is evident that the daily administration of 10 or 20 million units of citrovorum factor intramuscularly was insufficient to produce a satisfactory reticulocytosis in 4 of 5 patients with pernicious anemia in relapse. Three of these persons reached stationary blood levels below 12.0 g of hemoglobin per 100 ml and 4.0 million erythrocytes per cmm, respectively, after 35, 42, and 66 days, respectively. A fourth patient appeared to have attained near normal values when he died suddenly of a vascular accident. The fifth patient clearly showed a poor response to daily administration of 10 million units of citrovorum factor. In view of the intercurrent infection the efficacy of the 2 ml dose cannot be evaluated in this subject. Because of complicating associated disease clinical and subjective reactions were difficult to estimate. Improvement in appetite, strength, and well being appeared to follow in all instances but not so dramatically as with adequate amounts of liver extract, vitamin B_{12}, or folic acid. This is probably due to the inadequacy of 20 million units per day as an optimal dose. Significant increases in erythrocytes were observed in 3 patients when the citrovorum factor was increased to 40 million units a day. Further evidence of the inadequacy of the initial dose was shown in patient A. R. in whom leukopenia persisted until the medication was increased to 2 ml a day. Neurologic examinations were difficult to evaluate because of associated complicating diseases. In one case, however, a distinct improvement followed treatment with 20 million units a day.

Summary. The daily administration of 10 or 20 million units of citrovorum factor to 5 patients with pernicious anemia in relapse usually is followed by a submaximal reticulocyte response, suboptimal levels of hemoglobin and red blood cells, and moderate to good clinical remissions. In one case a theoretically maximal reticulocyte response followed the daily administration of 20 million units of citrovorum factor. Neurologic signs improved in one patient. Although the daily administration of 40 million units produced a further rise in erythrocytes in 3 cases, normal blood values were not attained.

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Effect of Photodynamic Action on the Viscosity of Desoxyribonucleic Acid.* (18396)

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Recent observations(1) have indicated the possibility that photodynamic action (the combined effect of radiations and sensitizing agents) is mutagenic as well as lethal to certain bacteria. Since genes are thought to contain desoxyribonucleic acid (DNA), it is conceivable that mutations occur when the native structure of the DNA is altered. Some strength to this contention is given by the reports that several mutagenic agents, such as ultraviolet radiations(2), X-rays(3,4), mustard gas and the nitrogen mustards(5,6), and peroxides(7) apparently are able to

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depolymerize highly viscous solutions of DNA *in vitro*. Even more dramatic were the observations made by Errera(8) and Limperos and Mosher(9). The former demonstrated that nuclei of chicken erythrocytes became less rigid on exposure to X-rays, and the latter that thymus DNA isolated from rats 24 hours after they had been irradiated with X-rays was almost completely depolymerized. The study reported here led to the conclusion that also photodynamic action can reduce the viscosity of DNA solutions.

Experimental. The preparation of the highly polymerized DNA used was purchased from the Worthington Biochemical Laboratory, where it was isolated essentially by the method of Mirsky and Pollister(10). The photodynamically active compounds tested were the dyes eosin (E) and methylene blue (MB), the slightly carcinogenic hydrocarbon 1,2-benzanthracene (BA), and the highly potent carcinogen 20-methylcholanthrene (MC). All substances were used at a concentration of 1:100,000, the dyes dissolved in water, and the hydrocarbons as colloidal suspensions prepared from 1:1000 acetone solutions. To determine the effect of photodynamic action DNA was irradiated in the presence of one of the photodynamically active compounds tested. Three types of control were used. One determined the effect of radiation alone, the second the effect of the compound alone, and the third the fate of the DNA in the absence of either radiation or sensitizer but otherwise under the same conditions as the exposed DNA. Test tubes containing the desired combinations were placed on a Burrell Wrist Action Shaker, and agitated to allow uniform exposure of the DNA. A source of radiation was placed exactly 10 cm from the center of the tubes. For E and MB this was a 100 watt light bulb; for the hydrocarbons a Hanovia quartz lamp screened with a Corning glass filter to transmit radiations near 3600 Å. In one experiment, designed to study the effect of BA and ultraviolet radiation, the samples were placed in quartz tubes and exposed to radiations from the unscreened quartz lamp at a distance of 25 cm from the center of the tubes. Heat produced by these sources was dissipated by a fan placed behind a radiator through which cold water circulated. During irradiation samples were removed at intervals, and their relative viscosity was determined with an Ostwald viscosimeter. The suspensions were allowed to equilibrate at 25°C for 5 minutes. An average of 3 readings was used in the calculations.

Results. Fig. 1 illustrates that BA in conjunction with "white" light causes a decrease in the relative viscosity of DNA. The behavior of the controls indicated that neither BA nor light alone had any depolymerizing effects during the experimental period. DNA in the absence of the chemical and protected from radiations by a cover (but otherwise treated exactly like the exposed DNA) also

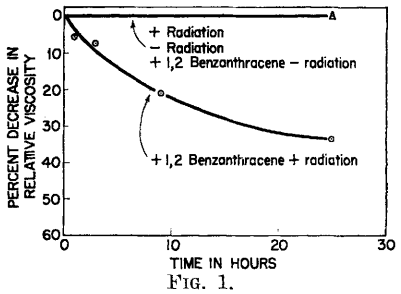

FIG. 1.

Effect of 1,2-benzanthracene and "white" light on the relative viscosity of desoxyribonucleic acid.

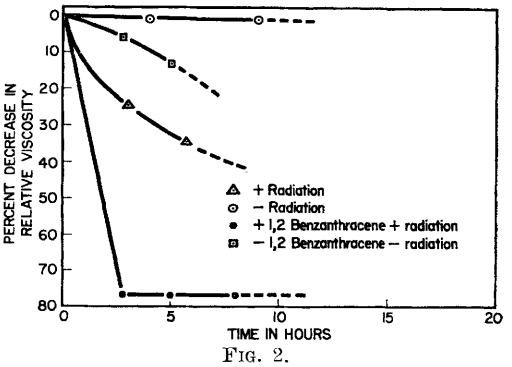

FIG. 2.

Effect of 1,2-benzanthracene and ultraviolet radiation on the relative viscosity of desoxyribonucleic acid.

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TABLE I. Effect of Photodynamic Action on the Viscosity of DNA.

Sensitizer*	Radiation*	Time to give 20% decrease in rel. viscosity, hr
Eosin	"White" light	7.3
Methylene blue	" "	55
1,2-benzanthracene	ca. 3600 Å	7.6
1,2-benzanthracene	ca. 2537 Å	0.7
None	ca. 2537 Å	2.4
20-methylcholanthrene	ca. 3600 Å	48

* In the time necessary to give a 20% decrease in relative viscosity by the sensitizer plus radiation the following decreases in relative viscosity (in %) were obtained in the various controls: no treatment, 0-1.7; "white" light, 0-3.5; 3600 Å, 0; eosin, 7.4; methylene blue, 5.8; 1,2-benzanthracene, 0; or 20-methylcholanthrene, 0.

remained unaffected. In the case of E and MB the controls showed a small decrease in viscosity, and this was most marked for the shielded samples that were shaken in the presence of the dyes. Some depolymerization may have occurred while the viscosity measurements were being made in the light.

Fig. 2 shows a similar experiment with BA and ultraviolet radiation (essentially at 2537 A). Ultraviolet radiation alone reduces the viscosity of DNA suspensions, but BA accentuates this effect very strikingly. Table I summarizes data that were taken from such graphs as shown in Fig. 1 and Fig. 2. No attempt was made in this study to find the optimum conditions for depolymerization. Such important factors as the affinity of the sensitizing substances for DNA, the wavelength of radiation used, etc. were not fully considered. The information in Table I, therefore, should be considered more as qualitative than as quantitative.

Comments. The observation that photodynamic action *in vitro* decreases the viscosity of DNA (which may mean that DNA becomes depolymerized) should be interpreted with caution. Although it is possible that such an alteration of the DNA present in genes is the cause of lethal and other mutations, such an explanation still awaits confirmation from experiments performed on living cells. It is known that carcinogenic hydrocarbons tend to be photodynamically active(11-17). This activity has been said(12) to depend on light-produced, water-soluble products of these hydrocarbons, and the possibility should be considered that such impurities are responsible also for the effects observed in this investigation. It would of course be premature to speculate on a possible connection between the carcinogenicity of certain hydrocarbons and their ability in the presence of radiation to decrease the viscosity of DNA suspensions.

Summary. Suspensions of desoxyribonucleic acid become less viscous when they are irradiated in the presence of photodynamically active compounds, such as eosin, methylene blue, 1,2-benzanthracene, or 20-methylcholanthrene. This apparent depolymerization may be involved in the lethal and possible mutagenic effects of photodynamic action.

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