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 Within 1 to 3 minutes, the convulsive seizure ensued and continued from 2 to 4 minutes until the inhaler was withdrawn.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

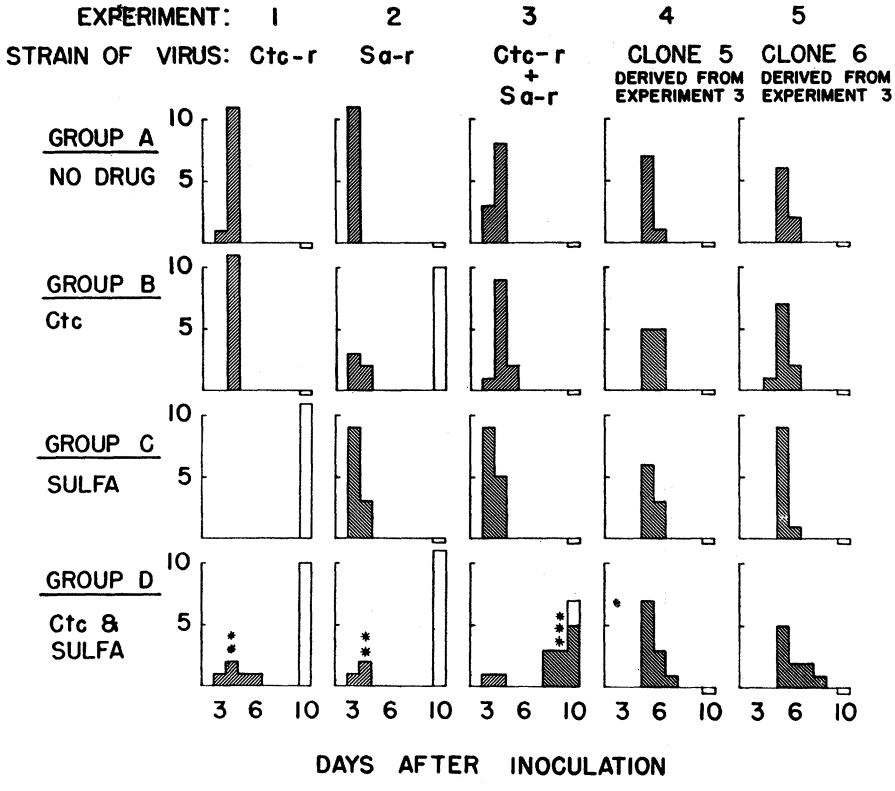

Fig. 1. Effect of chlortetracycline (Ctc) and sulfadiazine on infection of chick embryos with drug-resistant strains of psittacosis virus. The shaded bars represent deaths of chick embryos on the days indicated on the abscissa, and in the numbers indicated on the ordinate. Nonspecific deaths in the first 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).

for the harvests of experiment 3. Failure to recover virus in one case and evidence of low titer in two others suggest that these deaths may have been caused by the toxicity of the drugs used in this dosage, as was seen occasionally in drug controls of experiments not illustrated here. As was expected, the isolates were found to possess unchanged the drug resistance and susceptibility of the strain used in the experiment from which they were harvested.

Six infected yolk sacs, harvested from group-D eggs of experiment 3 and others, have been subjected individually to two serial "limit dilution" (*4*) passages in an attempt to provide clones of virus. The method was satisfactory in most instances, allowing passages to be made from individual eggs in which the probability was high that infection had occurred from a single infecting dose (one to four infected eggs in a group of ten or more, *5*). Since the assumption is valid that a single morphological unit of the virus (elementary body) represents a single infecting dose (*6*), the probability is good that actual clones were produced, and ten such preparations have been so named. Tests with two of them, derived from the harvests of experiment 3, are depicted as experiments 4 and 5 in Fig. 1. The dosages of virus and of drugs were reduced in these tests to avoid toxic deaths, but the results were essentially the same as those seen with previously used heavier doses and indicate resistance to both drugs.

Several hypotheses have been entertained during the course of this work to explain the results obtained. The possibility of a rapid mutation of one of the strains to the additional drug resistance had to be considered. This seems improbable in view of the absence of any evidence for such a mutation in experiments 1 and 2 and in additional similar tests not illustrated. The gradual acquisition of drug resistance previously observed during serial passages of single strains in the presence of drug (see *2*) would also render this explanation unlikely. Furthermore, mixed cultures of a drug-resistant and a drug-susceptible strain have not yielded evidence for mutation to a second drug resistance. The occurrence of a synergistic action, in terms of drug resistance, when the two strains were grown together was also considered. However, experiment 3 and others show that, when the two strains are placed together, double drug resistance is not seen immediately, as is the case with the isolates that possess the two characters, as illustrated in experiments 4 and 5. Furthermore, the fact that resistance to both drugs has been easily demonstrated in all ten isolates that had been passed at high dilutions under conditions favorable for the isolation of clones renders untenable any hypothesis involving persistence of both strains as separate entities.

Thus we are left with the evidence strongly favoring a combination of the two characters of drug resistance in a single strain as the correct interpretation of these results. We are deliberately using the word *combination* in a nontechnical sense while work is in progress to analyze this phenomenon and identify it in genetic terms (*7*).

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24 May 1957

A^{40}/K^{40} Ages of Micas and Feldspars from the Glenarm Series near Baltimore, Maryland

The inner zone of the central Appalachian Mountains is a belt of metamorphic and plutonic rocks which crop out in the Blue Ridge province and underlie most of the Piedmont province. In southeastern Pennsylvania and adjacent parts of Maryland, the oldest rock is the Baltimore gneiss, which forms domical uplifts surrounded and overlain by the Glenarm series. This series consists of a basal quartzite (Setters) overlain by a marble (Cockeysville) and a thick series of mica schists (Wissahickon and Peters Creek) of sedimentary origin but including in some places altered volcanics (*1*). The Glenarm group is locally cut by pegmatite, granite, and other plutonic rocks.

The age of the Glenarm series is not known; it is generally assigned to the pre-Cambrian (*2*), although some workers have considered it a eugeosynclinal facies of the older Paleozoic (*3*). The age of the Glenarm series is fundamental for the interpretation of the width and depth of the Appalachian geosyncline, its deformation, the participation of the basement in the deformation, and the role played by the intrusions. Here and there, younger slates are infolded in the Glenarm series (*4*). These have yielded late Ordovician fossils in the Quantico and Arvonia areas of Virginia, where they apparently lie unconformably on the metamorphic rocks and the granites which intrude them. The Glenarm rocks, proper, have yielded no fossils.

Despite detailed field and laboratory studies over a period of 50 years, the age of the Glenarm schists remains uncertain. It seemed appropriate, therefore, to determine the age of the minerals within the Glenarm rocks and their cross-cutting pegmatites (*5*). To this end, micas were collected from the Setters and Cockeysville formations, and feldspar and mica were collected from the cross-cutting pegmatites. Because the pegmatites postdate the Glenarm rocks, a dating of their mica or feldspar would establish a minimum age for the series near Baltimore. The age of the micas within the Glenarm rocks would establish the age of the metamorphism.

The age of a sample is given by the expression

$$t = \frac{1}{\lambda} \ln \left[1 + \frac{A^{40}}{K^{40}} \frac{(R+1)}{R} \right]$$

where

$$\lambda = \lambda_e + \lambda_\beta$$

and

$$R = \lambda_e / \lambda_\beta$$

λ_e and λ_β being the decay constants for electron capture and beta decay, respectively; A^{40}/K^{40} is the ratio of the number of radiogenic argon-40 atoms to the number of potassium-40 atoms now present in the sample; and t is the age in years.

Uncertainty in the values of λ_e and λ_β has to some extent limited the application of the method in solving geologic problems. It has been proposed (*6*, *7*) that the value of R is 0.085, assuming that $\lambda = 0.55 \times 10^{-9}$ yr^{-1}. These values for the constants R and λ are hereafter referred to as "decay constants I." This value for R was obtained by determining the A^{40}/K^{40} ratios in feldspars of known age. Subsequent work by Wetherill (*8*) has shown that the feldspars have lost radiogenic argon in comparison to micas and hence that decay constants I are empirical calibration constants which correct for argon loss from the feldspars investigated (*9*). In a recent paper by Wetherill *et al.* (*10*), it is shown that $\lambda_e = 0.0557 \times 10^{-9}$ yr^{-1} and that $R = 0.118$, assuming that $\lambda_\beta = 0.472 \times 10^{-9}$ yr^{-1}. The constants $R = 0.118$ and $\lambda = 0.528 \times 10^{-9}$ yr^{-1} are hereafter referred to as "decay constants II." These constants (II) are in reasonable agreement with those determined by counting methods.

The samples utilized, and the localities