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EFFECT OF HARVEST TIME OF RICKETTSIA RICKETTSII FROM DUCK EMBRY--ETC(U)
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Effect of Harvest Time of Rickettsia rickettsii from Duck Embryo
Cell Cultures on Rocky Mountain Spotted Fever Infection
in the Rhesus Monkey

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GERALD L. RUCH¹ AND DUANE E. HILMAS

United States Army Medical Research Institute of Infectious Diseases
Fort Detrick, Frederick, Maryland 21701

Running head: RMSF IN RHESUS MONKEYS

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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ABSTRACT

This study was undertaken to determine the optimum harvest time for infected duck embryo cell (DEC) cultures to yield the highest rickettsial titers and greatest infectivity for rhesus monkeys. A consistently reproducible Rocky Mountain spotted fever-infected monkey model for pathogenesis studies and testing vaccine is produced when the inoculum given intravenously contains $\geq 10^{6.0}$ chick embryo median lethal dose organisms. This is optimally attained with day-4 DEC culture harvest material.

The rhesus monkey has served as a suitable host for the study of the pathogenesis and pathophysiology of Rocky Mountain spotted fever (RMSF) (4,6,7,9). However, to establish a reproducible RMSF infection in subhuman primates, it has been essential to grow stock inocula of Rickettsia rickettsii of high yield while maintaining their infectivity for the host. This report describes the optimum harvest time for rickettsiae-infected duck embryo cell (DEC) cultures, both in terms of titer and infectivity for rhesus monkeys.

The Sheila Smith strain of R. rickettsii was propagated in embryonated chicken eggs according to the methods of Stoenner et al. (8). Seed stock was stored at 70°C as a 50% yolk sac slurry in sucrose-phosphate-glutamate (SPG) buffer (1). Dilutions were made in SPG buffer, pH 7.2. The preparation of DEC cultures and the techniques for rickettsial growth and harvest have been described earlier (3). Rickettsiae were harvested from several inoculated flasks daily for six consecutive days after infection.

Infectivity of each harvest obtained on these six days was determined by titration of each sample by serial 10-fold dilutions in the yolk sacs of 6-day-old embryonated chicken eggs and by intraperitoneal (i.p.) inoculation of Hartley strain male guinea pigs (350-400 g). Guinea pig rectal temperatures were measured daily for 14 days, scoring any temperature greater than 40.0°C as positive indication of infection. Guinea pigs were also examined for other clinical signs of RMSF, such as scrotal swelling and erythema of footpads, ears, and scrotum. The chick embryo median lethal dose (CELD₅₀) and guinea pig IP median infectious dose (GPIPID₅₀) were calculated by the method of Reed and Muench (5).

Twelve rhesus monkeys of either sex, 3.2 to 4.5 kg weight, were allocated into groups and inoculated intravenously (i.v.) with 1 ml of harvest material. Two monkeys were inoculated with material from each day's harvest and grouped as follows: Group I received rickettsiae harvested on day 1 or 2 ($10^{4.5}$ CELD₅₀), group II received rickettsiae harvested on day 3 or 6 ($10^{5.5}$ CELD₅₀), and group III received organisms harvested on day 4 or 5 ($\geq 10^{6.0}$ CELD₅₀). Monkeys were monitored daily for 16 days to record incubation period (time from inoculation to onset of fever $\geq 40.0^\circ\text{C}$), duration of illness (onset of fever $\geq 40.0^\circ\text{C}$ to death, or until temperature decreased to $< 40.0^\circ\text{C}$ in survivors), and time to death (time from inoculation to death). Pathological examinations were performed on all monkeys which died.

No difference in numbers of rickettsiae were apparent in stained (2) smears from the DEC cultures from days 3 to 6 postinfection, but peak titers of 6.2 and 7.5 logs occurred on day 4 by the respective CELD₅₀ and GPIPID₅₀ assay methods (Fig. 1). Rickettsial titers for day 3 were comparable to those for day 6 but were slightly less than those for days 4 and 5 (Fig. 1). RMSF infection was least severe, with 25% mortality, in monkeys which received $10^{4.5}$ CELD₅₀ of rickettsiae harvested from DEC cultures on day 1 or 2 (Table 1). Group II monkeys inoculated with $10^{5.5}$ CELD₅₀ of rickettsial organisms harvested from days 3 and 6 exhibited 50% mortality with a mean incubation period of 1.2 days (Table 1). The disease was 100% lethal only in those monkeys which received $\geq 10^{6.0}$ CELD₅₀ of rickettsiae from DEC culture material harvested on day 4 or 5 (Table 1). Duration of illness and time to death were generally shorter in this latter group (Table 1). All

monkeys that died exhibited lesions on histopathologic examination compatible with a diagnosis of RMSF.

In a subsequent experiment, a large number of DEC cultures were inoculated with R. rickettsii and harvested on day 4 postinoculation. The respective GPIPID₅₀ and CELD₅₀ titrations showed 7.5 and 6.3 logs of rickettsiae in this material, which is consistent with titers shown in Fig. 1. Twenty monkeys infected with this pool exhibited 100% morbidity and 90% mortality with nearly all deaths occurring between days 3 and 6 postinfection (4).

Clearly, the yield of rickettsial organisms depends upon the harvest time of inoculated DEC cultures. Stained smears do not distinguish between live and dead rickettsiae; therefore, they should not be used as a means of determining infectivity. Guinea pig and chicken embryo titrations indicate that materials harvested on day 4 or 5 yielded the highest titers and showed the greatest infectivity (shortest incubation period, highest mortality and earliest deaths) for rhesus monkeys. This is approximately 2 days earlier than the previously recommended harvest time for DEC culture material (3). Inocula from earlier (days 1 to 3) or later (day 6) harvest materials were not consistently infective for monkeys. With the exception of materials harvested on days 1 and 2, the guinea pig assay appears to be slightly more sensitive than the chicken embryo assay.

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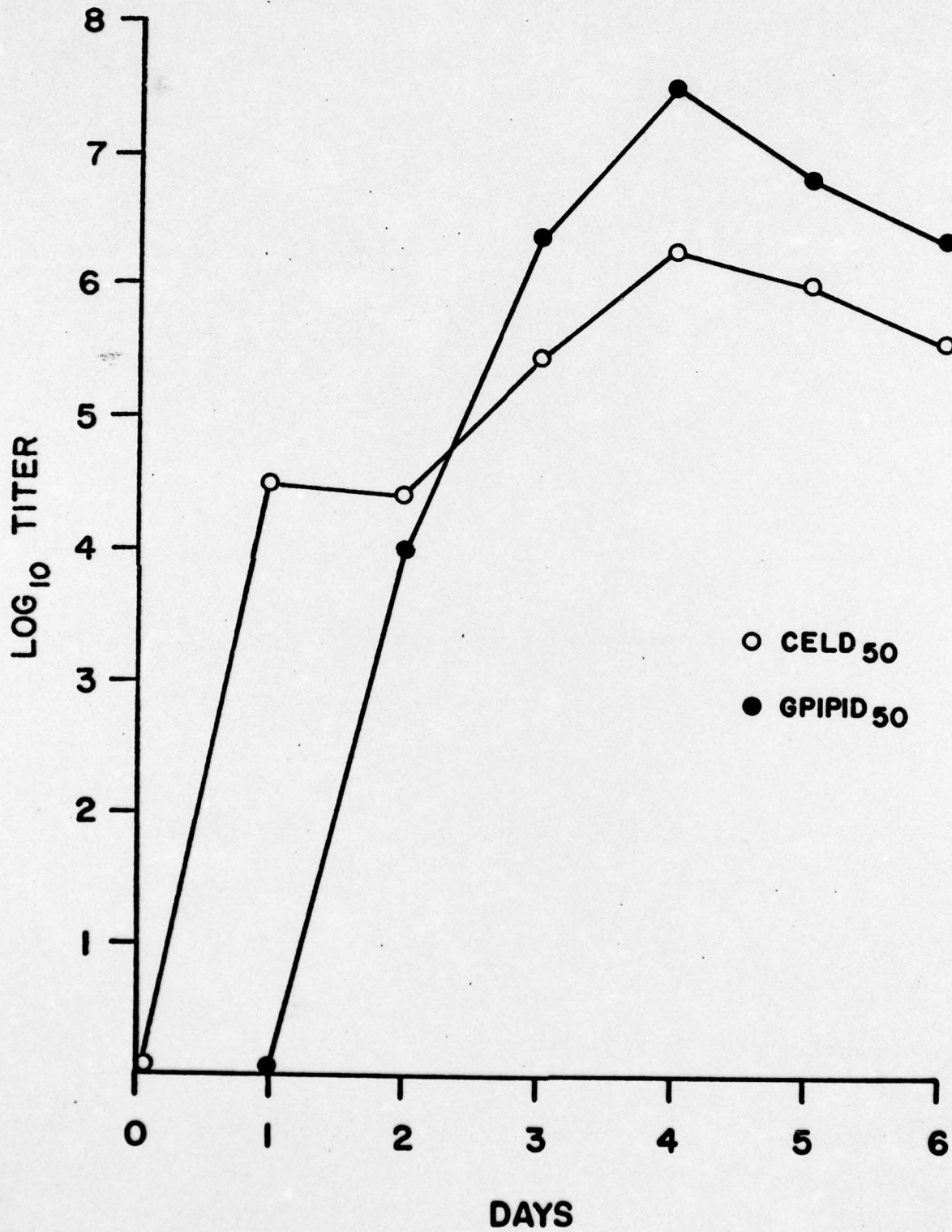
The pathogenesis of Rocky Mountain spotted fever in monkeys infected by inhalation. Arch. Pathol. 84:486-494.

TABLE 1. Dose response of R. rickettsii grown in DEC cultures and inoculated i.v. in rhesus monkeys (n=4)

Inoculum Log ₁₀		Days (mean)		
GPIPID ₅₀	CELD ₅₀	Incubation period	Time to death	Duration of illness in survivors
4.0	4.4-4.5	2,2,4,4 (3.0)	6,_,_,_	_,8,5,8
6.3	5.4-5.6	1,1,2,1 (1.2)	3,5,_,_	_,_,7,10
6.8-7.5	6.0-6.2	1,1,1,1 (1.0)	3,4,4,5 (4.0)	---

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FIG. 1. R. rickettsii titers by day postinfection in DEC cultures
assayed by two methods: CELD₅₀ and GPIPID₅₀.



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CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Effect of Harvest Time of <u>Rickettsia rickettsii</u> from Duck Embryo Cell Cultures on Rocky Mountain Spotted Fever Infection in the Rhesus Monkey		5. TYPE OF REPORT & PERIOD COVERED (9) Interim report
7. AUTHOR(s) Gerald L. Ruch and Duane E. Hilmas		6. PERFORMING ORG. REPORT NUMBER
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Army Medical Research Institute of Infectious Diseases SGRD-UIA-A Fort Detrick, Frederick, Maryland 21701		8. CONTRACT OR GRANT NUMBER(s)
11. CONTROLLING OFFICE NAME AND ADDRESS		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS (16) (17) 62776A 3M762776A841 00 008
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		12. REPORT DATE (11) 15 December 1976
		13. NUMBER OF PAGES 10 (12) (12 p.)
		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES Reprints bearing assigned AD number will be forwarded upon receipt. To be submitted for publication as a note in <u>Applied and Environmental Microbiology</u> .		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Rocky Mountain spotted fever Rhesus monkey Duck embryo cell culture Infectivity		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) This study was undertaken to determine the optimum harvest time for infected duck embryo cell (DEC) cultures to yield the highest rickettsial titers and greatest infectivity for rhesus monkeys. A consistently reproducible Rocky Mountain spotted fever-infected monkey model for pathogenesis studies and testing vaccine is produced when the inoculum given intravenously contains ≥ 10 ^{6.0} chick embryo median lethal dose organisms. This is optimally attained with day-4 DEC culture harvest material. 1000000		

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