STUDIES ON THE PSITTACOSIS-LYMPHOGRANULOMA GROUP

III. The Effect of Aureomycin on the Propagation of Virus in the Chick Embryo*

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It has been established that aureomycin has an inhibitory effect on the multiplication of the agents of the psittacosis-lymphogranuloma group. In experimental infection of laboratory animals and embryonated hens' eggs, this drug has been shown to suppress, but not completely inhibit, the multiplication of the viruses and to extend the survival time of the infected host (1, 2). Reports also indicate that it is of some therapeutic value in relieving clinical symptoms in human infections caused by these agents (3, 4).

A study of the growth cycle of meningopneumonitis virus in the allantoic cavity of the fertile hen's egg, reported elsewhere (5), strongly suggests that the pattern of multiplication of this agent is similar to that described for other viruses; *i.e.*, an early period of virus adsorption followed by a change into a non-infectious stage and a subsequent appearance of the new generation of virus. If the type of growth of this agent resembles that of true viruses, the question arises as to why this agent is susceptible to the action of antibiotics which have no effect on other viruses.

At present relatively little is known concerning the mechanism through which aureomycin inhibits the growth of the virus. Most of the work on this problem has dealt with the final increment of virus. It was the object of the present experiments to determine any possible effect which aureomycin might have on a particular phase of the growth pattern of meningopneumonitis virus in the chick embryo. It was felt that correlation of an observable effect with a specific phase of growth would be an aid in elucidating the mode of action of the drug on this agent.

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Methods and Materials

Virus.—The virus used throughout these studies was the California 10 strain of meningopneumonitis (MP) which has been adapted to the allantoic cavity of the chick embryo. Freshly harvested allantoic fluid of the 10th egg passage of the virus was used in all experiments.

Developmental Cycle.—The pattern of growth of MP virus in the presence of aureomycin was studied in the allantoic membranes of 8 ½ day fertile hens' eggs. To each embryo which had been infected with 0.2 ml. of the appropriate virus dilution by the allantoic route was given 1 mg. of aureomycin by the same route and at the times indicated in the various experiments. Eggs were incubated at 36°C. At the time intervals indicated the allantoic membranes from three living embryos were harvested. These membranes were pooled and washed 6 to 7 times in a broth-buffered water mixture (0.02 molar phosphate buffer). Depending upon the experiment, the washed membranes were (*a*) cut up and put in tissue culture or (*b*) ground in a mortar with sterile alundum to prepare a 20 to 40 per cent suspension for direct infectivity titration.

Titrations in Mice.—Serial tenfold dilutions were made from the ground preparation to be studied. Albino Swiss mice of the NIH strain weighing 12 to 15 gm. were inoculated with 0.03 ml. of the various dilutions. Five mice were used per dilution. The criterion for infectivity was death; the 50 per cent end-point (LD_{50}) was calculated by the method of Reed and Muench (6).

Titrations in Eggs.—Serial tenfold dilutions were made from the ground preparation to be studied. The various dilutions were inoculated in 0.2 ml. amounts into the allantoic cavity of 8 ½ day chick embryos. Six eggs were used per dilution. After 6 days' incubation at 36°C., smears were prepared from the allantoic fluid of each living egg and allowed to dry overnight. These were stained by the Macchiavello method; the criterion for infectivity was the presence of elementary bodies. The 50 per cent end-point (ID_{50}) was calculated by the method of Reed and Muench.

Tissue Culture Preparation.—Each membrane of a washed pool was cut into 5 or 6 pieces and placed in a sterile 250 ml. flask containing 50 to 60 ml. of Simms's ultrafiltrate. The flasks were stoppered with cotton and sealed by putting parafilm (grade M) tightly over the plugs. After the designated incubation time at 36°C., the pieces of tissue were removed from the flasks with a large bore pipette. They were then washed and ground in a mortar in preparation for infectivity titration.

Aureomycin.[1]—Three different lots of the drug were employed: lot No. 4691-165A which contained a glycinate buffer; lot No. 4423-1005 which was unbuffered crystals; lot No. 4423-10055 which was also unbuffered. The drug was stored in the refrigerator. In preparation for use, it was dissolved in sterile distilled water. It was injected in 0.2 ml. quantities in the allantoic cavity unless otherwise indicated.

Analysis of Methods

Toxicity of Aureomycin.—Based on mortality determinations, chick embryos appeared to tolerate 1 mg. of the drug when injected into the allantoic cavity; this was the quantity used in most experiments. In the mouse brain, 0.1 mg. of drug in the 0.03 ml. inoculum produced no deaths. The preparations used in these experiments carried an amount of drug which was well below this level.

Behavior of Aureomycin in Tissue Culture.—Three observations were made on the fate of aureomycin in cultures consisting of minced allantoic membrane in Simms's fluid:—

[1] This drug was generously supplied by Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

(*a*) There was no appreciable destruction of the drug in Simms's fluid over a period of 25 hours at 36°C.

(*b*) A small quantity of aureomycin was found to leave the membranes obtained from pretreated embryos and could be demonstrated in the culture fluid.

(*c*) Aureomycin added directly to Simms's fluid containing minced membranes from untreated embryos became associated in significant amounts with these membranes. The drug appeared to enter into the tissue following contact *in vitro*.

Washing of Allantoic Membranes.—It was necessary to determine the amount of washing which would remove the excess amount of aureomycin adhering to the membranes following introduction of the drug into the allantoic cavity. Assays were carried out on the consecutive wash fluids (broth-buffered water mixture) by the method of Dornbush and Pelcak (7). It was found that following 5 or 6 washings, the wash fluids contained no demonstrable aureomycin.

Effect of "Carried Over" Aureomycin on Titration of Infectivity.—Virus suspended in a menstruum containing 1 mg. of drug yielded on intracerebral inoculation of mice a titer that differed from the control titer by only 0.7 log or less. In view of this, one may assume that even the maximum amount of aureomycin that could possibly be carried over in a treated tissue would have no significant effect on titration.

Persistence of Aureomycin in the Chick Embryo.—Following inoculation of 1 mg. of aureomycin, the amount of drug in allantoic fluid decreased from about 150 γ/ml. in the first few minutes to 7 γ/ml. at 120 hours. The corresponding change in the allantoic membranes was from about 5 γ/ml. to about 1 γ/ml. However, the figures for the membranes are not exact, inasmuch as the assays were done on extracts and the total concentration of the drug in the membrane could not be determined. The drug was present in the blood stream, but its concentration varied from experiment to experiment and none was found 6 to 8 hours after treatment. Small amounts of aureomycin were detected in the liver. Aureomycin was not demonstrated in the brain or heart harvested at various intervals (8).

EXPERIMENTAL

Failure of Aureomycin to Affect "Inert" Virus.—Two types of experiments were designed. In one, aureomycin in several concentrations up to 1 mg. was mixed with serial tenfold dilutions of virus in normal allantoic fluid and allowed to remain at room temperature for 30 minutes and at 4°C. for 150 minutes. Thereafter, the mixtures were inoculated into chick embryos.

In the other experiment, 1 mg. of drug was added to MP virus diluted 1:5 and 1:1000 in distilled water. Following incubation at room temperature for 30 minutes and at 4°C. for 120 minutes, the preparations were centrifuged at 13,000 R.P.M. for 1 hour and the sediments were washed once by a second centrifugation at this speed. The sediments resuspended in distilled water were titrated in mice. In both experiments, there were no significant differences between the treated preparations and control preparations. Thus, aureomycin had no effect on the extracellular virus particle *per se*.

Relationship of Time of Administration of Drug to Virus Growth.—The lack of effect on inert virus suggested that the drug exerts its effect during the process of viral replication. In studying the effect of aureomycin on viral synthesis, it seemed important to determine first whether there might be a time in the

process of multiplication which was most sensitive to the action of the drug. This problem was approached in two different ways, by *in ovo* and tissue culture experiments.

(*a*) *In Ovo Studies.*—A group of chick embryos were infected with a 10^{-2} dilution of virus by the allantoic route; at various times after infection, groups of 5 eggs received 1 mg. of aureomycin also by the allantoic route. At 30 to 31 hours after infection, when the first infectious cycle presumably had been completed (5), three membranes from each series of eggs were harvested and titrated in mice.

In Fig. 1, it can be seen that regardless of the time at which aureomycin was given, the amount of infectious virus present at 31 hours in the membranes of treated eggs was less than that of the untreated controls (except possibly the 27 hour preparation in the series employing 10^{-3} virus). The most pronounced difference in titers was seen when the drug was given during the first 8 hours after infection, but it continued to be significant even when the drug was administered 11 hours after infection.

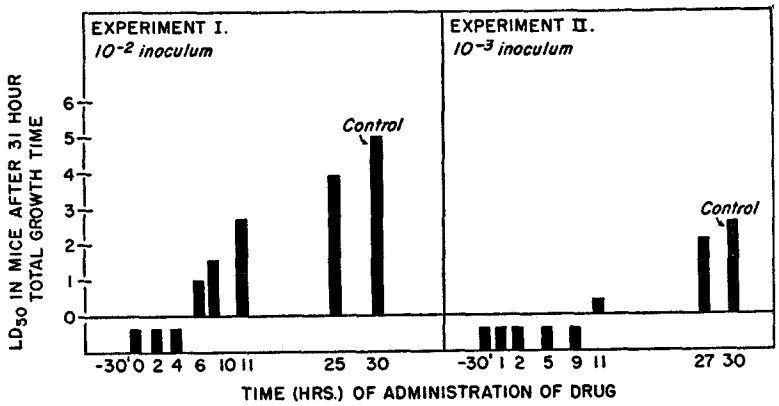

FIG. 1. The effect of aureomycin given at various time intervals on the first cycle of virus growth *in ovo*. The titer indicated at 30 hours was that of the controls which received no aureomycin.

It does not seem plausible that the observed results could be explained by inhibition of adsorption, for the effect could be achieved beyond the period of primary adsorption (9). The more reasonable explanation is that the drug was capable of inhibiting some other phase of virus multiplication.

Titrations in eggs had been found to be a more sensitive measure of infectivity than titrations in mice (5), the difference being in the order of 1 to 2 logs. Therefore, in an attempt to determine whether small amounts of virus (which may have escaped detection by the mouse method) developed by 31 hours when aureomycin was administered during the early hours of infection,

the same experiment was set up employing embryos for titration. For comparison, determinations were carried out at 30, 50, and 72 hours after infection.

As shown in Table I, results of titrations performed in eggs were less clear cut than results of those performed in mice. Reasons for this have been discussed previously (5). However, two facts emerge from this table: (1) Delay in the time of administration of aureomycin after infection seemed to allow for the appearance of more virus regardless of the incubation time. This is consistent with the data obtained in the mouse experiments. (2) Prolonged incubation of the infected embryos treated with aureomycin seemed to cause

TABLE I

Log Titers (ID_{50}) in the Membrane When Treated with Drug at Different Times

Time of inoculation of aureomycin	Time of titration (after infection)					
	30 hrs.		50 hrs.		72 hrs.	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
−10 min.	Negative	0.5	Negative	1.8	<1.0	0.6
+1 hr.	"					
2 hrs.	"	1.2	2.2	2.4	4.0	3.0
4 "	Undiluted		3.0	4.8		4.5
5 "					4.7	
6 "		0.4				
7 "	<2.0		3.5		5.3	3.4
8 "		<1.0		4.7		
12 "	3.0					
27 "	4.5					
28 "		4.2		4.8		
Control.....	5.6	4.6	6.2	6.0	7.5	6.4

the appearance of more virus regardless of the time of injection of the drug, with the possible exception of the −10 minute interval.

(*b*) *Tissue Culture Studies.*—The second approach to these experiments was to treat infected eggs (10^{-2} inoculum) with 1 mg. of aureomycin as in (*a*) but then to remove the membranes after 2 hours from the environment of both virus and drug *in ovo* and place them in tissue culture according to the technique described. 45 to 48 hours after infection the membranes were harvested from tissue culture and titrated in mice.

The results summarized in Fig. 2 show that when aureomycin was given at any time within the first 15 hours of infection, it was able, within 2 hours after inoculation into the egg, to become associated with the allantoic membrane in such a way as to inhibit virus growth in tissue culture. Because the same results were obtained with membranes treated as late as 15 hours (several hours after completion of primary adsorption) as with those treated as early

as 10 minutes, it would appear that inhibition of adsorption of the virus particle to the host cells plays at most a minor role in the mode of action of aureomycin. It seems that virus already adsorbed becomes in some manner "inactive" for this 48 hour growth period when exposed to the drug.

Additional experiments bearing on this point were designed. An attempt was made to obtain uniform virus populations as regards stage of development at given time intervals by interrupting adsorption at 1 hour.

A series of eggs were inoculated with 0.5 ml. 10^{-1} virus dilution in the allantoic cavity. After a 1 hour adsorption period, the allantoic membranes were harvested, washed, and put in tissue culture. At various times after the tissue cultures were set up, 1 mg. of aureomycin was added to them. These were harvested after 45 to 48 hours and titrated in mice.

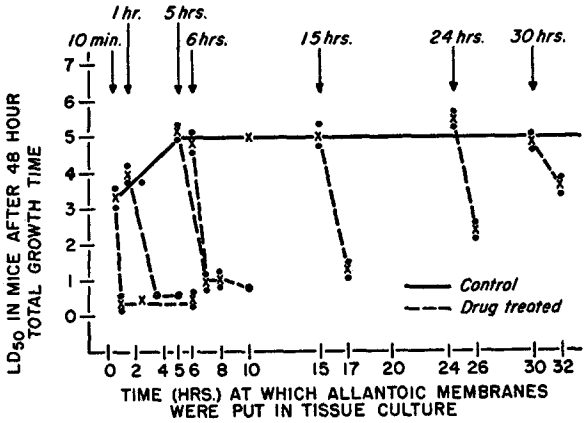

FIG. 2. The effect of aureomycin given at various time intervals during first cycle of virus growth—infection and treatment *in ovo* followed by transfer to tissue culture.

The results of such an experiment were comparable to those found in Fig. 2. When drug was added to the tissue culture at times corresponding to 2, 4, and 8 hours after infection of the egg, little or no virus developed during the 48 hour growth period. When drug was added 15 hours after infection, 1.2 logs of virus was found, and when it was added 24 hours after infection, 2.8 logs of virus was found. (These figures are averages of duplicate tissue cultures.)

Thus, the experiment indicates that the appearance of increased amount of virus with delay in time of administration of the drug could not be explained by continued virus adsorption since membranes were removed from the presence of virus 1 hour after infection. The results are consistent with previous observations that maximum inhibition of multiplication required administration of aureomycin during the early phases of virus growth. Furthermore, this experiment offers additional evidence that aureomycin can be absorbed *in vitro* by the membrane to inhibit virus replication.

It occurred to us that the progressive decrease in the effectiveness of aureomycin with time was due to its inability to affect virus which had passed a certain stage in development. Although final evidence in support of this hypothesis is not available at this time, a preliminary experiment suggested this to be true. In this experiment, aureomycin did not seem to affect infectious virus present in membranes in the later hours of the first cycle.

The data obtained in the tissue culture experiments and *in ovo* studies concerning the relationship of the time of administration of the drug to its effect on virus yield suggested that the maximum effect could be achieved only if aureomycin were given early but that the drug seemed to affect some step following adsorption. In order to test this hypothesis an experiment was de-

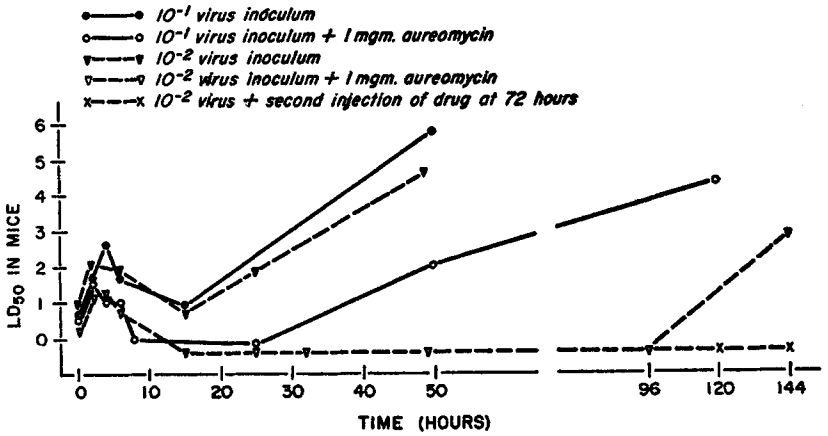

FIG. 3.The effect of aureomycin given at the time of infection on the growth pattern of MP virus in the allantoic membrane of the chick embryo.

signed in which a comparison was made of the growth curve of MP virus in treated and untreated embryos.

The Effect of Aureomycin on Virus Growth in Allantoic Membranes—Effect on Growth Curve.—It can be seen in Fig. 3 that when aureomycin was given at the time of infection, the most pronounced effect of the drug on the growth pattern seemed to be the extension of the "latent" period. There were noted some slight differences in the titers of treated and untreated membranes during the early hours. Even if these differences were attributed to a difference in the amount of virus adsorbed, they would not be sufficiently large to account for the prolonged latent phase. This fact seems to be an indication of a complex action of the drug, within the membrane, which inhibits the completion of the growth cycle.

The degree to which the "latent" period was extended by the presence of aureomycin seemed to be related to the amount of virus inoculated. When

10^{-1} inoculum was used, an increase in virus was first noted at 50 hours; when 10^{-2} dilution of inoculum was employed, the increase of virus in the membranes did not occur until about 120 hours (sometimes 96 hours) after infection. Thus, the time of appearance of new infectious virus was significantly different from that of 22 to 24 hours which was the case in the control membranes.

It would seem that as the concentration of aureomycin decreases, a certain critical level is reached which *cannot* keep virus in the non-infectious phase and thus the virus goes on to complete its cycle of multiplication. This possibility was tested by again injecting the eggs with 1 mg. of aureomycin at 72 hours after infection. It can be seen in Fig. 3 that this procedure caused a further delay in the appearance of new virus beyond 144 hours.

TABLE II

The Time of Appearance of New Virus in Treated Membranes in Tissue Culture

Time preparation of tissue culture (hrs. of growth in ovo)*	Time in tissue culture at titration	Total growth time at titration	Log titer in mice (LD_{50})
	hrs.	*hrs.*	
7	41	48	Negative
7	65	72	3.4
7A‡	65	72	Negative
72	72	144	3.2

* Eggs inoculated with 10^{-2} virus + 1 mg. drug at 0 hour.

‡ 1 mg. aureomycin added to tissue culture also.

Difference in the Time of Appearance of New Virus in Treated Membranes in Tissue Culture and in Ovo.—It has been noticed that membranes of eggs which had been inoculated with 10^{-2} dilution of virus and 1 mg. of aureomycin simultaneously, then transferred to tissue culture during the early hours of infection, developed a small amount of virus (approximately 0.5 log) after a 48 hour growth period. This was not found at corresponding times in growth cycle experiments (also using 10^{-2} dilution of virus) when direct titration of the membranes was made. Furthermore, as shown in Table II, membranes, if allowed to remain in tissue culture for another 24 hours (a total of 65 hours in tissue culture), yielded significant virus titers.

This suggested that the critical concentration of aureomycin in tissue culture may have been reached within 65 hours after the membranes were placed in tissue culture. It has already been mentioned that part of the aureomycin separates from the allantoic membranes in tissue culture. This would aid in reaching the critical low level of drug earlier than in the treated egg.

When 1 mg. of aureomycin was added to treated infected membranes in tissue culture (preparation 7A in Table II), the "latent" period was extended beyond the 72 hour period.

The reason for the effect of the addition of drug to the tissue culture was not determined. It may have been (*a*) that an additional amount of drug was taken into the membrane from the tissue culture fluid, or (*b*) that an equilibrium was established which prevented further "leakage" of the drug from the membrane. It was shown previously that aureomycin in Simms's fluid was able to become associated with the membrane.

It was evident from these experiments that the same virus material which was in some manner "kept" in the non-infectious phase by the action of aureomycin (48 hour tissue culture titration) was able to complete the cycle of multiplication after the concentration of the drug associated with the membrane was no longer sufficient to exert its effect (72 hour titration of identical tissue cultures).

Further Evidence for the Intracellular Effect of Aureomycin.—The data showing that aureomycin can affect virus after adsorption to the allantoic membrane (Fig. 2) and those showing the extension of the "latent" period in the growth cycle (Fig. 3), as well as those illustrating the lack of *in vitro* effect on the virus particle, suggest that the inhibiting effect of the drug was chiefly at the intracellular phase. Further experiments also lend supporting evidence to this hypothesis.

Normal chick embryos were injected with 1 mg. of aureomycin by the allantoic route. The membranes were removed from some of the embryos at 15 minutes and the remainder after 2 ½ hours from the time of administration of the drug. After washing in a broth-buffered water mixture, these membranes were placed in tissue culture (3 membranes per 50 ml. of Simms) and seeded with 2.5 ml. of the appropriate virus dilution. Control cultures of untreated membranes and virus were also set up. After a growth period of approximately 45 hours, the membranes were harvested, ground in a mortar, and titrated in mice.

The membranes exposed *in ovo* to 1 mg. of the drug for 2 ½ hours and seeded with 10^{-1} dilution of virus in tissue culture showed a 1 log difference in titer from that of the control (Table III). The membranes which had the same time of exposure to the drug but seeded with 10^{-3} dilution of virus exhibited a more striking difference in titer when compared with the controls. Thus, in these experiments there was an inverse relation between the amount of virus inoculum and the degree of inhibition of growth. Exposure of the membranes to the drug for a 15 minute period did not result in an inhibition of virus growth. In an experiment not reported in this paper in which aureomycin and virus were administered simultaneously, and the membranes were removed from the eggs at different intervals thereafter to be placed in tissue culture, it was found that 10 minutes was insufficient for "binding" of aureomycin to the membrane (no suppression of viral growth). An interval of 25 minutes was adequate.

Further evidence pointing to the intracellular action of aureomycin was obtained by putting virus on the ectodermal layer of the chorioallantoic membrane and the drug inside the allantoic cavity. When membranes of these

eggs were put in tissue culture, the resulting titers after 45 to 48 hour growth were similar to those presented in Fig. 2.

Presence of Virus in Various Tissues of the Chick Embryo.—It was mentioned earlier in this paper that aureomycin prolongs the survival time of the host infected with viruses of the psittacosis-lymphogranuloma group. Furthermore, aureomycin is present not only in the allantoic fluids and membranes, but also in the bloodstream and in small amounts in the liver. The question which arises is whether there might be a relation between the distribution of virus to various parts of the embryo and death; survival being the result of prevention of infection of certain vital organs.

TABLE III

Comparison of Amount of Growth in Treated and Untreated Membranes Seeded with Virus in Tissue Culture Log Titers (LD_{50}) in Mice

Dilution of seed	Membranes exposed to drug 2½ hrs.			Membranes exposed to drug 15 min.		
	Control	Treated	Difference	Control	Treated	Difference
10^{-1}	3.5	2.5	1.0	3.8	3.8	0.0
10^{-1}	3.8	2.8	1.0			
10^{-3}	2.6	0.3	2.3			
10^{-3}	1.8	0.2	1.6			

In preliminary experiments, liver, brain, plasma, and allantoic membranes of drug-treated eggs were titrated in mice to determine the amount of virus present.

In these experiments, 0.2 ml. of 10^{-2} dilution of virus inoculum and 1 mg. of aureomycin were administered to 10 day eggs simultaneously in the allantoic cavity. At the time intervals indicated, the organs from an average of eight living embryos were harvested, ground, and titrated.

The results of such an experiment as seen in Table IV show that in untreated eggs in which the titer of the allantoic membrane is increasing after 24 hours, virus appeared in both plasma and liver after 48 hours and in the brain 72 hours after infection. In the case of the treated embryos in which the titer of the membrane does not begin to rise until 120 hours after infection, no virus could be detected in the liver until 144 hours. As late as 192 hours after infection, no virus could be found in the brain tissue of treated embryos. Neither, however, was aureomycin found on assay of brain material. The above results were found in three separate experiments. However, similar experiments have not been done using dead embryos for purposes of comparison.

In two experiments there was an apparent disappearance of virus at 168 hours. The reason for this discrepancy has not been investigated and therefore

cannot be adequately explained. In one experiment, the 192 hour interval was also associated with a low titer.

At these time intervals, the embryos were 17 or 18 days old and one possible explanation might be the development of antibodies by the chick embryo.

TABLE IV

Presence of Virus in Various Tissues of Treated and Untreated Embryos Log Titers (LD_{50}) in Mice

Time	Treated embryos				Untreated embryos			
	Liver	Brain	Plasma	Membrane	Liver	Brain	Plasma	Membrane
hrs.								
3			<1.0				<1.0	
24	Negative	Negative	<1.0		Negative	Negative	<1.0	3.5
48	"	"			0.62	"	2.0	4.5
72	"	"			1.5	0.2		
90	"	"	<1.0		2.1	2.0		5.8
120	"	"	<1.0	1.5				
144	0.62	"						
168	Negative	"		Negative				
192	3.4	"		4.0				

DISCUSSION

In an attempt to determine the action which aureomycin exerts on the growth of a virus, it was necessary to consider the effect of the drug on extracellular virus, on adsorption of virus to host tissue, and on intracellular growth of the virus. The findings indicated that the drug had no *in vitro* effect on the MP virus particle itself; that is, aureomycin was not capable of altering the extracellular virus. Wong and Cox (2) previously had shown that this antibiotic had an *in vivo* rather than an *in vitro* effect on viruses of this group. In the experiments herein described, no significant effect of the drug on adsorption of the virus to host tissue seemed to be apparent. It is possible that there existed some slight differences in virus adsorption on treated and untreated membranes.

Aureomycin became associated with the host tissue. The fact that the drug was found in the bloodstream of the embryo early was suggestive that it was taken in the circulatory system and thence to the tissues. However, other experiments indicated that the *in vitro* exposure of normal allantoic membranes to aureomycin led to its association with the tissue and the suppression of virus growth. Therefore, it would appear that aureomycin may enter the membrane directly from the allantoic fluid as well as through the circulatory system. Regardless of the route by which the drug reached the cells, it seems that its prime action was on the virus-host complex, as evidenced by the extension of the "latent" period.

It was shown that aureomycin in amounts greater than 1 mg. was toxic and produced death in the chick embryo. It is possible that 1 mg. of the drug may alter the embryo's metabolism at some critical point essential for virus proliferation without producing obviously detectable damage to the host cells.

In experiments reported here, no direct microscopic examinations were made of the drug-treated tissue to determine whether the cells continued to multiply. However, comparisons of the rate of growth of allantoic membranes of treated and untreated eggs were carried out by marking the position of the membrane on the shell each day. There were no significant differences.

In our experiments it was found that complete inhibition of multiplication during the time interval corresponding to the first cycle of growth occurred only if aureomycin was administered during the first 6 to 8 hours. This would seem to indicate that after this time, virus synthesis had passed beyond the process or stage in development which could be blocked by the drug.

The nature of that part of the mechanism of virus production which is susceptible to aureomycin is not known. It could conceivably be protein synthesis. Cohen (10) has shown that protein formation is the earliest observable synthetic process during the latent period of multiplication of phage. A considerable amount of protein is found by the time synthesis of DNA commences. Our conjecture is in line with the recent observation of Gale (11) that aureomycin markedly inhibits protein synthesis. These considerations are speculative and the question of the nature of the intracellular action of aureomycin must await further experimentation.

Results obtained by other workers have indicated other substances which when given before a critical time in viral synthesis were able to inhibit the growth of a particular virus. Ginsberg and Horsfall (12) showed that capsular polysaccharide of Friedländer bacillus produced inhibition of the growth of PVM if given within the first ⅔ of the latent period of virus growth. Henle *et al.* (13) found that multiplication of influenza virus could be inhibited by the injection of homologous irradiated virus after the infection was in progress, thus suggesting that the irradiated virus might still enter the cell and interfere with some stage of the development of active virus. Using T_2 and T_6 bacteriophage, Foster (14) showed growth inhibition by adding proflavine during the first half of the latent period. Similar work has been done by Cohen and Fowler (15) who found that 5-methyltryptophane was capable of inhibiting T_2 multiplication also if given within the first half of the latent period.

In our studies, no attempt was made to confirm the findings of Gogolak and Weiss (16) that aureomycin causes a change in the morphology of certain developmental forms.

SUMMARY

The findings presented indicate that aureomycin could become associated with tissue of the chick embryo by both hematogenous distribution and direct adsorption.

Treatment of chick embryos infected with MP virus with 1 mg. of aureomycin by the allantoic route caused an inhibition of virus growth in the allantoic membrane.

The drug had no effect on "inert" virus, and appeared to have little effect on adsorption of virus to host tissue.

Complete inhibition of growth during the time interval corresponding to the first cycle of multiplication could be achieved only if the drug was administered within 6 to 8 hours after virus inoculation.

Partial inhibition of virus multiplication could be achieved even if the administration was delayed as late as 24 hours after infection.

In these experiments the chief role of the antibiotic appeared to be one of virustasis reflected in a prolongation of the latent period (non-infectious phase). The virus was able to resume its growth when a critical low level of the drug in the allantoic membrane was reached.

When infectivity titrations were carried out using various tissues and organs of treated and untreated embryos, it was found that no virus was detectable in the brains of treated embryos as late as 192 hours after inoculation of virus. This was in contrast with the findings in allantoic membranes and livers of such embryos; these organs showed virus at 120 and 144 hours, respectively. In untreated controls, virus appeared in membranes at 24 hours, in the liver at 48 hours, and in the brain at 72 hours.

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