STUDIES ON AVIAN TYPE TUBERCULOSIS

II. VILLEMIN TYPE TUBERCULOSIS IN RABBITS

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The pathological features of experimental avian type tuberculosis in rabbits are divided into three types, i. e., (1) Yersin type, (2) Villemin type, and (3) chronic Villemin type with articular involvement, according to their chronicity¹⁾. There are striking differences in the distribution of lesions and bacilli between the Yersin and Villemin types of the disease. Though there are no gross lesions, except marked splenomegaly, in Yersin type tuberculosis, there are numerous foci of large mononuclear cells and giant cells in the tissue sections of the liver, spleen, and bone marrow. Numerous bacilli are also demonstrated in the liver, spleen, and bone marrow, but only a few in the lung and kidney. On the contrary, the involvement of the liver, spleen, and bone marrow is quite inconspicuous, but tubercle formation in the lung and kidney is marked in the chronic Villemin type. The pathological features of Villemin type tuberculosis produced by the avian tubercle bacillus resemble those of experimental bovine tuberculosis in rabbits. Comparing the chronic Villemin type with the Yersin type, one may be suspicious whether these types are produced by the same microorganism, because of the extreme differences in pathological features shown between them.

In their previous paper¹⁾, the authors described in detail the course of the Yersin type as an extreme of experimental avian tuberculosis. The present paper deals with another extreme, Villemin type tuberculosis.

MATERIALS AND METHODS

Thirty normal albino rabbits, weighing about 2 kg, received intravenous injections of 0.001 mg of a culture of the Flamingo strain per kg of body weight. They were divided into two groups. In each group, one rabbit each was killed by air embolism 30 minutes, 1 week, 2 weeks, 18 days, 3 weeks, 25 days, 4, 6, 8, 10, 15, and 20 weeks after inoculation (a. i. for short). Liver, spleen, bone marrow, mesenteric lymph node, adrenal, lung, and kidney were subjected to quantitative culture for avian tubercle bacilli, as described in the previous paper¹⁾. Deaths occurred 19, 23, 24, 25, 118, and 185 days a. i. Quantitative cultures were made from the last two rabbits which died 118 and 185 days a. i., respectively. Because of the presence of large necrotic lesions in the lung of rabbits examined in the later stages (more than 15 weeks a. i.) of the experiment, special care was taken so that the lung sample for culture might represent the original distribution of lesions as much as possible. After weighed, the organs were fixed in 10% formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin, by the Ziehl-Neelsen method for acid-fast bacilli, and the silver impregnation method for reticulum fibers. Low-power microscopic pictures of the liver, spleen, lung, and kidney were projected on the focussing screen of a large photomicrographic camera and lesions were measured in five fields selected at random for size. Thus the percentage of lesions in each organ was calculated. All the animals

were examined for intradermal reactions to avian-, bovine-, and human-type tuberculin 6, 15, and 20 weeks a. i.

RESULTS

1. The fate of avian tubercle bacilli in the organs

The numbers of colonies isolated from the organs are shown in Table 1. The multiplication curves of bacilli are shown in Charts 1 and 2, together with schemata of bacillary distribution in individual animals. There is some difference in multiplication curve between the two groups. There is a peak between 3 and 4 weeks a. i., when the schema of bacillary distribution became characteristic of the Yersin type, though the absolute amount of bacilli was smaller than that in true Yersin type tuberculosis. The amount of bacilli decreased markedly in the liver, spleen, and bone marrow 8 to 10 weeks a. i., but decreased little, if any, in the lung and kidney. On the whole, it reached its minimum and the multiplication curves became astricted in this period, as shown in the charts. The schemata of bacillary distribution in this period show a rectangular pattern because of equalization in the bacillary amount among the organs. Though there was another general increase of bacilli 15 weeks a. i., the schemata of bacillary distribution remained rectangular in this period. The amount of bacilli again decreased generally 20 weeks a. i. The authors considered that the above-mentioned rectangular pattern of the schema, or the schema where the predominance of bacilli was shown in the lung and kidney, was characteristic of Villemin type tuberculosis.

Table 1. Numbers of Colonies Recovered from 100 mg of Each Organ

Time after inoculation	Rabbit No.	Liver	Spleen	Bone marrow	Lymph node	Lung	Adrenal	Kidney
30 minutes	K-220	82×10	170×10	10×10	0×10	47×10	0.2×10	0.2×10
	K-233	142×10	189×10	12×10	0×10	36×10	0.4×10	0.6×10
1 week	K-221	22×10^3	71×10^3	31×10^2	9×10	56×10	2×10	0.4×10
	K-234	27×10^4	43×10^3	51×10^2	24×10	87×10	11×10	1.8×10
2 weeks	K-222	12×10^5	11×10^5	17×10^4	11×10^3	26×10^2	9×10^2	5×10
	K-235	2×10^5	4×10^5	8×10^4	23×10^3	138×10^2	28×10^2	9×10
18 days	K-223	0.3×10^6	0.7×10^6	1.3×10^5	0×10^4	83×10^2	14×10^2	2×10
	K-236	1.3×10^6	1×10^6	1×10^5	9×10^4	123×10^2	4×10^2	10×10
3 weeks	K-224	29×10^4	0×10^5	8×10^5	0.7×10^4	7×10^3	1.7×10^3	12×10
	K-237	20×10^5	11×10^5	12×10^5	3×10^4	60×10^3	1×10^3	8×10
25 days	K-226	248×10^4	56×10^5	6×10^5	107×10^3	18×10^3	5×10^3	60×10
	K-238	122×10^4	9×10^5	5×10^5	46×10^3	12×10^3	36×10^3	85×10^2
4 weeks	K-227	161×10^5	41×10^5	19×10^5	377×10^3	43×10^3	3×10^3	32×10
	K-239	6×10^5	7×10^5	0×10^5	61×10^3	11×10^3	6×10^3	2×10
6 weeks	K-228	46×10^3	0.7×10^4	0.7×10^3	64×10^2	0×10^3	44×10^3	13×10^2
	K-240	7×10^3	14×10^4	5×10^3	5×10^2	2.3×10^3	2.3×10^3	0×10^2
8 weeks	K-229	11×10^3	0.7×10^2	64×10^2	1.3×10^2	15×10^3	9×10^2	7×10^2
	K-242	42×10^2	10×10^4	0×10^2	13×10^2	4×10^2	11×10^2	0×10^2
10 weeks	K-230	37×10	13×10^2	0.3×10^2	0×10^2	38×10^2	6×10^2	2×10^2
	K-243	23×10^2	24×10^2	2.3×10^2	0.7×10^2	0×10^3	0.7×10^2	30×10^2
15 weeks	K-231	271×10^2	36×10^3	143×10^2	316×10	45×10^3	340×10^2	40×10^3
	K-244	4×10^2	382×10^3	327×10^4	81×10	26×10^5	407×10^3	19×10^4
118 days	K-249*	109×10^2	84×10^3	16×10	213×10^2	82×10^4	19×10^3	78×10^4
20 weeks	K-232	113×10	77×10	3×10	1.3×10	29×10^4	0×10^2	0×10^2
	K-245	163×10^2	48×10	0.3×10	101×10^2	139×10^3	0×10^2	0×10^2
185 days	K-246*	31×10	5×10	0.7×10	6×10	270×10^2	1×10^2	0×10

* died

Chart 1.

The columns in the schema of an individual animal represent the amounts of bacilli in 100 mg each of liver, spleen, bone marrow, lymph node, lung, adrenal, and kidney from the left to the right.

Chart 2.

The columns in the schema of an individual animal represent the amounts of bacilli in 100 mg each of liver, spleen, bone marrow, lymph node, lung, adrenal, and kidney from the left to the right.

Therefore, they designated it as the Villemin type schema of bacillary distribution.

2. The clinical aspect of Villemin type tuberculosis

Febrile reaction was rather variable in the animals. Some of them began to show distinct febrile reaction, over 40°C, 2 weeks a. i., which continued for variable periods from only a few days to 3 weeks. Others showed only inconspicuous febrile reaction. Those animals which survived the full course of disease manifested intermittent slight febrile reaction from time to time.

Intradermal injection of avian-, bovine-, and human-type tuberculin was made 6, 15, and 20 weeks after inoculation. The results are shown in Table 2. Strongly positive re-

Table 2. Intradermal Tuberculin Reaction 6, 15, and 20 Weeks after Inoculation

Time after inoculation	6 weeks			15 weeks			20 weeks		
Type of tuberculin	A	B	H	A	B	H	A	B	H
Rabbit No.									
K-228	$\frac{18 \times 27}{23 \times 30}$ N	—	—						
K-229	$\frac{12 \times 13}{20 \times 22}$	$\pm$	—						
K-230	$\frac{18 \times 21}{22 \times 24}$ N	$\pm$	$\frac{8 \times 8}{9 \times 9}$						
K-231	$\frac{9 \times 9}{10 \times 10}$	$\pm$	—	$\frac{17 \times 20}{21 \times 22}$	$\pm$	—			
K-232	$\frac{18 \times 21}{21 \times 25}$	$\frac{15 \times 16}{19 \times 23}$	$\frac{9 \times 9}{10 \times 10}$	$\pm$	—	—	—	—	—
K-240	—	$\pm$	$\frac{6 \times 6}{7 \times 7}$				$\frac{—}{(15 \times 15)}$	$\frac{—}{(10 \times 10)}$	$\frac{—}{(7 \times 7)}$
K-242	$\frac{24 \times 26}{27 \times 29}$	$\frac{27 \times 27}{28 \times 28}$	$\frac{23 \times 23}{25 \times 25}$						
K-243	$\frac{20 \times 20}{26 \times 26}$ N	$\frac{24 \times 24}{25 \times 25}$	$\frac{10 \times 10}{15 \times 15}$						
K-244	$\frac{26 \times 24}{29 \times 27}$	$\frac{24 \times 20}{26 \times 22}$	$\frac{11 \times 11}{14 \times 14}$	—	—	—			
K-245	$\pm$	—	$\frac{8 \times 8}{10 \times 10}$	$\frac{—}{21 \times 22}$	$\frac{—}{15 \times 16}$	$\frac{—}{(15 \times 15)}$	$\frac{—}{(4 \times 4)}$	$\frac{—}{—}$	$\frac{—}{(3 \times 3)}$
K-246	$\frac{21 \times 24}{24 \times 26}$ N	$\frac{18 \times 20}{20 \times 22}$	$\frac{11 \times 11}{17 \times 16}$	$\frac{17 \times 21}{19 \times 23}$	$\frac{13 \times 14}{16 \times 18}$	—	$\frac{—}{16 \times 16}$	$\frac{—}{13 \times 13}$	$\frac{—}{10 \times 10}$
K-249	$\frac{16 \times 17}{17 \times 18}$	—	$\frac{5 \times 4}{6 \times 6}$	—	—	—			

The denominator represents the diameter of an erythematous area and the numerator that of an induration, both in millimeters.

"N" indicates necrosis and the figure in parentheses that related to weak reaction.

action was observed 6 weeks a. i., without exception. The reaction to avian tuberculin was most intense. The reaction decreased considerably in intensity 15 weeks a. i. It became almost negative 20 weeks a. i. The degrees of tuberculin reaction in animals killed 6, 15, and 20 weeks a. i. are shown in the upper columns of Charts 1 and 2.

3. Pathological findings

a. Gross findings

Splenomegaly of considerable degree was observed from 2 to 8 weeks a. i., as shown in Table 3. It seemed to be accompanied by the multiplication of bacilli.

In the liver, spleen, and the lung of rabbits which died 19 days a. i., minute grayish-

Table 3. Weights of Main Organs (g)

Time after inoculation	Rabbit No.	Liver	Spleen	Lung
30 minutes	K-220	116.7	1.5	14.2
	K-233	61.7	1.2	15.5
1 week	K-221	69.5	1.2	11.2
	K-234	97.7	1.7	16.0
2 weeks	K-222	87.0	3.7	12.7
	K-235	82.2	3.5	11.2
18 days	K-223	83.2	3.2	13.2
	K-236	80.0	3.2	10.3
3 weeks	K-224	116.2	2.7	13.2
	K-237	93.0	8.5	12.5
25 days	K-226	94.2	6.5	15.3
	K-238	133.7	4.0	16.7
4 weeks	K-227	93.5	2.2	8.5
	K-239	111.5	6.2	16.2
6 weeks	K-228	89.2	3.0	18.7
	K-240	88.0	3.0	8.8
8 weeks	K-229	85.2	1.2	11.2
	K-242	75.0	5.0	12.8
10 weeks	K-230	91.7	1.5	11.0
	K-243	74.2	1.7	9.7
15 weeks	K-231	114.5	6.2	16.0
	K-244	114.5	3.6	19.5
118 days	K-249*	41.0	1.3	13.0
20 weeks	K-232	46.0	1.7	18.5
	K-245	51.0	0.7	9.2
185 days	K-246*	41.2	0.6	9.7

* died

white lesions had already been produced. Similar lesions were found in the liver, spleen, and lung up to 25 days a.i. From 4 to 6 weeks a.i., the lesions developed to the size of pin head to poppy seed, being numerous in the liver, spleen, and bone marrow. Eight weeks a.i., a few foci of pin-head size appeared in the renal cortex, and tubercle formation became more intense in the lung. Ten weeks a.i., there were very few or no gross tubercles in the liver. The spleen shrank with a granular surface. In the lung, there were still a considerable number of foci of pin-point to pin-head size. A circumscribed yellowish necrotic lesion of soy-bean size was seen in the pulmonary apical lobe of one rabbit. Splenomegaly was again observed in rabbits killed 15 weeks a.i., together with numerous grayish-yellow minute foci of pin-head size in the liver and spleen. There were many miliary tubercles in the lung and kidney and a large yellowish necrotic lesion of hazel-nut size in the lung. Large necrotic areas were also found on the cut surface of the testes. These gross findings indicated the multiplication of bacilli in this period.

A rabbit which died 118 days a.i. showed still many foci of pin-point size in the liver, a marked involvement of the lung, and a few scattered foci of poppy-seed size in the kidney.

Twenty weeks, a.i., numerous conglomerated tubercles were observed in the lung. There were multiple articular lesions in two rabbits killed 20 weeks a.i. Such lesions were in the right 5th and left 9th costo-vertebral joints in one of them and in the right 9th costo-vertebral joint in the other.

In a rabbit which died 185 days a.i., there were a marked contraction in the liver and spleen, several large yellowish flat necrotic areas along the ventral borders of the lung, and a few minute grayish-white foci of pin-head size in the kidney. Multiple articular involve-

ment, i. e., abscess formation, was seen in its right carpal and left elbow joints.

b. Histopathological findings

One week a. i.: There were scattered foci of mononuclear cells in the liver, mainly at the periphery of the lobuli. Some of the mononuclear cells contained a few bacilli in their cytoplasm. A few pseudoeosinophils were also seen in the lesion. Kupffer cells became hypertrophic in general. In the spleen, small foci of large mononuclear cells were present in the red pulp and contained a relatively large number of bacilli. The lung had no circumscribed tuberculous lesion, but exhibited a diffuse thickening of the alveolar septa.

Two weeks a. i.: There were scattered circumscribed foci consisting of large mononuclear cells, giant cells, and a few pseudoeosinophils in the liver. Bacilli could be demonstrated easily in the large mononuclear cells and giant cells. The central part of each focus was necrotic. Similar lesions were seen in the spleen. There were small foci of large mononuclear cells also in the bone marrow. In the lung, there was somewhat circumscribed thickening of the alveolar septa due to the accumulation of mononuclear cells and pseudoeosinophils. Accumulations of large mononuclear cells with bacillary rosettes in their cytoplasm were seen in the lymphatic tissues of the intestine.

Eighteen days a. i.: The liver had many circumscribed lesions composed of large mononuclear cells and giant cells. These lesions were in the early stage of necrosis. Pseudoeosinophils were found in the necrotic areas in some cases. The lesions in the spleen and bone marrow mostly revealed central necrosis. The lung had many foci of large mononuclear cells which were in the early stage of central necrosis. A small number of bacilli could be demonstrated in the pulmonary lesions. Circumscribed accumulations of large mononuclear cells were found in the adrenal cortex of one rabbit. Bacilli were also demonstrated occasionally in the renal glomeruli. A rabbit which died 19 days a. i. exhibited essentially the same changes as rabbits killed 18 days a. i., in addition to accumulations of large mononuclear cells and pseudoeosinophils around renal glomeruli. A considerable number of bacilli were found in its renal lesions.

Three weeks a. i.: There were large circumscribed lesions in the liver. They showed distinct central necrosis associated with exudation of pseudoeosinophils. Large mononuclear cells and giant cells were somewhat loosely arranged in them and accumulations of erythrocytes found in some of them. Similar lesions were present in the spleen and bone marrow. In the bone marrow, the fat cells were reduced in number and abnormal cellularity was seen. A moderately large number of bacilli were scattered in the lesions of the liver, spleen, and bone marrow. There were many circumscribed accumulations of large mononuclear cells and giant cells in the lung. Early central necrosis was noticed in some of the pulmonary lesions.

Twenty-five days a. i.: There were many well-circumscribed large tubercles in the liver. They have a central necrotic area surrounded by spindle-shaped large mononuclear cells arranged in a radial position. In some tubercles, slits filled with red blood cells were seen in the central part. Similar lesions were found in the spleen and bone marrow. The latter showed an increased cellularity and a marked increase of megakaryocytes. A considerably large number of bacilli were scattered in the central part of the tubercles, showing no particular arrangement in the cytoplasm of any large mononuclear cell. Tubercles with central necrosis were also found in the lung. There were diffuse accumulations of large mononuclear cells in the lymphatic tissues of the intestine. These lesions contained bacilli showing a loose radial arrangement.

Four weeks a. i.: Many well-circumscribed lesions (tubercles) were found in the liver, spleen and bone marrow. Most of them were affected with central necrosis which was accompanied by the exudation of pseudoeosinophils. The large mononuclear cells and giant

cells in the lesions were not round in shape any longer, compared with those in the Yersin type, and were connected with one another with cytoplasmic processes. Bacilli were scattered throughout the lesion. There was no reaction, such as lymphocytic infiltration and fibrous encapsulation, around the tubercles. Only a few fat cells remained in the bone marrow, where megakaryocytes showed a great increase. The lung had large tubercle-like accumulations of large mononuclear cells, which contained only a few bacilli. Small foci of mononuclear cells were found in the adrenal cortex, where a few bacilli were harbored in mononuclear cells and capillary endothelial cells. There were accumulations of large mononuclear cells in the renal cortex of one rabbit, but no bacilli were demonstrated histologically in these lesions. Accumulations of large mononuclear cells were also observed in the lymphatic tissues of the intestine, as well as the mesenteric lymph nodes. Central necrosis was found in the lesions of the mesenteric lymph nodes.

Six weeks a.i.: Many of the tubercles in the liver were located near the periportal connective tissue and consisted of rounded large mononuclear cells and giant cells. The central part of each tubercle had undergone necrosis and its peripheral portion contained a small number of lymphocytes. Some of the tubercles had red blood cells in their central part. There were many foci of large mononuclear cells and giant cells both in the red and white pulp of the spleen. Some foci showed central necrosis. In one rabbit, the lesions in the red pulp penetrated the splenic capsule and reached the subserous tissue. In the bone marrow there were scattered circumscribed lesions, where central necrosis was not obvious. A very few bacilli were demonstrated in the lesions of the liver, spleen, and bone marrow. Well-circumscribed pulmonary lesions showed central necrosis and peripheral infiltration of lymphocytes. A relatively small number of bacilli were in the lesions of the lung. Accumulations of large mononuclear cells and giant cells were noticed in the renal cortex, which showed central necrosis and extended toward the medulla. Many disintegrated glomeruli and tubules were seen in these lesions. Together with many irregular accumulations of mononuclears and pseudoeosinophils which had undergone necrosis, small foci of mononuclear cells were found in the adrenal cortex, but they contained no bacilli. Accumulations of large mononuclear cells with central necrosis were also found in the lymphatic tissues of the intestine and mesenteric lymph nodes.

Eight weeks a.i.: There were large tubercles near the periportal connective tissue of the liver. The characteristics of these lesions were essentially the same as those seen 2 weeks before. A small number of bacilli were demonstrated in the necrotic area of the lesions. A few small foci of large mononuclear cells were also in the hepatic lobules. In the spleen, which was almost normal in size, there were a few small foci which consisted mainly of giant cells, in the red pulp. The reticulum cells of the red pulp became hypertrophic, having engulfed nuclear debris. The lymphatic follicles were almost normal in one rabbit. The spleen of the other rabbit showed considerable swelling and had lesions in many follicles. The lesions consisted of rounded large mononuclear cells and were affected with distinct central necrosis. Some of them had wide splits in their center, which contained pink-stained albuminous material. Only a few fat cells were in the bone marrow, where megakaryocytes had increased greatly in number and which had a few foci of large mononuclear cells. In the lung were seen a diffuse thickening of alveolar walls and many large tubercles with central necrosis. In the renal cortex, there were accumulations of large mononuclear cells and lymphocytes, in which many tubules had been trapped. The testes of one rabbit were severely affected and had widespread accumulations of large mononuclear cells and giant cells. Many seminiferous tubules and blood vessels were involved in the lesions which were affected with extensive necrosis. There were small foci of mononuclear cells in the adrenal cortex of one rabbit. Accumulations of large mononuclear cells were

found in the lymphatic tissues of the intestine and mesenteric lymph nodes.

Ten weeks a.i.: The lesions of the liver, spleen, and bone marrow were reduced markedly in size and number. There were only a few tubercles surrounded by lymphocytic infiltration near the periportal connective tissues of the liver. The hepatic lesions hardly showed a tendency to be necrotic. In the spleen, small foci of large mononuclear cells were present mainly in the red pulp. They were found also in the splenic follicles of one rabbit, but not in those of the other rabbit. The bone marrow was still rich in myeloid elements and had scattered foci of large mononuclear cells near the endosteum. Larger lesions in the bone marrow had a distinct lymphocytic zone in the periphery. Scattered foci of large mononuclear cells with central necrosis were observed in the lung. Accumulations of large mononuclear cells were seen in the alveoli. A small number of bacilli were demonstrated in the pulmonary lesions. In the kidney, there were large lesions extending from the cortex to the medulla. The central part of these lesions had undergone necrosis with bacilli demonstrable with ease. A large lesion with extensive necrosis was seen in the adrenal cortex of one rabbit. There were large-sized lesions with central necrosis in the lymphatic tissues of the intestine and mesenteric lymph nodes. The testes of one rabbit had a widespread accumulation of large mononuclear cells and giant cells. In this lesion were seen extensive necrosis and infiltration of small round cells, mainly of plasma-cell type, around blood vessels.

Fifteen weeks a.i.: Within the hepatic lobules there were only a few small foci which consisted of mononuclears and occasionally pseudoeosinophils or of large mononuclear cells and giant cells. The periportal connective tissues of the liver contained many distended canals, which were presumably lymphatic vessels and which were filled with large mononuclear cells and giant cells. The central portion of these accumulations was often necrotic or contained blood cells and albuminous material. A considerably large number of bacilli were demonstrated in the necrotic areas of these lesions. Similar lesions were found in the periportal connective tissues of a rabbit which died 118 days a.i. In this period, the involvement of the hepatic lymph node was far more advanced than that of the mesenteric lymph nodes. Lesions developed again in the spleen. They were many large tubercles with distinct central necrosis and contained a large number of bacilli. The bone marrow showed a marked cellularity and an increase of megakaryocytes in number. Many tubercles showing extensive necrosis were found in the bone marrow of only one rabbit. The involvement of the lung was far more extensive than that seen 5 weeks before. In addition to circumscribed tubercle-like lesions with central necrosis and peripheral lymphocytic infiltration, there were widespread caseous lesions. The latter consisted of necrotic large mononuclear cells and giant cells with which the alveoli were stuffed. Numerous bacilli were demonstrated in these lesions. In some places, a lesion broke through the bronchial walls, discharging caseous material into the bronchi. Large caseous lesions were present in the kidney, extending from the cortex to the medulla. In one rabbit, there was a caseous lesion just beneath the pelvic mucosa, and the renal pelvis was filled with caseous material. A small amount of caseous material was also found in the renal tubules and contained bacilli. Lesions with central necrosis and a considerable number of bacilli were seen in the lymphatic tissues of the intestine and mesenteric lymph nodes. Lesions with extensive necrosis and a large number of bacilli were noticed in the adrenal cortex of one rabbit. The testicular tissue of one rabbit had been almost entirely replaced by proliferated large mononuclear cells. Only a few seminiferous tubules with degenerated epithelium remained here and there. Multiple necrotic foci and areas of dense lymphocytic infiltration were seen in this lesion. Bacilli were demonstrated in a considerably large number in the necrotic areas of the testicular lesions. Some ducts of the epididymis were filled with large mono-

nuclear cells and giant cells.

Twenty weeks a.i.: There were a few circumscribed accumulations of large mononuclear cells in the liver. Some of them showed central necrosis. One rabbit had areas of focal necrosis in the hepatic cells. No tuberculous lesions were found in the spleen. In one rabbit, a marked hyaline degeneration was seen in the splenic follicles, some of which had been completely replaced by hyaline masses. In the other rabbit, the spleen exhibited marked general atrophy and its capsule and trabeculae were affected with fibrosis. The bone marrow of this rabbit had only a few foci of large mononuclear cells. No bacilli were demonstrated in the sections of the liver, spleen, and bone marrow, except in the hepatic lesions of one rabbit from which a few bacilli were detected. The lung had widespread caseous lesions with calcification. Lymphocytic infiltration was seen in the periphery of the lesions. Bronchioles and alveoli were filled with large mononuclear cells or caseous material. Circumscribed accumulations of large mononuclear cells were present here and there in the alveolar septa. In one rabbit, only slight infiltration of lymphocytes was seen in the interstitial tissue of the kidney. In the other rabbit, the submucosal lesion was extended to the renal pelvis, which was filled with caseo-purulent material. Bacilli were demonstrated in the caseous material. There were circumscribed accumulations of large mononuclear cells in the lymphatic tissues of the intestine and mesenteric lymph nodes. Small to moderately large numbers of bacilli were demonstrated in these lesions. The testes of one rabbit showed a diffuse proliferation of large mononuclear cells and giant cells. Dense lymphocytic infiltration was seen around the remaining seminiferous tubules in this lesion. Multiple necrotic foci were recognized in the testicular lesions. Caseous material filled the seminiferous tubules involved in the lesion.

In a rabbit which died 185 days a.i., the following histopathological changes were noted. A few small foci of mononuclear cells were in the hepatic lobules and thickened periportal connective tissues. No bacilli were demonstrated in the liver. No specific lesions were present in the spleen, except fibrosis. A few minute foci of large mononuclear cells were found in the bone marrow. There were wide areas of caseous necrosis along the ventral border of the lung. Large mononuclear cells and caseous material were moulded in the alveoli and bronchioles at the periphery of the caseous lesions. A small number of bacilli were demonstrated in the necrotic areas.

The proportion of the lesions of the liver, spleen, lung, and kidney in microscopic fields were calculated as mentioned above. The results are shown in the upper columns of Charts 1 and 2. There is a peak in the development of hepatic and splenic lesions about 3 to 4 weeks a.i. The lesions in the liver and spleen reached their minimum 10 weeks a.i. There was a considerable degree of development of secondary lesion 15 weeks a.i. At the end of the experiment, the development of lesion in the liver and spleen became minimum, having subsided greatly. The lesions in the lung and kidney, however, developed slowly, without showing any remarkable rise and fall. A rapid development of lung lesions was observed 15 weeks a.i. Thus, the lung became the main locus of lesion in Villemin type tuberculosis. The lesions in the kidney showed a definite, locally destructive nature, as described above, though the size of the lesion was rather small. As shown in the charts, the fate of the microscopic lesions in various organs went closely parallel with that of the bacilli.

DISCUSSION

Serial observations on Villemin type tuberculosis revealed that there were two peaks in the fate of bacilli, as well as in the development of lesions, and that both went parallel with each other. The first peak appeared about 3 to 4 weeks a.i. At this time, a rapid

multiplication of bacilli was observed in the liver, spleen, and bone marrow and the schemata of bacillary distribution in individual animals were of Yersin type. The absolute amount of bacilli, however, was smaller than that in the true Yersin type. The multiplication curves of bacilli went down to their minimum points within about 10 weeks a. i. and became astricted closely to each other. As a result, each schema of bacillary distribution showed a rectangular pattern at this time. The authors considered the schema of this pattern to be characteristic of Villemin type tuberculosis. Hence, they designated it as the Villemin-type schema of bacillary distribution. There was a second peak of bacillary multiplication and development of lesions 15 weeks a. i. Though there was a general increase in the amount of bacilli, the schema of bacillary distribution maintained its characteristic pattern of Villemin type tuberculosis.

There was a close correlation between the development of lesions and the fate of living bacilli. For 3 to 4 weeks a. i., the main sites of lesions were the liver, spleen, and bone marrow, as was the case with the Yersin type. After this period, a rapid diminution of lesions took place in the liver, spleen, and bone marrow, while lesions continued to develop slowly in the lung and kidney, without showing any noticeable rise and fall. As a result, the pulmonary lesions exhibited an obvious predominance in the later stages of the experiment.

Although the liver, spleen, and bone marrow showed a predominance in the distribution of lesions for 3 to 4 weeks a. i., the lesions of Villemin type tuberculosis differed considerably from those of Yersin type. Large mononuclear cells and giant cells were somewhat thinner and pricklier in lesions of the Villemin type than in those of the Yersin type. They never contained such numerous bacilli in lesions of the Villemin type as in those of the Yersin type. In Villemin type tuberculosis, lesions tended to be limited in number and were of large well-circumscribed tubercle-like structure. Central necrosis appeared in lesions of the Villemin type as early as 2 weeks a. i., but never did in those of the Yersin type. Exudation of pseudoeosinophils was seen sometimes in the necrotic areas. The appearance of lymphocytes in the lesion was considered to be characteristic of Villemin type tuberculosis.

The positive tuberculin reaction observed during the course of Villemin type tuberculosis indicates the increased resistance of the host animal. A small dose of the inoculum seems to have given plenty of time for sensitization of the animal. The modification of the pathological features described above seems to be the results of an increased resistance of the animal, particularly of the organs closely related to the reticuloendothelial system.

THOMAS²⁾ inoculated a large number of rabbits with various doses of bovine tubercle bacilli and observed two distinct phases of disease in the mortality rate, as well as in the nature and extent of the lesion. The duration of the first phase was approximately 3 months a. i. In these months the involvement of the spleen, bone marrow, and lymph node was extensive. In animals which died 3 months a. i., lesions were minimal in the liver, spleen, lymph node, and bone marrow, but were extensive in the lung and kidney. In the first three months a. i., there was a diffuse infiltration of the organs with numerous monocytes and epithelioid cells. THOMAS observed typical tubercle formation in the second phase of disease after diffuse lesions had regressed without showing caseation.

The differences between the Yersin and Villemin types of experimental avian tuberculosis are thus quite similar to those between the two phases of experimental bovine tuberculosis in rabbits observed by THOMAS. Yersin type tuberculosis seems to be an extreme on the acute side. Only a few reports have been made on the differences in the behavior of the organs against tubercle bacilli. LURIE^{3,4)}, in his study on quantitative culture, demonstrated the rapid multiplication of bovine and human tubercle bacilli in the spleen of the rabbit and the following rapid destruction of them from 2 to 4 weeks a. i. There was a

distinct predominance of bacilli in the lung and kidney 2 months a.i. In his successive studies, LURIE⁵⁾ demonstrated a close parallelism between the fate of bacilli and histopathological findings in certain organs. He claimed that mononuclear cells appearing in the lesions of the liver, spleen, and bone marrow destroyed tubercle bacilli more effectively than those in the lung and kidney, and that the lesions produced by bovine tubercle bacilli in the former group of organs disappeared completely 6 to 8 weeks a.i. He also demonstrated that the maturation of mononuclears into epithelioid cells followed their destruction of tubercle bacilli.

Accumulation of large mononuclear cells and giant cells was seen in the lymphatics of the periportal connective tissues of the liver 15 weeks a.i. This seems to indicate the exclusion of pathological cells from the hepatic lobules. Dissociation of large mononuclear cells and giant cells and formation of slits filled with blood cells are frequent findings in the hepatic lesion of Villemin type tuberculosis. Some of the dissociated cells may be carried away by the blood stream to the hepatic vein and posterior vena cava. Others may pass through Disse's spaces to the periportal lymphatics and portal lymph node, the same way taken by the particulate matter deposited in the liver⁶⁾. Indeed, more extensive lesions appeared in the portal lymph node than in the mesenteric lymph nodes in this period.

Widespread pulmonary lesions with extensive necrosis were observed in the later stages of the experiment. They are of caseous pneumonia in nature and differ from tubercle-like lesions or mere accumulation of large mononuclear cells and giant cells in the alveolar septa or interstitial tissues. In caseous pneumonic lesions, bacilli seem to multiply, without being disturbed by the host resistance. The predominance of bacilli in the lung and kidney in the later stages of disease is, therefore, due in part to the anatomical characteristics of these organs which allow the intracanalicular spreading process of the lesion.

SUMMARY

1. The course of Villemin type tuberculosis in rabbits produced by intravenous inoculation of a small dose of avian tubercle bacilli was traced up to 20 weeks a.i.

2. The fate of avian tubercle bacilli in various organs was traced by means of a quantitative culture method. Serial observation was made on the histopathology of Villemin type tuberculosis.

3. Differences in the histopathological characteristic of the lesion between Yersin and Villemin type tuberculosis was discussed.

REFERENCES

- 1) YAMAMOTO, S., ISHIDA, K. and K. FUJIWARA: *Jap. J. Vet. Sci.*, **24**, 169 (1962).
- 2) THOMAS, R. M.: *J. Exp. Med.*, **56**, 185 (1932).
- 3) LURIE, M. B.: *Ibid.*, **48**, 155 (1928).
- 4) LURIE, M. B.: *Ibid.*, **50**, 747 (1929).
- 5) LURIE, M. B.: *Ibid.*, **55**, 31 (1932).
- 6) KIYONO, K.: Vital Staining, Nanko-do, Tokyo (1928).
- 7) YAMAMOTO, S., ISHIDA, K., FUJIWAR, K. and A. SATO: *Tr. Soc. Path. Jap.*, **41**, Ed. Reg., 291 (1952).

鳥型結核に関する研究

II. 家兎の VILLEMIN 型結核について

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家兎の実験的鳥型結核症は感染菌量、従ってその経過に従い最も急性の YERSIN 型、VILLEMIN 型及び関節病変を伴った慢性の VILLEMIN 型の 3 型に分類し得る¹⁾。YERSIN 型に於ては家兎は約 1 カ月以内に斃死し著明な脾腫が認められるが肉眼的に結節形成は認められない。組織学的に Y 型では肝・脾・骨髓等に大単核細胞及び巨細胞の集簇が密発し、夥しい菌が之等細胞の胞体内に特異な放射状配列をとって証明される。一方 V 型では各臓器の結節形成が明らかとなり、病変は次第に肺及び腎に局限し、肝・脾・骨髓の病変は極めて軽度となる。すなわち Y 型と V 型を比較すると病変の占坐部位に著しい相違がある。

著者等は前報に於て家兎の実験的鳥型結核症の一極端病型である Y 型の経時的観察を行い、菌の消長及び病巣の組織発生を追究したが、今回は他の極端病型である慢性 V 型の経過を接種後 20 週迄観察した。

体重 2 kg 前後の白色家兎に鳥型結核菌 Flamingo 株 0.001 mg/kg を静脈内に接種し 30 分、1 週、2 週、18 日、3 週、25 日、4 週、6, 8, 10, 15 及び 20 週後各 2 頭を屠殺、各臓器の定量培養、病理組織学的検索を前報に記述した方法で実施した。尚今回は接種後 6, 15 及び 20 週にツベルクリン皮内反応を実施し、又各臓器に於ける病巣の拡がり大型顕微鏡写真撮影装置のピントグラスを用いて描画測定し、全視野に対する百分率を求めた。

菌の消長は Table 1 及び Chart 1, 2 に見る如く接種後 3 及至 4 週間のところに最初の山が見られ、この場合各動物の菌量模型は絶対量の少い Y 型を示す。8 及至 10 週後に於ては肝・脾・骨髓の菌量は著減を示すが、肺・腎のそれは減少を示さない。全体として菌量はこの時期に最小となり菌増殖曲線は収斂して来る。従って各動物毎の菌量模型図は各臓器間に差の少ない矩型を呈する様になる。接種後 15 週

では菌量は再び全般的に増加するが、菌量模型は矩型を保つ。肝・脾・骨髓に菌量が多く、肺・腎との間の傾斜が急峻で、梯型を呈する Y 型菌量模型¹⁾に対比させてこの様な矩型を呈し、各臓器間に差の少ない或いは肺・腎の菌量が肝・脾・骨髓のそれを上廻る様な菌量模型を V 型に特有のものとみなし、V 型菌量模型と称した。

接種動物は約 2 週間後から 40°C 以上の発熱を示すものがあったが、発熱の持続期間は数日及至 3 週間とまちまちで、又殆んど発熱を示さぬものもあった。ツベルクリン皮内反応は 6 週後にはいずれも陽性で、鳥型ツベルクリンに対する反応が最も著明であった。15 週後には反応は減弱して居るものが多く、20 週では極弱い反応が見られたに過ぎない (Table 2)。

病理所見としては、接種後 2 週間から脾腫が現われ、3 及至 4 週間ではかなり著明な脾腫を認める (Table 3)。この時期に一致して肝・脾・骨髓にかなり著明な病巣が形成されて居る。この時期はまた菌量曲線で、最初の山が見られた時期に一致して居り、菌の分布と同様、病変の占坐部位も又肝・脾・骨髓に概ね集中する。しかしこの場合病巣の性格は可成り Y 型のそれとは異って居る。すなわち病巣はよく局限し、数は少く且大型である。屢々病巣の大単核細胞は萎縮して居り、Y 型に見られる様な夥しい数の菌を見ることもない。又 Y 型では見られなかった病巣の壊死が既に接種後 2 週間から見られ、6 週頃から病巣周囲に淋巴球浸潤を認める様になる。V 型に於けるこの様な病巣の特異性は、経過中に見られたツベルクリン反応の陽転を以て表示される増強された個体の抵抗力の現れであり、少量の接種菌量が個体感作の時間的余裕を与えたものと考えられる。肝・脾・骨髓の病巣は其後減少し 10 週後には極めて軽微となる。一方腎皮質には 4 週から大単核細

胞の集簇が見られ、肺では18日からまとまった結節状の病巣が出現し、いずれも徐々に増強する。菌量曲線で第2の山が見られた接種後15週では再び肝・脾に病巣の発展が見られるが、肝病巣は主としてグリソン氏鞘にあって、小葉内には極少数の小型病巣が見られるに過ぎない。グリソン氏鞘の病巣は拡張した淋巴管内に見られ、それ迄に形成された病巣が、肝に沈着した異物と同様の経路によって⁶⁾肝外に排出される過程を示す所見として興味深い。肺の病巣はこの時期に飛躍的に増強し、結節性病巣の他に、大単核細胞及び乾酪物質が肺胞内に鑄型に入れた様につまって居る広範囲の乾酪性気管支肺炎を見る。又腎皮質の病巣は6週頃には髓質に達し、15週には之が腎盂粘膜を突破し、乾酪物質を腎盂内に放出する像を見る。10週から睪丸に壊死を伴った病巣が見られたが15週には殆んど全睪丸組織を占拠するに至る。20週で屠殺した2頭及び185日で斃死した家兎には多発性の関節病変があり、20週では肝の病変は極軽微で、脾には1例に淋巴濾胞の硝子化を認めたのみで結核性病巣を証明せず、肺・腎が主な病変占坐部位となる。

THOMAS²⁾ は家兎の家験的牛型結核症に於て斃死率及び病巣の性格上明らかに異なる2相を認め、第1相に於ては脾・骨髓・淋巴節病巣の優勢が見られて居るが、第2相では肺・腎の病変が重篤となることを認めた。実験的鳥型結核症のY型とV型の相違はTHOMASの云う結核症に於ける2相性が極端な形で現われたものと考えられる。又LURIE^{3,4,5)} は家兎の実験的結核症を定量培養法を用いて追究したが、菌の消長と病巣の進展の間に密接な関連を認め、肝・脾・骨髓病巣の単核細胞は肺腎のそれより菌の滅殺力が強く、肝・脾・骨髓の牛型菌は6及至8週で完全に一掃されると述べて居る。しかし肺・腎の病巣が抑制を受けることなく、進展を続ける様に見えるのは、一つには病巣が本実験の末期に見られた様に管内性に蔓延し、その結果菌が組織の抵抗を受けることなく増殖し得るという之等臓器の解剖学的特性によるものと考えられる。

以上家兎の実験的鳥型結核症の一極端病型であるVILLEMIN型の経時的観察を行い、定量培養法により主要臓器内の菌の消長を明らかにし、又病巣の組織発生を追究してYERSIN型との相違を検討した。

EXPLANATION OF PLATES

PLATE I

- Fig. 1. Scattered circumscribed lesions in the liver. 2 weeks (after inoculation; omitted for short). Hematoxylin and eosin stain (hereinafter omitted unless other stain is used). $\times 50$.
- Fig. 2. Higher magnification of Fig. 1 showing the detail of a lesion. The lesion consists of large mononuclear cells, giant cells, and a few pseudoeosinophils. Early necrosis is seen in its center. $\times 700$.
- Fig. 3. Accumulation of large mononuclear cells and giant cells in the liver. 25 days. Note spindle-shaped large mononuclear cells. $\times 500$.
- Fig. 4. Lesion of the liver similar to that in Fig. 3. 25 days. Tateda's reticulum stain. $\times 500$.

PLATE II

- Fig. 5. Accumulation of large mononuclear cells in the red pulp of the spleen. 25 days. Tateda's reticulum stain. $\times 500$.
- Fig. 6. Accumulation of large mononuclear cells in the lung. 25 days. $\times 200$.
- Fig. 7. Accumulations of large mononuclear cells in the lymphatic vessels (upper left and middle) of periportal connective tissue. 15 weeks. $\times 200$.
- Fig. 8. Two lesions of the bone marrow with central necrosis. 15 weeks. Note extreme cellularity, absence of fat cells, and an increase in number of megakaryocytes in the bone-marrow tissue. $\times 100$.

PLATE III

- Fig. 9. Spleen of a rabbit killed 20 weeks after inoculation. Note a marked deposition of hyaline substance in the splenic follicles. $\times 50$.
- Fig. 10. Large caseous pneumonic lesion in the lung. 20 weeks. $\times 50$.
- Fig. 11. Caseo-purulent masses in the renal pelvis. Submucosal lesion has broken into the renal pelvis. 20 weeks. $\times 50$.
- Fig. 12. Diffuse proliferation of large mononuclear cells and giant cells in the testes. Note dense infiltration of lymphocytes around the remaining seminiferous tubules. 20 weeks. $\times 50$.