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.

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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

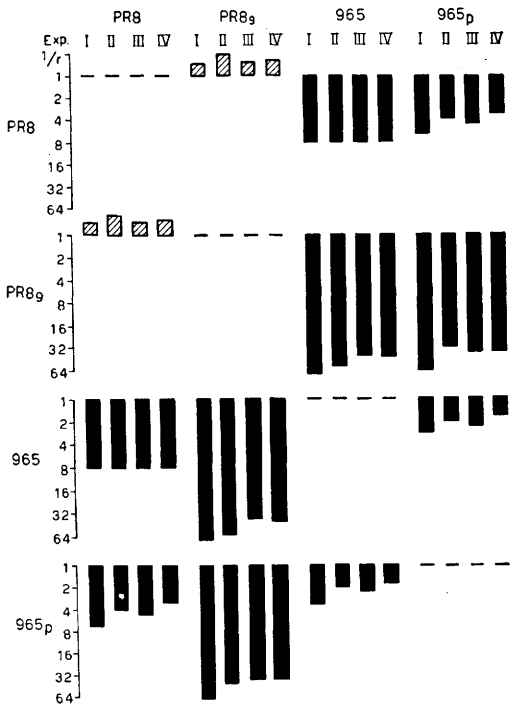

FIG. 1. Extent of cross-neutralization reactions with PR8, 965 and variants derived from them on serial passage *in ovo* in presence of heterologous immune serum. Exp. I, with immune serum; Exp. II, III and IV represent 6, 12, and 24, respectively, additional passages without serum.

additional serial passages in the absence of immune serum, were studied further. In the present study they were subjected to a total of 18 further passages *in ovo* in the absence of immune serum. Cross-serological experiments, by both the *in ovo* neutralization and hemagglutination-inhibition procedures, were carried out at the 6th and 18th additional passages. The objective was to determine if the new antigenic patterns which had appeared on passage in the presence of immune serum would persist throughout a total of 24 *in ovo* passages without serum.

The results of complete cross-neutralization reactions with PR8, 965 and the variant strains derived from them are shown in Fig. 1. The findings in 4 successive experiments during the passage series are illustrated for purposes of comparison. The data for Exp. I and II are taken from the earlier paper of Archetti and Horsfall(1); those for Exp. III and IV were obtained in the present study.

The ratio r represents the geometric mean value of the 2 heterologous titer ratios (r_1 and r_2) and was determined by means of the equation $r = \sqrt{r_1 \times r_2}$(1). In Fig. 1, the ratio r is shown as the reciprocal, $1/r$, and the extent of the antigenic difference between strains is indicated by the length of the blocks. Exp. I was carried out after 6 passages in the presence of immune serum; Exp. II after 6 additional passages without serum(1). Exp. III and IV were done after 12 and 24 passages, respectively, without serum. In all experiments, the same sera were used in each of the cross-neutralization reactions. These were prepared against the strains obtained in Exp. I(1).

It is clear from the results shown in Fig. 1 that the values of $1/r$ found in Exp. III and IV were not significantly different from those obtained in Exp. I and II. This evidence indicated that the antigenic differences, demonstrable by the neutralization procedure, which appeared after passage in the presence of immune serum, tended to persist through a series of 24 *in ovo* passages without serum.

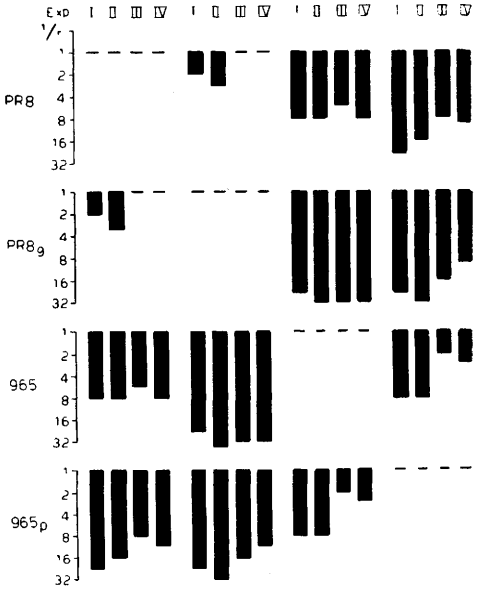

FIG. 2. Extent of cross-hemagglutination-inhibition reactions with PR8, 965 and variants derived from them on serial passage *in ovo* in presence of heterologous immune serum. Exp. I, with immune serum; Exp. II, III and IV represent 6, 12, and 24, respectively, additional passages without serum.

The results of cross-hemagglutination-inhibition reactions between PR8, 965 and the variant strains derived from them on passage with heterologous immune serum are shown in Fig. 2. For comparison, the results of 4 successive experiments during the passage series are presented. As in the case of Fig. 1, the data for Exp. I and II are taken from a previous paper(1), whereas those for Exp. III and IV derive from this investigation. The passage history of the strains employed in Exp. I to IV is described above. The sera were identical with those used in the cross-neutralization reactions.

It appears from the data shown in Fig. 2 that the antigenic differences between PR8 and $PR8_9$ disappeared after 12 passages in the absence of immune serum. However, the differences between 965 and 965_p remained demonstrable, by the *in vitro* procedure, through 24 passages without serum although they were less marked than after 6 such passages. This evidence supports that obtained in the cross-neutralization experiments.

Discussion. It was demonstrated previously that it is possible to select antigenic variants of influenza A virus strains by passage *in ovo* in the presence of immune serum against a somewhat different strain. The new antigenic patterns persisted for 6 serial passages in the absence of immune serum(1). On increasing the number of serial passages without serum to a total of 24, the antigenic differences between one parent strain (965) and its variant persisted, while those between the other parent strain (PR8) and its variant disappeared. The variant (965_p) which showed the persistence of antigenic properties different from those of the parent strain was that which manifested such differences both in neutralization and in hemagglutination-inhibition experiments. On the other hand, the variant ($PR8_9$) which appeared to revert on prolonged passage without serum was that which had shown antigenic dissimilarity only in hemagglutination-inhibition experiments.

Summary. Antigenic differences in influenza A virus strains which appear during serial passage *in ovo* in the presence of heterologous immune serum may remain demonstrable for at least 24 passages without serum.

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Pharmacological Studies on Irradiated Animals. II. Effect of Cortisone on X-Ray Mortality of Mice.* (19574)

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In continuation of our systematic pharmacological studies on irradiated animals, the effect of cortisone on the lethal effect produced by X-rays in total body irradiation of mice is here reported.

Materials and methods. Swiss male white mice of the Institute strain, 22 g ± 15% of body weight. were used. Irradiation was done as described previously(1). The radiation factors were: 200 kv, 15 ma, .25 Cu + 1.0 mm Al filtration, HVL = .8 mm Cu. In agreement with previously outlined rules for pharmacological studies on irradiated animals (2), the effect of a standard dose of cortisone of .25 mg (Cortone (Merck) 1 cc = 25 mg) was administered intramuscularly to mice exposed to various X-ray doses namely those of 410, 350, and 200 r/air, representing the LD 60 and 25/14 days and minimal lethal dose, respectively. Hormone injections were given immediately after exposure and continued to the 14th day after irradiation. For graphic

* The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.