STUDIES ON AVIAN TYPE TUBERCULOSIS

III. EFFECT OF SENSITIZATION ON YERSIN TYPE TUBERCULOSIS IN RABBITS (1)

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In the previous papers^{1,2)}, the authors described the fate of avian tubercle bacilli and the development of lesions in both Yersin and Villemin type tuberculosis in rabbits. Extreme differences in the distribution of the bacilli and lesions have been pointed out and discussed together with differences in the pathological characteristics of lesions between the two types of the disease.

The clinical aspects differ between the two types in that a positive intradermal tuberculin reaction develops not in the Yersin type, but in the Villemin type usually 4 to 6 weeks after inoculation. In the Villemin type, the bacilli in the liver, spleen, and bone marrow began to decrease in amount about this time, when skin sensitivity developed against tuberculin. If the sensitization of the host animal is responsible for the modification of the course of tuberculosis, similar sensitization to which an animal has been subjected prior to intravenous inoculation will also modify the course of Yersin type tuberculosis.

Though there was a gradual shift from Yersin to Villemin type in the distribution of bacilli and lesions in the course of Villemin type tuberculosis, the authors attempted to demonstrate the effect of sensitization on Yersin type tuberculosis in order to explain more clearly the differences in the distribution of bacilli and lesions between Yersin and Villemin type tuberculosis.

MATERIALS AND METHODS

For the sensitization of rabbits, they were injected subcutaneously with the Flamingo substrain at the hind leg. This substrain had been proved to be relatively low in virulence after a series of subcultures on artificial media. In spite of its relatively low virulence, the Flamingo substrain has also been proved to be capable of sensitizing rabbits by the subcutaneous route.

In the first experiment, the virulent Flamingo strain (0.05 mg/kg) was inoculated intravenously 1, 2, 3, and 4 weeks after, or simultaneously with, subcutaneous inoculation of the low virulent Flamingo strain (0.5 mg/kg).

Each group consisted of four rabbits, two of which were to be reinoculated with virulent bacilli and the others to serve for controls for subcutaneous inoculation. Since some animals died from diarrhea during the sensitization, the final number of animals used in the experiment was 2 (1 reinoculated) in the 4-week group, 3 (2 reinoculated) in the 3-week group, 3 (2 reinoculated) in the 2-week group, 4 in the 1-week group, 4 in the simultaneous-inoculation group, and 2 in the intravenous-inoculation control group. Intravenous inoculation was done in all the groups at the same time with the same bacillar suspension.

In the supplementary second experiment, six rabbits received subcutaneous inoculation of the low virulent Flamingo substrain (0.5 mg/kg). Of them, four were reinoculated intravenously with 0.1 mg/kg of the virulent Flamingo strain after 3 weeks. The other two

Chart 1. Body Temperature Curves and Schemata of Bacillary Distribution in Individual Animals in the First Experiment

The figures in parentheses under each rabbit number indicate an interval in weeks between subcutaneous and intravenous inoculations. "O" indicates intravenous inoculation simultaneous with subcutaneous inoculation and "Y" a control for intravenous inoculation.

The seven columns of a schema represent the amounts of bacilli in 100 mg each of liver, spleen, bone marrow, lymph node, lung, adrenal, and kidney from the top to the bottom.

rabbit for subcutaneous inoculation was killed at that time. In the 2-week and the 3-week groups, one rabbit each died of diarrhea 7 days after reinoculation. In the simultaneous-inoculation group, one rabbit died 21 days after inoculation and one control rabbit for subcutaneous inoculation of the same group was killed at that time. One of the control rabbits for intravenous inoculation died 25 days after inoculation. In the 1-week group, one rabbit died 26 days after reinoculation and one control rabbit for subcutaneous inoculation was killed at that time. In the simultaneous-inoculation group, the remaining rabbit

served for controls for subcutaneous inoculation without receiving challenge inoculation of the virulent strain. Two normal healthy rabbits were inoculated intravenously with 0.1 mg/kg of virulent bacilli at the time of reinoculation to serve for controls for intravenous inoculation.

During these experiments, body weight and temperature were recorded daily and the peripheral blood was subjected frequently to quantitative bacillary culture.

In the first experiment, the animals were examined for intradermal tuberculin reaction immediately before reinoculation and 1, 2, 3, and 4 weeks after reinoculation. In the second experiment, intradermal tuberculin tests were carried out in all animals immediately before reinoculation and in one animal 27 days after reinoculation.

In all animals used in the experiments, various organs were subjected to quantitative bacillary culture and histopathological examination in the same manner as described in the previous paper¹⁾.

RESULTS OF THE FIRST EXPERIMENT

1. Clinical findings

At the time of reinoculation, definitely positive intradermal tuberculin reaction was shown by rabbits which had received subcutaneous inoculation 2 to 4 weeks before. In the reinoculated rabbits, the reaction turned to be almost negative 1 week after reinoculation. Some of them manifested slightly intensified reaction thereafter (Table 1).

Onset of fever was earlier in the reinoculated rabbits than in the control rabbits for intravenous inoculation. This febrile reaction, however, was not always confirmed in all rabbits, since early death occurred to some reinoculated rabbits (Chart 1). Practically no febrile reaction was produced in all rabbits having received subcutaneous inoculation.

In the 4-week group, one rabbit died of diarrhea 6 days after reinoculation and one control

Table 1. Intradermal Reaction to Avian-type Tuberculin in the First Experiment

Period of sensitization	Rabbit No.	Before reinoculation	Weeks after reinoculation			
			1	2	3	4
4 weeks	K-183	$\frac{16 \times 16}{17 \times 17}$				
	K-184*	$\frac{-}{17 \times 17}$				
3 weeks	K-187	$\frac{21 \times 21}{24 \times 24}$				
	K-188	$\frac{10 \times 10}{17 \times 17}$	$\frac{\pm}{5 \times 5}$	$\frac{-}{(14 \times 14)}$	$\frac{-}{3 \times 3}$	$\frac{18 \times 20}{(21 \times 22)}$
	K-185*	$\frac{13 \times 13}{17 \times 17}$	$\frac{13 \times 13}{18 \times 20}$	$\frac{15 \times 16}{17 \times 19}$	$\frac{12 \times 15}{17 \times 21}$	$\frac{19 \times 19}{21 \times 21}$
2 weeks	K-190	$\frac{13 \times 13}{17 \times 16}$	$\frac{-}{4 \times 4}$			
	K-192	$\frac{21 \times 21}{24 \times 24}$	$\frac{6 \times 6 N}{15 \times 15}$	$\frac{\pm}{19 \times 19}$	$\frac{-}{21 \times 20}$	$\frac{23 \times 27 N}{(24 \times 28)}$
	K-189*	$\frac{10 \times 10}{12 \times 12}$	$\frac{24 \times 19 N}{27 \times 20}$	$\frac{17 \times 17}{18 \times 19}$	$\frac{18 \times 18}{19 \times 21}$	$\frac{20 \times 19}{23 \times 22}$
1 week	K-195	$\frac{5 \times 5}{8 \times 8}$	$\frac{7 \times 7}{9 \times 9}$	$\frac{12 \times 12}{2 \times 2}$		
	K-196	$\frac{7 \times 7}{8 \times 8}$	$\frac{4 \times 4}{13 \times 13}$	$\frac{18 \times 21}{(23 \times 23)}$	$\frac{21 \times 21}{25 \times 25}$	$\frac{21 \times 24}{-}$
	K-193*	$\frac{4 \times 4}{5 \times 5}$	$\frac{10 \times 10}{17 \times 17}$	$\frac{13 \times 13 N}{14 \times 14 H}$	$\frac{15 \times 15}{17 \times 17}$	
	K-194*	$\frac{5 \times 5}{7 \times 7}$	$\frac{20 \times 20}{23 \times 23}$	$\frac{17 \times 17}{18 \times 19}$	$\frac{16 \times 17}{18 \times 21}$	$\frac{19 \times 22}{23 \times 26}$

The denominator represents the diameters in mm of an erythematous area and the numerator those of an induration. A weak erythematous reaction is shown in parentheses.

* Control for subcutaneous inoculation.

** N: necrosis, and H: hemorrhage.

died 32 days after inoculation and one control rabbit was then killed. The other control rabbit for intravenous inoculation died 41 days after inoculation. In the 2-week group, one reinoculated rabbit and one control for subcutaneous inoculation were killed at this time for comparison. In the 1-week group, one reinoculated rabbit died 73 days after reinoculation and one control rabbit was killed at that time. In the 3-week group, one rabbit succumbed 211 days after reinoculation and one control rabbit was killed at that time.

Of the reinoculated rabbits in this experiment, some died of diarrhea soon after intravenous inoculation and a few survived a definitely longer period than the control rabbits for intravenous inoculation.

The results of culture of the peripheral blood are listed in Table 2. Though bacillema was almost continuous in the control rabbits for intravenous inoculation, it was inhibited apparently in some of the reinoculated rabbits (K-192 and K-195). It was markedly inhibited in those reinoculated rabbits (K-188 and K-196) which survived a long period of time. It was impossible to demonstrate any differences in the degree of bacillema among the five groups on account of early deaths among the reinoculated animals of the 3-week

Table 2. Number of Colonies Recovered from 0.1 ml of Peripheral Blood

Rabbit No.	Days after intravenous inoculation																
	1	3	5	7	10	13	15	17	19	22	24	26	29	31	34	36	38
K-183	18	0	1														
K-187	22	2	0														
K-188	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K-190	21	2	6														
K-192	3	3	1	0	0	1	0	0	0	4	3	0	0	1	17	8	25
K-195	24	3	0	0	0	5	6	3	10	21	17						
K-196	39		1	0	18	25	0	7	41	0	17	0	0	4	10	0	96
K-199	190	16	23	14	0	5	5	134	2	11	157	25	76	61			
K-200	10	0	0	0	0	3	0	4	36								
K-201*	10	0	2	25	29	89	22	9	36	66	700						
K-202*	15	6	2	8	10	16	16	12	9	9	4	41	18	13	3	10	0

	Days after intravenous inoculation														
	42	45	47	51	54	57	59	62	65	68	71	77			109
K-188	1	0	0	0	0	0	0.5	0.5	0	0	0	0			0
K-196	C	0	0	1	2	0	0	0	1	0	0				

* Control for intravenous inoculation.

C: Contaminated.

and the 4-week groups. A considerably high degree of bacillema was shown by the rabbits which had received subcutaneous and intravenous inoculation simultaneously. Therefore, simultaneous subcutaneous inoculation seems to have had little effect on the bacillema caused by intravenous inoculation.

2. Quantitative culture

The numbers of viable bacilli per 100 mg of each organ are shown in Table 3 together with the weights of the main organs. The schemata for the bacillary distribution in individual animals are shown in Chart 1 together with body temperature curves. The amount and distribution of bacilli in rabbits (K-183, K-187, and K-190) which died shortly after reinoculation were similar to those at the early stage of the Yersin type. Two rabbits (K-199 and K-200) which had received subcutaneous and intravenous inoculation simultaneously showed no significant differences from those died of the Yersin type. The amount of bacilli in the liver, spleen, and bone marrow in rabbit K-195 (of the 1-week group) which died one day later than rabbit K-201 (control for intravenous inoculation) was much smaller than in the control rabbit. The distribution of bacilli in rabbit K-192 (of the 2-week group) which was killed 41 days after reinoculation showed a definite pattern of Villemin type, while that in the control rabbit for intravenous inoculation which died at the same time was still of Yersin type. Rabbits K-188 and K-196, which survived a long period of time, or 211 and 73 days, respectively, exhibited a definite schema of bacillary distribution of Villemin type. In these cases, however, the period of survival was far beyond that in the case of Yersin type. Therefore, the Villemin-type bacillary schema shown in these cases should be considered to be the due result of a prolonged survival time.

The control rabbits for subcutaneous inoculation were also submitted to quantitative culture and all of their organs were cultured at the dilution of 10^{-1} . All the organs gave negative culture, except the spleens of two rabbits which were killed 32 and 33 days, respectively, after subcutaneous inoculation. The number of viable bacilli per 100 mg of the spleen in these rabbits were 1.3×10 and 0.7×10 , respectively. Accordingly, the distribution of bacilli from the site of subcutaneous inoculation to the visceral organs is considered to be negligible, so far as the quantitative culture of reinoculated bacilli is concerned.

Table 3. Number of Colonies Recovered from 100 mg of Organ Tissue and Weights of Main Organs

Period of sensitization	Rabbit No.	Number of Colonies Recovered from 100 mg of Organ Tissue						Survival time after i.v. inoculation	Weight of organ (g)			
		Liver	Spleen	Bone marrow	Lymph node	Lung	Adrenal		Kidney	Liver	Spleen	Lung
4 weeks	K-183	31×10^4	11.3×10^4	6×10^4	1.7×10^3	21.3×10^2	7.3×10^2	5.3×10	6 days	62	3.0	13
3 weeks	K-187	13.5×10^4	7.5×10^4	9.5×10^4	5×10^3	49×10^2	20×10^2	16.5×10	7 211	57.2	3.0	15
	K-188	8×10^2	1.7×10^3	5.3×10	4.3×10	3.3×10^2	3.3×10^2	6.7×10^3		66.5	0.5	14
2 weeks	K-190	149×10^4	246×10^4	28×10^4	9.5×10^3	82×10^2	6×10^2	23×10	7 41	57	1.0	10.7
	K-192	6×10^3	5.7×10^3	1.3×10^3	65×10^2	169×10^3	21×10^2	109×10^2		39.5	2.2	10.2
1 week	K-195	25×10^5	92×10^5	44×10^4	72×10^3	31×10^3	130×10^3	34×10^2	26 74	90.5	9.5	20.2
	K-196	25×10^3	37×10^4	0.3×10^3	4×10^2	8.3×10^4	1.7×10^2	1×10^3		37.5	1.0	12
none†	K-199	3×10^8	52×10^8	121×10^9	0.7×10^7	1.7×10^5	16×10^5	22×10^4	32 21	64.8	5.3	11.2
	K-200	66×10^4	137×10^3	290×10^3	210×10^2	45×10^2	30×10^2	50×10		112	5.5	28.2
	K-201*	78×10^8	145×10^8	48×10^8	2.7×10^7	9.7×10^5	6×10^5	1.7×10^4	25 41	73.7	4.7	12.8
	K-202*	0.3×10^8	2.3×10^3	1.7×10^8	13.3×10^7	22×10^5	8.7×10^5	3.7×10^4		49.2	5.0	17

† Received intravenous (i.v.) inoculation simultaneously with subcutaneous inoculation.

* Control for intravenous inoculation.

3. Pathological findings

A considerable degree of splenomegaly was already observed in two rabbits (K-187 and K-188) out of three which died approximately one week after reinoculation (Table 3). In the ordinary course of Yersin type tuberculosis, no splenomegaly has occurred at such an early period as this. Histopathological examination of these rabbits revealed accumulations of large mononuclear cells and giant cells in the liver, spleen, and bone marrow. Some of the splenic lesions showed central necrosis. In the ordinary course of Yersin type tuberculosis, no microscopic lesions have spread to such extent within such period of time as this. In the lesions of these reinoculated animals, however, bacilli could be demonstrated only in a small number.

Extensive microscopic lesions with early necrosis were seen in the liver, spleen, and bone marrow of rabbit K-200 which had received subcutaneous and intravenous inoculation simultaneously and which died 21 days after inoculation. There was not much difference between this rabbit and the control one for the Yersin type, except that early necrotic change were seen in the former.

The liver, spleen, and kidney of rabbit K-195 (of the 1-week group) which died 26 days after reinoculation were peppered with minute grayish-white lesions. When compared with the Yersin-type control (K-201) which died 1 day earlier than this rabbit, the microscopic lesions in the liver, spleen, and bone marrow were very extensive and showed definite central necrosis associated with exudation of pseudo-eosinophils. The amount of bacilli demonstrated in the tissue section, however, was much smaller in this rabbit than in the control one. There were lobular lesions with necrosis in the lung and intima granuloma in the pulmonary veins. Accumu-

lations of large mononuclear cells were also seen in the renal cortex. These kinds of lesions in the lung and kidney do not appear in Yersin type tuberculosis and are considered to be characteristic of Villemin type tuberculosis. In the adrenal and lymphatic tissues of this rabbit, there were much larger lesions with central necrosis than in those of the control rabbit.

In rabbit K-199, which had received simultaneous inoculation and which died 32 days after inoculation, numerous grayish-white tubercles of pin-head size were present in the spleen, bone marrow, and on the peritoneum. In addition to these gross tubercles, histopathological lesions similar to those of Yersin type tuberculosis and numerous bacilli were demonstrated in tissue sections prepared from this rabbit.

There were extensive microscopic lesions with necrosis in the liver, spleen, and bone marrow of the control rabbit for intravenous inoculation (K-202, which died 41 days after inoculation). These lesions contained relatively small numbers of bacilli, but diffuse lesions in the lung harbored considerable numbers of bacilli.

One (K-192) of the reinoculated rabbits of the 2-week group was killed 41 days after reinoculation, so that it might be compared with the above-mentioned control rabbit K-202. In the former, multiple necrotic lobular lesions were present along the ventral borders of the lung. Yellowish tubercles of poppy-seed size were found in the spleen and kidney. Histopathological examination revealed a few discrete minute lesions consisting of large mononuclear cells and lymphocytes in the liver, spleen, and bone marrow. Central necrosis was seen in these lesions, where no bacilli could be demonstrated. Accumulations of large mononuclear cells were shown in the renal cortex of this rabbit, but not in that of the control rabbit for intravenous inoculation.

There were two more rabbits which survived a long period of time after reinoculation. One (K-196) was from the 1-week group and died 73 days after intravenous inoculation. In this rabbit, numerous tubercles of pin-head size were found in the liver and lung, and a few of them in the kidney as well. Histopathological examination revealed a few, well circumscribed lesions at the periphery of the hepatic lobules. Central necrosis was seen in these lesions. In the bone marrow, there were a few accumulations of large mononuclear cells surrounded by lymphocytic infiltration. Lesions consisted mainly of giant cells were found in the red pulp of the spleen. In the lung there were widespread lesions with extensive necrosis which contained not a few bacilli. The renal cortex had large accumulations of large mononuclear cells and giant cells surrounded by layers of lymphocytes. The central part of these lesions had undergone necrosis and contained bacilli easily demonstrable.

Rabbit K-188 of the 3-week group died 211 days after reinoculation. At necropsy, there were yellowish necrotic lesions along the ventral border of the lung and numerous yellowish striations in the kidney extending from the cortex to the medulla. Besides, multiple joint involvement (abscess formation) was noted in the 1st, 3rd, 5th, and 6th costovertebral joints on the right and the 7th on the left side and the elbow, carpal, stifle, and tarsal joints on both sides. There was a yellowish miliary caseous lesion in the left cerebral hemisphere just beneath the meninges. Histopathologically, the periportal connective tissues of the liver were infiltrated with lymphocytes and a few small accumulations of shrunken large mononuclear cells and lymphocytes were in the periphery of the hepatic lobules. The inner part of the hepatic lobules was practically free from lesions. The spleen was atrophic and had no specific lesion, except scattered giant cells in the red pulp. In sections of the bone marrow, a few accumulations of large mononuclear cells were observed. The lung had necrotic lesions on the ventral border and a few circumscribed accumulations of large mononuclear cells surrounded by layers of lymphocytes. There were many wedge- or spindle-shaped lesions in the kidney extending from the cortex to the medulla. These renal lesions showed extensive central necrosis and were surrounded by thin layers of shrunken

large mononuclear cells. A large number of bacilli were contained in the necrotic areas of these lesions. Many glomeruli and tubules had been trapped in the lesions of the kidney. In some areas of this organ, widespread lesions with extensive necrosis occupied the inner zone of the medulla and caused dilatation of the collecting tubules. In the cerebral cortex, there was a caseous lesion surrounded by thin layers of large mononuclear cells. A large amount of nuclear debris was found in this lesion, where many necrotic arterioles were found. Perivascular cuffings of lymphocytic type were seen in the cerebral tissue around the caseous lesion. This was the only case in which the involvement of the central nervous system had been detected among a large number of rabbits used in the present series of experiments.

RESULTS OF THE SECOND EXPERIMENT

From the results of the first experiment, it was made clear that the effect of sensitization differed with the time intervals between subcutaneous and intravenous inoculation and that a definitely positive intradermal tuberculin reaction was shown 2 to 3 weeks after subcutaneous inoculation.

In order to supplement the information obtained from the first experiment, four rabbits were reinoculated intravenously with 0.1 mg/kg of virulent culture of the Flamingo strain 3 weeks after the subcutaneous inoculation. A period of observation was limited this time, being the same with the period for Yersin type tuberculosis.

1. Clinical findings

At the time of reinoculation, all animals showed a definitely positive reaction to the intradermal injection of tuberculin, as shown in Table 4.

There was a sudden pyrexia over 40°C in all animals on the second day of reinoculation, while no febrile reaction was observed in the control rabbits for intravenous inoculation at that time (Chart 2).

One of the reinoculated rabbits (K-212) was killed 14 days after intravenous inoculation. One of the control rabbits for subcutaneous inoculation (K-215) died of diarrhea on the

Table 4. Intradermal Reaction to Avian, Bovine, and Human Type Tuberculin
3 Weeks after Subcutaneous Inoculation (in the Second Experiment)

Rabbit No.	Type of tuberculin		
	Avian	Bovine	Human
K-211	$\frac{21 \times 17}{26 \times 24}$	$\frac{17 \times 17}{22 \times 22}$	$\frac{20 \times 17}{22 \times 21}$
K-212	$\frac{22 \times 25}{26 \times 30}$	$\frac{24 \times 24}{25 \times 25}$	$\frac{20 \times 17}{21 \times 21}$
K-213	$\frac{18 \times 18}{29 \times 24}$	$\frac{23 \times 19}{25 \times 22}$	$\frac{15 \times 20}{19 \times 23}$
K-217	$\frac{19 \times 22}{20 \times 23}$	$\frac{18 \times 20}{19 \times 21}$	$\frac{15 \times 15}{18 \times 18}$
K-215*	$\frac{14 \times 14}{10 \times 10}$	$\frac{\pm}{23 \times 22}$	$\frac{6 \times 6}{15 \times 15}$
K-216*	$\frac{\pm}{20 \times 20}$	$\frac{7 \times 7}{21 \times 21}$	$\frac{5 \times 5}{12 \times 12}$

The denominator represents the diameters in mm of an erythematous area and the numerator those of an induration.

* Control for subcutaneous inoculation.

next day. A control rabbit for intravenous inoculation (K-219) died 17 days after inoculation. Two reinoculated rabbits (K-211 and K-217) died 19 and 23 days after reinoculation, respectively. Another control rabbit for intravenous inoculation (K-218) died 28 days after inoculation. The remaining reinoculated rabbit (K-213) and a control for subcutaneous inoculation were killed at that time for comparison.

The results of quantitative culture of peripheral blood are shown in Table 5. There was a considerable degree of bacillema immediately after intravenous inoculation in the control rabbits for intravenous inoculation as well as the reinoculated rabbits. After that a lower degree of bacillema was observed in the latter.

2. Quantitative culture

The numbers of colonies recovered from the liver, spleen, bone marrow, lymph node, lung, adrenal, and kidney was listed in Table 6 together with the weights of main organs.

The amounts of living bacilli in the liver, spleen, and bone marrow of three rabbits, of which one was killed 14 days and the others died 19 and 23 days after reinoculation, were definitely smaller than those of rabbits at the similar stages of Yersin type tuberculosis. On the other hand, the amounts of bacilli in the lung, adrenal, and kidney of the three rabbits do not differ greatly from those of rabbits at the similar stages of Yersin type tuberculosis. Consequently, the schemata of bacillary distribution in those rabbits exhibited a rectangular pattern, which was considered to be characteristic of Villemin type tuberculosis²⁾ (K-211, K-212, and K-217 in Chart 2).

The amount of bacilli in a control rabbit which died 17 days after intravenous inoculation could not be estimated because of an accidental contamination of the culture media. The schema of bacillary distribution in another control rabbit was of typical Yersin type.

Rabbit K-213 was killed 28 days after reinoculation and compared with a Yersin-type control rabbit. It harbored a strikingly smaller amount of bacilli than the control. As shown in Table 6, the number of living

Chart 2. Body Temperature Curves and Schemata of Bacillary Distribution in Individual Animals in the Second Experiment

The seven columns of a schema represent the amounts of bacilli in 100mg each of liver, spleen, bone marrow, lymph node, lung, adrenal, and kidney from the top to the bottom.

Y: Control for intravenous inoculation.

SC: Control for subcutaneous inoculation.

Table 5. Number of Colonies Recovered from 0.1 ml of Peripheral Blood

Rabbit No.	Days after intravenous inoculation									
	0	2	5	7	9	12	13	15	19	24
K-211	177	0	4	0	0	8		4		
K-212	218	24	0	2000	2	0	0.4	0		
K-213	93	3	1	1	0	0		0	0	0
K-217	390	0	0	0	1	0		0	6	
K-218*	330	6	1	14	8	16		5	31	180
K-219*	800	1	0	2	10	1		69		

* Control for intravenous inoculation.

Table 6. Number of Colonies Recovered from 100 mg of Organ Tissue and Weights of Main Organs

Rabbit No.	Liver	Spleen	Bone marrow	Lymph node	Lung	Adrenal	Kidney	Survival time after i.v. inoculation**	Weight of organ (g)		
									Liver	Spleen	Lung
K-211	29×10^5	89×10^4	197×10^4	165×10^3	16×10^3	270×10^5	160×10^2	19 days	114	17.3	29
K-212	96×10^4	100×10^4	42×10^4	86×10^2	67×10^3	39×10^3	95×10^2	14 K	104.5	5.8	17.7
K-213	(0×10^4) $< 0.3 \times 10^3$	1.7×10^3	(0×10^4) $< 0.3 \times 10^3$	10×10^2	0.3×10^4	8×10^3	23×10^2	28 K	75.5	2.3	12.5
K-217	290×10^3	192×10^3	91×10^3	114×10^2	26×10^3	177×10^3	141×10^2	23	101	5.8	20.5
K-218*	106×10^3	15×10^3	25×10^3	2.7×10^3	7.3×10^5	3.3×10^7	26×10^4	28	88.5	8.0	15.5
K-219*	—	—	—	—	—	—	—	17	116.2	9.5	21.5

* Control for intravenous (i.v.) inoculation.

** K: killed.

bacilli per unit weight of the liver, spleen, and bone marrow of that rabbit was less than 10^{-7} of that of the control rabbit. In that rabbit, an early febrile reaction lasted for only a few days and bacillemia was definitely inhibited. If it had not been killed, it might have survived a long time.

Two control rabbits for subcutaneous inoculation were also subjected to quantitative culture. All organs cultured were negative for living bacilli at a dilution of 10^{-1} .

3. Pathological findings

Gross findings of rabbit K-212, which was killed 14 days after reinoculation, were similar to those of advanced Yersin type tuberculosis. No gross tubercles were observed in any of its organs, but it already showed conspicuous splenomegaly (5.8g). Supravital preparations of the liver and spleen of this rabbit revealed the presence of numerous large mononuclear cells and giant cells with rosettes of fine neutral-red-stained vacuoles. Histopathological examination revealed widespread lesions, consisting of large mononuclear cells and giant cells, in the liver, spleen, and bone marrow. Only a small number of bacilli could be demonstrated in these lesions. Many of the liver lesions had slits filled with red blood cells in their center. The bone marrow showed an extreme cellularity associated with an increased number of megakaryocytes and contained practically no fat cells. Accumulations of large mononuclear cells were also found in the adrenal and lymph node. The histopathological findings mentioned above were almost the same as those in advanced Yersin type tuberculosis and differed only in that only a few bacilli were demonstrable in the lesions. In the lung, however, there were numerous foci of large mononuclear cells. Tubercle-like protrusions (intima granuloma) were frequently noted in the pulmonary veins. The renal cortex contained many accumulations of large mononuclear cells. These lesions seemed to have started from glomeruli. Single large mononuclear cells laden with a considerable number of bacilli were occasionally found in glomeruli. Accumulations of large mononuclear cells were also present in the medulla of the ovary.

A control rabbit which died 17 days after

inoculation showed no gross tuberculous lesions other than splenomegaly.

Postmortem examination of rabbit K-211, which died 19 days after reinoculation, revealed the enormously enlarged spleen (weighing 17.3 g) with several pale areas of infarct. Its liver was peppered with numerous minute grayish-yellow lesions. There were scattered grayish-white tubercles of pin-point size in its renal cortex. Its lung was consolidated and beset with many grayish-white tubercles of pin-point size. Histopathological examination revealed widespread lesions in the liver and spleen. Early central necrosis and the participation of lymphocytes were noted in these lesions. Besides regular large mononuclear cells with abundant cytoplasm, shrunken large mononuclear cells were found in these lesions. Some of these lesions contained red blood cells in their center. In its spleen were observed specific lesions, sinuses highly engorged with red blood cells, and wide areas of coagulation necrosis. Only small numbers of bacilli could be demonstrated in the lesions of the liver and spleen. The hematopoietic tissue of its bone marrow was highly compressed by widespread lesions, which showed early necrosis. A considerable number of bacilli was demonstrated in the lesions of the bone marrow. In its lung, there were numerous conglomerate accumulations of large mononuclear cells, and early necrosis was seen in some lesions. Intima granuloma consisting of large mononuclear cells was found in the branches of the pulmonary veins. Accumulations of large mononuclear cells were found in the ovarian medulla. Nodular accumulations of shrunken large mononuclear cells with a few bacilli were found in its myocardium.

There was still a considerable degree of splenomegaly in rabbit K-217, which died 23 days after reinoculation. Numerous minute tubercles of pin-point size were found in its lung and perirenal adipose tissue and around its spermatic cords, but not in its liver and spleen. Histopathological examination, however, revealed numerous accumulations of large mononuclear cells and giant cells in its liver and spleen. Some lesions exhibited central necrosis and contained red blood cells. Only a small number of bacilli could be demonstrated in the lesions of its liver and spleen. Widespread lesions occupied its bone marrow, from which almost all fat cells had disappeared. A marked increase in megakaryocytes was also noted in this case. There were accumulations of large mononuclear cells and giant cells in its adrenal cortex and lymphatic tissues. Many minute foci of mononuclear cells and lymphocytes were found in its myocardium. A small number of bacilli was demonstrated in the myocardial lesions. Many well-circumscribed accumulations of large mononuclear cells were present in its lung. Bacilli were abundant in larger tubercles, which had Central necrosis associated with exudation of pseudo-eosinophils. Intima granuloma consisting of large mononuclear cells was often found in the pulmonary veins. Bacilli were occasionally found in the large mononuclear cells of the intima granuloma. Well-developed lesions with central necrosis were observed in its renal cortex and harbored a relatively large number of bacilli.

Rabbit K-213, which was killed 28 days after reinoculation, showed only a slight splenomegaly (2.3 g). On this day, the spleen of the control rabbit for Yersin type tuberculosis weighed 8 g. Scattered foci of pin-point or pin-head size were found in the kidney and lung of that rabbit. Yellowish necrotic lesions of soybean size were seen along the ventral border of the right diaphragmatic lobe of its lung. Histopathologically, most of the hepatic lesions were present near the thickened periportal connective tissues. These lesions consisted of large mononuclear cells and enormously large or single huge giant cells surrounded by lymphocytes. There were no well-developed lesions within the liver lobules, except a few accumulations of several large mononuclear cells. No bacilli could be demonstrated in the lesions of the liver. The red pulp of its spleen contained small accumulations consisting mainly of huge giant cells of Langhans' type. Hemosiderin granules were found in the cytoplasm of the giant cells together with a rosette of fine greenish-yellow

pigment granules. No bacilli were found in these lesions. There were only a few accumulations of large mononuclear cells in its bone marrow. Well-developed accumulations of large mononuclear cells and giant cells with central necrosis were noted in the adrenal cortex. Circumscribed accumulations of large mononuclear cells were found in the inter-ventricular septum of the heart and the ovarian medulla. Accumulations of large mononuclear cells were observed in the lymphatic tissue of the intestine. The lung had scattered well-circumscribed foci of large mononuclear cells with central necrosis. Some of these foci had distinct lymphocytic layers in their peripheral portions. Only a few bacilli were demonstrated in the lesions of the lung. Intima granuloma was also found in the pulmonary vein of this rabbit. There were multiple foci of various sizes in its renal cortex. The lesions of its kidney also exhibited central necrosis associated with exudation of pseudo-eosinophils and contained very scanty bacilli. Participation of lymphocytes was sometimes seen in the renal lesions.

The control rabbit which died 28 days after inoculation showed the typical feature of Yersin type tuberculosis. Its liver, spleen, bone marrow, and lymph node were occupied by numerous foci of large mononuclear cells and giant cells. Each focus contained numerous bacilli arranged in a radial pattern. No circumscribed tubercle-like lesions were present in its lung, which, however, showed a more conspicuous diffuse thickening of the alveolar walls than that of the reinoculated rabbits. There were no such organized renal lesions as observed in most of the reinoculated rabbits. Only single large mononuclear cells with bacilli were seen occasionally in the glomeruli. Central necrosis, which was quite common to the lesions in the reinoculated rabbits, was observed in no lesions of the Yersin type control.

DISCUSSION

The effect of sensitization on Yersin type tuberculosis was observed in rabbits which had received intravenous reinoculation 2 to 4 weeks after subcutaneous inoculation. These rabbits manifested a distinctly positive tuberculin reaction at the time of reinoculation.

In rabbits which had received intravenous inoculation simultaneously with subcutaneous inoculation, the effect of the subcutaneous inoculation was obscure.

Intravenous inoculation of virulent avian tubercle bacilli produced a prompt febrile reaction in rabbits which had shown a positive tuberculin reaction. In the second experiment of this study, a febrile reaction occurred, without exception, on the second day after reinoculation. In a normal rabbit which received the primary intravenous inoculation of avian tubercle bacilli, no febrile reaction usually occurred during a week after inoculation. The early febrile reaction suggested an accelerated response of the rabbit to the reinoculated bacilli.

Bacillemia, demonstrable continuously through the course of Yersin type tuberculosis, was apparently inhibited in some of the reinoculated rabbits.

Of the reinoculated rabbits, some died earlier, and others survived or were assumed to survive, if not killed, a definitely longer period of time, than the Yersin type control rabbits. Those which survived a long time revealed markedly inhibited bacillemia and showed only a slight febrile reaction.

The effect of sensitization was quite obvious on the multiplication of bacilli in the organs of reinoculated rabbits. In them, the distribution of bacilli was greatly modified and the schema of bacillary distribution close to that of Villemin type, even within the period of Yersin type, because of inhibited multiplication of bacilli in the liver, spleen, and bone marrow.

The accelerated response of the reinoculated rabbits was also proved in their pathological

findings. Marked splenomegaly was observed in them shortly after intravenous inoculation, though it had never occurred so early in ordinary Yersin type tuberculosis. Histopathological examination also revealed the appearance of widespread lesions in the liver, spleen, and bone marrow in very early stages following intravenous reinoculation. MEDLAR³⁾ observed an accelerated response and early death in a rabbit which had been vaccinated with human tubercle bacilli by the subcutaneous route and reinoculated with avian tubercle bacilli. He also noted modification of lesions in the vaccinated rabbit which survived a long time.

LURIE^{4,5)}, in his studies on quantitative culture with reinoculated rabbits, also observed early formation of lesions followed by a rapid destruction of reinoculated human and bovine type tubercle bacilli in the liver, spleen, and bone marrow.

The large mononuclear cells and giant cells in the lesions of reinoculated rabbits were indistinguishable from those in Yersin type tuberculosis in histological sections stained with hematoxylin and eosin. They differed from those in the Yersin type disease only in that a very few bacilli were demonstrable in their cytoplasm. The participation of lymphocytes in the lesions of reinoculated rabbits indicated the transformation of the lesions toward those of Villemin type tuberculosis²⁾. The gross tubercle formation which was considered to be characteristic of Villemin type tuberculosis was often found in the lung and kidney of reinoculated rabbits even within the period of Yersin type. Central necrosis, which had never been observed in the lesion of Yersin type, was also a frequent finding in the lesions of reinoculated rabbits.

Reinoculated rabbits which survived beyond the period of Yersin type showed the characteristics of Villemin type both in pathological features and distribution of bacilli in the organs. This should be considered, however, a mere result of the prolonged survival time. In other words, a relatively complete inhibition of bacillary multiplication in the early stages allowed the animals to take the course of Villemin type tuberculosis.

It is interesting to note that there was only a slight involvement of the liver, spleen, and bone marrow of a reinoculated rabbit (K-213) which was killed within the period of Yersin type (28 days after reinoculation). Further investigation is required to determine whether this phenomenon corresponds to the reduction of lesions in the liver, spleen, and bone marrow observed in the serial studies on Villemin type tuberculosis²⁾.

SUMMARY

1) The effect of sensitization on Yersin type tuberculosis in rabbits was investigated to explain the striking differences in the distribution of lesions and bacilli between Yersin and Villemin type tuberculosis.

2) The effect of sensitization was clearly observed in rabbits which had received intravenous reinoculation 2 to 4 weeks after the primary subcutaneous inoculation.

3) In well-sensitized rabbits, there was an accelerated response to reinoculated bacilli. The distribution of lesions and bacilli, as well as the pathological features of the lesions, were greatly modified to be close to those of Villemin type, even within the period of Yersin type tuberculosis.

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鳥型結核に関する研究

III. 家兎の YERSIN 型結核に対する前処置の影響について (1)

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家兎に鳥型結核菌の一定量以上を静脈内に接種すると YERSIN 型とよばれる敗血症型の病型を生じ、家兎は約 4 週以内に斃死する。この場合いずれの臓器にも肉眼的な結節形成は認められず、著明な脾腫を呈するだけであるが、組織学的には肝・脾・骨髓等に大単核円形細胞と巨細胞から成る繁殖性病巣が高度に発達し、菌も之等臓器の病巣に無数に証明される。YERSIN 型 (Y 型) に於ける肺・腎の病変は極めて軽度である。少量の鳥型菌を家兎の静脈内に接種して家兎が 1 ヶ月以上生存すると全身各臓器に肉眼的な結節形成を示す所謂 VILLEMEN 型結核 (V 型) が起り病変は次第に肺及び腎に局限して来る。従って Y 型と慢性の V 型を比較すると病変及び菌の分布が全く逆となって居る。著者等は前 2 報^{1,2)} に於て夫々 Y 型と V 型の経時的観察を行い病変及び菌の消長を追跡し、特に各臓器の菌量 (対数) を肝・脾・骨髓・腸間膜淋巴節・肺・副腎・腎の順に棒グラフで表示した場合、Y 型では肝・脾・骨髓に菌量が多く肺・副腎・腎に之が少いため、この菌量模型は逆梯型を呈し、V 型では之が各臓器間に差の少い矩型、或いは肺・腎の菌量の多い菌量模型が得られることを知った。之等を夫々 Y 型及び V 型の菌量模型図と呼んだ。

Y 型と V 型の相違の一つとして、Y 型ではツベルクリン皮内反応が陽転することなく斃死するのに対し V 型では之が陽転して居るという事実がある。V 型の経時観察で、接種後 4 週間以内では菌及び病変の分布上 Y 型に似た時期があることを知ったが²⁾、以後の病変・菌量の分布が V 型に移行するのは或いは個体の感作が影響して居るのではないかと考えられる。それならば、予め個体を感作しておけば Y 型が何等かの修飾を受ける可能性があるので、今回の実験を行った。

強毒鳥型結核菌 Flamingo 株の 0.05 mg/kg 静脈内接種前 4, 3, 2, 1 週及び静脈内接種と同時に夫々 4

頭の家兎に培養基継代により毒力の低下した Flamingo 株 0.5 mg/kg の皮下接種を行った。各群の半数を皮下接種の対照とし別に無処置の家兎 2 頭に強毒菌の静脈内接種を行った。感作の途中で下痢のため斃死するものがあつたので実際に強毒菌の静脈内再接種を受けたのは 4 週群 1, 3 週群～同時群各 2 頭で、皮下接種のみの対照は 4, 3 及び 2 週群各 1 頭、1 週及び同時群各 2 頭である。以上を第一実験とし、第二実験として 6 頭の皮下接種家兎を準備し内 4 頭に 3 週後強毒菌 0.1 mg/kg 静脈内接種を行った。別に 2 頭の無処置家兎に同量の静脈内接種を行って Y 型の対照とした。以下に両実験の結果をまとめて記述する。

1. ツベルクリン皮内反応は 4 乃至 2 週前に皮下接種を行った家兎では明瞭に陽転して居た (Table 1, 4)。ツベルクリン反応が陽性を呈した家兎では静脈内接種後対照の Y 型より早期に発熱が見られ、特に第二実験の再接種家兎では之が著明であつた (Chart 1, 2)。Y 型に於て全経過を通じて証明される菌血症は若干の家兎、特に生存期間が延長したものでは抑制されて居た (Table 2, 5)。

2. 第一実験では下痢のため再接種後 1 週間内外で斃死した家兎があつたが、又対照の Y 型が斃死した後長期間生存したものもあつた。第二実験では Y 型対照の斃死と共に再接種家兎を殺して検索したが、放置すれば長期間生存したであろうと思われる例があつた。

3. 臓器の定量培養の成績では早期に斃死したものでは Y 型の初期の菌量分布と大差はないが、それ以後 Y 型の期間内に斃死又は屠殺した 1~3 週群の再接種家兎では肝・脾・骨髓の菌量が対照或いは同時期の Y 型に比し著しく少く菌量模型図が V 型に近いものが見られた (Chart 1, 2, Table 3, 6)。静脈・皮下同時接種群では Y 型と大差のない菌量模型図が得られ、菌増殖に関しては同時皮下接種による大き

な影響はなかったものと考えられる。第一実験に於て対照の斃死後長期間生存したものの菌量模型は定型的なV型を呈したが、之は長期間生存したための当然の結果と考えられる。尚第一実験の皮下接種対照家兎の定量培養で接種後33日及び32日に屠殺したものの脾から夫々少量(0.7×10 及び 1.3×10)の菌が検出されたが他の臓器への菌の撒布は認められず、再接種家兎の定量培養では皮下接種菌の内臓への撒布は無視し得る程度と考えられた。

4. 再接種後1週間内外で斃死した家兎に既に相当程度の脾腫が認められた。正常家兎にY型を惹起すべき菌量を接種した場合にはこの様に早期の脾腫は認められず、ツ反応陽転家兎に於ける早期の発熱と同様再接種菌に対する促進された反応の現れと考えられる。之等の家兎では組織学的にも肝・脾・骨髓に相当高度の病巣が出現して居た。再接種家兎の病巣も大単核細胞及び巨細胞から成りH-E染色ではY型のそれと本質的な差は認められないが、之等細胞の胞体内にY型に見られる様な夥しい菌が見られないことは著しい相違点である。病巣の構造には、Y型の期間内に斃死又は屠殺した再接種家兎でも若干の相違点が指摘される。病巣周辺に淋巴球浸潤が

見られることはV型病巣の性格を示すものと考えられ、又Y型の期間内に既に各臓器の病巣に壊死が見られ、肺・腎に肉眼的病巣を生じた例がある。組織学的にも肺・腎にV型に見られたと同様の壊死を伴った大型病巣が見られる。Y型では決して見られない肺・腎の結節性病巣出現は感作によるY型のV型への修飾を最も明瞭に示した所見と考えられる。肺静脈枝にしばしば認められた内膜肉芽腫は再接種家兎に特有の病変と考えられる。長期間生存した家兎では1例に多発性関節病変が見られ、その他の臓器の病変分布及び性格も全く慢性のV型の特徴を呈した。このことは之等の家兎の菌量模型と同様、長期間生存したための当然の結果と考えられよう。Y型の期間内に殺した1例に肝・脾・骨髓の病変が極めて軽度のものが見られたことは興味深い。V型の経過中に見られた之等臓器の病変消退と同一の現象であるか否かは今後更に追究する必要がある。

以上弱毒鳥型菌の前処置により予め家兎のツ反応を陽転させておくと、再接種された強毒菌に対する家兎の反応、菌量及び病変の分布、病巣の性格が著しく修飾され、Y型を生ずべき期間内にV型の病変及び菌量模型が得られることが明かになった。

EXPLANATION OF PLATE

- Fig. 1. Well-circumscribed lung lesions in rabbit K-212, which was killed 14 days after reinoculation. Reticulum-hematoxylin-eosin stain. $\times 100$
- Fig. 2. Intima granuloma in the pulmonary vein of rabbit K-217, which died 23 days after reinoculation. Reticulum-hematoxylin-eosin stain. $\times 100$
- Fig. 3. Accumulation of large mononuclear cells in the renal cortex of the same rabbit as Fig. 2. This type of lesion has never been seen in Yersin type tuberculosis. Hematoxylin-eosin stain. $\times 100$
- Fig. 4. Well-developed accumulations of large mononuclear cells and giant cells in the adrenal cortex of rabbit K-213, which was killed 28 days after reinoculation. Hematoxylin-eosin stain. $\times 200$