo et al., 1962), or passed through the interstices of the sinuses of Billroth's chords, where they encountered free or mobilizable macrophages (Pictet et al., 1969). Compared with other bacterial infections, we feel that the participation of the reticular cells is a particular feature of our observations. They were present in large numbers very early in the lymph node and were apparently strongly stimulated, more, in fact, than the lymphoïd cells. Later, some seemed to undergo a differentiation. Mouse germinal centers are rich in reticular cells (Galindo et al., 1972; Schwarzendruber, 1966) which display phagocytic properties (Moe, 1964). They intervene in T-B lymphocytes interaction (Gutman et al., 1972) and are activated by T-stimulating (Capalbo et al., 1964), but not by B-stimulating (Nossal et al., 1968) antigens.

Though antigenic stimulation was probably continuous during the 10 days of the experiment, the response of lymphoïd cells was comparatively slow (Langevoort, 1973). The fact that bacteria undergo variable degradation within phagocytic cells may account for this observation; it might be that the bacteria observed inside cells at a given time after inoculation were, in fact, phagocytozed at different intervals, being continuously delivered from the site of inoculation. However, it has been observed that similar evidence of variable degradation was also found in vivo in macrophages, all infected at the same time (Oberti et al., 1974). Thus, the rate and the conditions of processing of bacterial structures probably play a prominent role in the pattern of the immune response. Working with a slow rate-dividing microorganism (*Histoplasma capsulatum*) Berry (1969) has shown that during the "stimulation" of the reticulo-endothelial system the phagocytic and microbicidal capacities of macrophages could be dissociated. It has been suggested that while most of the bone marrow-derived

monocytes produce a phagocytic system acting only as scavenger cells, the phagocytic cells implicated in immunological responses would represent only a part of the parenchymal macrophages and most of the reticular cells (Drewinko, 1973). The processing of brucella by this smaller cellular population could contribute to the characteristics of the response of infected animals.

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