

THE ENHANCEMENT OF THE EFFECT OF B.C.G. VACCINATION WITH HYALURONIDASE

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PART I. CLINICAL EXPERIMENTS

Introduction. The improvement of B.C.G. vaccination to lessen the abscess formation without impairing the immunizing action has been studied by many investigators. In 1928, Duran-Reynals^{1,2)} found that the infectiousness of smallpox virus was remarkably increased when the virus was added with the extract of mammalian testis. The same effect was also recognized by Mc. Clean (1931)³⁾. This active factor in the testis extract was called "spreading factor", and found also in sperm, spleen, certain bacteria, tumor tissues, snake-venom, etc. In 1939, Chain and Duthie⁴⁾ demonstrated that the hyaluronidase, which hydrolyzes the hyaluronic acid belonging to polysaccharide of connective tissue mucin, agrees with the spreading factor.

The present study was undertaken many years since, with a view of possible enhancement of B.C.G. infection by means of inoculation with the B.C.G. vaccin added with the spreading factor of Duran-Reynals. In the beginning, a spreading factor-containing testis extract for intradermic injection was difficult to be extracted intact and sterile. But, as hyaluronidase preparations suitable for the injection became available recently, the experiment was facilitated and completed.

Bergqvist reported in 1950⁵⁾ that the effect of B.C.G. vaccin in guinea pigs was enhanced when the hyaluronidase was added to the vaccin, and in 1951⁶⁾ that the effect of B.C.G. vaccination, in regard to the time required to turn positive tuberculin reaction and the extent of the reaction, was enhanced by the addition of hyaluronidase, based on the 2-months observation among the primary school pupils in Stockholm. A similar result was obtained by the present authors.

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INVESTIGATION METHODS

Two hundreds and forty-six pupils involving 150 boys and 96 girls with negative tuberculin reaction were selected for the investigation. They belonged to the first to fifth school years of primary school, and aged from 6 to 11. The 1st school-year pupils received no B.C.G. inoculation before, while the remaining 176 received single or several inoculations including the last one which was made 8 months before the present examination.

Above 246 pupils were divided into 3 groups unselectively. Seventy-seven pupils of Group A were inoculated with the B.C.G. vaccin added with 10 units of the spreading factor, 84 of Group B with the vaccin with 0.5 unit of the factor, and 85 of Group C, the control group, with B.C.G. vaccin alone. The pupils of the 1st school year, who received no B.C.G. inoculation and were of negative tuberculin reaction, were incidentally distributed evenly in 3 groups; 27 in Group A, 22 in Group B, and 21 in Group C.

The B.C.G. vaccin used was the dried vaccin prepared by the Antituberculous Association of Japan, and 0.1 cc (0.04 mg as bacteria) was inoculated intradermically to the left upper arm. The spreading factor used was "Alidase" imported from the United States.

As each school year had 3 classes—East, Middle and West—in the beginning, the West classes of each year were designated to Group A, the Middle classes to Group B, and the East classes to Group C, but, later, the classes were recomposed, and the examinations of tuberculin reaction for a year following inoculation were done without prepossession. The items of examination were the time required to turn positive reaction, the duration of positive reaction, the size of redness, the frequency of abscess formation, and the size of abscess.

RESULTS

The time and the rate to become positive reaction was indicated in Table 1. By 16th day, 81.8% of Group A, 67.9% of Group B, and 60.0% of Group C became positive, showing significant difference between Group A and Group C. However, no significant difference was noticed by 43rd day between Group A (15.6%), Group B (22.6%) and Group C (12.9%). By 79th day, the positive reaction was observed in none of Group A, 8.3% of Group B, and 18.8% of Group C, again showing significant differences between Groups A and B and Group C.

It was recognized that many pupils in Group A turned to positive early, followed by Group B, and the turn was slow in Group C. In the

total turn-rate, no significant difference was observed, though the rates of Group A (97.4%) and Group B (98.8%) were slightly higher than that of Group C (92.9%).

Table 1. Time required for positive-turn

Group	Addition of spreading factor	No. of pupils	Days after inoculation				Suspected positive	Negative
			16	43	79	133		
A	10 units	77	63* (81.8%)	12 (15.6%)	0*	0	2 (2.6%)	0
B	0.5 unit	84	57 (67.9%)	19 (22.6%)	7* (8.3%)	0	1 (1.2%)	0
C (Control)	none	85	51 (60.0%)	11 (12.9%)	16 (18.8%)	1 (1.2%)	4 (4.7%)	2 (2.4%)

* indicates significant difference.

The duration of positive tuberculin reaction was indicated in Table 2) The duration of 9~12 months was observed in 2.6% of Group A, 9.5% of Group B, and 5.9% of Group C, showing no significant difference between each group. The duration of 6~9 months was observed in 41.6% of Group A, 25.0% of Group B, and 25.9% of Group C. The difference was significant between Group A and Group C. The duration of 3~6 months was observed in 18.2% of Group A, 26.2% of Group B, and 17.6% of Group C, and that of within 3 months 37.7%, 39.3%, and 50.6%, respectively. No significant difference was noticed in these cases.

It was demonstrated that relatively long duration of positive reaction as 6~9 months was more frequent in Group A than in the control group. However, no significant difference was noticed in the duration of 9~12 months between Groups A and B and Group C.

Table 2. The duration of positive tuberculin reaction

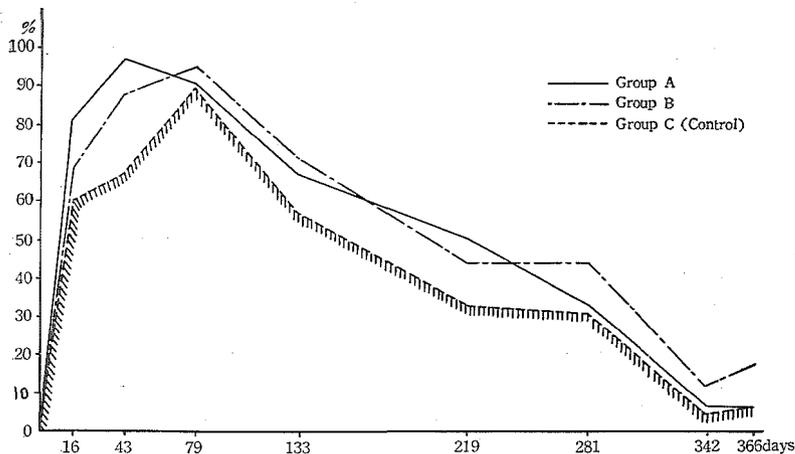
Group	Addition of spreading factor	No. of pupils	Duration of positive reaction			
			less than 3 months	3~6 months	6~9 months	9~12 months
A	10 units	77	29 (37.7%)	14 (18.2%)	32* (41.6%)	2 (2.6%)
B	0.5 unit	84	33 (39.3%)	22 (26.2%)	21 (25.0%)	8 (9.5%)
C (Control)	none	85	43 (50.6%)	15 (17.6%)	22 (25.9%)	5 (5.9%)

* indicates significant difference.

As stated above, the tuberculin reaction of each group becomes positive, and then turns to negative after different durations. The curves of positive reaction rates of 3 groups are shown in Fig. 1. The rates

of Groups A and B are constantly higher than that of the control group, being 50.6% in Group A, 44.0% in Group B, and 32.9% in Group C 219 days (7 months) after the inoculation. The difference is significant. The reaction was positive in half of the pupils of Group A, but only one-third in the control group. Therefore, it was recognized that the addition of spreading factor enhanced the effect of B.C.G. vaccination to turn the tuberculin reaction positive.

Fig. 1. Curves of positive rate of tuberculin reaction.



The sizes of redness of tuberculin reaction was illustrated in Table 3) The diameters of 10~14 mm are observed in 27.3% of Group A, 27.4% of Group B, and 31.8% of Group C, those of 15~19 mm in 53.2% of Group A, 53.6% of Group B, and 41.2% of Group C, and those of more than 20 mm in 16.9% of Group A, 17.9% of Group B, and 20.0% of Group C. The significant difference was noticed in no case.

Table 3. The size of redness of tuberculin reaction

Group	Addition of spreading factor	No. of pupils	Diameters of redness in mm				
			0~4	5~9	10~14	15~19	>20
A	10 units	77	0	2 (2.6%)	21 (27.3%)	41 (53.2%)	13 (16.9%)
B	0.5 unit	84	0	1 (1.2%)	23 (27.4%)	45 (53.6%)	15 (17.9%)
C (Control)	none	85	2 (2.4%)	4 (4.7%)	27 (31.8%)	35 (41.2%)	17 (20.0%)

The rates of abscess formation at the site of inoculation were shown in Table 4. The rates were 23.4% in Group A, 29.8% in Group B, and 14.1% in Group C after 16 days, and 92.2% in Group A, 84.5% in Group B, and 71.8% in Group C after 43 days. The differences between the

rates of Group A and Group C were significant. The rate of abscess formation with B.C.G. vaccin added with the spreading factor was concluded to be higher than that of the control group. However, the difference was insignificant after 79 days; the rate of Group A was 33.7%, Group B 20.2%, and Group C 27.1%. After 97 days, almost all the abscesses were cicatrized and healed in each group. The rates were 3.9% in Group A, 2.4% in Group B, and 1.2% in Group C, and no significant difference was found among these rates.

Table 4. The abscess formation at the site of inoculation.

Group	Addition of spreading factor	No. of pupils	Abscess formation after			
			16 days	43 days	79 days	97 days
A	10 units	77	18 (23.4%)	71* (92.2%)	26 (33.7%)	3 (3.9%)
B	0.5 unit	84	25* (29.8%)	71 (84.5%)	17 (20.2%)	2 (2.4%)
C (Control)	none	85	12 (14.1%)	61 (71.8%)	23 (27.1%)	1 (1.2%)

* indicates significant difference.

The size of abscess was shown in Table 5. The abscesses of more than 11 mm were formed in no case of Group A, in 3 cases of Group B, and in no case of Group C. Whereas, those of 5~10 mm were formed in 10 cases of Group A, in 12 of Group B, and in 5 of Group C; the difference was significant between Group B and Group C. The abscesses of less than 4 mm were found in 61 cases of Group A, in 60 cases of Group B, and in 64 cases of Group C; the differences being insignificant.

As a whole, the abscesses were formed in 92.2% of Group A, in 89.3% of Group B, and in 81.2% of Group C. The difference between Group A and Group C was significant.

Table 5. The size of abscess.

Group	Addition of spreading factor	No. of pupils	+++	++	+	-
			>11mm	10~5mm	<4mm	
A	10 units	77	0	10	61	6
			71 (92.2%) *			
B	0.5 unit	84	3	12	60	9
			75 (89.3%)			
C (Control)	none	85	0	5	64	16
			69 (81.2%)			

* indicates significant difference.

SUMMARY AND DISCUSSION

In the group received B. C. G. vaccin added with the spreading factor, the tuberculin reaction turned to positive earlier than in the control group, and the relatively long duration of positive reaction as 6~9 months was undoubtedly frequent. However, it seemed that the maximum duration of tuberculin allergy in man due to B.C.G. vaccin is not prolonged especially by the addition of spreading factor, because no significant difference was mentioned between both groups in regard to the duration of positive tuberculin reaction for 9~12 months. Thus, the total rates of positive-turn in both groups indicated no significant difference.

There was no significant difference in the size of redness in both groups. The abscess formation at the site of inoculation was more frequent in the group inoculated with the vaccin added with spreading factor, but almost all the abscesses were cicatrized by 97th day, and caused less trouble. Three cases in which the abscess remained relatively long were treated simply with local dusting of PAS powder and bandage. The abscess became dry within 3 days, and healed within a week.

If the trouble from abscess can be treated in the same manner, the inoculation of B. C. G. vaccin added with the spreading factor is expected to enhance the immunizing effect against tuberculosis. As the enhancing effect of spreading factor added to B. C. G. vaccin was supposed from the result of positive-turn, such inoculation method is considered to be useful, even if the abscess formation is prevented unsatisfactorily. It was considered that the infection with B. C. G. bacteria occurred effectively by the addition of spreading factor. Although changes in the nature of B. C. G. bacteria is improbable, further experiments will be made in order to clarify whether B. C. G. vaccin reaches deep into the body by spreading factor, as compared with the control.

As for the amount of spreading factor, the addition of 10 units to 0.1 cc of B. C. G. vaccin (0.04 mg bacteria) was slightly more effective than that of 0.5 unit, but the difference was not so significant.

CONCLUSION

- 1) In the group inoculated with B. C. G. vaccin added with the spreading factor, the tuberculin reaction turned to positive earlier and more frequent, and the positive reaction remained longer than in the control group. It was recognized that the addition of spreading factor enhances the effect of B. C. G. vaccin to turn the tuberculin reaction positive.

- 2) No significant difference was noticed in the size of redness of

tuberculin reaction.

3) The abscess formation at the site of inoculation was more frequent in the group inoculated with B. C. G. vaccin added with spreading factor, but all the abscess were cicatrized and healed without difficulty.

4) The addition of 10 units to 0.1 cc of B. C. G. vaccin (0.04 mg bacteria) was slightly more effective than that of 0.5 unit, but the difference was almost insignificant.

PART II. ANIMAL EXPERIMENTS

Introduction. An attempt to enhance the infection-effect of BCG vaccination by means of hyaluronidase was first made by Bergqvist⁵⁾, ⁶⁾ and then by Birkhaug⁷⁾, Oike⁸⁾, and the present authors. In the previous experiment⁹⁾ with primary school students, the authors have noticed the enhancing effect on tuberculin positivity in the group inoculated with BCG vaccin added with hyaluronidase.

In the present experiment, the BCG vaccin with hyaluronidase was inoculated to rabbits, and the state of bacterial invasion and the changes of body tissues were observed in order to clarify the mechanism of enhancing effect.

MATERIALS AND METHODS

Healthy rabbits weighing 1.49-2.25 kg were selected for the experiment, and each 0.1 cc of 1:5 diluted tuberculin was injected intradermically after the hair was removed. The injection sites were examined after 48 hours to ascertain the negativeness of tuberculin reaction.

The tuberculin-negative rabbits were divided into two groups; viz, the control group (BCG-group), and the group inoculated with BCG added with hyaluronidase (Hyaluronidase-BCG-group). In the BCG-group, each 1 cc (0.5 mg of bacteria) of BCG vaccin was injected hypodermically to depilated parts of both forelegs (frontal middle parts between elbow and shoulder joint). In the hyaluronidase-BCG-group, each 1 cc (0.5 mg of bacteria) of BCG vaccin added with 10 units of hyaluronidase was injected in the same way. The injections were made in the clean room in the least delay after the dried BCG vaccin was dissolved.

The dried BCG vaccin manufactured by the Anti-tuberculosis Association of Japan was used. The viable bacterial count by Ogawa's quantitative culture method was 21,400,000 cells per mg. The hyaluronidase used was 'Alidase' prepared in the United States.

The tuberculin reaction was examined 48 hours after the inoculation using 1:5 dilution of tuberculin manufactured by the Kitasato Institute

for Infectious Diseases.

The body was weighed before the inoculation of BCG vaccin, and on 29th, 74th, 95th, and 115th day after.

Each two rabbits of both groups were sacrificed by etherchloroform anaesthesia, on 11th, 23th, 29th, 56th, 74th, 95th, and 115th day after the BCG inoculation. The internal organs and lymphatic glands were observed macroscopically, and the lymphatic glands in 5 parts, lung, liver, spleen, and kidney were taken aseptically to perform the quantitative culture by Ogawa's method. Pathhistological observation was made on paraffinimbed preparations stained with haematoxylin-eosin, van Gieson's solution, or by Kumabe's staining method for tubercle bacilli. Lymphatic glands of cerevical, axillary and inguinal regions, and injection site of left side were utilized to make histological preparations, and those of right side for cultivation.

EXPERIMENTAL RESULTS

1) Tuberculin Reaction

The results are indicated in Table 6. On 11th day after the BCG inoculation, the redness with more than 10-mm diameter was observed in 2 rabbits (Nos. 6 and 11) (14.3%) of BCG-group, and in 8 (Nos. 16, 18, 21, 22, 23, 25, 27 and 28) (57.1%) of hyaluronidase-BCG-group; that is, more frequent and earlier in the latter, with significant difference.

On 29th day, the redness with more than 10-mm diameter was observed in all the rabbits of BCG-group, and all but one (No. 26) of hyaluronidase-BCG-group, and on 56th day, in all of both groups. Plates 1, 2, show the redness of tuberculin reaction of rabbits No. 8 (BCG-group) and No. 22 (hyaluronidase-BCG-group) on 56th day respectively. No remarkable difference was noticed between diameters of redness of both groups. The redness was relatively clear, and the diameters were measured without difficulty. On 95th day, all the animals of both groups still showed the redness of more than 10-mm diameters.

Table 6.

		BCG-group													
Animal No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Sex		♂	♀	♂	♂	♀	♀	♂	♀	♂	♂	♂	♀	♀	♀
Day of survival		11	11	23	23	29	29	56	56	74	74	95	14	95	115
Tuberculin reaction	11days after inoculation	6×6	7×6	0×0	0×0	5×4	11×12	7×6	7×6	9×7	6×8	16×15	7×6	7×7	9×9
	29 "					10×11	10×12	22×18	13×12	13×12	12×12	16×16		13×14	12×10
	56 mm "							13×14	17×13	22×18	16×18	13×14		17×16	13×14
	95 "											13×14		14×11	10×8
Weight in kg	Before inoculation	2.05	1.78	1.85	2.10	2.20	1.80	2.05	2.22	2.02	2.00	2.25	2.05	1.90	1.82
	29days after inoculation					2.46	2.05	2.30	2.45	2.26	2.41	2.66		2.36	2.10
	74 "									2.60	2.80	3.05		2.80	2.60
	95 "											3.20		3.10	2.75
115 "														2.75	
Course of death	Sacrificed	"	"	"	"	"	"	"	"	"	"	Death by accident	Sacrificed	"	
Site of BCG inoculation	BCG bacilli			+	+					+	+				+
Axillary glands	Swelling BCG bacilli	+ ₁₀	+ ₁₀	-	(+)	(+)	(+)	(#)	(+)	(##)	(##)	(#)	-	(#)	(#)
Cervical glands	Swelling BCG bacilli	-	-	-	-	-	-	-	-	-	(+)	-	-	-	-
Upper mesentricae	BCG bacilli	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Le. mesocolicae sinistra	BCG bacilli	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Inguinal glands	Swelling BCG bacilli	-	-	-	-	-	-	-	(+)	(+)	(+)	-	-	-	-
Lung	BCG bacilli	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Liver	BCG bacilli	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spleen	BCG bacilli	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kidney	BCG bacilli	-	-	-	-	-	-	-	-	-	-	-	-	-	-

		Hyaluronidase-BCG-group													
Animal No.		15	16	17	18	19	20	21	22	23	24	25	26	27	28
Sex		♀	♀	♂	♂	♂	♀	♀	♂	♀	♂	♀	♀	♂	♂
Day of survival		11	11	23	23	29	29	56	56	74	74	95	80	95	115
Tuberculin reaction mm	11days after inoculation	8×7	10×9	7×8	9×11	4×5	8×7	20×14	22×14	17×16	3×2	21×20	0×0	11×10	9×10
	29 "					14×14	11×11	18×22	16×11	16×14	18×16	18×21	4×4	19×18	9×10
	56 "							20×18	16×14	11×11	22×20	12×12	10×9	12×14	13×12
	95 "											14×12		11×11	14×12
Weight in kg	Before inoculation	1.81	2.22	2.00	2.00	2.22	1.49	2.09	2.05	1.82	1.72	2.00	1.80	1.99	1.90
	29days after inoculation					2.35	2.00	2.51	2.30	2.28	2.00	2.40	2.20	2.22	2.26
	74 "									2.90	2.45	2.80	2.40	2.60	2.60
	95 "											2.80		2.80	2.75
115 "														2.75	
Course of death		"	"	"	"	"	"	"	"	"	"	"	Death by accident	Sacrificed	"
Site of BCG inoculation															
	BCG bacilli			+	+					+	+				+
Axillary glands	Swelling			(+)	(+)	(++)	(+)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)
	BCG bacilli	+ ₂₀	+ ₃₀	-	-	-	-	-	-	-	-	-	-	-	-
Cerevical glands	Swelling									(+)	(+)				
	BCG bacilli	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Upper mesentricae	BCG bacilli	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Le. mesocolicae sinistra	BCG bacilli	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Inguinal glands	Swelling									(+)	(+)				
	BCG bacilli	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lung	BCG bacilli	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Liver	BCG bacilli	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spleen	BCG bacilli	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kidney	BCG bacilli	-	-	-	-	-	-	-	-	-	-	-	-	-	-

2) Body Weight

As indicated in Table 6, the body weight of every animals of both groups inclined to increase gradually without remarkable difference of weight gain between two groups.

3) Macroscopical Anatomical Findings

The results are shown in Table 6, where

(+) indicates the lymphatic glands which swell larger to some extent as compared with usual ones,

(+) indicates the lymphatic glands which swell distinctly, and

(#) indicates the lymphatic glands which swell remarkably.

In both groups, the swelling of local axillary glands was notable, appearing from 23 days after the inoculation, and reached largest after 74 days, and then inclined to decrease its size. Slight swelling was noticed after 56 and 74 days in inguinal glands, and after 74 days in cervical glands, but in other glands change was scarcely observed. The swollen lymphatic glands showed macroscopically no tubercle nor caseation.

On the site of BCG inoculation, development of ulcer was not noticed during the observation period of 115 days in both groups, but in the dissect examination made 11 days after the inoculation, hypodermic abscess with caseation were found. On 23rd day, the abscess became larger, and on incision, sporadic hemorrhages were noticed in some cases inside of caseated substances. Such an abscess formation was also found in the dissect examination made after 115 days, and the abscess were somewhat larger in hyaluronidase-BCG-group, containing cheese-like substances. In lung, liver, spleen and kidney, macroscopical tubercle, caseation, nor any significant change was found in both groups. The macroscopical findings in lung, liver, spleen, and kidney are indicated in Plates 3, 4, 5 and 6, and the weight of these organs in Table 7.

Table 7. Weight of Organs

Animal No.	BCG-group		Hyaluronidase-BCG-group	
	11	13	25	27
Lung	18.0gr	17.0gr	15.0gr	14.6gr
Liver	82.6gr	67.6gr	68.5gr	57.5gr
Spleen	1.6gr	1.4gr	1.2gr	1.1gr
Kidney	left	7.1gr	7.2gr	6.1gr
	right	7.1gr	7.1gr	6.0gr
Body weight	3.2kg	3.1kg	2.8kg	2.8kg

4) Quantitative Culture of Organs and Lymphatic Glands

In Table 6, the growth on culture media is represented as(+)and no growth as(-). The bacteria was cultured from axillary gland of all the animals sacrificed and dissected 11 days after the inoculation. The number of bacteria per 0.02 g of organs or glands was slightly larger in hyaluronidase-BCG-group as compared with BCG-group; viz. 10 in Nos. 1 and 2 of BCG-group (control), 20 and 30 in No. 15 and No. 16, respectively, of hyaluronidase-BCG-group. The bacteria was not cultured from other organs or lymphatic glands.

5) Histological Findings

i) Inoculation site of BCG vaccin

The histological examination made 11 days after the inoculation revealed that in BCG-group (Nos. 1 and 2) necrotic lesion surrounded by thick layer of larger cells of histiocytes was clearly circumscribed. Round-shaped cellular infiltrations composed mostly of lymphocytes were found in the ordinary tissues which enclose directly the circumscribed lesion mentioned above (Plate 7). In hyaluronidase-BCG-group (Nos. 15 and 16), similar circumscribed lesion was also formed. Although the lesion was larger in size than that in BCG-group, histiocytic layer was thinner, and surrounding ordinary tissues showed almost no reaction such as cellular infiltration (Plate 8).

After 23 days, the findings in BCG-group (Nos. 3 and 4) were almost the same as those after 11 days (Nos. 1 and 2). In hyaluronidase-BCG-group (Nos. 17 and 18), the findings were similar to those after 11 days (Nos. 15 and 16). In No. 18, the necrotic lesion had spread widely to musculature, however, cellular reactions were scarce in the surrounding tissues. No remarkable difference was observed between the findings after 23 days and those after 29 days in both groups.

After 56 days, in BCG-group (Nos. 7 and 8) the lesion became necrotic almost whole, formation of granulation tissues was remarkable in surrounding tissues, capillary vessels were newly formed, and cells, mostly histiocytes, appeared conspicuously (Plate 9). In hyaluronidase-BCG-group (Nos. 21 and 22), no granulation was observed in the surrounding tissues, of lesion by this time, and the circumscribing tendency was weak (Plate 10).

After 95 days, the granulation tissues became thicker in BCG-group (Nos. 11 and 13), the appearance of fibroblasts became noticeable, and in some parts, there formed connective tissue walls which divided the granulation tissues (Plate 11). In hyaluronidase-BCG-group (Nos. 25 and 27), round-shaped cellular infiltrations were observed in the surrounding tissues, but the formation of granulation was far slow as compared with control BCG-group (Plate 12). After 115 days, the findings of both groups were almost the same as those after 95 days.

Conclusively, in BCG-group, the circumscribed lesion was enclosed

by relatively thick granulation tissues in which increasing infiltration of histiocytic cells was noticeable and cells of epitheloid type was predominant. Whereas, in hyaluronidase-BCG-group, the circumscribed lesion was also formed, the size was larger, granulation inferior, development of epitheloid cells not so remarkable, compared with BCG-group, and the lesion adjoined directly the ordinary tissues.

ii) Lymphatic glands

The change in axillary lymphatic glands was not so remarkable. In BCG-group, there was a slight reticulosis, lymphatic tissue was apt to atrophied consequently or replaced by reticulum, and plasmatic exudates were found sporadically. The axillary lymphatic glands after 95 days is shown in Plate 13. respectively.

In hyaluronidase-BCG-group, relatively diffusive proliferation of reticulo-endothelial cells or reticulosis was noticeable, and the lymphatic tissues were becoming indistinct in their tubercle boundary, being replaced by the reticulo-endothelial cells. A few eosinophilic cells appeared. The axillary lymphatic glands after 95 days is shown in Plate 14. respectively.

In other lymphatic glands such as cerevical, inguinal, upper mesentrial and mesocolonal, no remarkable change was found in both groups.

iii) Lung

There was no noticeable change in lungs. Occasionally, incrassation of alveolar wall and blockade of alveolar cavity were observed, irrespective to the days after inoculation and other factors. No remarkable difference was found between both groups (Plates 15, 16).

iv) Liver

As the day went on after inoculation, there were usually swelling or clouding of liver cells, infiltration of round-shaped cells in stroma and fibrination. Such findings were somewhat more frequent in hyaluronidase-BCG-group than in BCG-group (Plates 17, 18, 19 and 20).

v) Spleen

The change was not remakable. The spleen of hyaluronidase-BCG-group contained generally more blood than that of BCG-group, and there was a tendency of fibrination of spleen venous sinus in the former. However, the difference between two groups was not distinct (Plates 21, 22).

vi) Kidney

In the kidneys of progressed stages, there were ocassionally swellings of glomerula, some of which were associated with swelling of epithelium

of renal tubules. No remarkable difference was noticed between BCG-group and hyaluronidase-BCG-group except infiltration of round-shaped cells in the latter (Plates 23, 24).

vii) Stained preparation for tubercle bacilli

Acid-fast bacteria were always found in inoculation sites of animals of both groups, but not in lymphatic glands nor internal organs.

Plate 1. The redness of tuberculin reaction No.8 (BCG-group)

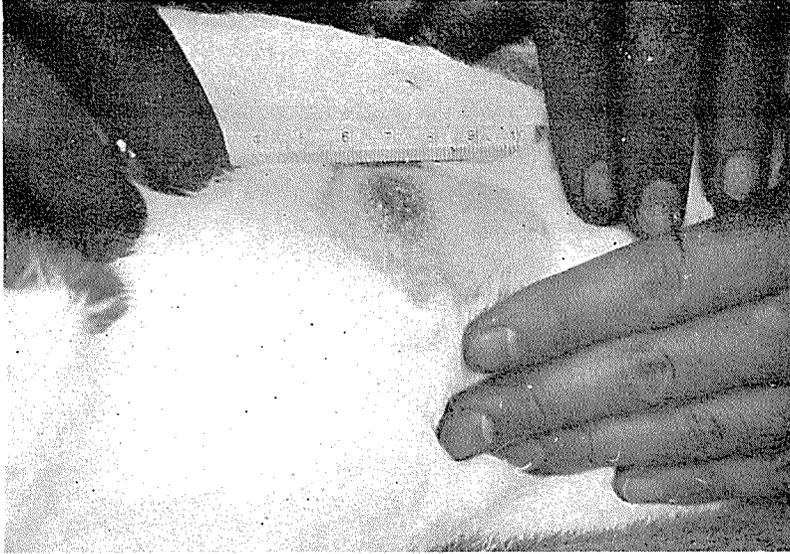


Plate 2. The redness of tuberculin reaction No.22 (Hyaluronidase-BCG-group)

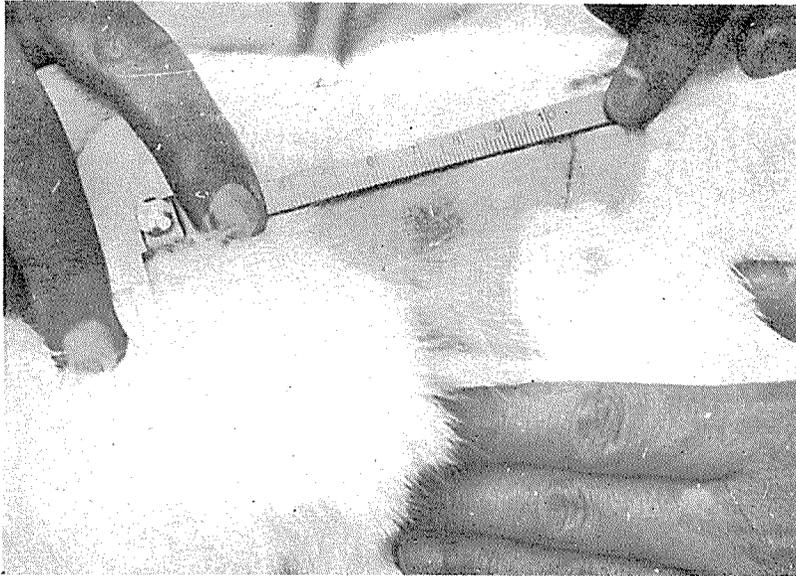


Plate 3. Lung

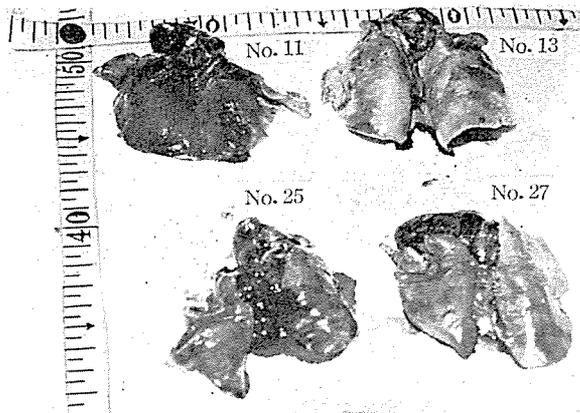


Plate 4. Liver

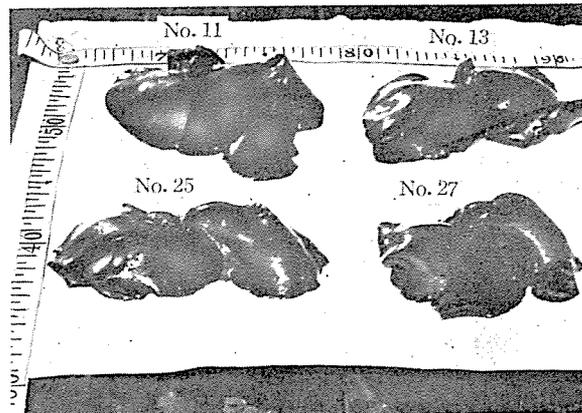


Plate 5. Spleen

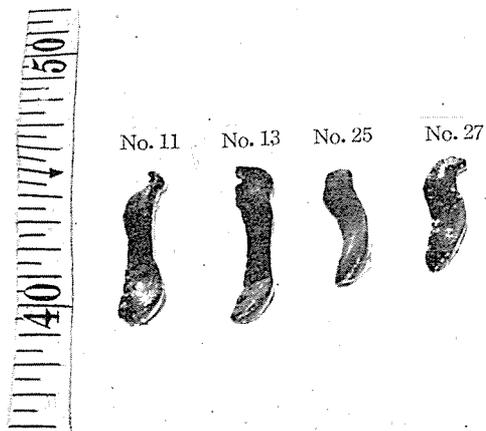


plate 6. Kidney

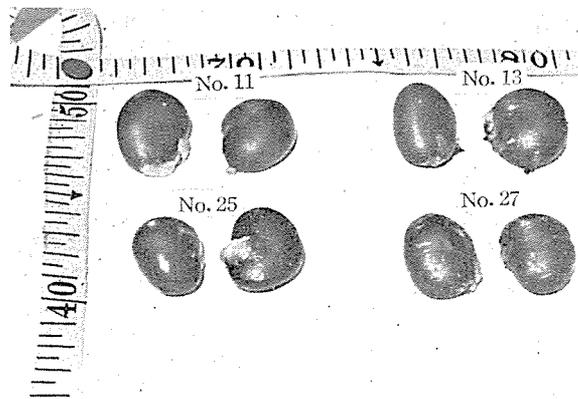


Plate 7. Site of inoculation after 11 days
(BCG-group)

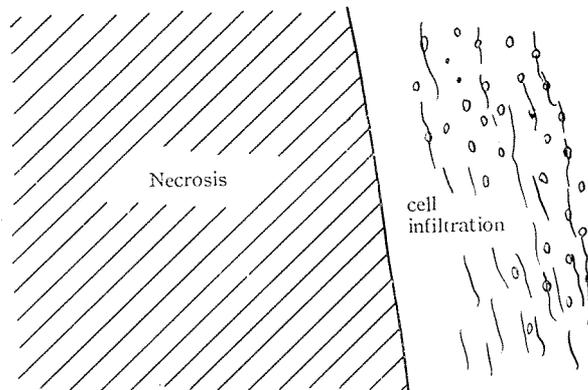
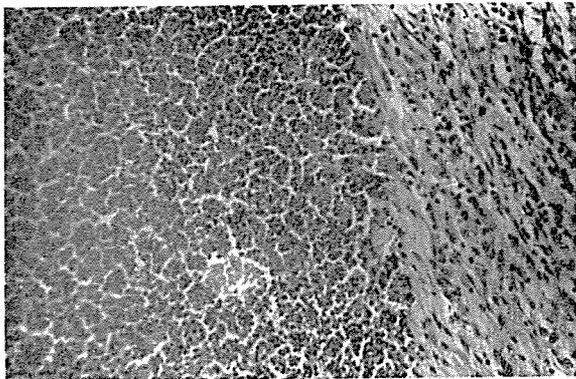


Plate 8. Site of inoculation after 11 days
(hyaluronidase-BCG-group)

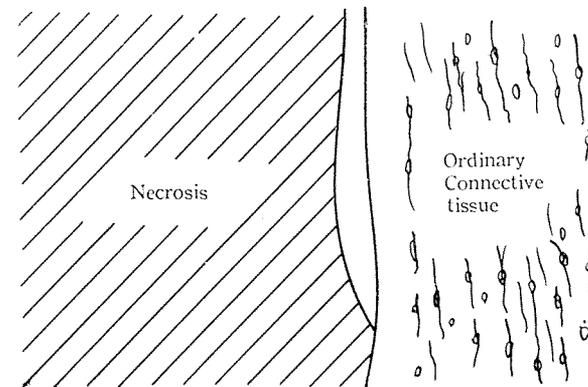
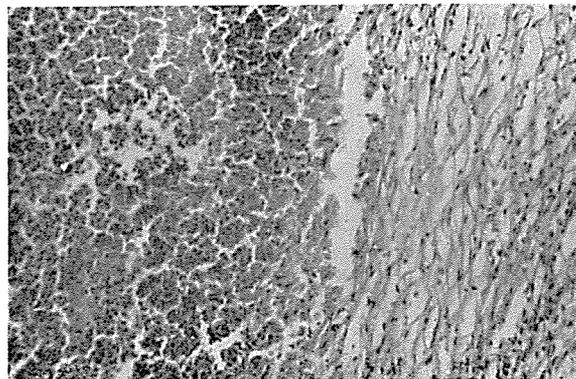


Plate 9. Site of inoculation after 56 days
(BCG-group)

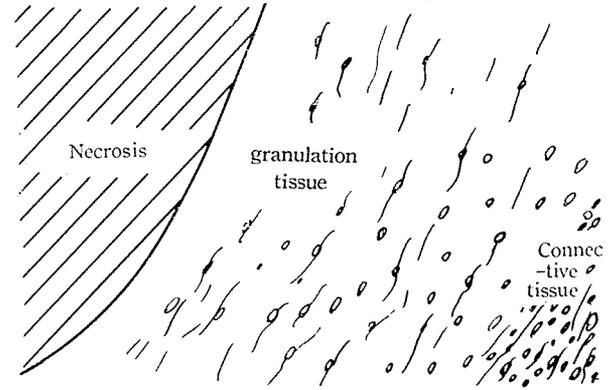
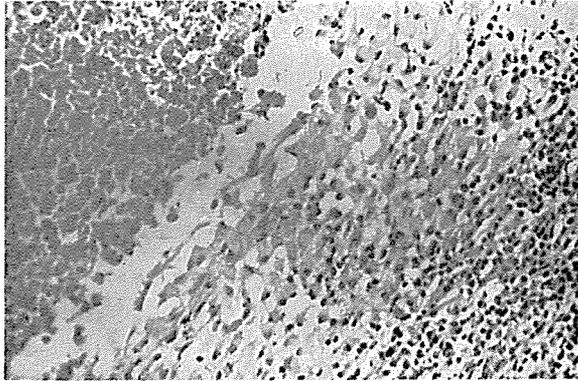


Plate 10. Site of inoculation after 56 days
(hyaluronidase-BCG-group)

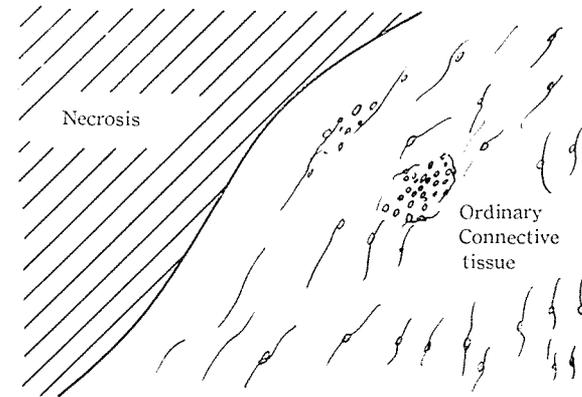
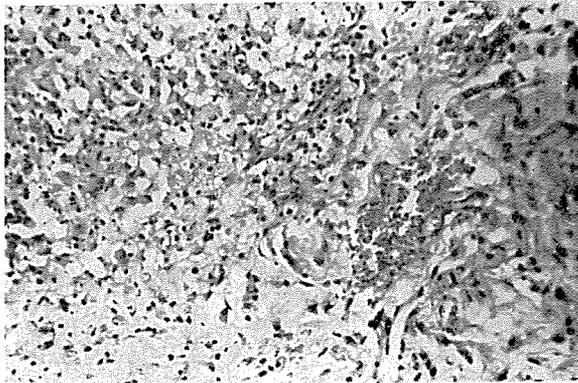
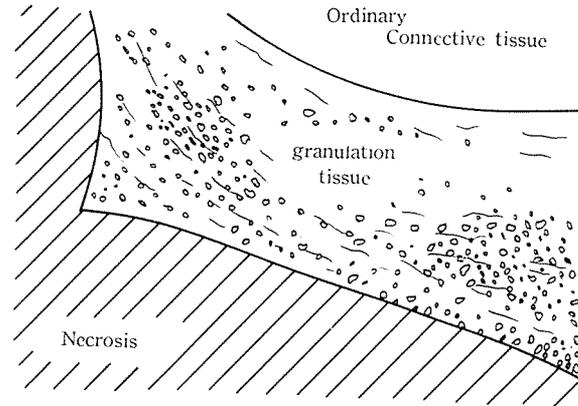
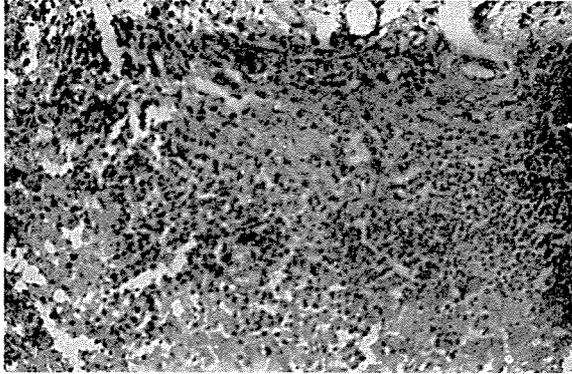


Plate 11. Site of inoculation after 95 days
(BCG-group)



No. 4

Plate 12. Site of inoculation after 95 days
(hyaluronidase-BCG-group)

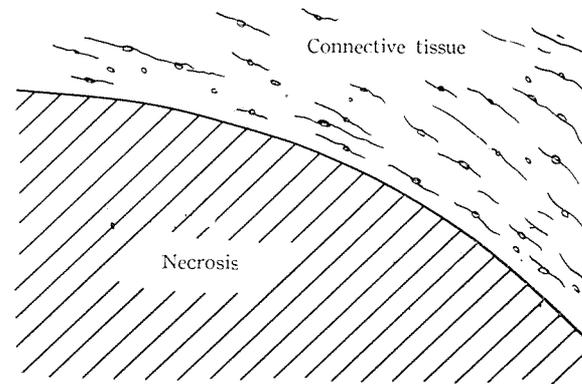
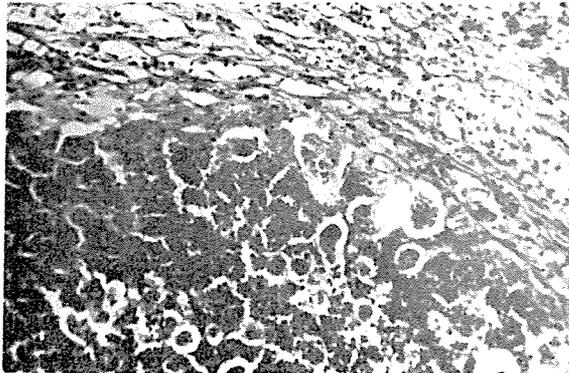


Plate 5

Plate 13. Axillary lymphatic gland after 95 days (BCG-group)

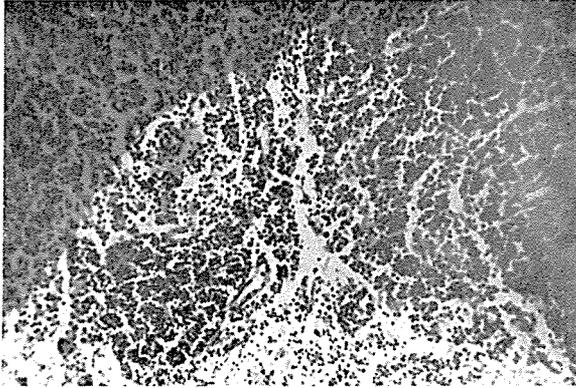


Plate 14. Axillary lymphatic gland after 95 days (hyaluronidase-BCG-group)

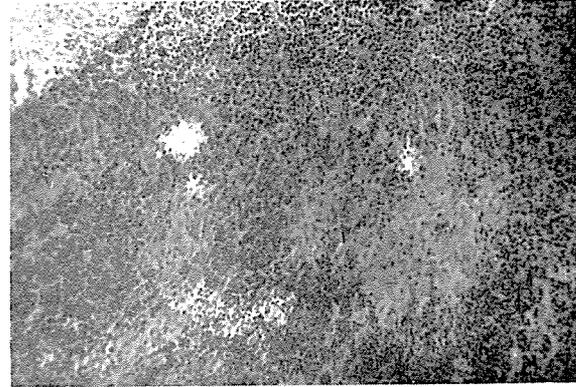


Plate 15. Lung after 56 days (BCG-group)

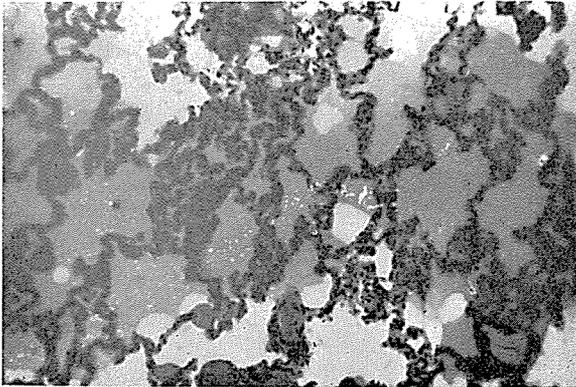


Plate 16. Lung after 56 days (hyaluronidase-BCG-group)

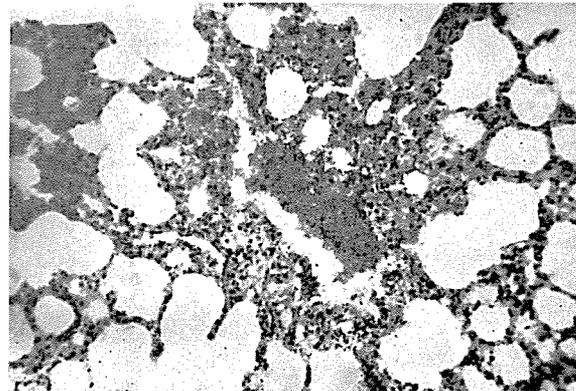


Plate 17. Liver after 11 days (BCG-group)

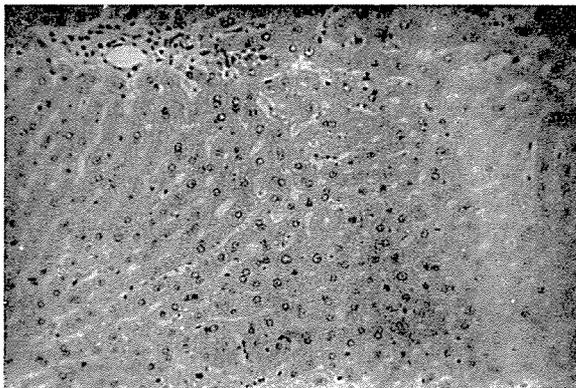


Plate 18. Liver after 11 days (hyaluronidase-BCG-group)

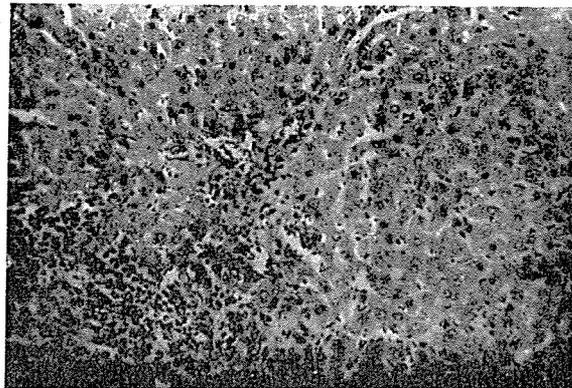


Plate 19. Liver after 56 days (BCG-group)

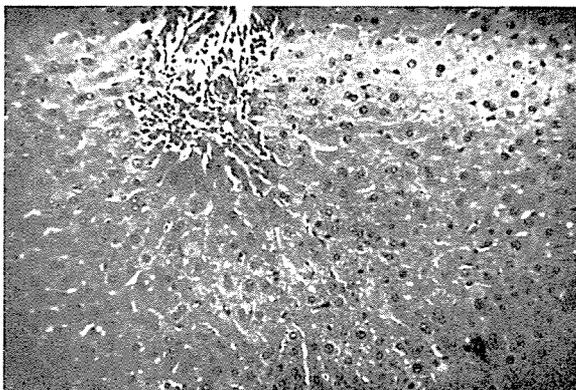


Plate 20. Liver after 56 days (hyaluronidase-BCG-group)

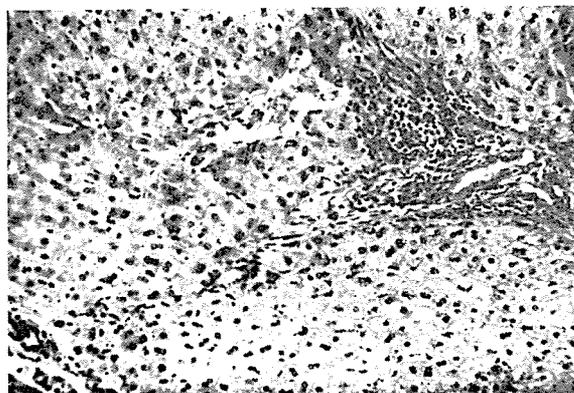


Plate 21. Spleen after 56 days (BCG-group)

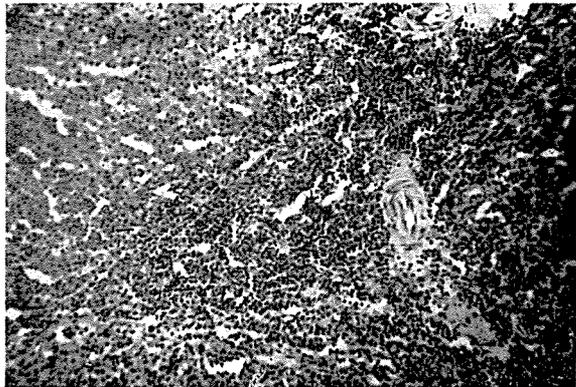


Plate 22. Spleen after 56 days (hyaluronidase-BCG-group)



Plate 23. Kidney after 56 days (BCG-group)

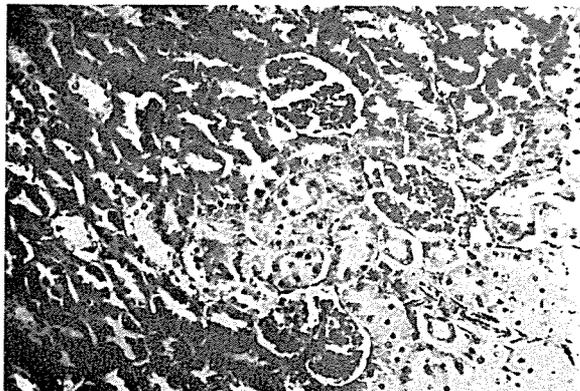
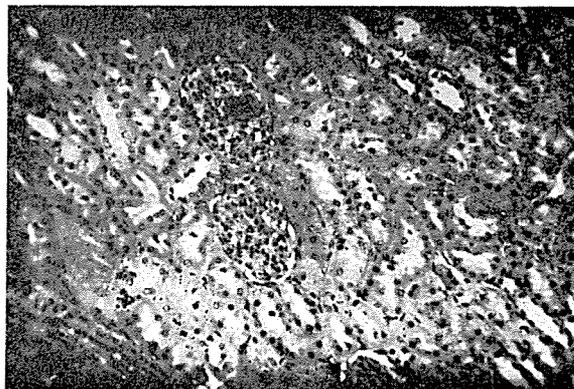


Plate 24. Kidney after 56 days (hyaluronidase-BCG-group)



SUMMARY AND DISCUSSION

The tuberculin reaction in hyaluronidase-BCG-group turned to positive earlier than in BCG-group. The animals with redness of more than 10-mm diameter in the earlier stages were determiningly more in hyaluronidase-BCG-group, the difference between two groups being significant. As for the tuberculin reaction with more than 10-mm redness, difference was scarcely noticed in their diameters between two groups. Using guinea pigs, Birkhaug also found that the tuberculin reaction turned to positive far earlier in the group inoculated with BCG vaccin added with hyaluronidase than in the group with BCG vaccin alone. In our experiment, the rabbits showed no decrease of appetite during the observation period of 115 days after the inoculation. The body weight inclined to increase gradually almost equally in both groups.

Macroscopical anatomical findings revealed remarkable swelling in local axillary lymphatic glands in both groups, but almost no change in other lymphatic glands, lung, liver, spleen and kidney. The development of ulcer at the site of inoculation was not noticed in both groups, but the dissect examination made 11 days after the inoculation revealed hypodermic abscess of with inside caseated. Such an abscess was still noticed in the dissect examination made on 115th day after the inoculation. The abscess was somewhat larger in hyaluronidase-BCG-group than in BCG-group. By the quantitative culture for acid-fast bacteria in organs and lymphatic glands, bacteria was obtained from the axillary glands of both groups on 11th day after the inoculation. The number of bacteria was somewhat larger in hyaluronidase-BCG-group than in BCG-group. No bacteria was cultured from other lymphatic glands and organs of any stage. There was no finding of bacteria which invaded beyond local lymphatic glands in either case of BCG plus hyaluronidase inoculation or BCG alone. Kato¹⁰⁾ reported that bacteria did not diffuse beyond local lymphatic glands even if far larger amount of BCG vaccin than that to man was inoculated to rabbits' forelegs hypodermically. The same fact was also noticed by Lange Lydtin and others¹¹⁾. Birkhaug inoculated BCG vaccin added with hyaluronidase to guinea pigs, and recognized that the numbers of bacteria obtained by the quantitative culture of lymphatic glands and organs were larger than those of control BCG-group.

In histological examination, the inoculation site of BCG-group had a circumscribed lesion which was enclosed by relatively thick wall of granulation tissue. In this granulation tissue, increased infiltration of histiocytes, and cells of epithelioid type were observed remarkably. Whereas, in hyaluronidase-BCG-group, the abscess were rather larger,

formation of granulation tissue was inferior, development of epitheloid cells not remarkable, and tendency of circumscription weaker.

In axillary lymphatic glands, slight reticulosis was observed in BCG-group, and relatively diffused proliferation of reticulo-endothelial cells (reticulosis), in hyaluronidase-BCG-group. The lymphatic tissues of the latter were becoming indistinct in their tubercle boundary, being replaced by the reticulo-endothelial cells. In other lymphatic glands, no difference was noticed between two groups. No change was observed in lungs of both groups. In the liver of hyaluronidase BCG-group, swelling or clouding of liver cells, infiltration of round-shaped cells, and fibrination were rather remarkable than in BCG-group. In kidney, swelling of glomerula and epithelium of renal tubules and slight infiltration of roundshaped cells were observed without remarkable difference between two groups. In spleen, too, difference between two groups was not distinct.

In the stained preparations for tubercle bacilli, acidfast bacteria were found in inoculation sites of animals of both groups until 115th day after inoculation, but not in lymphatic glands nor other organs.

B. Lange & K. Lydtin noticed acid-fast bacteria, by the histological examination, in the abscess at the site of inoculation for 3-4 months in guinea pigs and for 7-8 months in rabbits, using the animals inoculated BCG vaccin hypodermically. In our experiment, too, acid-fast bacteria were always present during the observation period of about 4 months. It is difficult to distinguish the number of acid-fast bacteria between two groups from the stained preparation. Okuhara¹²⁾ considered that there was a possibility to turn tubercle reaction positive even if the inoculation site was removed, the inoculation site which elapsed more than 7 days was less significant, and immunity or acquisition of tuberculin allergy was participated not only by inoculation site but by all organs. K. Birkhaug found that the tuberculin reaction turned to positive far earlier in the group inoculated BCG plus hyaluronidase than in BCG alone, even when the inoculationsite was removed 1, 4 and 24 hours after the inoculation. In our experiment, it is interesting that tuberculin allergy developed earlier in hyaluronidase-BCG-group than in control BCG-groups, the inflammation range at the inoculation site and the abscess were larger, encapsulation and formation of granulation tissue weaker, number of bacteria at the local lymphatic gland larger, the bacteria not invading into the internal organs beyond the lymphatic glands, and the histological change was rather severe.

CONCLUSION

- 1) In hyaluronidase-BCG-group tuberculin reaction turned to positive

earlier and showed more frequent the redness of more than 10-mm diameter than in BCG-group.

2) Body weight inclined to increase in both groups without difference in weight gain between two groups.

3) In macroscopical dissection findings, there was swelling in local axillary lymphatic glands in both groups, but no remarkable change was observed in other lymphatic glands, lung, liver, spleen and kidney. No ulcer was formed at the sites of BCG inoculation in both groups, but subcutaneous abscess were developed, the size being larger in hyaluronidase-BCG-group.

4) In quantitative culture for acid-fast bacteria in organs and lymphatic glands, the bacteria were cultured from axillary lymphatic glands of 11 days after the inoculation in both groups, the number being slightly larger in hyaluronidase-BCG-group than in BCG-group. No bacteria was cultured from other lymphatic glands and organs in any stage.

5) The histological examination of BCG inoculation sites revealed that, in hyaluronidase-BCG-group, abscess was larger, encapsulating tendency weaker, and granulation tissue inferior than in BCG-group. Histological changes in local lymphatic glands and liver were a little more severe in hyaluronidase-BCG-group, but no remarkable difference was noticed in spleen, kidney and other lymphatic glands of both groups.

6) In stained preparation for tubercle bacilli, acid-fast bacteria were detected in all inoculation sites in both groups. No bacteria was found in lung, liver, spleen and kidney.

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