RADIOBIOLOGY

Effect of Whole-body X-irradiation on ^{14}C-Leucine Incorporation into Proteins of Cell Nuclei of Regenerating Rat Liver

X-IRRADIATION has been shown to interfere with some biosynthetic processes in the cell nucleus of the regenerating liver. A disturbance of DNA synthesis[1-3] and of nuclear RNA turnover[4] was noted when animals were irradiated with moderate doses of X-rays in the early phase of liver regeneration (2–6 h after partial hepatectomy in the case of RNA), but no effect was observed after X-irradiation in the later stages within 24 h after operation. Similarly, an inhibitory effect of X-irradiation on the total protein turnover in the 23-h regenerating liver was reported to occur in animals exposed to lethal doses of X-irradiation 2–6 h after partial hepatectomy, but not in those irradiated 18 h after operation[5]. The present communication reports the effect of whole-body X-irradiation, at different time intervals within 24 h after partial hepatectomy, on amino-acid incorporation into proteins of cell nuclei isolated from a 24-h regenerating liver.

Animals used in this study were male Wistar rats of approximately 2 months and 160–170 g. Partial hepatectomies were performed according to Higgins and Anderson[6]. The rats were whole-body irradiated with a dose of 900 r. (measured in air), the X-ray machine operating at 220 kV and 15 m.amp., with 0·5 mm Cu and 1 mm Al. The target distance was 40 cm and the dose rate 82 r./min.

The liver was freed from erythrocytes by perfusion with cold physiological saline followed by 0·25 M sucrose–2 mM $CaCl_2$. The 10 per cent tissue suspension in 0·25 M sucrose–5 mM $CaCl_2$ was treated in a hand-operated ball type homogenizer[7] until almost all cells were broken. Nuclei were separated from other cell fractions by repeated centrifugation in a discontinuous density gradient of 0·25 M and 0·34 M sucrose, each solution containing 2 mM $CaCl_2$. Nuclear suspensions in isotonic sucrose were checked for purity by phase-contrast microscopy. Those containing more than 5 per cent whole cell contaminants were rejected. Nuclei were incubated for 90 min in the presence of ^{14}C-leucine (Fig. 1). After that time or at shorter intervals the reaction was stopped by adding an equal volume of 20 per cent trichloroacetic acid. Precipitated protein was purified according to Allfrey *et al.*[8]. Weighed aliquots were suspended in acetone and plated on Whatman No. 50 paper. The activity was counted in a gas flow counter (Nuclear, Chicago). The results were adjusted at infinite thickness.

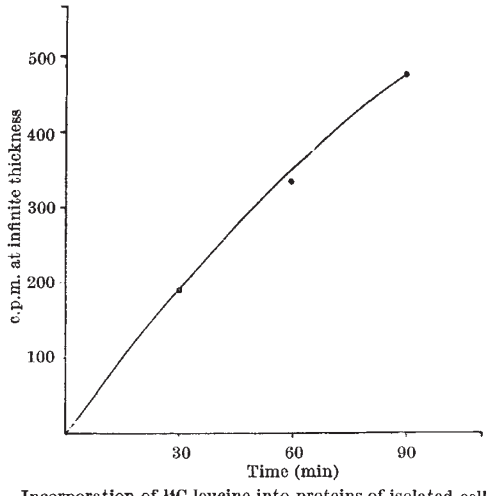

Fig. 1. Incorporation of ^{14}C-leucine into proteins of isolated cell nuclei of 24 h regenerating rat liver. Incubation at 37° C with constant shaking. Incubation medium: 1 ml. nuclear suspension, 1 μc. DL-1-^{14}C-leucine (6·4 mc./mmole), 0·20 M sucrose, 0·025 M sodium phosphate buffer (*p*H 7·3), 0·02 M glucose, 0·02 M NaCl, 5 mM $MgCl_2$, in a total volume of 2 ml.

The time course of ^{14}C-leucine incorporation into nuclear proteins during a 90-min incubation of nuclei isolated from a 24-h regenerating liver is shown in Fig. 1.

Irradiated rats received 900 r. of X-rays at 2, 6 and 16 h after partial hepatectomy. They were killed 24 h after operation. Each irradiated animal was paired with a sham-irradiated control for simultaneous incubation of nuclei. All animals were fasted from operation to killing. In these experiments the time course of ^{14}C-leucine incorporation was not followed, but only 90-min incubation values were recorded. The experimental results are summarized in Table 1.

Table 1. EFFECT OF 900-R. X-IRRADIATION ON ^{14}C-LEUCINE INCORPORATION INTO PROTEINS OF ISOLATED CELL NUCLEI OF 24-H REGENERATING LIVER

Time of irradiation after hepatectomy (h)	C.p.m. at infinite thickness Controls	Irradiated	*t*-test
2	454 ± 33	344 ± 23	$P < 0.05$
6	432 ± 30	326 ± 26	$P < 0.05$
16	501 ± 34	499 ± 33	No difference

For each time interval eight paired rats were used.

As shown in Table 1, ^{14}C-leucine incorporation into proteins of regenerating liver nuclei was inhibited by 20–25 per cent when irradiation was given 2 or 6 h after partial hepatectomy. No inhibition was observed when animals were irradiated 16 h after operation.

There is a striking similarity between the effect of X-irradiation on amino-acid incorporation at tissue level[5] and at nuclear level, suggesting that the mechanisms of nuclear and cytoplasmic protein synthesis are closely interdependent in growing liver cells.

NEDA ŠESTAN

Biology Division,
Institute 'Ruder Bošković',
Zagreb, Yugoslavia.

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Hereditary Cataract induced by X-irradiation of Young Rats

IN a previous investigation[1] we showed that about 5 per cent of the offspring of 8- and 17-day-old rats which had been subject to 50, 150, 300 and 500 r. of total-body X-irradiation were dwarfs. At weaning the body-weight of the dwarf animals was half that of the controls of corresponding age, but this difference between normal and dwarfed offsprings decreased with age. In the F_2 (from the F_1 dwarfed generations) almost the same percentage of dwarfed animals was observed, but, in addition, in F_3 some offspring were found with bilateral cataract.

The cataracts were observed in the F_3 generation of a 17-day-old female irradiated with 150 r. of total-body X-irradiation. At the age of 70 days, this female was mated with a control male and produced F_1 and F_2 progeny with normal eyes. In the F_3 generation about 25 per cent had cataracts although all animals were not affected at the same time: at weaning about 75 per cent of the mutant offsprings showed bilateral cataracts while one or both lenses of 25 per cent appeared to be normal. At 70 days of age, bilateral cataracts were present in all mutants. The breeding tests have established that this mutation is inherited as a simple recessive factor: the mating between cataractous male and female gave 100 per cent of cataractous offspring while the mating between