STUDIES ON EXPERIMENTAL BRUCELLOSIS

BRUCELLA ABORTUS INFECTION IN THE MALE GUINEA PIG AND EFFECT OF SENSITIZATION ON VARIOUS ORGANS

Katsumoto Ueda and Kiyoshi Imaizumi

Department of Veterinary Science, National Institute of Health, Tokyo

(Received for Publication March 31, 1969)

Brucellosis in domestic animals is characterized by an affection of the genital system, as well as a systemic infection\textsuperscript{10,21}. However, the relationship between the local infection (reproductive organs) and the systemic one (spleen, liver, lymph nodes) has not been fully analyzed so far. It is well known that experimental brucellosis of guinea pigs is characterized by a systemic infection of long duration\textsuperscript{4,7}. In this case, an affection of male reproductive organs, such as orchitis and epididymitis, also occurs frequently\textsuperscript{1,4,7,15}. These genital lesions are sometimes called metastatic foci\textsuperscript{1} since they are found in the late stage of the infection, whereas lesions in the liver, spleen and lymph nodes appear in the early stage. There are numerous reports on experimental brucellosis of guinea pigs, but no satisfactory work has been performed to elucidate any relationship between these two characteristic types of lesions of brucellosis.

A series of experiments was conducted to analyze the relationship between the systemic infection and the affection of the genital organs of male guinea pigs infected with \textit{Brucella abortus}.

MATERIALS AND METHODS

Animals: Male Hartley guinea pigs weighing 350 to 500 g were obtained from a commercial breeder. Five of them were kept separately in each metallic cage and maintained with commercial pellets, water, and a bit of vegetable.

Brucella strains: \textit{Brucella abortus} strain A 62 was used in the 1st experiment, and as a challenge strain in the 2nd experiment. This strain was isolated from the milk of an infected cow in 1952, and identified as \textit{B. abortus} type 1\textsuperscript{18}. Since then, it has been maintained on a nutrient agar containing inactivated horse serum at a concentration of 5\% (serum agar). This strain requires an increased CO\textsubscript{2} tension for its growth.

Furthermore, \textit{B. abortus} strain 19 maintained on the serum agar was used as a sensitizing strain in the 2nd experiment. Strain 19 is capable of growing in aerobic conditions.

Methods for infection: Strain A 62 grown on the serum agar for 48 hours was suspended in phosphate buffered saline (PBS), and made to appropriate dilutions with PBS. The inoculum was injected into the terminal vein of the left leg after removing a part of the skin over the site of injection. Each animal received 0.5 ml of the inoculum. Portions of the dilutions were inoculated on trypsinase agar plates (trypsinase

Table 1. Isolation of the Protein Fraction (BPF) from *B. abortus A 62*

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cells of A 62 (wet weight, 18.8 g)</td>
</tr>
<tr>
<td></td>
<td>Acetone 500 ml</td>
</tr>
<tr>
<td></td>
<td>Centrifuge 3,000 rpm 15 min.</td>
</tr>
<tr>
<td>2.</td>
<td>Precipitate</td>
</tr>
<tr>
<td></td>
<td>Distilled water 100 ml</td>
</tr>
<tr>
<td></td>
<td>Suspension</td>
</tr>
<tr>
<td></td>
<td>Alternate freezing and thawing (25 times)</td>
</tr>
<tr>
<td></td>
<td>Centrifuge 4,000 rpm 30 min.</td>
</tr>
<tr>
<td>3.</td>
<td>Precipitate</td>
</tr>
<tr>
<td></td>
<td>Distilled water 200 ml</td>
</tr>
<tr>
<td></td>
<td>Suspension</td>
</tr>
<tr>
<td></td>
<td>Sonic oscillation (2 hrs.)</td>
</tr>
<tr>
<td></td>
<td>Centrifuge 4,000 rpm 30 min.</td>
</tr>
</tbody>
</table>
| 4.   | Supernatant  
|      |  
| 5.   | Filtrate |  
|      | 50 % trichloracetic acid (5 % in final concentration) |  
|      | in refrigerator (2 hrs) |  
|      | Centrifuge 3,000 rpm 10 min. |  
| 6.   | Precipitate |  
|      | pH 7 with 0.5 N NaOH, distilled water to a volume of 200 ml |  
|      | 50 % saturated ammonium sulfate in refrigerator (overnight) |  
|      | Centrifuge 4,000 rpm 20 min. |  
| 7.   | Precipitate |  
|      | Phosphat buffer in a volume of 150 ml |  
|      | Dialysis against running water for 9 days |  
|      | Lyophilization |  
|      | Lyophilize (yields: 0.9 g) |  

Chart 1. Titration of BPF

soy agar; BBL). These plates were incubated under 10% CO₂ tension. The number of colonies developed was counted. The colonies were observed by the oblique light method⁹,¹⁷. All the colonies developed were confirmed to be of smooth type.

Sensitization of guinea pigs: In the 2nd experiment, some guinea pigs were sensititized by the pretreatment with an intradermal injection with strain 19. The organisms
grown on the serum agar were harvested after 48 hours' incubation period and suspended in PBS. Intradermal injection was made with the suspensions at the right leg of the guinea pigs. Each animal received 0.05 ml of the inoculum.

Quantitative culture method: Quantitative culture was made by the same way as the spleen count method which had ordinarily been used in the estimation of virulence of Brucella\(^{21}\) or of protective effect of immunization\(^{6,18}\). In this experiment, a piece of the organ was removed aseptically and made to a homogenate by adding 5 or 10 volumes of PBS in a glass homogenizer. Portions of 0.1 ml from appropriate dilutions of each homogenate were inoculated on serum agar plates and incubated at 37°C in an atmosphere containing CO\(_2\) at a concentration of 10%. After 4 to 7 days' incubation, the number of colonies developed was counted.

Serological examination: The tube agglutination test was performed according to the standard method recommended by the expert committee of the Ministry of Health and Welfare\(^{16}\). An antigen suspension adjusted to tube No. 3 of the McFarland nephelometer was prepared by diluting the Brucella abortus agglutination concentrate (prepared from B. abortus strain 99 in the National Institute of Animal Health, Tokyo).

The complement fixation test was performed by the method recommended by the Brucellosis Center in Japan\(^{14}\). A soluble antigen was extracted from B. abortus strain 99 with 2% phenol.

Intradermal skin test: A test antigen was prepared from strain A 62 cultivated on 0.5% glycerin agar containing 0.1% Tween 40 for 72 hours, as shown in Table 1. A crude Brucella protein fraction (BPF) was obtained. Titration of BPF diluted with PBS was made on guinea pigs infected with B. abortus. Intradermal injection of the infected animals with 1 or 10 \(\mu\)g of BPF provoked an intense reaction. The intact animals injected with the same doses exhibited only weak reaction when observed 24 or 48 hours after the injection (Chart 1).

The skin test was made by injecting BPF intradermally into the shaved abdominal skin. Each animal received a dose of 5 \(\mu\)g of BPF in 0.05 ml. The diameters of erythematous and indurated areas were measured 48 hours after the test injection.

Histopathological examination: The organs removed were fixed in 10% formalin. Paraffin sections 5 or 6 \(\mu\) in thickness were prepared and stained with hematoxylin and eosin.

RESULTS

1. Experiment 1

The guinea pigs used were divided into 4 groups of 20 or 25 animals each. The 1st group received \(1.4 \times 10^9\) organisms, the 2nd group \(1.5 \times 10^9\), the 3rd group \(2.0 \times 10^9\), and the 4th group \(1.5 \times 10^9\). Two to 4 (mostly 3) animals each were sacrificed on the 2nd, 4th, 7th, 14th, 21st, 28th, 49th, and 70th day after the infection. The animals were killed by bleeding by heart puncture. Some of the blood samples collected were used for detection of the organisms, and the remainder served for serological examinations. The main organs were weighed and examined for macroscopic lesions. Then, pieces of tissue were collected aseptically from the liver, spleen, kidney, lung, cervical lymph node, inguinal lymph node, portal lymph node, and a combined specimen of the testis and epididymis was also harvested. All of them served for the quantitative cultivation. The remaining portions of the organs were fixed in 10% formalin and subjected to the histopathological examination. Loopful samples of the bone marrow
Chart 2. Development of Lesions in Guinea Pigs Inoculated Intravenously with $4 \times 10^9$ Brucellae

- **Spleen**: Weight of spleen per kg of body weight increased over time, peaking around the 3rd week, then decreased. Fewer granulomatous lesions were observed.
- **Liver**: Slight increase in lesion severity observed.
- **Testis**: Moderate lesion severity, indicated by + symbols.
- **Epididymis**: Severe lesions, indicated by ++ symbols.

- **Observations**:
  - a) The number of granuloma more than 50 $\mu$m in diameter was counted in 2 specimens from different lobes of the liver of each animal. The area of the specimens counted was approximately 1.5 cm$^2$ per animal.
  - b) The severity of lesion is expressed following symbols: ++, nearly complete absence of spermatogenesis; +, partial absence of spermatogenesis in either or both testes of each animal.
  - c) The severity of lesion is graded as follows: severe (+ +) and mild (+) lesion in either (left or right) epididymis; the absence of lesion (−) in both (left and right) epididymides. The symbols in the figure: ●, cellular infiltration; ○, granuloma; and ×, abscess formation.

Chart 3. Fate of Organisms in Organs of Guinea Pigs Inoculated Intravenously

- **1st group**: (log) number of organisms per tissue (g) showed a decrease over time.
- **2nd group**: Similar trend as the 1st group.
- **3rd group**: (log) number of organisms per tissue (g) showed a slight increase over time.
- **4th group**: Similar trend as the 3rd group.

- **Symbols and lines in the figure**: ●—● spleen, ○—○ liver, ■—■ lung, ▲—▲ kidney, O—O cervical lymph node, △—△ portal lymph node, □—□ inguinal lymph node, and •—• heart blood. Each symbol indicates the mean of 3 or 4 animals.
- **Symbols**: ◊ indicates the testis and epididymis of an individual animal.
- **Signs**: −, +, ++, +++ indicate the roughly estimated degrees of bacterial population in the bone marrow (upper level) and in the urine (lower level) of an individual animal. Letter c indicates a contamination.
Studies on Experimental Brucellosis

(from the middle part of the left tibial marrow) and of urine (from the contents of the urinary bladder) were inoculated to serum agar plates in order to estimate the approximate bacterial population contained in the samples.

To test the development of the skin sensitivity, 25 guinea pigs, weighing 400 to 450 g, were infected intravenously with $1.0 \times 10^5$ organisms. Of them, 5 animals each were tested for skin sensitivity in the 4th, 5th, 6th, 7th, and 8th week after the infection, respectively.

a. Development of lesions in various organs

The changes frequently observed in various organs were as follows: splenomegaly, swelling of lymph nodes, scattering of minute grayish-white nodules in the liver, swelling, induration or abscess formation in the epididymis, and induration or atrophy of the testis. The larger the dose injected was, the earlier and the more intensively lesions developed, except those in the 1st group which showed nearly the same severity as those in the 2nd group.

Development of lesions differed with the kind of organs. Hepatic, splenic, and lymph-nodular lesions developed in the early stage and regressed thereafter (Figs. 1~6). On the other hand, epididymal and testicular lesions developed slowly, and fully developed lesions were observed in the late stage (Figs. 7~10). The differences in the course of infection among the organs were the most apparent in the 2nd group. The lesions of four representative organs in this group were graded and their development was illustrated in Chart 2. The weight of the spleen and the number of granulomata in the liver (as counted on microscopic preparations) reached a peak in the 3rd or 4th week of infection and regressed gradually thereafter. Testicular lesions were graded by the degree of retardation in spermatogenesis. Classification of epididymal lesions was made on the basis of three changes; that is, cellular infiltration, granuloma formation, and abscess formation. Each change was graded into 2 classes, severe and mild. As shown in Chart 2, these genital lesions were delayed in development and reached a peak in the 7th or the 10th week.

Although no results obtained are illustrated in the chart, lesions in the lymph nodes and bone marrow showed essentially the same course of development as those in the spleen and liver. Induration and abscess formation in the epididymis and atrophy of the testis became apparent macroscopically in the 7th or the 10th week. In the late stage of infection, macroscopic nodules with or without central suppuration were also found on the surface of the metacarpal bones, sternum, or ribs. The rate of incidence of these genital and bone lesions in the prolonged cases is shown in Table 2. The larger the dose employed, the higher the rate of incidence of lesions.

b. Fate of organisms in organs

The patterns of bacterial growth in all the groups are shown in Chart 3. The bacterial population in the main organs and lymph nodes reached a peak within the 1st or the 2nd week and maintained a high level up to the 4th week in all the groups, except the 4th group in which only a small peak was shown in the 7th week. The growth curve of bacteria declined gradually thereafter in these organs of every group. On the other hand, bacterial multiplication in the genital organs was delayed and reached a peak in the 3rd or the 4th week, as seen in the 2nd and 3rd groups. Although there was a slight decrease in bacterial population in the 7th week, a large amount of bacteria was recovered again from some animals of the 1st, 2nd, and 3rd groups in the 10th week.

The approximate bacterial population in the bone marrow during the course of
Table 2. Rate of Incidence of Genital and Bone-Articular Lesions after Intravenous Inoculation with Various Doses of B. abortus

<table>
<thead>
<tr>
<th></th>
<th>Genital lesion</th>
<th></th>
<th>Bone-articular lesion</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7th week</td>
<td>10th week</td>
<td>7th week</td>
<td>10th week</td>
</tr>
<tr>
<td>1st group (1.4x10^9)</td>
<td>3/3*</td>
<td>4/4</td>
<td>1/4</td>
<td>3/4</td>
</tr>
<tr>
<td>2nd group (1.5x10^9)</td>
<td>2/4</td>
<td>3/4</td>
<td>0/3</td>
<td>2/4</td>
</tr>
<tr>
<td>3rd group (2.0x10^9)</td>
<td>0/3</td>
<td>2/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>4th group (1.5x10^9)</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

* Denominator: The number of guinea pigs with lesions.
Numerator: The number of guinea pigs observed.

Table 3. Development of Skin Sensitivity in Guinea Pigs Inoculated Intravenously with 1.0x10^5 Brucella

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Weeks after inoculation</th>
<th>Extent of reaction measured at indicated hours after injection with 57 BPF per animal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 hours after injection</td>
</tr>
<tr>
<td>1</td>
<td>0*</td>
<td>6 x 7 x 3**</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>6 x 7 x 3</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>5 x 5 x 3</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>7 x 6 x 4</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>4 x 3 x 3</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>9 x 8 x 3</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>7 x 7 x 2.5</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>14 x 10 x 4</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>14 x 20 x 3.5</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>14 x 12 x 4</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>12 x 15 x 4</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>19 x 16 x 5</td>
</tr>
<tr>
<td>13</td>
<td>6</td>
<td>17 x 15 x 4.5</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>8 x 6 x 3</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>8 x 6 x 3</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>21 x 24 x 5.5</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>15 x 15 x 4</td>
</tr>
<tr>
<td>18</td>
<td>7</td>
<td>13.5 x 15 x 3</td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>20 x 24 x 5</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>21 x 23.5 x 6</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>8 x 11 x 4</td>
</tr>
<tr>
<td>22</td>
<td></td>
<td>14 x 20 x 6</td>
</tr>
<tr>
<td>23</td>
<td>8</td>
<td>18 x 25 x 5</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>20 x 18 x 5</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>16 x 22 x 5</td>
</tr>
</tbody>
</table>

* Before infection.
** Length, width, and thickness of the erythematous area in millimeters.

Infection was roughly parallel with that in the main organs, and that in the urine with that in the genital organs. Severe bacteremia was observed during the early period of several weeks. This period seemed to correspond to the period during which a high population of organisms was observed in the main organs.
c. Immunological findings

Observation was made on the development of serum antibody and of skin sensitivity after the infection. The agglutinin titer and complement-fixing titer of the serum were shown in Chart 4. The time of appearance and the rate of increase of both titers were roughly parallel with the injected dose in all the groups, except the 1st group. The antibody titer in the 1st group was nearly the same as that in the 2nd group. The skin test by the intradermal route elicited a marked reaction 6 to 8 weeks after the infection with $10^8$ brucellae (Table 3).

2. Sensitization with strain 19

Sensitization was performed with strain 19. Twenty-four guinea pigs weighing approximately 350 g were injected intradermally with $1.0 \times 10^8$ organisms at the left leg. Three animals each were sacrificed at certain intervals up to the 94th day. Detection of Brucella from the main organs and lymph nodes, quantitative cultivation of the spleen, left inguinal lymph node, testis, and epididymis, and macroscopic observation of lesions were carried out, as well as the serum agglutination and intradermal skin tests.

No changes were observed clinically, except swelling and erythema at the sites of injection. The erythema disappeared on the 10th day of infection. Serum agglutinin titer was elevated, and maintained a high level up to the 94th day at least. An intradermal skin test revealed an intensive reaction on the 17th day, and a marked reaction afterward.

Macroscopic examination showed negligible changes except in the left inguinal lymph node which was moderately enlarged when examined on the 14th and 21st day. On these days, scattered foci of large mononuclear cells were observed microscopically.
in this lymph node. They were diminished in number thereafter. A small number of small foci consisting of large mononuclear cells were found in the liver, spleen, and portal and cervical lymph nodes on the 14th and 21st day. A few small nodules were present in the spleen of guinea pigs sacrificed on the 56th and 94th day. No lesion was found at any stage in the sections of the lung, testis, and epididymis examined.

Isolation of organisms from these organs showed that the distribution of Brucella was mainly restricted to the left (injected side) inguinal lymph node and spleen. Quantitative cultivation of the spleen and left inguinal lymph node exhibited a peak of bacterial growth over a period from the 7th to the 14th day. The bacterial population was inclined to decrease thereafter.

3. Experiment 2

The results of the first experiment showed that there were differences in the development of lesions and the fate of organisms among the organs. As a result, it was revealed that there were two phases of the disease according to the period of infection; i.e., an early or a systemic phase and a late or a genital phase. It seemed that sensitizing conditions of animals accompanied by the primary infection might have some relations to the differences in the course of infection among the organs. In order to obtain some information on this point, an attempt was made to modify lesions in various organs by reinfection. Based on the results described above in section 2, animals were exposed to reinfection 4 weeks after intradermal inoculation with 10^7 cells of strain 19.

Forty-nine male Hartley guinea pigs weighing 400~450 g were provided. Thirtyone of them were pretreated with 1.4×10^7 organisms of strain 19 and the remainder served as controls. Four weeks later, all the animals, pretreated and intact, were divided into 3 groups. The animals of the 1st group (11 pretreated and 2 intact) received 4.0×10^8 organisms, those of the 2nd group (10 pretreated and 8 intact) 4.0×10^6, and those of the 3rd group (10 pretreat and 8 intact) 4.5×10^6.

A majority of the re-infected animals of the 1st group died from a shock. The other animals which had overcome the shock were sacrificed in the 11th week after challenge, as well as the controls of the same group. Four or 5 reinfected animals and 3 controls of both the 2nd and 3rd groups were subjected to autopsy in the 4th week. The remainder (5 reinfected and 5 control) of the 2nd and the 3rd group were autopsied in the 14th and the 15th week, respectively. The organs were carefully examined for gross lesions and weighed. Specimens were collected from them, immersed in 10% formalin, and subjected to histopathological examination.

a. Gross findings

Characteristic modification of lesions in the organs was the most apparent in the 2nd group. The main macroscopic lesions of the 2nd group are shown schematically in Chart 5.

In the 4th week after challenge, the spleen and lymph nodes of the reinfected animals were smaller in size than those of the controls. A few nodules 2 or 3 mm in diameter were present in the spleen of the reinfected animals, but no nodules were found in the controls. White semi-translucent miliary nodules approximately 1 mm in diameter were scattered in the liver of the reinfected. They were larger in size than those found in the controls. No apparent change was found macroscopically in the genital organs of the reinfected animals, whereas marked lesions occurred to the controls.
In the 14th week, swelling of the spleen and lymph nodes was still slighter in the reinjected group than in the control. Induration of the cervical lymph node and suppuration of small areas in the portal or inguinal lymph nodes were found in some of the animals, both reinjected and control. Atrophied testis and enlarged epididymis were found in 3 of the 5 reinjected animals, and in 4 of the 5 controls.

b. Histopathological findings

As the modification of lesions was most clearly shown in the guinea pigs of the 2nd group, microscopic observations were made on the lesions of this group.

Four weeks after challenge infection: Small foci composed of large mononuclear cells were scattered in the spleen and lymph nodes of reinjected animals. They were found in the red and white pulp of the spleen (Fig.12) and in the medullary cord of the lymph node. A small number of large granulomatous nodules with or without central necrosis were found in the spleen. Giant cells were occasionally found in these lesions. In the control animals, a proliferation of large mononuclear cells occurred diffusely in the spleen and lymph node. Few giant cells were found in these lesions.

Large granulomas composed mainly of mononuclear cells showing atrophy of the cytoplasm were scattered in the liver of reinjected animals (Fig.11). The number of nodules was a little smaller in these animals than in the control. Giant cells were found in almost all the nodules. Some nodules had small necrotic or supplicative areas at their center. The periphery of the nodules was circumscribed with a thin layer of spindle-shaped cells. A few small round cells were infiltrated in this peripheral layer. The liver of the control animal had scattered nodules smaller in size than those of the liver of the reinjected. Some of the nodules were affected with central necrosis or
suppuration.

The bone marrow of the reinfected animals had granulomas larger in size but smaller in number than those in the controls. The nodules in the reinfected animals occasionally had neutrophilic infiltration or necrosis in their central area. Giant cells were also frequently found in the reinfected animals. Small foci of large mononuclear cells were scattered in the bone marrow of the control. The appearance of giant cells, necrosis, or suppuration was scarcely found in the foci.

Small lesions of large mononuclear cells accompanied by a marked infiltration of small round cells were found in the cortex and intermediate zone of the kidney in two of the 5 reinfected animals. In the control animals, cellular infiltration with small foci of large mononuclear cells was found in the renal cortex and submucosal tissue of the renal pelvis.

The reinfected animals exhibited marked thickening of alveolar septa and scattering of small foci of large mononuclear cells in the lung. The control animals also showed thickening of alveolar septa and minute foci of large mononuclear cells, although these changes were lower in degree than in the reinfected animals.

Genital lesions were found in three of the 5 reinfected animals. There were foci of large mononuclear cells accompanied by infiltration of small round cells. On the other hand, the control animals exhibited the proliferation of histiocytic cells in the interstitial tissue of the interstitial tissue of the testis, the disturbance of spermatogenesis, marked cellular infiltration in the interstitial tissue of the epididymis and, in some cases, involvement of the epididymal ducts.

Fourteenth week after challenge infection: A few small well-circumscribed nodules were found in the spleen of three of the 5 reinfected animals. The other two showed no marked changes. The nodules were mainly composed of a few large mononuclear cells or giant cells (Fig.13). In the control animals, the spleen had numerous foci of various size. Giant cells were found occasionally. Well-developed granulomas with a necrotic minute central area were found in one control animal. They resembled those observed in the spleen of reinfected animals which were killed in the 4th week after the challenge.

Small foci of large mononuclear cells were scattered in the lymph nodes of three of the 5 reinfected animals which had lesions in the spleen. The other reinfected animals had few lesions. The cervical lymph nodes in one animal showed a marked increase in connective tissue and atrophy of lymphoid element (Fig.14). Solitary nodules with central suppuration, surrounded by connective tissue, were found in the portal and inguinal lymph nodes of two guinea pigs. Lesions in the control animals were still extended diffusely. In some animals, however, lesions tended to be circumscribed. In 2 controls, an increase in connective tissue was found in the cervical and inguinal lymph nodes. In the other 2 controls, large granuloma with central suppuration was present in the cervical and inguinal lymph nodes.

In 3 reinfected animals, small granulomatous nodules were formed in the interstitial tissue of the testis (Fig.15). Atrophy of the seminiferous tubules and spermatogenesis were also noticed. The epididymis had a marked lesion which mainly consisted of large mononuclear cells and small round cells (Fig.16). Some nodules showed accumulation of neutrophils at the center and were circumscribed with connective tissue. In the control animals, a marked increase in granulomatous tissue and, occasionally, an increase in fibrous element were seen in the interstitium of the testis. Atrophy of the seminiferous tubules and spermatogenesis were found simultaneously with these interstitial lesions. An extensive lesion consisting granulomatous tissue was
present in the epididymis. The peripheral adipose tissue and epididymal duct were
involved in some cases. Abscesses of various size were found frequently.

The pathological findings described above are summarized as follows. Observation
in the 4th week revealed that the effect of sensitization varied with the organ. The
lesions of the spleen and lymph node were moderate in the reinfected animals, but
severe in the control ones. The lesions of the liver and bone marrow were larger and
more developed in the reinfected than in the control. The Lung lesion was also more
obvious in the reinfected than in the control. The effect of sensitization on the renal
lesion was inconspicuous. Development of genital lesions was markedly depressed in
the reinfected animals. In the 14th week, lesions were observed in the liver, spleen, bone
marrow, and lymph nodes of both reinfected and control animals, although they were
lower in severity than those found in the 4th week. In the testis and epididymis, how-
ever, well-developed lesions appeared in both pretreated and control animals in this
late stage.

DISCUSSION

The first part of this experiment was carried out to clarify the development of
lesions and the fate of organisms during the course of infection. The results showed
that there were different patterns in bacterial growth and in development of lesions
according to the kind of organs when the inoculum was adequate in size (i.e., approxi-
mately 10⁶ organisms per animal). In the liver, spleen, and lymph node, peaks of
bacterial population and of extent of lesions were observed in an early stage, i.e., from
the 2nd to the 4th week. There was a decrease in bacterial population thereafter, and
the lesions were regressed gradually up to the 10th week. On the other hand, in the
testis and epididymis, the bacterial growth and development of lesions were delayed.
A high bacterial count and well-developed lesions were found in the late stage, i.e.,
from the 7th to the 10th week. Thus, after prolonged infection, a feature of disease
was manifested in the reproductive organs of the male guinea pig.

A preferential affection occurred to the genital system and its drainage lymph
nodes in the late stage of infection. This was revealed from an exhaustive survey con-
ducted on bovine brucellosis in Japan⁸, as well as from experimental infection of
dairy cattle with brucellosis performed by the Brucellosis Center in Japan.

The preferential affection of the genital system has attracted the interest of many
investigators¹²,¹⁹,¹⁰,²³, as a problem on “the affinity of Brucella organisms to the
genital system”. Bang et al.²⁴ stated an opinion from a different point of view. They
said, “The prevalent affection of the mammary gland and genital organs with bovine
brucellosis does not mean that these organs are particularly susceptible to this disease,
but that they cannot resist the infection so efficiently as the other organs.” They drew
this conclusion from the results of a serial observation on bacterial localization in the
organs of cattle. It seems, however, that there has been no satisfactory account to sustain
their conclusion, nor any effort to explain the mechanism underlying this phenomenon.
In the second experiment, the effect of sensitization on the lesions of various organs
was examined by reinfection of guinea pigs with Brucella abortus. This experiment
was undertaken on an assumption that the effect of sensitization on the course of
infection might differ with the organ, and that this characteristic difference in effect
among the organs might account for the late occurrence of such genital lesions as
mentioned in the first experiment.

Pathological observation reveal that the effect of sensitization differed with the
organ. The liver, bone marrow, and lung had more extensive lesions and the spleen and lymph nodes milder lesions than in the control. The lesions of all these organs were regressed in the late stage of infection. On the other hand, the initial development of genital lesions was inhibited in an early stage, but this inhibition was not complete, and well-developed lesions appeared in the late stage.

Many reports have been published on the fate of Brucella in reinfection, or the modification of lesions by sensitization, but none of them have particularly mentioned the fact that the effect of sensitization differed with the kind of organs. The results obtained from the second experiment showed that genital lesions developed progressively even in animals pretreated with an attenuated strain. It is evident that no infection can be inhibited from progressing in the genital organs even under sensitized conditions. This should be considered to be an important factor for the establishment of genital affection in the late stage of infection.

CONCLUSIONS

Male Hartley guinea pigs were inoculated intravenously with various doses of Brucella abortus strain A 62 to investigate the fate of this organism and the development of lesions. The results showed the presence of two phases in the disease according to the stage of infection. The first phase appeared in an early stage and was characterized by a systemic infection involving the spleen, lymph node, liver, and bone marrow. The second phase was one exhibited in the late stage. It was characterized by an affection of the testis and epididymis, diminution of bacterial population, and reduction of lesions in the organs which had shown an extensive affection in the early stage. In consequence, the guinea pigs manifested a feature of disease of the reproductive system after prolonged infection.

The second experiment showed that there was a difference in mode of modification of lesions between organs. More developed lesions of the liver, bone marrow, and lung were observed in the reinjected animals than in the control. Such lesions as these were reduced in number in the late stage. In the reinjected animals, lesions of the spleen and lymph nodes were milder than in the control both in the early and the late stages. On the contrary, genital lesions developed in the late stage of reinfection, although it was evident that the infection had been inhibited markedly in the early stage. The results of the second experiment also exhibited that no genital organs could inhibit progress of infection even under marked development of sensitivity.

REFERENCES

実験ブルセラ症に関する研究
雄モルモットの Brucella abortus 感染
および各臓器に対する感作の影響

上田雄幹・今泉浩
国立予防衛生研究所 狩猟部
（昭和44年3月31日受付）

家畜のブルセラ症は、乳房、生殖器系の疾患として重要であるが、同時に全身感染として、肝、脾、リンパ節などに顕著な病変を起こす。この全身感染は局所（生殖器）感染との関係において、詳しい解釈がされていない。他モルモットのブルセラ感作では、経口の感染を経て感染されるとともに、副睾丸炎、副睾丸腺炎が起こることが知られている。そこで著者らは、モルモットと Brucella abortus との組合せを用いて、両者の関係を解析する一連の実験を試みた。

第1実験（各臓器感作実験）では、静脈接種後、各臓器の変状および血中感作を経時的に10週まで調査した。第2実験（再感染実験）では、あらかじめ感作した (B. abortus No. 19 株) を皮内接種したモルモットに、各臓器の強毒株を皮内接種し、再接種後、早期（4週）と後期（14〜15 週）に、各臓器の変状と血中感作を調べ、感作の影響を観察した。実験動物には、Harlcy 雄モルモット、体重 200 g 前後のものを使用し、B. abortus の強毒株には A 62 株を用いた。接種材料は、馬肉清加寒天 48 時間培養マグの懸濁液を鞘腔接種した。

第1実験
臓器による感染経過の相違は、10 万 コ接種群で最も顕著であった (Fig. 2)。肝、脾、リンパ節、骨髄の変状は、早期 (3〜4 週) に発現し、その後後退した (Fig. 6〜11)。生殖器の変状は発現が遅れ、後期 (7〜10 週) に著明になった (Fig. 12〜15)。感作後期には、生殖器および骨 (中手骨、胸骨、肋骨) に肉眼的変状が出現し、その発現率は接種菌量が多いほど制限された (Table 2)。

臓器内菌量 (Fig. 3) は、脾、骨髄が最も多く、これらの臓器および骨髄では、2〜4 週に菌量の頂点が認められ、以後減少した。生殖器では、4 週でようやく菌量の頂点に達し、10 週後も極めて高い菌量がみられた。また尿からも、感染後期により菌が分離された。

再接種後、血中抗体価の上昇がみられ、長く持続した (Fig. 4)。感作後期には、皮内反応も顕著にみられた (Table 3)。

第2実験
前実験でみられた各臓器間の感染経過の相違が、各臓器の感作の影響の関係があるか否かをみるため、再感染時の各臓器の変状を調べた。変状の検査は、再接種菌量を約 10 万 コ群で最も顕著に観察された (Fig. 5)。

再接種後4週には、再接種群では、脾臓およびリンパ節腫脹が、対照群にくらべて軽度であるが、変状は軽く、しばしば巨細胞がみられた (Fig. 17)。再接種群の肝では、対照群にくらべて、数はやや少ないがよく発達した結節が発現し、結節の大単核細胞は萎縮し、巨細胞がほとんどどの結節に認められた。また、結節周囲に線維形成層がみられた (Fig. 16)。骨髄の結節もよく発達し、対照群ではほとんどみられない結節中心部の壊死または好中
球浸潤が、しばしばみられた。腎では感作の影響は不明瞭であった。肺では被胞肥厚の程度が、対照群にくらべて顕著であった。生殖器では、再接種群の副睾丸間隔に軽度の細胞浸潤、対照群の睾丸および副睾丸に著明な病変が認められた。

接種後14週には、肝、骨髄、脾、リラバンの病変は、再接種群、対照群ともに、4週例よりも消退していた（Fig. 18）。一方、再接種群の生殖器では、睾丸間隔に肉芽腫の出現、細管萎縮がみられ（Fig. 20）、副睾丸に発達した肉芽腫がみられた（Fig. 21）。対照群生殖器にも著明な病変がみられた。

要約と考察および結論

各群の感染実験の結果、臓器により、感染経過には相違があることが明瞭になった。すなわち、接種後早期（2〜4週）には、肝、脾、リラバン、骨髄などに着が多く、病変が顕著な全身感染が起こる。感染後期（7〜10週）には、これらの臓器では、病変が消退し、量も少なくなるが、菌増殖が緩慢で、徐々に発達した生殖器病変が治癒しないため、生殖器病の形を呈する。

ブルセラ感染の後期に、乳房、生殖器または付属リラバンからの菌分離頻度が高いことは、日本でブルセラ症センターの牛の調査成績、および感染実験の成績でも示されている。本菌が好

んで生殖器を侵すことについては、いわゆる生殖器親和性の問題として、多くの研究がある。

本症ではしばしば生殖器系が受されるのだけれど、生殖器がとくに感受性が高いというよりは、生殖器が、ほかの臓器のように効果的に感染を耐えこないためであると述べている。しかし、この見解の実験的根拠は、これまでになされていない。

感染後期には、血中抗体価の上昇、皮内反応の陽性などで知られるように、個体が感作状態にある。この感作の影響が、臓器により異なることがあるようにと思われたので、再感染時の各臓器の病変を調べた。その結果、再接種された動物の肝、骨髄、脾などでは、病変に偽陽性がみられ、やがて治癒し、脾、リラバンなどでは、病変は軽度であったが、生殖器では、対照群と同様の病変が発達することがわかった。モルモットを用いたブルセラ症の免疫実験は、おびただしい数にのぼるが、臓器別に感作の影響を含めたものは、ほとんどみられない。

本実験では、生殖器は、個体が感作されているにもかかわらず、その影響が充分に現われず、したがって免疫ということには、なりにくい臓器であることが示されたものといえる。これは、感染後期に生殖器病の形になること、密接な関係があること、と推察される。

Studies on Experimental Brucellosis
301
EXPLANATION OF PLATES

PLATE I

Fig. 1. A compact nodule in the liver. Four weeks after inoculation (hereinafter is shown the time only).
Fig. 2. Regressed lesion in the liver. Seven weeks.
Fig. 3. Proliferation of large mononuclear cells in the spleen. Four weeks.
Fig. 4. Regressed lesion in the spleen. Ten weeks.
Fig. 5. Diffuse proliferation of large mononuclear cells in the medullary cord of the cervical lymph node. Four weeks.
Fig. 6. Scattered foci of large mononuclear cells in the cervical lymph node. Ten weeks.
Fig. 7. Proliferative lesion in ductus recti of the testis. Ten weeks.
Fig. 8. Marked depression of spermatogenesis. Seven weeks.

PLATE II

Fig. 9. Cellular infiltration and edema in interstitial tissue and ducts of the epididymis. Four weeks.
Fig. 10. Well-developed granulomatous lesion with multiple suppurative areas in the epididymis. Ten weeks.
Fig. 11. A large nodule in the liver of a reinfected guinea pig. There are many giant cells and fibroplastic tissue in the periphery. Four weeks after reinfection (a.r.).
Fig. 12. Small lesions in the spleen of a reinfected guinea pig. Four weeks a.r.
Fig. 13. A nodule and a small focus in the spleen. Fourteen weeks a.r.
Fig. 14. Increased fibrous element in the cervical lymph node. Fourteen weeks a.r.
Fig. 15. Granulomatous lesion and disturbance of spermatogenesis in the testis. Fourth weeks a.r.
Fig. 16. Well-developed granuloma in the epididymis of a reinfected guinea pig. Fourteen weeks a.r.