

time samples of the serum all showed complete protective action in 0.5 cc. amounts against 5 mouse units of Follutein. The injections were continued for another 2 weeks, at the end of which time the rabbits showed multiple sores in the skin of the back, a phenomenon which had been previously noted in the animals injected with A.P.L from teratoma testis urine. All these animals continued to show a protective effect in their serum. A male rabbit injected for a month gave similar results. Twenty-seven mice tested with the sera after injection showed complete protection. Twelve control mice receiving Follutein alone all showed corpora lutea.

Cross protection experiments of this serum and the teratoma testis urine hormone were carried out. Three mice injected with the teratoma hormone alone showed corpora lutea while 9 receiving 4 mouse units of the hormone plus 0.5 cc. of serum immunized against the pregnancy hormone each showed no effect in the ovaries. Nine mice receiving four mouse units of "Follutein" plus 0.5 cc. of serum from the rabbits immunized against the teratoma hormone showed no evidence of luteinization while three control mice injected with "Follutein" alone showed corpora lutea.

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#### Successive Transmission of Virus of Lymphogranuloma Inguinale Through White Mice.

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(Introduced by Paul Reznikoff.)

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As part of a study of the properties of the virus of lymphogranuloma inguinale white mice were inoculated intracerebrally with bacteriologically sterile pus aspirated from an inguinal bubo and glandular material obtained from a case of lymphogranuloma inguinale. The pus and glandular material were diluted 1 in 5 with sterile distilled water and inoculated in 0.03 cc. quantities into each of 6 mice. The object of this section of the work was to ascertain whether or not the virus could be transmitted indefinitely in that manner. All of the inoculated animals died within an average of 11 days. The brain of one of these animals dying from lymphogranuloma inguinale was emulsified in 1 in 2.5 dilution of distilled water and inoculated intracerebrally in 0.03 cc. quantities into another batch of 6 white mice. All of these mice died within an

average of 7.0 days. A similar procedure caused the death of the next four generations of mice in an average of 5.4 days and by the time the eleventh generation was reached, the animals were expiring in four days. Similarly, there was an increased mortality rate with successive passage, 90 to 100% of all the inoculated animals succumbing in the seventh to the eleventh generations. The shortening of the period taken for the mice to die involved such frequent subpassage that the strength of the inocula of subsequent generations was reduced to a 1 in 5 dilution. The effect of the increased dilution was to make the average time taken for the mice to die longer and to decrease the mortality rate. Nevertheless, there continued to be an increase in the virulence of the virus so that by the 26th and 27th generations, when a 1 in 2.5 dilution was again employed, 100% of all the inoculated animals died in an average of 2.6 days. At the time of writing (35th generation) 80 to 100% of mice die in an average of 6.7 days as a result of an intracerebral inoculation of a 1 in 5 suspension of lymphogranulomatous mouse brain. Physiological saline, Tyrode's solution, ascitic fluid or infusion broth of pH 8.0 are diluents equally as good as sterile distilled water.

A control series of inoculations in which normal mouse brain was used instead of lymphogranulomatous mouse brain, did not produce any effect in mice.

Evidence of the presence of the virus of lymphogranuloma inguinale in the brains of the dead mice was furnished by the production of highly potent Frei antigens from these brains; the method of preparation of the mouse brain antigen was identical with that used for the preparation of Frei antigens from human lymphogranulomatous pus. The potency of the antigen appears to increase with successive passage of the virus. Normal mouse brains prepared and tested as Frei antigens do not produce any appreciable reactions.

Histological examination of the brains of the dead mice indicates that the virus produces a meningitis in which the predominant cell is of the lymphocyte type; polymorphonuclear leucocytes are comparatively sparse.

*Conclusion.* One strain of the virus of lymphogranuloma inguinale, upon intracerebral inoculation into white mice, has quickly developed a fixed virulence for these animals and appears to be capable of transmission indefinitely in this manner.

Frei antigens prepared from lymphogranulomatous mouse brains are specific and highly potent; the antigenic strength increases with successive mouse passage.