Effect of Small Amounts of Aureomycin and Terramycin on Virus of Feline Pneumonitis. (18804)

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(Introduced by F. M. Hanger.)
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The phenomenon of natural and acquired resistance of microorganisms to antibiotic agents is one of great theoretical as well as practical interest. First noted in connection with penicillin, acquired resistance of staphylococci was regarded as an adaptation by the organism to an environment containing sublethal concentrations of the drug. Later this was shown to occur in a step-wise fashion, and in all likelihood to depend on a selection of mutants occurring spontaneously in large bacterial populations, and involving multiple genetic steps. This is spoken of as the "penicillin type" of acquired resistance. Generally speaking it often tends to be of a low order of magnitude, and is not of great clinical significance. The second, or "streptomycin type" of acquired resistance is much more abrupt and may result in the emergence of a completely resistant form in consequence of a single genetic step. It is this "streptomycin type" of acquired resistance which is of great clinical importance, and has limited the therapeutic usefulness of that particular antibiotic. Parenthetically it may be remarked that the phenomenon of natural resistance of many strains of staphylococci and a few other microorganisms to penicillin associated with the production of the enzyme "penicillinase" has not been noted in connection with other antibiotics. With the development of new antibiotics, it has naturally been of interest to study the phenomenon of acquisition of bacterial resistance in each, and reports have already appeared in the literature concerning chloramphenicol, aureomycin and terramycin. These in general indicate, at least in connection with the latter two, that resistance when developed, is of a low order of magnitude, that it is of the "penicillin" rather than the "streptomycin" type, and unlikely to prove of much clinical significance. It may be remarked that in usual therapeutic concentrations, the effect of these new antibiotics appears to be mainly bacteriostatic rather than bactericidal. In addition to their antibacterial properties, these new agents also possess significant action against infections due to rickettsiae and large filterable viruses of the psittacosis-lymphogranuloma group. Here again, their action would appear to be rickettsiastatic and virustatic, rather than a killing one. In view, therefore, of the already reported appearance of bacterial resistance to these agents, it was deemed of considerable interest to inquire whether an analogous phenomenon could be established in connection with a virus. For this reason we have been led to perform the following experiments with aureomycin and terramycin, using the virus of feline pneumonitis as our viral agent, in an effort to determine whether acquired virus resistance to an antibiotic could be demonstrated.

Wong and Cox(1) first reported suppression of growth in embryonated eggs treated with aureomycin of viruses of the psittacosis-lymphogranuloma group, including feline pneumonitis. We(2) have studied both aureomycin and terramycin in the treatment of infection with feline pneumonitis following intranasal inoculation of mice, and it was observed that both agents were highly effective in preventing gross pulmonary lesions if treatment was begun early, and in reversing them when treatment was delayed. The experiments to be described below, wherein we endeavored to produce drug-resistant strains of feline pneumonitis virus, were performed by two methods —the passaging of virus serially in embryonated eggs with varying amounts of the anti-

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TABLE I. Intranasal Infection of Mice with 0.02 cc of Feline Pneumonitis Virus, 10^{-2} Dilution.

Control mice (untreated)	3+	*	1+		0		3+							
	3+		2+		0		4+							
	3+	pooled	3+		1+		4+							
	2+	→	3+	pooled	1+		4+							
	0		3+	→	2+		4+							
			3+		3+	pooled	4+							
					3+	→								
Aureomycin-treated mice	2+		±		0		0							
	1+		±		0		0							
	±	pooled	1+	pooled	0		0							
	3+	→	1+	→	1+		0							
	2+		0		1+	pooled	0							
	±		0		±	→	0							
							0							
Terramycin-treated mice	2+		0		0		1+		0		0			
	1+		±		1+		1+		±		0			
	2+	pooled	2+	pooled	1+		1+		1+		0			
	2+	→	2+	→	2+	pooled	2+		1+		0			
	0		2+		2+	→	2+	pooled	1+	pooled	0			
	0		2+		2+		2+	→	1+	→	0			

* Each symbol represents one mouse. The following code is employed: 4+ = nearly total pulmonary consolidation; 3+ = extensive consolidation; 2+ = approximately one-half consolidation, or innumerable focal lesions; 1+ = small areas of consolidation, or widely scattered focal lesions; ± = minimal gross pathologic change.

biotic agent to be tested, and the serial passage of virus intranasally in mice with subcurative doses of the antibiotic.

The Baker strain of feline pneumonitis virus employed was originally obtained through the courtesy of Dr. Geoffrey Rake in the form of a lyophilized yolk sac membrane. After 2 further egg passages and 3 more mouse passages, it was used to initiate the mouse experiments in a 10^{-2} lung suspension made up in nutrient broth. The 2 antibiotics studied, aureomycin (Lederle) and terramycin (Pfizer) were administered by subcutaneous inoculation in the form of the hydrochloride dissolved in nutrient broth. Following intranasal inoculation of 0.02 cc of virus, all mice were left 3 days untreated. Thereafter the experimental groups were treated for 4 days with a daily injection of 0.25 mg of antibiotic a dosage which appeared insufficiently large to cause complete regression of the lung lesions, although it had some effect. On the seventh day all mice were sacrificed, including controls, and the lungs passaged to fresh groups of mice. Table I gives the results of these experiments.

It can be seen from the table that the virus in the untreated mice appeared to increase in virulence with repeated passage, so that in the fourth 5 out of 6 mice showed nearly total pulmonary consolidation. By contrast, in the mice treated with 0.25 mg of aureomycin daily for four days beginning on the third day after intranasal inoculation, pulmonary lesions were less conspicuous, tended to diminish, and then completely disappeared in the fourth passage. A similar phenomenon occurred with terramycin administered in the same dosage, except that the effect of the antibiotic was less marked in the early passages, and complete disappearance of virus activity did not occur until the sixth passage. Under the conditions of this experiment, therefore, no evidence of the emergence of a resistant mutant was obtained. In fact the reverse occurred, for the virus, after apparently maintaining itself with some difficulty in an unfavorable environment, proceeded to die out.

After our failure to demonstrate the phenomenon of acquired virus resistance in mice, we undertook to see whether it could be produced when virus was cultivated in embryonated eggs with varying concentrations of aureomycin. In these experiments the virus was mixed with antibiotic and introduced by yolk sac inoculation in a 10^{-3} dilution of in-

TABLE II. Passage of Feline Pneumonitis Virus in Embryonated Eggs.

Passage	Dose	With aureomycin	Without aureomycin
1	.067	D6 D7*	
2	.067	D5 D7 D7 D7 D10	D4 D4 D5 D5 D5 D5 D6
3	.1	D4 D4 D5 D7 D7 D8 D8 †	D4 D4 D5 D5 D5
4	.1	D4 D11 D11 S	D5 D5 D6 D8 S
5	.1	D6 D7 D8 D9 D11 SS †	D6 D7 D7 D11 D13
6	.1	D4 D9 SS	D7 SSSSS
7	.1	D4 D6 D6 D11 SS	D6 D6 D6 D6 D7 D8 D9 S
8	.1	D5 D6 D6 D6 SS	D3 D5 SSS
9	.1	D7 D10 SSSSSS	D5 D5 D8 SS
			SSSS to mice‡ 00000
			D7 SSS to mice‡ 0000

* Each symbol represents one egg. D indicates embryo died, and the number the day of death. S indicates survival. Underlining of symbol indicates source of material for passage.

† Indicates source of material for other egg passages not included in table. All these proved ultimately negative for virus.

‡ Each symbol represents one mouse. 0 indicates no evidence of viral activity as manifested by pulmonary lesions.

fected yolk sac suspension. The volume of inoculation was 0.2 cc, of which 0.1 cc represented the virus, and 0.1 cc the antibiotic. It had previously been shown(1) that 1.0 mg of aureomycin was enough to suppress virus multiplication completely. Therefore in a long series of experiments we explored the effects of the following dosages: 0.034, 0.067, 0.1, 0.2, 0.25, and 0.4 mg. Eggs were candled each day, and embryos dying before the fourth day discarded. Death of the embryo after the fourth day was taken as presumptive evidence of virus activity, although occasional non-specific deaths may occur at any time. It was not possible to establish the cause of death by stained preparations, as elementary bodies are usually not demonstrable in the presence of even small amounts of antibiotic, an observation first made by Wong and Cox (1) and confirmed by our own experience. Thus the presence or absence of virus could only be determined by subsequent passage in eggs without antibiotic, or by intranasal inoculation in mice of undiluted suspension.

Table II gives the result of a typical experiment in which virus was passed through embryonated eggs in the presence of aureomycin. It will be observed that in this experiment the two initial egg passages were made in the presence of 0.067 mg of aureomy-

cin. Thereafter the dose was increased to 0.1 mg. One or more eggs in which death of the embryo took place were used as source of material for the next passage, and a control group of eggs (*i.e.*, without aureomycin) was also injected. At the end of the experiment residual virus activity was tested for by mouse as well as egg inoculation.

Altogether 12 experiments similar to that outlined in Table II have been performed, using the various dosages of aureomycin indicated above, and the results in every one have been almost identical. The virus seemed to survive in attenuated form for several passages, and then to disappear. For example, in the experiment outlined, there appeared to be little virus activity remaining after the fifth passage, and at the time of the ninth passage 2 successive attempts to demonstrate virus activity were completely negative.

Discussion and conclusions. The purpose of these experiments was to find out whether the phenomenon of acquired resistance could be demonstrated in the case of a filterable virus to the newer antibiotics. The virus of feline pneumonitis was employed, and the two antibiotics were terramycin and aureomycin. These two were used in subcurative dosages in mice following intranasal inoculation in one series of experiments, and aureomycin was studied in 12 experiments in varying dosages in embryonated eggs. In order to enhance the likelihood of the emergence of a resistant mutant, large inocula of virus were employed. Under the conditions of our experiments no evidence whatsoever of resistance could be demonstrated, but an interesting phenomenon was observed. In both the mouse series and the egg series virus seemed to persist through several passages in the presence of antibiotic in diminished amounts. Then, with no increase in the dosage of antibiotic, it died out. The significance of this finding is obscure. That it might be due to an actual increase in sensitivity of the virus to antibiotic is conceivable, although such an hypothesis is not proven by the data presented here.

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Cell Proliferation and Changes in the Large Granule Fraction During Hepatic Carcinogenesis.* (18805)

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Little quantitative study has been made of cell division during carcinogenesis, and in the few cases reported, mainly of skin carcinogenesis(1), estimates of cell proliferation were based on observed changes in the mitotic index. Such estimates are subject to error due to changes in the duration of the various phases of mitosis. An extreme example of such inaccuracy is given by the early impression that the main effect of colchicine is to stimulate mitosis, while later investigations showed its main effect to be the inhibition of cell division in the metaphase(2). The liver offers advantages for the study of cell proliferation in that it is possible to calculate the total number of cells present from the number of cells per g of tissue, a procedure introduced by Brues, Drury, and Brues(3) for the estimation of replacement of liver tissue during regeneration. Price, Miller, and Miller(4,5) have demonstrated a striking depression of the protein and pentosenucleic

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