amounts of potassium iodide. Moreover, iodine decreases the inactivation of TSH by thyroid tissue *in vitro*.

An inflammatory reaction in the thyroid glands of experimental animals has been produced by several technics; the most striking alterations follow radiation or auto-immunization procedures. Treatment of rats with appropriate amounts of I^{131}(9) leads to damage of thyroid epithelial cells and an inflammatory reaction not unlike the changes described above. So, too, the descriptions of auto-immune thyroiditis in several species(10) are certainly reminescent of the alterations we have observed here, so much so that we are currently attempting to produce thyroiditis in hamsters by injection of homologous thyroid tissue with an adjuvant and are examining the sera of animals with iodine thyroiditis for antibody to thyroglobulin. We are unable to explain the pathogenesis of the changes described herein. Certain possibilities such as the deleterious effects of accumulation of large amounts of iodine in a depleted gland, or production of excessive amounts of thyroid-active materials within the gland come to mind. Further study of this problem may help to understand the phenomena of *Jodbasedow*(2) and "iodine thyroiditis"(5) in man.

Summary. Administration of large amounts of iodine to hamsters which have hyperplastic goiters produced by an iodine-deficient diet or by a goitrogen results in a morphologic response which is different from that encountered when iodine is given in physiological amounts. An acute inflammatory reaction and a diminution of the accumulation of colloid are found, neither of which has been observed in animals treated with smaller amounts of iodine.

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Received July 28, 1959. P.S.E.B.M., 1959, v102.

Synthesis, Cytopathogenicity, and Modification of Avian Encephalomyelitis Virus (AEV) in Chick Kidney Cell Culture.* (25274)

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Multiplication of AEV in minced whole chicken embryo tissue culture has been reported(1). Owing to the low titer obtained the method is of limited value. This communication reports successful propagation of the virus in monolayer chick kidney cell culture, cytopathogenic changes produced in the cell culture, and modification of virulence of the virus for chicks.

* Aided in part by grants from Nat. Inst. Health and Am. Poultry and Hatchery Federation.

† Kindly supplied by Dr. Henry Van Roekel, Dept. of Vet. Sci., Mass. Agric. Exp. Station, Amherst.

Materials and methods. Virus. The seed virus was the brain homogenate from 6th chicken embryo passage. It had previously undergone 16 passages in chicks, after being received as the 135th chick-brain passage virus.† The titer of the virus was 10^{-5}/0.05 ml when inoculated intracerebrally into chicks. *Cell culture.* Monolayer chick kidney cell culture was prepared from 1-week-old chicks (2). *Passage of virus.* The growth medium (2) was replaced by 0.1 ml virus and 0.9 ml growth medium containing 0.07% sodium bicarbonate. Serial passage was made every 3

days up to 5th passage and every 2 days thereafter up to 10th passage. *Titration.* The 10th passage virus was titrated in 1-week-old chicks by intracerebral (IC) inoculation(3) and in a cell culture system using 4 chicks and 4 culture tubes for each virus dilution. *Identification of passage virus.* Four 1-week-old chicks were inoculated IC with 0.05 ml of the 10th passage virus. Brain, proventriculus, and ventriculus were taken for histopathologic study after definite signs of infection were recognized. Virus neutralization tests in a cell culture system were performed with 5 hyperimmune sera prepared by repeated intramuscular inoculation(4) of adult chickens with increasing dosages of chick brain virus and enhanced with embryo brain virus. The preinoculation sera were found by the *in vitro* virus neutralization test to be free of specific neutralizing antibodies. Five normal sera were included as controls. An equal volume of serum and virus dilution mixture was incubated at room temperature for 1 hour, and 0.2 ml of the mixture plus 0.8 ml of serum-free maintenance medium(2) were inoculated into each cell culture tube. Readings were made at 7th day of incubation.

Results. Virus synthesis and modification of virus virulence for chicks. The 10th cell culture passage virus titered 10^{-1}/0.05 ml in chicks and 10^{-8} in the cell culture system. Since the original seed virus titer in chicks was 10^{-5}/0.05 ml, there would be no virus carried over to the 6th passage. The virus titer in the cell culture can be considered high. Virus synthesis in the system is apparent, but the virus virulence for chicks was attenuated. *Cytopathogenicity.* There were no recognizable cytopathologic changes in the culture up to the 3rd passage. Starting from the 4th passage, definite changes became apparent. They consisted of contraction and rounding of infected cells, cytoplasmic granulation, and cytolysis (Fig. 1 and 2). The changes increased in intensity and incubation period shortened as successive passages were carried on. From the 6th passage the virus was harvested on the 2nd day of incubation. *Identification of passage virus.* All 4 inoculated chicks showed severe torticollis and histopathologic study revealed neuronal degeneration, gliosis and perivascular infiltrates in the brain, and lymphocytic aggregates in the proventriculus and ventriculus musculature which are pathognomonic for AEV infection(3,5). The hyperimmune sera neutralized 10^{7} to 10^{8} $TCID_{50}$, while normal sera neutralized only 10 to 50 doses. The virus was identified as AEV.

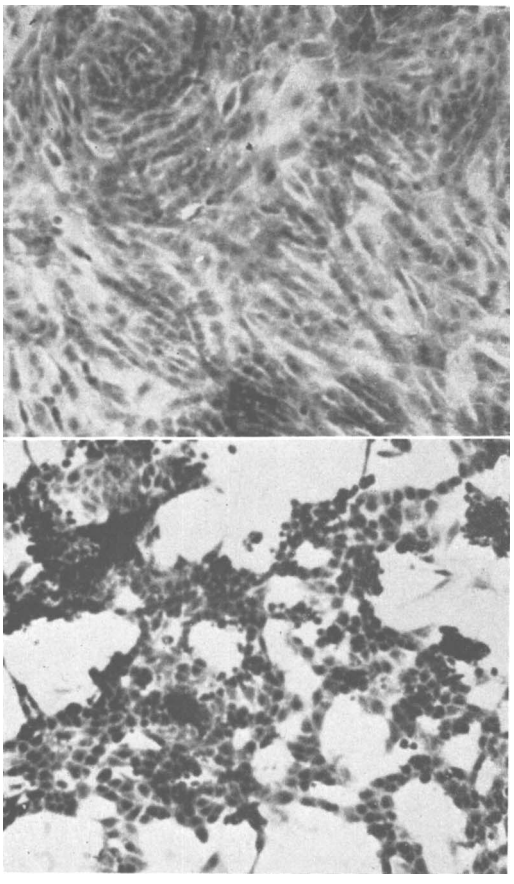

FIG. 1 (top). Chick kidney cell culture. Control. H & E, × 100.
FIG. 2 (bottom). Chick kidney cell culture, 48 hr post inoculation with AEV. H & E, × 100.

Discussion. AEV preparations for use in research and vaccine production have hitherto been supplied in the form of brain homogenate from infected chicks(3,6) or chicken embryos(7). These preparations contain a large quantity of foreign substances and may be difficult to prepare because the supply of susceptible chicken embryos is often curtailed by widespread occurrence of the infection among breeding stock(8). The cell culture virus may furnish a relatively pure stock for

both research and vaccine production. Attenuation of virulence of the virus for chicks by tissue culture may prove to be desirable for immunization purposes.

The diagnosis of an acute AE infection in chicks is thus far mainly based on clinical observations and histopathologic findings. Difficulty in differentiating AE from Newcastle disease and neurolymphomatosis on a histopathologic basis has been encountered. The diagnosis of inapparent infection in breeding flocks by embryo susceptibility test has been employed(8). The test requires 3 weeks to complete and is costly. Collecting representative eggs from large commercial farms and the shipment of them to laboratories may present a problem at times. A one-tube *in vitro* virus neutralization test using 100 $TCID_{50}$ might serve as a simple method for diagnosis of acute AEV infection in chicks and inapparent infection in adults. IC challenge and virus neutralization tests in chicken embryos have been used for evaluation of immunity in inoculated flocks(6,9). The former method is considered too severe and only a fraction of birds can be challenged; the latter method has the disadvantage of requiring a source of susceptible embryos. The virus neutralization test in a cell culture system may serve as a means of evaluating the immunity status of vaccinated flocks without the disadvantages mentioned.

Summary. Virus synthesis, cytopathogenicity, and modification of virulence for chicks, of AEV in chick kidney cell culture after 10 serial passages, have been described. The cell culture virus has been identified by histopathologic changes in inoculated chicks and virus neutralization tests in a cell culture system. The possible application of the findings are discussed.

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Received July 28, 1959. P.S.E.B.M., 1959, v102.

Plaque Assay of *Toxoplasma* on Monolayers of Chick Embryo Fibroblasts.*† (25275)

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The importance of *Toxoplasma gondii* as a parasite pathogenic for man and for many animal species is documented in voluminous literature dealing with various aspects of epidemiology, pathology, clinical findings, diagnosis and treatment(1,2). Relatively less is known about the organism as an independent entity. Its classification as a protozoon is based largely on morphological considerations (3). Although experimental infections of various animals and tissue cultures(2) have been studied in considerable detail, we know nothing about the attributes of the parasite responsible for its extremely fastidious requirements for survival and growth. To date, it has not been possible to propagate *Toxo-*

* Supported by research grants from NIAID, N.I.H., U.S.P.H.S.

† These studies formed part of a dissertation submitted by senior author to Faculty of Graduate School of Saint Louis University in partial fulfillment of requirements for degree of Ph.D. in Microbiology.

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