Effects of X-Radiation on Thymus Nucleic Acid

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Since the work of Muller in 1927, X-rays have become a tool in the production of chromosomal aberrations and artificial mutations in plant and animal cells. Although the effects of X-radiation have been investigated with regard to the organism as a whole and to its cellular and nuclear constituents, no clearly defined mechanism of action has emerged. The occurrence of nucleic acid, especially the desoxyribose type, in the chromosomes of all known cells warrants an investigation of the effects of X-rays on this substance.

Hollaender, Greenstein, and Jenrette (*3*) have shown that ultraviolet radiation produces a marked decrease in the structural viscosity and streaming birefringence of aqueous solutions of sodium thymus nucleate (TNA). Similarly, Sparrow (*4*) has demonstrated a fall in the viscosity of TNA upon X-radiation, the degree of change varying with the total dosage given. Since Sparrow's viscosity data were obtained at unknown velocity gradients, no conclusions can be reached regarding the structural viscosity of his solutions. In order to follow changes in structural viscosity it is necessary to measure the relative viscosity of the solution over a range of applied external pressures. When this is done, it is found that a marked decrease in structural viscosity occurs in TNA solutions irradiated with X-rays, similar to the change induced with ultraviolet radiation (see *2*), indicating an apparent decrease in the asymmetry of the nucleic acid particle with either type of irradiation.

Early in these studies it was noted that relative viscosity measurements at various pressures, made immediately at the end of the X-radiation period, cannot be compared because of the gradual decrease in the viscosity of TNA which occurs. This decay continues over a period of 18–24 hours, after which time the rate of fall in viscosity of the X-rayed sample approaches that of the control. Only then can comparative pressure studies be carried out. This continuous drop in viscosity subsequent to the cessation of X-radiation is shown in Fig. 1. The readings of relative viscosity taken 30 minutes after irradiation, allowing time for temperature equilibration, were: control, 22.9; 5-minute X-ray (28,000 r), 10.4; 10-minute X-ray (56,000 r), 7.3. The per cent of this first viscosity measurement is then plotted as a function of the time after irradiation.

Experiments were carried out on a dialysed solution of 0.7 per cent sodium thymus nucleate, prepared by the Hammarsten method from calf thymus. Viscosity was measured, relative to distilled water, in Bingham-Jackson viscometers at known pressures and at 30°C. X-ray dosage was delivered at the rate of 5,600 r-units/minute.

The rate of fall of the relative viscosity of TNA after the initial drop is markedly dependent upon temperature. If measurements of viscosity are carried out at 0°, the subsequent drop in viscosity with time is inhibited; both control and irradiated samples retain their viscosity as first measured. When brought up to 30°, after 5 hours at 0°, however, the per

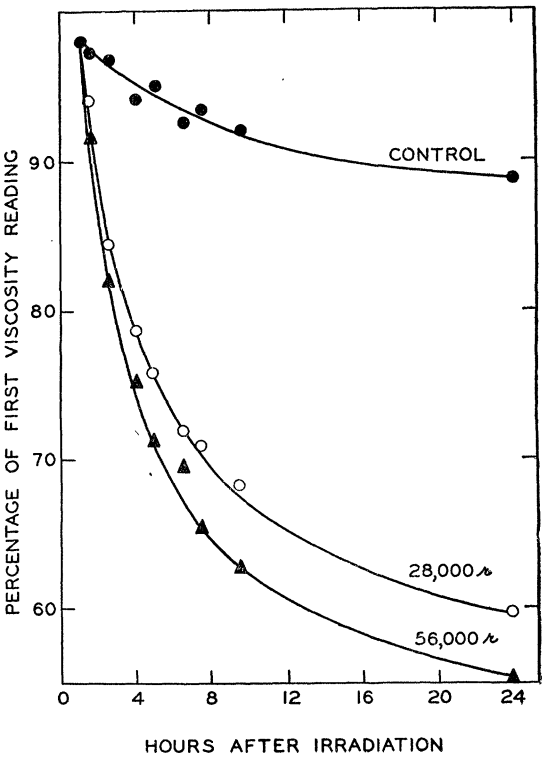

FIG. 1. Rate of decrease in viscosity of solutions of sodium thymus nucleate in terms of per cent of first reading at end of X-radiation period. Dialysed nucleate concentration was 0.7 per cent; temperature, 30°C.; external pressure, 18.4 cm. water.

cent drop in viscosity of the X-rayed sample is consistently greater than that of the control. Thus, at least two phenomena are observed: (1) the primary effect of X-rays to decrease the viscosity of TNA, which is proportional to the dosage, acts during the exposure to X-rays and is independent of the temperature; and (2) a secondary, progressive, presumably indirect effect is manifested only at the higher temperature of 30° and cannot be detected at 0°C.

It appears improbable that peroxide formed as a product of the X-radiation of water is concerned to any significant extent with the phenomena observed. Water was irradiated at 5,600 r/minute for 20 minutes and then added to a concentrated solution of nucleic acid. The drop in viscosity on standing was no different from that of a control diluted with the same

amount of untreated water. The addition of H_2O_2 to a solution of TNA in concentrations of 10^{-3} and 10^{-6}M caused neither an initial nor a continuous drop in viscosity. It appears likely that the secondary decay in the viscosity of the TNA is the result of progressive intramolecular alterations in the nucleic acid particle, which are set in motion by the primary effect of the X-radiation.

In order to ascertain the nature of the change brought about by X-radiation of TNA, a series of chemical and enzymatic studies were undertaken. These results indicate that there is no splitting off of ammonia or inorganic phosphate upon irradiation; neither are any titratable acid groups opened up. Spectroscopic studies on the dialysates of control and X-rayed samples, according to the technique described by Carter and Greenstein (*1*), show that there has been no splitting off of purine- or pyrimidine-containing fragments small enough to penetrate a cellophane membrane. Presumably this indicates that if depolymerization has occurred, the molecular weight of the product molecules is still greater than 10,000. The ultraviolet absorption spectra of the control and X-rayed nucleates are identical.

Purified desoxyribonuclease (McCarty's preparation) and rat tissue extracts behave in an exactly parallel fashion toward control and X-rayed TNA. The specificity of the substance, as determined by its susceptibility to enzyme action, is in no way altered by the irradiation. Whatever the way in which the particle has been changed by X-radiation (whether by depolymerization or rearrangement involving a change in shape), the change in the axial ratio of the particle has not involved a splitting of primary linkages or a rearrangement of the spatial configurations necessary for its susceptibility to enzymatic attack.

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An Improved Oxygen Absorption Bulb for the Carpenter-Haldane Gas Analysis Apparatus

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One of the most time-consuming aspects of basal metabolism determination by the open-circuit method of Lewis (*2*) is the Carpenter-Haldane process (*1*) of gas analysis. Though this method yields excellent results, it is most tedious and laborious, particularly the process of oxygen absorption by an alkaline pyrogallol solution. To insure complete absorption of oxygen, the analyst must pass the gas sample into the pyrogallol tube about 25 times while he watches a weird little windshield wiper "burp" a rubber bulb to facilitate automatic circulation of the pyrogallol solution from the reservoir.

Numerous efforts toward a more rapid absorption procedure proved that the old Hempel tube method was highly applicable. The Carpenter oxygen absorption tube was replaced by a simple bulb with a capillary stem arranged so that it could be easily shaken by hand, and the original 800-cc. pyrogallol reservoir was replaced by a small 200-cc. bulb fitted with a water seal. The windshield wiper circulator was found unnecessary when the small volume of solution was more frequently renewed.

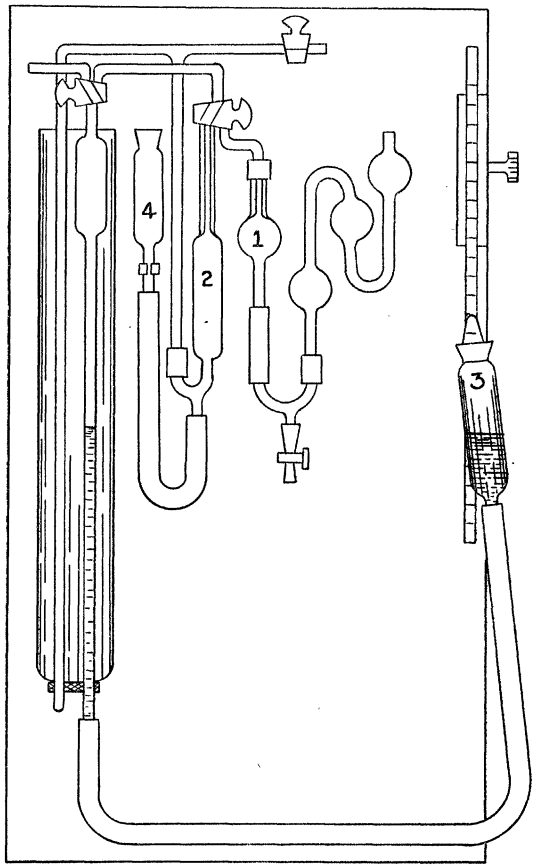

Fig. 1. Oxygen absorption bulb (1), KOH tube for CO_2 absorption (2), mercury leveling reservoir (3), and KOH reservoir (4).

Using this modified Hempel tube, the analysis of a gas sample is much the same as that by the Carpenter method. After CO_2 absorption in KOH solution, the gas sample is passed into the "Hempel" tube. The stopcock to this tube is closed, and the tube is shaken for about 10 seconds. This procedure is repeated 6 times, a burette reading being taken on the 5th and a check reading on the 6th. Thus, oxygen is completely absorbed after a total of 6 contacts with the pyrogallol solution.

With this improvement, the time necessary for a complete analysis of oxygen and carbon dioxide was reduced from the previous average of 30 minutes to about 15 minutes, a time decrease which looms more significant as the number of analyses increases.

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