9% levels. If Mg were required merely to form part of the structure of new tissue, at least as much weight would be gained on a 14% protein intake as on a 9% protein intake with Mg-deficient rations.

Comparative studies of mineral supplements, conducted on a 7% protein intake (about 120 mg N daily, the quantity employed by Frost and Sandy(3) in studying Mg-deficiency), may fail to reveal injurious effects of deficient mineral rations, because such effects may only become evident when N-intake is sufficient to assure a normal or optimal rate of protein synthesis.

Summary. Adult protein-depleted rats receiving a complete mineral supplement gain over twice as much in 10 days on a 14% level of protein intake as on a 7% (120 mg N daily) level, but those receiving a Mg-deficient ration gain over twice as much on a 7% level of protein intake as on a 14% level. These results indicate that the Mg requirement for normal protein metabolism increases during protein synthesis as protein intake is increased from 7% to 14% and that the increased Mg requirement is not merely for the structural inclusion of Mg in new tissue. A 7% level of protein intake permits only a markedly subnormal rate of protein synthesis in a previously depleted animal, but 9% and especially 14% levels of protein intake insure more normal rates of protein synthesis and reveal the markedly deleterious effects of magnesium-deficient rations on protein synthesis within 10 days.

The author is indebted to Dr. Edith E. Sproul for the opportunity and facilities to perform the experimental work reported herein.

1. Menaker, W., and Kleiner, I. S., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 377.

2. Cannon, P. R., Frazier, L. E., and Hughes, R. H., *Metabolism,* 1952, v1, 49.

3. Frost, D. V., and Sandy, H. R., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 102.

4. McCollum, E. V., and Orent, E. R., *J. Biol. Chem.,* 1931, v92, xxx (Proceedings).

Received November 24, 1953. P.S.E.B.M., 1954, v85.

Human Body Lice. IV. Direct Serial Passage of Typhus Rickettsiae by Oral Infection.* (20814)

HENRY S. FULLER.† (Introduced by J. C. Snyder.)

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

In connection with projected studies(1) of mutations in microorganisms which propagate in arthropods, methods for performing serial passages are necessary. This paper describes a simple method for serial passage of rickettsiae in lice and presents the results of its application to experimental studies of typhus.

Materials and methods. The origin and maintenance of our normal louse colony has been described(2). When lice were selected for these experiments, they were insects of the third instar, starved for 18 to 24 hours before the infective meal was offered. Experimental infections were initiated by using yolk sac propagated organisms of the Wilmington and Florida(3) strains of *Rickettsia mooseri* and the E strain(4) of *R. prowazeki.* Infection of lice was accomplished by a chicken skin membrane technic(5), and infected lice were fed daily upon normal rabbits(6).

Serial passage. Two or more infected lice

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

† Formerly Assistant Professor of Microbiology, Present address: Department of Entomology, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C. For technical assistance in this study, the writer is pleased to express his appreciation to Elizabeth B. Eustis, Patricia F. Cottam, and Doris L. Simon.

were examined daily for the presence of rickettsia-like organisms in smears of intestinal tissue stained by Macchiavello's method. When numerous rickettsiae were seen, it was probable that any single louse of the group contained sufficient organisms to act as a donor in passage. Accordingly, 3 or 4 recently emerged adult lice were allowed to cling to the fibers of a sterile cotton swab. Since the integument of recently emerged lice had been exposed for the shortest possible time to bacterial contamination, the use of such insects was preferable. In order to reduce the numbers of surface contaminants, lice were washed by plunging the swab into a 2 ml portion of sterile K7G(7) at room temperature and rotating it rapidly for 1 minute. This procedure was repeated with 10 to 15 fresh portions of sterile liquid. The washed lice were placed in a dry sterile petri dish awaiting dissection. Lice were dissected under aseptic precautions. A single insect was placed in a small drop of K7G(7) on a chilled slide which rested on a covered petri dish containing cracked ice. A small portion of intestinal tissue was teased free and smeared on the surface of the slide, while the remaining intestinal tissue and contents were aspirated through a 23 gauge needle attached to a 1 ml syringe containing diluent. The contents of this syringe were kept chilled.

A prospective donor louse was regarded as suitable if numerous rickettsiae, but no bacteria, were seen in the smear preparation. The contents of the corresponding syringe were ejected into a small chilled tube and aspirated several times in order to macerate the infected tissues. The resulting suspension was mixed with defibrinated or heparinized blood of a normal rabbit. This mixture was then offered by the membrane technic to lice of the next consecutive passage. After recipient lice had fed to repletion, a portion of the meal was often used for injection of animals to detect viable rickettsiae. The remainder of the meal was frozen in an ampoule and stored at −70°C. Surviving lice of a given passage were maintained alive until rickettsiae had been seen in smears of lice of the next passage. At that time, lice of the previous passage were sacrificed, frozen, and stored at −70°C.

TABLE I. Complement Fixation Patterns of Sera of Cotton Rats Inoculated with Viable *Rickettsia mooseri* after Louse Passage.*

Strain	Louse passage	Denominator of highest serum dilution in which complement was fixed by specific antigen: Murine	Epidemic
Wilmington	3rd	320	40
"	19th	640	40
Florida	2nd	480	30
"	7th	640	80

* Complement fixation tests were performed according to the method of the Army Medical Service Graduate School(18).

Results. The Wilmington strain of *R. mooseri* was maintained for a total of 95 days through 19 consecutive louse passages. Lice of a given passage were incubated for 3 to 6 days at 32°C before the strain was passed to the next group. The tissues of a single louse were mixed with diluent and blood each time, the total volume of meal offered to recipient lice being 1.2 to 1.3 ml. The dilution thus effected made it unlikely that 19th passage lice contained surviving rickettsiae of the original yolk sac material. Evidence of multiplication of rickettsiae was provided by the massive numbers of organisms in smear preparations.

In an essentially similar manner, the Florida strain of *R. mooseri* was maintained for a total of 43 days through 8 consecutive louse passages. The serologic patterns of cotton rats inoculated with these 2 murine typhus strains after louse passage were typical of *R. mooseri* in the complement fixation reaction (Table I).

The E strain of *R. prowazeki* was maintained for a total of 79 days through 5 louse passages.‡ Although infected lice of the final passage contained rickettsiae which evoked typical serologic patterns in test animals, studies of the ability of the strain to evoke a febrile reaction in guinea pigs were unsatisfactory, owing to the atypical reactions of control animals.

Additional experiments were designed to test the ability of *R. mooseri* to multiply in lice which were nourished on serum instead of

‡ These passages were performed by Peter Skaliy while a student in this Department.

blood containing formed elements. Lice which had ingested a 10% suspension in K7G of yolk sac infected with the Wilmington strain were subsequently nourished on single daily meals of Seitz-filtered human serum, which was offered by the membrane technic. Smears of the intestines of these lice provided visual evidence of multiplication of rickettsiae after three days. A second series of lice ingested a meal composed of the intestines of 2 lice of the 7th passage of the Wilmington strain, suspended in a mixture of 0.4 ml K7G and 1 ml serum. During the first 2 days thereafter, the lice fasted at 27°C. On each subsequent day, they were offered meals of normal serum and maintained at 32°C. Visual evidence of multiplication of rickettsiae was provided by smears of lice incubated for 3, 4, and 5 days following ingestion of the infective meal. The cloudy, moth-eaten appearance of the cytoplasm of the intestinal lining cells suggested that the lice were suffering from nutritional deficiency. All lice but one were dead by the 5th day. No change in murine properties of the rickettsiae was demonstrated by the serologic patterns of cotton rats inoculated with a suspension of this 5th day louse. Further passages in lice nourished on serum were not attempted.

Discussion. By means of the present technic, passage of rickettsiae from donor to recipient lice is accomplished without the interposition of a different infected host species. Rickettsiae are administered by the oral route, as opposed to the method of rectal injection. The only serious technical problem encountered in the present study was the death of recipient lice following ingestion of a meal containing bacterial contaminants from the tissues or surfaces of donor lice. Previous studies of serial passage of rickettsiae in lice have employed the technics of intra-rectal injection(8-14), inoculation into the body cavity of the insect(13,15), or the feeding of lice by a bleb technic(4,16).

It is of interest that no fundamental change in the properties of a strain of rickettsiae has been reported after serial louse passages. The derivation of the E strain from the Madrid strain(4) of *R. prowazeki,* however, demonstrates that the properties of rickettsiae are not inflexible. A further example consists of the changes in virulence of *R. rickettsi* observed during passages in suitable animals (17). Hence the possibility of changes in properties of rickettsiae during passage in arthropods merits further study.

Summary. A simple feeding technic for serial passage of rickettsiae in human body lice is described. Consecutive louse passages are not interrupted by growth of the organisms in other host species. Application of the method to serial passages of epidemic and murine typhus rickettsiae is reported. No changes in properties of the strains were noted during louse passage. The multiplication of murine typhus rickettsiae in lice nourished only on human serum is reported.

1. Snyder, J. C., *Postgraduate Med.,* 1953, v13, 331.

2. Fuller, H. S., *Am. J. Hyg.,* 1953, v58, 188.

3. Rickard, E. R., *Am. J. Trop. Med.,* 1951, v31, 306.

4. Perez Gallardo, F., and Fox, J. P., *Am. J. Hyg.,* 1948, v48, 6.

5. Fuller, H. S., Murray, E. S., and Snyder, J. C., *Pub. Hlth. Rep.,* 1949, v64, 1287.

6. Snyder, J. C., and Wheeler, C. M., *J. Exp. Med.,* 1945, v82, 1.

7. Bovarnick, M. R., Miller, J. C., and Snyder, J. C., *J. Bact.,* 1950, v59, 509.

8. Weigl, R., *Beitr. z. Klin. d. Infektionskr. u. z. Immunitätsforsch.,* 1920, v8, 353.

9. da Rocha-Lima, H., and Sikora, H., in Abderhalden, E., *Handbuch der biologischen Arbeitsmethoden,* Abt. XII, Teil 1, 1925, 769.

10. Bacot, A., and Segal, J., *Brit. J. Exp. Path,* 1922, v3, 125.

11. Weigl, R., *C. R. Soc. Biol., Soc. Polon. Biol.,* 1930, v103, 823.

12. Weyer, F., *Z. Hyg. u. Infektionskr.,* 1952, v135, 280.

13. ———, *Acta Tropica,* 1952, v9, 170.

14. ———, *Am. J. Trop. Med. Hyg.,* 1952, v1, 809.

15. ———, *Z. Tropenmed. u. Parasitol.,* 1950, v2, 40.

16. Murray, E. S., and Snyder, J. C., *Am. J. Hyg.,* 1951, v53, 22.

17. Price, W. H., *Science,* 1953, v118, 49.

18. Scoville, A. B., Jr., Bennett, B. L., Wertman, K., and Gauld, R. L., *Am. J. Hyg.,* 1948, v47, 166.

Received November 30, 1953. P.S.E.B.M., 1954, v85.