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Early Development in Monkeys of Cutaneous Resistance to Reinfection with *Rickettsia tsutsugamushi.* (30286)

J. A. MORRIS
Division of Biologics Standards, National Institutes of Health, USPHS, Bethesda, Md.

In the late 1940's the U. S. Army Scrub Typhus Research Unit in Malaya reported a striking difference in incidence of eschars in volunteers infected with scrub typhus who had received chloramphenicol prophylactically and in those who had not. In one experience, 4 subjects who did not receive chloramphenicol during a field trial developed eschars while an equal number of men who were given the drug during and after exposure failed to show such lesions. To explain the role of prophylaxis in eschar formation Smadel *et al* (1,2) postulated that during the suppression of growth of rickettsiae by the drug, sufficient local immunity developed in the skin to prevent subsequent development of an eschar. However, no experiments were performed by the group in Malaya to test the validity of the hypothesis. In 1951 studies were undertaken to elucidate this point by determining in monkeys the reactions to multiple intradermal inoculations of scrub typhus rickettsiae administered intermittently over periods of 1 and 2 weeks. The findings are presented now, even though the data are not extensive, in the hope that the observations will add information on the effects of the immune processes in development of skin lesions in scrub typhus.

Materials and methods. Nine young adult rhesus monkeys were inoculated into the anterior abdominal wall with 300 mouse ID_{50} of the Karp strain of *Rickettsia tsutsugamushi* contained in 0.2 ml of infected yolk sac suspension (63rd egg passage). Infectivity titrations were performed in mice each time an aliquot of infectious yolk sac pool was thawed for use. Group 1 monkeys received 3 inoculations at 3-day intervals; Group 2 monkeys received 4 inoculations, the second being given 6 days after the first and the third and fourth following at 3-day intervals thereafter; Group 3 monkeys also received 4 inoculations, the second being given 9 days after the first and the remaining 2 inoculations thereafter at 3-day intervals (Table I, column 3). The animals were examined each day for 17 days after the initial inoculation for developent of dermal lesions and for occurrence of fever. All monkeys were bled on days 0 and 31 for serum for use in serologic tests and a single monkey (Monkey 13) was bled on the second day of fever to obtain blood for rickettsial isolation.

Results. Development of specific skin lesions. Fig. 1 presents detailed results obtained in Monkey 12 and is illustrative of the dermal reactions obtained in the 3 animals in Group 1. On day 4 a lesion appeared at the site of the first injection and consisted of a small, slightly raised, erythematous area. By the sixth day the lesion had progressed to a necrotic eschar and scab. Healing began on the ninth day and on the seventeenth day, the scab had fallen off leaving a healed, slightly raised scar. A lesion at the site of the second injection was discernible 3 days after administration of the inoculum. It appeared overnight and on the first day of its appearance had reached its maximum size; this lesion did not progress to necrosis. It is seen in Fig. 1 that 6 days elapsed between inoculation and formation of a maximum-sized eschar at the site of the

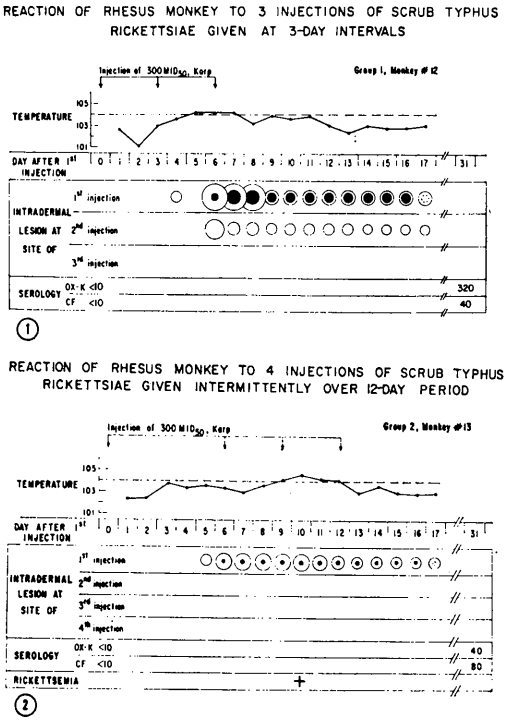

FIG. 1 and 2

first injection; only 3 days passed between injection and maximum development of the lesion at the site of the second inoculation. The basis for this rapid development of the lesion at the second injection site is unknown; possibly it could be a manifestation of dermal sensitivity directed towards specific rickettsial antigens. No lesion occurred at the site of the third injection which was administered 6 days after the first. The partial suppression of the lesion at the site of the second injection and the complete suppression of development of a lesion at the third site suggest the presence in the skin of this monkey of an immune factor which was active as early as the third day after initial exposure to *R. tsutsugamushi*.

Fig. 2 presents findings in Monkey 13 which are illustrative of results obtained with animals in Group 2. Except for size, the lesion at the site of the first injection in this monkey was strikingly like that at the place of the first injection in Monkey 12. No lesions, however, developed at the second, third and fourth injection sites. Absence of lesions in all but the site of the first injection is taken as evidence that as early as day 6 following the intradermal injection of scrub typhus rickettsiae, there were present in the skin of this monkey immunity factors sufficient in amount to suppress completely development of skin lesions at the site of the second, third and fourth injections. This hypothesis is supported by the data for the remaining 7 animals included in the study. In Table I it is seen that specific skin lesions developed at 2 injection sites only when the first two inoculations were given 3 days apart. When an interval of 6, 9, 12 or 15 days separated the first from subsequent injections, only a single lesion developed, and this one at the site of the first injection.

Rickettsial isolation findings. *R. tsutsugamushi* was recovered from the only blood specimen examined for the presence of rickettsiae (10-day sample obtained from Monkey 13), (Fig. 2). This specimen, when diluted

TABLE I. Summary of Findings in Monkeys Inoculated Intermittently with Scrub Typhus Rickettsiae, Strain Karp.

Group	Monkey	Inoculation schedule—day inoculated	First inoculation*	Second inoculation*	Third inoculation*	Fourth inoculation*
1	10	0,3,6	++	++	—	
	11		++	+	—	
	12		++	+	—	
2	13	0,6,9,12	++	—	—	—
	14		++	—	—	—
	15		+	—	—	—
3	16	0,9,12,15	++	—	—	—
	17		++	—	—	—
	18		+	—	—	—

* 300 mouse ID_{50} contained in 0.2 ml.
— Absence of lesion.
\+ Erythema and papule.
++ Necrosis and black eschar.

1:1, induced fatal scrub typhus infections in 4 of 6 inoculated mice and produced immunity to challenge with *R. tsutsugamushi* in the 2 survivors. It is noted that if antibody directed against *R. tsutsugamushi* was present in the blood of Monkey 13 on day 10, this antibody was present in an amount insufficient to suppress rickettsemia in this animal. However, in spite of the occurrence of rickettsemia on day 10, no lesion developed at the site of the second injection where one would have been expected to occur if immunity factors were not present and active in the skin.

Observations on febrile, serologic and challenge responses. With one single exception, all monkeys responded to the inoculum with development of Weil-Felix and complement-fixing antibodies. Failure of one animal to respond serologically (Monkey 11 in Table I) suggests that this monkey might have escaped systemic infection; this belief is substantiated by the finding that this animal experienced only a single day of fever (day 4) after rickettsial exposure, whereas all other monkeys underwent 2 or more days of fever. All 9 animals resisted a challenge inoculum of 10,000 mouse ID_{50} of strain Karp administered 77 days after administration of the first inoculum. All remained afebrile and free of specific dermal lesions. It is noted that even though Monkey 11 might have escaped infection of sufficient intensity to elicit formation of detectable antibodies following initial exposure to *R. tsutsugamushi,* the 3 injections of rickettsiae administered on days 0, 3 and 6 were enough to render this monkey immune to the relatively massive challenge inoculum administered 2½ months later.

Discussion and summary. These findings provide evidence that immunity factors were active in the skin in the immunologic defense of rhesus monkeys experimentally infected by the intradermal route with scrub typhus rickettsiae, 6 days after exposure to the rickettsiae and several days before circulating antibodies had reached levels high enough to rid the blood of organisms. In 3 groups of monkeys, development of specific dermal lesions was completely suppressed at sites of the second, third, and fourth injections of scrub typhus rickettsiae when an interval of 6 days separated the first from subsequent injections, while in a single group of monkeys (Group 1) given 3 injections at a 3-day interval, lesions occurred at 2 injection sites. Although the role of circulating antibodies in the state of resistance to superinfection in the skin is unknown, the fact that presence of the immune state in the skin of a single monkey was established at the time this animal was circulating rickettsiae in the blood favors the suggestion that other immunity factors besides circulating antibody were at work in the dermal resistance of monkeys inoculated by the technique employed.

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Effects of Urea and Some Organic Solvents on Myosin A.* (30287)

GEORGE KALDOR† AND SANDRA WEINBACH (Introduced by B. W. Volk)
Department of Physical Chemistry, Isaac Albert Research Institute, Jewish Chronic Disease Hospital, Brooklyn, N. Y.

A relatively large number of compounds is known to cause activation or inhibition of myosin A-ATPase depending upon their respective concentrations in the incubation mixture. Some of these modifiers, such as ethylene diamine tetraacetic acid (EDTA), p-

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† Present address: Dept. of Physiology, Woman's Med. Coll. of Pa., Phila., Pa.