The effect of X-irradiation on the uptake of nucleic acids and protein precursors by isolated rabbit-liver, appendix and thymus nuclei

In a previous paper[1] it was shown that X-irradiation of isolated rat-liver and calf-thymus nuclei inhibited the uptake of [8-^{14}C]adenine and [2-^{14}C]phenylalanine *in vitro*, as determined by the autoradiographic technique of FICQ[2]. In the present work, a study was made of the effect of X-irradiation on the uptake of various precursors of the nucleic acids and proteins by isolated nuclei of rabbit liver, thymus and appendix.

The liver, thymus and appendix tissues were obtained from young albino rabbits (1000–1200 g body weight). The nuclei were prepared from a homogenate of tissue in 1 vol. 0.5 *M* sucrose and 8 vol. 0.25 *M* sucrose–0.002 *M* $CaCl_2$[3]. One half of the nuclear preparations was left as control while the other half received 300 R X-irradiation, in petri dishes (7.5 cm diameter) 15 cm from an Aeromax 12 tube which was working at 85 KV and 5 mA. The irradiation was for 90 sec with constant stirring.

The nuclei were incubated in a medium containing 0.1 *M* sodium phosphate, pH 7.3, in 0.25 *M* sucrose, 0.1 *M* glucose (containing 8.16 mg NaCl and 5.07 mg $MgCl_2 \cdot 6H_2O$ per ml) and 2 μC of radioactive precursor. The mixture was incubated at 37° with gentle shaking. Smears of the incubation mixture were made on prepared microscope slides after 30-, 60-, 90- and 120-min incubation and were set up for autoradiography as described by LOGAN, FICQ AND ERRERA[4]. After exposure of the autoradiograms, the photographic emulsion was developed and fixed, and the smears stained with Unna stain. The slides were examined microscopically and several series of 200 nuclei classified. The number of microscopically clean nuclei showing 0, 1, 2, 3, etc., tracks were counted and the total number of tracks in 200 nuclei determined.

The precursors used were [8-^{14}C]adenine, [6-^{14}C]orotic acid, [2-^{14}C]uracil, tritiated thymidine and [2-^{14}C]phenylalanine. The results are shown in Fig. 1–5 respectively. It can be seen that the nuclei of all of the tissues examined showed marked inhibition in their uptake of all of the precursors after X-irradiation *in vitro* with 300 R.

[8-^{14}C]Adenine (Fig. 1) was taken up by liver, appendix and thymus nuclei. X-irradiation inhibited the uptake of adenine by approximately 30 % in appendix and thymus nuclei and by approximately 45 % in liver nuclei, after 90-min incubation.

[6-^{14}C]Orotic acid (Fig. 2) was also taken up by the nuclei of all three tissues. X-irradiation inhibited the uptake of orotic acid by approximately 30 % in thymus nuclei, 40 % in liver nuclei and by almost 50 % in appendix nuclei after 120-min incubation.

[2-^{14}C]Uracil (Fig. 3) was taken up almost exclusively by the nuclear RNA under the incubation conditions used. This was shown by treating duplicate nuclear smears with ribonuclease prior to setting up for autoradiography. After such treatment the nuclei showed no traces of radioactivity. All three types of nuclei showed uptake of uracil. X-irradiation gave rise to 23 % inhibition in uptake in appendix nuclei, 43 % in liver nuclei and 53 % in thymus nuclei.

[^{3}H]thymidine (Fig. 4) was taken up entirely by the DNA of the nuclei. When the smears were treated with deoxyribonuclease prior to setting up for autoradiography, all radioactivity was removed. Appendix nuclei showed the greatest inhibi-

Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

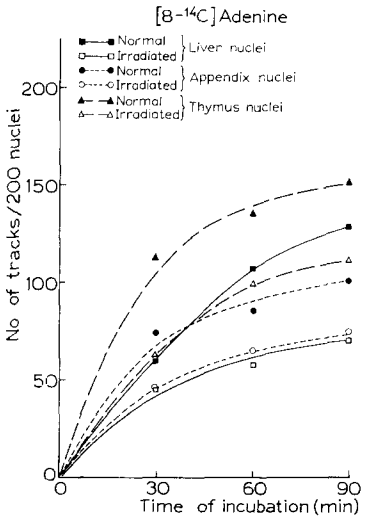

Fig. 1. The effect of 300 R of X-irradiation on the uptake of [8-^{14}C]adenine by isolated rabbit-liver, -appendix and -thymus nuclei. 1 ml nuclear suspension; 0.5 ml 0.25 *M* sucrose–0.1 *M* Na phosphate pH 7.3; 0.4 ml 0.1 *M* glucose (containing 8.16 mg NaCl and 5.07 mg $MgCl_2$.6 H_2O per ml); 0.1 ml [8-^{14}C]adenine (2 μC and 0.124 mg adenine).

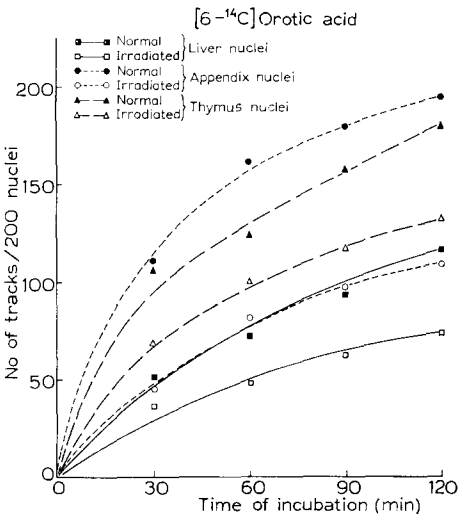

Fig. 2. The effect of 300 R of X-irradiation on the uptake of [6-^{14}C]orotic acid by isolated rabbit-liver, -appendix and -thymus nuclei. 1 ml nuclear suspension; 0.5 ml 0.25 *M* sucrose–0.1 *M* Na phosphate, pH 7.3; 0.4 ml 0.1 *M* glucose (containing 8.160 mg NaCl and 5.07 mg $MgCl_2 \cdot 6\ H_2O$ per ml); 0.1 ml [6-^{14}C]orotic acid (2 μC and 0.175 mg orotic acid).

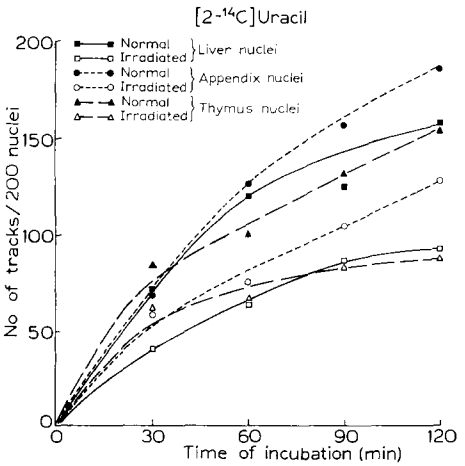

Fig. 3. The effect of 300R of X-irradiation on the uptake of [2-^{14}C]uracil by isolated rabbit-liver, -appendix and -thymus nuclei. 1 ml nuclear suspension; 0.5 ml 0.25 *M* sucrose–0.1 *M* Na phosphate, pH 7.3; 0.4 ml 0.1 *M* glucose (containing 8.16 mg NaCl and 5.07 mg $MgCl_2 \cdot 6\ H_2O$ per ml); 0.1 ml [2-^{14}C]uracil (2 μC and 0.0486 mg uracil).

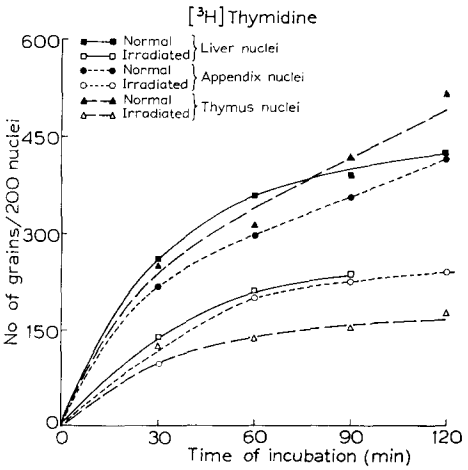

Fig. 4. The effect of 300 R of X-irradiation on the uptake of tritiated thymidine by isolated rabbit-liver, -appendix and -thymus nuclei. 1 ml nuclear suspension; 0.5 ml 0.25 *M* sucrose–0.1 *M* Na phosphate, pH 7.3; 0.4 ml 0.1 *M* glucose (containing 8.16 mg NaCl and 5.07 mg $MgCl_2 \cdot 6\ H_2O$ per ml); 0.1 ml tritiated thymidine (2 μC and 0.0242 mg thymidine).

tion, 67 % after 120-min incubation, thymus nuclei showed 50 % inhibition after 120-min incubation, while liver nuclei showed a 50 % inhibition after 120-min incubation.

Fig. 5 shows the uptake of [2-^{14}C]phenylalanine by the nuclear proteins of normal and irradiated nuclei. Liver nuclei showed the greatest inhibition after irradiation (50 % after 90-min incubation), while after 120-min incubation thymus nuclei showed 40 % and appendix nuclei 35 % inhibition.

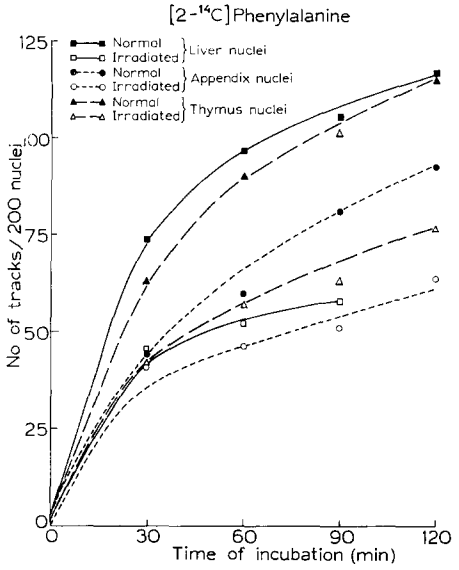

Fig. 5. The effect of 300 R of X-irradiation on the uptake of [2-^{14}C]phenylalanine by isolated rabbit-liver, -appendix and -thymus nuclei. 1 ml nucleus suspension; 0.5 ml 0.25 *M* sucrose–0.1 *M* Na phosphate, pH 7.3; 0.4 ml 0.1 *M* glucose (containing 8.16 mg NaCl and 5.07 mg $MgCl_2 \cdot 6\ H_2O$ per ml); 0.1 ml [2-^{14}C]phenylalanine (2 μC and 0.040 mg phenylalanine).

In some instances, portions of the incubation mixtures after 120-min incubation were used for the chemical isolation of RNA and DNA, which were then subjected to measurement of radioactivity. In spite of the differences clearly shown up in autoradiograms, no significant differences were found between the activities of the components isolated from control and irradiated nuclei. The reasons for this anomaly are still under investigation.

The results obtained by autoradiography show that isolated rabbit liver, thymus and appendix nuclei are capable of taking up known precursors of RNA, DNA and proteins. X-irradiation of the isolated nuclei inhibits the uptake of the precursors. The amount of uptake and the degree of inhibition obtained varies with the precursor and also with the tissue used.

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