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EFFECTS OF ^{60}Co GAMMA RADIATION ON THE DISTRIBUTION, CATABOLISM AND INCORPORATION OF ^{125}I-5-IODO-2'-DEOXYURIDINE INTO INTESTINAL AND SPLENIC DEOXYRIBONUCLEIC ACID IN MICE

by

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180 MeV protons and ^{60}Co gamma radiation produce similar effects on the incorporation of the thymidine analogue ^{125}I-5-iodo-2'-deoxyuridine (^{125}IUdR) into DNA of the small intestine and the spleen in mice (JOHANSON & LARSSON 1972). Two hours after ^{60}Co gamma irradiation at 400 rad, for example, the incorporation of ^{125}IUdR into DNA was 13 per cent of the controls.

GITLIN et coll. (1962) using ^{131}IUdR and whole body counting in a large crystal reported that 2 hours after irradiation of mice at 416 R 250 kV roentgen radiation the retention of ^{131}I was 12 per cent of the normal value. The result was confirmed and extended by JOHANSON (1972) and reflects a more marked depression of the DNA synthesis than has been demonstrated with ^{3}H-thymidine or other DNA precursors used (cf. MITCHELL 1966). The marked effect on the

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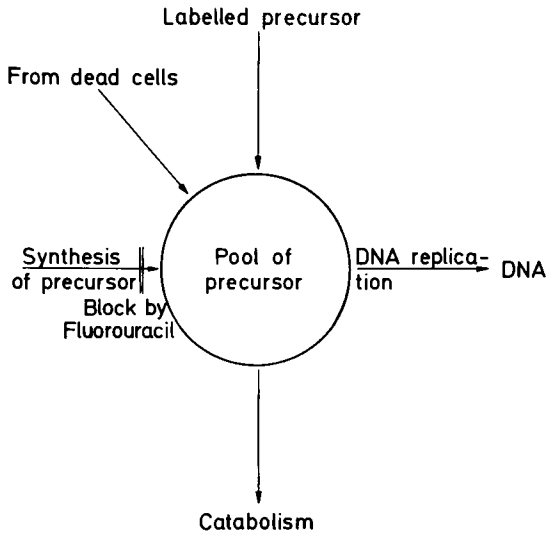

Fig. 1. Modified four-factor model (five-factor model) for the metabolic pathways of thymidine (QUASTLER 1963). The new factor included is thymidine from dead cells.

incorporation of ^{125}IUdR after irradiation may depend upon several factors. The following effects have been investigated: (a) disturbance of precursor distribution, (b) disturbance of precursor catabolism, and (c) increase in the thymidine pool due to the break-down of DNA from dead cells.

An experiment was designed in which the kinetics of the distribution of ^{125}IUdR in the blood stream, the small intestine and the spleen was examined (a). The appearances of the catabolic products in the blood were examined (b). It is known that IUdR is mainly broken down to iodouracil (IU) and further to iodide (PRUSOFF et coll. 1960, HAMPTON & EIDINOFF 1961). Fluorouracil was used in an experiment to increase the incorporation of ^{125}IUdR into DNA (c). Fluorouracil blocks the synthesis of thymidine de novo (HEIDELBERGER et coll. 1957, DANNEBERG et coll. 1958) and in this way decreases the thymidine pool. A mouse given fluorouracil is probably a more sensitive indicator than the normal mouse for changes in the thymidine pool (Fig. 1) which is smaller than in the untreated animal.

Material and Methods

Animals. Male NMRI mice weighing 26 to 28 g were given water ad libitum but had no access to food from 20 hours before irradiation.

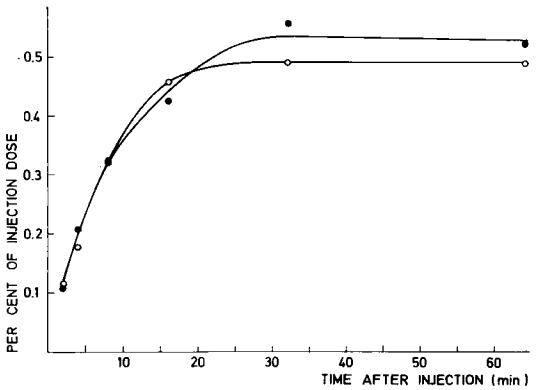

Fig. 2. The total activity of ^{125}I in 100 μl blood after intraperitoneal injection of 10 μCi ^{125}IUdR. ○ control, ● irradiated.

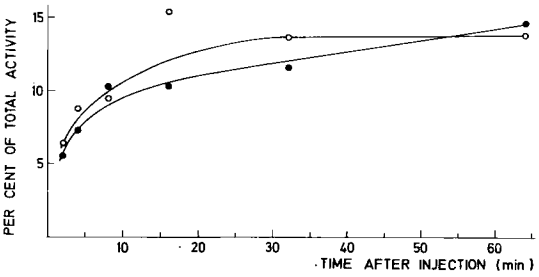

Fig. 3. Percentage of the total activity of ^{125}I in blood, insoluble in cold 0.2 N perchloric acid. ○ control, ● irradiated.

Gamma irradiation. Irradiation was applied with a ^{60}Co source as previously described (JOHANSON & LARSSON 1972) at a dose rate of 56 rad per minute. The control mice were sham irradiated.

Administration of ^{125}IUdR and fluorouracil. Ten μCi ^{125}IUdR (Radiochemical Centre, Amersham) and 0.1 μmole carrier IUdR (Sigma Chem. Co.) in 1 ml 0.9 % NaCl were injected intraperitoneally two hours after irradiation or sham irradiation. The mice in one experiment were given an intraperitoneal injection of 5×10^{-5} mole fluorouracil (Schuchardt, München) in isotonic saline 10 min before the injection of ^{125}IUdR.

Preparations of organs. Groups of 3 control and 3 irradiated mice were killed by decapitation, 2, 4, 8, 16, 32 or 64 min after injection of ^{125}IUdR. Of the blood collected, 100 μl were transferred with a heparinized pipette to a plastic tube with 2 ml water for measurement of the total activity of ^{125}I. The radioactivity of the precipitate was determined after deproteinization with perchloric acid (PCA) and centrifugation. Another sample of blood was transferred to a test-tube with 500 μl water and the sample was stored at —20° C until analyzed by thin-layer chromatography after deproteinization with PCA. The spleen and

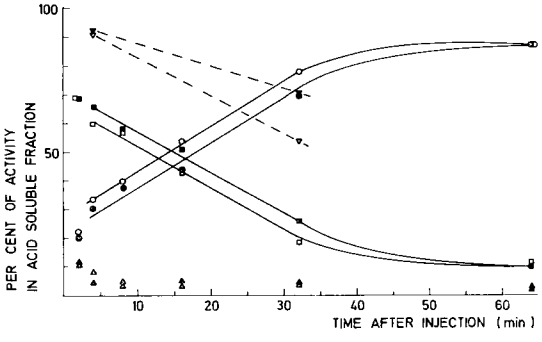

Fig. 4. Percentage of ^{125}I activity in the 'acid soluble fraction' of blood and of peritoneal fluid as ^{125}IUdR, ^{125}IU and ^{125}I$^-$. Blood: ○ 0 rad ^{125}I$^-$, ● 200 rad ^{125}I$^-$, □ 0 rad ^{125}IUdR, ■ 200 rad ^{125}IUdR, △ 0 rad ^{125}IU, ▲ 200 rad ^{125}IU. Intraperitoneal fluid: ▽ 0 rad ^{125}IUdR, ▼ 200 rad ^{125}IUdR.

10 cm of the jejunum were also removed as quickly as possible and the content of the intestine was flushed out with ice-cold isotonic saline. Both organs were frozen on solid CO_2 and kept at —20° C until analyzed. A sample of the peritoneal fluid was stored at —20° C until analyzed by thin-layer chromatography after deproteinization with PCA. The mice in the fluorouracil experiment were killed by cervical dislocation 90 min after injection of ^{125}IUdR and the spleen and 10 cm of the jejunum were prepared as described above.

Extraction and estimation of nucleic acids. Low molecular weight compounds were extracted with ice-cold 0.2 N PCA, the 'acid-soluble fraction', and the nucleic acids with 0.5 N PCA at 90° C for 60 min, the 'nucleic acid fraction'. The extraction procedure has been described in more detail elsewhere (Johansson & Larsson). The DNA was estimated by the diphenylamine method modified by Burton (1956) with calf thymus DNA (Sigma Chem. Co.) as a standard. The absorbency was measured at 600 nm in a Zeiss PMQ II spectrophotometer.

Radiometry. The activity of ^{125}I was measured in a well-type NaI(Tl) crystal with 0.2 μCi ^{125}IUdR in 1 ml solution as a standard. A quantity called 'specific activity' was calculated from

$$\frac{\text{radioactivity recorded (c.p.m.)}}{\text{absorbency at 600 nm}}$$

Thin-layer chromatography. The distribution of radioactive components in the deproteinized blood and peritoneal fluid was analyzed by thin-layer chromatography with Merck TLC-plates silica gel F 254 and isopropanol: chloroform 20/80 (v/v) as the solvent (Fox & Prusoff 1966). Non-radioactive samples were run with IU and IUdR (Sigma Chem. Co.) in parallel to the radioactive

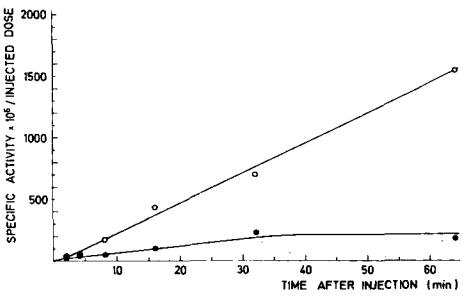

Fig. 5. Relative activity of ^{125}I in the 'nucleic acid fraction' of the small intestine after injection of $^{125}IUdR$. ○ control, ● irradiated.

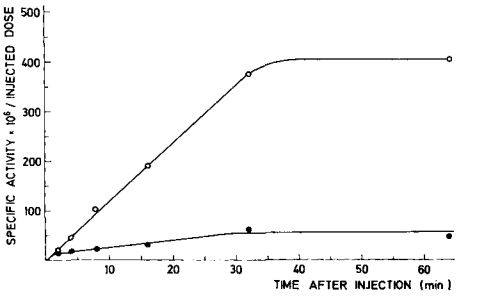

Fig. 6. Relative activity of ^{125}I in the 'nuleic acid fraction' of the spleen after injection of $^{125}IUdR$. ○ control, ● irradiated.

sample. The position of the spots were located under UV light. The chromatography of the catabolic products of $^{125}IUdR$ on thin-layer plates with the solvent system employed permitted excellent separation of the iodide, iodouracil, and iododeoxyuridine. A suitable area was scraped off and its activity of ^{125}I determined.

Results

The appearance of ^{125}I in the blood. The total radioactivity in 100 μl blood at various times after injection appears in Fig. 2. The activity increased up to 32 min and then reached a plateau at about 5 per mille of the injected ^{125}I activity in 100 μl of blood. Assuming that the blood volume of a 27 g mouse be 1.5 ml (BERNSTEIN 1966) the corresponding total activity of ^{125}I in the blood was about 7.5 per cent of the injected dose; about 10 to 15 per cent of this activity was insoluble in 0.2 N PCA (Fig. 3).

The catabolism of $^{125}IUdR$. The activity of ^{125}I in the 'acid-soluble fraction' of blood is mainly in the chemical form of IUdR, IU and I^- (PRUSOFF et coll. 1960, HAMPTON & EIDINOFF 1961). A linear decrease in the activity of the IUdR occurred from 4 to 32 min after injection, 60 per cent remaining unchanged at 4 min and about 20 per cent at 32 min (Fig. 4). The IU concentration was always low and the I^- concentration increased inversely compared with that of the IUdR, indicating a rapid breakdown of IU to I^-, in accordance with previous observations (HUGHES et coll. 1964). About 5 per cent higher radioactivity was evident in the IUdR fraction of the irradiated mice than in the control mice. The ^{125}I activity remaining as $^{125}IUdR$ was higher in the peritoneal

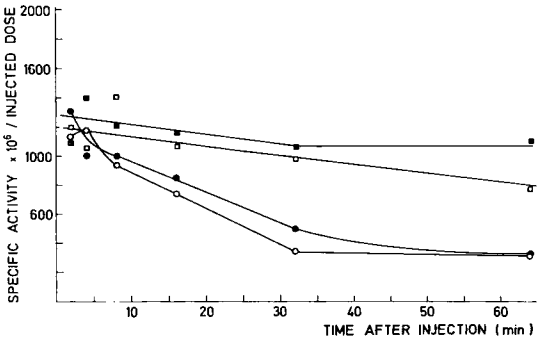

Fig. 7. Relative activity of ^{125}I in the 'acid soluble fraction' from the small intestine and the spleen after injection of ^{125}IUdR. Spleen: ○ control, ● irradiated. Small intestine: □ control, ■ irradiated.

fluid than in the blood. Four minutes after injection, over 90 per cent of the radioactivity was still in the form of IUdR in the peritoneal fluid (60 to 70 per cent in the blood). At 32 min about 70 per cent of the radioactivity in the irradiated mice and 55 per cent in the control mice remained as IUdR (20 per cent in the blood).

Incorporation of ^{125}IUdR into jejunal and splenic DNA. The incorporation of ^{125}IUdR into DNA is presented in Figs 5 and 6. The incorporation in the spleen was almost completed 32 min after injection both in the irradiated and control groups. The rate of incorporation was constant during the first 32 min. The incorporation rate of ^{125}IUdR in the small intestine was also constant, in the irradiated group for 32 min and in the control group for at least 64 min. Only small changes in the relative activity in the 'acid soluble fraction' of the small intestine were present from 2 to 64 min after injection. A 75 per cent decrease in the relative activity in the spleen was recorded during the same period (Fig. 7).

Effects of fluorouracil on the incorporation of ^{125}IUdR into DNA. Fluorouracil increased the incorporation of ^{125}IUdR into DNA in the control group with 54 per cent in the small intestine and 17 per cent in the spleen (see Table) and 2 hours after irradiation with 200 rad with 95 per cent in the small intestine and 42 per cent in the spleen. The results indicate an enhancing effect of fluorouracil on the incorporation of ^{125}IUdR into DNA after irradiation.

Discussion

The route of injection of IUdR influences the period during which the precursor is available for the synthesis of DNA. After an intraperitoneal injection of ^{125}IUdR the maximum concentration of ^{125}I activity in the blood appeared

Table

Incorporation of ^{125}IUdR into DNA during a 90 minute period in the small intestine and the spleen of mice 2 hours after 200 rad with and without fluorouracil (FU) treatment. Mean of 6 mice and standard error (SE) of mean.

	Dose (rad)	'Acid soluble fraction'		'Nucleic acid fraction'	
		Relative activity	SE	Relative activity	SE
Small intestine					
FU treated	0	795	60	1859	321
FU treated	200	862	148	326	64
No FU	0	878	100	1211	315
No FU	200	768	95	167	24
Spleen					
FU treated	0	362	30	230	48
FU treated	200	433	74	34	4
No FU	0	329	34	197	115
No FU	200	286	52	24	5

at 32 min (Fig. 2). A uniform distribution of injected ^{125}I (12.8×10^6 c.p.m.) in the whole mouse would give 47 000 c.p.m. or 0.36 per cent of injected activity in a 100 μl blood sample. The value obtained 64 min after injection was 63 000 c.p.m. (0.49 per cent) in the control and 67 000 c.p.m. (0.52 per cent) in the irradiated mice. There was no significant difference in the ^{125}I-activity in the blood of control and irradiated mice (Fig. 2), a t-test on the 32 and 64 min groups pooled together giving $0.2 > p > 0.1$. A difference of at least 2 000 c.p.m./100 μl blood could be explained by the higher incorporation of ^{125}IUdR into DNA in the control group. The ^{125}I-activity in the blood that was not soluble in the cold PCA (Fig. 3) was probably associated with the plasma proteins and cell fragments. Small amounts of ^{125}IUdR may have been incorporated into DNA of cells which had then entered the blood stream.

Once in the blood stream, IUdR is rapidly catabolized, particularly in the liver. The first step is the cleavage of the nucleoside to iodouracil and deoxyribose (PRUSOFF et coll. 1960). This appears to be a rate-limiting enzymatic reaction (HUGHES et coll. 1964), an assumption corroborated by the present observation that there was only a small amount of iodouracil in the blood (Fig. 4). The next step is the deiodination of iodouracil which begins with reduction to give 5,6-dihydro-iodouracil resulting in spontaneous loss of iodide (BARRETT & WEST 1956, GRISOLIA & CARDOSO 1957, NEWMARK et coll. 1962).

The half-life of ^{125}IUdR in the blood appears to be about 3 min (HUGHES et coll. 1964).

The incorporation of IUdR into intestinal DNA after intravenous injection is thought to be completed within 20 min (HUGHES et coll.). The present results indicate that after an intraperitoneal injection a pool of ^{125}IUdR is produced in the peritoneal fluid and is available for transport via the blood stream. This would increase the period of ^{125}IUdR incorporation into DNA to about 30 min in the spleen and about an hour in the small intestine. It is possible that the periods are still longer after subcutaneous injection, since a higher retention of ^{131}IUdR in mice has been reported after subcutaneous than after intraperitoneal injection (ROTENBERG et coll. 1962).

Only small differences appear to exist in the kinetics of the ^{125}IUdR distribution in the blood, the small intestine and the spleen as well as in the catabolism of ^{125}IUdR in the control and irradiated mice. The differences observed might be explained by the decreased incorporation of ^{125}IUdR into DNA in the irradiated mice.

The results of the fluorouracil experiments (Table) indicate that an increase in the thymidine pool due to breakdown of DNA from dead cells in the small intestine and in the spleen 2 hours after 200 rad ^{60}Co gamma radiation are of minor importance. It is known that fluorouracil blocks the synthesis of thymidine de novo (the conversion of the methyl group in the formation of thymidine) to produce a decrease in the thymidine pool (HEIDELBERGER et coll. 1957, DANNEBERG et coll. 1958). It seems reasonable that the incorporation of ^{125}IUdR into DNA would be less in fluorouracil-treated mice than in control mice if there were a flow of thymidine from dead cells into the DNA synthesizing cells.

An overproduction of precursor for DNA may of course occur in each cell due to a disturbed balance between synthesis of the precursor and its incorporation into DNA.

SUMMARY

The effect of 200 rad ^{60}Co gamma radiation on the kinetics of the distribution and catabolism of ^{125}IUdR was investigated in mice. No significant changes were observed. Fluorouracil injected before ^{125}IUdR increased its incorporation into DNA. This increase was more marked at two hours after irradiation, indicating that changes in the thymidine pool due to flooding of thymidine from dead cells are of minor importance.

ZUSAMMENFASSUNG

Die Wirkung von 200 rad ^{60}Co Gamma-Strahlung auf die Kinetik der Verteilung und des Katabolismus von ^{125}IUdR wurde bei Mäusen untersucht. Es wurden keine signifikanten Veränderungen beobachtet. Fluorouracil, das vor ^{125}IUdR injiziert wurde, steigerte dessen

Einbau in die DNS. Dieser gesteigerte Einbau war zwei Stunden nach Bestrahlung stärker ausgeprägt, was darauf hinweist, dass Änderungen im Thymidin-Pool durch Einstrom von Thymidin von getöteten Zellen von untergeordneter Bedeutung ist.

RÉSUMÉ

L'auteur a étudié l'effet de 200 rad de radiation gamma du ^{60}Co sur la cinétique de la distribution et sur le catabolisme de ^{125}IUdR sur des souris. Il n'a pas observé de modification significative. Le fluorouracile injecté avant la ^{125}IUdR augmente son incorporation dans l'ADN. Cette augmentation est moins marquée deux heures après l'irradiation, indiquant que les modifications du pool de thymidine dues à l'inondation par la thymidine provenant des cellules mortes sont d'importance mineure.

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