STUDIES ON THE PSITTACOSIS-LYMPHOGRANULOMA GROUP

II. A Non-Infectious Phase in Virus Development Following Adsorption to Host Tissue*

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The pattern of development of meningopneumonitis virus in the allantois of the chick embryo was described in a recently published report from this laboratory (1). The growth cycle of this virus was determined by titrating allantoic fluids and membrane suspensions, obtained at various intervals after the inoculation of virus, for infectivity in mice and chick embryos. The development of virus in allantoic membranes was characterized by the following pattern: (*a*) an increase in infectivity during the first few hours following inoculation; (*b*) a decrease in infectivity which was rather marked during the next 6 hours, and which then continued at a reduced rate until about the 22nd hour of incubation; (*c*) an increase in infectivity due to the new generation of virus. A typical growth curve in membranes is included in Fig. 4 of this report (lower curve).

The present report concerns the first two phases of virus development and the data presented suggest that: (*a*) the initial increment of virus infectivity was due to the adsorption of virus from the allantoic fluid to the allantoic membrane and not to immediate virus reproduction; (*b*) the decrease of virus infectivity, following this initial increase, was due to a change in the virus from an infectious to a non-infectious state.

Since *in vitro* methods were employed as a tool in this study, it was necessary to show that the same type of growth pattern occurred when infected allantoic membranes were placed in tissue culture. The accuracy of such methods of culture was also studied and will be discussed.

Methods and Materials

Some of the methods were essentially the same as previously described (1) and these will only be outlined briefly. Tissue culture methods not previously employed will be discussed more thoroughly.

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The Virus.—The virus used throughout these studies has been the Cal 10 strain of meningopneumonitis. Freshly harvested allantoic fluid containing abundant virus (about 10^8 mouse LD_{50}/ml.) was used at all times.

Developmental Cycle.—8½ day old chick embryos were inoculated with 0.2 ml. of appropriately diluted virus by the allantoic route and incubated at 36°C. At certain intervals following inoculation, three living embryos were selected at random and the allantoic membranes were harvested, pooled, washed in five changes of a broth-buffered water mixture and either (*a*) prepared for immediate titration, or (*b*) placed into tissue culture fluid.

Immediate Titration of Membranes.—40 per cent suspensions were prepared in the broth-buffered water mixture and serial tenfold dilutions were titrated in mice using the intracerebral route of inoculation. The end-points (LD_{50}) were determined by the method of Reed and Muench.

Preparation of Tissue Cultures.—Each washed membrane was cut into 5 or 6 pieces and a pool of 3 membranes was placed in Simms's serum ultrafiltrate,[1] the volume of fluid employed depending on the length of the incubation period. For cultures incubating less than 20 hours, 40 ml. of fluid was used; when longer periods were required, 50 to 60 ml. was used. To allow for the proper volume-surface ratios, 125 ml. Erlenmeyer flasks were employed with 40 ml. of fluid; 250 ml. Erlenmeyer flasks with 50 to 60 ml. quantities. The cotton-stoppered flasks were then sealed with parafilm[2] (grade M) and incubated at 36°C. Tissue cultures were prepared in duplicate for each time interval of the developmental cycle.

Titration of Tissue Cultures.—The time during the growth cycle at which tissue cultures were prepared and the time at which they were harvested varied with different experiments and will be described in detail as each experiment is discussed. When harvested, the tissues were washed twice in the broth-buffered water mixture and 40 per cent suspensions were titrated in mice as described previously.

Analysis of Experimental Methods

The Accuracy of Tissue Culture Titers.—Preliminary experiments had indicated that virus was able to multiply in tissue culture and the titers recorded in the experiments that follow did not represent merely survival of virus. Furthermore, the methods were sufficiently accurate to reflect different final titers which were related to the amount of virus employed in the initial inocula. This is reviewed in the two experimental procedures which follow.

Procedure 1.—The first experiment was entirely an *in vitro* procedure. Tissue cultures were prepared with normal allantoic membranes of 8½ day old embryos which were washed and placed in Simms's ultrafiltrate—3 membranes in 57 ml. Simms's fluid. To each culture was added 3.0 ml. of one of the following virus dilutions: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}. After 48 hours' incubation the tissues were harvested, washed, and 40 per cent suspensions were titrated in mice.

The results of two separate *in vitro* experiments are listed in Table I. The agreement between the initial inocula and the resulting titers was quite good. It should be noted, however, that this procedure was not exactly comparable to the procedures employed in our studies. With the inoculation of virus into the tissue cultures, adsorption to both ectoderm and entoderm of the allantoic membrane resulted. Inoculation of virus *in ovo* would allow adsorption onto the entoderm only. With this in mind, the next experimental procedure was employed.

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[2] Marathon Corporation; Menasha, Wis.

Procedure 2.—In this experiment the different virus dilutions were inoculated *in ovo*, using 0.2 ml. of the virus dilution *via* the allantoic route. After 4 hours of incubation, the infected allantoic membranes were washed and tissue cultures were prepared; two cultures for each dilution employed. One set of cultures was harvested (as above) after 48 hours, the other after 72 hours, and titrations were performed in mice.

The resulting titers in 48 and 72 hours are presented in Table II. Although agreement was not as close as given in Table I for the *in vitro* inoculation series, these values show the same general trend. The values marked with an asterisk do not agree with the others in the respective columns; the others show good agreement.

TABLE I

Effect of the Virus Dilution Inoculated in Vitro and the Resulting Titers in the Membranes after 48 Hours

Virus dilution inoculated	Resulting titers (LD_{50})	
	Experiment 1	Experiment 2
10^{-1}	4.10	4.68
10^{-2}	2.94	3.50
10^{-3}	2.00	2.38
10^{-4}	0.84	1.7

TABLE II

Effect of the Virus Dilution Inoculated in Ovo and the Resulting Titers in the Membranes in Tissue Cultures (48 and 72 Hours)

Virus dilution inoculated	Resulting titers (LD_{50})	
	48 hrs.	72 hrs.
10^{-1}	5.62	4.50*
10^{-2}	4.62	4.38
10^{-3}	3.17	3.00
10^{-4}	3.38*	1.62

In subsequent experiments of the same type, similar results were obtained with an occasional culture demonstrating a final titer not in agreement with the other values in the series.

In view of the occasional discrepancies, many close time intervals were employed with duplicate cultures at each interval. (For example, in Fig. 4, seven postadsorption time intervals and 14 cultures were employed to determine a fixed level of virus in the infected tissues.)

The Pattern of Growth in Tissue Cultures.—In the experiments which follow, the virus was inoculated into the allantoic cavity of the chick embryos. Following the removal of the infected membranes from the embryos, the remainder of the experimental procedures was performed in tissue cultures prepared with the infected tissues. Therefore, it was necessary to demonstrate that the change in the environment did not affect the pattern of the developmental cycle.

Procedure.—8½ day old embryos were incoulated by the allantoic route with 0.2 ml. of a 10^{-1} dilution of MP virus and were incubated at 36°C. 4 hours following inoculation, tissue cultures were prepared with the harvested and washed membranes (as described in detail in the section on methods). At each indicated interval the tissue from two cultures was harvested, washed twice, and 40 per cent suspensions were titrated in mice. The indicated time intervals refer to hours after the original inoculation of the chick embryos.

Fig. 1 shows the change of infectivity in the membranes in tissue culture. The usual decrease in virus activity occurred during the first 20 hours, and as new virus appeared, the titer began to increase in about the same manner as *in ovo*. It seemed, then, that the development of virus continued in approximately the same fashion when the infected tissues were removed from the embryos and placed in Simms's ultrafiltrate.

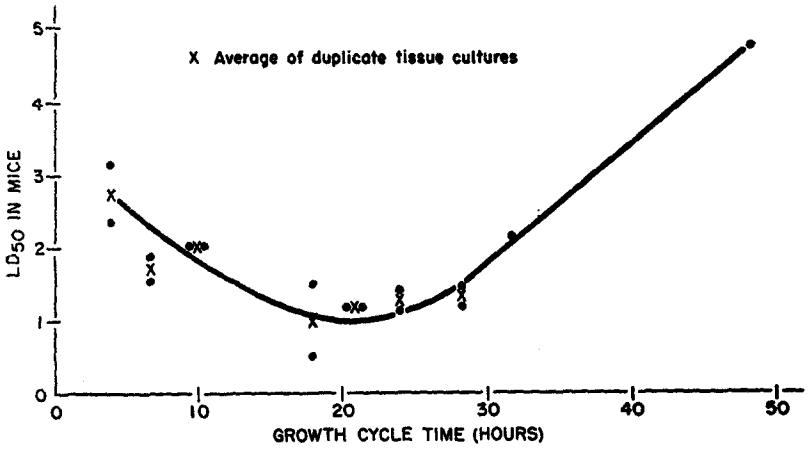

FIG. 1. The developmental cycle of virus in tissue culture following adsorption of virus *in ovo*.

EXPERIMENTAL

The Initial Increment of Infectivity in Allantoic Membranes. A Test for Immediate Multiplication.—The increased infectious titers of allantoic membrane suspensions during the first few hours following inoculation *in ovo* could represent either continued adsorption of virus or adsorption followed by immediate virus growth (lower curve; Fig. 4). The problem was resolved by determining the pattern of development resulting under conditions which excluded prolonged adsorption.

For this purpose, membranes were harvested from chick embryos before the period of increment was completed and placed in a virus-free environment; *i.e.*, tissue culture fluid. The remainder of the developmental cycle could be traced without the possibility of additional adsorption, and any increase in titer after this time could be interpreted as immediate reproduction of virus. However, if a decrease in viral infectivity followed transplantation of tissue

from embryo to tissue culture, it would seem the initial increment occurring *in ovo* was due to continued adsorption and not immediate reproduction.

The results of the following experiment supported the hypothesis that the increased infectivity of allantoic membranes during the first few hours following virus inoculation was due to continued adsorption of virus.

Experiment 1.—8½ day old embryos were inoculated by the allantoic route with 0.2 ml. of a 10^{-1} dilution of MP virus and incubated at 36°C. Membranes were harvested at two intervals following inoculation, *i.e.* at 2 hours and at 4 hours, and several tissue cultures were prepared at each interval as previously described. At indicated intervals after these cultures were prepared, the tissues were harvested and titrated for infectivity. The indicated time intervals refer to hours after the original inoculation of the chick embryos.

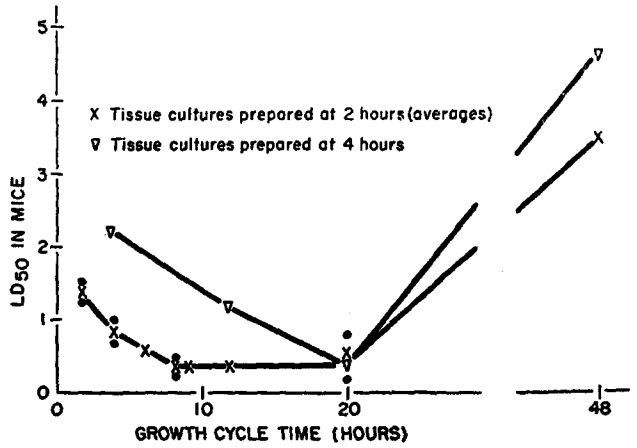

FIG. 2. Influence of the length of exposure of membrane to virus on the early phases of development of the latter and on the amount of virus developing in 48 hours.

Fig. 2 shows the resulting development cycles. After the tissue cultures were prepared with the infected membranes both curves demonstrated a decrease in virus activity until the new generation of virus appeared after 20 hours. However, two important differences were noted which seemed to indicate that the early increment *in ovo* was due to adsorption.

(*a*) Tissue cultures in the 2 hour series did not increase in titer between 2 and 4 hours, whereas tissues remaining *in ovo* showed a definite increase during this interval (compare the first point in each curve).

(*b*) By 48 hours, the culture from the 2 hour series developed a titer of $10^{-3.6}$, as compared with a titer of $10^{-4.8}$ for the culture in the 4 hour series. As will be indicated in Experiment 4 which follows, such differences in titer did not occur when membranes, used to prepare tissue cultures, were harvested from embryos after the adsorption period has ended. Therefore, it appeared that adsorption was still occurring *in ovo* between 2 and 4 hours.

Other experiments employing an undiluted virus inoculum, or virus diluted $10^{-2.0}$, yielded similar results. Regardless of when the period of increment was interrupted, no further increases in membrane infectivity occurred in an environment free of extracellular virus until the formation of new virus after 20 hours.

The Effect of Virus Dilution on the Duration of Adsorption.—It was observed in the course of many growth experiments that the duration of the initial increment of virus activity in membranes varied from one experiment to another, depending on the titer of the original virus preparation and the dilution employed.

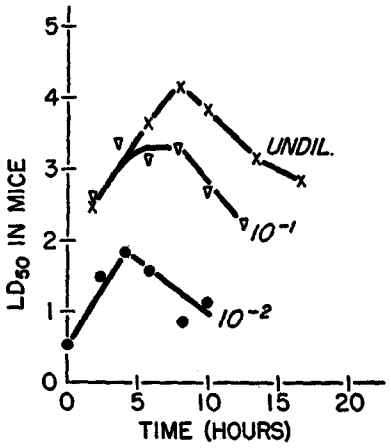

FIG. 3. The effect of virus dilution on the duration of virus adsorption.

Usually the original virus preparation when freshly harvested contained about 10^8 LD_{50} per ml. When 0.2 ml. of undiluted material (2×10^7 LD_{50}) was inoculated into chick embryos *via* the allantoic route, virus adsorption persisted for 8 to 10 hours. With a dilution of 10^{-1} this period was shortened to 6 to 8 hours (at times, 4 hours); with a 10^{-2} dilution, 4 to 6 hours. Further dilution (10^{-3} and 10^{-4}) did not shorten the adsorption period below 4 hours.

In Experiment 2, virus inocula of three different dilutions (undiluted, 10^{-1}, and 10^{-2}) were employed and the early portion of the cycle was followed in the allantoic membranes (Fig. 3). In this particular experiment the adsorption period for undiluted virus extended for 8 hours; for 10^{-1}, between 4 and 8 hours; and for 10^{-2}, 4 hours. The 4 hour value for the 10^{-1} preparation was unusually high when compared with the values of previous experiments, but otherwise these results resembled those formerly observed when different dilutions were employed in many separate experiments.

The possible implications of these observations are discussed later.

Non-Infectious Virus during the Initial Stages of Growth. The Absence of Virus Elution from Host Tissue.—Since the usual diminution of virus activity in tissue was observed during the early phases of virus development *in vitro* (as *in ovo*) it was possible to use the described tissue culture methods to determine the cause of the decrease of infectivity following adsorption. The possibility that elution from host tissue was responsible for this decrease was investigated. Had elution taken place, the virus could have been recovered from the surrounding culture fluid. Therefore, the following experiment was performed.

Experiment 3.—Fluids were saved from cultures (prepared in Experiment 1) following the harvest of tissues at 9, 12, and 20 hours. These fluids were subjected to centrifugation at 1500 R.P.M. for 15 minutes and the supernatant fluid was then recentrifuged at 13,000 R.P.M. for 60 minutes to concentrate the virus. The sediments of both centrifugation procedures were resuspended in a volume of broth-buffered water mixture equal to that in which the tissues were suspended (2.0 to 4.0 ml.) and titrated in mice.

No virus in titrable amount was ever recovered except in one culture in which less than 0.5 log of virus was found, far less than the amount necessary to account for the decreasing infectious titer of allantoic membrane suspensions. Thus, the decrease of infectivity was not caused by elution of virus.

Evidence for the Presence of Non-Infectious Virus.—Another possible explanation was that virus had undergone a change from an infectious to a non-infectious state. If such a change had occurred, the infected membranes transferred from the chick embryo to Simms's fluid at different intervals during the latent stage should have similar potentialities for producing virus even though the infectious titers in the host tissue were progressively decreasing at the time. This was assuming that only an insignificant amount of adsorption occurred during the latent period. The following experiment was designed to investigate this possibility.

Experiment 4.—8½ day old chick embryos were inoculated by the allantoic route with 0.2 ml. of a 10^{-2} dilution of MP virus and incubated at 36°C. At various intervals after inoculation three allantoic membranes were harvested, washed in 6 changes of a broth-buffered water mixture, ground, and suspended for immediate titration in mice. Duplicate tissue cultures were prepared with other membranes harvested at the same intervals using three membranes in 60 ml. of Simms's ultralfitrate in 250 ml. Erlenmeyer flasks. These tissue cultures were allowed to incubate at 36°C. until about 48 hours from the time of original inoculation of chick embryos so that the virus was in the same stage of the growth cycle when all the tissue cultures were harvested. The tissues were washed twice in a broth-buffered water mixture and 40 per cent suspensions were titrated in mice.

The results of a typical experiment are shown in Fig. 4. The lower curve represents the titration of the infected membranes immediately following their removal from the chick embryos and is the usual one observed for the developmental cycle of MP virus in the allantoic membrane.

The upper curve represents the titration of the membranes in the tissue cultures prepared at the intervals indicated on the abscissa and harvested and titrated at the 48th hour of total growth. In this curve one can see that the tissues removed from embryos after 4 hours developed approximately the same titers, regardless of the immediate infectious titer, indicating that no loss of virus had occurred during the latent period. Had the virus been eluted from the membranes *in ovo*, one would have expected progressively lower titers to result in cultures prepared at different stages of the latent period. Thus, the data suggested that the decrease in infectivity represented a change of the virus from an infectious to a non-infectious form.

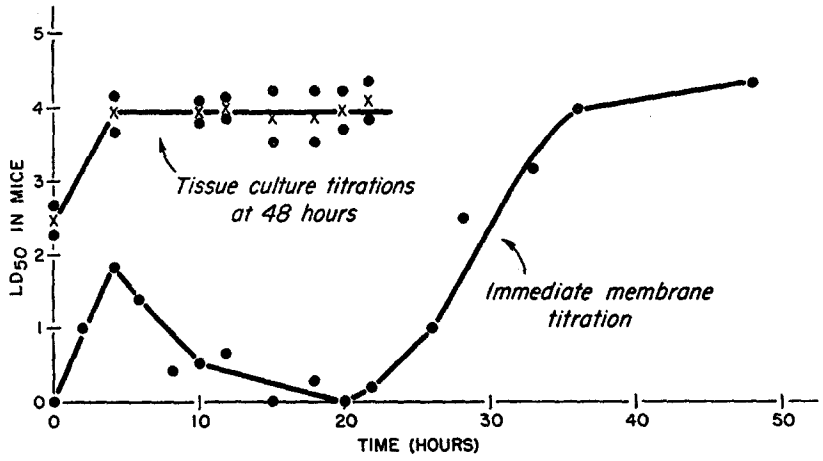

FIG. 4. The potential for virus development of host tissues harvested during the latent period.

Tissue cultures prepared at 0 hour developed less virus ($10^{-2.5}$) than those prepared at 4 hours ($10^{-4.0}$), since after 4 hours *in ovo* more virus had been adsorbed onto the allantois and could participate in propagation. After 4 hours no further increase was observed in the tissue cultures indicating that adsorption had essentially ended. This was in agreement with other results which indicated that tissue cultures, prepared while adsorption was still occurring, developed progressively higher titers as the adsorption time *in ovo* increased. One example of this was previously described in Experiment 1 where the 4 hour culture developed more virus in 48 hours than the 2 hour culture.

DISCUSSION

An analysis of the developmental cycle of MP virus was aided by the use of tissue culture methods. The pattern observed *in vitro* was similar to that developing *in ovo*. Therefore, it was possible to study the initial phases of the

cycle—the adsorption and the latent periods—in tissue culture preparations of infected allantoic membranes.

Virus Adsorption.—The initial increment of infectivity observed in membrane suspensions following virus inoculation *in ovo* seemed to be due to prolonged adsorption and not to immediate virus reproduction (Experiment 1).

The length of the adsorption period varied with the virus dilution employed (Fig. 3) and was quite prolonged when compared with the adsorption time observed with other viruses. For instance, Delbrück (2) reported that adsorption of bacteriophages on host cells occurs within a few minutes. Adsorption onto the ectoderm of the chorioallantois of chick embryos proceeds rapidly for both *herpes simplex* (3) and psittacosis viruses (4), most of the virus being adsorbed within 1 hour.

(*a*) In our studies, perhaps dilution of the virus in the allantoic fluid of the embryo caused adsorption to be prolonged but the same finding would be expected with other viruses as well. However, with the inoculation of influenza virus (5) and psittacosis virus (4) by the allantoic route, the adsorption was still quite rapid. The discrepancy between the adsorption time of psittacosis and MP viruses does not necessarily reflect basic differences in the two viruses but may have been due to different experimental procedures used in the determinations. For instance, with psittacosis virus, infectivity titers of membranes were not reported and the conclusion was based on differences observed in the titers of allantoic fluid at various times after inoculation.

(*b*) Factors other than dilution by the allantoic fluid may have contributed to the prolonged adsorption. The role of univalent cations (6) and organic cofactors (7) in bacteriophage adsorption seems well established, and these may participate in virus adsorption in the chick embryo as well. Puck and coworkers (8) have investigated the quantitative aspects of interactions between bacteriophages and host cells and stated that the rate of interaction of these viruses and cells in chemically defined media can be adjusted to any desired value between zero and the maximum theoretically possible rate by control of the ionic constitution of the media. Also, the addition of ions in great excess may cause a decrease in the rate of interaction.

Although observations with bacteriophage systems cannot be directly projected on animal virus systems, it is possible that the differences in adsorption time with various dilutions of MP virus were related to an optimal ionic concentration. The undiluted allantoic fluid virus preparation (adsorption time, 8 to 10 hours) might have contained too few or too many of the necessary ions for adsorption. Dilution in broth could shorten this period by either the addition of the necessary ions or by diluting out an inhibiting concentration present in the original preparation.

The amount of virus adsorbed was not determined under our experimental conditions. Thermal inactivation of the virus (at 36°C.) accounted for the

change in the infectious titer observed in the allantoic fluids (1), and therefore one could not determine the amount of virus adsorbed by comparing the original titer of the fluid with that resulting after adsorption had occurred. Neither could the highest titer observed in membranes at the end of the adsorption period be used to determine the total amount of virus adsorbed since a dynamic turnover to a non-infectious state may have been occurring during the period of adsorption. This phenomenon of "inactivation" may have been overshadowed by the higher rate of adsorption.

The Latent Period.—The reduction of the virus titer in the allantoic membranes subsequent to adsorption was next investigated and the data seemed to support the belief that the observed decrease in titer (both *in ovo* and *in vitro*) represented the change of infectious virus to a non-infectious state. A non-infectious phase has been suggested in the developmental cycles of other viruses and many theories have been presented to explain the disappearance of virus infectivity; *e.g.*, (*a*) thermal inactivation of virus during incubation; (*b*) elution of virus or its distribution to other tissues of the host; (*c*) inherent differences in the growth pattern of adapted and unadapted strains of the same virus; (*d*) inadequate preparation of the specimen (or host) before titration for infectivity; (*e*) combination of the virus with a host component, the resulting complex being non-infectious; (*f*) inactivation of the virus by host enzymes; (*g*) disintegration of the particle after infection of the host cell. (These last three factors may all be steps in the same developmental cycle as well as individually active mechanisms.)

When one reviews the results obtained with meningopneumonitis virus it seems that several of these explanations may be excluded as possible reasons for the disappearance of virus infectivity.

(*a*) Two facts seemed to repudiate the belief that inactivation was due to the temperature of incubation. Observations on thermal degradation of virus infectivity as published in the previous paper of this series (1) indicated that inactivation seemed to follow a slope representing a first order reaction. Furthermore, thermal degradation could account for a decrease of approximately one log of virus activity in a 24 hour period. It is quite clear in the lower curve in Fig. 4 that such results were not observed with the virus associated with the allantoic membranes. Here, two different rates of inactivation were observed. The decrease which occurred immediately after adsorption was relatively rapid (almost one and one-half logs in a period of 6 hours); thereafter, a much slower rate of inactivation resulted (about one-half log in a period of 12 hours). This latter diminution might represent thermal inactivation of the remaining infectious virus since the rate of inactivation was in better agreement with that expected to occur due to the incubation temperature. However, it is quite obvious that the inactivation subsequent to adsorption was too rapid to be attributed to thermal degradation alone.

(*b*) Distribution to other tissues of the host—The presence of non-infectious

virus was suggested in the developmental cycle of the Lansing strain of poliomyelitis (9) and for WEE virus (10) following the intracerebral inoculation of mice. However, it has been claimed by Cairns (11) that about 95 per cent of any intracerebral inoculum passes into the circulation of the mouse and that the observed decreased titers of brain suspensions could be due to this unavoidable wastage.

Therefore, one could postulate that the observed decrease of MP virus infectivity during the latent stage *in ovo* could be accounted for by distribution to various other tissues, or to an elution of the virus into the allantoic fluid. Obviously this distribution could not occur in tissue cultures prepared with infected membranes, in which similar decreases in titer were observed. In this case one might have expected that the virus had eluted from the membrane to the surrounding culture fluid. However, all attempts to recover virus from the fluid by high speed centrifugation have indicated that no appreciable amount of virus was present.

(*c*) Unadapted strains of virus may show a different developmental pattern in a new host from that shown by adapted strains in growth curve experiments. It has been indicated that after intranasal inoculation of mice, the lungs showed decreasing infectious titers before any multiplication was observed when *unadapted* strains of influenza were employed (12), but not when *adapted* strains were used. Therefore, the presence of a non-infectious virus stage during normal virus growth was not clearly demonstrated with these viruses, and the decreasing titer may be only a special manifestation during growth with an unadapted strain.

However, it is unlikely that this point affected our experiments since a strain of MP virus well adapted to growth in the allantoic cavity of the chick embryo was employed.

(*d*) The fact that virus in given tissue suspension is unable to infect other cells may only be a manifestation of inadequate treatment of the specimen or the host before titration. In suspensions of mouse lung infected with PVM, a non-infectious host-virus complex developed which, when treated with an enzyme, liberated infectious virus (13). Shope (14) has indicated that swine influenza virus is present in a masked, non-infectious form in the lungworm and to induce infection must be rendered active by a provocative stimulus applied to the swine carrying the lungworm.

Evidence has been presented (15–17) which indicates that a non-infectious form of rabbit papilloma virus is present in tumors of domestic rabbits. However, following preliminary treatment of the skin of the test animal in ways that rendered it hyperplastic (18), the presence of infectious virus could be demonstrated in extracts of papillomas that yielded no growth when inoculated by ordinary means, and which for this reason were supposed to contain no virus.

One might suspect that the tissues in our studies had not been emulsified

sufficiently before titration to liberate intracellular virus, but this factor alone could not account for the decreasing infectious titers of MP virus; the titers continued to decrease with time during the latent period, even though the technique remained the same. If the lack of proper preparation of the tissue was responsible, then a factor other than disintegration of the host cells was involved.

(*e*) The disappearance of PVM in mouse lung suspensions has suggested a latent phase in the development of that virus (19). Volkert and Horsfall (13) found that this virus was bound to a host component (probably protein) and *as such* could not infect a new host. This complex could be dissociated by trypsin or anti-PVM immune serum. When such complexes were inoculated into normal lungs, enzymes present in mouse lung could dissociate the complex liberating infectious virus which could then initiate the disease. However, under these conditions the process of adsorption required 4 to 5 times the normal length of incubation in the host.

(*f*) Further investigations with PVM (20) have shown that sulfhydryl groups, as in glutathione, or as in concentrated suspensions of mouse lung tissue, may adversely affect this virus with a resulting loss of infectivity. Therefore, disappearance of MP virus during the latent period might be a result of inactivation of the virus by host enzymes, or a combination of the virus with a host component.

(*g*) Studies on bacteriophage growth have suggested that there is a disruption of the infecting particle in the host cell. Putnam and Kozloff (21) and Lesley *et al.* (22) have indicated that on lysis of cells infected with P^{32}-labelled viruses, the greater part of the isotope was found in the medium as acid-soluble phosphorus. Since 50 per cent of this fraction was of an inorganic nature, and since 95 per cent of the phosphorous found in purified phage is present in desoxypentose nucleic acid (23), the DNA of these particles must have undergone an extensive breakdown.

It has not been determined whether disintegration by host enzymes or the development of a non-infectious host-virus complex was responsible for the latent phase in the developmental cycle of MP virus. However, it seems likely that a change from the infectious to the non-infectious state was a manifestation of the normal growth process of the virus. This was confirmed by experiments in which tissue cultures prepared with membranes harvested at different intervals during the latent period developed similar titers, indicating similar potentialities for producing virus.

An alternate explanation would be that the minute amount of virus which was still infectious at the end of the latent period was the virus responsible for propagation, but preliminary investigation of this hypothesis seemed to indicate that it was not in accordance with the observed results. It will be recalled that in Experiment 1 cultures were prepared at 2 and at 4 hours,

when different amounts of virus were present in the infected tissue. By the end of the latent period, however, the amount of infectious virus was the same in both series of tissue cultures. If only the infectious virus remaining at the end of the latent period participated in multiplication, then both series should have been expected to develop the same amount of virus in 48 hours. This did not occur; on the contrary, the amount of virus at 48 hours was related to the amount of virus present in tissues before the non-infectious phase (Fig. 2).

Results of investigations with other members of the psittacosis-lymphogranuloma group might also support the belief that a non-infectious developmental form exists in host tissue, and these have been discussed in a previous report (1). (For psittacosis virus in mouse spleens, see reference 24, and for lymphogranuloma venereum in the yolk sac of the chick embryo, see reference 25.)

With influenza virus, Henle (26) had previously indicated that non-infectious virus was present as a normal feature of virus growth. The same has been suggested for vaccinia virus (27).

SUMMARY

The pattern of growth of meningopneumonitis virus *in vitro* seemed to be similar to that occurring *in ovo* and thus the initial stages of development, the adsorption and the latent periods, were investigated by the use of tissue culture procedures.

The initial increment of infectivity in allantoic membrane suspensions following virus inoculation *in ovo* was due to prolonged adsorption of virus and not to immediate virus reproduction. The length of the adsorption period varied with the virus dilution employed.

The reduction of virus titer in allantoic membrane suspensions subsequent to adsorption was due to a change of infectious virus to a non-infectious form and this seemed to be a part of the normal developmental cycle of the virus.

The possible causes for both prolonged virus adsorption and for the subsequent development of a non-infectious form are discussed.

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