STUDIES ON HOST-VIRUS INTERACTIONS IN THE CHICK EMBRYO-INFLUENZA VIRUS SYSTEM*

XI. The Effect of Partial Inactivation of Standard Seed Virus at 37°C. upon the Progeny

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In the preceding paper of this series (1), certain aspects of the von Magnus phenomenon (2, 3) have been analyzed. It was found that the yields of non-infectious hemagglutinin of influenza virus (NIHA) upon serial transfers of undiluted infected allantoic fluid depended to some extent on the circumstances of passage. Relatively little NIHA was obtained when precautions were taken to avoid accumulation of inactivated virus in the seeds; *i.e.*, when passages were made after short periods of incubation or when virus was used for transfer which had been collected during 2 hour intervals from eggs deembryonated shortly before or at the time when the maximal rates of liberation of virus from infected cells had been established (4). Although the von Magnus phenomenon was still apparent to some extent in these instances it was considerably more pronnounced with seeds which were obtained under conditions favoring inactivation of infectious virus during the *in ovo* incubation or on storage. The presence of inactivated "complete" virus in the inoculum was felt, therefore, to be a contributing factor to NIHA production.

The present study was undertaken in order to determine to what extent the use of infectious virus exposed *in vitro* to 37°C. for various periods of time duplicates in its results those obtained with seeds derived from serial passages of undiluted allontoic fluids. It will be shown that with such heated standard seeds many of the aspects of the von Magnus phenomenon can readily be reproduced. A brief summary of these findings has been included in a previous report (5). Significant differences between heated standard and undiluted passage seeds have also become apparent, indicating that the NIHA found in the latter does not solely represent inactivated "complete" virus.

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Methods and Materials

The PR8 strain of influenza A virus was used exclusively in these experiments. Most of the technics employed have been fully described elsewhere: the production of seed virus (1, 6); the inoculation of chick embryos and the harvest of allantoic fluids and membranes (6, 7); the titrations for infectivity and hemagglutinating activity (6, 8); growth curves in the intact chick embryo (7, 8) or in deembryonated eggs (9).

Heating of Virus at 37°C.—Infected allantoic fluids containing 500 units of penicillin and 100 μg. of streptomycin were incubated at 37°C. in rubber-stoppered Erlenmeyer flasks. Aliquots were then removed at given time intervals and stored at 4°C. until all samples were available. The formation of precipitates in the course of incubation could largely be avoided by either prior dialysis of the allantoic fluids in the cold room against 20 volumes of phosphate buffered saline solution of pH 7.0, or by employing allantoic fluids of embryos not older than 13 days at the time of harvest.

Other technical details are given in the text when required.

EXPERIMENTAL

Inactivation of Standard Virus at 37°C.

Incubation *in vitro* at 37°C. of infected allantoic fluids resulted in a gradual loss of the infectious property of the virus. In native allantoic fluids the rate of inactivation proceeded as a rule in an exponential fashion as is apparent from Fig. 1 B. The 3 experiments listed were conducted with allantoic fluids collected from embryos of different ages as indicated in the legend. In all of them the infectivity endpoints fell reasonably well on straight lines. However, it was noticed on occasion that initially the loss in infectivity was more pronounced, with a sharp break occurring in the inactivation curve after 24 or 48 hours of incubation as seen for instance in Fig. 1 A. It was suspected at first that this might denote the presence in the preparations of a mixed population of virus particles exhibiting different susceptibilities to heat. However, when such preparations were dialyzed overnight *vs.* 20 volumes of phosphate-buffered saline (pH 7.0) prior to incubation, such a break in the inactivation curve did not occur. The hemagglutinating property remained, as a rule, unaffected over a period of incubation up to 8 days, but in some instances, as seen in Fig. 1, a rise in hemagglutinin titer became apparent after the 4th or 5th day. While the reasons for this rise are obscure, it may be pointed out that similar observations have been made following exposure of virus to ultraviolet light (10) and sonic vibration (11).

The data presented in Fig. 1 indicated that the rate of decline of the infectious property on incubation at 37°C. may vary to some extent from preparation to preparation, probably owing to differences in the composition of the allantoic fluids. In most instances, however, the results were in fairly close agreement as seen in Table I, which summarizes the data. The average loss in infectivity in non-dialyzed allantoic fluids amounted to 1.09 $\log_{10}$ units per day or 0.045

$\log_{10}$ units per hour, with a range from 1.56 to 0.95 and 0.065 to 0.040, respectively. The rates of inactivation in dialyzed preparations seemed to be some-

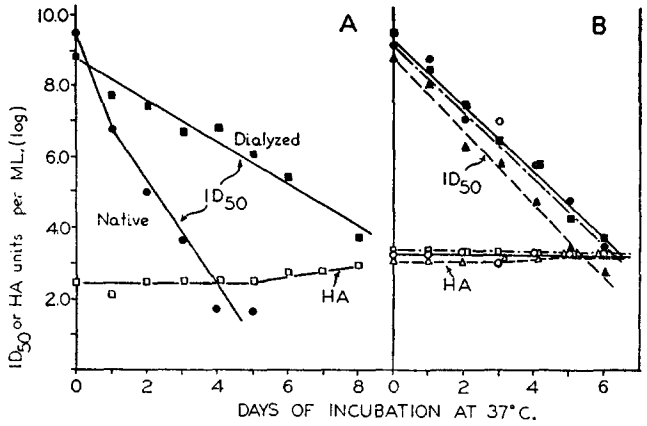

FIG. 1. Rates of inactivation of standard virus preparations *in vitro* at 37°C. A. Comparison of the rates in a native and dialyzed allantoic fluid. B. Comparison of the rates in allantoic fluids derived from 11-, 12-, and 13-day-old chick embryos, respectively.

TABLE I

Average Rates of Inactivation of Influenza Virus in Allantoic Fluid at 37°C.

Experiment No.	Dialysis	Period of incubation at 37°C.	ID_{50}/ml.		Loss in ID_{50}/ml.		
			Initial titer	Final titer	Total	Per day	Per hr.
		days	*log*	*log*	*log*	*log*	*log*
1	−	4	10.6	6.8	3.8	0.95	0.040
2	−	6	8.9	2.8	6.1	1.01	0.042
4	−	6	9.6	2.6	7.0	1.16	0.048
5	−	5	8.9	3.1	5.8	1.16	0.048
6	−	5	9.5	1.7	7.8	1.56	0.065
7	−	6	9.2	3.5	5.7	0.95	0.039
8	−	6	9.5	3.7	5.8	0.97	0.040
9	−	6	8.8	2.8	6.0	1.00	0.042
10	−	2	9.6	7.5	2.1	1.05	0.044
					Average....	1.09	0.045
5A	+	5	9.1	4.8	4.3	0.86	0.036
6A	+	8	8.8	3.8	5.0	0.63	0.026
					Average....	0.74	0.031

what lower. Accordingly, the half-life at 37°C. of the infectious property of the PR8 strain used in these experiments amounted to approximately 6.5 hours. These results are at variance with those reported by Horsfall (12) indicating a

half-life of only 2.5 hours for the PR8 strain maintained in his laboratory. The slight variations in the rates of inactivation in the present study did not influence the results of the passage experiments to be reported below and in the paper to follow (13) as long as the analyses were based upon the residual infectivity titers and the hemagglutinin concentrations of the seeds.

The rate of inactivation of extracellular virus in the allantoic cavity appears to be of the same order as that seen *in vitro*, as was observed also by Horsfall (12). Following infection of the embryos with standard seeds (10^0 to 10^{-6}) and incubation for periods up to 6 days, maximal infectivity levels were obtained in the allantoic fluids within 48 hours. Thereafter, the titers declined at an average rate of 0.95 $\log_{10}$ units per day.

Passages of Standard Seeds Incubated for Various Periods of Time at 37°C.

A number of passage experiments with heated standard seeds were carried out. The technic employed was as follows.

Standard virus preparations, exposed to 37°C. *in vitro* for periods up to 6 or 7 days, were injected allantoically in 0.5 ml. amounts either undiluted or in dilutions 10^{-1}, 10^{-2}, and 10^{-3} using 5 or 6 11-day-old chick embryos per group. The allantoic fluids of these eggs were harvested after incubation for 24 hours and titrated for infectivity as well as hemagglutinating activity. In some instances, allantoic fluid was harvested from additional groups of embryos (*a*) 2 hours after infection in order to establish the level of non-adsorbed seed virus at the onset of the experiment, and (*b*) after 48 hours of incubation.

The results of a representative experiment are summarized in Table II. It should be pointed out that the yields recorded represent values per milliliter. In order to arrive at the total yield the figures would have to be increased by approximately 0.9 $\log_{10}$ units, taking 8 ml. of allantoic fluid as the average amount per egg. Considering the undiluted passages first, it is obvious that infectious virus was produced in all instances. However, the yields decreased sharply with prolongation of the exposure of the seed to 37°C. Delay of harvesting to the 48th hour usually did not increase the yield of infectious virus; on the contrary, some loss in infectivity was seen in most instances, indicating inactivation of liberated virus on prolonged incubation. An increase in hemagglutinins was likewise noted in all passages by the 24th hour except when the seed had been heated for 6 days or longer. In these cases significant rises in hemagglutinin titers over the base line values, determined 2 hours after inoculation, were often no longer detectable. The yields of hemagglutinin also decreased as the period of heating of the seed was increased, but the decline was of a vastly smaller order as compared to the infectivity data. These differences in yields of infectious virus and hemagglutinins are reflected in the ID_{50}/HA ratios of the harvests which fell to levels as low as $10^{1.5}$ in the experiment presented, and in others even to about $10^{1.0}$. It should be noted that the ratios of the yields never fell below those of the seeds, but rather tended to show higher values than the inocula. These relationships, which point to a significant difference between

heated standard and undiluted passage seeds, will be discussed further in the paper to follow (13).

On 10-fold dilution of the heated seeds the 24 hour yields were of the same order as those obtained on undiluted passage. When more dilute inocula were employed more infectious virus was produced, the hemagglutinin titers were somewhat low and the ID_{50}/HA ratios reached standard values. In these instances, maximal titers often had not been reached in 24 hours.

It is apparent then that harvests derived from passages of heated standard seeds in low dilution contained a considerable proportion of non-infectious

TABLE II

Passage of Standard Seed Heated at 37°C. for Various Periods of Time

Inoculum					Results of passage (24 hrs. of incubation)		
Incubation at 37°C.	ID_{50}	HA	ID_{50}/HA	Dilution	ID_{50}/ml.	HA/ml.	ID_{50}/HA
days	*log*	*log*	*log*		*log*	*log*	*log*
0	9.2	3.1	6.1	10^{0}	9.8	3.4	6.4
				10^{-1}	8.8	3.3	5.5
				10^{-2}	8.7	2.8	5.9
				10^{-3}	>9.1	3.3	>5.8
3	6.2	3.1	3.1	10^{0}	7.3	3.1	4.2
5	4.0	3.1	0.9	10^{0}	5.3	3.0	2.3
				10^{-1}	5.3	2.4	2.9
				10^{-2}	7.7	1.3	6.4
				10^{-3}	8.5	2.2	6.3
6	3.4	3.4	0.0	10^{0}	4.3	2.8	1.5

hemagglutinins. Within limits the proportion of NIHA in the yields increased with an increase in the length of exposure of the seed to 37°C. although the total obtained gradually declined. Little or no NIHA was found if the seeds were diluted 100- or 1000-fold prior to passage. If harvests derived from inoculation of heated seeds were passed serially without dilution and without additional exposure to 37°C., the results were comparable to those seen in undiluted passage series with intermittant rises and falls in the ID_{50}/HA ratios of the consecutive harvests (2). Thus, heated standard seed is capable of reproducing some of the aspects of the von Magnus phenomenon.

Growth Curves with Heated Standard Seeds

The experiments recorded thus far were restricted to assays of virus material liberated during an arbitrary incubation period following injection of heated

standard seeds. It was essential to study dynamically not only the appearance of virus components in the allantoic fluids but in the membranes as well. Fig. 2 summarizes the results of a growth curve experiment in the intact chick embryo. In the upper sections data are presented which were obtained with a standard seed preparation heated for 5 days at 37°C. Allantoic fluids were collected from groups of embryos at the stated intervals and the results of the assays are found on the left side, whereas those recorded for the corresponding

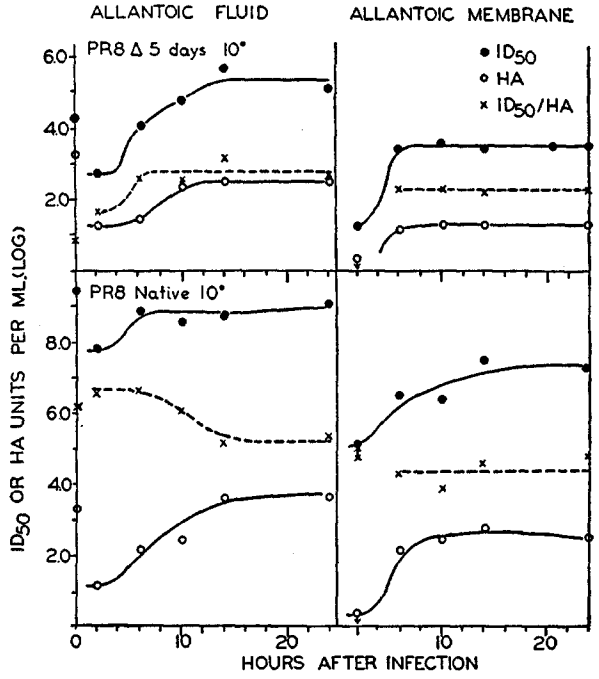

FIG. 2. Growth curves obtained in the intact chick embryo with standard seed before and after heating at 37°C. for 5 days.

membrane suspensions are given on the right. The inoculum, as indicated on the ordinate, contained $10^{4.3}$ ID_{50} and $10^{3.4}$ HA units per ml., *i.e.*, an ID_{50}/HA ratio of $10^{0.9}$. It can be seen that both infectivity and hemagglutinins began to rise in the allantoic fluids above the 2 hour levels of non-absorbed seed between the 5th and 6th hours. The titers reached their maxima by the 14th hour and remained at these levels until termination of the experiment in 24 hours. The ID_{50}/HA ratio in the allantoic fluid began to rise simultaneously with the liberation of virus material but the levels reached remained well below those observed with the unheated standard seed (lower, left section of the figure). The data obtained with membrane suspensions presented an essentially similar

picture except that maximal titers were attained earlier and the ID_{50}/HA ratios were somewhat lower than in the corresponding allantoic fluids. Such differences in ratio levels between allantoic fluids and membranes were apparent also in the growth curve with standard virus. These will be discussed further in a subsequent report (14).

As was shown previously (9, 4), growth curve experiments in intact chick embryos offer certain handicaps, particularly with respect to the onset of detectable release of progeny and to the extent of the liberation period. These difficulties have been overcome largely by the application of the deembryonation technic (15, 9). The removal of residual non-adsorbed seed virus in the process of deembryonation permitted detection of liberation at an earlier stage, and removal of the progeny at regular intervals (differential growth curve) revealed that liberation extended at a nearly constant rate over periods in excess of 30 hours (4). At the same time the technic was thought to prove more satisfactory in determining the actual composition of the progeny with respect to the relative concentrations of infectious virus and non-infectious hemagglutinins, since the 1 or 2 hour interval between harvests would tend to minimize inactivation of liberated "complete" virus. Consequently, the differential growth curve technic was employed in further experiments with heated standard seeds.

Four groups of chick embryos were inoculated with seeds which had been exposed to 37°C *in vitro* for 1, 2, 4, and 5 days. 1 hour after injection, deembryonation was performed according to the technic described (9). The medium added after deembryonation contained 2.5 per cent RDE[1], in order to remove most of the seed virus superficially adsorbed onto the membrane and to destroy remaining cell receptors. After 2 hours, the RDE-containing medium was removed and the membranes were washed once prior to addition of fresh glucosol solution without RDE. Following 30 minutes of further incubation on the rotating machine, aliquots of fluid were withdrawn from each egg and pooled according to groups. These samples served as base lines. From the 5th hour on, the media were exchanged at 2-hourly intervals without intermittent washing, until termination of the experiment 21 hours after infection of the eggs.

Since the media derived from the series inoculated with seeds heated for 4 and 5 days either revealed no hemagglutinins or only low levels of them by the usual technic of assay, a more sensitive method was adopted for this experiment, using 2 ml. volumes of the preparations and 0.5 ml. of a 0.5 per cent suspension of chicken red cells. The sensitivity of the tests was thereby increased by a factor of 10. This factor was employed for adjusting the results to those of the standard technic, but in order to avoid fractions in presenting the data, they are expressed not as units per milliliter but per egg, 10 ml. of medium being used.

The data presented in Fig. 3 indicate that the pattern of liberation of virus material following infection with heated seeds principally corresponds to that reported for standard virus (4) in that after a given period a nearly constant rate of release is established which is maintained over many hours. Although

[1] RDE, receptor-destroyiug enzyme of *Vibrio cholorae* was kindly supplied by Dr. Richard Haas of the Behring Werke, Marburg, Germany.

in this experiment no untreated seed was employed, a representative differential growth curve obtained under the same conditions with standard seed has been added to the figure for comparison. The infectivity values recorded for the 3 hour collections represent presumably superficially adsorbed seed virus which was removed from the membrane by the action of RDE. The washing procedure

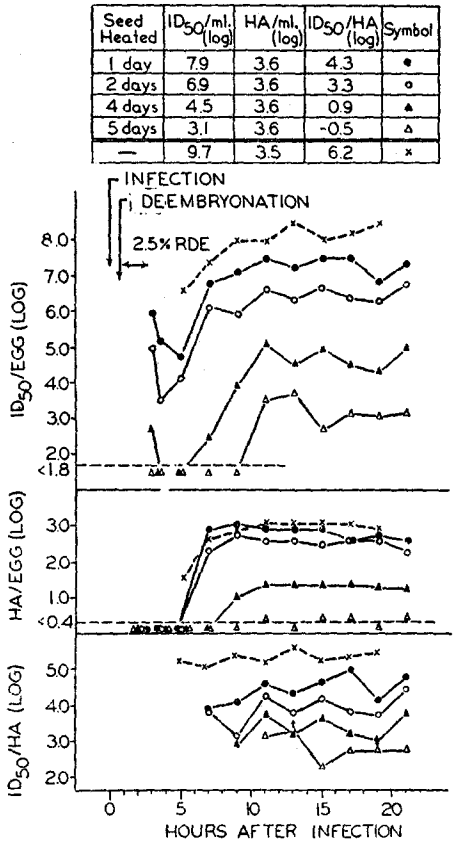

FIG. 3. Differential growth curves obtained in deembryonated eggs with standard seed heated at 37°C. for 1, 2, 4, and 5 days.

thereafter in two of the groups did not eliminate all virus in the medium (3½ to 5 hours) which constituted presumably contamination with residual seed and in part some progeny. Thereafter, liberation became pronounced in the series injected with seeds heated at 37°C. for 1, 2, and 4 days, respectively, whereas with the inoculum exposed to heat for 5 days the rise in both infectivity and hemagglutinins was slightly delayed. The ID_{50} and HA levels attained in the various groups decreased with an increase in the period of heating of the

seed. However, the ID_{50}/HA ratios plotted in the bottom part of the chart revealed that there were also differences in the relative composition of virus material produced inasmuch as the ratios fell to lower levels upon prolongation of the exposure of the inocula to 37°C. It can be noted that the seeds heated for 1 and 2 days, with ratios of $10^{4.3}$ and $10^{3.3}$, respectively, produced yields which revealed average ratios of the same magnitude, namely, $10^{4.4}$ and $10^{3.9}$. As the period of heating was extended to 4 or 5 days, the average ratio of the progeny showed a more than 100-fold increase over that of the seed.

These experiments showed then that as soon as production and liberation of virus become detectable, low ratio material, *i.e.* NIHA, appears in the tissues or is released therefrom. Furthermore, once the level of maximal liberation has been attained, approximately equal amounts of the same type of virus material are released from the cells of the allantois during successive 2-hour periods until termination of the experiments.

Comparison of Heated Standard and Undiluted Passage Seeds in Differential Growth Curves

Although the above data show that heat-inactivated standard virus resembles in some respects undiluted passage seeds the results do not imply that the non-infectious hemagglutinins they contain are necessarily identical nor that the effects they exert on the progeny are exactly the same. Comparative growth curve experiments in deembryonated eggs were undertaken, therefore, with undiluted passage seeds and heated standard virus preparations of comparable infectivity and hemagglutinin titers, in view of establishing any possible differences in their capacities to yield non-infectious hemagglutinins. One experiment may serve as an example.

Several undiluted passage preparations were obtained by serial transfer of standard seeds and a second passage (UP 2) was found to correspond most closely with respect to infectivity and hemagglutinin titers to a standard virus which had been inactivated *in vitro* for 2 days (STΔ2 ds.). In addition to these two preparations the untreated standard seed (ST), undiluted and diluted 100-fold, was also used in the experiment as controls. Of the four preparations, comparable infectivity titers were revealed by ST 10^{-2}, STΔ2ds., and UP 2 and similar hemagglutinin levels were exhibited by STΔ2ds., UP 2, and ST 10°. Deembryonation was performed 1 hour after infection in the usual manner. The medium was exchanged for the first time 2 hours later (3 hours after infection) and at 2-hourly intervals thereafter up to the 19th hour. Subsequently, the liberated virus was permitted to accumulate until the 31st hour at which time the experiment was discontinued. The medium contained 1 per cent RDE throughout the experimental period. Its 2-fold purpose was to remove most of the virus which had been superficially adsorbed by the cells of the chorio-allantoic membrane and to prevent re-adsorption of newly liberated virus onto remaining susceptible cells in the ST 10^{-2} series.

The results of this experiment are presented in Fig. 4. The data obtained with individual collections at 2-hourly intervals are recorded on the left-hand side of the chart. It will be seen that in all except the ST 10^{-2} series hemagglu-

tinins became detectable between the 5th and 7th hours and comparable levels had been reached by the 9th hour. In the ST 10^{-2} series liberation of detectable quantities of hemagglutinins were not expected. According to the dilution factor of the seed only 1 per cent of the amounts measured in the undiluted ST series should have been found, if the RDE present in the medium had protected the remaining susceptible host cells. In that case the values should have fallen below the sensitivity of the method of assay. Yet by the 15th hour hemagglu-

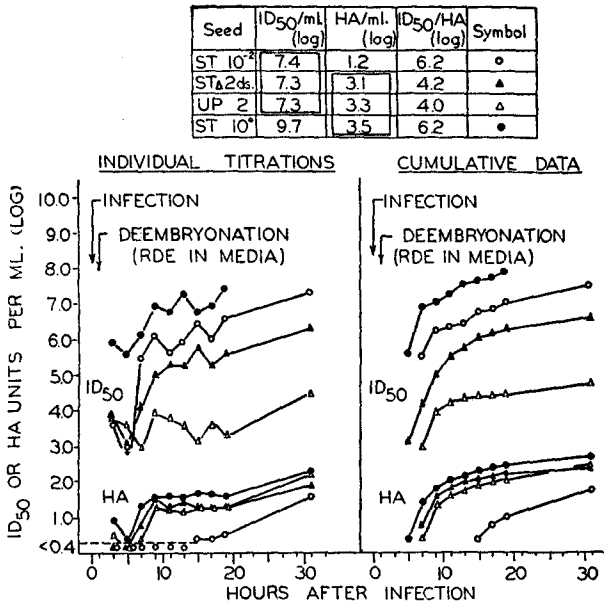

FIG. 4. Comparison of differential growth curves obtained in deembryonated eggs with heated standard and undiluted passage seeds of similar infectivities and hemagglutinin concentrations.

tinins rose and increased up to the 31st hour. Although it is possible that the concentration of RDE was too low to exert a protective effect, it is also possible that it became partially inactivated at 37°C. since large batches of replacement medium were kept in readiness for exchanges in the warm room.

The infectivity titers had risen in all groups except the UP 2 series by the 7th hour and in the latter case by the 9th hour. This delay may only be apparent, however, since the threshold level of residual seed may have prevented detection of an earlier rise. The most important difference is revealed on comparing the infectivity levels produced in the STΔ2d. and UP 2 series. In the former, the titers were higher by about 2 $\log_{10}$ units and this relationship prevailed throughout the duration of the experiment. These differences are again

reflected in the ID_{50}/HA ratio which, in the case of the STΔ2d. series compared to that of the seed with an average value of $10^{4.3}$. The average ratio produced by UP 2 was $10^{2.2}$, or about 1.8 $\log_{10}$ units lower than that of the seed. On the other hand, the yields from the 2 standard virus groups exhibited high ratios amounting to $10^{5.6}$ and $10^{6.2}$ for the ST 10^{0} and ST 10^{-2} series, respectively. The calculated cumulative curves shown on the right hand side of Fig. 4 revealed the same differences as the individual values except that some of the fluctuations encountered with individual titrations are cancelled out on cumulation of the data. Other growth curves undertaken with similar preparations have confirmed the findings that heated standard virus and undiluted passage preparations of comparable infectivity and hemagglutinin titers will give rise to progenies which differ in their composition with regard to the amount of infectious virus present. Such comparisons were possible only within relatively narrow ranges of seed infectivities since in undiluted passage series the ID_{50} concentrations rarely fell below 10^{6}.

DISCUSSION

The data presented indicate that inactivation of the infectious property of influenza virus exposed to 37°C. *in vitro* proceeds in an exponential manner as has been established previously for higher temperatures (16). In native allantoic fluid the ID_{50} titers decreased by about 1.1 $\log_{10}$ units per day, corresponding to a half-life of approximately 6½ hours, and a somewhat slower decline was noted with dialyzed allantoic fluids. However, different lines of the PR8 strain may well vary in their susceptibility to thermal inactivation, since Horsfall reported recently a half-life of only 147 minutes for the PR8 strain employed in his studies (12). The hemagglutinating activity was much more stable than the infectious property at 37°C. and undiminished HA titers were recorded even after exposure for 7 or 8 days.

As a result of inactivation of 37°C. the capacity of standard virus to produce infectious progeny on passage in low dilution decreased roughly in proportion to the loss in infectivity of the inoculum. However, its ability to yield hemagglutinins was affected to a considerably lesser degree. Thus, within limits, progressive heat inactivation resulted in relatively increasing proportions of NIHA to infectious virus in the yield. These changes will be discussed in greater detail in the subsequent paper of this series (13).

The standard virus preparations, heated for a few days, resembled in many respects undiluted passage seeds (2, 3, 1). On transfer in high concentrations they both yielded considerable quantities of non-infectious hemagglutinins, but on dilution standard virus was produced. Harvests obtained following inoculation of heated seeds on further serial undiluted passage continued to yield large amounts of NIHA. With either type of seed the appearance of NIHA in the tissues or its release into the allantoic fluids or medium of deembryonated

eggs coincided with the first signs of activity, and once maximal liberation had been established it was released at a nearly constant rate for many hours. Yet, there are also considerable differences between the 2 types of seeds with respect to the progenies they produce. As a rule, the ID_{50}/HA ratios of the harvests derived from heated seeds corresponded to those of the inocula or were higher if the seed had been heated for long periods. Undiluted passage seeds, on the other hand, frequently produced a progeny with ID_{50}/HA ratios lower by as much as 2 $\log_{10}$ units than those of the inocula (1–3). The yields of standard seeds incubated at 37°C. for 5 to 6 days may reveal ID_{50}/HA ratios as low as 10^1 or 10^2, *i.e.* considerably lower than those obtained, as a rule, with undiluted passage seeds in this laboratory (1), although von Magnus recorded similarly low values in his passage series (2). Failure to observe equally low ID_{50}/HA ratios with undiluted passage seeds may be explained by the fact that inoculation of such seeds containing less than 10^7 ID_{50} (2nd or 3rd undiluted passage seeds) nearly always resulted in the liberation of relatively greater amounts of infectious virus, and less hemagglutinins, and correspondingly ID_{50}/HA ratios obtained under these conditions reverted toward standard values (1). The most convincing evidence of differences between the two types of seeds was obtained in comparative differential growth curves in deembryonated eggs with selected inocula possessing nearly identical concentrations of infectious virus and hemagglutinins. The rate of release of hemagglutinin was essentially the same with both types of inocula but only about $\frac{1}{50}$ to $\frac{1}{100}$ the amount of infectious virus was liberated following inoculation of the undiluted passage seeds as compared to the injection of heated standard virus. Accordingly, the ID_{50}/HA ratios differed over a 50- to 100-fold range.

These considerations point to certain qualitative differences between heated standard virus and the dominant components in undiluted passage seeds. The latter apparently develop under conditions of overwhelming infection of the host tissue and possibly represent the product of an incomplete or aberrant infectious cycle. The results of injection of various types of seeds will be influenced, therefore, by the presence in the inoculum of non-infectious hemagglutinins arising either as a result of inactivation of infectious virus or from an incomplete cycle of multiplication. Moreover, it has been suggested that NIHA derived from undiluted passages may exhibit varying degrees of "incompleteness" which manifest themselves in the effect they exert on the yields in further passages, in their sedimentation constants, and in other properties (see reference 1). Similarly, standard virus particles exposed to 37°C. for increasing lengths of time undergo progressive degradation associated with the eventual loss of the ability to yield NIHA on passage. Thus the conditions encountered in these various types of passages are highly complex and interpretation of the results must, of necessity, remain limited to suggestions. These problems will be dealt with further in the paper to follow(13).

SUMMARY

The role of inactivated influenza virus in the von Magnus phenomenon has been studied by exposing standard virus preparations *in vitro* to 37°C. for periods up to 6 or more days. The rate of inactivation of the infectious property of the line of PR8 virus employed was found to be approximately 1.1 $\log_{10}$ unit per day, denoting a half-life of 6½ hours. The rate of inactivation was similar in the allantoic cavity of chick embryos.

On allantoic passage of such heated seeds without dilution it was seen that with a decrease in the infectivity of the inocula proportionately less infectious virus was found in the harvests. The yields of hemagglutinins were much less affected, and thus ID_{50}/HA ratios of as low as $10^{1.0}$ were observed in the 24 hour harvests. The ratios of the yields always equalled or were higher than those of the inocula. On 100- or 1000-fold dilution of the heated seeds standard virus was obtained.

Growth curves in intact chick embryos or in deembryonated eggs (differential yields) revealed that non-infectious hemagglutinins appeared in the tissues or were liberated therefrom as soon as any virus activity became detectable. Furthermore, once maximal liberation had been established, infectious virus and non-infectious hemagglutinins were released for extended periods of time at nearly constant rates and in unchanging proportions, the latter depending upon the seed employed.

Heated standard virus and undiluted passage seeds (von Magnus), selected on the basis of similar ID_{50} and HA concentrations, failed to yield similar results in differential growth curves in deembryonated eggs. Although the hemagglutinin titers in the 2-hourly harvests were nearly identical, the undiluted passage seeds produced as little as 1 per cent of the infectious virus which was derived from the heated inocula. Thus considerable differences exist between the 2 types of seeds.

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