Rickettsialpox. II. Recovery of *Rickettsia akari* from mites, *Allodermanyssus sanguineus*, from West Hartford, Conn.*† (19685)

ELIZABETH B. EUSTIS AND HENRY S. FULLER. (Introduced by John C. Snyder.)

From the Department of Microbiology, Harvard School of Public Health, Boston.

During December, 1951, and January, 1952, 7 cases of rickettsialpox occurred in persons exposed in an apartment house located in West Hartford, Conn.(1). Samples of mites collected by Dr. M. E. Rindge from the walls of incinerators in the basement of the building concerned were received in this laboratory through the courtesy of Dr. John R. Paul, Professor of Preventive Medicine, Yale University School of Medicine.‡ The mites arrived in a tightly stoppered screw cap bottle which was packed in solid carbon dioxide. It is the purpose of the present paper to report the recovery and characterization of *Rickettsia akari* from these frozen mites by two different methods.

Materials and Methods. The laboratory procedures used for the primary recovery of this strain in mice and in developing chick embryos differed in no important respects from those previously described(2). These involved (a) preparation of a suspension of mites ground in sucrose PG(3); (b) inoculation of mice with portions of this suspension by the intra-abdominal route; and (c) injection of the yolk sacs of developing chick embryos with mite suspension, treated with antibiotics to suppress the growth of bacterial contaminants presumably present upon the surfaces of the mites. The criteria for the recognition of *R. akari* which were used in our previous publication(2) were followed in this investigation.

Observations. After the vial containing mites had been quickly thawed at 37°C, it was chilled in an iced bath. Three mites were mounted and identified by one of us (H.S.F.) as *Allodermanyssus sanguineus* (Hirst), and additional specimens were preserved for future reference. The remaining 20 to 25 mites, mainly females, were ground in chilled sucrose PG. A portion of the resulting suspension was used for inoculation of mice, the remainder being shell frozen at −72°C (no. 5559) and used subsequently for strain recovery by inoculation of chick embryos.

1. *Recovery by inoculation of albino laboratory mice*: Each of 6 test mice received 0.2 ml of mite suspension by the intra-abdominal route; one of them was sacrificed on the eighth day following inoculation because it appeared to be slightly sick. Rickettsia-like objects were noted in smears of peritoneal exudate and spleen. A suspension of ground liver and spleen in sucrose PG was used for inoculation of several second-passage mice, and for storage at −72°C (no. 5560B). Further serial passages were not performed. Five first passage mice and 4 second passage mice subsequently survived intra-abdominal challenge doses of the MK strain(4) administered 35 and 27 days respectively following injection of the immunizing inocula.

2. *Establishment in chick embryos of the strain recovered in mice*: The strain recovered in mice was passed to chick embryos by the following procedure. On the eighth day following inoculation with mite suspension, an intact, anesthetized, apparently well, first passage mouse was injected intra-abdominally with 2 ml sucrose PG. The liquid was withdrawn, reinjected and again withdrawn to obtain peritoneal washings from an intact mouse

* This study was sponsored by the Commission on Virus and Rickettsial Diseases, Armed Forces Epidemiological Board, and was supported in part by a contract with the Office of The Surgeon General, Department of the Army, and in part by grants from Lederle Laboratories, American Cyanamid Company, and the Division of Research Grants of the National Institutes of Health, U. S. Public Health Service.

† It is a pleasure to acknowledge the technical assistance of Misses Judith C. Miller and Avis H. Ofstrock, as well as the encouragement and guidance of Dr. John C. Snyder.

‡ The following individuals were instrumental in securing the material and data for this study: Dr. M. E. Rindge, and Dr. James C. Hart (Director), Bureau of Preventable Diseases, and Dr. F. L. Mickle, Conn. State Dept. of Health; Dr. C. E. McPartland, West Hartford, and Dr. E. Nichols of Hartford.

under aseptic conditions. The material in the syringe was used for inoculation of seven-day-old eggs, the yolk sac of each of 7 developing embryos receiving 0.25 ml peritoneal washings. Rickettsia-like objects were seen in smears of each of the yolk sacs of embryos found dead on the sixth and seventh days following inoculation, as well as in each of the yolk sacs of 3 living embryos sacrificed on the seventh day. These infected yolk sacs were pooled, ground with alundum, diluted to 50% with sucrose PG, and centrifuged for 5 minutes at 900 RPM to facilitate separation of gross particles and yolk cake. The resulting suspension (no. 5568) was used for (1) inoculation of 60 eggs from which a yolk sac pool was subsequently made (no. 5564); (2) titration in mice; and (3) subsequent challenge of mice in a reciprocal cross-immunity test with the MK strain. When pool No. 5568 was titrated at dilutions of 10^{-1} through 10^{-7}, by inoculating 0.25 ml doses by the intra-abdominal route to 10 mice for each dilution, the numbers of survivors on the 15th day were 0, 4, 8, 4, 7, 9 and 9 respectively.

When the survivors were challenged 20 days later with at least 10 certainly fatal doses of the MK strain by the intra-abdominal route, all mice survived, giving evidence of solid immunity through 10^{-7}. Although no immunizing endpoint was obtained, therefore, the results indicated the presence of at least 10^6 immunizing doses and approximately one certainly fatal dose in each 0.25 ml portion of 10% suspension.

A further point related to the response of mice to *R. akari* concerns the possibility of a toxic effect in mice similar to that produced by concentrated suspensions of *R. prowazeki*, *R. mooseri*, and certain strains of *R. tsutsugamushi* (syn.: *R. orientalis*). Accordingly, groups of mice were inoculated by the intravenous route (tail vein) with 0.25 ml portions of yolk sac pool no. 5564 in the following dilutions: 20, 6, 2, and 0.6%. Inasmuch as the first deaths were observed on the fourth day, and these in the 20% group, it was inferred that no toxic effect had been demonstrated. Organisms failed to grow in broth or blood agar cultures of any of the yolk sac materials.

3. *Recovery of* R. akari *by direct inoculation of chick embryos with mite suspensions*: The original suspension of mites in sucrose PG (no. 5559) was thawed; a 0.25 ml portion was removed, mixed with 0.25 ml normal yolk sac suspension to act as a nutrient, and incubated for 2 hours at 36°C, in order to provide circumstances conducive to division of bacteria presumably present. After this preliminary incubation, 0.25 ml portions of solutions in K7G(3) in penicillin, 200 units per ml, and streptomycin, 200 mcg per ml, were added and the resulting mixture was incubated for 1.5 hours at 36°C. Inoculation of 4 chick embryos was then performed, the yolk sac of each receiving 0.2 ml of incubated suspension, followed shortly by 0.2 ml of a 2% solution of methyl cellulose containing approximately 11 mg of sulfadiazine.

A sick embryo was sacrificed on the seventh day following inoculation; examination of yolk sac smears revealed rickettsia-like objects, but no bacteria were noted, and no growth of organisms was observed in broth or blood agar cultures of the material. When the yolk sacs of the 3 remaining eggs were harvested on the eighth day following inoculation, rickettsia-like objects were seen in smears of one living and 2 dead embryos. Eight mice were inoculated with a 1% suspension of the egg sacrificed on the seventh day; all were found dead on the sixth and seventh days. Examination of smears of peritoneal exudate, and cut surfaces of liver and spleen revealed large numbers of rickettsia-like organisms within mononuclear cells, and the gross findings were consistent with rickettsialpox infection in albino laboratory mice. It was concluded that a rickettsial strain had been recovered from mites by the relatively simple and rapid procedure of direct inoculation of chick embryos.

4. *Specific complement fixing antibodies produced in response to the Hartford strain*: The serologic evidence recorded for human sera in Table I indicates a response of the Hartford patient to a member of the Rocky Mountain spotted fever group of rickettsiae, and when considered in the light of the pathogenicity of the Hartford strain for mice, it provides evidence in favor of rickettsialpox.

5. *Reaction of Hartford yolk sac antigen with MK immune serum to fix complement*: A rickettsial suspension derived from a yolk

TABLE I. Observations on Complement Fixation Reaction in Characterization of the Hartford Strain of *Rickettsia Akari*.

Serum	Highest serum dilution in which complement is fixed by the specific antigen			
	Rickettsialpox MK	Rickettsialpox Hartford	Epidemic typhus (soluble)	Q fever
Human				
Hartford, rickettsialpox, 5th wk after onset	1:320	1:640	0	0
MK immune	1:320	1:320	0	0
Q fever immune	0	0	0	1:320
Normal	0	0	0	0
Guinea pig				
MK immune	1:320	1:320	0	0
" "	1:320	1:320	0	0
Epidemic typhus immune	0	0	1:320	0
" " "	0	0	1:640	0
Murine " "	0	0	1:320	0
Normal	0	0	0	0

Tests were performed by the method of the Army Medical Department Research and Graduate School(5), modified by the use of a magnesium buffer as diluent(6). The antigens were used at a constant dilution of 2 units. 0 = negative at 1:10.

sac pool (no. 5564, representing the second egg passage of the strain recovered by direct inoculation of eggs with mite suspension) was used for preparation of antigen by Method 1 of Topping and Shepard(7), as modified by Boyd(8). Results of parallel complement fixation tests in which this antigen and antigen prepared from the MK strain were performed, using various sera, are summarized in Table I. These observations are taken to indicate that antigens prepared from the Hartford and MK strains are indistinguishable in the complement fixation test as performed.

6. *Reciprocal cross immunity in mice between the Hartford and MK strains of* R. akari. The principles and methods used were similar to those described in a previous paper (2) concerned with characterization of strains of *R. akari*. The Hartford mite strain pool (no. 5568) used in immunizing and challenging mice has been described. On the basis of the titration, it was believed that each 0.25 ml portion of 10% suspension would kill mice between the fifth and eighth days following inoculation of non-immune animals by the intra-abdominal route, hence the use of this dose for challenge. It is possible that loss of titer had occurred, inasmuch as 4 of 16 normal mice survived inoculation with this dose. The MK pool (no. 5530) used for immunizing and challenging mice consistently killed animals which received 0.25 ml of 10% suspension by the intra-abdominal route, and data obtained independently indicated that this corresponded to at least 10 certainly fatal doses. The MK immune mice were inoculated with 0.25 ml of a 10^{-5} dilution of this pool as an immunizing inoculum. The mice were challenged on the 20th day following administration of the immunizing doses. Survivors were recorded on the 15th day as indicated in Table II. The results are interpreted as revealing solid and complete reciprocal cross immunity between the Hartford and MK strains as tested in mice. This fact is regarded as the final piece of evidence that the Hartford strain is indistinguishable from *R. akari*.

Discussion. The recovery of a strain of *R. akari* from *Allodermanyssus sanguineus* (Hirst), collected in West Hartford, Conn.,

TABLE II. Summary of Cross Immunity Tests in Mice with Hartford and MK Strains.*

Immunizing strain	Challenge strain Hartford	Challenge strain MK
Hartford	11/12	38/38
MK	14/14	12/12
Normal controls	4/16	0/20

* Ratio: Survivors to total challenged.

is of interest from several standpoints. It establishes an additional locality record for this rickettsial species which had been positively identified previously only in New York and Boston. It is to be expected that additional strain recoveries will extend the known distribution of *R. akari*. The present strain recovery provides evidence that the patients diagnosed as having rickettsialpox were exposed in an environment where the hazard of infection was present. Finally, this strain recovery, indicating the presence of the causative agent in mites collected in a natural environment, provides additional evidence for the vector role of the house mouse mite, the species previously incriminated by the studies of Huebner, Jellison, and Pomerantz(9) in New York.

The recovery of the present strain by inoculation of mice was supplemented by isolation accomplished by direct inoculation of developing chick embryos with mite suspension which has been appropriately treated. This simple method of strain isolation may be applicable to other problems involving recovery of rickettsiae or viruses from potentially infected arthropods.

Summary. The recovery of a strain of *Rickettsia akari* from mouse mites, *Allodermanyssus sanguineus* (Hirst), collected in West Hartford, Connecticut, is described. The evidence for its identification as *R. akari* is presented, and certain implications of these findings are discussed.

1. Rindge, M. E., *Conn. Health Bull.*, 1952, v66, 73.
2. Fuller, H. S., Murray, E. S., Ayres, J. C., Snyder, J. C., and Potash, L., *Am. J. Hyg.*, 1951, v54, 82.
3. Bovarnick, M. R., Miller, J. C., and Snyder, J. C., *J. Bact.*, 1950, v59, 509.
4. Huebner, R. J., Stamps, P., and Armstrong, C., *Pub. Health Rep.*, 1946, v61, 1605.
5. Plotz, H., Bennett, B. L., Wertman, K., Snyder, M. J., and Gauld, R. L., *Am. J. Hyg.*, 1948, v47, 150.
6. Mayer, M. M., Osler, A. G., Bier, O. G., and Heidelberger, M., *J. Exp. Med.*, 1946, v84, 535.
7. Topping, N. H., and Shepard, C. C., *Pub. Health Rep.*, 1946, v61, 701.
8. Boyd, H. M., *J. Lab. Clin. Med.*, 1951, v38, 313.
9. Huebner, R. J., Jellison, W. L., and Pomerantz, C., *Pub. Health Rep.*, 1946, v61, 1677.

Received May 26, 1952. P.S.E.B.M., 1952, v80.

Effect of Glucose on Oxygen Consumption and Respiratory Quotient of Normal and Denervated Muscle.* (19686)

CHARLES M. GRUBER, JR., KWANG SOO LEE, AND ZINAIEDA LASHICHENKO.
From the Department of Pharmacology, Jefferson Medical College, Philadelphia, Pa.

The oxygen consumption of denervated muscle was first studied by Langley and Itagki(1). They compared the oxygen content of arterial and venous blood from innervated and denervated extremities of anesthetized cats. Since that time several workers have studied the oxygen consumption of denervated muscle *in vivo*(2,3) and *in vitro* (4.5). These various reports are not in agreement with each other as to the extent of the increase in oxygen consumption by the denervated muscle. Changes in various respiratory enzyme systems following denervation have been reported by Knowlton and Hines (4) and Humoller *et al.*(6,7). The experiments upon which this report is based were done with the hope of clarifying the effect of denervation on the oxygen consumption and respiratory quotient of muscle tissues. The influence of glucose in the perfusate on the oxygen consumption and the respiratory quotient of muscle was also investigated.

Method. One hundred young white rats weighing between 100 and 120 g were used. Unilateral phrenectomy was performed under ether anesthesia by destroying one phrenic nerve in the neck. Ten to 14 days after sec-

* This investigation was made possible by a grant from Geigy Co., Inc.