Experimental Study of Intraaxillary Vaccination of B. C. G.

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(Received for publication, April, 19, 1947)

Yoshio Takahashi,¹⁾ in 1943, made public intraaxillary vaccination of B.C.G. Iwao Kanno²⁾, afterwards, suggested intraaxillary inoculation as a method of vaccination of B.C.G. more rational from the clinical point of view than the other methods. He reported the school boys and guinea-pigs did not suffer injury and the local lesion through the vaccination performed in this manner, and reacted positively to tuberculin more rapidly and more strongly than when the intracutaneous method was employed. He had 115 school boys who were vaccinated with 0.06 mg. of B.C.G. intraaxillary and 86 (74.8 per cent) had become tuberculin positive 22 days after the vaccination. In 111 there were no local reactions, and in 4 (3.5 per cent) the regional lymph glands were swollen. He saw 110, 45 days after the vaccination, and found that 110 (100 per cent) had become tuberculin positive. In 104 there were no local reactions, and in 6 (5-4%) the regional lymph glands were swollen. He saw 110, 102 days after the vaccination, and found that 110 (100 per cent) had become tuberculin positive. In 108 there were no local reaction, and in 2 (1.8%) the regional lymph glands were swollen. If we want to do the intraaxillary vaccination, we must order the one who is to be vaccinated to place his hand on his occiput. By this behaviour, a hollow will be formed in the axillary region, into the bottom of which we prick a needle of a syringe deeply back-upwards about five centimeters, and inject B.C.G. 0.1 mg. in 1 cc.

I wanted to resolve the questions of why the local lesion did not appear by this method. Let us think about why the local lesion will appear. Abscessformation is, first, more likely to occur in a naturally infected person (Koch's phenomenon). Second, a great individual variation in susceptibility and individual difference in the resistance to B.C.G. virus should

be considered. Third, the homogeneity, the density, the quantity of the emulsion and the place in which B.C.G. is injected should be considered. According to Rosenthal's³⁾ microscopical studies, the cutaneous and subcutaneous lymphatics, soon after inoculation was of great significance. Following the multiple puncture method, the lymphatics contained a few polymorphonuclear leucocytes which had phagocytosed tubercle bacilli. On the other hand, following a large single intracutaneous dose, the lymphatics were widely dilated and stuffed with cells and bacteria- amounting to a partial obstruction.

EXPERIMENT.

I would, therefore, examine by which way and how rapidly B.C.G. would be absorbed, and where it would stay. It was determined, preparatorily, how many mg. of B.C.G. in the blood and the lymph could be isolated. This was tried according to the method of cultivation which was reported by Takashi Katakura, Sutemi Oka, Tamura and Ishikawa⁴⁾ and known as the sulphuric acid pepsin method in Japan. The emulsion of B.C.G. was put into 3 cc of blood mixed with 18 cc of 0.1% saponin solution, containing calcium chloride at the ratio of 0.25%, and left to stand for three hours. Then it was centrifuged at a ratio of three thousand revolutions per a minute for thirty minutes, and the supernatant fluid was thrown away. Distilled water was poured into the sediment. This was centrifuged once more in the same manner, after being washed. The supernatant fluid was thrown away and a few drops of 4% sulphuric acid solution containing pepsin was dropped into the sediment, and it was left to stand for thirty minutes and then inoculated into the Oka-Katakura medium, which was reported by Sutemi Oka and Takashi Katakura.⁵⁾

I succeeded in isolating 1/10,000,000 mg. of B.C.G. in 3 cc of the blood (Table I).

TABLE I.
Results of bloodculture (preparatory).

No.	mg. of B.C.G.	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷
1	##		2 colonies	1 colony	1 colony
2	##		4 colonies	1 colony	0
3	##		3 colonies	2 colony	0
4	##		4 colonies	2 colony	1 colony
5	##		3 colonies	2 colony	0

Then dogs were used for study. Five mg. to 28 mg. of B.C.G. were injected into the axillary region of dogs, which weighed 6 kg. to 20 kg. Their lymph, which was obtained from ductus thoracicus, and the blood, which was obtained from V. jugularis, were cultivated according to the sulphuric acid pepsin method, after the inoculation of B.C.G. A sterilized canula was directly inserted into ductus thoracicus near V. jugularis to obtain lymph. The lymph was centrifuged at a ratio of 3,000 revolutions a minute, and the supernatant fluid was thrown away. A few drops of sulphuric acid solution containing pepsin was added to the sediment and left to stand for thirty minutes and inoculated into Oka-Katakura medium. These were placed into a incubator kept at 38.0°C for two months. The blood, obtained sterilely from V. jugularis, was inoculated into Oka-Katakura medium according to the sulphuric acid pepsin method and placed in the incubator kept at 37.0°C for two months. As Table II shows, it was not possible to isolate B.C.G. from the lymph after the intraaxillary vaccination, but from the blood, it was possible to isolate 18 minutes to 32 hours after injection. The lymph vessels of the axillary region show a

TABLE II.

Results of blood-, lymphculture after intraaxillary vaccination.

No. of anim.	1		2		3		4	5
Weight (Kg)	9		9		7		12	14
Quant. of B.C.G. (mgr.)	5		18		28		28	28
	Lymph	Blood	Lymph	Blood	Lymph	Blood	Blood	Blood
Before Vacc.	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)
11'								
15'								
18'								
25'						(#)	(#)	82col. (#)
30'	(—)	(—)	(—)	60col.	(—)			
1 h	(—)	2col.	(—)	50col.	(—)	50col.		
2 h	(—)	(—)	(—)	6col.	(—)	11col.	(#)	35col.
3 h	(—)	(—)	(—)	10col.	(—)	23col.		
4 h					(—)	8col.		
7 h					(—)	8col.		
9 h					(—)	5col.		
11 h					(—)	18col.		
16 h					(—)	3col.		
19 h					(—)	15col.		
32 h					(—)	(—)		

variation⁶⁾⁷⁾, so they sometimes flow into ductus thoracicus and sometimes into V. subclavia and V. jugularis. It, therefore, does not mean that B.C.G., which was injected into the axillary region, was not absorbed by the lymph vessels (and unable to be isolated in the lymph, which is obtained from ductus thoracicus). Wallgren⁸⁾ has seen, after the intracutaneous vaccination, an enlargement of the regional lymph glands, followed by caseous degeneration, suppuration, and fistula formation. It is obvious that B.C.G. is spread by way of the lymphatics, from the primary focus to the regional lymph glands. Following the multiple puncture method, Rosenthal³⁾ has reported that the lymphatics contained a few polymorphonuclear leucocytes, which had phagocytosed tubercle bacilli. On the other hand, following a large single intracutaneous dose, the lymphatics were widely dilated and stuffed with cells and bacteria. Therefore, in spite of the fact that B.C.G. is not isolated from the lymph, it should spread by way of the lymphatics. S. Katsura⁹⁾ has reported that paratyphoid B bacilli would be absorbed directly by blood vessels. And according to my experiments, in the shortness of time and the number of B.C.G. isolated, B.C.G. must be absorbed directly by blood vessels as well as by lymphatics.

Neither was B.C.G. isolated in the blood which was obtained from V. jugularis after subcutaneous injection near the axillar.

B.C.G. was not isolated in the blood, which was obtained from V. jugularis, either after intracutaneous vaccination of the same dose the intraaxillary and subcutaneous vaccination near the axillar.

These experiments seem to show that B.C.G. would be absorbed more easily and more rapidly from intraaxillary than from subcutaneous or intracutaneous injection.

Next, rats were slaughtered after intraaxillary, subcutaneous, and intracutaneous vaccination of 15 mg. of B.C.G., and the viscera was crushed in mortars, and some 4% sulphric acid solution was poured into them. They were centrifuged at a ratio of 3,000 revolutions a minute after being left to stand for 30 minutes. The supernatant fluid was thrown away, and the sediment was inoculated into Oka-Katakura medium. They were placed in a incubator kept at 37.0°C for two months. In the case of the intraaxillary vaccination, B.C.G. could be isolated from the lung, heart, spleen and kidney, but not from the liver, 10 minutes after injection (Table III).

In the case of the subcutaneous or intracutaneous vaccination, B.C.G. could not be isolated from the lung, heart, spleen, kidney or liver 10 minutes to 3 hours and 30 minutes after injection of the same does as the former.

In the case of the intraaxillary vaccination, there may be multiple foci of quantities of bacilli, which call forth tissue responses that are accompanied by good drainage by the way of patent lymph channels. Following

TABLE III.

Results of visceraculture after intraaxillary vaccination.

No. of animal	Quant. of B.C.G.	Time of Kill	Lung	Heart	Spleen	Kidney	Liver
1	15mg.	10'	1col.	1col.	2col.	2col.	(-)
2	15mg.	10'	1col.	1col.	2col.	2col.	(-)
3	15mg.	10'	1col.	1col.	1col.	1col.	(-)
4	15mg.	15'	1col.	1col.	1col.	1col.	(-)

the subcutaneous or intraneous vaccination of the same amount of vaccine, there may be only one local lesion which, because of the massive concentrated does of bacilli, cause local tissue destruction that is further enhanced by the partial blockade of the lymph channels.

Next, I observed the mode of the absorption of a mono-sodium salt solution of phenolsulphthalein (Sankyo) after intraaxillary, intracutaneous, and subcutaneous injection of 0.6% solution, which applied to man. This dye is absorbed by way of the blood vessels, after injection into the serous cavity, and excreted very well through kidney.¹⁰⁾ The urine excreted was collected, and the "phthalein" excreted was estimated colorimetrically after injection of one measured cubic centimeter of the solution. The quantity excreted was estimated, using the Dunning colorimeter. One tenth of the urine excreted was rendered alkaline by the addition of a little solution of sodium hydroxide, and the alkaline urine was diluted with water to make it measure one hundred cubic centimeters. The open ampule, containing the diluted alkaline urine, was then placed in the center hole of the colorimeter box and compared with the number test ampules until the one that most nearly matched the specimen in color was found.

The quantity of this excreted dye was estimated 3 to 4 hours after being injected. This dye was absorbed intraaxillary first, intracutaneously second, and subcutaneously third.

Five cubic centimeters of a 2% solution of methylene blue (Grübler) was observed after intraaxillary and subcutaneous injection of man. The standard solution was made by me. A 0.0001% solution of this dye was found in the urine. If the excreted contains methylene blue, it appears blue. It seems to be absorbed by way of the blood vessels and excreted in the urine after injection.¹⁰⁾

This dye was estimated from the urine which was excreted 3 hours after the injection. According to my experiments, methylene blue, when injected intraaxillary, was found in the urine, but, when injected subcutaneously, it could not be found (Table IV).

Next, 10 cc of a 2% solution of Congo-red (Grübler) was observed after intraaxillary and subcutaneous injection of a dog. The standard

TABLE IV.
The mode of the absorption of methylene blue.

Number of Experiment		I		II		III	
Time	Mode of Injection	Subcut	Intraax.	Subcut	Intraax.	Subcut	Intraax.
30'		(—)	0.0378%	(—)	(—)	(—)	0.0154%
1 h		(—)	0.0345%	(—)	0.0093%	(—)	0.0082%
2 h		(—)	0.0555%	(—)	0.0074%	(—)	0.0060%
3 h		(—)	0.0350%	(—)	0.0060%	(—)	0.0055%
Total		(—)	0.1628%	(—)	0.0227%	(—)	0.0351%

solution was made by me also. A 0.0001% solution of this dye was found in the blood. If the blood contains this dye, the serum appears red. This can be confirmed by adding a few drops of hydrochlorid acid. If the serum contains this dye, it become blue. This colloid dye seems to be absorbed by way of the lymphatics only and passes into the blood vessels. If it is injected venously, it stays in the blood longer, and it is not excreted in the urine. Five cubic centimeters of blood was procured, and its serum was observed to estimate the amount of congored. This dye, when injected intraaxillarily, was absorbed more than injected subcutaneously (Table V).

TABLE V.
The mode of the absorption of congored.

Time	Subcut	Intraax
12'		0.0015%
25'		0.0025%
35'	0.0007%	0.0020%
40'	0.0005%	
1 h	0.0003%	0.0020%
2 h	(—)	
3 h	(—)	0.0020%

Last, 2 cc of a 2% solution of indigocarmin was observed after intraaxillary and subcutaneous injection of man. The standard solution was made by me also. A 0.0001% solution of this dye was found in the urine. This dye, when injected, is excreted in the urine. If the urine contains this dye, it appears blue. The molecule of this dye is a cross between a crystalloid and a colloid and seems to be absorbed both by way of the lymphatics and blood capillaries. The urine, which was excreted 3 hours and 30 minutes after the injection, was examined to estimate this dye.

This dye, when injected into intraaxillary region, was found in the urine, but, when injected subcutaneously, it could not be found (Table VI).

TABLE VI.
The mode of the absorption of indigocarmin.

Number of Experiment		I		II	
Time	Mode of Injection	Subcut	Intraax	Subcut	Intraax
30'		(-)	(-)	(-)	(-)
1 h					0.066%
1h30'		(-)	0.280%	(-)	
2 h					(±)
2h30'		(-)	0.175%	(-)	
3 h					(-)
3h30'		(±)	(-)	(-)	

DISCUSSION.

I must first answer the question of how B.C.G., inoculated intra-axillarily will be absorbed. According to my experiments, B.C.G. could be shown in the blood obtained from V. jugularis 18 minutes after injection. It seems to be absorbed directly by blood capillaries as well as by the lymphatic capillaries.

I must, secondly, answer the question of how rapidly B.C.G., inoculated intraaxillarily, will be absorbed. B.C.G. was able to be isolated in the heart, lung, kidney, and spleen of rats 10 minutes after the intra-axillary vaccination.

I must, thirdly, answer the question of where B.C.G. will stay. According to many experiments, it stays in the lung, kidney and spleen. It shows according to my experiments that B.C.G. and other dyes, when inoculated into the intraaxillary region, were and more rapidly absorbed than when they were inoculated subcutaneously and intracutaneously. We can easily suppose that the intraaxillary region has more well-developed lymph-vessels than subcutaneous and intracutaneous sites. In the intraaxillary region there is a large hole which has a spongy construction, so that when substance is injected into the intraaxillary region, it spreads throughout the entire hole and is absorbed easily. Therefore, there is no doubt that B.C.G. vaccinated intraaxillarily, and absorbed by the blood capillaries and lymph capillaries, is absorbed so easily and readily, that the local lesion does not appear.

SUMMARY.

I can resolve the question of why the local lesion does not occur when B.C.G. is vaccinated intraaxillarily. The reasons are the following:

1. B.C.G., which is inoculated into the intraaxillary region, seems to be absorbed by way of the blood capillaries as well as lymphatic capillaries.

2. In the intraaxillary region there is a large hole which has a spongy construction and well-developed lymph vessels and blood vessels.

3. The substances (bacteria, dyes) which are inoculated into the intraaxillary region, are absorbed so readily, rapidly, and easily that the local lesion does not occur.

References.

- 1) Takahashi, Igaku to Seibutugaku., 1943, **4**, 7.
  - 2) Kanno, Shimano, Nihon-Igaku, 1944 (No. 3393), II.
  - 3) Rosenthal, Amer. Rev. Tbk., 1939, **39**, 128.
  - 4) Katakura, Oka, Tamura and Ishikawa, Tohoku-Igaku-zasshi, 1939, 71.
  - 5) Oka, Katakura, Nihon-rinsho-kekkaku, 1940, **1**, 829.
  - 6) Ellenberger Baum, Handbuch d. Verg. Anatomie d. Haustiere. 16, Aufl. Berlin, Springer, 1926.
  - 7) Ellenberger Baum, Anatomie d. Hundes, Berlin, Parey, 1891.
  - 8) Wallgren, Jour. of Amer. Med. Association 1928, 1876.
  - 9) Katsura, The Tohoku Journ. Exper. Med., Vol. IV, No. 1, April, 1928, **4**, 58.
  - 10) Katsura, The Tohoku Jour. Exper. Med., 1924, **5**, 294.
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Fig. 1. The mode of the absorption of phenolsulphthalein.

Fig. 2. The mode of the absorption of phenolsulphthalein.