Effect of Aureomycin on Viruses of the Psittacosis-LGV Group in Embryonated Eggs and Mice. (18393)

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The efficacy of aureomycin(1) in the treatment of bacterial and viral diseases made it desirable to obtain additional data on the effect of this antibiotic on viruses of psittacosis-LGV group.

Preliminary reports by Wong and Cox(1) showed that aureomycin, in mice and eggs infected with some strains of the psittacosis-LGV group, was effective in counteracting the lethality of the virus. Considerable significance therefore has been attached to the antiviral activity of this drug. Should the drug show similar activity for other psittacosis-LGV strains, a useful adjunct or substitute for either penicillin or sulfadiazine therapy (2) would be indicated. This paper will present the results of such a study.

Materials and Methods. Virus Strains. Ten representative strains from both mammalian and avian sources were included in the studies. The 6BC, Borg, Gleason, S-F and P-207 viruses were employed in animal studies, while the Cal 10, LGV, 12xN, Baker and Nigg viruses were included in the egg experiments. The source of these strains has been presented previously(2).

Egg Studies. Groups of fertile eggs, 8 or 9 days old, were inoculated by the yolk sac route with (1) 0.25 ml of a M/15 phosphate buffer solution (pH 7.0), or (2) 0.25 ml of buffer solution containing 0.5 mg of aureomycin per egg. Two to 4 hours later both groups of eggs were injected by the yolk-sac technique with 0.25 ml of 10-fold broth dilutions of the viral strain under test, using 20 eggs for each dilution. Yolk sac material was employed as the source of virus in all tests. The eggs were candled daily for 10 days and deaths recorded. At intervals the yolk sacs of dead eggs were examined microscopically by the Macchiavello technique for virus. The effect of the drug on the infection was evaluated by comparing the titers of the virus in LD_{50}(3), in the 2 series.

Animal Studies. Groups of 80 or more white mice, weighing 15-18 g were inoculated intracerebrally with 0.03 ml of virus suspension in nutrient broth representing (1) 50 mouse intracerebral LD_{50} or (2) 1000 mouse intracerebral LD_{50}. The groups were then subdivided into lots of approximately 15 mice each for treatment with aureomycin which was started 48 hours following virus inoculation and continued for 5 consecutive days. Daily subcutaneous inoculations of 0.25 ml of aureomycin solution were given representing 0.5 mg, 0.1 mg, or 0.02 mg of drug and the test observed for 30 days unless otherwise noted. Control groups of animals received either virus or drug alone. In some experiments the treatment was repeated at 9 and 19 day intervals following virus inoculation to provide more extensive treatment.

Concomitant studies were undertaken to determine the concentration of aureomycin in blood, urine and tissues of mice receiving aureomycin treatment as described. Approximately 24 hours after each aureomycin injection, 3 mice were sacrificed, the organs ground separately in sterile distilled water and the tissue suspensions analyzed by an inhibition technique(4).* The carrier state of drug treated animals in each group was checked at various times. Three mice were sacrificed, the brains removed aseptically and 10% emulsions prepared in sterile nutrient broth. The suspensions were centrifuged at 1000 rpm for 5 minutes and the supernatant fluids re-

1. *Annals of New York Academy of Sciences, Aureomycin,* 1948, v51, 175.

2. Meiklejohn, G., Wagner, J., *et al., J. Immunol.,* 1946, v54, 1.

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

4. Forgacs, J., Herring, A. S., *J. Bact. Abst.,* May 1949, 8.

* The lowest range of sensitivity of the test is considered to be 0.3 μg of drug per ml of fluid.

TABLE I. Effect of Aureomycin on Growth of 6BC Virus in Eggs.

Dilution of viral inoculum	Results in untreated eggs	Results in treated eggs*		
	Survival ratio†	Survival ratio‡	Yolk sac smear of surviving eggs	LD_{50} titer of surviving eggs
10^{-2}	0/16†	9/14	+	$10^{-8.4}$
10^{-3}	0/16	15/20	+	$10^{-8.3}$
10^{-4}	0/18	14/16	+	$10^{-8.3}$
10^{-5}	0/18	15/17	+	$10^{-6.9}$
10^{-6}	0/18	17/20	—	$10^{-4.0}$
10^{-7}	0/18	16/20	—	$10^{-3.8}$
10^{-8}	0/18	15/17	—	$10^{-2.0}$
10^{-9}	8/14	13/13	—	$10^{-2.0}$

* A single yolk-sac inj. of 0.5 mg of drug 2-3 hr following virus inoculation.

† LD_{50} endpoint of control titration $10^{-8.8}$.

‡ LD_{50} endpoint of test titration $10^{-2.8}$.

§ This calculation, although not statistically valid, represents a rough estimation of the endpoint.

inoculated intracerebrally into groups of 10 normal mice. The animals were observed for 21 days and the deaths recorded. Irregular deaths were checked for virus by microscopic examination of brain tissues.

Experimental. Experiments with Eggs. Table I shows results obtained when aureomycin was tested against the 6BC strain. The drug was extremely effective in controlling the infection as evidenced by comparing the titer of the virus in drug treated embryos (LD_{50} $10^{-2.8}$) with the titer of the same virus in untreated control embryos (LD_{50} $10^{-8.8}$). This indicates a neutralization of at least 1.0 x $10^{6.0}$ LD_{50}'s of virus. Since it was evident that a considerable number of eggs would survive in all of the drug treated groups, it was desirable to determine whether the virus inoculated into these embryos was killed by the drug or merely inhibited in its growth. At the end of the observation period 2 or more eggs were selected at random from among the surviving drug treated eggs in each virus dilution group. The yolk sacs from these eggs were pooled and titrated for virus by inoculation of serial dilutions into eggs by the yolk sac route. The results of these tests are also shown in Table I.

Yolk sacs of treated eggs surviving infection following inoculation with 6BC virus dilutions $10^{-2.0}$, $10^{-3.0}$ and $10^{-4.0}$ yielded virus titers of $10^{-8.4}$, $10^{-8.3}$ and $10^{-8.3}$ respectively. These titers approximate those seen in heavily infected yolk sacs, *e. g.*, control titration ($10^{-8.8}$) in Table I. As the amount of virus inoculated into the drug treated embryos decreased (dilutions of $10^{-5.0}$, $10^{-6.0}$ and $10^{-7.0}$) the titer of virus in the surviving eggs correspondingly decreased to $10^{-6.9}$, $10^{-4.0}$ and $10^{-3.8}$ respectively. In eggs given virus dilution $10^{-8.0}$ none of the control embryos survived while 15 of 17 drug treated eggs lived, with a subsequent yolk sac passage titer of less than $10^{-2.0}$.

Microscopic examination of stained yolk sac smears of surviving drug treated eggs showed elementary bodies in groups inoculated with virus dilutions $10^{-2.0}$ through $10^{-5.0}$, but not in groups receiving dilutions $10^{-6.0}$ or greater. It can be seen (Table I) that, at the dilution point between positive and negative smears, the titer of virus in surviving eggs dropped from $10^{-6.9}$ to $10^{-4.0}$. These data point out the advisability of employing titration methods rather than microscopic examination for evaluating drug efficacy in embryos.

The consolidated results of similar data for all 10 virus strains in aureomycin treated embryos are shown in Table II. All the virus strains followed a pattern similar to that described in detail for the 6BC strain. Only in those strains usually considered to be most infectious; *i.e.*, 6BC, Borg, Gleason, S-F, P-207 and Cal 10, was it possible to demonstrate high titer virus in surviving drug treated embryos. Correspondingly LGV, 12xN, Baker, and Nigg strains in most instances yielded virus titers so low as to suggest complete inhibition of growth in all dilutions tested.

Experiments with Mice. The results of these studies are presented in Table III. As was expected, not all of the virus strains had the same degree of susceptibility to the drug. In general, however, all were affected by 0.5 mg of aureomycin administered over a 5 day period. This is evident by the extended average day of death (A.D.D.) of treated ani-

TABLE II. Effect of Aureomycin on Growth of Psittacosis Strains in Eggs.

Virus strain	Titer of virus LD_{50} (negative log) Untreated eggs (control)	Drug treated eggs	LD_{50} (negative log) titer of drug treated eggs Surviving on dilutions							
			10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
6BC	8.8	2.8	8.4	8.3	8.3	6.9	4	3.8	<2	<2
Borg	8.5	3.7	8.5	8.5	8.5	8.4	8	3.5	3.5	1
Gleason	8.0	2.4	8.3	8.3	8.3	4.6	<2	<2	<2	<2
S-F	6.7	<2	>8.5	>8.5	8.2	4.6	4.9	<2	<2	
P-207	7.3	2.4	>7.5	7.5	3.9	3.9	<2	<2	<2	
Cal 10	7.1	<2	>8.5	8	6	6	<2	<2	<2	<2
LGV	3.5	<2	3.4	<2	<2	<2				
12xN	5.4	<2	<2	<2	<2	<2				
Baker	5.66	<2	<2	<2	<2	<2	<2			
Nigg	5.2	<2	<2	<2	<2	<2				

TABLE III. Effect of Aureomycin in Mice Inoculated Intracerebrally with Psittacosis Virus Strains.

Strain	No. intracerebral LD_{50} inoculated	Survival ratio of mice After 5 day drug treatment with: 0.5 mg/dose Surv./total	0.5 mg/dose A.D.D.*	0.1 mg/dose Surv./total	0.1 mg/dose A.D.D.	0.02 mg/dose Surv./total	0.02 mg/dose A.D.D.	No treatment Surv./total	No treatment A.D.D.
6BC	50	4/12	15	0/14	13	0/12	6	0/12	5
	1000	7/12	14	0/13	9	0/12	5	0/12	5
Borg	50	12/12	>30	4/13	9	0/12	5	0/12	6
	1000	10/12	25	5/15	9	1/12	4	0/12	4
Gleason	50	6/12	13	0/13	7	0/12	5	0/12	5
	1000	8/12	17	0/14	9	0/10	4	0/12	5
S-F	50	20/20	>30	19/20	>22	8/20	8	0/34	7
	1000	9/20	23	15/20	19	2/20	8	0/31	7
P-207	50	2/12	15	0/13	12	0/12	5	0/12	6
	1000	10/12	25	0/12	18	0/11	4	0/12	5

* A.D.D.—Average day of death of animals dying on each drug treatment level.

mals as compared to untreated. The Borg and S-F strains appeared particularly susceptible, while the Gleason, 6BC and P-207 strains, although not showing a particularly impressive survival ratio for the 0.5 mg series, did show a marked extension in the average day of death (A.D.D.). In none of the tests did a dosage of 0.02 mg of drug appreciably alter the course of the disease. Fig. 1 and 2 present graphically the results observed when the 3 doses of aureomycin were tested against the Borg strain. Similar results were obtained for the other viruses.

It was observed that mice given aureomycin treatment and surviving infection with strain 6BC continued to harbor virus in brain tissues examined 21 days after inoculation. The development of a similar carrier state in animals infected with viruses of this group has been observed frequently in therapy studies employing such compounds as sulfadiazine, penicillin and chloromycetin. The results of testing all groups of surviving mice at the end of 30 days for evidence of living virus indicated that a carrier state was established in aureomycin treated animals inoculated with the 6BC, Borg, Gleason, S-F and P-207 virus strains, living virus being recovered from all groups of treated mice. There was no evidence to suggest strain differences from the results of these carrier studies. The action of aureomycin therefore appears to be similar to penicillin, chloromycetin and other chemotherapeutic compounds effective against viruses of the psittacosis-LGV group.

Distribution of Drug in Mice. Tests were conducted to determine the distribution of aureomycin in the tissues of normal mice following a series of drug treatments similar to those employed in the therapeutic studies. The results of these determinations are presented in Table IV. Aureomycin levels are

TABLE IV. Distribution of Aureomycin in Animal Tissues Following Repeated Subcutaneous 0.5 mg Injections of Drug.

No. of days of drug treatment	Results of assays of*					
	Blood†	Liver‡	Spleen	Kidney	Lung	Brain
1	0.3	7.5	TR§	5.0	TR	0
2	TR	7.6	0	7.7	0	0
3	0	4.6	0	TR	0	0
4	0	3.4	0	1.0	TR	0
5	TR	TR	TR	7.1	TR	0

* Values expressed as average of 3 individual samples.
† Blood level expressed as μg/ml.
‡ Tissue levels expressed as μg/g.
§ Trace quantities 0.3 μg/g.

expressed as the average of 3 individual samples. Only in liver and kidney were significant levels of aureomycin consistently detected. Blood samples following one day treatment yielded levels of approximately 0.3 μg ml. with traces to negative levels thereafter. Spleen, lung and brain samples were essentially negative in all tests.† These results are particularly significant since, in the therapy studies, mice were infected by the intracerebral route and were benefited by drug treatments. There was no evidence for cumulative drug levels in any of the animal tissues when treated and sampled at 24 hour intervals.

Multiple Five Day Treatment Periods. Fig. 3 shows the survival ratio of 3 groups of 20 or more animals, all infected intracerebrally with 1000 mouse IC LD_{50} of 6BC virus, and each treated with drug. Group 1 received daily intraperitoneal injections of 0.5 mg of aureomycin beginning on the second day following virus infection and terminating 5 days later. Group 2 received the above schedule plus an additional 5 days treatment beginning on the ninth and extending to the thirteenth day. Group 3 received both the above schedules plus an additional 5 days treatment beginning on the nineteenth day and extending to the twenty-fourth day. It can be seen that the percentage of mice surviving in any group was directly influenced by the number of drug treatments. While only 30% of mice lived following the first treatment, the survival index was raised to 65% and 91%, respectively, by the second and third schedules. It should be noted, too, that in each of the 3 groups there was 6 to 9 days lapse between the cessation of treatment and the beginning of deaths.

Accompanying groups of mice were checked periodically to determine how long the virus remained viable in the brain following drug treatment. The brains of three mice from each group were removed, each ground to make a 10% emulsion in nutrient broth and reinoculated intracerebrally into 10 mice which were observed for 21 days. In 2 of the 3 groups, living virus was recovered 69 days following inoculation, while after 83 days no virus was recovered from any of the groups.

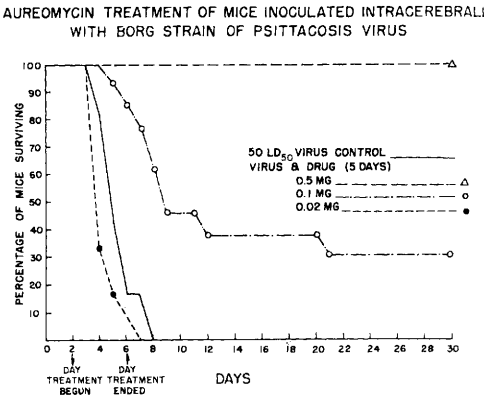

FIG. 1.

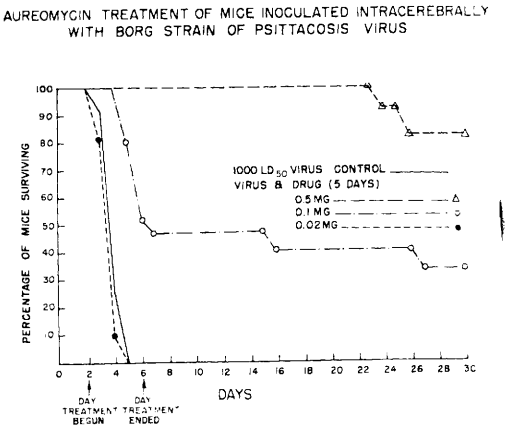

FIG. 2.

† The results of more detailed studies on the tissue distribution and excretion of aureomycin in animals receiving repeated doses will be reported by Dr. J. Fongacs, Camp Detrick, Md. in a later publication.

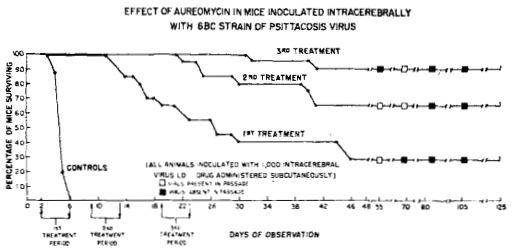

FIG. 3.

Discussion. The data indicate that aureomycin had a definite therapeutic effect in both eggs and mice infected with several virus strains of the psittacosis-LGV group. The results obtained in the egg studies seem particularly significant since large numbers of treated embryos survived until time of hatching while a majority of untreated controls died. These observations are in contrast to those previously reported for the psittacosis-LGV viruses tested against penicillin and sulfadiazine(2), where the treated embryos showed significant delay in death but seldom survived the prehatching observation period.

All the virus strains injected in high dilution were nearly completely inactivated by aureomycin. As the virus concentration increased, this inactivation was manifested only by the survival of drug treated embryos but not by the titer of the virus in them. These data suggest a quantitative relationship between the concentration of virus and the dosage of aureomycin used. With strains 6BC, Borg, Gleason, S-F, P-207 and Cal 10, virus was present in high titer in surviving drug treated embryos, while strains LGV, 12xN, Baker and Nigg yielded titers so low as to suggest almost complete inactivation by the drug in dilutions tested. These results are understandable since, in normal titrations of the strains, the former group yield titers far in excess of those observed for the latter.

The results of studies in mice were in agreement with those obtained in chick embryos. Treatment of infected animals with 0.5 mg doses of drug not only extended the A.D.D., but markedly reduced the percentage of deaths. Gradations in these therapeutic effects were demonstrated by using lesser amounts of drug. It is of particular interest that the drug was not detectable in brain tissues even though beneficial effects were apparent after intracerebral virus inoculation. This was true even after five successive daily injections of drug. Thus, it seems that drug concentrations of less than 0.3 μg per ml were sufficient to prevent death, but not to sterilize brain tissue although it is possible that aureomycin was present in higher concentrations than indicated, but masked by some substance present in brain tissue.

Summary. 1. Aureomycin was effective, in both embryonated eggs and in mice, in controlling experimental infections with 10 strains of virus in the psittacosis-lymphogranuloma venereum group. 2. Drug activity was evidenced in both the comparative titers of treated and untreated embryos, and in the titer of the virus recovered from these treated embryos. 3. In mice, multiple doses of 0.5 mg of drug lengthened significantly the life span of treated groups of animals (Borg, S-F, Gleason, 6BC, P-207), as compared to untreated controls. Gradations in these therapeutic effects were demonstrated by lesser amounts of drug. 4. The survival of treated mice as compared to untreated controls was markedly increased by repeated cycles of drug treatment.

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