were tested for their LH and FSH content by the injection of such material, following crude extraction, into 21-day-old male and female rats. It was found that the pituitaries from the females kept in the light were the more potent in producing ovarian development, whereas the pituitaries from females kept in the dark were the more potent in producing seminal vesicle growth. These results indicate that the pituitaries of females kept in the dark produce more luteinizing hormone than do those of the females kept in the light.

A study of the ovaries of females kept in the light or dark for 175 days also indicated an increased LH secretion in the dark, the ovaries being larger and heavily luteinized under such conditions, whereas those from animals in the light were almost entirely follicular.

Males kept in the light from the twenty-first day to the one hundred and seventy-fifth day of life differed from males kept in the dark for the same period in that the pituitaries, testes, and seminal vesicles of the former were larger, the pituitaries being almost twice as large, the testes 30% heavier, while the seminal vesicles were 3 times as large. The pituitaries of these animals have not yet been tested for LH and FSH.

From the results here presented it appears that the balance of the 2 gonadotropic hormones is changed under varying light conditions.

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Effect of Prolonged Passage in the Chick Egg on the St. Louis Encephalitis Virus.

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Cultivation of the virus of St. Louis encephalitis on the chorio-allantoic membrane of the chick embryo was reported by Harrison and Moore,[1] who carried one strain through 7 passages and another through 10. The titer of the virus in the chorio-allantoic membrane was not determined. They also demonstrated the presence of the virus in the brain, liver and spleen of the chick embryo, but did not determine the titer of the virus in these organs.

[1] Harrison, R. W., and Moore, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 359; *Am. J. Path.*, 1937, **13**, 361.

Schultz, Williams and Hetherington[2] reported 3 series of experiments in which they carried the virus of St. Louis encephalitis on the chorio-allantoic membrane of the developing chick through 15, 16, and 22 passages, respectively. In the first 2 series, the chorio-allantoic membrane was not infective (presumably for mice) in dilutions above 10^{-1}. In the 3rd series, however, the infective dilution increased from 1×10^{-1} at the 5th passage to 2×10^{-3} at the 17th passage and to 2×10^{-4} at the 22nd passage. The chick embryo brain at the 17th passage was infective in a dilution of 2×10^{-4}, a slightly higher dilution than the membrane from the corresponding passage.

Both Harrison and Moore, and Schultz, Williams and Hetherington used for the original inoculation of the chick eggs virus grown by the flask culture method, while we have used as the original inoculum infected mouse brain virulent for mice in a dilution of 10^{-6}. The Hubbard strain of virus used in these experiments was isolated in mice from a patient dying in the 1937 outbreak of encephalitis in St. Louis.

We have carried the virus through 68 passages on the chorio-allantoic membrane of the chick. The virus has been passed at 3 or 4 day intervals. The membranes were ground and diluted with nutrient broth pH 7.4. Amounts of 0.1 cc and 0.15 cc of a 1:3 dilution were used for transferring the virus to the chorio-allantoic membrane of eggs incubated for 10 to 12 days. For injecting eggs we used the artificial air sac technic, a modification of the Woodruff-Goodpasture method introduced by Burnet.[3]

There has been no evidence of increasing adaptation of the virus to the chorio-allantoic membrane. Membranes from early passages (1st, 2nd, 4th, and 29th) contained at 4 days enough virus to kill mice regularly when 0.03 cc amounts of 10^{-2} dilutions were inoculated intracerebrally. Mice receiving the 10^{-3} dilution died irregularly. When tested after 62 passages, the amount of virus in the membrane 4 days after inoculation was the same, killing 4 of 4 mice in a dilution of 10^{-2} and 1 of 4 in a dilution of 10^{-3}. Membranes tested at 6 and 10 days after inoculation show no increase in virus over the amount present at 4 days.

The virus content of the chick embryo brain from the corresponding passage was titrated on several occasions at the same time as the membrane. In the early passages it contained enough virus on

[2] Schultz, E. W., Williams, G. F., and Hetherington, A., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 799.

[3] Burnet, F. M., Med. Research Council, Special Report, Series No. 220, 1936.

the 4th day to kill mice uniformly in dilutions of 10^{-3}. After 62 passages the brain material 4 days after inoculation of the membrane still killed mice uniformly in dilutions only as great as 10^{-3}. However at 10 and 11 days after inoculation, the brain seems to contain slightly more virus than at 4 days since the majority of the mice receiving the 10^{-4} dilution of virus die. The membrane at this time is not infective in greater dilutions that at 4 days. The presence of a slightly greater amount of virus in the embryo brain than in the chorio-allantoic membrane has been reported by Schultz, Williams and Hetherington also.

The strain of virus used for the inoculation was originally infective for mice in dilutions of 10^{-6}, when infected mouse brain was used as the source of the virus. Presumably the virulence of the virus for mice has not been altered by passage on the egg membrane. This is demonstrated by the fact that the brains of mice, dying as the result of inoculation with the egg membrane from the 66th and from the 67th passages were still infective for mice in dilutions of 10^{-6}. The difference in the virus concentration of the chorio-allantoic membrane and that of the mouse brain as titrated in mice is apparently due to the difference in propagation of the virus in these media rather than to any change in virulence of the egg cultured virus for the mouse.

Chick embryos apparently are little affected by the virus. The great majority of those which are not used earlier for passage or for study live until approximately the time of hatching and no gross anatomical changes have been observed. However, only a few chicks hatch successfully.

Although the amount of virus in the brain 4 days after inoculation of the membrane is sufficient to kill mice in dilutions of 10^{-3} and at the time of hatching in dilutions of 10^{-4}, only slight microscopic lesions have been observed in the chick brains and even these appear in only a few chicks. The brain of one embryo 4 days after inoculation of the membrane showed two small foci of glial proliferation, one near the cortical surface and one in the midline near the ventricles. In the first focus there were also a few wandering cells, and in the meninges near by there was a cluster of mononuclear cells near a vessel. In the brains of 2 other chicks killed at the time of hatching, having been inoculated 11 days previously, several small clusters of wandering cells were seen near vessels in the meninges. No cellular infiltration about vessels in the brain has been observed.

On the 3rd or 4th day after the clear supernatant fluid from the membrane-broth mixture has been inoculated on the egg membrane,

the membrane is grossly edematous and slightly opaque. There is a very fine stippling or granulation of the surface. At a later time the edema lessens, the membranes appear thinner and have a slight but uniform opacity. Neither at 4 days nor later have we seen gross ulcerations of the membrane except when it has been suspected that the membrane was traumatized at the time of inoculation.

Microscopically, at 4 days the edema of the mesodermal layer of the membrane is apparent. There is some proliferation of fibroblasts and there are foci of cellular infiltration, chiefly mononuclear, which tend to be concentrated about blood vessels.

The ectodermal layer of cells shows focal proliferation, which may become diffuse at times. The surface cells of these foci of proliferation become necrotic, but this degeneration does not extend into the mesoderm to form an ulcer. It is the microscopic foci of ectodermal proliferation and degeneration which give the gross appearance of fine granulations. In places, small epithelial pearls extend downward into the mesodermal layer under or near the foci ectodermal proliferation and there is proliferation of fibroblasts about these downgrowths. An increase in thickness of the entodermal layers may also occur. No specific cellular inclusions have been observed.

Summary. After 68 passages on the chorio-allantoic membrane of the chick there is no increased proliferation of the virus on the membrane. The virulence of this virus after cultivation in eggs for 9 months has been demonstrated to be the same for the mouse as that of the original inoculum of the eggs. The virus content of the chick embryo brain is consistently slightly greater than that of the corresponding chorio-allantoic membrane. The majority of the chicks live until the time of hatching and show no gross changes attributable to the virus and only slight and inconstant microscopical changes in the brain. The membranes show a gross edema and fine granulation of the surface. Microscopically, they show chiefly edema and cellular infiltration of the mesoderm and foci of ectodermal proliferation with superimposed necrosis of the superficial layers.