from original greater availability. Mice with either type of hemoglobin appear to be normal and healthy, and both alleles must have been present in wild mice to account for the observed strain distribution.

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References and Notes

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2. The symbol for the allele determining single-type hemoglobin is Hb^1; that for the allele determining diffuse-type hemoglobin is Hb^2 (personal communication from S. Glücksohn-Waelsch).
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New Type Sedative and Soporific Drug

Abstract. Trimethoxybenzoyl-glycine-diethylamide induced in dogs and cats normal sleep without preceding ataxia. A five- to ten-fold increase of the soporific dose resulted in restlessness and disorientation instead of sleep. In man, oral doses of 500 to 1500 mg caused sedation or drowsiness, or both, in half the cases. No spindling or drug-induced artifacts were found in electroencephalographic recordings.

Drugs commonly used for hypnotic action in the practice of medicine in human beings are ineffective when similarly applied in dogs and cats. These species show ataxia accompanied frequently by frank excitement during induction. Similarly, on spontaneous awakening or active arousal, a similar period of disorganized activity results. In the course of an investigation of new compounds with action on the central nervous system, trimethoxybenzoyl-glycine-diethylamide (Riker 548; proposed generic name, trimeglamide) demonstrated the ability to induce in animals a state of somnolence which could not be distinguished from the physiologic state of sleep. This somnifacient action was neither preceded nor followed by the above-mentioned skeletal muscle involvements. No other drug known to us will produce this phenomenon.

In dogs and cats, the oral soporific dose of trimeglamide was 50 mg/kg; this dose had a latency of 30 to 90 minutes and a duration of 2 to 6 hours. When asleep, the animals could be aroused easily by sound or touch, and they would respond in a normal manner to external stimuli. If left alone, the animals would fall asleep again within a few minutes. There were no indications of skeletal muscle involvement, and no gross abnormalities were detected in neurological examinations. Effects on blood pressure and heart rate were also absent. Rate and depth of respiration remained unchanged.

Larger doses (100 mg/kg) only prolonged the soporific action in cats. In dogs, the soporific effects were also longer lasting and, in addition, some side effects appeared: emesis in about 10 percent of the animals and some muscle twitching and occasional slight ataxia. In addition, a few animals showed definite signs of hyperactivity prior to falling asleep. Raising the dose to 500 mg/kg did not increase the central depressant activity in the dogs nor did it produce sleep, hypnosis, or anesthesia (*1*). Instead, after a brief period of drowsiness or somnolence, a stimulant effect was superimposed upon the soporific action. This state was characterized by restlessness and purposeful locomotion (even though slight ataxia was present in some animals), unusual inquisitiveness, but also some apparent disorientation. Minor obstacles such as a chair leg or a small carton would completely stop the animals, and no attempt would be made to go around or remove the obstacle. Furthermore, the animals frequently would attempt to crawl into almost inaccessible places. As far as could be ascertained, there was no impairment of vision, hearing, taste, or smell. The drug effect gradually disappeared within 2 to 6 hours.

The absence of hypnosis or anesthesia at five to ten times the effective soporific dose in dogs is rather unique and distinguishes trimeglamide from such presently used sedatives as barbiturates, chloral hydrate, methyprylon, ethchlorvynol, and others.

In man, single or repeated oral doses of 500 to 1500 mg caused sedation or drowsiness, or both, without any side effects in about half of over 200 patients thus tested (*2*). Oettinger (*3*) has described the effect of trimeglamide on electroencephalographic recordings. The administration of 2 to 8 mg/kg to 51 children or adults produced in the majority of patients a feeling of relaxation and pleasant tiredness. Electroencephalographic recordings showed neither spindling nor any drug-induced artifacts and no changes in alpha frequency or alpha index.

The lack of hypnosis or anesthesia and of undesirable side effects or artifacts is a distinct advantage for a sleep-inducing drug. In the case of trimeglamide, this advantage is of particular interest since the acute toxicity is very low. Dogs, cats, and mice have tolerated single oral doses of 500, 770, and 2000 mg/kg, respectively. Chronic administration to dogs (35 mg/kg day for 9 months) and rats (100 mg/kg day for 6 months) did not produce signs of drug toxicity during the test or on histopathological examination.

The prolongation of barbiturate-induced sleeping time is considered an index of general central nervous system depression. In mice, sleep induced by pentobarbital sodium (65 mg/kg, intraperitoneal) was increased from 64 ± 11 to 135 ± 21 minutes ($\pm$ standard error) by premedication with 300 mg/kg of trimeglamide given orally 30 minutes before the test. With a subthreshold dose of pentobarbital sodium (30 mg/kg, intraperitoneal) a sleeping time of 13 minutes in 1/20 control mice was increased to 36 ± 9 minutes in 14/40 mice premedicated 30 minutes before the test with an oral dose of 300 mg/kg of the drug. In dogs, with thiopental sodium as the anesthetic, trimeglamide, when given perorally at 20 mg/kg 30 minutes before the test, increased the sleeping time from 20 ± 9 minutes to 46 ± 15 minutes. No apparent effect on respiration was observed. Conversely, premedication with the same dose significantly reduced the amount of thiopental required to abolish the swallowing reflex from 16.8 ± 2.8 mg/kg to 11.3 ± 2.9 mg/kg in a cross-over experiment with eight dogs.

Trimeglamide has anticonvulsant effects in mice against supramaximal electroshock (monophasic rectangular wave, 60 cy/sec, 8.3 msec pulse duration, 0.2 sec shock duration delivered through ocular electrodes). The oral ED_{50} of 510 mg/kg given 30 minutes before the test was about one-sixth of the acute LD_{50}. The drug resembled in this respect other general central nervous system depres-

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.

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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.
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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.

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