gram. This coincidence was obtained with five different solvent systems: butanol–propionic acid–water (*10*); propanol–ammonia–water (*9*); butanol–formic acid–water (*11*); *n*-butanol (*12*); and water at *p*H 10 (*13*). The radioactivity could have come only from the cytosine, establishing the conversion of cytosine to uracil. The maximum yield of uracil (Table 1) is 3.7 percent at 2×10^6 rads. This is significantly larger than the maximum yield of hypoxanthine (1.9 percent at 5×10^6 rads) observed in the deamination of adenine (*1*).

The biological significance of the deamination of purines and pyrimidines has been widely recognized (*14*). Gierer and Mundry (*15*) have shown that nitrous acid treatment of tobacco mosaic virus RNA, or intact tobacco mosaic virus, actually results in the formation of mutants. The alteration of the amino-acid sequence in the protein of the mutant virus has indeed been strikingly demonstrated (*16*).

As cytosine occurs both in DNA and RNA, the conversion of cytosine to uracil may have more biological implications than the deamination of adenine. This change would give rise to an unnatural base in DNA and, if replication occurs, a cytosine-guanine base pair would ultimately be replaced by an adenine-thymine pair. With RNA, it is conceivable that the altered nucleic acid undergoes identical replication. In either case, an altered base would presumably lead to a change in protein composition (*17*, *18*).

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Molecular Changes in Deoxyribonucleic Acid Induced by X-rays

Abstract. A chromatographic study of molecular changes in DNA after in vitro exposure to x-rays revealed little effect on the DNA macromolecule when either intact red cells of frog or DNA isolated from these cells was exposed to 1000 r. Increasing the dose to 10,000 r resulted in a significant shift from gradients of lower molecular weight to the higher gradients. These data suggest that cross-linking of DNA occurred at the higher radiation level.

Several lines of investigation have shown that the physical and chemical properties of deoxyribonucleic acid (DNA) are profoundly altered by radiation. Main chain scission (depolymerization) of the DNA molecule has been reported as an important action subsequent to ionizing radiation (*1*). Intra- and intermolecular cross-linking of DNA has been proposed as another basic effect of radiation. Alexander and Stacey (*2*) exposed sperm heads to ionizing radiation and observed cross-linking of the DNA. Recently these results were confirmed and extended (*3*). Borenfreund *et al.* (*4*) have reported both aggregation and degradation of DNA molecules from human leukocytes on exposure to tritium.

Our study (*5*) was directed at understanding more about the basic changes occurring in the DNA molecule in an isolated cell type on in vitro exposure to ionizing radiation. The standard procedure of ECTEOLA (*6*) anion exchange fractionation analysis was used as a sensitive indicator of physicochemical changes in the DNA macromolecule. Evidence is presented which suggests the possibility that cellular DNA may increase in molecular weight when it is exposed to ionizing radiation.

Blood was obtained by ventricular puncture of curarized bullfrogs (*Rana catesbiana*). In order to eliminate many of the errors in the isolation and fractionation of DNA, simultaneous experiments were performed under exactly the same conditions. Each experiment consisted of two aliquots of cells, a control sample and an x-irradiated sample with all preparatory and analytical procedures accomplished at 4°C. Blood samples or DNA isolates were exposed to ambient temperatures only during the radiation procedure. X-irradiation was performed at room temperature with either blood cells or the DNA solution layered 3 to 5 mm deep in a thin plastic dish. Samples were irradiated in an 85 kv (peak) x-ray unit with an "Aeromax" T12 tube at 10 ma. This unit gives a relatively "soft" beam with a half value layer of 1 mm Al. All doses were precalibrated with a Victoreen roentgen meter (*7*).

After x-irradiation of the experimental sample, paired experimental and control samples were centrifuged at low speed (115*g*) for 15 minutes. Buffy coat and plasma layers were carefully removed by pipetting. The packed erythrocytes (approximately 3 cm^3) were then transferred into vessels containing 50 ml of distilled water which caused hemolysis and resulted in the formation of a thick red gel. Precipitation and resolution of the deoxyribonucleoprotein, by the technique described by Mirsky (*8*), was repeated three times and yielded a clear white viscous precipitate free from discoloration by hemoglobin.

Protein was removed from the deoxyribonucleoprotein by chloroform-octanol treatment (*9*), which yielded a protein-free DNA solution as determined by the Weichselbaum biuret test (*10*). The fractionation pattern obtained depended in part on the length of treatment. In two early experiments at 1000 r (Fig. 1, *A* and *B*) an increased amount of DNA of lower molecular weight (gradient 2) was obtained after 22 hours of treatment. Subsequently, it was found that approximately 8 hours of treatment gave a more uniform and reproducible product. In all subsequent experiments deproteinization was completed within 5 to 8 hours.

After deproteinization and centrifugation (1020*g*), DNA was precipitated

from the aqueous layer by addition of 95 percent ethanol. DNA samples were washed in 70 percent ethanol, dissolved in 0.01*M* phosphate buffer at *p*H 7, and stored at 4°C for 24 to 48 hours before being placed on ECTEOLA anion exchange columns (*11*). The DNA was eluted from the columns by five different linear gradients as described by Bendich *et al.* (*12*), and the fractions were collected automatically (*13*). Approximately 160 4-ml fractions were collected from each column. The optical density of each fraction at 260 mμ was determined with a Beckman DU spectrophotometer.

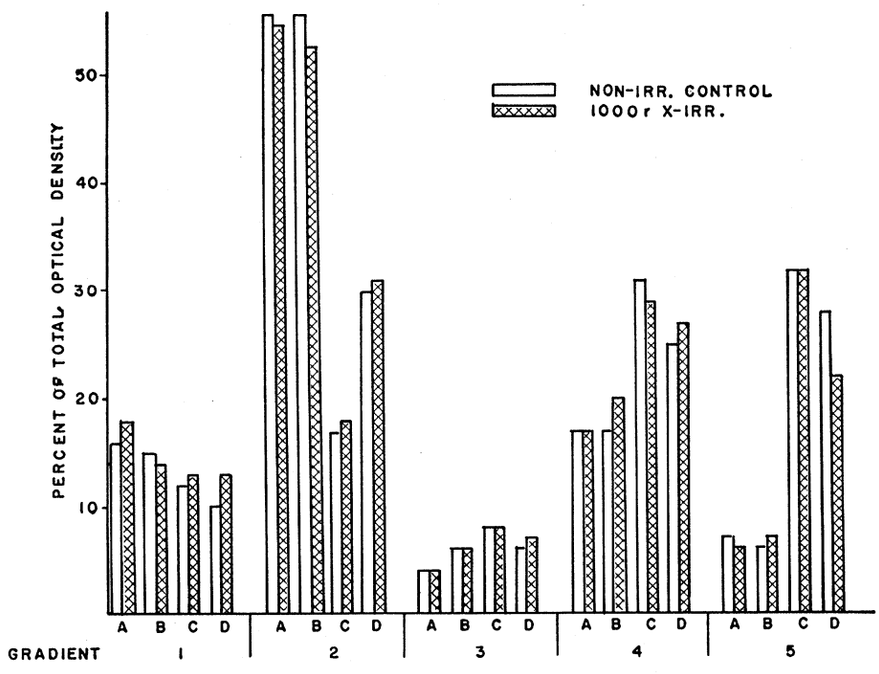

Fig. 1. Gradient distribution of fractionated DNA isolated from nucleated frog erythrocytes x-irradiated in vitro with 1000 r. *A, B, C,* and *D* are the results of individual paired experiments. Prolonged chloroform-octanol treatment in *A* and *B* accounts for the increased material in gradient 2 as compared to *C* and *D*.

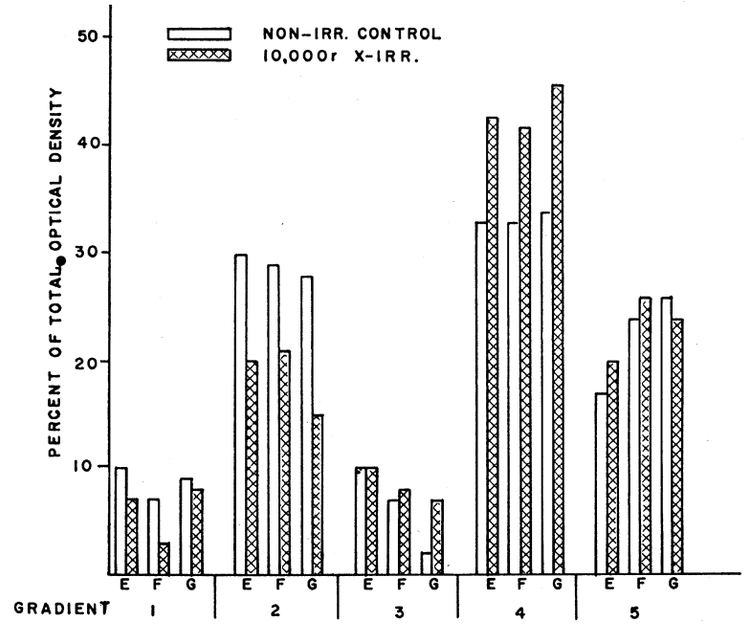

Fig. 2. Gradient distribution of fractionated DNA isolated from nucleated frog erythrocytes x-irradiated in vitro with 10,000 r. *E, F,* and *G* are the results of individual paired experiments.

The 20 consecutive tubes from each of the five gradients which gave the highest total optical density were used for the comparison of the DNA specimens. The optical density of each gradient was expressed as a percentage of the total optical density for all five gradients.

Rosenkranz (*14*) determined that ECTEOLA anion exchange fractionation in which eluents of increasing salt concentration were used first, followed by solutions of increasing *p*H, separated DNA on the basis of molecular weight (based on sedimentation data). The DNA of lower molecular weight is eluted first (gradient 1) and the heavier fractions of DNA come off in later gradients. Chromatographic procedures can thus be used to prepare fractions of DNA of graded sedimentation coefficients. This fractionation is the basis for the interpretation of our data.

It appears that, within the parameters of this type of fractionation analysis, ionizing radiation had no apparent effect on the DNA molecule when the nucleated red blood cells were irradiated in vitro with 1000 r (Fig. 1). Nor did we find any change in the molecular weight of isolated DNA irradiated at a dose of 1000 r.

When the red cells were exposed to 10,000 r, however, there was a significant shift of DNA from gradient 2 to gradient 4 in the irradiated samples in each set of simultaneous experiments (see Fig. 2). This shift may be interpreted as an overall increase in the molecular weight of the eluted DNA. In another set of simultaneous experiments the isolated DNA, rather than the red cells, was irradiated at 10,000 r. A similar shift of gradients was noted, suggesting that polymerization had occurred.

The principal changes in DNA produced by ionizing radiations are main-chain scission, cross-linking, and disruption of secondary structure of the macromolecule. The results of this study indicate that the cross-linking mechanism may occur in living cells exposed to x-irradiation at doses of 10,000 r. It has not been determined whether a primary or secondary effect is reflected by these data (*15*).

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6. ECTEOLA anion exchange cellulose with tertiary amine exchange groups on the solka-floc cellulose lattice. Purchased from Biorad Laboratories, Richmond, Calif.
7. The target distance for 1000 r was 17 cm, and the dosage was delivered at 370 r/min. Target distance for 10.000 r was 9 cm, and the dosage was delivered at 1480 r/min.
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Flight Behavior of a Fly Alighting on a Ceiling

Abstract. Salient features in the maneuvers executed by a house fly (*Musca domestica*) in ascending to land on the underside of a horizontal surface have been observed in photographs exposed in a continuous-writing high-speed framing camera. Details of the action and the instrumentation required to record it are described.

The high-speed camera has been widely used by entomologists to study the flight mechanism of insects (*1*). In recent years engineers have become increasingly interested in the maneuvering ability demonstrated by many kinds of insects. The difficult maneuver of landing on a ceiling is of interest to both the entomologist and the engineer, but the spontaneity of the action makes it a difficult one to record by conventional high-speed motion picture techniques. Eyles (*2*) studied the problem and concluded that the fly "performed a 'half roll' in alighting, coming to rest at a slight angle to the direction of flight." More recently, Curran (*3*) reported an entirely different maneuver in which the fly made surface contact with its forefeet first, then swung its other four feet into contact with the ceiling.

To study this problem further, through high-speed photography, I chose a continuous-writing drum-type framing camera (Beckman and Whitley Dynafax), which exposes 224 frames on a 33-inch length of film supported on the inside of a rotating drum of 11-inch diameter (*4*). The camera was operated at 9500 frames per second. Exposure illumination of as much as 300,000 ft-ca was provided by an electronic light source (Beckman and Whitley model 357). This source produces a rectangular pulse of light 22.35 msec in duration, corresponding to one rotation of the camera drum. The exposing light was triggered by the fly when its body eclipsed a horizontally collimated light beam focused on a phototube. The height from the ceiling of the electric-eye trigger could be adjusted to permit initiation of illumination from the exposing light source at various stages of the action. Experiments were conducted indoors at a low ambient-light level, so the camera could be operated continuously for several minutes with the lens uncovered without exceeding the exposure threshold of the film.

House flies (*Musca domestica*) were introduced into the bottom of a light-tight box, whereupon they were attracted upward toward the light transmitted by a translucent ceiling illuminated from the opposite side by a 40-watt lamp. The camera was focused on the illuminated ceiling through a glass port in the side of the box. Experiments were conducted with two ceiling surfaces: galvanized window screen and tracing paper. Selected frames from a typical film strip are shown in Fig. 1.

A blinding flash is required to photograph the action at short exposure time, and there is reason to suspect that the fly's mechanism of reaction to this stimulus may interfere with the normal performance of its landing maneuver. To eliminate doubt on this point, confirming experiments were performed with sunlight as a source of light for exposure. A conventional high-speed camera of roll-film type was mounted inside the box, with the lens aimed vertically toward the back-lighted ceiling. Operated at 650 frames per second, this camera requires 2 seconds to reach running speed and then continues to run for 5½ seconds until the film is expended. It had been previously determined under simulated operating conditions that the chances of recording a fly's landing during the operating time of the camera were one in five, at a 0.05 significance level, if six flies were released into the box simultaneously with the starting of the camera. On the basis of these preliminary data five 100-

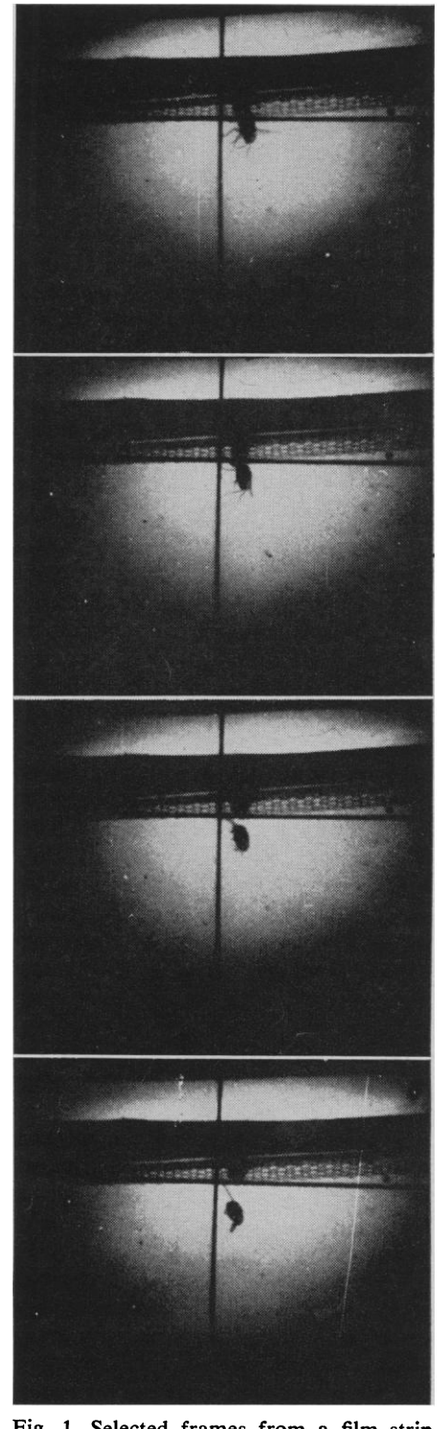

Fig. 1. Selected frames from a film strip showing the approach phase of the landing maneuver. The cross-hairs visible in the photographs serve as a reference for velocity measurements.