sants such as methylparafynol, methyprylon, or ethchlorvynol.

To test for the development of tolerance, pentobarbital-induced sleeping time was determined in mice as described previously after a single or five daily oral doses of 300 mg of trimeglamide per kilogram. The sleeping times (ten mice/group) were 74 ± 10 minutes for the control, 174 ± 32 minutes after a single dose, 112 ± 14 minutes after repeated doses. Thus some degree of tolerance had developed under these conditions in mice.

In dogs, trimeglamide had a mild protective effect against apomorphine-induced emesis. The ED_{50} of intravenously administered apomorphine hydrochloride was increased from 8.3 ± 0.6 to 11.4 μg/kg after three oral doses of 36 mg/kg day. No tolerance developed to this antiemetic effect during 7 months of daily drug administration.

GEORG CRONHEIM
JAMES T. GOURZIS*
I. M. TOEKES
Research Division, Riker Laboratories, Northridge, California

References and Notes

1. In the present discussion hypnosis is defined as a state of deep sleep from which the animals are aroused only with difficulty. However, when they are aroused, the animals appear normal but are reluctant to move around even though no ataxia is detectable.
2. Unpublished reports from several investigators to the Medical Department, Riker Laboratories, Inc.
3. L. Oettinger, Jr., and H. Sjaardema, *J. Nervous Mental Disease*, in press.
* Present address: Department of Pharmacology, University of Manitoba, Winnipeg.

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Development of Resistance of Influenza B Virus to Polysaccharides

Abstract. Algal polysaccharide obtained from carrageenin protects 80 to 100 percent of chicken embryos against fatal infections with the Lee strain of influenza virus. This report describes the rapid emergence of a stable variant of this virus which is resistant to the protective action of this polysaccharide.

In the absence of any practical chemotherapy for viral diseases (except for the diseases caused by the agents of the psittacosis-lymphogranuloma group that are no longer classified with the true viruses), published information concerning the development of drug resistance of viruses is scarce. Ginsberg and Horsfall (*1*) reported the development of a variant of mumps virus resistant to the antiviral action of the capsular polysaccharide of *Klebsiella pneumoniae*, but the resistant character of this mutant did not persist after three to five passages in the absence of the polysaccharide.

Table 1. Effect of algal polysaccharide treatment in chicken embryos infected with influenza B virus (Lee strain) or with a resistant variant (infections and treatment by the allantoic route).

Viral strains	No. of LD_{50}'s	Treatment*	Survivors/ total	Average survival time (days)
Parent	10	Algal polysaccharides (400 μg)	10/10	> 10
Parent	100	Algal polysaccharides (400 μg)	10/10	> 10
Parent	10	Saline	0/10	4.3
Parent	100	Saline	0/10	4.2
Variant	10	Algal polysaccharides (400 μg)	0/10	4.3
Variant	100	Algal polysaccharides (400 μg)	0/10	4.0
Variant	10	Saline	0/10	4.5
Variant	100	Saline	0/10	4.2

* One hour after infection.

We recently observed that 40 μg or more of algal polysaccharide derived from carrageenin or *Gelidium cartilagenium* protected 80 to 100 percent of 10-day-old chicken embryos against fatal infection with the Lee strain of influenza B virus if the embryos were treated within 8 to 10 hours after infection with 100 median lethal doses (LD_{50}) of the virus. This offered an opportunity to study the development of variants resistant to the protective action of this polysaccharide.

In the experiments described below the algal polysaccharide was obtained by acetone precipitation of aqueous extracts of carrageenin. The viral strains employed were the egg-adapted Lee strain of influenza B virus (designated as the parent strain) and a variant of it resistant to this polysaccharide. The variant strain was produced by two passages of the parent strain in 10-day-old embryos in the presence of the algal polysaccharide at a dose (400 μg) which was 10 times that required to protect 80 to 100 percent of the embryos. For this purpose each tenfold dilution (10^{-5} to 10^{-9}) of the parent strain was injected intra-allantoically into ten embryos and, after 1 hour, the eggs were injected by the same route with the polysaccharide. After 48 hours' incubation at 36°C, the eggs were chilled and the individual allantoic fluids were tested for the presence of viral hemagglutinins.

In the 10^{-5} dilution group the fluids of two of the ten eggs tested were positive, whereas none of the fluids of the eggs infected with higher dilutions of virus caused detectable hemagglutination. One of these positive allantoic fluids was passed again in fertile eggs similarly treated with the polysaccharide. At 48 hours, viral hemagglutinins were present in 10/10, 6/10, and 1/10 of the fluids from the 10^{-7}, 10^{-8}, and 10^{-9} dilution groups, respectively. The virus present in the positive allantoic fluid from the highest dilution group was designated as the resistant variant; it was then distributed into several ampules and was stored at −60°C.

The parent and variant strains were found to be similar with respect to virulence for chicken embryos, rate of multiplication in fertile eggs, and serological character. Their respective responses to the action of algal polysaccharide are shown in Table 1. It may be seen that treatment with polysaccharide protected all of the embryos infected with the parent strain of influenza Lee virus but that the variant strain was completely resistant to the action of the polysaccharide. There was no significant difference in the average survival times between the embryos infected with the variant strain and the saline-treated embryos infected with the parent strain. Similar results were obtained in a second series of experiments.

The persistence of the resistant character of the variant strain in the absence of the algal polysaccharide was demonstrated in two separate experiments in which it was passaged at high concentration ($10^{5.5}$ LD_{50}) in untreated chicken embryos every 24 hours. The drug-resistant character remained unchanged following 12 such serial transfers in the absence of the polysaccharide.

This stable polysaccharide resistance may be a useful finding in studies of viral genetics. The rapid development of the resistance indicates that any chemotherapy for influenza may be complicated by the emergence of drug-resistant variants.

PAUL GERBER*
EUGENE ADAMS
Squibb Institute for Medical Research New Brunswick, New Jersey

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* Present address: Division of Biological Standards, National Institutes of Health, Bethesda, Md.

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