Acta Radiologica Oncology 24 (1985) Fasc. 4

FROM THE DEPARTMENT OF MEDICAL RADIATION BIOLOGY, KAROLINSKA INSTITUTET, S-104 01 STOCKHOLM, SWEDEN.

RNA AND PROTEIN SYNTHESIS OF IRRADIATED EHRLICH ASCITES TUMOUR CELLS

I. In vivo investigations related to the cell cycle

S. SKOG, B. TRIBUKAIT and G. SUNDIUS

Abstract

The effects of roentgen irradiation on the incorporation of ^{3}H-uridine and ^{14}C-leucine into RNA and protein and the RNA and protein contents of in vivo growing Ehrlich ascites tumour cells were studied. The results were related to changes in the composition of cells in cell cycle and compared with the synthesis of RNA and protein in cell material from various parts of the cell cycle obtained by means of elutriator centrifuging. The incorporation expressed by the ratio between acid insoluble/acid soluble activity was unchanged for RNA during the observation period up to 24 hours after a dose of 5.0 Gy. The ratio for protein was markedly decreased between 4 and 24 hours. This decrease was partly due to a decrease of the pool size of leucine as studied by changing the amounts of ^{14}C leucine used. From these studies, the existence of at least two pools, an expandable and a non-expandable fixed pool can be concluded. There were no differences in the decrease of protein-synthesis between cells from the various parts of the cell cycle. The RNA and protein contents of the irradiated cells from various parts of the cell cycle corresponded to those of non-irradiated cells except for G_1/early S-phase cells at 15 and 24 hours after irradiation. Possible reasons for this discrepancy are discussed.

While the effect of irradiation on DNA synthesis is well known, changes in the metabolism of other macromolecules, such as RNA and protein, are more uncertain, in spite of extensive studies (1–4, 6–13, 15, 17–23, 26, 31, 32, 34, 35). Generally, following in vivo irradiation (4–40 Gy) of normal mammalian cells (1–4, 6–8, 11, 13, 17, 18, 20–22, 32, 34) and tumour cells (9, 10, 15, 19, 23, 26, 31) both stimulation and inhibition of RNA and protein synthesis have been reported. According to the few studies available, synthesis of m-RNA (3, 22) and t-RNA (18) seems to be inhibited while r-RNA synthesis may be stimulated (2).

These contradictory results may partly be explained by irradiation induced transient changes in the cell cycle distribution resulting in an accumulation of cells in G_2 (25). Since synthesis rate and cellular contents of RNA and protein increase during the cell cycle (16), irradiation induced changes in the cell cycle composition also change these parameters.

In the present investigation the RNA and protein synthesis rate and contents of irradiated in vivo growing Ehrlich ascites tumour cells were studied. The results from asynchronously growing cells were compared with fractions of cells from various parts of the cell cycle obtained by elutriator centrifuging. The cell cycle composition of these fractions and of the unfractionated growing cells at various times after irradiation was determined using flow-cytometry.

Material and Methods

Cell material. 3×10^{7} 10-day-old hyperdiploid Ehrlich ascites tumour cells were transplanted intraperitoneally into 3-month-old female NMRI mice.

Cell counting and viability studies. The cell number was counted microscopically using a Bürker

---

Accepted for publication 12 February 1985.

chamber. Dead cells were determined by dye exclusion using 0.2 per cent Lissamine Green B.

Irradiation procedure. At day 4 after transplantation, the mice were whole-body irradiated with 5.0 Gy by 250 kV roentgen rays, operating at 15 mA with 0.5 mm Cu added filtration, SSD 50 cm. The dose rate was 1.33 Gy/min. The dosimetry based on lithium fluoride dosimetry was controlled routinely by a Simplex-Universal Dosimeter.

Cell fractionation. The non-irradiated and irradiated animals were killed by cervical dislocation and the tumour cells were removed. After washing twice in TRIS-buffer (TRIS 0.1 mol/l, NaCl 0.07 mol/l, EDTA 0.005 mol/l, pH=7.5, 290 mmol (mOsm)) and centrifuged at 250 × g for 5 min at 4°C, the cells were fractionated by elutriator centrifugation as described previously (29). About 5×10^8 cells in TRIS buffer supplemented with 5 per cent foetal calf serum at 4°C, were added to the system (a Beckman JE-G elutriator rotor with a Beckman J 21 B centrifuge) with an initial pump speed of 12 ml/min. The rotor speed was held at 2780 ± 10 rpm. Twenty-one fractions of 100 ml were isolated after increasing the flow rate in steps of 2 ml/min. The degree of purity was high (29, 30) with fractions of G_1, mid S-phase and G_2+M cells of 90, 80 and 70 per cent, respectively.

Determination of the cell cycle composition of the cell fractions. The relative number of G_1, S-phase and G_2+M cells in each fraction was determined using cellular quantitative DNA analysis by means of flow cytometry as described previously (33). Ethanol fixed cells were washed in TRIS buffer together with RNAse (1.0 mg/ml). After pepsine treatment (5.0 g (1000 IU/g)/l in 0.055 mol/l HCl) individual cell nuclei were stained with ethidium bromide (2.5×10^{-5} mol/l). The DNA contents of the nuclei were analyzed using a flow-cytofluorometer (ICP 11, Phywe; Göttingen, W. Germany, now Ortho Instruments, Westwood, Mass., USA) with a maximum flow rate of 1000 cells/s. The fluorescent measurements were stored and sorted with the aid of a 256 channel analyzer. The proportion of cells in the different cell cycle phases was determined from the area of the histograms, assuming a Gaussian function for the G_1 and the G_2+M maxima and attributing the remaining part of the DNA histogram to the cells of the S-phase.

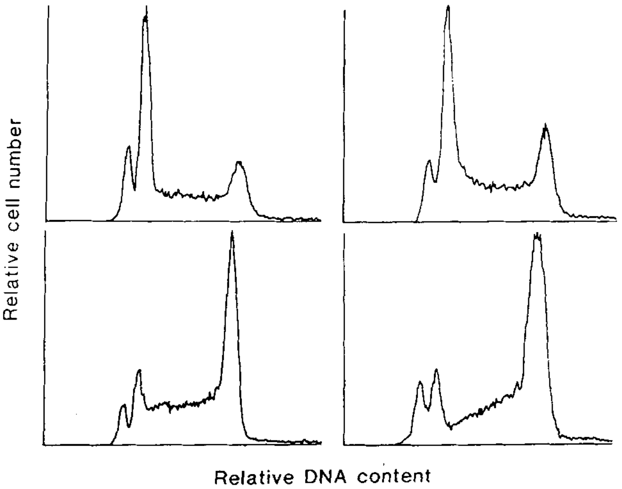

Fig. 1. DNA histograms of non-irradiated cells (a, top left) and cells 4 hours (b, top right), 15 hours (c, bottom left) and 24 hours (d, bottom right) after a dose of 5.0 Gy. The peak to the left corresponds to normal mouse cells, the highest peak to the left and the peak to the right to G_1 and G_2+M cells, respectively. Between these peaks are the S-phase cells.

Synthesis rate and the contents of RNA and protein of in vivo growing cells. In order to measure the RNA and protein synthesis rate the cells were double-labelled in vivo by intraperitoneal injection of ^{3}H-uridine (1.0 TBq/mmol) and ^{14}C-leucine (2.1 GBq/mmol). In the experiments with unfractionated cells 37, 74, and 148 kBq ^{3}H-uridine and 74, 148, 222 and 296 kBq ^{14}C-leucine with an isotope time between 2.5 and 20 min were used. These various quantities of ^{3}H-uridine and ^{14}C-leucine were chosen in order to study possible changes of the uridine and leucine pools. In the fractionation experiments, 74 kBq ^{3}H-uridine and 74 kBq ^{14}C-leucine with an isotope time of 10 min were used.

The labelled ascites cell suspension was removed from the animals and the cells separated from the ascites fluid by centrifuging at 250×g for 5 min at 4°C. The ascites fluid was precipitated with 0.2 mol/l PCA and the activity in the PCA soluble fractions measured. The cell material was subdivided into two parts and precipitated in 0.2 mol/l PCA. After washing twice in 0.2 mol/l PCA, the sediments of the first part were dissolved in 3 ml of 0.3 mol/l KOH for RNA measurements and the other part dissolved in 1 ml 0.5 mol/l NaOH for protein determination. For the RNA analysis, RNA was hydrolysed at room temperature for 24 hours. After further precipitation of DNA with 0.2 mol/l PCA, the PCA soluble fraction was mixed with orcinol reagents (1 : 1) for hydrolysis at 100°C for 40 min and then extracted with isoamyl alcohol. The RNA was measured spectroscopically at 675 nm after refrigeration to room temperature (5). The protein con-

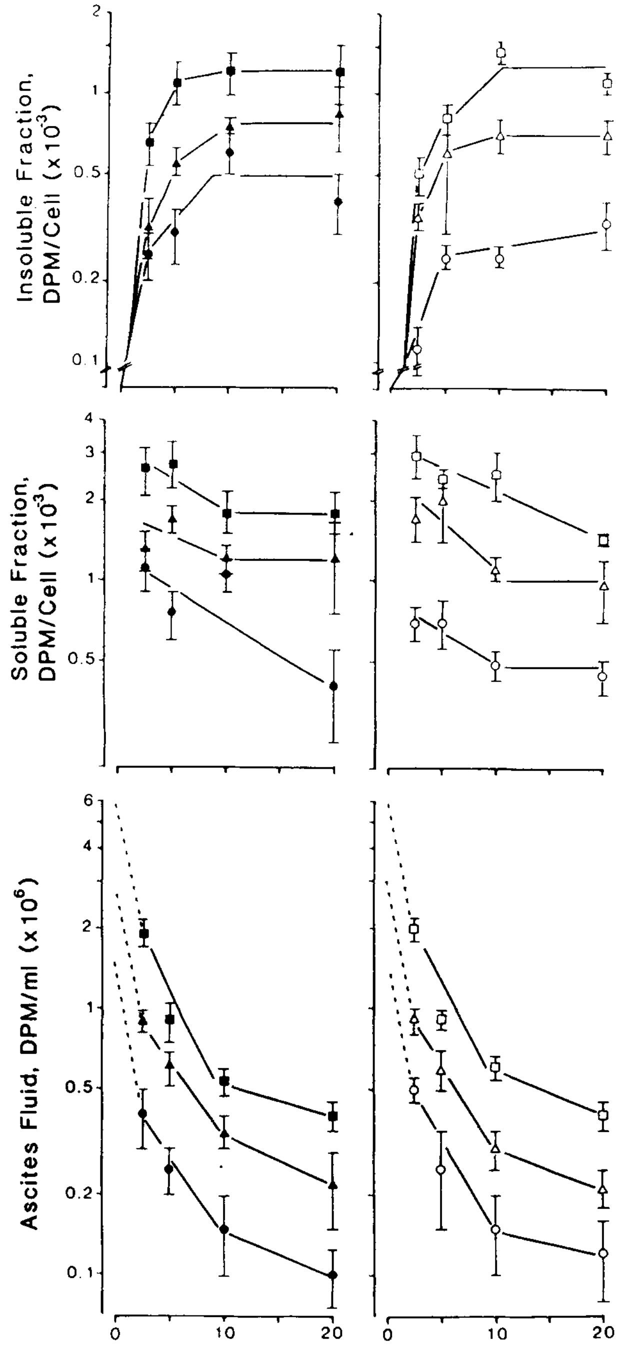

Fig. 2. Time curves of ^{3}H-uridine activity in the PCA insoluble and soluble cell fractions and the PCA acid soluble fraction of the ascites fluid of non-irradiated animals (left) and animals 4 hours after a dose of 5.0 Gy (right). Mean values of 4 to 6 experiments ±SEM. 37 kBq (●, ○), 74 kBq (▲, △), 148 kBq (■,□).

tents were measured according to LOWRY et coll. (14). The ^{3}H-uridine and ^{14}C-leucine activity of the insoluble PCA fractions of the cells and the soluble PCA fractions of the cells and the ascites fluid were separated using the Intertechnique oxidizer, and the ^{14}C-activity was measured in oxifluore and the ^{3}H-activity in biofluore supplemented with 30 per cent econofluore (Intertechnique, SL 30).

Results

Cell cycle distribution. Four days after transplantation, during the exponential growth of the tumour, the ascites cell suspension contained about 90 to 95 per cent of hyperdiploid tumour cells; the percentages of G_1, S-phase and G_2+M cells were 38±2, 48±2 and 14±1, respectively. The remaining part contained tumour cells with higher degrees of ploidy and normal mouse cells (Fig. 1 a).

After irradiation no increase in the total number of cells was found for about 35 hours. At that time the total number of cells started to increase with a rate comparable to the non-irradiated cells. One hour after irradiation no changes were observed in the distribution of the cells in cell cycle. After 4 hours a slight decline was found in the relative number of G_1 cells with corresponding increase in S-phase and G_2+M cells (Fig. 1 b). At 15 and 24 hours the number of G_2 cells was further elevated (Fig. 1 c, d), and the number of G_1 cells reached its minimum value.

Kinetics of ^{3}H-uridine and ^{14}C-leucine uptake. Figs 2, 3 and 4 show the uptake of ^{3}H-uridine and ^{14}C-leucine from the ascites fluid into the cellular pool and further into the RNA and the protein of non-irradiated cells and cells 4 hours after irradiation. The amounts of uridine injected varied between 3 and 15×10^{-11} mol and for leucine between 3.5 and 14×10^{-8} mol. From these values and the known ascites volume the activities at zero time have been calculated.

There were no differences for ^{3}H-uridine between non-irradiated and irradiated cells except for the lowest concentration at which the concentration in the soluble and insoluble fraction was lower in the irradiated animals. Plateau values for the incorporation of ^{3}H-uridine were reached after 5 to 10 min (Fig. 2). The activity from the ascites fluid decreased exponentially with a $t_{1/2}$ of 1.0 min up to 2.5 min, a $t_1/_2$ of 7.0 min between 2.5 and 10 min and a $t_{1/2}$ of 28 min between 10 and 20 min. This pattern of decrease is possibly also true for the activity of the soluble fraction or the intracellular pool.

The incorporation of ^{14}C-leucine into the protein of non-irradiated cells reached plateau values between 5 and 10 min (Fig. 3). Maximum values were reached at a concentration of 10.5×10^{-8} ml. The activity from the ascites fluid decreased biphasic

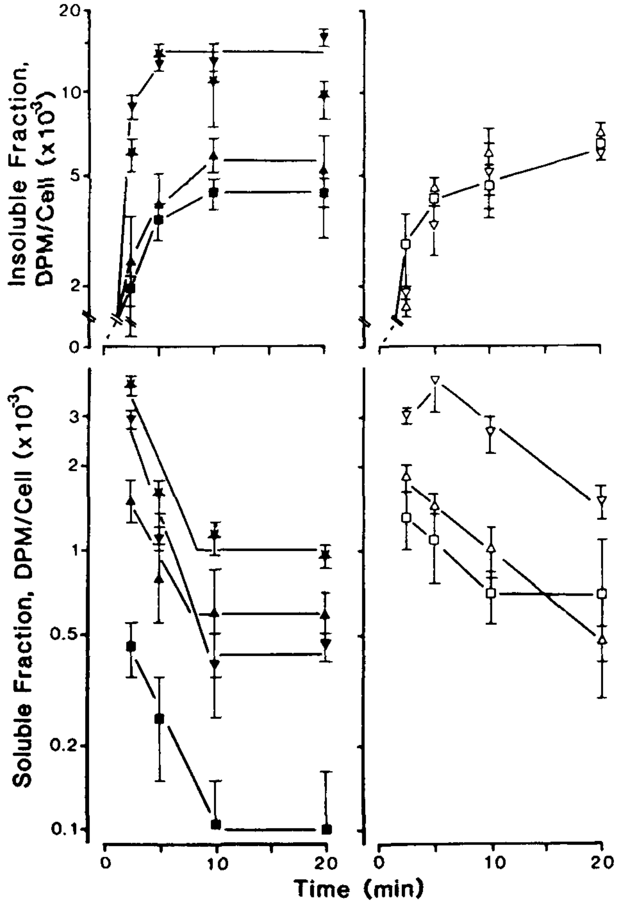

Fig. 3. Time curves of ^{14}C-leucine activity in the PCA insoluble and soluble cell fractions of non-irradiated animals (left) and animals 4 hours after a dose of 5.0 Gy (right). Mean values of 4 to 6 experiments ± SEM. 74 kBq (■, □), 148 kBq (▲, △), 222 kBq (▼, ▽) and 296 kBq (*).

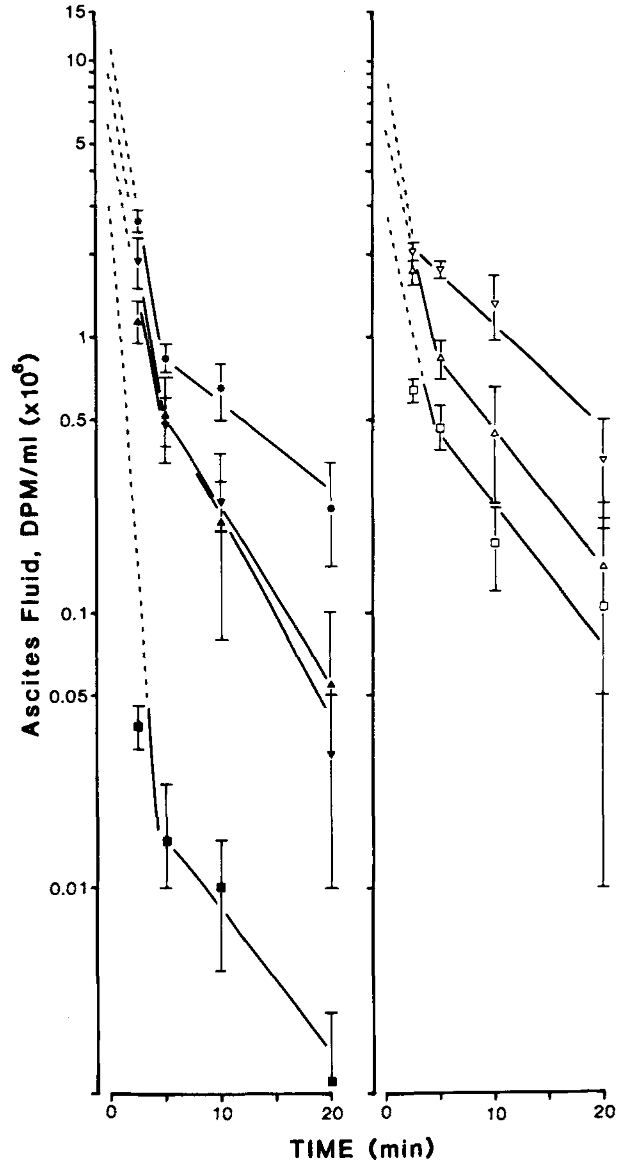

Fig. 4. Time curves of ^{14}C-leucine activity in the PCA soluble fraction of the ascites fluid of non-irradiated animals (left) and animals 4 hours after a dose of 5.0 Gy (right). Mean values of 4 to 6 experiments ± SEM. 74 kBq (■, □), 148 kBq (▲,△), 222 kBq (▼, ▽) and 296 kBq (*).

exponentially with a $t_{1/2}$ of 1.0 min during the first 5 min and at a $t_{1/2}$ of 6.0 min after this time. The activity of the intracellular pool declined exponentially up to 10 min and was then constant at about 10 per cent of the initial activities. Compared with the non-irradiated cells, the following differences were found for irradiated cells: 1) The incorporated activity into the protein increased rapidly up to 5 min as in the non-irradiated cells but continued to increase further at a slower rate up to 20 min. 2) The incorporation reached maximum values already after the lowest concentration injected, 3.5×10^{-8} mol. 3) The amount of the activity in the pool and the ascites fluid 2.5 min after administration of the ^{14}C-leucine was somewhat higher. 4) The activity from the intracellular pool declined at a lower rate with a $t_{1/2}$ of 9.3 min compared with that of non-irradiated cells with a $t_{1/2}$ of 3.3 min. No constant levels were observed as in the controls.

RNA and protein synthesis in unfractionated cells. Based on the results of the kinetic studies for the following investigations one, 4 and 24 hours after irradiation, a fixed isotope time of 10 min was chosen. The activity of ^{3}H-uridine and ^{14}C-leucine in the soluble and the insoluble PCA fractions of the cells and in the soluble PCA fraction of the ascites fluid of non-irradiated hosts increased linearly with increasing amounts of the precursors added (data not shown). There were no differences between 4- and 5-day-old cells and no differences in the cell cycle composition.

In the experiments on irradiated cells there were

Table 1

Amounts of ^{3}H-uridine and ^{14}C-leucine activity in the insoluble and the soluble fraction of non-irradiated and irradiated cells. Mean values of 4 to 6 experiments ± SEM

	Incorporated activity (DPM/cell×10^{-4})					
	Insoluble		Soluble		Insoluble/soluble	
	RNA	Protein	RNA	Protein	RNA	Protein
Non-irradiated	7±1	49±8	10±1	2±1	0.7±0.1	25±2
1 hour	7±1	30±10	9±1	2±1	0.8±0.1	15±2
4 hours	7±1	50±10	11±1	7±2	0.6±0.1	7±1
24 hours	9±1	27±10	13±2	3±1	0.7±0.1	9±1

Table 2

RNA and protein synthesis rate expressed as incorporated amount of ^{14}C-leucine and ^{3}H-uridine into non-irradiated and irradiated unseparated cells and into cells from the various parts of the cell cycle. The values are expressed per cell and as a per cent of the injected activity. Mean values of 3 to 4 experiments ± SEM

	RNA synthesis (DPM/cell×10^{-11})				Protein synthesis (DPM/cell×10^{-10})			
	Non-irrad.	4 h	15 h	24 h	Non-irrad.	4 h	15 h	24 h
Unseparated cells	10±0.4	7±1	9±1	14±1	11±1	6±0.2	7±1	9±0.4
G_1 cells	7±1	4±1	3±0.2	5±0.3	7±1	2±0.2	3±0.2	2±0.4
S-phase cells								
Early	10±1	5±0.4	6±1	8±0.3	10±1	4±0.2	5±0.3	6±0.2
Middle	10±1	5±0.4	11±1	12±0.4	12±1	4±0.4	9±1	7±0.3
Late	12±1	6±1	9±1	14±1	14±1	4±0.2	9±1	8±1
G_2+M cells	13±1	8±1	9±0.4	13±0.4	18±1	5±0.2	7±0.3	9±0.2

no deviations from control values for ^{3}H-uridine uptake and incorporation irrespective the amount of uridine given and the time of observation after irradiation. In Table 1, therefore, the results of only one concentration of ^{3}H-uridine (7.4×10^{-11} mol) is given demonstrating no change in the ratio insoluble/soluble activity. In corresponding experiments using ^{14}C-leucine both changes in ^{14}C-leucine uptake and incorporation were found at all ^{14}C-leucine concentrations studied and the ratio insoluble/soluble activity decreased significantly one, 4 and 24 hours after irradiation. In Table 1, the representative results are given from experiments using 3.5×10^{-8} mol leucine.

Cell cycle related RNA and protein synthesis. The experiments on separated cells were performed following injection of 3.5×10^{-8} mol ^{14}C-leucine and 7.4×10^{-11} mol ^{3}H-uridine at a fixed isotope time of 10 min. Only the insoluble activity was measured. The reason was that the amount of soluble activity decreased by about 50 per cent in the course of the elutriation separation. In an attempt to minimize the effect of variations in the various control animals, experiments on irradiated animals were run in parallel with experiments on controls. The results are shown in Table 2.

For these cell cycle related studies the fractions of cells separated by elutriator centrifuging were characterized by their average DNA contents as determined by the centroid of the DNA histograms. Each fraction was placed into the cell cycle according to this value. Fractions containing G_1 or G_2+M cells with the same mean DNA contents were placed according to their fraction number, i.e. early, middle or late G_1 or G_2 +M.

In non-irradiated cells, the ^{3}H-uridine incorporation doubled from early G_1 to early S-phase. There were about unchanged values during S-phase and a slight further increase during G_2. The ^{14}C-leucine incorporation during the cell cycle increased about 2.5 times; this increase was more or less continuous with a somewhat lower rate during S-phase. These results are in correspondence with earlier experience (28).

One hour after irradiation, the incorporation of ^{3}H-uridine into the various cell cycle phases was the

Table 3

Amount of RNA and protein of unseparated non-irradiated and irradiated cells and of cells from the various parts of the cell cycle. Mean values of 3 to 4 experiments ± SEM

	RNA/cell (pg)			Protein/cell (pg)		
	Non-irrad.	15h	24 h	Non-irrad.	15 h	24 h
Unseparated cells	18±1	20±1	26±2	171±15	210±41	256±10
G_1 cells	11±1	9±1	10±1	103±6	89±5	42±10
S-phase cells						
Early	16±1	13±1	16±1	130±6	84±6	62±6
Middle	18±1	22±1	17±1	149±9	146±9	166±12
Late	20±1	22±1	16±1	219±9	152±6	161±17
G_2+M cells	22±2	19±1	24±1	234±10	259±13	254±9

same as in the controls (data not shown). High values in some experiments could not be confirmed in repeated experiments. The ^{3}H-uridine incorporation at 4 hours was significantly ($p<0.01$) reduced by about 50 per cent. At 15 hours the reduction was about 30 per cent in all cell cycle phases. The ^{3}H-uridine incorporation was normal at 24 hours. The ^{14}C-leucine incorporation was normal one hour after irradiation (data not shown) but was reduced by at least 50 per cent in all cell cycle phases between 4 and 24 hours after irradiation.

Cell cycle related RNA and protein contents. The RNA content of non-irradiated cells doubled during the cell cycle in accordance with earlier results (28) (Table 3). There was a rapid increase during G_1 up to early S-phase, a slow increase during S-phase and a higher increase during late S-phase/G_2. The protein content was doubled with somewhat higher rates during G_1 and G_2 (Table 3). Neither RNA nor protein content changed following irradiation significantly, except for protein content of G_1 and early S-phase cells at 15 and 24 hours after irradiation ($p<0.01$) (Table 3).

Dicussion

In the present investigation of in vivo growing cells we studied the incorporation of radioactive precursors for RNA and protein synthesis. Changes in incorporation may reflect real changes in the synthesis rate of RNA or protein. They may, however, also reflect changes in the cellular uptake or changes in the size of the cellular uridine and leucine pools. Furthermore, since cellular uptake and incorporation are dependent on the position of the cell in the cell cycle, irradiation induced changes of these parameters may be due to changes in the cell cycle composition, particularly at times exceeding 12 hours following irradiation. We therefore studied the cell cycle composition after irradiation and also the incorporation into cells from various parts of the cell cycle.

After injection of the precursors into the ascites fluid, part of each precursor is taken up by the tumour cells while the remainder part leaves the ascites and enters the body of the animal. The decline in the activity of ^{3}H-uridine and ^{14}C-leucine in the ascites showed some differences. The decline in ^{3}H-uridine showed several components; the first rapid part up to 2.5 min mostly reflects the cellular uptake which is followed by phosphorylation in the cell and incorporation into RNA.

Apparently, only part of the ^{3}H-uridine taken up by the cell is avaliable for RNA synthesis since a constant level of activity in the cellular pool is found 10 min after injection, while the incorporation into RNA had reached its maximum level after 5 to 10 min. This constant amount of activity in the pool was about 10 per cent of the injected activity, as calculated from the total number of cells found at that time. About half of the activity injected was incorporated into the RNA of the tumour cells. The slow decline in the activity from the peritoneal cavity 10 min after injection thus only reflects the uptake by the body. Irradiation was without effect on these parameters.

The decline in the ^{14}C-leucine activity from the peritoneal cavity was biphasic; the first rapid component corresponds, as for ^{3}H-uridine, mainly to the cellular uptake in the pool and incorporation into protein, the second component to the outflow into the body. As for RNA, two types of pools were also found, one of them not available for protein synthesis as indicated by constant activity without further incorporation into protein 10 min after injection. The higher amount of activity in the ascites fluid and in the cellular pool 2.5 min after injection in irradiated animals indicates a lower protein synthesis either due to direct inhibition of protein synthesis or, indirectly due to a decrease in the size of the pool available for protein synthesis. In irradiated animals with higher pool activities and lower rates of decline, constant pool activities have not been reached 20 min after injection.

In the experiments using ^{3}H-uridine, the injection of 3 to 15×10^{-11} mol resulted in a proportional increase in the incorporation into RNA and the uptake into the pool. In the ^{14}C-leucine experiments, working in a range of 3.5 to 14×10^{-8} mol, saturation of the incorporated activity in controls was reached at 10.5×10^{-8} mol. Assuming a two-pool system for leucine with an expandable and a fixed pool, the fixed pool would have reached saturation at this concentration. In irradiated animals this fixed pool had already saturated at a concentration of 3.5×10^{-8} mol. Thus, a decrease in the size of the fixed pool can be considered. In addition, the reduced rate at which the incorporated activity reaches a plateau value tends to indicate a real decrease in the protein synthesis. This conclusion is also indicated by the reduced rate of decline in the pool activity.

The existence of two pools of amino-acids, a non-expandable and an expandable pool was discussed earlier for liver cells (24) and an increased amount of ^{14}C-leucine in the cellular pool of irradiated liver cells has also been observed (17). Based on the present finding of a non-available pool of uridine for RNA synthesis, a similar subdivision of RNA pools as for amino-acids is suggested. Since, in the present study, the amounts of uridine used did not reach the saturation level for RNA synthesis, further studies are needed to establish whether uridine pools are really unaffected by irradiation.

An effect on protein synthesis or pool changes was already indicated one hour after irradiation and is thus independent of changes in cell cycle composition.

For the cell cycle related studies of RNA and protein, fixed amounts of ^{3}H-uridine and ^{14}C-leucine were injected into the animals and the cells were removed from the peritoneal cavity after an isotope time of 10 min. Since plateau values for incorporation into RNA and protein were reached at about this time, no significant changes in the incorporated activity can be expected during the following 60 min after which time period the cells were elutriated at 4°C.

A comparison between unseparated cells and cells after separation showed both for RNA and protein a loss in the amount of these compounds by about 15 per cent due to elutriation and a corresponding loss in the incorporated activity. The pool activity, on the other hand, decreased markedly during separation. The relationship between insoluble/soluble activity cannot, therefore, be applied to the expression of incorporated activity, and the evaluation of the irradiation experiments was therefore based only on control experiments carried out at the same time. Out of four control experiments, three showed about the same incorporated activity in RNA but the fourth only about half of these values. However, the relative increase during G_1 and late part of S-phase/G_2 was the same as in the other experiments. Based on these data, the low values of ^{3}H-uridine incorporation into RNA 4 hours after irradiation are conspicuous for an irradiation effect. An extensive loss of activity is excluded by the comparison between the cell material before and after separation and is also supported by the unchanged total amount of RNA. For the studies of protein synthesis in which the controls were in good mutual correspondence, the irradiation effect was most marked 4 hours after irradiation without any specific cell cycle related inhibition.

It has been found earlier in this cell system and at an irradiation dose of 5 Gy that a significant proportion of cells in G_1 24 hours after irradiation belonged to non-viable cells (27). Such cells may contain less protein and may, therefore, explain the significant decrease in the protein content of cells in G_1—early S-phase found at that time.

ACKNOWLEDGEMENT

The investigation was supported by the Cancer Society of Stockholm.

Request for reprints: Dr Sven Skog, Department of Medical Radiation Biology, Karolinska Institutet, P.O. Box 60204, S-104 01 Stockholm, Sweden.

REFERENCES

1. Abdel-Halium M. N.: Polyribosome distribution in regenerating livers of irradiated adrenalectomized rats. Int. J. Rad. Biol 32 (1977), 309.

2. — The role of cortisone acetate in liver RNA synthesis of gamma-irradiated rats. Int. J. Rad. Biol. 35 (1979), 235.
3. — Effects of ionizing radiation and partial hepatectomy on messenger RNA synthesis. Int. J. Rad. Biol 35 (1979), 245.
4. ARIYAMA K., FAUSTO N., TAMVAKOPOULOS E. and VAN LANCKER J. L.: Effects of X-radiation on amino acid incorporation into regenerating liver protein. Rad. Res. 42 (1970), 528.
5. CERIOTTI G.: Determination of nucleic acids in animal tissues. J. Biol. Chem. 214 (1955), 59.
6. FLORY W. and NEUHAUS O. W. Induced transport of amino acids in rat liver after whole-body irradiation. Rad. Res. 68 (1976), 138.
7. KEDROVA E. M. and RODINOV W. M.: The effect of ionizing radiation on nuclear cell proteins. Changes in SH-group content in proteins synthesized in late interphase. Int. J. Rad. Biol. 16 (1969), 359.
8. KILBERG M. S. and NEUHAUS O. W.: The role of stimulated amino acid transport in promoting glycogenesis in the irradiated rat. Rad. Res. 66 (1976), 597.
9. KILLANDER D., RIBBING C. H., RINGERTZ N. R. and RICHARDS B. M.: The effect of X-radiation on nuclear synthesis of protein and DNA. Exp. Cell Res. 27 (1962), 63.
10. KLEIN G. and FORSBERG A.: Studies on the effect of X-rays on the biochemistry and cellular composition of ascites tumours. Exp. Cell Res. 6 (1954), 211.
11. VAN LANCKER J. L., ARIYAMA K. and CHMURA C.: The effect of whole-body X-irradiation on the incorporation of ^{14}C-lysine in nonproliferating and proliferating tissues. Rad. Res. 44 (1970), 68.
12. LAUGHLIN T. J. and TAYLOR J. H.: The effects of X-rays on DNA synthesis in synchronized Chinese hamster ovary cells. Rad. Res. 83 (1980), 205.
13. LEHNERT S. and OKADA S.: Nucleoprotein changes in regenerating liver nuclei after X-irradiation. Int. J. Rad. Biol. 8 (1964), 75.
14. LOWRY O. H., ROSEBROUGH N. Y., FASS A. C. and RANDELL R. J.: Protein measurement with the folin phenol reagent. J. Biol. Chem. 193 (1951), 265.
15. MATSUDAIRA H., SEKIGUCHI T. and NAKAMURA C.: Effects of X-irradiation on the synthesis of rapidly labelled RNA in an experimental tumour of the rat. Int. J. Rad. Biol. 13 (1967), 21.
16. MITCHINSON J. M.: The biology of the cell cycle. Cambridge University Press, London 1971.
17. MUKERJEE H. and GOLDFEDER A.: Protein biosynthesis in the liver of X-irradiated mice. Int. J. Rad. Biol. 25 (1974), 445.
18. OLEINICK N. L., BIAGLOW J. E. and RUSTAD R. C.: Radiation-induced fluctuations of the polysome content in adequately oxygenated Tetrahyema pyriformis. Int. J. Rad. Biol. 36 (1979), 555.
19. ONTOKO J. A. and MOREHEAD W. R.: Histone synthesis after inhibition of deoxyribonucleic acid replication. Biochim. Biophys. Acta 91 (1964), 658.
20. PATIL M. S., NARASIMHAN S. and PRADHAN D. S.: Synthesis of DNA and chromosomal proteins in the rat thymus following whole-body X-irradiation. Int. J. Rad. Biol. 34 (1978), 367.
21. — PRADHAN D. S. and SREENIVASAN A.: Effect of whole-body irradiation on transcription in the rat thymus. Int. J. Rad. Biol. 27 (1975), 363.
22. PITOT H. C., PERAINO C. and LAMAR C.: Comparative effects of actinomycin and ionizing radiation on enzyme synthesis in mammalian cells in vivo. Rad. Res. 33 (1968), 599.
23. RINGERTZ N. R.: The effect of X-radiation on nuclear histone content. Exp. Cell Res. 32 (1963), 401.
24. SEGLEN P. O. and SOLHEIM A. E.: Valine uptake and incorporation into protein in isolated rat hepatocytes. Nature of the precursor pool for protein synthesis. Eur. Biochem. 85 (1978), 15.
25. SCHMITT G., BRUCKSCH K. D., EWEN K., PREUN A., RÖTTINGER E. M. and SEEBER S.: Dose-response of specific activities of high molecular weight nuclear and ribosomal RNA of Novikoff hepatoma ascites cells. The effect of 300-kV X-rays, ^{60}Co γ-rays, 43-MV X-rays, 43-MeV electrons, and 10-MeV neutrons. Rad. Res. 70 (1977), 530.
26. — REINECKE V., HÜDEPOHL G. and SEEBER S.: The effects of various kinds of sparsely-ionizing radiation on total cell RNA and preribosomal nuclear RNA of Novikoff hepatoma ascites cells after in vivo labelling. Int. J. Rad. Biol. 32 (1977), 229.
27. SKOG S. and TRIBUKAIT B.: Irradiation induced cell death as related to cell cycle. Acta radiol. Oncology 24 (1985), 87.
28. — — Discontinuous RNA and protein synthesis and accumulation during cell cycle of Ehrlich ascites tumour cells. Exp. Cell Res. (1985), (in press).
29. — — and SUNDIUS G.: Energy metabolism and ATP turnover time during the cell cycle of Ehrlich ascites tumour cells. Exp. Cell Res. 141 (1982), 23.
30. — — — Energy metabolism and ATP turnover time during the cell cycle in roentgen irradiated Ehrlich ascites tumour cells. Acta radiol. Oncology 22 (1982), 369.
31. STEUENS R. H., OSBORNE J. W., SHANER J. P. and OBERLEX L. W.: Protein kinase activities in X-radiation-induced adenocarcinoma of rat small bowel. Rad. Res. 76 (1978), 95.
32. TOKARSKAYA V. I., SKOTNIKOVA O. I. and UMANSKY S. R.: The effect of radiation on processing of nuclear RNA and chromatin ribonuclease activity in rat liver and thymus. Int. J. Rad. Biol. 27 (1975), 533.
33. TRIBUKAIT B., MOBERGER G. and ZETTERBERG A.: Methodological aspects on rapid-flow cytofluorometry for DNA analysis of human urinary bladder cells. *In*: Pulse-cytophotometry. Part I, p. 50, European Press, Medikon, Ghent 1975.
34. UCHIYAMA T., FAUSTO N. and VAN LANCKER J. L.: Metabolic alterations after total-body doses of X-irradiation. The effects of X-irradiation on rapidly labeled RNA. Arch. Biochem. Biophys. 110 (1965), 191.
35. WALTERS R. A. and ENGER M. D.: Effects of ionizing radiation on nuclei acid synthesis in mammalian cells. Adv. Rad. Biol. 6 (1976), 1.