Immunization of Mice with Ultraviolet Killed Tuberculosis Vaccines.* (18323)

ALBERT MILZER, SIDNEY O. LEVINSON, AND MILDRED B. LEWIS.

From the Department of Bacteriology and Virology, and the Michael Reese Research Foundation, Michael Reese Hospital, Chicago, Ill.

There is increasing evidence that ultraviolet inactivated vaccines can be prepared for a number of viruses which are significantly more antigenic than heat or chemically inactivated control vaccines. We have prepared an effective irradiated rabies vaccine which is consistently more immunogenic than control phenolized vaccines(1) and which is widely used at present for both human and canine vaccination. We have also prepared an irradiated poliomyelitis vaccine which produces significant immunity to intracerebral challenge inoculation in vaccinated mice(2) and monkeys(3). Effective irradiated vaccines for the viruses of lymphocytic choriomeningitis(4), St. Louis encephalitis(1), influenza(5) and psittacosis(6) have also been prepared. Relatively few publications have appeared on the preparation of irradiated bacterial vaccines. In 1946 we prepared an irradiated polyvalent bacillary dysentery vaccine which was successful in the active immunization of mice(7) but not of man(8) for reasons other than the method of sterilization. Since the inception of our work we have considered the application of our ultraviolet irradiation technic to the production of a tuberculosis vaccine because of our previous success with the viruses and pathogenic bacteria mentioned above. In the meantime, Olson *et al.*(9) and more recently Sarber *et al.*(10) have prepared ultraviolet killed human type tuberculosis vaccines which have antigenic values at least equal to BCG vaccine on the basis of guinea pig vaccination tests. Paterson *et al.*(11) have prepared an irradiated vole bacillus vaccine which also confers a degree of protection in guinea pigs comparable to that obtained from BCG.

Since all of the reported studies with irradiated tuberculosis vaccines thus far have been confined to the use of guinea pigs, we considered it worthwhile to report the following experiments in which line 1 dba mice were vaccinated with irradiated tuberculosis vaccines. A standard BCG vaccine was used as a positive control vaccine in the comparative active immunity experiments.

Procedure. Mice. Line 1 dba mice weighing 12-15 g obtained from the Roscoe Jackson Memorial Laboratories were used throughout these studies. The mice were fed a diet consisting of Purina dog checkers and housed in glass jars with metal tops.

Irradiated Vaccine. The H37Rv strain of *Mycobacterium tuberculosis* var. *hominis* was used both for the preparation of irradiated vaccines and the challenge inoculation. This strain suspended in 5% gastric mucin consistently produces macroscopic lesions in dba mice when 0.001 mg moist weight is inoculated

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intraperitoneally or subcutaneously(12). The vaccine was prepared by growing the H37Rv strain on Petragnani's media slants incubated at 37°C for 14 days. The cultures were then harvested, weighed, and a suspension in sterile distilled water was prepared and ground for 30 minutes in a ball mill. The suspension was next diluted to contain 1 mg of moist weight of bacilli per cc and filtered through 120 mesh bolting silk cloth in order to remove gross particles and other debris. The suspensions were checked microscopically for purity after staining by the Ziehl-Neelsen technic and irradiated immediately with the Oppenheimer-Levinson type of quartz cell ultraviolet irradiation equipment(1). Careful preliminary titration studies were made to determine the least amount of irradiation necessary to kill the suspensions, and it was found that exposures for 0.5 second would consistently sterilize the vaccines with minimal antigenic destruction. After removing samples for sterility and safety tests, 1:10,000 merthiolate was added as a preservative and the vaccines were stored in the refrigerator at 3-5 C.

Sterility and safety tests. Most lots of irradiated vaccine consisted of 100 to 200 cc quantities of suspension. Each lot of vaccine was tested for sterility by seeding 20 tubes of Petragnani's media, 20 tubes Dubos media and 3 tubes of thioglycollate media with 0.5 cc of undiluted vaccine per tube. Thus 10 to 20% of the total volume was cultured. Simultaneously control samples of suspension before irradiation were tested for viability. All media were incubated at 37°C for 6 months unless growth was apparent sooner. Safety tests were carried out by inoculating 3 guinea pigs weighing 200 to 300 grams with 5 cc of undiluted irradiated vaccine intraperitoneally for each batch of vaccine. The guinea pigs were observed for 3 months and then sacrificed and examined for the presence of lesions. Ten dba mice were also inoculated both subcutaneously (0.5 cc) and intraperitoneally (0.5 cc) with irradiated vaccine mixed with equal parts of gastric mucin. The mice were sacrificed at intervals of 10 to 20 days and examined for lesions.

BCG vaccine. Freshly prepared standard BCG vaccine obtained through the courtesy of Dr. S. R. Rosenthal of the Tice Laboratory of the Chicago Municipal Tuberculosis Sanitarium was used in comparative immunogenic potency tests. The BCG vaccine was less than 5 days old in all experiments.

Results. Comparative active immunization tests were carried out by vaccinating line 1 dba mice with ultraviolet killed H37Rv and freshly prepared, viable BCG vaccines. The irradiated vaccines were at least one month old at the time of the first vaccination, and the BCG vaccine less than 5 days. Groups of mice were given 4 subcutaneous injections of 0.5 cc (0.5 mg) of each vaccine at weekly intervals. The challenge inoculation was given 30 days after the last dose of vaccine and consisted of a saline suspension of a 14-day-old culture of the H37Rv strain grown on Petragnani's media. Different groups of vaccinated and control mice were challenged with 0.025, 0.01 and 0.001 mg moist weight of bacilli suspended in equal parts of 5% gastric mucin inoculated both subcutaneously (0.5 cc) and intraperitoneally (0.5 cc). All animals were sacrificed 30 days after the challenge dose and examined carefully for macroscopic lesions. Impression smears were routinely made of all suspicious lesions and stained by the Ziehl-Neelsen technic.

A summary of the results obtained in several comparative antigenicity tests of ultraviolet killed H37Rv bacilli and living BCG vaccines in vaccinated mice is shown in Table I. Lesions in the Table refer to gross tubercle-like lesions ranging in size from 2 to 4 mm at the site of inoculation, liver, spleen or in the peritoneum. Impression smears of gross lesions in positive mice showed the presence of large numbers of acid-fast bacilli. The results shown in Table I indicate that both the irradiated and BCG vaccines produce a statistically significant† increased resistance to experimental tuberculosis in dba mice. The differences in average number of gross lesions

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TABLE I. Comparative Antigenicity of Irradiated H37Rv and BCG Vaccines in dba Mice.

Vaccine	Challenge dose, mg	No. mice with lesions / No. mice inoculated	%	Avg No. lesions per mouse
Irrad.	.025	16/36	44.4	1.9
BCG	.025	23/39	59	2.8
Controls	.025	37/37	100	3.1
Irrad.	.01	9/24	37.5	2.4
BCG	.01	13/24	54.2	3.1
Controls	.01	22/22	100	3.3
Irrad.	.001	11/36	30.6	1.6
BCG	.001	20/39	51.3	2.8
Controls	.001	37/37	100	2.8

TABLE II. Antigenicity of Various Lots and Storage of Irradiated Tuberculosis (H37Rv) Vaccines.

Vaccine	Storage time, mo.	Challenge dose, mg	No. mice with lesions / No. mice inoculated	%	Avg No. lesions per mouse
UV Lot #1	1	.025	5/12	41.6	2.2
		.001	4/12	33.3	1.6
#2	1	.025	6/12	50	2.0
		.010	4/12	33.3	3.0
		.001	4/12	33.3	2.0
#3	1	.025	6/12	50	2.5
		.010	5/12	41.6	2.1
		.001	4/11	36.3	2.1
#1	12	.025	6/12	50	2.5
		.010	5/12	41.6	2.0
		.001	4/11	36.3	2.1

in each mouse is not significant, although the maximum number of lesions in the ultraviolet vaccinated mice was 3 compared with 5 in the BCG and control groups. The irradiated vaccine consistently was slightly superior to BCG with the various challenge doses given.

Table II shows the uniformity in antigenic response obtained with 3 different lots of ultraviolet vaccine which were killed by irradiation for 0.5 second. Table II also shows the results obtained in storage experiments of 3 lots of irradiated vaccine after 1 month at 3-5°C as well as one lot (No. 1) which was stored for 12 months without significant loss in potency. All of these vaccines contained 1:10,000 merthiolate as a preservative. Further experiments on storage of the irradiated vaccines are in progress.

Discussion. The results obtained in the present studies indicate that a completely killed ultraviolet H37Rv tuberculosis vaccine gave as good or slightly superior results to BCG in comparative active immunity tests of vaccinated dba mice. These results are in agreement with those obtained by others (9,10) using guinea pigs as the test animal. Our experiments confirm those of Sarber *et al.* (10) that the irradiated tuberculosis vaccine is relatively stable when stored in the refrigerator at 3 to 5°C for several months.

A killed antigenic tuberculosis vaccine offers several advantages over BCG. First, since BCG contains living organisms to which a preservative cannot be added there is always the potential hazard of contamination with a pathogen. Furthermore, Vorwald *et al*(13) have recently reported that BCG produced a progressive and fatal tuberculosilicosis in silicotic guinea pigs. Finally, the known instability of BCG is a great disadvantage for general use of the vaccine.

No attempt was made to test the effectiveness of the irradiated vaccine against heterologous strains of virulent tubercle bacilli.

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No attempts were made to test the antigenicity of ultraviolet-killed BCG or heat killed virulent tubercle bacilli vaccines because of the ineffectiveness of such vaccines previously reported by others(9,10).

Summary. Several lots of ultraviolet-killed virulent human type tubercle bacilli (H37Rv) vaccine were prepared which were equal or slightly superior in antigenic potency to a standard BCG vaccine on the basis of dba mouse vaccination tests. The irradiated vaccine preserved with 1:10,000 merthiolate was stable and retained its immunogenicity for as long as 12 months after preparation.

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Amount of Luteal Tissue Required for the Maintenance of Pregnancy in the Rat.* (18324)

R. C. KELSEY, AND ROLAND K. MEYER.

From the Department of Zoology, University of Wisconsin, Madison.

Gustav Born first suggested that the corpora lutea maintain the uterus in a condition compatible for pregnancy(1). Fraenkel and Cohn(2), Fraenkel(3,4) and Corner(5) confirmed this hypothesis in the rabbit. Harris (6) and Parkes(7) found that ovariectomy always produced resorption or abortion in mice. Loeb(8) showed that pregnancy in guinea pigs was terminated if the corpora were removed before the end of the second day but was uninterrupted if removed after the end of the second day. That ovariectomy causes abortion in a high percentage of guinea pigs was demonstrated by Herrick(9), but ovarian transplants reduced the possibility of abortion. Courrier(10) suggested that endocrine secretions of the placenta may prevent some abortions which would normally be expected. Hartmann(11) suggested that man, the guinea pig, the horse, and the monkey are exceptions to the dependence of successful gestation on the presence of luteal secretions. Similar work on the rat by Harris and Pfiffner(12), Nelson and Haterius(13), Johnson and Challans(14,15), Pencharz and Long(16), Hain(17) and Zeiner(18) demonstrated that in a majority of the cases pregnancy was terminated within 48 hours after bilateral ovariectomy. Preliminary studies of lutein tissue required to maintain pregnancy in the rat done in our laboratories, indicate that the rat is able to parturate having only

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