plained by either assuming that a hemagglutinating unit of virus had not yet been accumulated or by the alternate hypothesis offered by Henle and Henle(4) who suggest that the formation of influenza virus proceeds through a development of immature forms or building block. The contentions of these workers can be extended to the present study since it can be seen that while the infectivity titers of the 16th, 18th and 24th hr samples remain stationary, the hemagglutinating titers continue to rise. This paradox almost bespeaks a maturation process that endows the virus with its hemagglutination property, and is an event which lags behind the development of its infectivity.

Summary. 1. Data are presented to support the contention that the growth of Newcastle disease virus in the developing chick embryo occurs in cycles of 4 hr. The apparent 4-hr latent period in the production of virus following the inoculation of an infective dose does not correspond to the lag phase in the multiplication of bacterial cells, but rather indicates an intercellular development of virus particles that cannot be determined by any of the present technics for measuring viral activity. The maximum adsorption of virus onto susceptible cells occurs within 10 minutes after its introduction into the embryonated egg. 2. The existence of an unequal development of infective and hemagglutinating titers during the growth of Newcastle disease virus is described and an attempted explanation for this discrepancy is made on the hypothesis that the hemagglutination unit results from a maturation of the infectious particles and cannot therefore be detected early in its growth.

1. Blumenthal, H. W., Greif, D., Pinkerton, H., and DeWitt, R., *J. Exp. Med.*, 1950, v91, 321.
2. Henle, W., *J. Exp. Med.*, 1949, v90, 1.
3. ———, *J. Exp. Med.*, 1949, v90, 13.
4. Henle, W., and Henle, G., *J. Exp. Med.*, 1949, v90, 23.
5. Hoyle, L., *Brit. J. Exp. Path.*, 1948, v29, 390.
6. Bang, F. B., PROC. SOC. EXP. BIOL. AND MED., 1947, v63, 5.
7. ———, *J. Exp. Med.*, 1948, v88, 233.
8. Burnet, F. M., and Ferry, J. D., *Brit. J. Exp. Path.*, 1934, v15, 56.
9. Cunha, R., Weil, M. L., Beard, D., Taylor, A. A., Sharp, D. G., and Beard, J. W., *J. Immunol.*, 1947, v55, 69.
10. Doyle, T. M., *J. Comp. Path. and Therap.*, 1927, v40, 144.
11. Burnett, F. M., *Aust. J. Exp. Biol. Med. Sci.*, 1942, v20, 81.
12. Henle, W., and Henle, G., *Am. J. Med. Sci.*, 1944, v207, 717.
13. Salk, J. E., *J. Immunol.*, 1944, v49, 87.
14. Reed, L. S., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.
15. Stulberg, C. J., Schapira, R., and Todd, H. C., Abstracts of papers M1:68, Soc. of Amer. Bacteriologists, 1950, 50th General Meeting.

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Response of the Baby Chick to West Nile Virus. (19572)

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The virus employed in this study was the B 956 strain of West Nile virus furnished by the American Type Culture Collection in Washington, D. C. This virus was obtained by the above institution from Dr. K. C. Smithburn(1) of the Rockefeller Foundation Laboratories in New York, who had isolated this virus strain from a patient in Uganda, Africa and had passed this virus 25 times in Swiss albino mice by intracerebral inoculation. This virus has been cultivated in chick embryos by other investigators(2). At this laboratory the above-mentioned virus was passed 4 additional times in mice by the intracerebral route before initiating the present study.

Methods and results. Three-week-old Swiss albino mice (Webster strain) were employed.

This strain of mice was the one recommended by Webster(3) as being especially susceptible to neurotropic viruses. The baby chicks employed were 1-day-old and were furnished by a local farm in the state of Maryland.

Each of 6 mice were inoculated intracerebrally with 0.03 ml of a 10% suspension of West Nile virus of the 29th intracerebral mouse passage. The mice showed involuntary motor reactions on the 4th day. When these symptoms of central nervous system involvement appeared, the mice were sacrificed and the brains were removed aseptically. The pool of infected mice brains was ground with alundum and diluted to a 10% suspension with physiological saline. The suspension was then subjected to 5 minutes centrifugation in a horizontal centrifuge at 2,000 r.p.m. The supernatant was used as the inoculum for initiating the virus experiment in baby chicks. The virus titrated 10^{-6} in Swiss albino mice inoculated intracerebrally. The supernatant material was injected intracerebrally into 6 unvaccinated mice and into 6 mice which had been immunized previously with the 25th mouse passage of West Nile virus. Immunization was accomplished by intramuscular injections using high dilutions of the virus over a period of several weeks. After 6 days the unvaccinated mice showed symptoms of central nervous system involvement, while the immunized mice showed no nervous symptoms during a 30-day observation period. This confirmed the virus to be West Nile virus.

Six 1-day-old baby chicks were injected in the right frontal lobe with 0.05 ml of the supernatant from the virus-bearing mouse brain suspension, and 6 Swiss albino mice were injected intracerebrally with 0.03 ml of the same suspension. From 3 to 6 days after injection the baby chicks showed irritability, extreme nervous tremors, and paralysis. When nervous symptoms developed, the brains were removed aseptically, ground with alundum and diluted to a 10% suspension with physiological saline. This suspension was passed to 6 baby chicks and 6 Swiss albino mice by intracerebral injection. The mice showed West Nile nervous symptoms between 6 and 8 days after inoculation and were discarded. The West Nile virus was carried serially through 8 passages in the baby chick by intracerebral inoculation as shown in Table I. A 10% suspension of virus-bearing chick brain from each passage was injected intracerebrally into 6 Swiss albino mice in order to determine the presence of West Nile virus. All of the mice succumbed to the disease. The incubation periods are shown in Table I. Three hundredth ml of a 10% suspension of virus-bearing chick brain of the 8th intracerebral passage was injected intracerebrally into 6 unvaccinated mice and into 6 mice which previously had been immunized with the 25th mouse passage of West Nile virus. Immunization was accomplished as previously described. After 6 days the unvaccinated mice showed symptoms of central nervous involvement, while the immunized mice showed no nervous symptoms during a 30-day observation period. This confirmed the virus in the brain material of the 8th intracerebral chick passage to be West Nile virus. Throughout the 8 intracerebral baby chick passages, the chicks showed typical symptoms such as paralysis, nervous tremors, malaise, and death.

TABLE I. Response of Baby Chicks and Swiss Albino Mice Inoculated Intracerebrally with West Nile Virus.

Passages	No. chicks exposed	No. chicks showing symptoms	Min to max incubation period,* days	
			Chickens	Mice
1	6	6	3-6	6-8
2	6	4	3-5	6-8
3	6	4	3-5	3-5
4				
5				
6	12	12	4-6	3-5
7				
8				

* Incubation period = time between exposure to virus and onset of symptoms.

Twenty-eight healthy 1-day-old baby chicks were divided into 7 groups of 4 chicks each. Chicks of the first 5 groups were administered 0.06 ml of a 10% suspension of the virus-bearing chick brain of the 7th intracerebral baby chick passage by one of the following methods: intraperitoneal, intradermal, intramuscular, intralingual, and intracardiac injections. Five-hundredths ml of the same

TABLE II. Response of Baby Chicks to West Nile Virus of 7th Intracerebral Chick Passage.

Route of inoculation	No. chicks exposed	No. chicks showing symptoms	Min to max* incubation period, days
Intraperitoneal	4	3	5–10
Rectal	4	4	
Intradermal	4	3	
Intracardiac	4	3	
Intramuscular	4	4	
Intralingual	4	4	
Intranasal	4	4	

* Incubation period = time between exposure and onset of symptoms.

inoculum was instilled intranasally in chicks of the 6th group and rectally in chicks of the 7th group.

After inoculation all groups were observed twice daily for nervous symptoms characteristically found in baby chicks infected experimentally with West Nile virus. The results of this group exposure are given in Table II.

Summary. A strain of West Nile virus isolated by Dr. K. C. Smithburn from a patient's serum in Africa and passaged intracerebrally in Swiss albino mice has been successfully transmitted intracerebrally to 1-day-old White Leghorn chicks. The virus was carried intracerebrally through 8 serial passages in 1-day-old baby chicks. Virus-bearing brain suspensions from chicks of each passage proved to be infective for Swiss albino mice when inoculated intracerebrally. Virus from a chick-brain suspension of the 7th intracerebral passage was transmitted successfully to baby chicks by the following routes of exposure: intraperitoneal, intradermal, intramuscular, intracardiac, intralingual, intranasal and rectal. Baby chicks showed typical symptoms of West Nile disease between 5 to 10 days post-inoculation.

1. Smithburn, K. C., Hughes, T. P., Burke, A., and Paul, J., *Am. J. Med.*, 1940, v20, 471.
2. Taylor, R. M., *J. Immunol.*, 1952, v68, 473.
3. Webster, L. T., and Dawson, J. R., Jr., PROC. SOC. EXP. BIOL. AND MED., 1935, v32, 570.

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Further Studies on Antigenic Variation of Influenza A Virus. (19573)

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In a previous paper(1) it was shown that antigenic variants of influenza A virus strains emerge during serial passage *in ovo* in the presence of immune serum against antigenically different but related strains. It was also demonstrated that the modified strains retained their new antigenic patterns in the absence of immune serum.

It was of interest to determine how long the new antigenic properties would persist on serial passage in the absence of the selective environment used to obtain the variants. This report is concerned with the latter problem.

Materials and methods. Viruses. Two strains of influenza A virus, PR8 and 965, and the variants obtained from them on passage with heterologous immune serum(1) were used. The variant strains had been through 6 passages in the absence of immune serum before the present experiments were begun. In the experimental section, the lines of a particular strain, *e.g.*, PR8, are identified by subscripts as follows: original strain = $PR8_o$; control passage strain = $PR8_c$; heterologous immune serum passage strain = $PR8_9$ (subscript corresponding to the strain, *i.e.*, 965, used to produce the immune serum). Virus titrations, immune serum preparation, neutralizing antibody titrations, and hemagglutination-inhibiting antibody titrations were carried out exactly as in the previous study(1). In the serial passages, the inoculum consisted of 0.2 cc of a 10^{-3} dilution of infected allantoic fluid.

Experimental. The variant strains $PR8_9$ and 965_p which had been through 6 serial passages *in ovo* in the presence of the heterologous immune serum, and then through 6