

EFFECTS OF CELLULAR CONSTITUENTS OF MYCOBACTERIA ON THE RESISTANCE OF MICE TO HETEROLOGOUS INFECTIONS

I. PROTECTIVE EFFECTS*

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Injection of the somatic constituents of Gram-negative bacilli into mice is known to alter profoundly the resistance of these animals to heterologous bacterial infections. Depending upon the dose of material injected and the time of injection, the outcome of this treatment can be either an increase or a decrease in resistance to infection. Both types of effects have been brought about by injecting intraperitoneally whole bacterial bodies, crude extracts of endotoxins, preparations of cell walls, or purified fractions consisting essentially of lipopolysaccharides. The biphasic effect on resistance has been observed by using for challenge infection either Gram-negative pathogens such as virulent *Escherichia coli* or *Salmonella typhosa* (1, 2), and even more strikingly, virulent strains of *Staphylococcus aureus* and *Mycobacterium tuberculosis* (3).

We have now established that some cellular constituents of mycobacteria also are capable of exerting a dual influence on heterologous experimental infections in mice. It will be shown in the present paper that injection of mycobacteria or extracts of them can increase the resistance of mice to experimental infection with *Staph. aureus* or with *Mycobacterium fortuitum*. In contrast, it will be shown in the following paper that a sudden and profound depression of resistance to infection with these bacteria can be brought about by injecting the same materials under other conditions (4).

The information available does not warrant any statement with regard to the chemical nature of the cellular components of mycobacteria responsible for the phenomena to be described in this and the following paper. Nor is there much knowledge concerning the tissue responses set in motion by these materials, beyond the fact that changes in the activity of the reticulo-endothelial system appear to be involved (5). For these reasons, we have deemed it wiser to postpone a general discussion of the mechanisms through which mycobacterial constituents alter resistance to infection until more chemical

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and histopathological data have been accumulated. It seems worth pointing out at this time, however, that the results obtained in the present study probably have a direct bearing on certain puzzling phenomena recently reported by other investigators.

There is now extensive experimental evidence that the presence of one type of infection can alter profoundly the course of another superimposed infection with an unrelated etiological agent. While a complete review of this topic cannot be presented here, we shall single out the fact—especially relevant to our findings—that some of the most striking examples of non-specific resistance to infection have been observed in investigations with tubercle bacilli (6-10). It has been established, for example, that preexisting tuberculosis markedly influences the course of infection with *Brucella abortus* in guinea pigs, and that, on the other hand, mice infected with *Brucella abortus* exhibit heightened resistance to experimental tuberculosis. It is also of very great interest that the monocytes obtained from rabbits vaccinated either with BCG or with an appropriate *Brucella* antigen display in tissue culture systems a much greater resistance to the cytotoxic effect of both tubercle bacilli and *Brucella melitensis* than do the cells derived from normal animals (11). Taken together, these facts suggest that injection of certain components of mycobacteria (and of other bacteria) can result in a type of response that alters the susceptibility of tissue cells to infection with unrelated organisms both *in vivo* and *in vitro*.

Materials and Methods

The attenuated culture of *Mycobacterium tuberculosis* BCG used in these experiments was originally obtained from Dr. J. Aronson of the Henry Phipps Institute in Philadelphia; the characteristics of this strain as observed in our laboratory have been extensively described in recent publications (12). The stock culture was maintained by monthly transfer in tween-albumin liquid medium; a 7 day old culture was used for injection into mice.

For production of large amounts of cells, the BCG culture was grown for 3 to 5 weeks in the form of surface pellicles in the medium devised for this purpose by Dubos and Middlebrook (13), or in one similar in composition to that described by Hirsch (14), but without cholesterol or charcoal. During the first phase of the study, the fully grown culture was killed with 2 per cent phenol, centrifuged, the cells washed with water and acetone, and then air-dried. The centrifuge was a completely enclosed, basket type unit, designed to avoid exposure of the operators to aerosols of bacilli. The amount of bacillary material recovered (acetone-washed and air-dried) was of the order of 3 gm. per liter. In more recent experiments, the culture was centrifuged, washed with water and acetone in the centrifuge, without preliminary killing with phenol.

As indicated in the text, a few of the experiments were carried out with bacillary material kindly provided by Dr. Gladys Hobby of Chas. Pfizer and Company, Brooklyn. This material was prepared from the strain of BCG used in our own laboratory and supplied by us to Dr. Hobby. The culture was grown in a medium of composition similar to ours, but it was violently agitated in a mechanical shaker throughout incubation. According to Dr. Hobby, the yields of bacillary material obtained by this technique were very much higher than those obtained by surface incubation, as practiced in our laboratory.

Methanol Extracts.—The methanol extracts were prepared from the acetone-washed bacterial cells by techniques similar in general outline to those described in an earlier publication (15). In some experiments, the operation was carried out by continuous Soxhlet extraction. In most instances, extraction was carried out in several separate steps by prolonged agitation of the bacilli in an excess of methanol first at 37°C., then at 55°C., and finally at 65°C. Two consecutive extractions were carried out at each temperature, usually with 1000 ml. of methanol per 50 to 100 gm. of bacteria.

At the end of each extraction, the methanol-soluble material was passed without changing the temperature through a sintered glass filter. The solvent was then distilled off in a Craig evaporator; the methanol-soluble material remaining after evaporation of the solution was emulsified in water. Finally, the water was eliminated by lyophilization in ampules which were then sealed. The yields of the three methanol-soluble fractions were approximately as follows. Soluble at 37°C., 12 per cent of original bacillary weight; soluble between 37° and 55°C., 3.5 per cent; soluble between 55° and 65°C., 1 per cent.

Mycobacterium fortuitum.—Several strains of this bacterial species have been extensively studied in our laboratory. By intravenous injection of the proper dose, all proved capable of producing in mice a fatal disease with marked localization in the kidneys. The characteristics of this disease have been recently described (16, 17). The experiments reported in the present paper were carried out with the Penso strain of *Mycobacterium fortuitum*, first isolated by Dr. G. Penso (18), and kindly supplied to us by Dr. Ruth Gordon of the Institute of Microbiology, New Brunswick, New Jersey (19).

The mouse infection tests with *Mycobacterium fortuitum* were carried out with cultures 2 to 7 days old (as indicated in the text) grown in liquid tween-albumin medium.

Large quantities of cells of this organism were produced by surface growth, using techniques similar to those employed for the production of BCG cells (see above).

Staphylococcus aureus.—All experiments were carried out with the Giorgio strain. Its cultural characteristics and behavior in mice have been described in an earlier publication (20). Cultures 12 to 18 hours old, in peptone broth, were used for infection tests.

Pertussis Vaccine.—This was a commercial preparation, distributed for human use by Lederle Laboratories, Pearl River, New York. It contained approximately 60 billion cells per ml. Its effects on heterologous bacterial infections have been reported in recent publications (10, 21).

Mice.—All experiments were carried out with albino mice of the so called Rockefeller Institute Swiss strain, produced in the animal house of The Rockefeller Institute.

The mice were obtained within a few days after weaning; *i.e.* when approximately 4 weeks old. The sexes were separated, the animals randomized in groups of 5 or 8, and placed in metal cages on metal grids. They were fed pellets and given water *ad lib.* The pellets were antibiotic-free and supplied by Rockland Farms, New City, New York (Rockland mouse diet).

Vaccination was either by the intraperitoneal route, or in some instances by the subcutaneous route. Challenge infection was by the intravenous route (caudal vein). The infective doses and the materials used for vaccination were administered in a final volume of 0.2 ml., following proper dilution with pyrogen-free physiological saline. In order to obtain uniform suspensions, the materials to be tested were homogenized in a teflon tissue grinder of the type described earlier (22).

During the last 6 months of experimentation, an epizootic occurred in the mouse colony of The Rockefeller Institute. Several of the animals became obviously ill, or died, particularly during the period immediately following the intraperitoneal injection of the test materials. Although two strains of Gram-negative bacilli were repeatedly isolated during the epizootic, the observations made were not sufficient to establish their etiological significance. In an attempt to minimize the spread of the epizootic, all animals received terra-

mycin in their drinking water immediately upon receipt from the animal house and during 1 week after vaccination. The preparation used was animal formula soluble powder, distributed by Chas. Pfizer and Company. This material was generously given to us by Dr. Gladys Hobby. It was diluted to a final concentration of 3 per cent in tap water. Use of the drug was always discontinued several days before challenge infection.

RESULTS AND COMMENTS

1. *Effect of Vaccination with Living and Killed Cells of BCG on the Survival Time of Mice Infected with Staphylococci.*—

It has been repeatedly shown, in this laboratory and elsewhere, that vaccination of mice with living BCG or with killed cells of these organisms in-

TABLE I
Comparative Effects of Living BCG Vaccine and of Killed BCG Cells on Susceptibility of Mice to Staphylococcal Infection

Materials injected (i.p.)	Interval between treatment and infection	Cumulative deaths at indicated days (d.) after infection*			
		3 d.	6 d.	12 d.	17 d.
	<i>days</i>	(out of 8 mice)			
BCG living † 0.1 ml.	13	1	5	7	8
BCG killed § 2.5 mg.	"	0	0	2	3
Saline	"	3	7	8	
		3 d.	6 d.	9 d.	12 d.
		(out of 8 mice)			
BCG living † 0.1 ml.	19	1	5	6	7
BCG killed § 2.5 mg.	"	0	1	1	2
Saline	"	5	7	8	

* 0.05 ml. of overnight culture of *Staph. aureus* (Giorgio) injected i.v.

† 8 day old culture in tween-albumin medium.

§ Bacilli washed with acetone and air-dried.

creases their resistance to experimental tuberculosis. In the course of the control experiments involved in these studies, the unexpected finding was made that vaccination with BCG also enhanced in a lasting manner the resistance of mice to staphylococcal infection. This protective effect is illustrated in the following experiments.

Mice (in groups of 8) received by the intraperitoneal route either 0.1 ml. of an 8 day old culture of BCG in tween-albumin medium, or 2.5 mg. of a suspension of phenol-killed, acetone-dried cells of BCG, prepared in our laboratory as described under Materials and Methods. The antigens were injected in a final volume of 0.2 ml., the volume being made up with pyrogen-free physiological saline. Control animals received saline only.

All animals were infected by the intravenous route with 0.05 ml. of an overnight culture of staphylococcus, half of them 13 days and the other half 19 days after receiving BCG. Deaths were recorded daily. The findings are presented in Table I.

As seen in Table I, mice vaccinated with 0.1 ml. of living BCG survived staphylococcal infection somewhat longer than did the control animals. The increase in survival time was even more striking in animals that had received 2.5 mg. of killed BCG.

The observed ability of killed BCG cells to increase resistance to staphylococcal infection was confirmed by vaccinating mice with 1 mg., 0.3 mg., or 0.1 mg. of either one of two preparations obtained by different techniques in different culture media. The cells of one preparation (BCG R.I.) had been obtained by still culture in our laboratory at The Rockefeller Institute. The cells of the other preparation (BCG Pf. No. 1) had been obtained by agitated

TABLE I A
Comparative Ability of Two Different Preparations of Killed BCG Cells to Increase Resistance to Staphylococcal Infection

BCG preparation injected (i.v.)		Cumulative deaths at indicated days (d.) after infection*		
		3 d.	7 d.	14 d.
	mg.	(out of 8 mice)		
R.I.	1	0	0	0
	0.3	0	1	2
	0.1	1	1	3
Pf. No. 1	1	0	1	2
	0.3	0	0	0
	0.1	1	2	2
Saline		3	3	5

* 0.05 ml. of overnight culture of *Staph. aureus* (Giorgio) injected i.v.

culture in the laboratories of Chas. Pfizer and Co., and thoroughly extracted with hot acetone before use. (See under Materials and Methods for further technical details.) As shown in Table I A, both preparations proved able to increase markedly the resistance of mice to staphylococcal infection.

The following experiment shows that the protective activity of the killed bacilli was retained even after heating at neutral, alkaline, or acid reactions.

Killed BCG cells, washed with acetone, were resuspended in water in a concentration of 50 mg. per ml. Aliquots of the bacillary suspension were adjusted to pH 3.0, 7.0, or 9.0 with HCl or NaOH. The suspensions were heated at 80°C. for 30 minutes at these reactions. They were then injected intraperitoneally into mice in amounts corresponding to 1.0, 0.3, or 0.1 mg. of bacillary bodies after proper dilution in a mixed phosphate buffer solution (M/15) at pH 7.0. Other groups of mice were vaccinated with unheated killed bacilli resuspended in saline. Two groups of control animals received either saline, or buffer solution.

Two weeks after vaccination, the animals were infected by the intravenous route with 0.05 ml. of *Staph. aureus*. The results are presented in Table II.

The results presented in Table II illustrate that a state of increased resistance to staphylococcal infection could be elicited by injecting into mice amounts of killed BCG cells as small as 0.1 mg.; the bacillary material re-

TABLE II
Effect of Heating on Ability of Mycobacterial Constituents to Elicit Resistance to Staphylococcal Infection

Materials injected (i.p.)	mg.	Cumulative deaths at indicated days (d.) after infection			
		5 d.	9 d.	13 d.	17 d.
		(out of 10 mice)			
BCG unheated	1	2	3	5	5
	0.3	4	6	7	7
	0.1	2	6	6	6
BCG heated pH 7.0	1	2	3	4	6
	0.3	4	8	8	8
	0.1	8	8	9	9
BCG heated pH 3.0	1	2	2	2	2
	0.3	2	3	5	5
	0.1	7	8	9	9
BCG heated pH 10.0	1	2	3	5	5
	0.3	1	4	4	5
	0.1	4	6	7	8
<i>Myc. fortuitum</i> unheated	1	2	5	6	6
	0.3	1	1	2	2
	0.1	5	9	9	9
<i>Myc. fortuitum</i> heated pH 7.0	1	3	5	6	8
	0.3	3	6	10	
	0.1	4	7	7	8
Saline		5	8	10	
Buffer		5	8	10	

tained its protective activity even after heating for 30 minutes at acid or alkaline reactions.

Further evidence of the stability of the protective material is provided by the following experiment.

A suspension of killed BCG bacilli, prepared in our laboratory as described under Materials and Methods, was adjusted to pH 10.5 with NaOH and washed at this pH four times for 12 to 18 hours at 37.5°C. Following this treatment, an aliquot of the suspension of washed

cells was adjusted to pH 3.0 with HCl and maintained at this pH at 37.5°C. for 18 hours before centrifugation. The different fractions in amounts of 1 mg. were injected by the intraperitoneal route into mice. Infection was carried out intravenously with 0.05 ml. of staphylococcus culture 2 months after vaccination (Table III).

As seen in Table III, the protective effect elicited by the BCG cells against staphylococcal infection was still fully evident 2 months after treatment. The cells retained their activity even after prolonged washing at pH 10.5. It appeared, however, that much of the activity was lost when the cells first treated at alkaline pH were subsequently washed at acid reactions (See also Table VIII).

2. *Protective Effect of Extracts of BCG against Staphylococcal Infection.*—

As shown in earlier publications from this laboratory, the resistance of mice to experimental tuberculosis could be increased by vaccinating them under the

TABLE III
Lasting Effect of BCG Cells Washed with NaOH on Susceptibility of Mice to Staphylococcal Infection

Materials injected (1 mg. i.p.)	Interval between treatment and infection	Cumulative deaths at indicated days (d.) after infection*				
		3 d.	5 d.	8 d.	12 d.	16 d.
	<i>mos</i>	(out of 8 mice)				
BCG killed	2	0	0	2	3	4
BCG washed 4 × at pH 10.5	"	1	2	3	3	4
BCG washed 4 × at pH 10.5, then once at pH 3	"	3	5	5	5	6
Saline	"	3	5	6	7	8

* 0.05 ml. *Staph. aureus* (Giorgio) injected i.v.

proper conditions with methanol extracts of BCG cells (15, 21). The following experiments illustrate that these extracts could also increase resistance to staphylococci.

Methanol extracts of BCG were prepared as follows. Twenty gm. of BCG cells were extracted twice with 1500 ml. of methanol for 18 hours at 37°C. The cell residue was separated by filtration and again extracted twice with methanol at 55°C. Finally, the cell residue from the 55°C. extraction was extracted twice at 65°C. The 55° and 65°C. methanol extracts were passed through sintered glass filters and the solvent evaporated in a Craig evaporator. The methanol-soluble material was emulsified in water and was then desiccated from the frozen state by lyophilization. The dry material was resuspended in saline in a teflon grinder for injection into mice. A methanol extract prepared at 55°C. in the laboratories of Chas. Pfizer and Company was also tested.

Mice received by the intraperitoneal route either 1 or 0.3 mg. of these extracts. A group of animals receiving pertussis vaccine was included in the test.

All animals were infected by the intravenous route with 0.05 ml. of staphylococcus culture 2 weeks after vaccination. The survival times are presented in Table IV.

The results presented in Table IV confirm an earlier finding that pretreatment with pertussis vaccine can markedly increase the survival time of mice infected with staphylococcus (3). Of the three samples of methanol extracts tested in this experiment, only the fractions obtained at 55°C. exhibited

TABLE IV
Protective Effect of Methanol Extracts of BCG and of Pertussis Vaccine on Susceptibility of Mice to Staphylococcal Infection

Materials injected (i.p.)	Interval between treatment and infection	Cumulative deaths at indicated days (d.) after infection*			
		4 d.	6 d.	11 d.	
	<i>mg.</i>	<i>days</i>	(out of 10 mice)		
Methanol extract 55°C.	1.0	14	4	6	8
“ “ “	0.3	“	6	9	10
“ “ 60°C.	1.0	“	8	9	10
Methanol extract Pf.†	0.3	“	4	6	9
Pertussis vaccine‡		“	4	5	8
Saline		“	10		

* 0.05 ml. of *Staph. aureus* (Giorgio) injected i.v.

† Preparation kindly supplied by Dr. Gladys Hobby of Chas. Pfizer and Co., Brooklyn.

‡ 0.05 ml. of commercial vaccine preparation distributed by Lederle and Co., Pearl River, New York.

TABLE V A
Effect of Killed Cells of BCG and of Myco. Fortuitum on Susceptibility of Mice to Staphylococcal Infection

Materials injected (1 mg. i.p.)	Interval between treatment and infection	Cumulative deaths at indicated days (d.) after infection*			
		3 d.	5 d.	10 d.	15 d.
	<i>days</i>	(out of 8 mice)			
BCG	19	2	4	6	8
<i>Myc. fortuitum</i>	“	4	7	7	8
Saline	“	8			

* 0.05 ml. overnight culture of *Staph. aureus* (Giorgio).

significant protective activity. It is of interest to mention in passing that the 55°C. fraction has been found in earlier experiments capable of eliciting a state of increased resistance to mouse tuberculosis (15, 21).

3. Protective Effect against Staphylococcal Infection Exerted by Suspensions of Killed Cells of *Myc. fortuitum*.—

We have found in a number of preliminary experiments (unpublished) that a protective effect similar to that exerted by killed cells of BCG can be elicited

4. *Protective Effect of Suspensions of Living BCG Vaccine and of Killed Cells of Myco. fortuitum against Infection with Myco. fortuitum.*—

Myco. fortuitum is a strongly acid-fast but rapidly growing microorganism which produces in mice a disease localized in the kidneys similar to that pro-

TABLE V C
Effect of Killed Cells of BCG and of Myco. Fortuitum on Susceptibility of Mice to Staphylococcal Infection

Materials injected (i.p.)	Interval between treatment and infection	Cumulative deaths at indicated days (d.) after infection*			
		3 d.	7 d.	14 d.	21 d.
		(out of 8 mice)			
BCG killed	15	0	1	2	5
“ “	“	0	1	3	4
“ “	“	0	0	3	5
<i>Myco. fortuitum</i> killed	“	0	2	5	5
“ “ “	“	0	2	5	6
“ “ “	“	1	3	7	8
Saline	“	3	5	7	8

* 0.05 ml. overnight culture of *Staph. aureus* (Giorgio).

TABLE V D
Protective Effect of Killed Cells of BCG and Myco. Fortuitum, Injected Subcutaneously, against Staphylococcal Infection of Mice

Materials injected (in 0.2 ml. s.c.)	Interval between treatment and infection	Cumulative deaths at indicated days (d.) after infection*			
		7 d.	10 d.	14 d.	20 d.
		(out of 8 mice)			
BCG cells	15	1	2	2	3
“ “	“	1	1	3	3
<i>Myco. fortuitum</i>	“	2	2	2	2
“ “	“	2	2	5	6
“ “	“	2	3	4	6
Saline	“	2	3	4	5

* 0.05 ml. overnight culture of *Staph. aureus* (Giorgio).

duced by staphylococci (16, 17). It is of interest, therefore, that the cell constituents of mycobacteria, which have been found to elicit increased resistance against infection with staphylococci, are also effective against *Myco. fortuitum*. The protective effect of vaccination with living BCG, and with killed BCG cells against *Myco. fortuitum* is illustrated in Table VI, which presents the results of an experiment similar in design to the first one reported in the present paper.

TABLE VI
Comparative Effects of Living BCG Vaccine and of Killed BCG on Susceptibility of Mice to Myco. Fortuitum

Materials injected (i.p.)		Interval between treatment and infection	Cumulative deaths at indicated days (d.) after infection*			
			4 d.	8 d.	11 d.	20 d.
		<i>days</i>	(out of 8 mice)			
BCG living†	0.1 ml.	13	0	0	1	3
BCG killed‡	2.5 mg.	"	0	0	0	0
Saline		"	4	6	8	
			6 d.	13 d.	20 d.	
			(out of 8 mice)			
BCG living†	0.1 ml.	25	1	4	6	
BCG killed‡	2.5 mg.	"	1	1	2	
Saline		"	3	7	8	

* 0.1 ml. of 2 day old culture of *Myc. fortuitum* injected i.v.

† Eight day old culture in tween-albumin medium.

‡ Bacilli washed with acetone and air-dried.

TABLE VII
Effect of Killed Cells of BCG or of Myco. fortuitum on Survival Times of Mice Infected with Myco. fortuitum

Materials injected (i.p.)		Interval between treatment and infection	Cumulative deaths at indicated days (d.) after infection*					
			5 d.	10 d.	15 d.	20 d.	5 d.	30 d.
		<i>days</i>	(out of 8 mice)					
BCG killed	2.5	16	0	0	0	2	4	4
" "	1.0	"	0	0	1	1	4	6
<i>Myc. fortuitum</i>	2.5	"	0	0	0	2	2	2
Saline		"	1	2	6	7	8	
BCG killed	2.5	35	0	0	3	4	4	4
" "	1.0	"	0	1	3	3	4	4
<i>Myc. fortuitum</i>	2.5	"	0	1	3	3	3	3
Saline		"	2	3	5	5	5	5

* 0.05 ml. *Myc. fortuitum* (Penso) administered i.v.

As shown in Table VII, marked prolongation of life following intravenous infection with *Myc. fortuitum* also resulted from vaccination with cells of BCG or *Myc. fortuitum* killed and acetone-washed. A similar result could be obtained by injecting smaller amounts of these killed cells or of methanol extract of BCG (Tables VIII and IX).

As observed in an earlier experiment with staphylococci (Table III), treatment of BCG cells with HCl likewise decreased protection against *Myc. fortuitum* (Table VIII). In the experiment presented in Table IX, the extract

TABLE VIII
Effect of Killed BCG on Susceptibility of Mice to Myco. fortuitum

Materials injected (i.p.)	Weight change*		Interval between treatment and infection	Cumulative deaths at indicated days (d.) after infection†			
	mg.	gm.		4 d.	9 d.	18 d.	27 d.
BCG acetone-dried	2.5	-10	22	0	3	5	7
" " "	0.8	+4	"	1	1	8	9
BCG acetone-dried, then	2.5	-43	"	0	0	2	4
acid-washed	0.8	-24	"	1	3	15	15
Saline		+18	"	12	16	18	22

* The figure indicates total weight change (in grams) for 24 mice 1 day after injection
† 0.05 ml. *Myc. fortuitum* (Penso) administered intravenously 22 days after treatment

TABLE IX
Lasting Effect of Killed BCG Cells, Methanol Extract, and Methanol Residue on Susceptibility of Mice to Myco. fortuitum

Materials injected (i.p.)	Interval bet. treat. and inf.	Cumulative deaths at indicated days (d.) after infection*				
		3 d.	6 d.	10 d.	17 d.	24 d.
BCG killed	32	0	4	4	5	6
" "	"	0	1	2	3	4
Methanol extract	"	2	5	7	7	8
" "	"	3	7	8		
Bacterial residue	"	1	3	3	7	8
" "	"	1	2	3	3	5
Saline	"	6	6	6	7	7

* 0.1 ml. of 5 day old culture of *Myc. fortuitum* (Penso) injected i.v.

had been prepared by prolonged extraction of the BCG cells in boiling methanol. It can be seen that the bacterial residue left after this exhaustive extraction still retained a great deal of protective activity.

5. *Effect of Vaccination with Killed Cells of BCG or of Myco. fortuitum on the Fate of Staphylococci in the Organs of Mice.*—

Mice were treated by the intraperitoneal route with 2.5 mg. of killed cells of *Myc. fortuitum*. Ten weeks after this treatment, they were infected by the intravenous route

with 0.05 ml. of an overnight culture of *Staphylococcus aureus* (strain Giorgio). The animals were sacrificed 1 day, 3 days, or 6 days after infection. The numbers of living staphylococci present in their organs at the time of sacrifice were determined by quantitative bacteriological techniques previously described (22). The results for the spleen, liver, and kidneys are presented in Table X.

Although limited in extent, the results presented in Table X show that fewer staphylococci could be recovered from the spleen, liver, and kidneys of vaccinated mice than from the organs of control animals. Similar results have

TABLE X
Effect of Vaccination with Killed Cells of *Mycobacterium fortuitum* on Fate of Staphylococci in the Organs of Mice

Materials injected (i.p. 10 wks. before infection)	Time after infection	No. of staphylococcal colonies recovered at indicated times after infection											
		Spleen				Liver				Kidney			
	days												
<i>Mycobacterium fortuitum</i>	1		*				*				‡		
	Saline	132	43	100	160	30	80	100	100	0	28	36	61
Saline	"	170	210	270	600	70	80	160	490	14	82	∞	∞
			*				*				§		
<i>Mycobacterium fortuitum</i>	3	0	0	0	2	0	0	1	26	1	5	95	?
	Saline	1	2	4	8	1	22	45	70	140	∞	∞	∞
<i>Mycobacterium fortuitum</i>	6		*				*						
	Saline	0	2	7	26	0	0	0	110	0	20	74	100
	"	0	0	26	40	0	120	∞	∞	10	150	∞	∞

* Figures to be multiplied by 10^8 to give numbers of colonies recovered per total spleen or liver.

‡ Figures to be multiplied by 10^6 to give numbers of colonies recovered per total kidney.

§ Figures to be multiplied by 10^7 to give numbers of colonies recovered per total kidney.

|| Figures to be multiplied by 10^8 to give numbers of colonies recovered per total kidney.

been obtained in other experiments in mice vaccinated with killed BCG cells and subsequently infected with *Mycobacterium fortuitum*. It seems best, however, to postpone presentation of these findings until quantitative studies have been carried out on animals followed for more prolonged periods of time after vaccination and after challenge infection.

SUMMARY

Vaccination with living attenuated tubercle bacilli (BCG) was found to increase the resistance of mice to infection with virulent staphylococci.

An even more striking protective effect could be elicited by intraperitoneal or subcutaneous injection of small amounts (0.1 mg. or more) of killed BCG cells.

The killed BCG cells retained most of their protective activity after prolonged heating at acid, neutral, or basic reactions—and after extraction with acetone, methanol, and NaOH (at pH 10.5). Some protective activity could be recovered in a fraction soluble in methanol at 55°C.

The protective effect against infection manifested itself in a prolongation of survival time following infection, and also in the fact that smaller numbers of staphylococci were recovered from the organs of infected mice. Both types of effects were still evident 10 weeks after vaccination.

Injection by the intraperitoneal route of killed cells of BCG, or of methanol extracts of them, elicited in mice a high level of protection against intravenous injection of *Myc. fortuitum*.

A protective effect quantitatively and qualitatively similar to that elicited by BCG, resulted from the intraperitoneal or subcutaneous injection of killed cells of *Myc. fortuitum*.

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