Contributions of Shibasaburo Kitasato to the Improvement of Smallpox Vaccine in Japan

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After a seven-year stay in Robert Koch's laboratory Kitasato returned to Japan in 1892, and became the Adviser in 1893 and the Director in 1902 of the Smallpox Vaccine Institute in Tokyo.

Kitasato contributed to the improvement of smallpox vaccine in Japan by his achievement in bacteriology. His contribution will be briefly introduced in this paper.

I. Introduction of the tuberculin test for quarantine of cows used for smallpox vaccine production

As is well known, Kitasato was the most trusted coworker of Koch in the study of tuberculin, and he himself was well acqainted with tuberculin and its applications. Therefore, he introduced the tuberculin thermal test for calves used for smallpox vaccine production in Japan (1). Use of tuberculin test could complement clinical diagnosis of tuberculosis of living cows under preliminary quarantine.

II. Initiation of the use of phenol to kill bacteria in smallpox vaccine

On May 14 1896, a grand centenary of Jenner's discovery of vaccination was held at Ueno Park in Tokyo with more than 6,000 persons in attendance (2).

On this occasion, Kitasato dedicated a paper to Jenner (3). This paper reported his experiment on the sterilization of bacteria contaminating glycerol smallpox vaccine and concluded that phenol was

the most effective chemical for the purpose.

Kitasato's original report is briefly abstracted below: *Heating* of smallpox vaccines to a temperature of 60° C which is necessary to kill bacteria is simultaneously destroyed the potency of vaccines.

Filtration of smallpox vaccines through Chamberland-filters resulted in bacteria-free filtrates, but their potency was simultaneously lost. *Chemicals* such as thymol, salicylic acid and boric acid were added to smallpox vaccines in various concentrations. The solubility of these chemicals was so small that vaccines were highly diluted to reduce their potency. After surveying many other chemicals, phenol was found to be the most suitable one for the purpose.

(1) Sterilization test of phenol

Water suspensions of *Strept. pyogenes; Staphyl. albus, aureus* and *citreus; Pseud. aeruginosa;* bouillon broth cultures of *Ery. rhusiopathiae* and *Past. multocida;* and heart blood of a guinea pig which died just before from artificial inoculation of *Bac. anthracis* were mixed in equal volumes with normal glycerol vaccine. To these samples phenol was added at a concentration of 0.66–0.80% and they were stored in a cold room. When examined after 5–7 days all specimens were found to be sterile, but spores of *Bac. subtilis* were sometimes present.

(2) Influence on potency

1) Test by inoculation into calves (3–3.5 months old)

phenol(%) / storage(days)	0.50	0.66	0.80
6	·	·**	—**
27	—	—	·
40	·	·	—
64	—	—	·
72	—*	—	·

— : Takes were not influenced.
* : A very small number of living bacteria was found.
** : Living bacteria were not found.
· : Potency test was not performed.

2) Test by human inoculation

Phenol (%)	Storage (days)	Initial of vaccinees	On one arm: Vaccine (phenol added)	On the other arm: Vaccine (ordinary)
0.50	40	S (♂)	5*	5
	40	S (♂)	5	5
	70	M (♂)	5	5
	70	Y (♂)	5	5
	97	K (♂)	4	5
	97	S (♂)	4	5
	97	H (♀)	4	4
0.66	86	K (♀)	4	3
	86	K (♂)	2	4
	104	T (♀)	2	1
	104	O (♂)	1	2
	105	I (♂)	2	3
0.80	10	I (♀)	4	5
	10	M (♂)	2	4
	10	T (♀)	5	5
	30	T (♀)	5	3
	30	O (♀)	4	5
	30	T (♂)	4	4

* Number of positive takes among five inoculations

Conclusions

(1) Bacteria-free smallpox vaccines were prepared by the addition of phenol to ordinary glycerol vaccine at a concentration of 0.66–0.80 % and by keeping them in a cold room for seven days.

(2) After one hundred days of storage, phenol-added vaccines maintained sufficient potency for practical use.

(3) Bacteria-free smallpox vaccines caused no unpleasant, severe inflammations in the vaccinee, which were often seen with the use of ordinary vaccines.

III. Development of a procedure for maintenance of the potency of seed vaccine virus

At that time, serial passage of vaccine virus in calves were considered almost impossible over 3-4 generations. Kitasato suspected that bacteria contaminating smallpox vaccine made serial passages of vaccine virus difficult (3).

Shinkichi Umeno, one of the assistants of Kitasato, studied serial passages of vaccine virus using bacteria-free seed virus and concluded that vaccine virus could be serially passaged for many generations without diminishing its potency, when the following prerequisites are fulfilled (4):

(1) The area of the skin to be inoculated must be limited proportionally to the body weight of the calves.
(2) The seed vaccine virus used for inoculation of the calves must be diluted to a certain concentration.
(3) Bacteria-free seed vaccine virus must be used for the inoculation.

Discussions

Use of phenol was gradually adopted for the production of smallpox vaccine, not only in Japan but also in various vaccine institutes in foreign countries. Such authorities as H.A. Gins in Berlin and E. Paschen in Hamburg introduced the phenol procedure as a method conventionally used in Japan, " . . ,wie es in Japan geschiet, . . ."(5). Even in modern times, phenol has been recommended by WHO to kill bacteria contaminating vaccinial dermal pulp (6).

An authentic procedure for maintenance of the potency of seed vaccine made the provision of necessary amount of seed virus possible and resulted in 1902 the closing of the Osaka Smallpox Vaccine Institute, one of the two governmental institutes.

The smallpox vaccine produced under the strict surveillance of Kitasato was of high quality and was often exported to other Asian countories on request.

In the Russo-Japanese War, carried out where smallpox was then

endemic, only three hundreds and sixty two cases and thirty five deaths of smallpox per one million Japanese soldiers were reported (7).

W. Osler introduced the statistics from Japan, which showed strikingly the efficacy of vaccination in Japan (7), (8).

References

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