insect Ringer's solution in a glass chamber. When carbon dioxide was added, the isolated spiracles opened; when oxygen was added, they closed. That is, the isolated spiracles behaved in relatively normal fashion for many hours as independent effectors. In hanging drops of a suitable medium (*6*), they functioned for as long as 3 days.

Further experiments were performed on pupae bisected parasagitally in Ringer's solution so that one side had spiracles with intact connections to the central nervous system, while the other side had no connections to the central nervous system. When a stream of carbon dioxide was directed at a spiracle, it opened; when oxygen was applied, it closed. This was true both of spiracles connected to the central nervous system and of those not connected to it.

To see whether carbon dioxide was acting by virtue of its acidity, Ringer's solutions of different *p*H were added to the spiracle. When a spiracle was flooded with Ringer's solution of *p*H 5 or less, it opened; when basic Ringer's of *p*H 9 was added, it closed. Ringer's of intermediate *p*H gave variable results. Both innervated and denervated spiracles responded similarly to changes in *p*H. The *p*H's to which the spiracle responded were extreme compared with the normal *p*H variations expected in the insect. Therefore, it is unlikely that carbon dioxide is acting as an acid. Further evidence that *p*H does not serve as the normal stimulus for the spiracles stems from the fact that spiracles placed in a phosphate buffer at *p*H 7 continued to respond to both carbon dioxide and oxygen.

In all these experiments, the "receptors" sensitive to changes in gas and acidity appeared to be localized only in the region of the spiracle, for when these agents were applied locally to other parts of the pupa, particularly to parts of the central nervous system, the spiracles failed to respond.

The specific function of each part of the spiracular apparatus was studied in preparations in Ringer's solution. Parts of the apparatus were excised individually or in combinations, and the spiracle was stimulated. These experiments revealed that when the spiracular nerve was cut as close to the muscle as possible and the nerve stump carefully dissected out of the muscle, and when all the parts of the apparatus were removed except the naked bars, lever, and closer muscle, the muscle still responded to carbon dioxide and oxygen. It is clear that the autonomy of the spiracle is a function of the closer muscle: the muscle remains contracted without stimulation of the central nervous system, not for hours but for months. Furthermore, since repeated histological study has failed to reveal ganglion cells in or near the muscle, we must conclude that this muscle can contract without any nervous stimulation whatever—that is, the spiracle muscle is spontaneously excitable. This spontaneous excitability is reflected in electric recordings which showed that the denervated muscle continued to produce action potentials. Indeed, even when most of the muscle fibers were cut away, the few remaining fibers continued to produce rhythmic action potentials at a rate of 12 to 15 per second (*7*). In the intact insect, of course, the spiracular muscle is normally controlled by the central nervous system.

From these experiments we have concluded that, while the spiracles are normally coordinated by the central nervous system, they can behave like independent effectors sensitive to carbon dioxide and to oxygen. In the normal as well as in the denervated condition, the receptor mechanism sensitive to these gases seems to be located within the muscle itself. The spiracular muscle, so far as we are aware, is unique among insects and probably among higher animals in exhibiting sustained contractions in the apparent absence of nervous stimulation.

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New Pharmacoconvulsive Agent

In our investigations concerning the anesthetic action of aliphatic fluorinated ethers of low molecular weight (*1*) the anesthetic properties of Fluoromar (R) (trifluoroethyl vinyl ether) were observed. These appeared sufficiently promising to warrant a study of its effect on man (*2*). Further observations among these compounds revealed that perfluorodiethyl ether was devoid of anesthetic activity. On the other hand, hexafluorodiethyl ether ($CF_3CH_2—O—CH_2CF_3$) was found to elicit violent convulsions upon inhalation in many species of laboratory animals.

Hexafluorodiethyl ether is a colorless mobile liquid which emits a mild, pleasant ethereal odor. Its boiling point is 63.9°C, and its specific gravity is $1.41\frac{20°}{4°}$. It is very insoluble in water but soluble in alcohol.

White rats that were exposed to the vapor of hexafluorodiethyl ether in concentrations as low as 30 ppm (wt/vol.) convulsed violently within 30 seconds. There were marked clonic and tonic seizures, and there was some degree of emprosthotonus. The convulsions stopped promptly when the agent was removed from the inspired air. Repeated exposures on subsequent days did not appear to produce injury to the animals, as shown by studies of blood chemistry and by histological examination of the lungs, brain, liver, kidneys, and bone marrow.

The convulsive seizure is readily prevented in the rat, dog, and monkey by Pentothal Sodium, ether, or Fluoromar anesthesia, but not by mephenesin. Under Pentothal anesthesia, hexafluorodiethyl ether caused no change in the pattern of the electrocardiogram and only a slight depression in blood pressure and a mild tachycardia in dogs during the cortical dysrhythmia.

The compound did not produce hypoglycemia in the rabbit. Studies in dogs demonstrated that it did not inactivate cholinesterase.

Electroencephalographic studies in dogs and monkeys under succinylcholine-Pentothal Sodium anesthesia were performed. The inhalation of hexafluorodiethyl ether produced marked cortical dysrhythmia without involvement of the skeletal muscle. The multiple spike and three- to four-per-second slow-wave discharge which first occurs was soon followed by high-voltage multiple spiking similar to that of the myoclonic type *petit mal* epilepsy and to the cerebral pattern elicited by Metrazol injections.

We considered the agent as possibly useful as a pharmacoconvulsive drug for shock therapy in mentally disturbed patients. Among the factors which suggested its use were the apparent harmless nature of repeated exposures to animals, the rapid onset of the seizures, the ease of control of depth and duration of the seizures, and the similarity of the cortical dysrhythmia to that evoked by Metrazol. Accordingly, four patients who were suffering with mental disturbances in which electroshock therapy was indicated were subjected to inhalation of hexafluorodiethyl ether. The agent was placed in a plastic inhaler dispersed on cotton. The inhaler was of the type em-

ployed for the administration of the generally used nasal decongestants. Volumes of 1 to 3 ml were employed. The patients were not anesthetized, and the inhaler was placed in one of the nostrils. Within 1 to 3 minutes, the convulsive seizure ensued and continued from 2 to 4 minutes until the inhaler was withdrawn. The seizure episode resembled that of electroshock therapy. Unconsciousness supervened following the convulsion for from 5 to 17 minutes in three patients. The first patient was allowed to inhale the compound only until a few myoclonic facial twitches occurred.

The four patients recovered uneventfully. There appeared to be no clouding of memory. Two of the patients who were combative assumed a more cooperative attitude.

It is possible that this comparatively simple procedure of the inhalation of hexafluorodiethyl ether might be found useful in the treatment of certain types of mentally ill patients.

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Combination of Characters (Drug Resistance) in a Single Strain of Psittacosis Virus

The susceptibility of psittacosis virus and other viruses of the same group to chemotherapeutic drugs and the existence of several stable, drug-resistant strains provide valuable tools for genetic studies in this group of viruses. The experiments reported here were performed with two strains of psittacosis virus, one resistant to sulfonamides (*1*), strain Sa-r, and the other resistant to chlortetracycline (*2*), strain Ctc-r. When strain Ctc-r is introduced into the yolk sac of 7- or 8-day embryonated eggs in standard doses, it multiplies in the presence of 0.15- to 0.5-mg doses of chlortetracycline given via the air sac (*3*) essentially as well as in the absence of the drug and kills the embryo on the 4th day or later, depending on the dosage. Sulfadiazine, in amounts of 1.5 to 5.0 mg administered in similar fashion, will completely protect the inoculated embryos from death for the usual 10-day observation period. The converse is true of strain Sa-r, since it is susceptible to the antiviral action of chlortetracycline but possesses a high degree of resistance to sulfadiazine. These facts are illustrated in egg groups A, B, and C of experiments 1 and 2, Fig. 1. The failure of chlortetracycline to overcome completely the infection with strain Sa-r (experiment 2, group B) is due to the very heavy dose of virus (5 percent) used in this particular experiment.

When strains Ctc-r and Sa-r were grown together in the yolk sac of embryonated eggs, substrains were repeatedly isolated that possessed resistance to both drugs. One such experiment, No. 3 (Fig. 1), was chosen as an illustration because of its conciseness. This experiment was performed by mixing equal parts of 5-percent emulsions of the two strains and injecting them into the yolk sacs of four groups of eggs that received drugs as indicated in Fig. 1. The results in groups A, B, and C were as expected, since both strains would multiply in group A, strain Ctc-r in group B, and strain Sa-r in group C eggs. Virus growth, as determined by embryo deaths, was almost completely suppressed or delayed in group D for the first 7 days of observation. This also was expected because, in this group, each virus of the mixture would encounter a drug to which it is susceptible. However, on the eighth, ninth, and tenth days, 11 deaths occurred in the 13 embryos remaining. When yolk sacs harvested from the three embryos that died on the ninth day were used individually for inoculation of eggs in experiments of the same design as experiment 3 (but not shown in Fig. 1), all deaths in double drug groups (D) occurred before the seventh day, indicating that the material passed possessed resistance to both drugs. Three other experiments similar to No. 3 have been performed, and in each case harvests of individual eggs (total, 18) have possessed levels of resistance to both drugs similar to those seen individually in the original strains.

As is indicated in Fig. 1, harvests were also made from embryos of groups D in control experiments 1 and 2. These were tested in the same manner as described

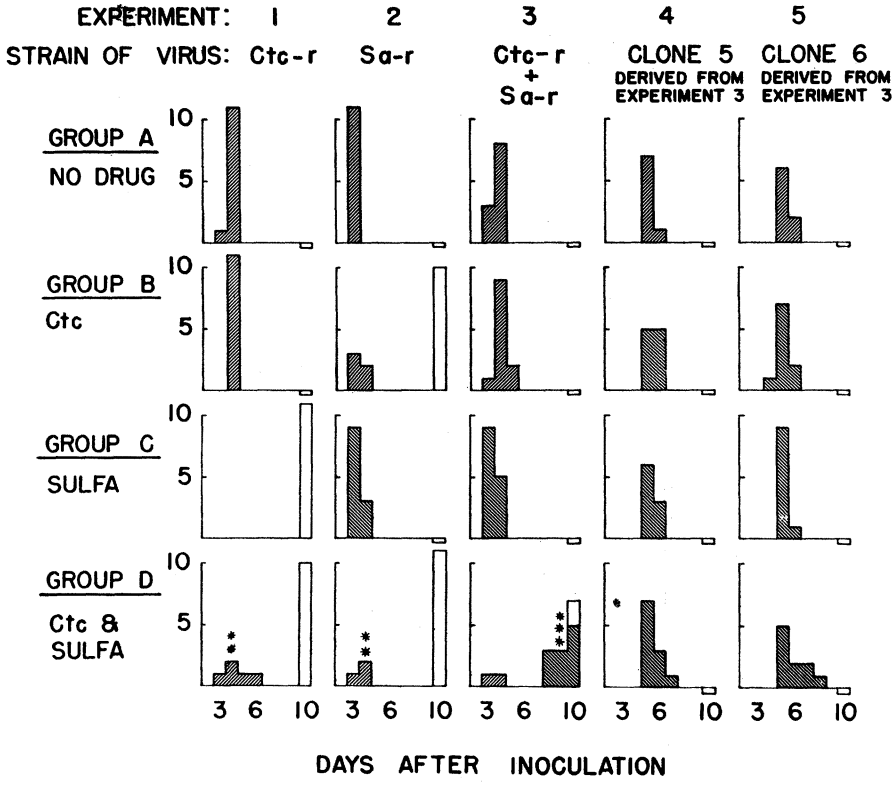

Fig. 1. Effect of chlortetracycline (Ctc) and sulfadiazine on infection of chick embryos with drug-resistant strains of psittacosis virus. The shaded bars represent deaths of chick embryos on the days indicated on the abscissa, and in the numbers indicated on the ordinate. Nonspecific deaths in the first 2 days are omitted. Open bars represent embryos surviving on the tenth day; where there were no survivors, a short bar is placed below the abscissa. Virus dosage: 0.25 ml of 5-percent emulsion in experiments 1, 2, and 3; 0.1 percent in experiments 4 and 5. Drug dosage: 0.5 mg of chlortetracycline and 5.0 mg of sulfadiazine on days 0, 2, and 4 in experiments 1, 2, and 3; 0.3 and 1.5 mg, respectively, on days 1 and 3 in experiments 4 and 5. Asterisks represent yolk sacs harvested on day indicated and subjected to further tests (see text).