# Effect of Aureomycin, Chloramphenicol and Para-Aminobenzoic Acid on Myxoma and Vaccinia Viruses in the Egg.* (18293)

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Aureomycin and chloramphenicol (Chloromycetin ®) have proved effective in the treatment of both experimental and clinical infections caused by rickettsiae and viruses of the psittacosis-lymphogranuloma venereum group(1-3). The large size of the pox viruses, also of the herpes and myxoma viruses, approximating that of the rickettsiae, made it seem worthwhile to evaluate the effect of antibiotics against these agents. Recently, herpes simplex virus has been studied by Baldridge and Blank(4), who demonstrated that its activity *in vivo* and *in vitro* is not inhibited by aureomycin.

The present report deals with our observations on the effect of aureomycin, chloramphenicol and para-aminobenzoic acid, both singly and in combination, on the infections of the chorioallantoic membrane of the embryonated egg with the viruses of infectious myxoma and vaccinia.

*Method and materials.* Crystalline chloramphenicol (Chloromycetin Lot No. 131334), which was kindly supplied by the Research Laboratory of Parke, Davis and Co., was dissolved just before use in sterile distilled water. In the case of aureomycin the antibiotic for intravenous administration containing 100 mg aureomycin hydrochloride and 50 mg sodium glycinate in a vial was dissolved in sterile physiologic saline. Para-aminobenzoic acid was made up in physiologic saline to a concentration of 2 mg or 4 mg per ml and autoclaved at 10 pounds for 15 minutes. The myxoma virus used was in its forty-first or forty-second chorioallantoic passage. The vaccinia virus was egg-adapted from the dermal strain by 11 serial passages on the chorioallantoic membrane in the laboratory of Dr. G. J. Buddingh of the Louisiana State University Medical School. Virus inoculations were made onto the dropped chorioallantois. In the case of myxoma virus, groups of 9 to 18 11- or 12-day eggs were employed in each experiment and the inoculum varied from 10 to 1000 infectious doses in 0.1 or 0.2 ml of distilled water. In the case of vaccinia virus, groups of six 12-day chick embryos were used in each experiment and the embryos were incubated for two days before the infected chorioallantoic membranes were examined. The inoculum consisted of 1000 infectious units in 0.2 ml physiologic saline. *In vitro* tests were performed by diluting the lyophilized virus-infected tissue to $10^{-2}$ by weight with solutions containing the desired amount of drug; the mixture was then allowed to stand in the refrigerator for varying periods of time, generally 2 or 4 hours. Serial 10-fold dilutions were prepared and 3 or 4 eggs were inoculated with each dilution. The *in vivo* effect of the drug used was thus largely eliminated. For the *in vivo* tests, the desired doses of drug in solution were injected onto the chorioallantois or into the yolk sac of the embryos. Several different treatment schedules were used to detect the possible prophylactic or therapeutic action of the agents under study. For each experiment, an equal number of control embryos was infected with the same virus preparation used in the test, except that distilled water or physiologic saline was given instead of the drug solution. The system of recording lesions by the gross pathology and number of plaques on the membrane was used(6).

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TABLE I. *In vitro* Effect of Chloramphenicol and Para-Aminobenzoic Acid on Myxoma Virus.

| Approximate No. of infectious units | Chloramphenicol 2000 γ/ml for 24 hr | |
|---|---|---|
| | Treated | Control |
| 1 × 10¹ | 3+* | 3+ |
| 1 × 10² | 1+ | 1+ |
| 1 × 10³ | D | D |
| | Chloramphenicol 1000 γ and PABA 1000 γ for 4 hr | |
| 1 × 10¹ | 3+ | 4+ |
| 1 × 10² | 1+ | 1+ |
| 1 × 10³ | D | D |

* Mean value for lesions on 3 or more chorioallantoic membranes.
4+: Many discrete lesions and confluent lesions.
3+: >50 discrete lesions and confluent lesions.
2+: >50 discrete lesions.
1+: <50 discrete lesions.
D = Doubtful.

TABLE II. Growth of Myxoma Virus on Chorioallantois of Eggs Treated with Aureomycin, Chloramphenicol and PABA.

| Approximate No. of infectious units | Chloramphenicol 1000 γ and PABA 2000 γ/egg | |
|---|---|---|
| | Treated | Control |
| 1 × 10¹ | 3+* | 3+ |
| 1 × 10² | 3+ | 3+ |
| 1 × 10³ | D | D |
| | Aureomycin 1000 γ and chloramphenicol 400 γ/egg | |
| 1 × 10¹ | n.t.† | n.t. |
| 1 × 10² | 3+ | 3+ |
| 1 × 10³ | n.t. | n.t. |
| | Aureomycin 200 γ and PABA 1000 γ/egg | |
| 1 × 10¹ | n.t. | n.t. |
| 1 × 10² | 4+ | 3+ |
| 1 × 10³ | n.t. | n.t. |

* Mean value for lesions on 3 or more chorioallantoic membranes.
4+: Many discrete lesions and confluent lesions.
3+: >50 discrete lesions and confluent lesions.
2+: >50 discrete lesions.
1+: <50 discrete lesions.
n.t. = not tested.

*Results and discussion.* A series of 24 therapeutic trials, both *in vivo* and *in vitro*, against myxoma virus infection in the chick embryo was performed. In Tables I and II are summarized the data of a few typical tests. In no experiment could any difference between control and treated membranes be detected, either as to the size or the number of the lesions. In one experiment, not included in the table, in the course of which the membranes were examined every 24 hours after infection, chloramphenicol did not appear to exercise any influence on the course and appearance of the lesions on the membranes.

Our investigations with the vaccinia virus were limited to *in vivo* studies. In agreement with the findings of others(7-9), our preliminary experiments showed that none of the three agents studied, when used singly, was active against the vaccinia virus. Table III presents the results of experiments in which the embryos were infected with vaccinia virus and treated with combinations of the therapeutic agents. In one trial, in which aureomycin and para-aminobenzoic acid were used in combination, the mean value for the lesions on the treated membranes was less than that for the controls. This difference was not statistically significant; moreover, this combination of therapeutic agents failed to delay the death of embryos following infection.

Baldridge and Blank(4) showed that, when the antibiotic was injected onto the chorioallantois, aureomycin is more toxic for the chick embryo than for most other laboratory animals, and that there is a significant dif-

TABLE III. *In vivo* Effect of Aureomycin, Chloramphenicol and Para-Aminobenzoic Acid on Vaccinia Virus.

| Combinations of therapeutic agents | Mean No. of lesions on 6 chorioallantois | |
|---|---|---|
| | Treated | Control |
| Chloramphenicol 500 γ and PABA 1000 γ/egg | 9 | 7.3 |
| Aureomycin 2,000 γ and chloramphenicol 400 γ/egg | 17 | 15.2 |
| Aureomycin 2,000 γ and PABA 800 γ/egg | 9.8* | 15.2 |

* According to Student's "t" test, the probability is greater than 0.1.

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TABLE IV. Toxicity of Aureomycin and Chloramphenicol for Embryonated Eggs.

| Dose in μg | Route | Aureomycin | Chloramphenicol |
|---|---|---|---|
| 1000 | Yolk sac | 8/24* | 2/21 |
| 800 | Chorioallantois | 17/17 | |
| 200 | " | 6/12 | 2/18 |

* Numerator: No. of embryos dead within 24 hr after treatment. Denominator: Total No. of eggs injected.

ference in toxicity between different lots. We also found our aureomycin preparation to be toxic for the chick embryo. As can be seen from Table IV, all of the embryos which were treated with 800 μg of aureomycin per egg by the chorioallantoic route died, while most of the embryos tolerated 1,000 μg or 2,000 μg per egg given into the yolk sac. This finding might well be correlated with the observations made by Lépine and his associates(10) that the growth of certain proliferating cells was definitely retarded by 100 μg of aureomycin and chloramphenicol per ml of tissue culture medium, and completely inhibited by 1,000 μg per ml. Moreover, Womack and his associates(11) demonstrated the presence in egg yolk of a substance which inhibits deterioration of aureomycin activity. The number of embryos treated was too small to evaluate the relative toxicity of aureomycin and chloramphenicol for the chick embryo, although aureomycin seemed to be a little more toxic than chloramphenicol.

*Conclusion.* A study of aureomycin, chloramphenicol and para-aminobenzoic acid in myxoma virus and vaccinia virus infection in the chick embryo failed to show any chemotherapeutic or chemoprophylactic action of these agents, either when they were used alone or in combination. In agreement with others, we also found that aureomycin, when introduced onto the chorioallantois, appeared to be toxic for the chick embryo.

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## Influence of Estradiol on $P^{32}$ Uptake by the Uterus. (18294)

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The object of this investigation was to secure increased concentration of a radioactive isotope in a target organ of the female genital tract. Evidence has been adduced that the weight response of target organs in treated animals may be influenced in an unexpected manner by varying the proportions of the constituents of the hormonal mixtures without changing the total equivalent doses(1,2). In order to elucidate the mechanism of such reactions experiments employing radioactive phosphorus ($P^{32}$) were designed. In the light of the observation that the rate at which new tissue is formed is one of the factors that influences the uptake of phosphorus(3), it seemed reasonable to assume that the uptake of $P^{32}$ by a target organ, such as the

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