Distribution and Persistence of Aureomycin in the Chick Embryo.* (20173)

E. G. ALLEN, A. J. GIRARDI, M. M. SIGEL, AND M. KLEIN.

From the Children's Hospital of Philadelphia, Department of Preventive Medicine and Public Health, School of Medicine, University of Pennsylvania, and the Department of Bacteriology, Temple University School of Medicine, Philadelphia, Pa.

In connection with our investigation of the effect of aureomycin on meningopneumonitis virus in chick embryos(1), it was felt desirable to determine the distribution and persistence of the antibiotic in this host following inoculation into the allantoic cavity. This report summarizes the findings obtained in these determinations.

Materials and methods. Chick embryos were inoculated with 1 mg of crystalline aureomycin by the allantoic route, and at various intervals thereafter tissues, allantoic fluids and plasma were harvested. For assay of the drug content in *allantoic fluids,* a sample of a pool of fluids from 3 eggs was used. *Allantoic membranes* were usually removed from the same eggs from which fluid samples had been taken, washed 6 to 7 times in broth-buffered water mixture and triturated in a Ten Broeck grinder. The resulting suspension could be removed from the grinder with a pipette without the addition of diluent. *Organs* were ground in a mortar with saline. *Plasma* was obtained by collecting blood from 12- to 13-day embryos (younger embryos were found to be unsatisfactory for bleeding with syringe and needle) in an equal volume of 2% sodium citrate. The bleeding of the embryos was done by making a window in the shell over a large vessel of the allantoic membrane. Sterile mineral oil was used to make the shell membrane transparent, and blood could be drawn from the vessel without entering the allantoic cavity. All preparations were frozen immediately and kept at −20 to −25° C until the assays were done. At the time of assay an equal volume of saline was added to the suspensions of membranes and organs and extraction of aureomycin was allowed to proceed at room temperature for 30 to 60 minutes. The assay was done on the saline extract. All figures are presented as γ per ml. Conversion to γ per g was not warranted because the values are not absolute values but rather approximations (complete extraction was not possible). The *zero hour* interval in Table I actually involved 2 to 5 minutes to carry out the harvesting procedure. Each egg at this interval was inoculated and harvested immediately. This was true of the embryos which were bled also; the shell windows were prepared beforehand. The method of assay, that of Dornbush and Pelcak(2), was sensitive to $\pm$ one 2-fold dilution step.

Experimental. It can be seen in Table I that the amount of drug present in the allantoic fluid immediately after inoculation (0 hour) was only slightly less than the expected theoretical amount based on dilution of one mg of drug in the allantoic fluid of the egg. Though there appeared to be some variation in the actual values (2-fold variations are considered within limits of experimental error), the general picture was one of a progressive decrease in the amount of the drug with time. The difference between concentrations of aureomycin at time zero in the fluids of 9-day and 11-day embryos can be explained by the larger amount of allantoic fluid in the 11-day eggs. It is interesting, however, that the rate of loss was much greater in the 11-day-old embryos. The difference is especially significant in view of the fact that the increase in amount of allantoic fluid and hence progressive dilution of the drug in the course of growth is greater in the embryos which are 9 days at time of treatment, than in those which are 11 days old.

The changes in the amount of drug associated with the allantoic membrane in relation to time seemed to be similar to those observed with fluids but on a much lower scale. As mentioned under *materials and methods,* the figures in Table I for the amount of drug per ml of membrane or organ do not represent

* This work was supported by a Contract between the Office of Naval Research, Department of the Navy, and The Children's Hospital of Philadelphia.

TABLE I. Concentration of Aureomycin in Embryo Tissues.*

Time (hr)	Allantoic fluid† (γ/ml) 9 day	11 day	Allantoic membrane† (γ/ml) 9 day	11 day	Plasma‡ (γ/ml) 11 day			Organs (γ/ml) Embryo	Liver 11 day	Brain 11 day	Heart
0	150	75			6.2	.2	6.2				
1	125		5.2				0				
2		45		1.7	1.5	.1	12.5	0	.2	0	0
3	50						12.5				
4				1.0	12.5	<.1	0				
6	150	20	4.0	1.7		<.1	1.5	1.5	.2	0	0
8	100	12				<.1					
20		7			0						
25	50	7	6.3	1.5	0	<.1		0.37	.2	0	0
48	40	2	7.0	.5				0	0	0	0
72	12		4.0								
96		2		.37				0	0	0	0
120	7		1.5								
144		0.75		.2					0	0	0

Sensitivity varied from 0.05 to 0.1 γ/ml.

* In most instances, crystalline aureomycin + glycinate buffer was used (unbuffered crystalline aureomycin was used in two instances with essentially the same results).

† Determinations on fluids and membranes were carried out in several experiments, and most figures represent the average of two or three results.

‡ Because of irregularities, three individual experiments (corrected for dilution factor) are shown.

the exact amount of the drug which is associated with the tissue. However, it can be seen that a continuous decrease in amount of aureomycin occurred with the increase in time.

The results of assays on plasma appear somewhat irregular. It would seem, however, that drug was present in the blood stream but for a short time.

Of the organs tested, the liver was the only one which showed any detectable amount of aureomycin. This was an exceedingly small quantity. No organs other than the brain, heart and liver were harvested because of the difficulty in distinguishing them in these young embryos.

Summary. Data were presented on the distribution and persistence of aureomycin in the allantoic fluids and tissues of 9- and 11-day-old embryos following injection of the drug into allantoic cavity. The drug was found associated with allantoic fluid, membrane, blood and liver, but not with brain or heart. The amount of drug found following injection decreased with an increase in time.

1. Allen, E. G., Girardi, A. J., Sigel, M. M., and Klein, M., *J. Exp. Med.*, in press.

2. Dornbush, A. C., and Pelcak, E. J., *Ann. N. Y. Acad. Sci.*, 1948, v51, 218.

Received March 4, 1953. P.S.E.B.M., 1953, v82.

Influence of Oxygen Tension on Tetrazolium Reduction by Epiphyseal Cartilage of Rachitic Rats *in vitro*.* (20174)

HOWARD H. HIATT† AND EPHRAIM SHORR.

From the Russell Sage Institute of Pathology, the Department of Medicine, Cornell University Medical College and The New York Hospital, New York City.

Little is known concerning oxidative processes in cartilage. Oxygen uptake has been demonstrated(1-3) but in amounts so small as to be comparable only with the oxygen consumption of mammalian erythrocytes. Articular cartilage of the horse and of the rabbit has been shown to reduce methylene

* Aided by a grant from the National Institutes of Health of the U. S. Public Health Service.

† Work done under Post-Doctorate Fellowship of the National Institutes of Health, U. S. Public Health Service.