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# Studies on Typhus Rickettsiae Cultivated in Yolk Sac of Developing Chick.

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Although Barykine, *et al.*,[1] first reported the use of the yolk sac as a new culture medium for the classical type of typhus rickettsiae in 1936, little attention was paid to this method until Cox[2] showed conclusively that egg yolk is indeed an excellent medium for the growth of all types of rickettsiae. Since that time, a great deal of interest has been aroused in the practical application of this method, particularly for the production of an immunizing agent.[3, 4, 5] However, it appears to us that in order to make full use of this valuable procedure, a better understanding of the characteristics of rickettsiae so cultivated is desirable. In view of this, the results of preliminary studies carried out with local murine typhus strains are hereby reported.

The simple technic originally employed is followed in principle. Special attention was paid to the localization of the rickettsiae in the yolk sac, the successful cultivation from the blood of infected animals, and to the virulence of the organisms so cultivated for the white mice.

1. *Localization of the rickettsiae:* In spite of the statements made by previous workers[2, 6] that no intracellular organisms were found, it seems to us more reasonable to expect typhus rickettsiae to grow intracellularly in the yolk sac as in other culture media. By examinations of smear of the suspension of yolk sac cells, which float on the surface on brief centrifugation rickettsiae can invariably be demonstrated inside the cells in positive cultures. It is true that some extracellular organisms can also at times be found but they can be regarded as having been liberated from cells. This is in agreement with the recent findings of Rake and his co-workers[7] in their cultivation experiments with virus of lymphogranuloma inguinale.

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1 Barykine, W., *et al., Proc. 2nd Internat. Congress Microbiology,* 1936, 322; *Bull. Office Internat. Hyg. Publ.,* 1938, **30**, 326.

2 Cox, H. R., *U. S. Pub. Health Rep.,* 1938, **53**, 2241.

3 Cox, H. R., *U. S. Pub. Health Rep.,* 1939, **54**, 1070.

4 Otto, R., and Wohlrab, R., *Z. f. Hyg. Infekt.,* 1939, **122**, 220.

5 Zinsser, H., Plotz, H., and Enders, J. F., *Science,* 1940, **91**, 51.

6 Rake, G., *et al.,* PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 332.

7 Tchang, J., and Mathews, G. B., *Chinese Med. J.,* 1940, **57**, 47.

2. *Isolation of typhus rickettsiae from the blood:* As the yolk sac affords a suitable medium for massive proliferation of rickettsiae it was considered of interest to see if the less heavily infected blood of typhus guinea pigs would, as in the case of more heavily infected blood of spotted fever animals,[2] initiate successful cultures. As the hemoglobin of the cells would color the contents of the egg, and seems to cause the death of the embryo, the plasma has been used with some success. In 8 trials 4 successful cultures were obtained. It is apparent that the small number of typhus rickettsiae present in the circulating blood of infected animals could initiate cultures in a certain percentage of cases.

3. *Virulence of the rickettsiae cultured in yolk sac for white mice:* Although occasional strains of murine typhus have been found to be pathogenic for white mice[8, 9] the experience in general seems to indicate that normal white mice are resistant to the inoculation of infected materials ordinarily employed and an inapparent infection was the rule. In the present investigation, both freshly isolated and passage murine strains were employed. The latter strains, "S" and "N", have not produced any demonstrable infection in white mice during several years' observation in this laboratory. However, after one or more passages in the egg yolk sac, 20 out of 28 mice inoculated with "S" rickettsiae, and 6 out of 9 with "N" succumbed to the infection. The incubation period varied from 3 to 7 days and numerous intra- and extracellular rickettsiae were variably found in the peritoneal exudate. The identity of the typhus nature was made by negative culture on ordinary laboratory media and severe infection on reinoculation into guinea pigs with the peritoneal exudates. Thus it can be conclusively stated that murine typhus rickettsiae, after brief cultivation in the yolk sac, acquire a virulence, not only for the susceptible guinea pig,[10] but also for the naturally resistant white mice.

*Comment and Summary.* By paying special attention to a few fundamental characteristics, significant findings were noted which may have important practical implications. The concentration of the organisms in the cells of the yolk sac which can easily be separated by centrifugation would suggest a simple method for the removal of much undesirable inert materials contained in the yolk sac during the preparation of vaccine. The presence of these substances has been the main objection for employing this vaccine in human prophylaxis. Successful initiation of cultures from the blood of in-

8 Okamoto, Y., *Kita. Arch. Exp. Med.*, 1937, **14**, 99.

9 Giraud, P., and Panthier, R., *Bull. Soc. Path. Exot.*, 1939, **32**, 404.

10 Parker, R. R., personal communication.

fected animals naturally leads to its possible application for diagnosis in patients, when test animals usually employed such as guinea pigs and white rats were not available. Preliminary studies in this connection have offered some indication as to its practical use. The fact that death is regularly produced in white mice by the yolk sac cultivated rickettsiae gives us an additional and convenient animal which may be employed for typhus studies. By its use, immunological and chemotherapeutic studies can be facilitated and extended.

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## Urinary Excretion of Vi-Phage and *B. typhosus* Following Phage-Inoculation in a Typhoid Carrier.

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Following the finding of Vi-phage specific for V form of *B. typhosus,* the influence of phagotherapy on typhoid infections has gained new interest. Asheshov, Wilson and Topley[1] have demonstrated the protective effect of a Vi-phage on experimental typhoid infection in mice when the phage was given not later than 4 hours after infection. In the same experiment a non-specific phage, which could lyse both V and W forms *in vitro,* was found to be ineffective. Later, Fisk[2] also showed the protective action of a typhoid phage on experimental typhoid infection in mice. He, however, did not indicate whether the phage employed was a Vi-phage or not. In view of the lack of information on the influence of Vi-phage in human infection it is considered desirable, in this communication, to present some of our observations made on the treatment of a case of a urinary carrier of *B. typhosus* with Vi-phage and non-specific phages.

The patient (CCL), male, aged 42, with a history of symptoms of pyelitis, cystitis, and urethritis for at least 18 years, was first seen 3 years ago when *B. typhosus* was detected in his urine. Roentgenological examination then suggested the presence of bilateral renal calculi. In the succeeding 3 years, the patient was given vigorous local treatment with various urinary antiseptics such as argyrol, and oral medication with sulfanilamide and mandelic acid, but the

1 Asheshov, I. N., Wilson, J., and Topley, W. W. C., *Lancet,* 1937, **1**, 319.

2 Fisk, R. T., PROC. SOC. EXP. BIOL. AND MED., 1939, **38**, 659.