neonatally thymectomized BALB/c mice and found ectopic thymic tissue in 7 of 13 animals. Although the presence of such ectopic thymus in our BALB/c mice might have accounted for the absence of "Wasting disease," the removal of all thymic tissue from the mediastinum significantly increased the susceptibility of the mice to the oncogenic activity of adenovirus 12. Another explanation for the absence of "Wasting disease" in our animals might be the absence of bacterial infection in the thymectomy wounds because of careful aseptic technique. Azar has recently presented evidence that the wasting in neonatally thymectomized Sprague-Dawley rats may be related to such infection(12).

Summary. Newborn BALB/c and C3H/HeN mice inoculated subcutaneously with human adenovirus 12 developed tumors only when thymectomized at birth. Tumors developed in 5 of 30 BALB/c and 3 of 18 C3H/HeN thymectomized mice. These results suggest that neonatally thymectomized animals may be useful in evaluation of the oncogenic potential of viruses which have not produced tumors when injected into non-thymectomized animals.

1. Trentin, J. J., Yabe, Y., Taylor, G., *Science,* 1962, v137, 835.

2. Huebner, R. J., Rowe, W. P., Lane, W. T., *Proc. Nat. Acad. Sci.,* 1962, v48, 2051.

3. Huebner, R. J., Rowe, W. P., Turner, H. C., Lane, W. T., *ibid.,* 1963, v50, 379.

4. Yabe, Y., Samper, L., Bryan, E., Taylor, G., Trentin, J. J., *Science,* 1964, v143, 46.

5. Rabson, A. S., Kirschstein, R. L., Paul, F., *J. Nat. Cancer Inst.,* 1964, v32, 77.

6. Vandeputte, M., Denys, D., Jr., Leyten, R., De Somer, P., *Life Sciences,* 1963, v7, 475.

7. Malmgren, R. A., Rabson, A. S., Carney, P. G., *J. Nat. Cancer Inst.* in press.

8. Miller, J.F.A.P., *Lancet,* 1963, v5, 43.

9. Habel, K., *J. Exp. Med.,* 1962, v115, 181.

10. East, J., Parrott, D. M. V., Chesterman, F. C., Pomerance, A., *ibid.,* 1963, v118, 1069.

11. Law, L. W., Dunn, T. B., Trainin, N., Levy, R. H., Wistar Inst. Symp. Mono. No. 2, 1964.

12. Azar, H. A., Abst. of 61st Meeting of Am. Assn. Path. and Bact., 1964.

Received June 15, 1964. P.S.E.B.M., 1964, v117.

Interaction Between the Soluble Antigen of PR8 and NWS Virus.* (29536)

ROBERT E. LOUIE† AND JACOB FONG
Department of Bacteriology, University of California, Berkeley

Work on multiplicity reactivation(1,2), recombination between ultraviolet irradiated virus and live virus(3), recombination between heat inactivated virus and live virus (4,5,6,7), and recombination between incomplete and complete virus(8) has shown that non-infective forms of a virus can enter a cell and contribute to the formation of new virus particles. These works indicated that inactivation of the infectious property of a virus need not mean that whatever information these virus particles contain is lost or is inaccessible to the cell.

The present investigation is an attempt to ascertain whether the non-infective nucleoprotein (soluble antigen) of influenza A (PR8) virus can transmit information to a susceptible cell and upon subsequent infection of this cell by a heterologous virus contribute to the formation of either genetically or phenotypically mixed particles.

Materials and methods. Viruses. Two egg-adapted strains, PR8 and NWS, of influenza type A were used. They were prepared by suballantoic inoculation of 10- to 11-day-old embryonated eggs with 0.05 ml of a 10^{-4} dilution of virus in beef-heart infusion broth. After 40-48 hours' incubation at 35.5°C, the infected allantoic fluids were harvested and stored in ampoules in a —70°C electrical deep freeze.

* This research was supported in part by a grant (E-3971) from U.S.P.H.S.

† This work constitutes part of the thesis submitted by R.E.L. to the Graduate Division, University of California.

Preparation of the PR8 soluble antigen (PR8-SF). The ether extraction of the soluble antigen from PR8 virus was done according to the method of Lief and Henle(9). Virus concentrated by adsorption and elution from chicken red cells was mixed with a one-half volume of Squibb's anesthetic ether for 2 hours at room temperature. The ether-virus mixture was then transferred to a separatory funnel to allow its separation into a lipid phase and an aqueous phase. The aqueous phase was drawn off and nitrogen gas bubbled through it to rid it of excess ether.

The aqueous phase which contains the hemagglutinating antigen and soluble antigen of the virus was now absorbed 3 times with a 20% suspension of chicken red cells and 3 times with a 20% suspension of guinea pig red cells to remove the hemagglutinating antigen. After the last absorption the supernatant fluid was tested for the presence of hemagglutinins against chicken and guinea pig red cells. If hemagglutinins against either or both types of cells were still present, the fluid was reabsorbed with the respective type of red cell until no hemagglutinins were detected.

Preparation of normal allantoic fluid (NAF). Allantoic fluids from uninfected 10- to 11-day-old embryonated eggs were carried through the same processes used in the concentration of virus from infected allantoic fluids and the extraction of the soluble antigen from concentrated virus fluids.

Anti-PR8 and anti-NWS sera. Antisera against PR8 and NWS were produced by the method described by Baron and Jensen (3). Rabbits were given intravenous injections of 2 ml of infected allantoic fluid for 3 consecutive days every 2 weeks. Three such immunizing courses were given. Fourteen days after the last injection, the animals were bled by cardiac puncture. The sera were cross absorbed with their respective heterologous virus and heated to 56°C for ½ hour to inactivate any residual virus.

Antiserum against the soluble antigen (Anti-SF serum). Antiserum against the soluble antigen of PR8 was prepared by the method of Lief and associates(10). Guinea pigs under light ether anesthesia were given 0.1 ml PR8 stock virus in each nostril. Two to 3 weeks later they were inoculated intraperitoneally with 1 ml of PR8 soluble antigen. One week later the animals were bled by cardiac puncture. Because antiserum thus prepared contained antibodies against the whole virus, the serum was absorbed twice with concentrated suspensions of PR8 and subsequently heated to 56°C for ½ hour to inactivate any residual virus.

Quantitative hemagglutination test. Serial 2-fold dilutions in normal saline were made of the material to be tested. 0.2 ml of a 1% suspension of chicken red cells was added to 0.4 ml of each dilution. The tubes were read by the pattern method after a 45-minute incubation period at room temperature.

In vitro double neutralization test. This test was performed by substituting a 1:200 dilution of one of the antisera (anti-PR8 or anti-NWS) for saline used in the dilution blanks of the standard hemagglutination test. Both sera had previously been cross absorbed with its heterologous virus. The criterion, established by Fraser(11), used to judge a sample to be doubly neutralized was that the sum of its titers in the 2 antisera be less than 50% of its titer in saline. More specifically, in view of the observations that the hemagglutination titer of a virus may be one tube lower when titrated in heterologous serum (Table I) and that a one tube difference in

TABLE I. Hemagglutination Tests of Stock Viruses Performed in Saline, Anti-PR8, and Anti-NWS Sera.

Stock viruses	Saline	Anti-PR8 (1:200)	Anti-NWS (1:200)
PR8	640*	20	640
NWS	160	80	10
Mixture of PR8 and NWS†	320	80	160

Tests were done using saline, anti-PR8 serum (diluted 1:200), and anti-NWS serum (diluted 1:200) as dilution blanks in the standard hemagglutination titration.

* Titers are expressed as the reciprocal of the highest dilution of virus causing complete or partial agglutination of chicken red blood cells.

† Equal volumes of stock PR8 and stock NWS viruses were mixed.

titer in the hemagglutination test is within experimental error, a sample was judged doubly neutralized if each of its titers in the 2 antisera was at least 3 tubes lower than its titer in saline. This criterion was used mostly in reference to the titers in anti-PR8 serum since the titers in anti-NWS were usually 20 or less.

Infectivity tests of the soluble antigen. All soluble antigen preparations used in the experiments were tested for the presence of infective virus in order that there be no equivocation as to whether the observed results were due to residual virus or the soluble antigen. Each preparation of the soluble antigen was tested on the same day it was used in an experiment. A number of 12- to 14-day-old embryonated eggs, equal to the number which received soluble antigen in the experiment, were inoculated intravenously with the same amount of this material. These eggs were incubated at 35.5°C for the same length of time the soluble antigen remained in the experimental eggs. Afterwards the allantoic fluids were harvested and pooled. The chorioallantoic membranes were collected, ground with sterile sand, and suspended in 1 ml of beef-heart infusion broth per membrane. This suspension was clarified by centrifugation. The 2 pools, allantoic fluid and membrane extract, were inoculated into a sufficient number of 10- to 11-day-old embryonated eggs (0.5 ml per embryo) to use up all of each fluid. After 42-48 hours' incubation at 35.5°C, the allantoic fluids were tested for the presence of hemagglutinins. In a few instances, hemagglutinins were found in the eggs inoculated with the membrane extract but not in the eggs which received the allantoic fluid. The experiments in which the soluble antigen preparation contained infective virus were discarded; only those experiments employing non-infectious soluble antigen are reported here.

Intravenous inoculation of embryonated eggs. Under transillumination, the outline of a straight and prominent blood vessel on the chorioallantois of a 12-day-old embryonated egg was traced on the shell. The direction of blood flow in this vessel was marked by observing the direction of convergence of the tributary vessels. This area of the shell was cleansed with 70% ethyl alcohol. Overlying this blood vessel an elliptical piece of shell (1 cm × 0.5 cm) was cut out with a small hand drill and separated from the shell membrane with a dissecting needle. Embryonated eggs thus prepared were reincubated and the intravenous injections made the following day. To perform the intravenous injection, the egg was laid on its side on a piece of modeling clay with the cut portion of the shell uppermost. A drop of light mineral oil may be placed on the exposed shell membrane to facilitate visualization of the blood vessel under transillumination. For the inoculation a 27-gauge needle was used. The inoculum was injected in the direction of blood flow. Care must be taken when inserting the needle so that there is only a very slight angle between the needle and the blood vessel. To prevent bleeding afterwards, the needle should be withdrawn at exactly the same angle at which it was inserted. Eggs which bled excessively were discarded.

Results. In ovo neutralization tests with anti-PR8 and anti-NWS sera. As a preliminary to studies on the interaction of the PR8 soluble antigen and NWS virus, tests were performed to determine the neutralizing capacity and specificity of the two antisera, anti-PR8 and anti-NWS. Prior to use in any of these tests, each of the 2 antisera was cross absorbed with its heterologous virus.

In ovo serum neutralization tests were performed using a constant dilution (1:10) of antiserum against serial 10-fold dilutions of the virus. Equal volumes of virus dilution and antiserum were mixed and incubated at room temperature for ½ hour prior to suballantoic inoculation of 10- to 11-day-old embryonated eggs. The allantoic fluids were tested for the presence of hemagglutinins after 42 to 48 hours' incubation at 35.5°C. Anti-PR8 and anti-NWS sera were thus tested against their homologous and heterologous virus. In addition, each virus was titrated in the presence of a 1:10 dilution of normal rabbit serum. The results of these titrations (results not shown) revealed that

the titers of PR8 and NWS were the same in the presence or absence of normal rabbit serum, thereby indicating that non-specific inhibitors were not present in rabbit serum used at a 1:10 dilution. The 2 antisera, anti-PR8 and anti-NWS, were capable of neutralizing 10^9 and 10^6 EID_{50} respectively of the homologous virus. When directed against its heterologous virus, each of the 2 antisera showed some slight degree of cross neutralization. Anti-NWS decreased the titer of PR8 virus by 0.5 log unit and anti-PR8 decreased the titer of NWS by 0.8 log unit. These amounts of cross neutralization by the 2 antisera can be neglected if the condition is made that any *in ovo* double neutralization of virus progeny by these 2 antisera shall exceed 0.8 log unit.

Interaction between PR8-SF and NWS. Previous efforts to effect an interaction between the soluble antigen of PR8 and NWS virus by intra-allantoic inoculation of embryonated eggs produced negative results. In the following experiments embryonated eggs were inoculated intravenously with the two agents. This procedure was based on the hypothesis that inhibitors of the soluble antigen are present in the allantoic fluid and that by administration of the soluble antigen and NWS intravenously, the soluble antigen would not come in contact with these hypothetical inhibitors.

To effect an interaction between the soluble antigen and NWS, nine 12-day-old embryonated eggs were inoculated intravenously with 0.25 ml of the soluble antigen. After incubation at 35.5°C for 1 hour these eggs were given 0.25 ml of a 1:4 dilution of NWS by the same route and reincubated for 10 hours. The allantoic fluids were subsequently harvested and pooled. This pooled fluid, called the primary fluid, was divided into 3 aliquot parts. Ten- to 11-day-old embryonated eggs were inoculated intra-allontoically with the entire first portion; 0.5 ml inoculum was used per egg. Anti-PR8 and anti-NWS sera were added (in a final dilution of 1:20) to the second and third portions respectively. These were allowed to stand at room temperature for ½ hour. Embryonated eggs were inoculated with these 2 aliquots in the same manner as the first aliquot. After incubation for 42-48 hours the allantoic fluids were tested for the presence of hemagglutinins.

The results of this first passage of the primary fluids (results not shown) were that the first group of eggs, inoculated with the fraction of the primary fluid without antiserum, showed development of hemagglutinins in 12 out of 15 eggs. The 12 positive allantoic fluids were pooled and later used again for titrations. In the second group of eggs, given the fraction of primary fluid containing anti-PR8 serum, 5 out of 15 eggs showed the presence of hemagglutinins. Of the eggs in the third group, inoculated with the fraction of primary fluid with anti-NWS, only 1 out of 13 showed the presence of hemagglutinins.

Since the PR8 soluble antigen was non-infective, the only infective virus present in the primary fluid would be NWS if no interaction had occurred between it and the soluble antigen. If this were the case, the virus would be neutralized by anti-NWS serum and not by anti-PR8 serum. However, while anti-NWS serum did neutralize practically all of the virus present in the primary fluid, anti-PR8 serum also neutralized this virus to some extent. Based on this interpretation of the data, the virus in the primary fluid was assumed to have characteristics of both NWS and PR8 as the result of some type of interaction between the PR8 soluble antigen and NWS virus.

To confirm and quantitate the preceding results, the pooled positive allantoic fluids from the first group of eggs were titrated in triplicate. The 3 titrations were done without antiserum and in the presence of a 1:10 dilution of anti-PR8 and anti-NWS sera.

The results of the 3 titrations (Table II) indicated that the virus titer was decreased considerably by the 2 antisera. As compared to the amount of virus detected (7.44×10^7 EID_{50}/ml) in the absence of antiserum, there were only 8.24×10^4 EID_{50}/ml and 1.28×10^2 EID_{50}/ml in the presence of anti-PR8 and anti-NWS sera respectively. The

TABLE II. *In ovo* Double Neutralization Test on First Passage Fluids from the Interaction of PR8-SF and NWS.

	Virus titer (EID_{50}/ml)*
No antiserum	7.44×10^7
Anti-PR8	8.24×10^4
Anti-NWS	1.28×10^2

* EID_{50} = 50% egg infective dose.

sum of the latter two virus titers not adding up to the total amount per milliliter was interpreted as confirmation of the preceding observation, namely that the virus in the primary fluid was doubly neutralized by anti-PR8 and anti-NWS sera.

Effect of antisera on interaction of PR8-SF and NWS. Groups of three 12-day-old embryonated eggs were inoculated intravenously (0.25 ml per embryo) with one of the following preparations: SF, SF and anti-PR8 serum, SF and anti-SF serum, and normal allantoic fluid (NAF). The dilutions of anti-PR8 and anti-SF sera in the SF preparations were 1:20 and 1:8 respectively. These were allowed to stand at room temperature for ½ hour before use. The inoculated eggs were incubated at 35.5°C for 1 hour and subsequently inoculated intravenously with 0.25 ml of undiluted NWS virus. After further incubation for 10 hours the allantoic fluids were harvested and passaged in embryonated eggs in an attempt to isolate double neutralizable virus in the region of limited dilution. For this purpose, 5-fold dilutions of the fluids were made and inoculated into the allantoic cavity of a sufficient number of eggs to use up all the fluid of each dilution, each embryonated egg receiving 0.5 ml inoculum. After the usual incubation period, those hemagglutinin-containing fluids from the 50% end point region were tested for the presence of doubly neutralizable virus by the *in vitro* double neutralization test.

In ovo passage of the allantoic fluids from eggs inoculated with NAF and NWS yielded a total of 18 hemagglutinating fluids. Double neutralization tests performed on these fluids showed no doubly neutralizable particles to be present. Tests for doubly neutralizable particles in the 21 hemagglutinating fluids from the passage of allantoic fluids of eggs inoculated with SF, anti-PR8, and NWS were likewise negative.

Passage in embryonated eggs of undiluted and 5-fold dilutions of allantoic fluids from eggs inoculated with SF, anti-SF, and NWS yielded 11 hemagglutinating fluids. Double neutralization tests performed on these fluids revealed that fluid #2 was doubly neutralizable (Table III). This fluid had titers of 1:128 in saline and 1:16 in both anti-PR8 and anti-NWS sera. It was from an embryonated egg which had received an undiluted inoculum.

Nine hemagglutinating fluids resulted from the *in ovo* passage of allantoic fluids of eggs inoculated with SF and NWS. Of these nine, 3 fluids, #18, #19, and #20 were doubly neutralized by anti-PR8 and anti-NWS sera (Table IV). Fluids #18 and #19 were from embryonated eggs which had received undiluted inocula; fluid #20 was from an embryonated egg which had received an inoculum diluted 1:5.

Second passage of doubly neutralizable fluids. The fluids which showed double neu-

TABLE III. Double Neutralization Tests on Hemagglutinin-Containing Fluids from the Passage of SF/anti-SF, NWS Primary Fluids.

	Reciprocal of hemagglutination titer*		
Fluid No.	In saline	In anti-PR8	In anti-NWS
1	4	—	—
2†	128	16	16
3	<2	—	—
4	<2	—	—
5	32	16	2
6	128	64	8
7	<2	—	—
8	512	256	8
9	256	64	—
10	64	64	—
11	256	256	—

Embryonated eggs were inoculated intravenously with a mixture of SF and anti-SF, followed 1 hr later with NWS. After 10 hr incubation the allantoic fluids, designated SF/anti-SF, NWS primary fluids, were passaged in embryonated eggs. The hemagglutinin-containing fluids from this passage were tested for presence of doubly neutralizable particles.

* Hemagglutination titers were determined using saline, 1:200 dilution of anti-PR8 and 1:200 dilution of anti-NWS sera as dilution blanks in the standard hemagglutination titration.

† This fluid was saved and used for second passage.

— = No hemagglutination titer detected.

TABLE IV. Double Neutralization Tests on Hemagglutinin-Containing Fluids from Passage of SF/NWS Primary Fluids.

Fluid No.	Reciprocal of hemagglutination titer* In saline	In anti-PR8	In anti-NWS
12	16	8	2
13	<2	—	—
14	<2	—	—
15	128	64	8
16	512	256	—
17	32	16	—
18†	128	16	—
19†	256	32	—
20†	512	64	—

Embryonated eggs were inoculated intravenously with SF followed 1 hr later with NWS. After 10 hr incubation the allantoic fluids, designated SF/NWS primary fluids, were passaged in embryonated eggs. The hemagglutinin-containing fluids from this passage were tested for presence of doubly neutralizable particles.

* See footnote (*) of Table III.

† These fluids were saved and used for second passage.

— = No hemagglutination titer detected.

tralization by anti-PR8 and anti-NWS sera were passaged in eggs a second time for reisolation of virus progeny showing this characteristic. Ten- to 11-day-old embryonated eggs were inoculated by the allantoic route with 10-fold dilutions of fluids #2, #18, #19, and #20. Fifteen eggs were used per dilution, each egg receiving 0.05 ml inoculum. After the usual incubation period the allantoic fluids were tested for hemagglutinins. Double neutralization tests were performed on the positive fluids from the 50% end point region. The results (not shown) indicate that none of the positive fluids tested was doubly neutralized by anti-PR8 and anti-NWS sera. The double neutralization property of these fluids was apparently lost in this second passage.

Discussion. The first study on the interaction between the soluble antigen of PR8 and NWS virus showed that the soluble antigen inoculated into embryonated eggs followed one hour later by NWS produced viral progeny which gave indication of being doubly neutralized by anti-NWS and anti-PR8 sera. *In ovo* double neutralization tests performed on the once passaged viral progeny confirmed the indication that the viral progeny in the primary fluid was doubly neutralized by the 2 antisera.

Studies on the effect of antisera (anti-PR8 and anti-SF) on the production of doubly neutralizable virus showed that the mixing of the soluble antigen with anti-PR8 serum prior to inoculation resulted in the production of virus which was not doubly neutralizable by the two antisera. If the working hypothesis upon which these interaction studies are based is correct, namely that the doubly neutralizable virus was the result of interaction between NWS virus and the soluble antigen, then the mixing of the soluble antigen and anti-PR8 serum should have had no effect on the soluble antigen. This mixture and NWS should have also produced doubly neutralizable virus. However, as stated above, this was found not to be the case. This apparent discrepancy may be accounted for if it is considered that the anti-PR8 serum, produced in rabbits by repeated inoculations of infective allantoic fluid, probably contained antibodies against the soluble antigen of PR8 as well as antibodies against the whole virus.

Reacting a mixture of SF and anti-SF with NWS *in ovo* resulted in production of doubly neutralizable virus in one of the infective fluids. This one doubly neutralizable fluid may have arisen due to the dissociation of the anti-SF-soluble antigen complex before or after its inoculation into the embryo, and not to residual PR8 in the soluble antigen preparation. One observation in support of this contention is that only one out of 11 fluids in the SF/anti-SF, NWS series was doubly neutralizable, whereas in the SF/NWS series, 3 out of 9 fluids tested were doubly neutralizable. If doubly neutralizable virus were indeed due to residual PR8 and NWS interaction, a more equal percentage of doubly neutralizable fluids would be expected in these 2 series. To eliminate a further possibility that the doubly neutralizable virus was due to interaction of NWS and some unknown factor in the suspending medium of the soluble antigen, NWS and NAF were reacted *in ovo*. This did not result in any doubly neutralizable fluids.

Second passage of these doubly neutralizable fluids was unsuccessful. None of the resultant hemagglutinin-containing fluids tested possessed this property. The loss of these doubly neutralizable particles upon passage may have been due to an inherent instability or to the fact that they were not true genetic recombinants but were the result of phenotypic mixing. The phenomenon of phenotypic mixing, described for bacteriophage by Delbrück and Baily(12), Novick and Szilard(13), and later used by Hirst (14), Gotlieb and Hirst(15), and Hirst and Gotlieb(16) to explain some of their results on the passage of doubly neutralizable virus, may be applicable to these findings. According to Hirst and Gotlieb(16), these phenotypically mixed particles could be passaged 10 times provided a large inoculum was used. The use of small or dilute inocula caused a reversion of these to the 2 parental types.

The doubly neutralizable fluids #2, #18, #19, and #20 (Tables III and IV) were from eggs inoculated with 0.5 ml of undiluted or 1:5 dilutions of the primary fluid. Passage of these fluids, using a small inocula (0.05 ml per egg), resulted in the loss of the double neutralization property. These results seem compatible with those of Hirst and Gotlieb (16), namely, passage of doubly neutralizable fluids using large inocula causes retention of this property, whereas passage of these fluids using small inocula causes a loss of this property.

Since the soluble antigen preparations used in these experiments were non-infective, the mechanism involved in the formation of doubly neutralizable particles needs consideration. In preliminary experiments employing the fluorescent antibody technique, soluble antigen particles were detected in the nuclei of chorioallantoic membrane endodermal cells of embryos inoculated with the soluble antigen. With the passage of time, however, no hemagglutinating antigens were detected. The infection of soluble antigen-containing cells with NWS virus and the production of doubly neutralizable particles may indicate that in some way the NWS virus had caused a "maturation" of the intranuclear soluble antigen to infective virus. The simultaneous maturation of the PR8 soluble antigen and the replication of NWS virus could then result in formation of phenotypically mixed particles.

An alternative explanation is that the soluble antigen-containing cells may also contain small amounts of hemagglutinating antigen which were not detected by the fluorescent antibody technique. Subsequent infection and replication of NWS in these cells may then incorporate these PR8 hemagglutinating antigens into the NWS progeny. However, with such a mechanism it is inconceivable how the doubly neutralizable particles could survive even one passage.

Summary. The soluble antigen of PR8 virus was interacted *in ovo* with NWS. The passaged allantoic fluids were doubly neutralized by anti-PR8 and anti-NWS sera. Second passage of these doubly neutralizable particles resulted in a loss of this character. The hypothesis was presented that the doubly neutralizable particles were the result of phenotypic mixing when NWS virus in some way brought about the "maturation" of the intranuclear PR8 soluble antigen.

1. Henle, W., Liu, O. C., *J. Exp. Med.*, 1951, v94, 305.

2. Barry, R. D., *Virology,* 1961, v14, 389.

3. Baron, S., Jensen, K. E., *J. Exp. Med.,* 1955, v102, 667.

4. Burnet, F. M., Lind, P. E., *Aust. J. Exp. Biol. Med. Sci.,* 1954, v32, 133.

5. Fraser, K. B., *Virology,* 1959, v8, 544.

6. Kilbourne, E. D., *ibid.,* 1960, v11, 291.

7. Kilbourne, E. D., Murphy, J. S., *J. Exp. Med.,* 1960, v111, 387.

8. Burnet, F. M., Lind, P. E., Stevens, K. M., *Aust. J. Exp. Biol. Med. Sci.,* 1955, v33, 127.

9. Lief, F. S., Henle, W., *Virology,* 1956, v2, 753.

10. Lief, F. S., Fabiyi, A., Henle, W., *J. Immunol.,* 1958, v80, 53.

11. Fraser, K. B., *Brit. J. Exp. Path.,* 1953, v34, 319.

12. Delbrück, M., Baily, N. T., Jr., *Cold Spring Harbor Symp. Quant. Biol.,* 1946, v11, 33.

13. Novick, A., Szilard, L., *Science,* 1951, v113, 34.

14. Hirst, G. K., *Cold Spring Harbor Symp. Quant. Biol.,* 1953, v18, 25.

15. Gotlieb, T., Hirst, G. K., *J. Exp. Med.,* 1954, v99, 307.

16. Hirst, G. K., Gotlieb, T., *ibid.,* 1953, v98, 53.

Received June 15, 1964. P.S.E.B.M., 1964, v117.