Effect of Ultra-violet Light on Deoxyribonucleic Acid in Rat Thymocytes

THE amount of deoxyribonucleic acid recovered from ultra-violet light irradiated intact rat thymocytes has been found to be significantly less than the amount recovered from non-irradiated controls. The thymus glands of twenty normal 150-gm. Wistar-strain male rats were removed immediately after decapitation of the animals, placed in 140 ml. of Ringer's solution and homogenized for 30 sec. in a Waring blendor. The resultant suspension of thymocytes was removed to a dark-room and divided into two equal portions, one of which served as a control. The other was placed in an 80-ml. quartz-bottom van Tieghem cell. While being constantly agitated by a slowly rotating stirrer, in order to ensure uniform exposure of all the cells, the suspension was irradiated from below for 30 min. with ultra-violet light ($3{\cdot}4 \times 10^8$ ergs). The ultra-violet source, used without filters, was an air-cooled, quartz-spiral mercury resonance lamp (Hanovia), which, according to a Hanovia ultra-violet meter, Model *AV* 971, emits about 25 per cent of the total output in the ultra-violet below $\lambda = 2800$ A. The irradiated and control suspensions were left in the dark-room to avoid possible side effects due to photo-reactivation. A period of 5½ hr. was allowed to elapse before extraction of deoxyribonucleic acid, in order to provide sufficient time for possible depolymerization of the acid induced by the radiation. Thymocyte viability for a period of 5½ hr. after homogenization and irradiation was verified by means of tissue culture. Deoxyribonucleic acid was extracted according to the Limperos modification[1] of the Mirsky–Pollister method[2], dissolved in distilled water and then lyophilized. The weight of deoxyribonucleic acid obtained from irradiated tissue homogenates (seven replicate experiments) was only 52·9 per cent ($S.E._{mean}$ 4·6) of the weight of that obtained from the non-irradiated controls. Nitrogen and phosphorus content were determined on aliquot portions made up on the basis of percentage weight of deoxyribonucleic acid. Adjustment to equal concentrations of the acid was based on phosphorus content. No appreciable differences between irradiated and control samples were found in relative viscosities, structural viscosities, or sedimentation rates.

Thymine was determined according to the method of Day[3] on the supernatant fluids (Ringer's solution and the 0·14 *M* saline extraction fluids) collected during the initial stages of the extraction process. The greater proportion of loss of deoxyribonucleic acid calculated on the basis of thymine content of the supernatant fluids from the irradiated tissue homogenates occurred during the 5½ hr. period following irradiation before the extraction of deoxyribonucleic acid.

Depolymerization of deoxyribonucleic acid *in vitro* by prolonged exposure to ultra-violet radiation was demonstrated by Hollaender *et al.*[4]. A fall in nuclear absorption of mouse lymphocytes, thymocytes and stem cells of bone marrow during such irradiation was reported by Bradfield[5] and was suggested by him to be due to depolymerization of the acid with immediate extranuclear leakage of the acid breakdown products.

The small amount of deoxyribonucleic acid recovered from irradiated tissue homogenates and the large amount of thymine present in the Ringer's supernatant fluids indicate that, as a result of ultra-violet irradiation, partial fragmentation of the polymerized deoxyribonucleic acid molecule occurred. Since no appreciable differences were found between the physico-chemical characteristics of the deoxyribonucleic acid recovered from the controls and from the irradiated homogenates, apparently not all the deoxyribonucleic acid in the irradiated homogenates was depolymerized. There was a slight loss of the deoxyribonucleic acid from the controls attributable to mechanical injury. The major loss of the acid from the irradiated homogenates, however, must have been due to ultra-violet radiation.

S. J. DE COURCY, JUN.
J. O. ELY
M. H. ROSS

Biochemical Research Foundation,
Newark, Delaware.
Feb. 16.

[1] Limperos, G., and Mosher, W. A., *Amer. J. Roentgenol. Radium Ther.*, **63**, 691 (1950).
[2] Mirsky, A. E., and Pollister, A. W., *J. Gen. Physiol.*, **30**, 117 (1947).
[3] Day, E. D., and Mosher, W. A., *J. Biol. Chem.*, **197**, 227 (1952).
[4] Hollaender, A., Greenstein, J. P., and Jenrette, W. V., *J. Nat. Cancer Inst.*, **2**, 23 (1941).
[5] Bradfield, J. R. G., Farad. Soc. Discuss., No. 9, 481 (1950).

Deoxyribonucleic Acid Content of the Cell Nucleus and Mitosis

THE fact that the deoxyribonucleic acid content in the nuclei of a given tissue remains constant at least between certain limits suggests that mitosis must necessarily be preceded, accompanied or followed by a synthesis of deoxyribonucleic acid. The moment of this synthesis is still under discussion.

The blastula of *Artemia salina* L. was chosen as material for the study of the problem. At the moment of the invasion of the blastocœl by the yolk granules[1] the nuclei remain near the surface of the germ and present semi-synchronous divisions, so that blastulæ practically devoid of mitosis and others with numerous nuclear divisions are easily found. After telophase, most of the newly formed nuclei can be recognized by their volume. The two little nuclei swell progressively during early interphase to the volume of the interphase nuclei.

It seemed to us that a comparison between the amount of deoxyribonucleic acid in nuclei of blastulæ devoid of mitoses and those with numerous mitoses could give some information upon the deoxyribonucleic acid content during nuclear division. The histochemical method of L. Lison[2] was used for this purpose. The calculated mean optical density of the nucleus multiplied by its surface gives the amount of deoxyribonucleic acid expressed in arbitrary units. The reported figures have only relative values. Those brought together in each of the histograms (*A*, *B*) correspond with nuclei found in sections of a female fixed *in toto* (alcohol 9, formalin 1), which were brought on to the same slide to ensure a uniform Feulgen reaction. In the histograms the relative amounts of deoxyribonucleic acid are plotted logarithmically as abscissæ and the relative frequencies of the classes comprised between two successive figures of the horizontal scale are plotted as ordinates. As reference, the spermatozoids