moved from the animals and collected in physiological salt solution. A total of 18,729 fleas were thus assembled in 355 pools, which were stored at 3°C. for approximately 10 to 11 months since suitable facilities to examine them were not available. In 1937 the insects collected from the squirrels of the same area were pooled in 155 lots, carefully washed with salt solution, ground in a mortar and then injected subcutaneously into guinea pigs. Of the 155 pools so tested 6 produced fatal plague infections within 6 to 8 days. Two of the locations in the County where infected fleas were found in 1936 were recognized as the same colony or series of burrows proven to harbor diseased squirrels in the summer of 1916.

These and similar observations indicate that sylvatic plague persists probably indefinitely in an area once invaded and that the gross anatomical examinations fail to detect rodent infections. Squirrels, just as rats, may harbor *P. pestis* without visible lesions. The biology of these latent infections is the subject of further investigations. Although the viability of *P. pestis* in stock cultures for many years is well known, the fact that the bacteria remained alive in the dead fleas soiled with a variety of microörganisms *(B. coli, B. proteus,* Cocci, etc.) held at ice box temperature is indeed noteworthy.

9843 P

Antiviral Substances to the Virus of Encephalitis (St. Louis Type) in Serums Collected in California.

B. F. HOWITT. (Introduced by K. F. Meyer.)

From the George Williams Hooper Foundation, University of California Medical Center, San Francisco, and the California State Department of Health.

During the summer of 1937 reports were obtained of an outbreak of acute encephalitis in the San Joaquin Valley region in California. A number of fatal cases were recorded and while brain material was received from 3 of these, no virus was recovered. Monkeys and mice were inoculated but all remained negative, even after repeated serial passages in the mice. Nasopharyngeal washings were also taken from 6 patients still hospitalized but no virus was recovered. Since the histopathology of the human material was typical of an acute encephalitis, serum was obtained through the courtesy of the Health Departments of both Fresno and Tulare

counties from the available cases of 1937 and from 2 diagnosed in 1936.

Serums from 29 people were tested for neutralizing ability against the viruses of lymphocytic choriomeningitis (l.c.m.) and the St. Louis type of encephalitis,* respectively. The blood was taken at different lengths of time, 15 days to 13 months, after the onset of the disease, the majority being obtained from 2 to 3½ months after the onset. The patients varied in age from 3 to 66 years, but the largest group was from 17 years upward. There were 10 under 16 and 19 over that age. All except 3 had recovered without particular after-effects. The 3 exceptions showed cerebral symptoms and their serums failed to neutralize the St. Louis virus.

Blood was also obtained from 6 doctors and nurses who had been in contact with the patients during the outbreak but who had not contracted the disease. Two of the nurses reported having had poliomyelitis, one in Fresno in 1935 and the other in Los Angeles in 1934. Serums from a group of 9 nurses and students connected with the University of California were also tested.

The neutralization tests were performed as follows: A 10% mouse brain suspension was diluted in a mixture of equal parts of hormone broth and sterile distilled water to a 1-100 and occasionally 1-250 dilution, respectively, for the l.c.m. virus and to 1-10,000 and 1-50,000, respectively, for the other strain. Equal parts of each serum and of each dilution of virus were mixed, placed at 37°C. for one hour and then kept overnight in the icebox before injection of 0.03 cc. intracerebrally into mice. Both positive and negative serums were always included in each series.

It was found that no serums neutralized the l.c.m. virus, but that of the 29 from encephalitic patients, 16 or 55.1% gave positive neutralization against the St. Louis virus and 13 or 44.9% were negative. According to districts: of the 12 serums from Fresno 4 were positive, including one from a case diagnosed in 1936, while from Tulare 12 of the 17 serums gave positive neutralization. In the latter region the diagnosis of "polio-encephalitis" was largely made for these cases from whom the serums were obtained, headache and general malaise being usual symptoms. No residual paralysis was noted for this group.

Of the 6 serums from contacts only one neutralized the St. Louis virus and that was from the nurse that had been diagnosed as having had poliomyelitis in 1935 with some paralysis from which a good

* The former virus was kindly sent by Dr. C. Armstrong of the National Institute of Health and the latter by Dr. L. T. Webster of the Rockefeller Institute.

recovery was made. The serum was removed about 2 years after the onset. All of the non-contact serums were negative.

McCordock, Smith and Moore[1] reported another outbreak of encephalitis in the St. Louis region in 1937 while Brodie[2] describes having tested the l.c.m. and the St. Louis viruses, respectively, against serums from patients in New York who had recovered from non-paralytic poliomyelitis in 1935. All failed to neutralize either type of virus. It might seem, therefore, from the results recorded here that the virus of acute summer encephalitis has been traveling west rather than east after the 1933 epidemic.

Summary. Of 29 serums from patients diagnosed as having had acute encephalitis or a polio-encephalitis, respectively, in one region of California none neutralized the virus of lymphocytic choriomeningitis, while 16 or 55.1% gave a positive test with the St. Louis type of encephalitic virus.

Appreciation is extended to Dr. E. R. Zumwalt and his staff at Tulare, to Dr. H. M. Ginsburg and the group at Fresno for their kind coöperation and the many courtesies offered and to Dr. C. Hawley for the collection of many of the serums.

9844 P

Synthesis of the Antihemorrhagic Vitamin by Bacteria.

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Fish meal and rice bran which have been exposed to the action of microörganisms contain the antihemorrhagic vitamin-K.[1] This factor has also been demonstrated in the feces of chicks on a K-free diet.[2] The present work is a preliminary investigation of the production of the vitamin by bacterial synthesis.

It was again found that the putrefaction of ether-extracted, K-free fish meal was accompanied by the formation of appreciable

[1] McCordock, H. A., Smith, M. G., and Moore, E., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 288.

[2] Brodie, M., *J. Inf. Dis.*, 1937, **61**, 139.

[1] Almquist, H. J., and Stokstad, E. L. R., *J. Biol. Chem.*, 1935, **111**, 105.

[2] Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1936, **12**, 329.