TABLE I. Effect of Infusion of Epinephrine at Different Rates on Potassium Concentration at Times Available for Comparison.

| Rate of epinephrine infusion ($\mu$g/kg per min) | No. of animals | Potassium concentration (meq/liter) changes at varying intervals (min) during epinephrine infusion | | | | | Ref. |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 4 | 6 | 20 | |
| 1.5 | 9 | 0.3 | 0.9 | 1.2 | 0.4 | | Fig. 1 |
| 2.0 | 8 | 0.7 | 1.6 | 1.7 | 1.0 | —0.7 | Fig. 2 |
| 5.0 | 8 | | 4.6 | | | —1.02 | (8) |
| 5.0 | 4 | 3.6 | | 3.2 | 2.0 | | (7) |

$[K^+]$. The magnitude of the early increase in $[K^+]$ and the time from the beginning of infusion to the return of $[K^+]$ to control levels appear to be related to the rate of infusion of epinephrine. Further study is needed to determine how the later decrease in $[K^+]$ is related to the rate of infusion.

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## Complement Fixing Antibody Response of Man to Yolk Sac-Grown Rocky Mountain Spotted Fever Vaccine* (32976)

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The first Rocky Mountain spotted fever (RMSF) vaccine was made from infected tick tissues in 1924 (1). It protected animals against virulent challenge, elicited antibodies in man, and lowered mortality in vaccinated persons who subsequently developed disease (1–3). In 1939 a vaccine was made from infected yolk sacs of embryonated hen's eggs (4) and in 1948 manufacture of the tick vaccine was discontinued. The assumption of efficacy in man of the yolk sac vaccine rests on (a) the field and laboratory data obtained with the earlier tick vaccine, (b) the capacity of the yolk sac vaccine to protect guinea pigs against lethal challenge with *Rickettsia rickettsi*, and (c) the fact that "Rocky Mountain spotted fever vaccines prepared from infected yolk sacs and tick tissue were about equally active in producing immunity in guinea pigs" (5). There is a paucity of published data on the serological response of man to the yolk sac type of spotted fever vaccine; no information is available on its efficacy in preventing

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TABLE I. Groups of Volunteers in Vaccine Study and Schedules of RMSF Vaccination for Each Group.

| Group | No. of subjects | History of exposure to RMSF antigens | Primary vaccination schedule | | Booster schedule | |
|---|---|---|---|---|---|---|
| | | | No. of doses | Days between doses | No. of doses | Weeks between last dose and booster |
| IA | 8 | None | 3 | 7 | 1 | 24 |
| B | 3 | Vaccinated 6–17 years earlier | 3 | 7 | 1 | 24 |
| IIA | 14 | None | 1 | — | 1 | 4 |
| B | 17 | None | 1 | — | 1 | 8 |
| C | 14 | None | 1 | — | 1 | 12 |
| D | 10 | None | 1 | — | 1 | 16 |
| E | 13 | None | 1 | — | 1 | 24 |

or modifying the disease among exposed persons (6).

This study was designed to measure the spotted fever group complement fixing (CF) antibody response of nonimmune and of previously vaccinated subjects to different immunization regimens with a commercially available Rocky Mountain spotted fever vaccine. It is unlikely that the spotted fever group CF antibodies are directly responsible for specific immunity to RMSF but in the absence of a more feasible *in vitro* test whose correlation with protection has been established, the group CF antibodies may serve as a rough guide to some immunogenic properties of RMSF vaccine.

*Materials and Methods. Vaccine.* A single lot of commercial RMSF vaccine (Lederle lot No. 092-288)[2] was used throughout the study. In all cases, a single dose consisted of 1.0 ml of vaccine administered subcutaneously according to the schedules outlined in Table I. The CF antigen titer of this vaccine was 2 to 4 when tested against human and guinea pig RMSF convalescent serum in block titrations.

*Subjects.* The subjects in Part I were departmental personnel undergoing routine immunization for the prevention of laboratory infections; those in Part II were healthy young adult medical students who desired vaccination against RMSF.

[2] Supplied through the courtesy of Dr. Herald Cox of the Lederle Laboratories.

*Serologic methods.* Sera were stored at —20°C; later all specimens from a given group were tested simultaneously in the CF test. The CF test was performed by the microtiter technique of Takatsy (7) as modified by Sever (8) and Casey (9).

Rickettsial antigens, either "soluble" or "complete" as indicated, were prepared by ether treatment of yolk sacs (10,11) infected with *R. akari,* Hartford strain, or *R. rickettsi,* Sheila Smith strain. Normal yolk antigen was prepared from uninfected yolk sacs of the same age in the same manner as soluble antigen.

All sera were tested with the group reactive *R. akari* soluble antigen; 2 units were employed in Part I and 8 units in Part II. The generally low titers encountered suggested the possibilities that (i) the *R. akari* group antigen might not cross react optimally with RMSF sera, and (ii) larger antigen doses might be required to detect early antibodies, as in typhus fever (12). Accordingly, all sera from the fifth bleed of all groups in Part II were tested with *R. akari* "complete" antigen (2 units) and *R. rickettsi,* Sheila Smith, "complete" antigen (8 units). The results with these tests did not differ significantly from those obtained in the original tests with *R. akari* soluble antigen; hence, only the latter are presented in the tables.

Since both the RMSF vaccine and the serologic antigens contained egg proteins, 76 sera from the fifth bleeding in Part II were

TABLE II. Complement Fixing Antibody Response to 3-Dose Initial RMSF Vaccine Regimen Followed by a Single Booster Dose at 6 Months.

| Subject | CF titer on day | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0[a] | 7[a] | 16[a] | 25 | 31 | 38 | 45 | 85 | 120 | 151 | 189[a] | 203 | 231 |
| Group IA. No prior RMSF vaccine | | | | | | | | | | | | | |
| PR | <2 | <2 | 4 | 8 | 8 | 4 | 4 | 4 | —[b] | — | 2 | 2 | 2 |
| IF | <2 | <2 | 2 | 2 | 2 | 4 | — | 2 | — | — | 2 | 2 | 2 |
| SB | <2 | <2 | 2 | 16 | 8 | 8 | 8 | 2 | — | — | 2 | 4 | 4 |
| MP | <2 | <2 | 2 | 8 | 8 | 8 | 8 | 2 | — | — | 2 | 8 | 8 |
| MG | <2 | <2 | 8 | 8 | 8 | 16 | 16 | 4 | — | — | 4 | 8 | 8 |
| GK | <2 | <2 | <2 | 2 | 2 | 2 | <2 | 2 | — | — | <2 | — | 2 |
| LS | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2[a] | 2 | <2 | <2 | <2 |
| RR | <2 | <2 | <2 | <2 | <2 | <2 | <2 | — | 2[a] | 8 | 2 | 2 | 2 |
| Group IB. RMSF vaccine 6–17 years previously | | | | | | | | | | | | | |
| CW | <2 | 2 | 16 | 8 | 16 | 16 | 16 | 4 | — | — | 4 | 4 | 4 |
| BS | <2 | 4 | 16 | 8 | 8 | 8 | 8 | 4 | — | — | 2 | 8 | 4 |
| GC | <2 | 4 | 16 | 64 | 16 | 16 | 16 | 16 | — | — | — | — | — |

[a] One-ml dose of vaccine was administered.

[b] No specimen.

also titrated in the CF test with normal yolk sac antigen. Eight sera gave CF titers of 4 or greater with *R. akari* antigen alone, 2 sera with both *R. akari* and normal yolk sac antigen and 3 sera with normal yolk sac antigen alone. Therefore some positive titers may have been due to yolk sac antibody.

All titers are expressed as the reciprocal of the initial serum dilution in which a 50% or greater fixation of complement was observed. Because of the difficulty in evaluating a titer of 2, serologic conversion was considered to have occurred only when titers increased from less than 2 to 4 or greater. Only titers of 4 or greater were considered to be positive and only a 4-fold or greater increase in titer was considered a significant rise.

*Results. Part I. Antibody response to 3-dose vaccine series followed by a single booster at 6 months in subjects with and without prior vaccination history* (Table II). Five of the 8 subjects who had no history of prior RMSF vaccine (Group IA) developed CF titers of 4 or greater following the initial 3-dose course of vaccine. Antibodies were first detected on day 16, reached maximum titers by day 25 and declined after day 45. In contrast, all 3 subjects who had previously received RMSF vaccine in the distant past (Group IB) developed antibodies which were detectable by day 7 and maximum by day 25; subsequently, they declined slowly. Surprisingly, the response of both groups to a booster dose at 6 months was poor. Thus, only two subjects, both in Group IA, displayed a 4-fold or greater rise in CF antibody titer and in neither instance did the titer exceed that following the primary dose.

Two of the 3 subjects who failed to develop a significant primary CF antibody response received an extra 1.0-ml dose of vaccine at 120 days as well as the booster dose at 6 months. Only one developed a transient antibody rise following the 120-day dose and neither displayed significant response to the 6-month booster. Thus, some subjects failed to develop more than a very transient antibody response even with 5 doses of vaccine over a 6-month period.

Six subjects from Group IA were available 1 year after the "6-month" booster, i.e., about 18 months after the primary series. Each received a second booster dose of the same lot of vaccine. Serum specimens were obtained prior to and 2 weeks after the booster dose. Only two of the six subjects showed a fourfold or greater rise in spotted fever group CF antibody titer. The serum from one of these also reacted with normal yolk sac antigen to about the same titer, casting doubt upon the

TABLE III. Frequency Distribution of Spotted Fever Group Complement Fixing Antibody Titers in Volunteer Human Subjects Following Single Dose Primary Vaccination and a Booster after Various Intervals.[a]

| Vaccine | Group | Weeks after primary dose | No. of subjects with titer of | | | | |
|---|---|---|---|---|---|---|---|
| | | | <2 | 2 | 4 | 8 | 16 |
| IIIA | Primary | 0 | 13 | 1 | 0 | 0 | 0 |
| | | 3 | 9 | 3 | 2 | 0 | 0 |
| | Booster | 4 | 9 | 3 | 2 | 0 | 0 |
| | | 6 | 9 | 4 | 1 | 0 | 0 |
| | | 8 | 11 | 1 | 2 | 0 | 0 |
| B | Primary | 0 | 17 | 0 | 0 | 0 | 0 |
| | | 3 | 8 | 4 | 2 | 3 | 0 |
| | Booster | 8 | 9 | 4 | 2 | 2 | 0 |
| | | 10 | 9 | 4 | 3 | 1 | 0 |
| | | 12 | 10 | 3 | 3 | 1 | 0 |
| C | Primary | 0 | 12 | 2 | 0 | 0 | 0 |
| | | 3 | 8 | 2 | 3 | 0 | 1 |
| | Booster | 12 | 11 | 2 | 1 | 0 | 0 |
| | | 14 | 10 | 3 | 1 | 0 | 0 |
| | | 16 | 11 | 2 | 1 | 0 | 0 |
| D | Primary | 0 | 10 | 0 | 0 | 0 | 0 |
| | | 3 | 3 | 4 | 3 | 0 | 0 |
| | Booster | 16 | 10 | 0 | 0 | 0 | 0 |
| | | 18 | 7 | 2 | 1 | 0 | 0 |
| | | 20 | 9 | 0 | 1 | 0 | 0 |
| E | Primary | 0 | 12 | 1 | 0 | 0 | 0 |
| | | 3 | 10 | 2 | 0 | 1 | 0 |
| | Booster | 24 | 13 | 0 | 0 | 0 | 0 |
| | | 26 | 12 | 0 | 1 | 0 | 0 |
| | | 28 | 13 | 0 | 0 | 0 | 0 |

[a] Values below the horizontal broken lines were obtained after the booster dose. Values to the right of vertical broken line represent significant titers.

specificity of the apparent antibody response. Thus, even after an interval of 1 year following the last dose of vaccine, the group CF antibody response to a booster dose of vaccine was not enhanced.

*Part II. Antibody response to single primary dose of vaccine followed by a single booster at different time intervals* (Table III). A study was designed to determine if a single primary dose of the available RMSF vaccine would prepare subjects to give a booster response to a subsequent dose of vaccine administered at various intervals between 1 and 6 months after the primary dose. Thus, all subjects were given a primary dose of RMSF at the same time. Then groups of 10–17 subjects each were given a booster dose at 4, 8, 12, 16, or 24 weeks.

Only about 22% (15/68) of all subjects developed a CF titer of 4 or greater by 3 weeks after the primary dose. Particularly striking, however, is the fact that no significant booster response was detected regardless of the time which had elapsed since the primary dose. Thus, 7/68 had a titer of 4 or greater at the time the booster was given, whereas only 8/68 had titers of 4 or greater 2 and 4 weeks after booster.

*Summary and Conclusions.* The spotted fever group complement fixing antibody response was used as a measure of the immunogenicity of a commercially available killed Rocky Mountain spotted fever vaccine in human subjects. A single-dose primary course of vaccine elicited a detectable antibody response in only about 22% of 68 subjects, whereas a 3-dose primary course stimulated the development of complement fixing antibodies in about 63% of eight subjects. The low antibody titers attained may be a function of the relatively low complement fixing antigen content of the vaccine.

A booster dose of vaccine given between 1 and 6 months after the primary course failed to elicit an antibody response in the majority of subjects. A second booster dose, given a year after the 6-month booster dose, also failed to cause a significant response. On the other hand, three subjects who had last received RMSF vaccine several years prior to this study developed a typical secondary antibody response upon revaccination. These same subjects, however, failed to show an antibody response to an additional dose of vaccine 6 months later.

The interval between doses or courses of the currently available vaccine required for eliciting an optimal secondary CF antibody response is unknown, but the results of this

study suggest that it may be greater than one year.

It is unknown if the uniformly low CF antibody response in man to the commercial RMSF vaccine reflects an equally low degree of protection afforded against the disease. Minimum requirements for potency of RMSF vaccine lots released for distribution include demonstration of capacity to protect guinea pigs against challenge with virulent organisms (5). Studies of the protective effect of the vaccine in man would seem desirable to evaluate its efficacy and to establish the degree of correlation of protection with laboratory tests.

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## Protein and Carbohydrate Composition of Cecal Contents of Gnotobiotic Rats and Mice* (32977)

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One of the peculiarities of certain germfree animals is the enlarged cecum which can average up to 20% of the body weight. In conventional animals this part of the intestinal tract is about 1% of the body weight and its "normality" is attributed to the resident microbial flora. Previous studies have indicated that the material accumulating in the gnotobiotic cecum is higher in water content than that from conventional animals (1), and contains high molecular weight mucins (2). Recently Combe *et al.* (3–5) have fractionated the cecal contents of gnotobiotic rats and have demonstrated the presence of muco-proteins and large increases in free amino acids and urea over that found in the conventional cecum.

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The present investigation was initiated to provide information concerning the total protein and carbohydrate content of cecal material obtained from the gnotobiotic rat and mouse. Such base line information is necessary in order to evaluate the phenomena responsible for cecal enlargement and could lead to a judicious selection of microbes with which to reduce cecal size.

*Materials and Methods. Animals.* Gnotobiotic CDF rats and CD-1 Swiss mice, were obtained from the Charles River Breeding Laboratories (North Wilmington, Massachusetts) and were housed in plastic germfree isolators. Comparable aged conventional animals of the same strain were kept in the animal room. Gnotobiotic rats and conventional rats were fed autoclaved diet L356 (General Biochemicals Corp.) (6) and water *ad libitum*. The gnotobiotic mice were fed Purina diet 5010 and conventional mice were