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EFFECT OF STORAGE AT 37°C ON IMMUNIZING POWER OF DRIED BCG VACCINE *

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SYNOPSIS

Experiments were carried out to determine the effect of storage at 37°C on the immunizing power of dried BCG vaccine. Vaccines were prepared with sodium glutamate and with sucrose, and were preserved for 6 months at 5°C and at 37°C. The preserved vaccines were then injected into four groups of 12 tuberculin-negative guinea-pigs; a fifth group of 12 non-vaccinated animals acted as controls. Six weeks after inoculation, all surviving animals were given a challenge dose of virulent human tubercle bacilli. After a further six weeks the guinea-pigs were killed, and an examination was made of the macroscopic and histological changes produced in the lymph-nodes and viscera.

No significant difference in the tuberculous changes induced by the challenge infection was observed among three of the groups of vaccinated animals—namely, the two inoculated with the sodium glutamate vaccines and the one inoculated with the sucrose vaccine preserved at 5°C. The fourth vaccinated group showed greater changes than the other three, indicating that the immunizing power of the sucrose vaccine had decreased markedly during storage for 6 months at 37°C. The non-vaccinated control group, however, showed the most conspicuous changes of all the five groups.

Introduction

The production of a dried BCG vaccine which can withstand storage at a temperature of over 30°C is a matter of considerable importance from the point of view of the carrying-out of successful vaccination campaigns in places where facilities for preserving the vaccine at a low temperature are not easily available. As reported by Edwards, Palmer & Magnus,[1] when the storage temperature rises above 30°C, liquid vaccine loses its

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[1] Edwards, L. B., Palmer, C. E. & Magnus, K. (1953) *BCG vaccination: studies by the WHO Tuberculosis Research Office, Copenhagen*, Geneva, p. 65 (*World Health Organization: Monograph Series*, No. 12)

allergenic potency very rapidly; and even with the dried vaccine so far employed some decrease in antigenicity within a few months is unavoidable.[2] Since BCG vaccination is practised in many places where the storage temperature does rise above 30°C, it is highly desirable that the stability of the present mass-produced dried vaccine be enhanced to such a degree that it can, if necessary, be safely preserved at room temperature for several months, even in tropical districts.

Cho & Obayashi [3] have recently studied the effect of various adjuvants on the preservability of dried BCG vaccine at 37°C. Their results showed that, when sodium glutamate was used as adjuvant, the dried vaccine could withstand a temperature of 37°C for 6-8 months without appreciable loss of viability. In the present paper, some experiments carried out on guinea-pigs to determine the immunizing power of vaccines prepared with sodium glutamate and with sucrose after preservation for 6 months at 37°C are described.

Method

Preparation of the vaccines

Using 9-day-old second-generation Sauton (S_2) cultures of BCG, a 20 mg/ml bacillary suspension was prepared, was diluted to 10 mg/ml with a 1% solution of sodium glutamate or of sucrose, and was dispensed into ampoules in 0.5-ml quantities. Lyophilization was carried out by the method employed in routine production, using a chamber-type desiccator.[4] The resultant vaccines were preserved for 6 months either at 5°C or at 37°C and were then inoculated into guinea-pigs.

Culture tests were carried out on the two kinds of vaccine immediately after drying and after storage.

Inoculation of vaccines

Sixty tuberculin-negative guinea-pigs weighing approximately 280 g each were divided into five groups, each consisting of 12 animals. These animals were inoculated with the different vaccines as follows:

Animal group	*Type of vaccine*	*Storage temperature*
1	1% sodium glutamate	5°C
2	1% sodium glutamate	37°C
3	1% sucrose	5°C
4	1% sucrose	37°C
5	non-vaccinated controls	

[2] Obayashi, Y. (1955) *Dried BCG vaccine*, Geneva, p. 137 (*World Health Organization: Monograph Series*, No. 28)

[3] See article on page 657 of this number of the *Bulletin*.

[4] Obayashi, Y. (1955) *Dried BCG vaccine*, Geneva, p. 23 (*World Health Organization: Monograph Series*, No. 28)

The dried vaccines were reconstituted to a concentration of 0.2 mg/ml with sterilized physiological saline, and 0.5-ml (0.1-mg) quantities were then injected subcutaneously into the left side of the abdomen of the animals.

The degree of allergy induced in each animal was determined every two weeks by means of an injection of 1 mg of Old Tuberculin (OT).

Challenge inoculation

Six weeks after inoculation with the dried vaccines, all the surviving immunized animals and the non-vaccinated controls (53 in all) were inoculated subcutaneously, in the right side of the abdomen, with a 0.01-mg dose of a 3-week-old Sauton culture of virulent human-type tubercle bacilli (strain KH_1). This dose produced 2.52×10^7 viable units (inoculum, 10^{-6} mg) after 4 weeks' incubation on Ogawa's egg medium.

Autopsy

Six weeks after the challenge inoculation, the 53 surviving animals were killed and subjected to autopsy, the macroscopic and histological changes produced in the lymph-nodes and viscera being assessed. The criteria of macroscopic changes are as follows: The degree of enlargement of lymph-nodes is designated by plus signs: + for rice-grain size; ++ for soya-bean size; +++ for green-pea size; and ++++ for horse-bean size or larger. The number of tubercles in an organ is designated by the same signs: + for an organ in which tubercles are found with some difficulty; ++ for one in which tubercles are found easily but do not number more than 10; +++ for one containing numerous tubercles; and ++++ for one in which the tubercles are exceedingly numerous.

In addition to the foregoing examinations, quantitative culture tests were carried out on the livers of the animals, in order to determine the degree of multiplication of the tubercle bacilli used in the challenge inoculations. A portion of the liver weighing approximately 0.3 g was excised and ground thoroughly in a porcelain mortar. The ground material was then diluted with a 1% solution of sodium hydroxide and inoculated on five slants of Ogawa's medium in 100-mg and 10-mg quantities. At the end of four weeks' incubation at 37°C, the number of colonies was counted.

Results

The results of the culture tests made on the two types of vaccine are shown in Table I.

The number of viable units was found to be slightly greater in the sodium glutamate vaccine than in the sucrose vaccine, both immediately after drying and after preservation for 6 months at 5°C, and very much greater after preservation for 6 months at 37°C.

TABLE I. CHANGES IN VIABILITY DURING PRESERVATION

Type of vaccine	Viable units in 1 mg of bacilli		
	immediately after production	after 6 months at 5°C	after 6 months at 37°C
1% sodium glutamate	3.8×10^7	4.13×10^7	4.7×10^6
1% sucrose	1.8×10^7	1.27×10^7	0.7×10^4

TABLE II. CHANGES IN BODY-WEIGHT OF ANIMALS *

Group no. \ Weeks after vaccination	0	2	4	6 (challenge infection)	7	8	10	12
1	283	324	367	393	413	434	458	469
2	284	323	362	404	423	438	460	463
3	282	336	374	390	425	428	452	467
4	285	347	360	424	458	450	477	477
5 (control)	280	334	366	398	426	426	452	450

* Indicated by average body-weight (g) of respective groups

TABLE III. CHANGES IN TUBERCULIN REACTION

Group no. \ Weeks after vaccination	0	2	4	6 (challenge infection)	7	8	10	12
1	0/0	16/19	19/22	19/20	24/24	23/23	27/27	29/29
2	0/0	15/17	18/19	19/20	23/23	24/24	28/28	29/29
3	0/0	15/17	22/23	20/20	24/24	23/23	26/26	28/28
4	0/0	10/12	18/21	20/20	25/25	23/23	29/29	30/30
5 (control)	0/0	0/0	0/0	0/0	17/17	20/20	29/29	30/30

The reactions were read 24 hours after the intracutaneous injection of 1 mg of OT.

The upper and lower figures indicate the average diameters (mm) of induration and erythema, respectively.

The changes in the average body-weight of the animals in each of the five groups are shown in Table II. As can be seen, the weights did not fluctuate but increased steadily during the experimental period.

The results of the tuberculin tests after inoculation of the vaccine and after the challenge inoculation are given in Table III. At the first test (i.e., two weeks after vaccination), the reactions were found to be a little more intense in groups 1, 2, and 3 than in group 4, but this difference was no longer perceptible in the subsequent tests. As to the reactions after the challenge inoculation, they were observed to be stronger in the vaccinated groups than in the control group up to the second week, but after that the difference was insignificant.

The results of the macroscopic findings at autopsy are shown in Table IV and Fig. 1. There was very little difference in the degree of tuberculous involvement between groups 1, 2, and 3, but group 4 (inoculated with sucrose vaccine stored at 37°C) showed more marked changes, both in the lymph-nodes and in the viscera. The non-vaccinated control group, however, revealed the most conspicuous changes of all the five groups.

The same trend was observed in the histological findings. In the control group the ulcer at the site of inoculation was large and the tuberculous infiltration penetrated deep into the subcutaneous tissue, and sometimes even into the muscle. Tubercles, often showing necrosis at the centre, were found in abundance both in the central part and on the margin of the ulcer. In groups 1, 2, and 3 the ulcer was small, and the tuberculous infiltration was not so widespread as in the control group. Tubercles were not found in the deep part of the ulcer and tubercles with necrotic centres were rarely observed. In group 4 the size of the ulcer varied: in some animals it was as large as in the control group; in others, it was smaller. The extent of tuberculous infiltration in the central part of the ulcer in group 4 was similar to that in the control group.

The swelling of the draining lymph-node near the site of inoculation was large both in group 4 and in the control group. In these groups, the lymph-node contained a great deal of softened necrotic tissue and conglomerated tubercles. In the other groups, the swelling of the lymph-node was smaller, and although some necrosis and tubercles were found in all cases, a considerable area of normal lymphoid tissue still remained.

In the control group, large tubercles were found in abundance in the histological specimens of viscera, such as the liver, spleen, and lung, and of the portal lymph-node; necrosis was usually observed at the centre of the tubercles in the spleen and in the portal lymph-node. The proliferation of bile ducts—a phenomenon accompanying the development of tuberculous infection in guinea-pigs—was observed only in the control group.

TABLE IV. MACROSCOPIC FINDINGS AT AUTOPSY OF GUINEA-PIGS 6 WEEKS AFTER INFECTION WITH VIRULENT HUMAN TUBERCLE BACILLI

Group no.	Animal no.	Local lesion	Lymph-nodes													Viscera				
			L knee fold	R knee fold	L inguinal	R inguinal	L axillary	R axillary	L cervical	R cervical	retro-sternal	retro-peritoneal	portal	bronchial	lung	liver	spleen	kidney	weight of spleen (grams)
1	1	U	(++)	(++)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.1
	2	S	(+)	(+++)	—	—	—	—	++	+	—	+	—	—	—	—	+	—	0.8
	3	U	(+)	(+++)	—	—	—	—	—	—	—	(++)	(+)	—	+	—	+	—	1.0
	4	U	(+)	(++)	—	—	—	—	—	—	—	++	++	—	—	—	+	—	0.8
	5	U	+	(++)	—	—	—	—	—	—	—	+	+	—	—	—	+	—	0.85
	6	S	+	(++)	—	—	—	—	—	—	—	+	+	—	—	—	—	—	0.9
	7	U	+	(+++)	—	(+)	—	—	—	—	—	+	—	—	—	—	—	—	0.85
	8	U	+	+++	—	+	—	—	—	—	—	+	+	+	—	—	+	—	0.9
	10	U	—	(+++)	—	—	—	—	—	—	—	—	+	—	—	—	—	—	0.7
	11	U	+	++	—	+	—	—	—	—	—	+	—	—	—	—	—	—	0.8
	12	U	(++)	(++++)	—	—	—	—	—	—	(++)	+	(++)	—	—	—	+	—	1.4
2	14	U	(+)	(++++)	—	+	—	—	—	—	—	++	(+++)	—	—	—	+	—	1.0
	15	U	—	(++)	—	—	—	—	—	—	—	+	—	—	—	—	—	—	1.05
	16	U	—	(+++)	—	—	—	—	—	—	—	—	+	—	—	—	—	—	0.5
	17	U	+	(+++)	—	—	—	—	—	—	—	(+)	(+)	+	+	—	+	—	0.85
	18	U	(+)	(++)	—	+	—	—	—	—	—	—	—	—	—	—	—	—	1.0
	19	U	+	(+++)	—	—	—	—	—	—	—	—	+	—	—	—	—	—	0.85
	21	U	++	(+++)	—	—	—	—	—	—	—	—	(+)	—	—	—	—	—	0.9
	22	U	(+++)	(+++)	—	+	—	+	—	—	—	++	(++)	+	—	—	+	—	1.3
	23	U	(++)	(+++)	—	(+)	—	—	—	—	—	+	(++)	+	+	—	+	—	1.1
	24	U	—	(+++)	—	(+)	—	—	—	—	—	(+++)	(+++)	—	—	—	—	—	1.1
3	25	S	—	(+++)	—	+	—	—	—	—	—	+	+	—	—	—	—	—	0.8
	26	S	+	(++)	—	—	—	—	—	—	—	+	—	—	—	—	—	—	0.5
	27	U	+	(+++)	—	—	—	—	—	—	—	+	(++)	—	—	—	—	—	0.8
	28	U	—	(+++)	—	(+)	—	—	—	—	—	(++)	—	—	—	—	—	—	0.85
	29	U	(++)	(+++)	—	—	—	—	—	—	—	+	+	—	—	—	—	—	0.75
	31	U	(+++)	(+++)	—	+	—	—	—	+	—	—	(+)	+	—	—	+	—	0.7
	32	U	+	++	—	—	—	—	—	—	—	—	+	—	—	—	—	—	1.3
	33	U	(++)	(++)	—	+	—	—	—	—	—	(+)	+	—	—	—	—	—	0.8
	34	U	++	(+++)	—	—	—	—	—	—	—	+	(++)	—	—	—	—	—	0.75
	35	U	+	(+++)	—	(+)	—	—	—	—	—	+	(++)	—	—	—	—	—	0.75
	36	U	+	(+++)	—	—	—	—	—	—	—	+	(+)	—	—	—	+	—	0.7
4	37	U	(+++)	(++++)	—	(++)	—	—	—	—	—	(++)	+	—	—	—	—	—	0.6
	39	U	(+++)	(++++)	—	(+)	—	—	—	—	+	(+)	(+++)	(++)	++	++	+++	—	1.3
	40	U	(++)	(++++)	—	(+)	—	—	—	(+)	—	+	(++)	+	+	+	++	—	1.05
	41	U	(++)	(+++)	—	+	—	+	—	—	—	(+++)	(++)	—	—	—	—	—	0.85
	42	U	(++)	(++++)	—	(++)	—	—	—	—	(+)	(++)	(+++)	+	+	++	+++	—	1.5
	44	U	(+)	(++++)	—	—	—	—	—	—	—	+	(++)	—	—	—	+	—	0.85
	45	U	(+)	(++)	—	+	—	—	—	—	—	—	—	—	—	—	—	—	1.05
	46	U	++	(+++)	—	+	—	+	—	—	—	(+)	(++)	+	++	++	+++	—	1.6
	47	U	—	(++++)	—	—	—	—	—	—	—	+	(++)	—	—	—	+	—	1.25
	48	U	+	(++++)	—	+	—	—	—	—	—	+	(++)	+	+	+	++	—	1.1
5	49	U	(++)	(++++)	—	+	—	+	—	(+)	—	(++)	(++)	+	+	++	++	—	1.0
	50	U	(++)	(++++)	—	(++)	+	—	(++)	—	(++)	++	(+++)	+++	+++	+++	++++	—	1.9
	51	U	(++)	(+++)	—	(+)	—	—	—	—	+	(++)	(++)	+	++	+++	++++	—	1.4
	52	A	(++)	(++++)	—	+	—	+	—	—	—	(++)	(+++)	+	+	+++	+++	—	0.95
	53	U	(+)	(+++)	—	(+)	—	—	—	—	+	++	(++)	+	+	+++	++++	—	1.5
	54	U	(+++)	(+++)	—	(++)	—	—	+	—	+	++	++	+	++	++	+++	—	1.1
	55	U	+	++++	—	+	—	—	—	—	+	(+++)	(++)	+	+	++	+++	—	1.2
	56	U	(+++)	(+++)	—	+	—	+	(+)	—	+	++	(+++)	++	+++	+++	++++	—	2.8
	58	U	(+++)	(++++)	—	(+)	+	—	+	—	—	(+++)	(++)	(+++)	++	+++	+++	—	1.2
	59	U	++	(+++)	—	+	—	—	—	—	+	+	(+++)	+	+	++	+++	—	1.9
	60	U	(++)	(++++)	—	+	—	—	—	—	+	(++)	(+++)	++	++	++	++++	—	1.4

L = left; R = right

Local lesions:
A = abscess
S = scab
U = ulcer

Lymph-nodes:
+ rice-grain size
++ soya-bean size
+++ green-pea size
++++ horse-bean size or larger

Viscera:
+ tubercles found with some difficulty
++ tubercles found easily but not exceeding 10
+++ numerous tubercles
++++ very numerous tubercles

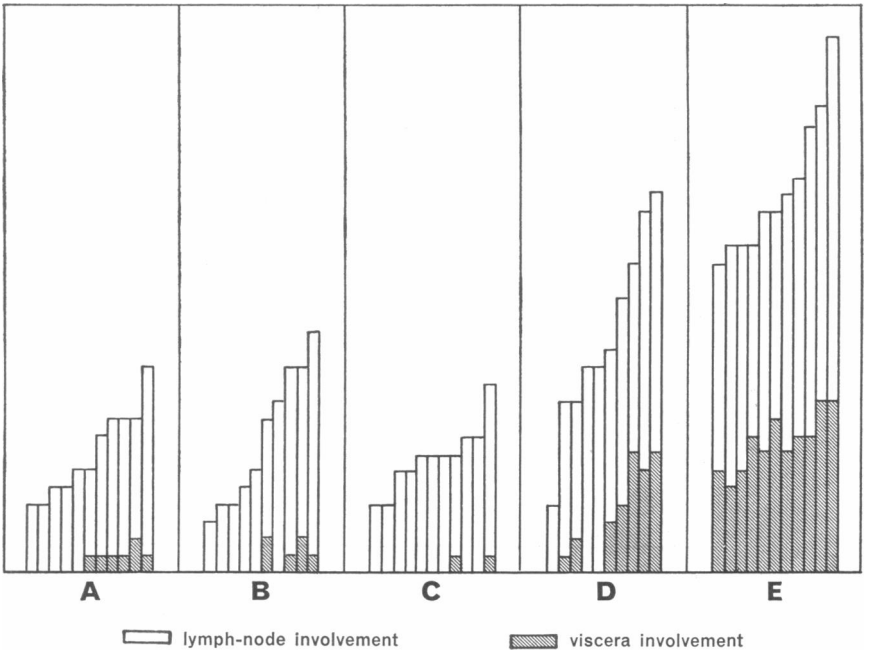
Plus signs in parentheses indicate caseation

The tuberculous changes in the viscera of group 4 were similar to those observed in the control group, except that, in some animals, necrosis was not found in the tubercles of the spleen.

In groups 1, 2, and 3, tubercles in the liver, spleen, and lung were very scarce and small. Necrosis at the centre of the tubercles in the spleen was rarely observed in these groups. In groups 2 and 3, the changes in the portal lymph-node were somewhat more marked than the changes in the other viscera; conglomerated tubercles, with necrosis at the centre and small encapsulated caseous foci surrounding them, were observed, but the changes were slighter than those in the control group. In some animals of group 1, there was no tuberculous change in the histological specimens of the portal lymph-node; in others, the changes were similar to those in groups 2 and 3.

The results of the quantitative culture tests carried out on the excised livers are shown in Table V. Again, the same trend was observed as in the

FIG. 1. DEGREE OF INVOLVEMENT OF LYMPH-NODES AND VISCERA IN VACCINATED AND NON-VACCINATED GUINEA-PIGS 6 WEEKS AFTER INFECTION WITH VIRULENT HUMAN TUBERCLE BACILLI



A = group 1 : inoculated with 1 % sodium glutamate vaccine preserved for 6 months at 5°C
B = group 2 : inoculated with 1 % sodium glutamate vaccine preserved for 6 months at 37°C
C = group 3 : inoculated with 1 % sucrose vaccine preserved for 6 months at 5°C
D = group 4 : inoculated with 1 % sucrose vaccine preserved for 6 months at 37°C
E = group 5 : non-vaccinated controls

The height of the columns indicates the degree of involvement of lymph-nodes and viscera expressed by the total number of plus signs (for explanation of signs, see text, page 673).

TABLE V. RESULTS OF CULTURE TESTS ON EXCISED LIVERS

Group no.	Animal no.	Viable units from 10 mg of liver	Group no.	Animal no.	Viable units from 10 mg of liver	Group no.	Animal no.	Viable units from 10 mg of liver	Group no.	Animal no.	Viable units from 10 mg of liver	Group no.	Animal no.	Viable units from 10 mg of liver
	1	0		14	0.7		25	3.0		37	0		49	5.4
	2	0		15	0		26	0		39	64.0		50	81.4
	3	0		16	0		27	0		40	5.4		51	83.4
	4	0		17	0		28	0		41	0.7		52	12.0
1	5	0	2	18	0.4	3	29	0	4	42	3.4	5	53	63.0
	6	0.4		19	0		31	0		44	0		54	6.4
	7	0		21	0		32	0		45	0		55	17.0
	8	0		22	0		33	0		46	17.0		56	70.0
	10	0		23	0		34	0		47	0		58	13.0
	11	0		24	0		35	0		48	10.0		59	39.0
	12	0					36	0					60	29.4

macroscopic and histological findings, the number of viable units of tubercle bacilli produced being greatest in the control group, somewhat smaller in group 4, and much smaller in groups 1, 2, and 3.

Discussion

The experiment described in this paper has revealed that dried sodium glutamate vaccine retains its immunizing power far more effectively during preservation at 37°C than does dried sucrose vaccine. While the immunizing power of the latter vaccine decreased markedly during 6 months' storage at 37°C, no significant difference was detected in the tuberculous changes induced by the challenge infection in the animals immunized with dried sodium glutamate vaccines preserved at 5°C and at 37°C for 6 months, all of the animals showing far slighter changes than either the non-vaccinated controls or the animals which had been immunized with dried sucrose vaccine preserved at 37°C for 6 months. This, however, may not necessarily mean that the antigenicity of dried sodium glutamate vaccine is completely unaffected by preservation at a high temperature, since the culture test showed that some decrease in viability occurred during preservation at 37°C. But it can be stated that, with the sodium glutamate vaccine, the decrease in antigenic potency resulting from such preservation is so slight that it is scarcely detectable by ordinary immunizing experiments on guinea-pigs.

Since the immunizing power of BCG vaccine is considered to depend mainly on the activities of living bacilli, it would seem natural to try to estimate the immunizing power of a vaccine indirectly by determining the number of living bacilli present. However, an exact comparison, by the culture test, of the numbers of living bacilli in two given lots of BCG vaccine is possible only if the two lots have a similar degree of bacillary aggregation—a condition practically never realized. Particularly when two kinds of dried vaccine have been prepared with different adjuvants, as in the present experiment, is the cultivation test alone insufficient for comparing preservability. It is for this reason that the present experiment on the immunization of guinea-pigs was carried out.

As has already been pointed out, our immunizing experiment showed that the dried sodium glutamate vaccine retained its antigenicity during preservation at 37°C far better than did the dried sucrose vaccine at present in routine use—a finding which agreed well with the results obtained in culture tests.

As to the efficacy of the sodium glutamate vaccine in human subjects, an experiment is now in progress and it is hoped that the results will be published in the near future.

RÉSUMÉ

Dans leurs précédentes expériences, les auteurs avaient constaté que le vaccin BCG desséché préparé avec du glutamate de sodium peut garder une viabilité suffisante pendant 6 à 8 mois de conservation à 37°C. Afin de déterminer si le vaccin ainsi conservé garde également son activité allergène, ils l'ont inoculé à des cobayes. Pour comparaison, ils ont inoculé à d'autres cobayes le vaccin BCG desséché préparé avec du saccharose, qui est utilisé au Japon.

Soixante cobayes ont été répartis en 5 groupes égaux. Le groupe 1 a reçu du vaccin au glutamate de sodium, conservé à 5°C; le groupe 2, le même vaccin conservé à 37°C; le groupe 3, le vaccin au saccharose conservé à 5°C; le groupe 4, le vaccin au saccharose conservé à 37°C. Le groupe 5 servait de témoin. Au bout de 6 semaines, on a administré à tous les cobayes survivants une dose de bacilles tuberculeux virulents. Six semaines plus tard, on les a sacrifiés.

L'examen des ganglions lymphatiques, la recherche des tubercules dans les viscères et la culture des broyats de foie sur milieu d'Ogawa ont donné des résultats concordants. Les lésions les plus fréquentes et les plus graves, les bacilles tuberculeux les plus nombreux, se trouvaient dans le groupe témoin et dans le groupe 4 (vaccin au saccharose conservé à 37°C). En revanche, le vaccin au glutamate de sodium conservé à 37°C s'était montré aussi efficace que le même vaccin conservé à 5°C. Ainsi, la conservation à 37°C pendant plusieurs mois affaiblit nettement l'activité allergène du vaccin BCG au saccharose, mais ne diminue pas de façon sensible celle du vaccin BCG au glutamate.

Les auteurs étudient maintenant l'efficacité de ce dernier vaccin chez l'homme.