Failure of Parenteral Vaccines to Protect Monkeys Against Experimental Shigellosis. (32087)

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In previous studies we have rendered monkeys resistant to experimental shigellosis by prior oral administration of living attenuated shigella bacilli(1-4). Killed vaccines administered parenterally have not been successful in protecting human beings against the disease under field conditions(5-8). It was of interest to us, therefore, to determine whether or not parenteral immunization would render monkeys resistant to experimental challenge under our conditions of assay and these experiments constitute the basis for this report.

Materials and methods. Bacterial cultures: Virulent *Shigella flexneri* 2a strain M42-43 has been previously described(4). It was maintained in the lyophilized state, and a new ampule was opened for each experiment. This was the only strain used in this study.

Immunization procedures: Vaccines consisting of either dead or living bacteria were employed. A heat-killed vaccine was prepared from strain M42-43 by suspending agar-grown cells in saline to a density of 1×10^9 live bacteria per ml, heating in a boiling water bath for 30 minutes, and adding formalin to a final concentration of 0.25% to the cooled product. Acetone-killed and dried (AKD) cells were prepared as described by Landy (9). A vaccine from these bacteria was made by suspending them in saline in a concentration of 20 mg per 50 ml. This is equivalent to approximately 2×10^9 organisms per ml. Live vaccines were prepared from strain M42-43 by suspending living, agar-grown cells in saline in a concentration of 1×10^9 bacteria per ml. All vaccines were injected subcutaneously. The animals which received the dead vaccines were inoculated at weekly intervals. The first 3 doses consisted of 0.1 ml of the heat-killed cells; the fourth dose was 0.5 ml of the same product, and the fifth dose was 1.0 ml of the AKD preparation. The animals receiving the living vaccine were injected 4 times at intervals of 3 to 4 days. The first 3 doses were 0.1 (1×10^8 living cells) and the final dose was 1 ml (1×10^9 living cells).

Serological tests: The monkeys were bled before immunization and 5 days after the last vaccine dose was injected. At the same time blood was also obtained from control animals. Serum antibody levels were determined by the passive hemagglutination test (3).

Morphological studies: Tissues for morphological study were taken at autopsy from monkeys which were anesthetized with 2.5 mg/kg of phencydidrine hydrochloride (Sernylan, Parke Davis & Co., Detroit, Mich.). They were processed for histological and fluorescent antibody examination by procedures previously described(3).

Results. In the first experiment 17 monkeys were injected with 4 doses of the heat-killed cells followed by a single dose of the AKD bacteria. They, together with 18 control animals, were challenged 10 days later with 5×10^{10} living *S. flexneri* 2a strain M42-43 administered by stomach tube. This was the same challenge level used in our previous studies with oral vaccines. At the time of challenge, 7 vaccinated and 8 control animals were randomly selected to be sacrificed 48 hours post-challenge in order to compare the tissue responses of the two groups.

Of the 8 control animals which were sacrificed 48 hours post-challenge one had formed stools, one had pasty stools, 3 had diarrhea, and 3 had dysentery (blood and inflammatory cells in diarrheal stools). Histological and fluorescent antibody studies on the intestines of these animals revealed that all animals had lesions which ranged from mild to severe and which contained specifically fluorescing dysentery bacilli. Of the 10 control animals which were kept for observation, one had formed stools, 3 had diarrhea and 6 had dysentery. Six of these animals died.

Of the 7 animals vaccinated with the killed vaccine and sacrificed 48 hours after challenge, one had pasty stools, and 6 had dysentery. Microscopic examination revealed that

TABLE I. Signs of illness in control monkeys and in monkeys injected subcutaneously with either killed or living *S. flexneri* 2a vaccines and then challenged orally with *S. flexneri* 2a.

Vaccine	Vaccine Group			Control Group		
	No. with diarrhea	No. with dysentery	Total ill / Total challenged	No. with diarrhea	No. with dysentery	Total ill / Total challenged
Killed	1	14	15/17*	6	9	15/18*
Living	1	6 (4 died)	7/10	0	4 (4 died)	4/6

* Seven vaccinated and 8 control animals were sacrificed 48 hr after challenge for other studies. Six of the remaining 10 animals in each group died.

all had lesions containing specifically fluorescing dysentery bacilli. In the 10 animals of the vaccinated group which were retained for observation, one had formed stools, one had diarrhea and 8 developed dysentery. Six animals in this group succumbed to the infection. These results are summarized in Table I.

A second experiment was conducted in which 10 animals were vaccinated by the subcutaneous route with a vaccine made from living bacteria. Ten days after the last vaccine dose, these animals and 6 control monkeys were challenged orally with the same strain used for immunization. In the vaccinated group, one animal had diarrhea, 6 developed dysentery and 4 died. Of the 6 controls, 4 had dysentery and these 4 animals succumbed to the infection. The results of this study are summarized in Table I.

The serum antibody response of vaccinated and control monkeys is summarized in Table II. Sixteen of seventeen animals which received the killed product and all of the 10 monkeys injected with the living cells had a 4-fold or greater rise in titer as measured by the passive hemagglutination test. None of 11 controls which were bled at the same time experienced a rise in antibody levels.

Discussion. Parenterally administered antidysentery vaccines are not commonly used. Aside from the criticism that some preparations cause unacceptable reactions when injected, the most compelling reason for their neglect is that they have failed to confer protection on man when used under field conditions(5-8).

TABLE II. Serum Antibody Response of Monkeys Which Were Injected Subcutaneously with Either Killed or Living *S. Flexneri* 2a Vaccines and Bled 5 Days After the Last Vaccine Dose.

Group	No rise	2-fold rise	4-fold or greater rise
Killed vaccine	0/17	1/17	16/17
Living vaccine	0/10	0/10	10/10
Controls*	11/11	0/11	0/11

* Control animals were bled at the same time as the vaccinated monkeys.

We have been able to confer resistance on monkeys against experimental dysentery by prior oral administration of living attenuated bacteria(1-4). There is, of course, a very real possibility that the experimental infection which we produce in the laboratory bears little or no resemblance to the disease as it occurs in nature. Should this be the case, it is also possible that the protection which we have achieved in the laboratory might not be reproduced under field conditions. While all of these possibilities must be considered, a final answer cannot be had until oral vaccines are tested under conditions of natural exposure.

What we have tried to do in the present study is to determine whether or not we could obtain results in the laboratory which were similar to those already accumulated by several groups in field studies, *i.e.,* to test the efficacy of parenteral vaccines under conditions of laboratory assay. It might be noted here that injected vaccines have conferred a degree of protection on mice experimentally infected with shigellae(10). However, the results which we have obtained in rhesus monkeys indicate clearly that this natural host is not protected against an experimental infection with either dead or living vaccines injected subcutaneously, and that rises in serum antibody levels are not associated with increased resistance.

In the case of killed vaccine, both heat-killed and acetone-killed and dried products were injected into the same animals. The signs of illness in both the vaccinated and control animals were similar, and the tissue re-

sponse in those animals which were sacrificed was equally severe in both groups. The latter finding is in contrast to previous observations made on animals which received living vaccine by the oral route.

The living vaccine was employed to rule out the possibility that our inactivating procedures had destroyed a labile protective antigen. The animals tolerated the living vaccine well, but even under conditions where the same living, virulent organism was used to immunize as was employed to challenge, evidence of protection was not apparent. Thus, parenterally administered preparations conferred no protection, therefore reproducing under our conditions of laboratory assay the results obtained in field studies.

Summary. Monkeys injected subcutaneously with either a combination of heat-killed or acetone-killed *S. flexneri* 2a vaccines or with vaccines made from living, virulent *S. flexneri* are not rendered resistant to experimental oral challenge with the homologous organism.

The technical assistance of E. J. Tapley and G. White is gratefully acknowleged.

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Received February 16, 1967. P.S.E.B.M., 1967, v125.

Chorionic Gonadotrophin in the Blood and Urine of Pregnant Rhesus Monkeys (*Macaca mulatta*).* (32088)

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Chorionic gonadotrophin has been demonstrated in the urine of pregnant rhesus monkeys(1,2). These studies indicate, however, that monkey chorionic gonadotrophin (MCG) is excreted for only a limited period of pregnancy. The main object of the present study was to develop a sensitive biological test for MCG in order to detect early pregnancy and to measure daily fluctuations in urinary chorionic gonadotrophin.

Materials and methods. Twenty-four-hour urine samples from feral pregnant monkeys were collected in glass jars packed in ice. The urine was filtered through glass wool and either extracted immediately or frozen if extraction was to be done later.

A modification of the procedure described by Bradbury *et al*(3) and Albert(4) for concentrating the gonadotrophic hormones was used for urine extraction. The 24-hour urine volume was recorded and the pH adjusted to 4.5 with 20% HCl. Five ml of a 20% suspension of acid washed Kaolin were thoroughly mixed with the urine and the mixture centrifuged for 5 minutes. The supernatant was discarded and the precipitate washed by adding 10 ml of distilled water and recentrifuging. The supernatant was again discarded and 5 ml of 0.1 N NaOH added to the precipitate, mixed thoroughly and centrifuged for an additional 5 minutes. The supernatant was saved and the pH adjusted to 7.0 with 1% HCl.

Twenty-one-day-old female rats (Holtzman strain) were used as bioassay animals and

* This study was supported in part by Grants I T I HD 104-01 and FR-0167, Nat. Inst. Health, and by The Ford Foundation.