took to verify their findings, Loeb and his associates[2] have reported that the same organ, when transplanted subcutaneously, causes the carcinoma rate to be *increased.* We have not attempted any transplantation experiments, but our data concerning the results of subcutaneous injection of the hormone may apply to some extent to Loeb's new interpretation of anterior pituitary function. Cramer[3] has presented an elaborate theory of the genesis of mammary carcinoma in which the pituitary takes an important rôle as an antagonist of the disease. His theory would appear to be premature.

Summary. Long continued subcutaneous injection of an anterior pituitary preparation which contained thyrotropic hormone and some gonadotropic hormone did not definitely affect the incidence of mammary carcinoma in female mice of the RIII strain.

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Typhus Rickettsia Isolated from Mice and Mouse-Fleas During an Epidemic in Peiping.*

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It is now generally recognized that "endemic" or murine typhus has its reservoir in rats and is transmitted to man by rat-fleas, particularly *Xenopsylla cheopis.*[1, 2] However, in South Queensland, Australia, circumstantial evidence strongly incriminates mice, rather than rats, as the reservoir, although actual proof of infection among these rodents or their ectoparasites is as yet lacking.[3, 4] Lépine and Lorando,[5, 6] in Athens, examining rats as well as mice trapped in the premises where cases of typhus had occurred, found that whereas rats were frequently infected with typhus Rickettsia, mice were

2 Loeb, L., and Kirtz, M. M., *Am. J. Cancer,* 1939, **36**, 56.

3 Cramer, W., *Am. J. Cancer,* 1940, **38**, 463.

* We are indebted to Dr. A. B. D. Fortuyn for identification of the species of mice and to Dr. H. F. Hsü for that of fleas.

1 Biraud, Y., and Deutschman, S., *League of Nations Epidem. Rep.,* 1936, **15**, 99.

2 Zinsser, H., *Am. J. Hyg.,* 1937, **25**, 430.

3 Wheatland, F. T., *Med. J. Australia,* 1926, **1**, 261.

4 Strickland, C., *Far East. Assn. Trop. Med. Trans. Ninth Congress,* 1927, **2**, 517.

5 Lépine, P., *Compt. rend. Soc. de biol.,* 1934, **117**, 848.

6 Lépine, P., and Lorando, N., *Bull. Soc. path. exot.,* 1936, **29**, 285.

uniformly free from the infection. Sparrow,[7] on the other hand, recovered 2 typhus strains from the pooled brain emulsions of 10 out of 300 mice *(Mus musculus gentilis)* caught at random from different households in Tunis. Brigham[8] also obtained a typhus strain from a field-mouse *(Peromyscus polionotus poliomotus)* in the rural district of southern Alabama where cases of typhus had developed under conditions which made the rat a "highly improbable causative factor." It is clear from the above reports that hitherto the association of typhus-infected mice and mouse-fleas with the disease in man has not been conclusively demonstrated, and hence the rôle of mice as another reservoir of "endemic" typhus can not be considered as settled.

In March, 1940, a typhus epidemic occurred in a small household in this city, affecting successively 9 out of 10 members of the family. In all of them, the clinical symptomatology was characteristic; 2 were admitted into this hospital and showed, in addition, a positive Weil-Felix reaction.† They admitted that they harbored lice through the greater part of the year and not infrequently encountered fleas in their clothings and beddings. Rats were scarce, but the house was heavily infested with mice. Four mice *(Mus wagneri)* were captured, and 12 fleas collected from these animals were identified as *Leptopsylla musculi.* By inoculating separately the brains of the mice and 10 of the fleas into the peritoneal cavity of 2 guinea pigs, 2 typhus strains were recovered. Each strain was passed through guinea pigs for 11 generations. In most of the animals, the infection was manifested by fever (40.0-41.5°C), lasting from 2 to 9 days, after an incubation period of from 2 to 11 days. In those sacrificed 10 to 14 days after the onset of fever, typhus nodules were either absent or scanty in the brains. In some of the animals, the tunica vaginalis exhibited various degrees of congestion and exudation, but in none was a scrotal swelling observed. After 4 and 5 passages in guinea pigs, the 2 strains were transferred to albino rats and have been carried separately from rat to rat for 10 generations to date. They produced in the rats a definite febrile reaction (38.1-39.5°C), lasting in most cases from 2 to 8 days, after an incubation period of from 4 to 10 days. Some of the animals killed on the 10th to the 18th day of infection gave a positive Weil-Felix reaction at titers varying from 1:10 to 1:640. The brains of rats of

[7] Sparrow, H., *Arch. Inst. Pasteur,* Tunis, 1935, **24**, 435.

[8] Brigham, G. D., *U. S. Pub. Health Rep.,* 1937, **52**, 659.

† From one of the cases, a third typhus strain was obtained from body lice in the garments, which so far does not seem to differ experimentally in any important respect from the mouse and the flea strains herein reported.

the 1st and 8th generations of each series were inoculated into guinea pigs. All of the 4 animals used developed typical fever; the one inoculated intraperitoneally with the brain of a rat of the 8th generation of the flea series showed a moderately pronounced scrotal swelling, and Rickettsiae were grown from the tunica vaginalis in Maitland culture and Zinsser agar tissue media.[9]

Comment. In 1938, one of us (Zia),[10] examining over 100 mice trapped at random from different households in this city, failed to isolate typhus Rickettsia from any of these animals. The fact that the present strains were recovered from 4 mice and their fleas in a house in which an epidemic had developed naturally suggests a causal relationship between the disease in men and the infection in mice. 1. If the disease in men were historic typhus, the epidemic having started from a human carrier, it would be indeed difficult to conceive, in view of the experimental observations of Nicolle and Giroud[11, 12] how the mice could have acquired the infection from man. 2. We are, therefore, inclined to think that the epidemic was of murine origin, the infection being conveyed accidentally from mice to man by mouse-fleas and thereon, independent of mice and mouse-fleas, from man to man by human body lice. Although the mouse-fleas, *L. musculi,* ordinarily do not attack man, it is not inconceivable that they may do so occasionally, when men live in close association with mice, especially if many of the mice should die in an epizootic, as during the years 1925 and 1928 in South Queensland. Moreover, as the number of mouse-fleas we examined was small, we are not certain that the mice were not infested with some other fleas which may leave their normal host more readily than *L. musculi.* In this connection it is worthy of note that a number of workers have shown that the Rickettsia of murine typhus is present in the urine of infected rats, and have suggested that the disease may be conveyed to man by ingestion of contaminated food without the mediation of fleas.[13-16] 3. It is, of course, possible that the initial case of the epidemic had contracted the disease from rats through rat-fleas, the mice being only secondarily involved in the course of infection among

[9] Zinsser, H., FitzPatrick, P., and Wei, H., *J. Exp. Med.*, 1939, **69**, 179.

[10] Zia, S. H., unpublished observations.

[11] Nicolle, C., and Giroud, P., *Compt. rend. Acad. d. sc.*, 1934, **199**, 1169.

[12] Nicolle, C., and Giroud, P., *Arch. Inst. Pasteur*, Tunis, 1935, **24**, 47.

[13] Marcandier, A., and Pirot, R., *Bull. Soc. path. exot.*, 1933, **26**, 349.

[14] Nicolle, C., Giroud, P., and Sparrow, H., *Arch. Inst. Pasteur*, Tunis, 1934, **23**, 1.

[15] Le Chuiton, F., Berge, C., and Pennanéac'h, J., *Bull. Soc. path. exot.*, 1936, **29**, 831.

[16] Le Chuiton, F., Pirot, R., Berge, C., and Pennanéac'h, J., *Bull. Acad. Med.*, 1938, **119**, 175.

rats, but this does not seem very probable, since mice were greatly preponderant over rats in the house, and from the observations of Lépine and Lorando[5, 6] and Sparrow,[7] it appears that natural transmission of typhus infection from rats to mice in the same households does not commonly occur. It must be said, however, that in general, inasmuch as rats are considerably more flea-infested than mice, and the common rat-flea, *X. cheopis,* attacks man much more readily than the common mouse-flea, *L. musculi,* rats are probably much more important than mice as reservoir of "endemic" typhus.

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Cultivation of *Clostridium tetani* in Unfertilized and Developing Fertilized Hens' Eggs.

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The employment of fertilized egg has proven of value for the cultivation of some bacteria and a number of filterable viruses. The chorio-allantoic membrane has been mostly used as the site of infection. Since the cultivation of anaerobic microörganisms in unfertilized and developing fertilized eggs has not been recorded, the preliminary results of such a study with an obligatory anaerobe, *Cl. tetani,* is herewith communicated.

Technic. A pure toxigenic strain of *Cl. tetani,* recently isolated from a patient, was used. Cultures in cooked meat tube after 48 hours' growth were used as the inoculum. Unfertilized and developing fertilized hens' eggs of different age were employed. After the position of the embryo and the air sac was identified by transillumination and marked off and the shell opposite the air sac was sterilized with tincture of iodine, a small opening was made with a carborundum disc attached to a dental drill, leaving the inner shell membrane intact, which was later cut with a small knife after resterilization with 70% alcohol and heat. A loopful of inoculum was then introduced through this opening to the center of the yolk sac, and in the case of fertilized eggs toward a point at some distance from the embryo. The shell aperture was then closed with melted hard paraffin. The eggs were then incubated at 37°C. Different batches of them were taken out and examined grossly and by