for 3 days before final reading for sterility or contamination was made. About half of the tubes were usually found to be slightly contaminated after an exposure of 6 hours' duration. Complete sterilization was consistently obtained after an irradiation time of 20 hours. While considerably less time may be sufficient for tubes not purposely and so highly contaminated, the safe practice has been adopted of exposing tubes of any size to the ultraviolet light for 24 hours. The metal screw caps are placed in petri dishes and sterilized separately by any heat. They are then attached with the aid of sterile forceps to the threaded tops of the sterilized tubes, preferably before removing the tubes from the cabinet.

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Lymphocytic Choriomeningitis: Two Human Fatalities Following an Unusual Febrile Illness.

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Human infections with the virus of lymphocytic choriomeningitis may assume a number of different clinical forms, *viz.*, aseptic meningitis,[1] meningo-encephalomyelitis,[2] anterior poliomyelitis[3] and grippe.[4, 5] Furthermore, unrecognized infection with this virus appears to be common since many individuals have in their serum neutralizing antibodies for the agent.[5] Nevertheless, acute illness proved to be caused by the virus of choriomeningitis has been reported in less than a hundred individuals and none of these died.[5] Fatalities have been reported among patients assumed to have been infected with this virus;[6] however attempts to isolate the agent from

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autopsy material were not made, or the results of such attempts were open to question, or the identity of the virus which was recovered was not clearly established. This note is published in order to call attention to a hitherto unrecognized type of severe acute illness associated with the virus of choriomeningitis. A detailed report of the case records of our 2 patients will be given at a later date.

Important clinical and pathological findings in the records of the 2 adult males who died after a severe febrile disease of 12 and 18 days' duration, respectively, may be summarized as follows: The illness of the first patient, a laboratory worker, who had no known contact with the virus of choriomeningitis, was characterized at the onset by fever, malaise, generalized aches, sore throat, vomiting, cough and leukopenia (W.B.C. 2400). Fever rose to 104-105.8°F (by mouth) and continued unabated until death. The white blood cell count rose gradually to 11,350 during the course of the disease; abnormal leucocytes were not encountered in stained smears. A tentative diagnosis of typhoid fever was abandoned when the results of bacteriological studies of blood and stool specimens failed to substantiate it; the terminal clinical diagnosis was "hyperpyrexia of unknown cause." The findings on postmortem examination were those to be expected in a severe febrile disease but the immediate cause of death was not apparent. Patches of bronchopneumonia were present; in a number of areas the usual polymorphonuclear leucocyte exudate was found but in some portions the exudate was composed in part or wholly of mononuclear cells. There was no histopathological evidence of meningitis, choroiditis or encephalitis.

The second individual assisted at the autopsy of the first patient but he sustained no known injury during the procedure. Eight days later he developed malaise and low grade fever; these continued for several days when he was found to have leukopenia (W.B.C. 2500) and mild pharyngitis. The patient was hospitalized because he was thought to have a mild upper respiratory infection in addition to his recognized chronic active pulmonary tuberculosis. He became increasingly ill and died 17 days later. During the course of the disease the pharyngitis increased in intensity and ultimately became necrotizing in character. Hyperpyrexia developed and continued until a day before death. A diffuse erythematous rash appeared for several days. Bleeding from the mucous membranes developed and was accompanied later by hemorrhages into the skin and from the external auditory canal. The white blood cell count rose progressively and terminally it reached 33,000 per cu mm with

immature forms constituting 13% of the total. Acute leukemia and "a virus infection" were considered as possible clinical diagnoses at the time of exitus. On postmortem examination numerous hemorrhages were encountered; these included a subdural hematoma and a large clot in one renal pelvis. Extensive necrotizing tonsillitis and pharyngitis were present. Several areas of hemorrhagic consolidation were found in the lungs as well as foci of chronic ulcerative tuberculosis. Histologically the acute pneumonic process was characterized by infiltrations of mononuclear cells in the alveolar septa, by proliferation of alveolar cells and by an exudate consisting of large pale-staining mononuclear cells mixed with red blood cells and fibrin. Microscopic study revealed the presence of loose cuffs of lymphocytes about small vessels in several organs; although not conspicuous the infiltrations were found most often in the periportal spaces of the liver, in the meninges and in the brain substance.

It is of interest that lumbar puncture was not done on either patient. There appeared to be no clinical evidence to warrant such a procedure.

The virus of choriomeningitis was isolated from postmortem blood and from brain tissue of the first patient, and from lung and brain tissue of the second patient. Suspensions of each specimen were inoculated into 2 normal guinea pigs and also, in most instances, into 6 mice. All of the inoculated normal guinea pigs developed fever within a few days and, unless sacrificed for passage material, they subsequently showed the usual signs of severe lymphocytic choriomeningitis[1] and all died. Practically all of the mice injected with human material became sick 7 to 10 days later but about 15% of them ultimately recovered; the remaining mice died in convulsions or were sacrificed when moribund.

The infectious agent recovered from the various tissues of the two patients was identified in each instance as the virus of choriomeningitis. This was done by demonstrating the specific soluble antigen of choriomeningitis[7] in extracts prepared from splenic tissue of guinea pigs of the initial and of each subsequent passage. Furthermore, the agent isolated from each tissue was shown to be incapable of producing obvious disease when inoculated either into guinea pigs or into mice known to be immune to the W.E. strain[1] of the virus of choriomeningitis, while portions of the same tissue suspensions uniformly produced a fatal disease in normal animals. Finally, those mice which survived infection with the newly-isolated strain of virus were later found to resist 1000 M.L.D. of the W.E.

[7] Smadel, J. E., and Wall, M. J., *J. Bact.*, 1941, **41**, 421.

strain given intracerebrally. It may be mentioned that the histopathological lesions in the central nervous system of guinea pigs and mice that succumbed to infection with the virus isolated from the 2 patients were more severe than those generally encountered in animals infected with the virus of choriomeningitis;[1] not only an extensive choriomeningitis was present but also a true encephalitis.

The occurrence of disease in all guinea pigs and most mice inoculated with the human material clearly indicated that the virus of choriomeningitis had been present in the inocula and that it had not been encountered adventitiously.† In order to remove any doubt about this a portion of each tissue was taken from storage at –70°C and after fresh suspensions had been prepared they were again inoculated into normal guinea pigs or mice. The virus was reisolated and reidentified from each specimen.

Summary. The virus of lymphocytic choriomeningitis was isolated from the tissues of two patients who died following an undiagnosed acute systemic disease.

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Morphological Structure of the Virus of Vaccinia

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Elementary bodies of vaccinia which represent the infective unit of the disease[1] are relatively large, *i. e.*, 235-250 mμ[2] in diameter. They respond to osmotic influences,[3] are antigenically complex,[4] and possess certain chemical and biologically active constituents in common with bacteria and mammalian cells.[5] The virus of vaccinia,

† The virus of lymphocytic choriomeningitis was also isolated by Dr. R. S. Muckenfuss, of the Department of Health, New York City, from tissue of the second patient mentioned in this report.

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