When New Zealand white or chinchilla rabbits were used for this test, it was found they were less sensitive than the Dutch or Belgian breeds. They required an injection of 20 or 30 units of parathormone per kg to react positively. Six chinchillas and 4 New Zealand whites all gave negative reactions when injected with 10 units per kg. With a dose of 20 units per kg of parathyroid extract only 3 of 5 chinchillas and 1 of 3 New Zealand white rabbits reacted positively, while 2 of each of these strains gave positive reactions only with a dose of 30 units per kg.

It is advisable, therefore, to determine the sensitivity of rabbits to be used for the Hamilton and Schwartz test. The animals should be at least 5 months old and they should be kept on a diet whose Ca:P ratio is one or more for several days before use.

11472 P

Agent of Lymphogranuloma Venereum in the Lungs of Mice.

MORRIS F. SHAFFER, GEOFFREY RAKE AND CLARA M. MCKEE

From the Squibb Institute for Medical Research, New Brunswick, N. J.

It has been shown[1] that the agent of lymphogranuloma venereum readily initiates a fatal infection when introduced into the yolk-sac of the developing chicken embryo, in contradistinction to the well known low-grade character of the infection which results when the virus is placed on the chorio-allantois. In the former site the minute "granulocorpuscles"[2] which are believed to represent elementary bodies of the agent are found in enormous numbers. With this source of abundant virus at hand the possibilities of intranasal infection in mice were investigated, as has also been done recently by Schoen[3] who employed virus propagated in the Ehrlich mouse sarcoma.

Two strains* of the lymphogranuloma venereum agent were

1 Rake, G., McKee, C. M., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 332.

2 Miyagawa, Y., Mitamura, T., Yaoi, H., Ishii, N., Nakajima, H., Okanishi, J., Watanabe, S., and Sato, K., *Jap. J. Exp. Med.*, 1935, **13**, 733.

3 Schoen, R., *C. R. Acad. Sci.*, 1939, **208**, 772.

* One strain was obtained through the courtesy of Dr. Wm. L. Fleming, the School of Hygiene and Public Health, Johns Hopkins University. The second was obtained through the courtesy of Dr. Marion Howard, Department of Medicine, Yale University.

studied in Swiss mice weighing usually less than 10 g. Ground suspensions of yolk-sacs heavily infected with virus were diluted tenfold with broth and centrifuged to throw down tissue fragments. 0.03 to 0.05 ml of supernatant was inoculated intranasally under light ether anesthesia. The mice which within 48 to 72 hours were manifestly ill with signs of marked respiratory involvement, were sacrificed; some died during this period. The former at autopsy showed, in one or more lobes, areas of semi-translucent gray-red consolidation varying in extent; the dead mice showed hemorrhagic consolidation of nearly all of the lung tissue. On microscopic examination of smears made by streaking a fragment of consolidated lung on a slide, fixing the film in methyl alcohol and staining with Giemsa stain, numerous elementary bodies could be seen lying free or within monocytic cells. In histological sections the picture was one of pneumonia, varying in degree but often very intense. This was both interstitial, with an accumulation of fluid and cells in the walls of the alveoli, and lobular with filling of the alveoli with fluid, monocytes and neutrophils. In the cytoplasm of certain cells, apparently the lining cells of the alveoli, there could be seen in most lungs large vacuoles filled with elementary bodies or larger inclusions both of which stained purple with Giemsa. In addition, clumps of elementary bodies were seen lying free in the alveoli and bronchioles. The microscopic changes during the development of the lung lesions will be described in greater detail elsewhere.

Serial intranasal passages in mice under light ether anesthesia were readily effected by sub-inoculation of broth suspension of affected lung tissue taken on the 2nd to 4th days after infection. With one strain 39 such lung-passages have been attained; with the other strain, 24 passages. Mice given intranasally 10^{-2} dilution of grossly diseased lung tissue usually died within 5 days with almost total pneumonic consolidation. Fatal illness was less frequent in animals receiving 10^{-3} dilution; many of these mice exhibited a transient malaise but eventually recovered. If sacrificed between the 3rd and 5th days, however, macroscopic lung lesions were found in most mice receiving 10^{-3} dilution. Lesions were not found in mice receiving 10^{-4} and 10^{-5} dilutions. Nevertheless, at the end of 4 days' infection virus was present in considerable amount in the lungs of mice receiving 10^{-4} dilution as could be demonstrated on serial intranasal passage, and it seems certain that multiplication had occurred.

The possibility that the lesions obtained in the lungs were due to organisms of the pleuro-pneumonia group or to infection with the latent virus of mouse pneumonia[4] was excluded, in the first case by

cultures on suitable media and in the second, by cross-neutralization tests with anti-sera supplied through the courtesy of Dr. F. L. Horsfall.

In the case of both strains, inoculation of 1 ml of 10^{-7} and occasionally 10^{-8} dilution of consolidated lung tissue into the yolk-sacs of 5- or 6-day eggs incubated at 36° C sufficed to bring about death of the developing chicken embryos after several days' infection. The yolk-sacs of these eggs examined immediately after death were bacteriologically sterile but showed innumerable elementary bodies in Giemsa-stained smears. These findings not only confirm the microscopic observations as to the presence of large amounts of virus in the lungs of mice intranasally infected with lymphogranuloma venereum agent but also serve to reëmphasize the delicacy of the yolk-sac technic as compared with all other methods at present available for the detection of the virus. The obvious usefulness of the pulmonary infection† of mice in immunological experimentation on lymphogranuloma venereum is being further explored.

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Complement Fixation Test in Lymphogranuloma Venereum.

CLARA M. MCKEE, GEOFFREY RAKE AND MORRIS F. SHAFFER

From the Squibb Institute for Medical Research, New Brunswick, N. J.

Although it is generally acknowledged that the cutaneous test with the Frei antigen is of great value in establishing the diagnosis of infection with the etiological agent of lymphogranuloma venereum, many workers have sought to devise other procedures which might be employed as corroborative evidence. The serological technic most widely explored in this connection has been complement fixation but, using a variety of antigens, most investigators have been unsuccessful in their attempts to demonstrate a specific reaction (for literature see[1]). Nearly all the reports of positive findings are justly open to criticism on such grounds as inadequately detailed description of the method, lack of controls, or incomplete data on the results.

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[4] Horsfall, F. L., and Hahn, R. G., *J. Exp. Med.*, 1940, **71**, 391.

† Workers should bear in mind the possible hazards involved in the use of the intranasal technic where high concentrations of virus are concerned.

[1] Melczer, N., and Sipos, K., *Arch. f. Dermat. u. Syph.*, 1937, **176**, 176.