Incomplete Growth Cycle of Influenza Virus in Mouse Brain. (17966)

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Stuart-Harris(1) and Francis and Moore (2) were able to propagate the WS strain of type A influenza virus in mouse brain. Once adapted to this organ, the virus multiplied and produced fatal encephalitis after intracerebral inoculation. On the other hand, repeated attempts to propagate other strains of influenza virus in mouse brain have failed (2,3,4). Yet, despite the absence of readily demonstrable neurotropic tendencies, active preparations of several egg-adapted strains have been found to have two striking effects after intracerebral inoculation in mice: (a) "toxicity"(3), and (b) the ability to interfere with certain unrelated neurotropic viruses, *e.g.*, equine encephalomyelitis viruses(4). Both effects, especially "toxicity," depend on the inoculation of relatively large amounts of virus.

It was thought that a possible explanation of the interference between influenza and unrelated viruses might be found in the form of common cellular receptors. In studies on the effect of infection with influenza viruses on the amount of virus hemagglutination inhibitor (VHI) extractable from a variety of tissues, no relationship between VHI and equine encephalomyelitis virus could be detected(5). It was found, however, that mouse brain was a particularly rich source of VHI. Progressive destruction of VHI was noted after intracerebral inoculation not only of the neurotropic WS variant (WS–N) but also, to a lesser degree, of the non-neurotropic WS, PR8, or Lee strains(5). It appeared difficult to reconcile this observation with the fact that the latter strains did not multiply, but, on the contrary, progressively decreased in titer in the mouse brain. The measurable decrease in VHI, which was assumed to be due to progressive destruction of cellular receptors of the brain, suggested some viral activity which could not be accounted for by the amount of infective virus present.

Reports to the effect that after inoculation of influenza virus into the allantoic cavity of chick embryos, the appearance of newly formed, infectious virus was preceded by that of specific complement-fixing antigen and viral hemagglutinin(6,7) stimulated the idea that influenza virus might undergo in the mouse brain an incomplete cycle of reproduction in which the development of virus particles did not proceed beyond the hemagglutinating to the infectious stage. This hypothesis has been borne out by experimental findings.

Materials and methods. Virus strains: The PR8 and WS strains of type A, and the Lee strain of type B influenza virus were propagated in the allantoic cavity of 11-day chick embryos. Eggs inoculated with infected allantoic fluid, diluted 10^{-6}, were incubated for 2 days at 36°C, then chilled overnight at 4°C before harvest. Freshly harvested virus was used, which was sometimes concentrated by centrifugation for 1 hour at 13,000 r.p.m. in the cold and resuspension of the sediment in a reduced volume of the supernatant. The neurotropic variant of the WS strain (WS–N) was originally obtained from Dr. T. Francis, Jr., and has been maintained by intracerebral passage in mice. Stock virus suspensions consisted of 20% infected mouse brain in 50% heated normal rabbit serum in saline.

Cholera vibrio filtrates: Culture 35A3 of

1. Stuart-Harris, C. H., *Lancet*, 1939, v1, 497.
2. Francis, T., Jr., and Moore, A. E., *J. Exp. Med.*, 1940, v72, 717.
3. Henle, G., and Henle, W., *J. Exp. Med.*, 1946, v84, 623.
4. Vilches, A., and Hirst, G. K., *J. Immunol.*, 1947, v57, 125.
5. Schlesinger, R. W., to be published, see Abstracts of papers, *Soc. Am. Bact.*, 49th Gen. Meeting, 1949, p. 94.
6. Hoyle, L., *Brit. J. Exp. Path.*, 1948, v29, 390.
7. Henle, W., and Henle, G., *J. Exp. Med.*, 1949, v90, 23.

V. cholerae (Inaba) was obtained from the National Institute of Health, Bethesda, Md. Berkefeld N filtrates of 18 hour beef infusion broth cultures were used as source of "receptor-destroying enzyme" (RDE)(8). Such preparations were capable of destroying, in 1 hour at 37°C, all hemagglutinin inhibitors demonstrable in mouse brain extracts(5).

Infectivity titrations: Ten-fold serial dilutions of test materials were inoculated into 11- or 12-day-old eggs. Fluids were harvested after 48 hours' incubation at 36°C and tested for hemagglutinin (HA). Infectivity titers were estimated on the basis of HA production and will be expressed as EID_{50} per gram of tissue or per ml of fluid(9). Intracerebral titers of the WS–N strain are also given in terms of LD_{50} per gram of infected brain tissue.

Hemagglutinin titrations: To 0.5 ml of each 2-fold serial dilution of test material, 0.5 ml of a 0.4% (sometimes 0.3%) suspension of fowl erythrocytes (RBC) was added. All dilutions were made in saline or, when the test material contained RDE, in saline containing 1 or 2% sodium citrate(10). The tests were read as soon as the cells in control tubes had settled in a clearly demarcated button, usually after 1 hour(11). Agglutination was read as either complete or partial, with partial agglutination usually limited to one tube. The endpoint was either the highest dilution giving complete HA or the logarithmic mean between the highest dilution giving partial HA and the next lower dilution. Titers will be expressed as the inverse log of the final concentration (after addition of cells) of test material.

Partial purification by adsorption and elution. Because the presence of tissue components tended to obscure the sedimentation pattern in HA titrations, it was desirable to eliminate them. Fowl RBC to a final concentration of 1.5% were added to the test material. If the latter contained RDE, sodium citrate to a final concentration of 1% was added. The mixture was kept at 4°C for 1 hour to allow adsorption, then the cells were sedimented and resuspended in the desired volume of RDE. Incubation in the 37°C waterbath for 60 to 90 minutes resulted in elution of all adsorbed HA.

Complement-fixation tests: Crude 1:4 mouse brain suspensions in saline were centrifuged at 13,000 r.p.m. for 1 hour, and the clear supernatant was used as C-F antigen. Presumably, these preparations contained mainly the "soluble" antigen(12). 0.1 ml of each 2-fold antigen dilution and 0.1 ml of a constant dilution of type-specific or of control human serum were mixed with 0.2 ml of complement dilution representing 1.5 to 2 units. After incubation for 1 hour at 37°C, 0.2 ml of a 1.5% sensitized sheep erythrocyte suspension was added. The tests were read after further incubation for 30 minutes at 37°C. The highest dilution of antigen giving partial fixation was chosen as endpoint. If the highest positive dilution gave complete (4+) fixation, the endpoint was considered as 0.15 log higher. Antigen titers will be expressed in terms of final concentration of brain tissue before addition of the hemolytic system.

Experimental. "Unmasking" of virus hemagglutinins after digestion of brain inhibitors by cholera vibrio filtrates: Previous experiments had revealed VHI of high titer in mouse brain extracts(5). While infection with the neurotropic strain, WS–N, reduced the titer progressively to about 5% of the normal value by the 3rd to 4th day after inoculation, there remained enough VHI in the tissue to inhibit any demonstrable hemagglutinin in brains which contained 10^8 LD_{50} or more of the virus. Since it had been found (5) that brain VHI, like red cell receptors and inhibitors from other sources, could be destroyed by the receptor-destroying enzyme (RDE) in cholera vibrio filtrates(8), the effectiveness of RDE in "unmasking" hemag-

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9. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

10. Stone, J. D., *Austr. J. Exp. Biol. and Med. Sc.*, 1947, v25, 137.

11. Salk, J. E., *J. Immunol.*, 1944, v49, 87.

12. Wiener, M., Henle, W., and Henle, G., *J. Exp. Med.*, 1946, v83, 259.

TABLE I.
"Unmasking" of PR8 Virus Hemagglutinin from a Mixture of Virus and Mouse Brain by RDE.

Test material	Medium	Log HA titer* after overnight storage at 4°C	Log HA titer* after overnight storage at 37°C with RDE	Log, EID/ml*	EID/HA ratio (log)
PR8 AF 1:50	Saline	3.9	3.9	10.2	6.3
" " "	25% NMB	<2.0	3.8	10.2	6.4

* Titers are expressed as inverse log of final dilution of original allantoic fluid concentrate (centrifuged 1 hr at 13,000 rpm, sediment resuspended in 1/10 vol. supernate).

TABLE II.
Infective, Hemagglutinating, and Complement-fixation Antigen Titers of Mouse Brains Harvested 3 Days After Intracerebral Inoculation of the Neurotropic WS strain.

Intracerebral inoculum LD_{50}/0.03 ml (log)	Titers* (log) of brain tissue				
	LD_{50}/g	EID/g	HA	C-F antigen	Ratio EID/HA
6.0	7.2	8.5	2.3	1.5	6.2
5.0	8.0	8.0	2.3	1.65	5.7
3.0	8.5	8.0	2.1	1.5	5.9
1.0	7.7	8.2	1.5	0.9	6.7

* Titers are expressed as inverse log of the final concentration of brain tissue (by wet wt).

glutinins in mixtures of virus and mouse brain suspensions was tested. To a 1:4 suspension of homogenized normal brain tissue in saline was added 1/50 volume of PR8-infected allantoic fluid. A portion of the mixture was kept in the refrigerator. To the remainder, equal parts of RDE were added, and the mixture was kept in the 37°C waterbath overnight. Similarly, virus diluted 1:50 in saline was incubated with RDE at 37°C overnight. The next day, all samples were tested for hemagglutinin (HA).

Table I shows that RDE under these conditions failed to affect the HA titer of the virus diluted in saline. However, while no HA was detectable in the untreated brain-virus mixture, overnight incubation in presence of RDE "unmasked" HA to full titer. Further tests revealed that incubation of brain-virus mixtures with RDE for 1 hour was sufficient to obtain 100% recovery of known amounts of virus HA, even without addition of Ca ions(13).

Infective, HA, and C-F antigen titers in mouse brains after intracerebral inoculation of the WS–N strain. Treatment of brain suspensions with RDE was used in several experiments with the WS–N strain in an attempt to determine whether HA could be demonstrated also in tissue infected *in vivo*.

In a typical experiment, mice were inoculated intracerebrally with varying amounts, ranging from 10^1 to 10^6 LD_{50}, of WS–N stock virus. On the third day, 5 to 7 mice of each group were sacrificed. Their brains were pooled and 25% suspensions were prepared. Portions of each suspension were used for the titrations summarized in Table II. All of the groups yielded titers of the same order of magnitude in each type of test, indicating that the yield at the peak of virus multiplication was not affected by the size of the intracerebral seed inoculum. Of particular interest, in the light of the data to be described below, is the fact that the EID/HA ratio was about 10^6 in each instance. Thus, after digestion of infected brain tissue with RDE, it was possible to show that this strain of virus has retained the EID/HA ratio characteristic of the WS and other strains of influenza virus grown under optimal conditions in the allantoic cavity of chick embryos.

Infective, HA, and C-F antigen titers in mouse brain after intracerebral inoculation of non-neurotropic strains of influenza virus.

13. Burnet, F. M., and Stone, J. D., *Austr. J. Exp. Biol. and Med. Sc.*, 1947, v25, 227.

TABLE III.
Infective, Hemagglutinating, and Complement-fixation Antigen Titers of Mouse Brains Harvested at Varying Intervals After Intracerebral Inoculation of PR8-infected Allantoic Fluid.

	Titers (log)				
		Hemagglutinin after overnight at			
Test material	EID/g	4°C	37°C with RDE	C-F antigen	Ratio EID/HA
Seed virus (all. fluid)	10.2	3.9	3.9	n.t.†	6.3
M.B. 1 hr*	7.2	<.9	<1.8	<0.9	>5.4
" 5-6 hr	7.1	<.9	1.95	1.05	5.15
" 24 "	6.0	<.9	3.0	1.5	3.0
" 48 "	5.1, 5.3‡	<.9	3.45	1.8, 1.5‡	1.75

For explanations see Table II.
* M.B. = Mouse brain.
† n.t. = not tested.
‡ Duplicate figures indicate tests on two separate brain pools.

Entirely different results were obtained with brains of mice inoculated intracerebrally with the non-neurotropic WS, PR8, or Lee strains. As had been previously found by others(3,4). only a fraction of the inoculum could be recovered as infectious virus from brains harvested within 1 or 2 hours after inoculation. This fraction amounted to about 1 to 10% of the amount theoretically recoverable if one assumed that all of the inoculum remained unaltered within the brain.* This initial drop in recoverable infectious virus was followed by further progressive decrease in titer during subsequent days (Table III). The infective titer of brain suspensions was not increased by incubation with RDE. In contrast, as shown in Table III, treatment with RDE revealed the presence of HA in samples in which none was demonstrable without treatment. Since the seed virus had an HA titer of $10^{-3.9}$, the maximum theoretical yield at 1 hour (without virus multiplication) would have been $10^{-2.8}$. The actual titer was less than $10^{-1.8}$ (lowest dilution tested). However, HA was demonstrable after 5 to 6 hours. Two days after inoculation the titer was 4-fold higher than the theoretical maximum and at least 45-fold higher than at 1 hour. The significance of this increase is underlined by the shift in the EID/HA ratio from $10^{6.3}$ for the allantoic fluid inoculated to $10^{1.75}$ for the brains harvested 2 days after inoculation. Simultaneously with the increase in HA, specific complement-fixing antigen became demonstrable in the mouse brains.

Production of viral hemagglutinin in mouse brain in a single cycle of multiplication. Fig. 1 shows that after intracerebral inoculation of PR8 virus, hemagglutinin was not demonstrable at 1 to 3 hours but began to appear after 4 hours. It increased rapidly up to 9 hours, after which there was no appreciable further rise. The maximum titer was about 100-fold higher than the initial amount. Similarly, after intracerebral inoculation of the PR8 or WS strains, any significant increase in C-F antigen was completed within about 8 hours after inoculation. In contrast to the findings for the type A strains, it was found that the constant period preceding any demonstrable

* One mouse brain weighs approximately 0.4 g and has a volume of about .4 ml *in situ*. Inoculation of .03 ml into this organ would therefore result in 13-fold dilution of the inoculum. Hence, the maximum amount theoretically recoverable before multiplication begins would be 1.1 log less than the inoculum. Initial losses of 90 to 99% of the theoretically recoverable virus have been described for equine encephalomyelitis virus in mouse brain (14) and also for influenza viruses in the chorioallantoic membrane of the chick embryo(15). Since in the case of the mouse brain it is impossible to control the amount of leakage or to separate adsorbed from free virus, nothing can be surmised concerning the nature of this initial loss.

14. Schlesinger, R. W., *J. Exp. Med.*, 1949, v89, 491.

15. Henle, W., *J. Exp. Med.*, 1949, v90, 1.

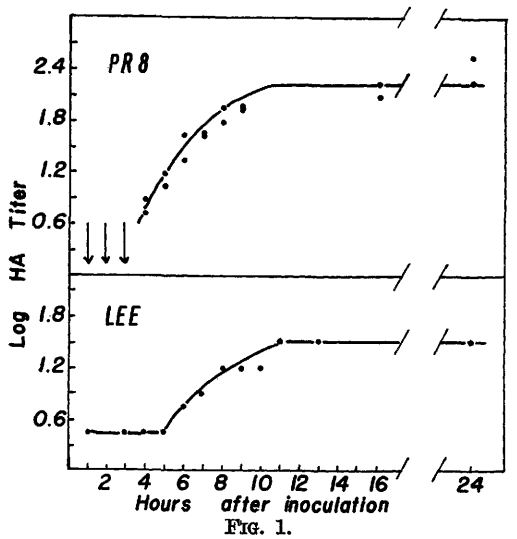

FIG. 1.

Rise in HA titer in mouse brain after intracerebral inoculation of allantoic fluids infected with PR8 or Lee virus.

increase was at least 6 hours when the Lee strain was used for intracerebral inoculation. Fig. 1 shows that the highest titer was reached after 11 hours and was maintained up to at least 24 hours after inoculation.

These results, especially the characteristic difference between the PR8 and Lee strains, led to the tentative conclusion that the HA and the C-F antigen were products of a process analogous to a single growth cycle in the allantoic membrane(16) which, in the mouse brain, did not go on to the completion of infectious virus particles. On passage to other mice, the HA failed to further reduplicate itself, just as it failed to infect chick embryos. In this respect, the material derived from mouse brains appeared to be similar to the hemagglutinating, but non-infectious "500 S component" present in allantoic fluid of eggs inoculated with PR8 virus in high concentration(17). However, while the allantoic membrane is known to support the propagation of fully infectious virus, especially after inoculation of more dilute seed virus(18), this was not true for non-neurotropic strains in mouse brain. In fact, if the cells involved in this incomplete cycle of multiplication were inherently unable to support the development of *complete* virus particles, then the inoculation of graded amounts of virus should result in proportional differences in yield of hemagglutinin and C-F antigen. This expectation was fulfilled in several tests with the WS and PR8 strains. An example is illustrated in Table IV. In this experiment, the inocula differed by a factor of 4, and so did the yields at 48 hours after inoculation. Only in the range of low seed dilutions were the yields nearly identical (see the first two lines in Table IV). Presumably in this range a maximum number of cells is saturated with virus. An attempt to estimate the actual increase of HA units in mouse brain is defeated by the uncertainty concerning the number of seed particles involved in infection. Thus, while after inoculation of various dilutions of PR8 virus the increase over the theoretical maximum yield was only 13-fold (Table IV), the increase over the amount present at 1 to 3 hours after inoculation of the same undiluted allantoic fluid was about 100-fold (Fig. 1). In other experiments, with the PR8 or WS strain, increases of up to 38-fold over the theoretical maximum were observed. Since this figure is based on the highly unlikely assumption* that each infectious unit injected is capable of initiating multiplication, the increase may be assumed to be much higher and well in the range of the yields given by Henle *et al.*(16) for "one-step" growth experiments in eggs. Variations in maximum yield reflecting variations in the intracerebral seed inoculum were found also for C-F antigen. However, because of its low titer under the most favorable conditions, the range of seed dilutions giving rise to *any* demonstrable C-F antigen in mouse brain was too narrow to be conclusive by itself.

Properties of the "incomplete" virus. The hemagglutinin recovered from mouse brain is adsorbed onto and agglutinates fowl and human type O erythrocytes. It elutes spontaneously and, as already mentioned, is removed from red cells rapidly and quantitatively by RDE. It appears to be antigenic in

16. Henle, W., Henle, G., and Rosenberg, E. B., *J. Exp. Med.*, 1947, v86, 423.

17. Gard, S., and von Magnus, P., *Ark. Kemi, Miner. och Geol.*, 1947, v24b, No. 8.

18. Henle, W., and Henle, G., *Am. J. Med. Sc.*, 1944, v207, 705.

TABLE IV.
Effect of Varying the Amount of PR8 Virus Inoculated Intracerebrally on the Yield of Hemagglutinin in Mouse Brain 48 Hours After Inoculation.

Intracerebral inoculum—PR8 allantoic fluid		Yield in M.B. at 48 hr (log)		
Dilution	Log HA titer of inoculum	Theoretical max.*	Actual	Min. increase†
Undil.	3.15	2.05	2.55	0.5‡
1:4	2.55	1.45	2.55	1.1
1:16	1.95	.85	1.95	1.1
1:64	1.35	.25	1.35	1.1
1:256	.75	.00	.75	>0.75
1:1024	.15	.00	<0.3	—

For explanations see Table II.
* See footnote 1.
† Min. increase = Difference between actual yield and theoretical max.
‡ Rate of hemagglutinin production after inoculation of the same undiluted allantoic fluid was shown in Fig. 1, which showed that the actual increase was at least 100-fold over the amount demonstrable up to 3 hr after inoculation.

that intravenous inoculation into a rabbit of 10 ml of WS mouse brain HA, partially purified by adsorption-elution and centrifugation, resulted in production of specific HA-inhibiting antibody. The preparation used as antigen contained a small proportion of residual infectious WS virus, presumably not enough to account for the antigenicity(19). Further work on the antigenic specificity of mouse brain hemagglutinins is in progress.

Preliminary attempts have been made to differentiate the HA in mouse brain from fully developed virus, as it occurs in allantoic fluid, by means of centrifugation in a sucrose gradient according to the method described by Pickels(20). This method has failed to reveal a significant difference in sedimentation pattern, indicating that there is no gross difference in size. The similarity in size between the incomplete and the fully developed virus adds further weight to the conclusion that the HA present in mouse brain is not a degradation product of the inoculum but a product of a "stunted" growth process.

Further evidence in this direction has been obtained in recent experiments in which PR8-infected allantoic fluids of identical HA titers but differing infectious titers, obtained by the method of von Magnus(21) (serial passage in eggs with undiluted allantoic fluids), were inoculated into mice. The production of HA in the brains of these mice depended upon the *infectious* titer of the inoculum, not on the HA titer. Since the total amount of sedimentable material in such preparations parallels the HA rather than the infectious titer (17), it would appear that only particles capable of infecting cells and of self-duplication are capable of inducing the incomplete growth cycle in mouse brain. Details of this experiment will be reported in a subsequent paper.

It may be assumed that the C-F antigen from infected mouse brain is of the "soluble" type. The antigens were prepared without treatment with RDE and under conditions which eliminated most of the detectable hemagglutinin by centrifugation. No adequate complement-fixation tests have as yet been done with the sedimentable material.

Discussion. Mouse brain tissue has hitherto been considered as insusceptible to infection with viruses of the influenza group, with the exception of the neurotropic variant of the WS strain(1,2). The findings reported in this paper necessitate a partial revision of this belief. While mouse brain does not support the complete reduplication of unadapted strains, it nevertheless enables them to develop through certain phases of their repro-

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20. Hughes, T. P., Pickels, E. G., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1938, v67, 941.

21. von Magnus, P., *Ark. Kemi, Miner. och Geol.*, 1947, v24b, No. 7.

ductive cycle which have been demonstrated to precede the completion of infectious particles in the allantoic endothelium of the chick embryo(6,7). After intracerebral inoculation of non-neurotropic strains, HA and C-F antigen appear and increase in spite of progressive decrease in infectious titer. The conclusion that this phenomenon results from a reproductive process rather than from viral degradation is based on the following considerations:

(a) The constant period preceding increase, and the rate and duration of the rise in hemagglutinin titer in mouse brain correspond for each strain to those characteristic for it in one-step growth experiments in eggs(16);

(b) the HA and C-F antigen titers remain for at least 48 hours at the levels reached at the end of the single rise period, without evidence of qualitative change, while the infectious titer continues to decrease;

(c) the yield of HA and C-F antigen in mouse brain varies in proportion to the amount of seed virus, as has been found also for the yield of infectious virus in one-step growth experiments in eggs(16,7);

(d) only fully infectious particles, but not the "500S" component(17), present in certain infected allantoic fluids, give rise to incomplete virus;

(e) the HA from mouse brain is of similar or of the same size as the complete virus in allantoic fluid.

It has already been mentioned that a valid estimate of the number of incomplete particles arising from each infecting particle cannot be made. The data reported leave no doubt, however, that the increases in HA titer are significant and may well be within the range of the yields estimated by Henle *et al.*(16) for single growth steps in eggs.

What are the factors responsible for this stunted reproduction? The work on the neurotropic variant of the WS strain shows that the process of adaptation has enabled it to utilize the cells of the mouse brain in much the same way as egg-adapted strains use the allantoic endothelium. More detailed comparison between the egg-adapted WS strain and its neurotropic analogue may give a clue concerning the nature of this adaptive process. At this stage, only a general working hypothesis can be developed, *i.e.*, that the infected cells lack some specific component or components which "fully susceptible" cells of other tissues contribute to the incomplete virus particles in order to make them infectious.

The demonstration of incomplete virus reproduction in mouse brain may throw new light on the mechanism of the "toxicity" of non-neurotropic influenza strains after intracerebral inoculation(3). It is likely that the integrity of infected cells is equally impaired whether the endproduct of virus multiplication is complete or "stunted" virus. Thus, even in the absence of multiplication in the classical sense, *i.e.*, increase in *infectious* titer, the "toxicity" appears to be in fact a manifestation of a *bona fide* infection. Since the latter is limited to a single infectious cycle, it is not surprising that "toxic" signs appear only if the inoculum contains a large number of active virus particles, enough to infect simultaneously a large number of cells.

The type of cells which support the incomplete growth can only be surmised. The manifestations of viral "toxicity"(3) strikingly resemble the acute convulsive seizures seen in infection of mice with a number of neurotropic viruses, *e.g.*, equine encephalomyelitis, and suggest impairment of neuronal elements. By the same token, one may assume that the ability of non-neurotropic influenza viruses to interfere with equine encephalomyelitis and other neurotropic viruses (4) is due to viral activity affecting the same cells. Indeed, recent experiments have suggested that in mouse brains heavily infected with W.E.E. virus the yield of influenza hemagglutinin is lower than in normal mouse brains(22).

Some possible wider implications of the findings suggest themselves. Thus far, no viruses other than the influenza strains mentioned have been tested for their ability to induce the incomplete growth cycle, nor have conclusive tests been done with organs other than the mouse brain. Such tests are in progress with a view toward possible explanation of the "toxicity" of influenza viruses when given by routes other than the intracere-

22. Schlesinger, R. W., unpublished observations.

bral in mice(23) and in rabbits(24). Influenza virus and the other hemagglutinating viruses lend themselves particularly well to this type of investigation, because both, the soluble complement-fixing antigen and the hemagglutinin, can be measured apart from the infectivity as specific phases of viral activity. It is fascinating to speculate upon the possibility that intermediate stages in the reproductive cycle of other viruses may also become recognizable.

Summary. Mouse brain, previously considered as insusceptible to infection with non-neurotropic strains of influenza virus, has been found to support a single cycle of viral reproduction in which hemagglutinin and complement-fixing antigen increase without concomitant rise in infective titer. Rather, the infective titer decreases progressively in mouse brain. The newly formed, incomplete virus is non-infectious, but is produced only if the intracerebral inoculum contains fully infectious virus. The yield of incomplete virus is proportional to the amount of infectious virus inoculated. In mouse brain, as in the allantoic membrane, the constant period preceding a rise in hemagglutinin titer is longer for the Lee than for the PR8 strain. These findings appear to have significance in relation to the "toxicity" of non-neurotropic influenza viruses and to their ability to interfere with unrelated neurotropic viruses, and to the problem of viral adaptation to experimental hosts.

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Absence of Digitalis-like Cardiac Effects in the Action Pattern of Several Simple Lactones.* (17967)

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The cardiac glycosides are currently being subjected to investigative studies aimed at recognition of various relations of chemical structure and biological activity. Interest has centered on the lactone ring moiety and degradation of this component has been described as abolishing the typical digitalis effects(1). Further, various series of the simple lactones have been described from several laboratories as exhibiting positive inotropic effects when tested with cold-blooded hearts(1-10). At the same time, one of these reports by Chen *et al.*(3) presents evidence that a lactone producing digitalis-like effects on frog-heart preparations fails to exhibit such effects when tested on mammalian hearts. This observation made by electrocardiographic recordings in cats has been

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