tained with little decrease for as long as 10 and 15 days.

The Eastern strain of equine encephalomyelitis has been grown with a dilution activity of 10^{-7} in whole minced chick embryo in serum-ultrafiltrate at room temperature and 37° C.

A vaccine, containing very small amounts of protein, prepared from the clear supernatant fluid of serum-ultrafiltrate cultures protected guinea pigs against 1,000 to 10,000 m.l.d. doses of virus (Western strain).

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Characteristics of a Fixed Rabies Virus Cultivated on Developing Chick Embryos.

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In a preceding paper[1] we reported the cultivation of rabies virus in developing chick embryos in 2 series through 9 and 6 successive passages respectively. Since then, one series has been carried through 16 subcultures; the second series has been passaged through 47 subcultures during a period of 18 months and is still being maintained. The purpose of this note is to summarize our observations on the behaviour of this virus to different hosts after prolonged cultivation in the developing chick embryo.

Technic. A fixed virus, presumably derived from the original Pasteur strain, was used for initiating the chick embryo cultures. The first inoculation was made by dropping 0.1 cc of a 10% saline emulsion of infected mouse brain on the allantois immediately over the embryo using the Burnet[2] technique. The same technique was used for subcultures: pieces of the inoculated allantois, or preferably the brains of the embryos of 3 to 6 infected eggs, were ground in a sterile glass mortar and enough saline added to make a 10% emulsion. Each passage material was titrated intracerebrally in mice in tenfold dilutions. In the first 12 passages, 5-day-old embryos were infected; subsequently 6-day-old embryos were used because they were more resistant to the manipulations involved. Passages were usually made 6-11, sometimes up to 14, days after the inoculation.

The infection in the chick embryo. The embryos of earlier as well

1 Kligler, I. J., and Bernkopf, H., *Nature*, 1939, **143**, 899.

2 Burnet, F. M., Sp. Rep. Ser. Med. Res. Coun. Lond., No. 220, 1936.

as later passages developed normally and usually remained alive up to hatching time; in later passages the embryos were sometimes smaller than normal but showed no other apparent defect in development. Virus was already found in embryo brain 4 days after infection and usually reached a maximum after 9 days and usually continued at this concentration until time of hatching; however, virus was not detectable in the brain of inoculated embryos which hatched. The adaptation of the virus to the embryo medium is indicated by the fact that, whereas, original mouse passage virus could not infect embryos 8 days old or over with regularity, and passages were usually negative, virus of the 40th egg passage was transmitted in 3 successive subcultures through 10-day-old embryos.

Embryo brain always gave a higher titer of virus than other tissues; it was usually positive in dilutions of 1:1,000 to 1:10,000. Apparently virus multiplication in the chick embryo is limited; the multiplication of the virus in the chick embryo proceeds 15 days as against 7 days in the mouse, yet the titer in mouse brain is 10 to 100 higher than that in chick embryo brain. The allantois titer was usually lower (1:100 to 1:1,000), but occasionally reached the same titer as the corresponding brain.

The presence of virus in the allantois points to multiplication of this strictly neurotropic virus in a membrane which is supposed to be free of nerve tissue,[2] and raises the question whether fixed rabies virus is able to multiply in embryo tissues other than nerve tissue. Two attempts to cultivate the virus in flask cultures[3, 4] with allantois instead of mouse embryo brain failed to give evidence of multiplication. The virus titre of liver and heart of infected embryos was consistently low (1:10, in one experiment 1:100).

The low pathogenicity of the virus for the developing chick coincided with the scanty histopathological changes found in the infected embryo brain. Degenerative changes of the nerve cells are sometimes observed in brains fixed with Bouin and stained with Hämatoxylin-Eosin or Giemsa; the meninges are frequently richer in cells than normal and there may be slight perivascular infiltration around the smaller blood vessels, the endothelial cells of which appear swollen. In many sections it is difficult to see any changes at all. Negri bodies were never found. No specific changes were noted in the allantois.

Infectivity for mice. Prolonged passage of the virus through chick embryos did not appreciably modify its virulence for mice when injected intracerebrally. Virus of the last chick embryo passages caused the same incubation period of 6-7 days and the same course

[3] Webster, L. T., and Clow, A. D., *J. Exp. Med.*, 1937, **66**, 125.

[4] Bernkopf, H., and Kligler, I. J., *Brit. J. Exp. Path.*, 1937, **18**, 481.

of infection as did the original mouse virus. Mice injected intraperitoneally with 1 ccm of a 10% embryo brain emulsion of different passages died in varying percentages according to their age: of 69 mice, 6 weeks old, 7 died, while of 26 mice, 4 weeks old, 5 died of the infection. The surviving mice showed varying degrees of immunity to a subsequent intracerebral infection and complete immunity to an intraperitoneal infection with the original mouse passage virus. This fact was used to demonstrate the serological identity of the chick embryo passage virus with the original fixed virus. Ten 4-weeks-old mice were injected intraperitoneally 0.25 ccm of 10% chick embryo brain emulsion of the 45th passage, 11 animals of the same age served as controls. Ten days later both groups of mice received intraperitoneally 0.25 ccm of a 1:50 brain emulsion of a mouse infected with rabbit brain fixed virus; all the treated animals survived, whereas 7 of the 11 controls died of the infection.

Infectivity for rabbits. In contrast with the unchanged virulence of chick embryo passage virus for mice, that for rabbits was greatly reduced. Brain emulsions of chick embryos of different serial passages were injected intracerebrally into rabbits and simultaneously titrated in mice. Sixth passage virus still produced the disease after the usual incubation period of 6 days, death following on the 8th day. The rabbit injected with brain of the 37th passage died after 13 days. Virus of the 40th passage produced the first signs of trouble after 11 days, paralysis set in after 16 and death after 17 days; mice injected with the brain suspensions of this rabbit were paralysed after 7 days and died 3-4 days later. Virus of the 44th passage with a mouse titre of 1:100 produced no signs of illness in rabbit. Two rabbits injected with 47th passage virus showed the first signs of paralysis after 11 and 13 days respectively; death following on the 14th and 15th day. (Table I). A guinea pig inoculated intracerebrally with the 47th passage embryo brain developed paralysis after 7 days

TABLE I.
Intracerebral Virulence Test of Chick Embryo Passage Virus in Rabbits.

Passage and dilution of chick embryo brain inoculated	Titre i.c. in mice	Onset of paralysis in rabbits after .... days	Death of rabbits after .... days	Remarks
Passage 6 1:10	1:1000	7	8	
" 37 1:100	1:1000	12	13	
" 40 1:5	1:1000	16	17	Mice inj. i.c. with rabbit brain paralysed after 7 days
" 44 1:10	1:100	—	—	Well after 2 mo.
" 47 1:5	1:500	11	14	
" " "	1:500	13	15	

and died 2 days later, the normal course with fixed virus. On the other hand, rabies fixed virus cultivated in Maitland medium with mouse embryo brain shows no decrease in intracerebral virulence for rabbits; the 47th subculture on mouse embryo brain which had the exceptionally low titre of 100 intracerebral mice units, produced complete paralysis in a rabbit 8 days after inoculation. The reduced virulence for rabbits of the chick embryo virus appears, therefore, to be a specific modification.

Summary. After 47 passages through the developing chick embryo, rabies fixed virus shows no enhanced virulence for the chick embryo, as judged by the complete development of the embryo, the relatively low virus titre in the embryo brain and the scanty pathological changes in the brain. The virulence of the chick embryo passage virus for mice and guinea pigs remained unchanged, while that for rabbits appeared reduced considerably, as demonstrated by the prolonged incubation period, and duration of the infection. The specific antigenic character of the chick embryo virus remained unchanged during 47 passages over a period of 18 months.

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Identity of Prolactin with Water Drive Factor in *Triturus viridescens.**

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Reinke and Chadwick have shown that the factor which induces the water drive in the red eft (land phase) of *Triturus viridescens* originates in the anterior lobe of the hypophysis and, further, that this factor acts independently of the thyroid glands and gonads in effecting the water drive.[1] Indicating the possibility that the water drive factor is similar to or identical with the growth-promoting hormone, Chadwick[2] was able to induce the water drive with injections of a commercial extract of the anterior pituitary which assays

* The Prolactin used in these experiments was very generously provided by Dr. H. W. Rhodehamel of the Eli Lilly Research Laboratories.

1 Reinke, E. E., and Chadwick, C. S., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 691; Reinke, E. E., and Chadwick, C. S., *J. Exp. Zool.*, 1940, **83**, 223.

2 Chadwick, C. S., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 509.