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

from which they were collected, are presented in column 1 of Table 1. Samples PE-2, PE-3 and PE-4 are fresh microclines. Sample PEM-1 is from very large sheets of muscovite. All of the aforementioned samples were taken from pegmatites which were observed to cross-cut rocks of the Glenarm series.

Samples PEM-2 and PEM-2-f2 are both muscovites taken from the Setters quartzite. The first of these was taken from small pegmatitic blebs, about 1 inch in size, included in the quartzite. The second sample was extracted from the quartzite proper and is mica which was oriented parallel to the foliation and which appeared to be concentrated at the free surfaces of these structures.

Sample PEM 3-1 is a phlogopite which was extracted from the Cockeysville marble. This phlogopite occurs in somewhat concentrated patches and as more sparsely disseminated grains throughout the marble at the Arundel Quarry.

Sample BGBi-1 is a fresh biotite extracted from the Baltimore gneiss. This biotite was an integral part of the gneissic texture of the Baltimore gneiss. The age obtained was 319 million years. This result indicates that the period of metamorphism during which the gneissic texture was developed in the rock of the Towson dome was contemporaneous with the metamorphism of the Glenarm series. This date is in disagreement with the pre-Cambrian age usually assigned to the Baltimore gneiss.

The results obtained for these materials are presented in Table 1. Column 3 gives the radiogenic A^{40} in cubic centimeters (S.T.P.) per gram. In all the experiments reported, the argon extracted was more than 90 percent radiogenic. The isotopic analyses were made in J. H. Reynolds' laboratory at Berkeley, California. The techniques used in the A^{40}/K^{40} age determinations have been described in an earlier publication (*6*). All the argon extractions reported in Table 1 were done using NaOH flux. Argon was also extracted from samples BGBi-1 and PEM-1 by induction heating of the samples to 1500°C in a molybdenum crucible without any flux. The radiogenic argon yields were respectively 0.954×10^{-4} and 1.37×10^{-4} cm^3 (S.T.P.) per gram. A comparison of these data with the results obtained with NaOH flux as presented in Table 1 shows that the two extraction procedures give results in good agreement.

In column 5, the ages t_I of the microclines are given. These ages were calculated using decay constants I, which correct for argon loss from the feldspars. In column 6 are tabulated the ages t_{II} of the micas. These ages were calculated using the absolute decay constants II.

The results obtained are in reasonably good agreement. The age of sample PEM-2-f2 is obviously low. The total spread of ages indicated is 20 percent. This is much greater than the analytic error of the A^{40}/K^{40} determinations. In this study it is clear that the A^{40}/K^{40} method does not "resolve" the more refined sequential relationships in conformance with the known relative ages of the metamorphics and cross-cutting pegmatites.

From the preceding data, it is concluded that both the age of metamorphism of the Glenarm series and the time of pegmatite injection was about 350 million years ago, corresponding to the end of the Ordovician period on the Holmes time scale. The absolute age is thus seen to coincide with the ages of pegmatites in New England and North Carolina. This evidence supports the view that the whole eastern Appalachian region was "simultaneously" subject to vigorous igneous and metamorphic activity about 350 ± 20 million years ago.

The thesis that the Glenarm series is pre-Cambrian because of its degree of metamorphism is untenable on the basis of the data presented here. These A^{40}/K^{40} dates do not, however, preclude the possibility that this series was originally deposited during pre-Cambrian time.

The general agreement obtained on materials from such contrasting lithologies as the Cockeysville marble, the Setters quartzite, and the cross-cutting pegmatites indicates that it is sometimes possible to determine the ages of micas formed during profound metamorphic episodes. Such micas do not appear to show any effect of inheritance of argon.

Table 1. A^{40}/K^{40} ages of samples.

Sample and Locality	K (%)	Radiogenic $A^{40} \times 10^4$ cm^3 (S.T.P.)/g	A^{40}/K^{40}	Age (10^6 yr) $t_I \times 10^{-6}$	Age (10^6 yr) $t_{II} \times 10^{-6}$
PE-2 Microcline. Pegmatite, Notch Cliff crossing of Maryland and Pennsylvania Railroad 2 mi southwest of Glenarm, Md.	11.25	1.25	0.0163	344	
PE-3 Microcline. Pegmatite, Glenarm Road 0.4 mi northeast of intersection with Cromwell Bridge Road	10.85	1.22	0.0165	347	
PE-4 Microcline. Pegmatite, 0.7 mi south of Henryton, near Henryton Road	11.22	1.34	0.0175	367	
PEM-2 Muscovite. Pegmatite blebs in Setters quartzite from rustic quarry near Cromwell Bridge Road 3.5 mi northeast of Towson, Md.	8.50	1.19	0.0206		338
PEM-2-f2 Muscovite. Setters quartzite from same location as sample PEM-2	8.36	1.06	0.0186		309
PEM-1 Muscovite. Pegmatite on Baltimore and Ohio Railroad tracks about 0.3 mi east of Daniels, Md.	8.60	1.34	0.0229		372
PEM-3-1 Phlogopite. Cockeysville marble from Arundel Corporation Quarry 2.5 mi west of Lake Roland	8.51	1.14	0.0196		324
BGBi-1 Biotite. Baltimore gneiss from road cut, Charles Street Ave., Towson, Md., between Malvern Ave. and Chesapeake Ave.	7.24	0.951	0.0193		319

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13 May 1957

Some Histochemical Observations on Human Dystrophic Muscle

A number of histochemical techniques have been applied to biopsy specimens of human muscle taken from 32 patients suffering from muscular dystrophies and a variety of other muscular diseases. Control muscle was obtained partly by biopsy, at post mortem, and within 1 hour of death from two young, healthy men.

The following histochemical tests were employed: succinic dehydrogenase, cytochrome oxidase, cholinesterase, 5-nucleotidase, acid and alkaline phosphatase, Periodic acid-Schiff reaction, fat, disulfide and sulfhydryl groups, and amino groups. Figure 1 shows some of the results.

Succinic dehydrogenase activity varied considerably between different control muscles and between individual fibers in any one muscle. It varied independently of the cytochrome oxidase reaction. In pathological muscle there was a decrease in activity of succinic dehydrogenase, suggesting a lowered aerobic metabolism. Where substantial replacement of muscle by connective tissue occurred, there was, as might be expected, a considerable reduction of the over-all reaction for this enzyme.

Gomori's (*1*) modification of Koelle and Friedenwald's (*2*) method showed, both in control and in pathological muscle, positive classical end-plates, "terminaisons en plaque" (*3*), and the elongated scattered type of end-plate described by Coërs as "terminaisons en grappe." We were also able to confirm the findings by Couteaux in 1953 and Gerebtzoff in 1956 (quoted by Gerebtzoff, *4*) that there were cholinesterase-positive reticulated or parallel-guttered structures sitting like caps over the ends of the muscle fibers—that is, at the musculotendinous junctions. Another cholinesterase-positive structure consisted of a series of parallel gutters situated in various positions along the length of the muscle fibers. We call these "cake-frill" endings. Their appearance suggests that they, like the endings of the musculotendinous junctions, are stretch receptors. Muscle spindles gave a positive reaction, mainly in the end-plates situated at the poles of the spindles, but occasionally also in some of the spirally arranged sensory nerve fibers.

There was very little evidence of decrease of cholinesterase activity in dystrophic muscle, but where there was considerable replacement of muscle by connective tissue, as for example in facioscapulohumeral or familial dystrophy, isolated and apparently intact end-plates attached to remnants of muscle fibers were seen which gave a normal cholinesterase reaction. Atrophying muscle fibers showed a strong positive reaction in the muscle substance itself.

We could obtain little evidence from our work that muscular dystrophy and related diseases were associated with either physical or enzymatic breakdown of neuromuscular transmission.

Nerves and some blood vessels are the only positive elements in normal human muscle. In dystrophies characterized by atrophy of muscle fibers and their replacement by connective tissue, cells, fibers, and capillaries of the latter gave a strong 5-nucleotidase reaction and appeared to be actively invading and destroying the substance of the muscle fibers. Atrophying fibers also gave a strong reaction for the enzyme.

Since 5-nucleotidase dephosphorylates adenosine monophosphate (which is a starting point for the resynthesis of adenosine triphosphate), this result may be of some significance. Inhibitors of 5-nucleotidase may be of use in the treatment of these conditions, and so might also the anti-snake-venom serums prepared from venoms containing 5-nucleotidase.

Acid phosphatase in normal muscle is present in peripheral nerves, Golgi material and nuclei of both muscle fibers and connective tissue cells, and in adipose tissue. The small amount of acid phos-

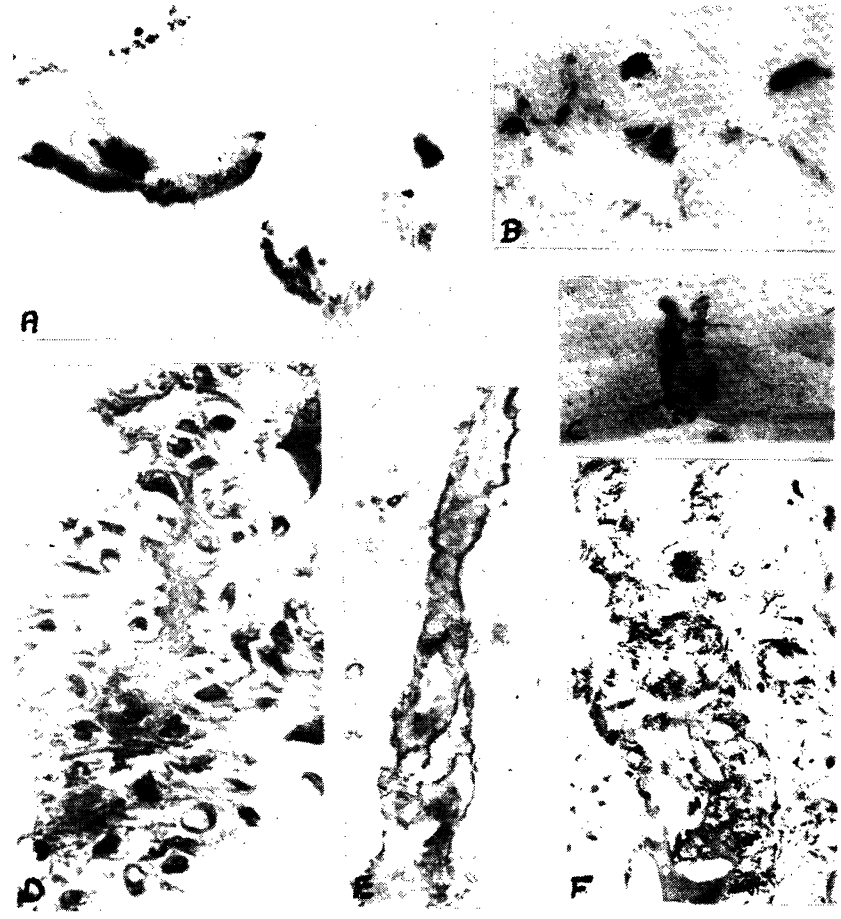

Fig. 1. *A*, Acetyl cholinesterase in structures situated at musculotendinous junctions; *B*, acetyl cholinesterase in numerous small end-plates from a case of dystrophia myotonica; *C*, acetyl cholinesterase in two "cake-frill" type structures in adjacent muscle fibers; *D*, 5-nucleotidase in an atrophied degenerating fiber and in the cells involved in its destruction, from a case of doubtful diagnosis (polymyositis or pseudohypertrophic muscular dystrophy); *E*, alkaline phosphatase in a degenerating muscle fiber and in fine connective tissue fibers wound around it, from a case of familial dystrophy; *F*, acid phosphatase present in high concentration in a muscle fiber undergoing necrosis and in the cells involved in this necrosis (the muscle was taken from a case of familial dystrophy).