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EFFECT OF WHOLE BODY IRRADIATION ON DIFFERENT TISSUES

Experiments with ^{14}C leucine in the rat

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The uptake of a labelled aminoacid and the subsequent elimination of the tracer in normal and irradiated tissues of mammals has been compared for evaluating alterations that lead at the biochemical level to radiation injury. The few references found in the literature about this subject mostly concern the small intestine (LIPKIN & QUASTLER 1962, LIPKIN et coll. 1963, EDWARDS et coll. 1964, SASSEN et coll. 1965, REUTER et coll. 1967, ALTMANN 1976). Preliminary experiments were performed to determine a possible difference in uptake of labelled aminoacid, injected at different hours of the day. Subsequently, at the time of maximum uptake thus found, the labelled molecule was injected and its uptake and elimination observed through the following 72 hours.

These experiments were repeated in animals whole-body irradiated 2 h before injection of ^{14}C leucine. A dose of 8 Gy appears to be the maximum dose that causes an intestinal sublethal syndrome (BECCIOLINI et coll. 1972) and was therefore used. The small intestine, kidney, skin, plasma and parotid gland (CREMONINI et coll. 1979) were examined.

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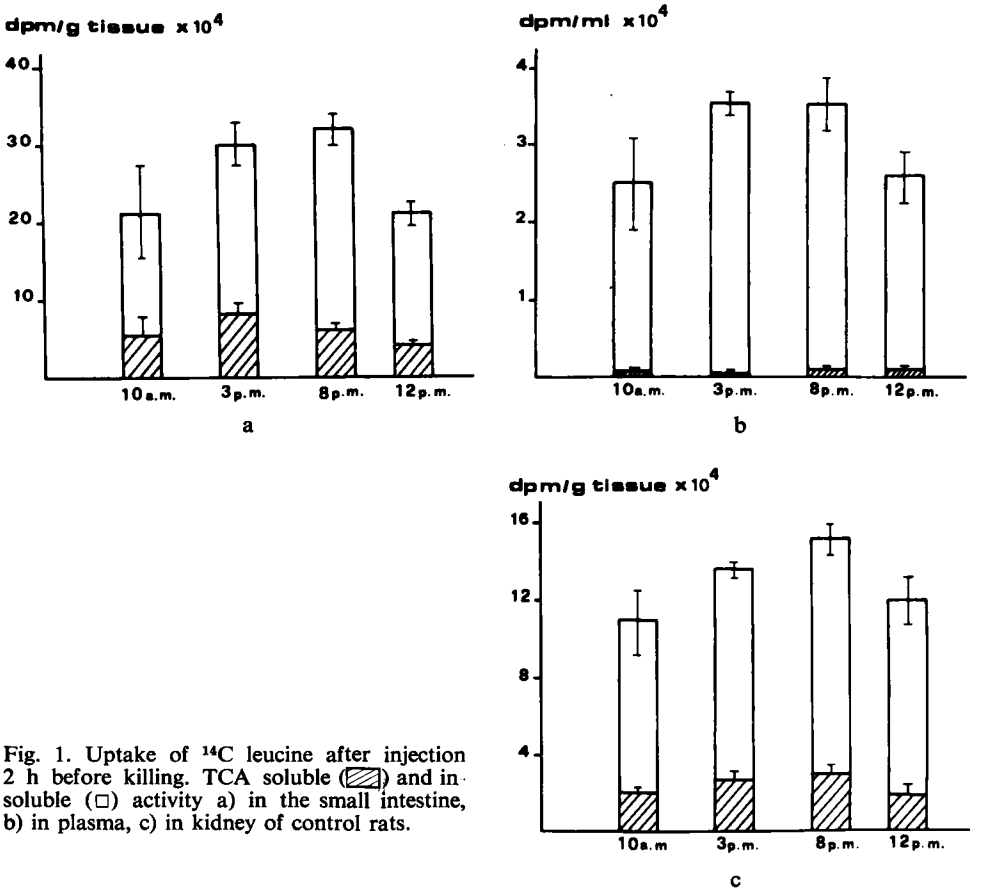

Fig. 1. Uptake of ^{14}C leucine after injection 2 h before killing. TCA soluble (▨) and insoluble (□) activity a) in the small intestine, b) in plasma, c) in kidney of control rats.

Material and Methods

A total of 92 female Wistar rats, 10 to 12 weeks old and weighing 170 to 180 g, were used. Before and during the experiment the animals were kept at a light/darkness (L/D) cycle, 6.30 a.m.:6.30 p.m., and fed ad libitum with standard diet.

The animals were injected intraperitoneally with U^{14}C leucine (Radiochemical Centre, Amersham, England, specific activity 10 μCi/mmol (370 kBq)). This route of administration (unlike the oral, intraluminal, etc. routes) guarantees homogeneous uptake of labelled aminoacid in the small intestine and in the other tissues examined (ALPERS 1972).

For the preliminary experiment 12 rats were used to determine the different uptakes of the ^{14}C leucine during the 24 h cycle: groups of 3 animals were injected with 3 μCi (111 kBq) of labelled aminoacid respectively at 8 a.m., 1 p.m., 6 p.m. and 10 p.m., and killed 2 h later.

A second group of 40 animals, sham irradiated, was used for the determination

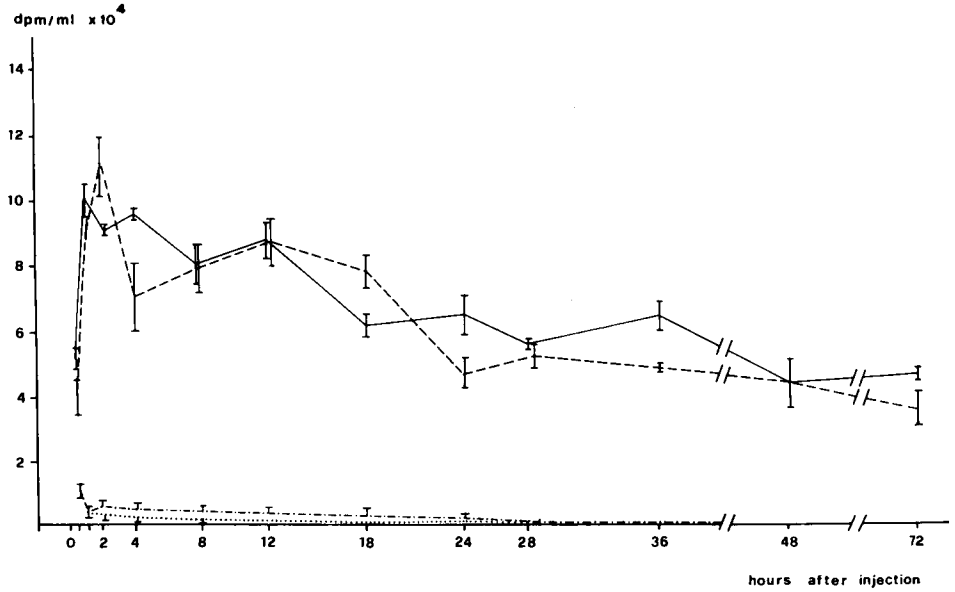

Fig. 2. Plasma activity at different times after injection of ^{14}C leucine in controls and in animals irradiated 2 h before injection. TCA insoluble fraction: controls (——), irradiated animals (– – –); TCA soluble fraction: controls (—·—), irradiated animals (· · · ·).

of uptake and elimination of the tracer. Between 5.30 and 6.30 p.m., 7 μCi were injected and groups of 3 to 4 animals were killed at 0.5, 1, 2, 4, 8, 12, 18, 24, 28, 36, 48 and 72 h after injection.

A third group of 40 animals was irradiated with γ rays from ^{60}Co to a dose of 8 Gy between 3.30 p.m. and 4.30 p.m. ^{14}C leucine was injected 2 h later and the animals killed at the same intervals as the controls.

The rats were killed under ether anaesthesia, by heart resection after blood sampling from the left ventricle. The small intestine, sectioned into 5 equal parts, kidneys, parotid glands and some areas of clean shaven dorsal skin were removed. Every tissue was washed with cold saline (NaCl 0.9 %) and then kept at $-25°C$ until the activity assay.

For the autoradiographic observations parts of left kidneys, pieces of skin and two little segments of the small intestine, corresponding to the proximal and distal jejunum, were cut off and fixed in Carnoy. A Kodak NTB2 liquid emulsion was used for the sections and developed after 10 days.

Different tissues were homogenized in a Potter-Elvejhem homogenizator, in an ice bath, with distilled water. The five tracts of small intestine were homogenized at 10 per cent w/v, and the kidney and skin at 5 per cent.

Parts of each homogenate or plasma were homogenized a second time with an equal volume of trichloroacetic acid (TCA) at 10 per cent and then centrifuged. TCA soluble activity was assayed in the supernatant by using Bray's mixture (BRAY

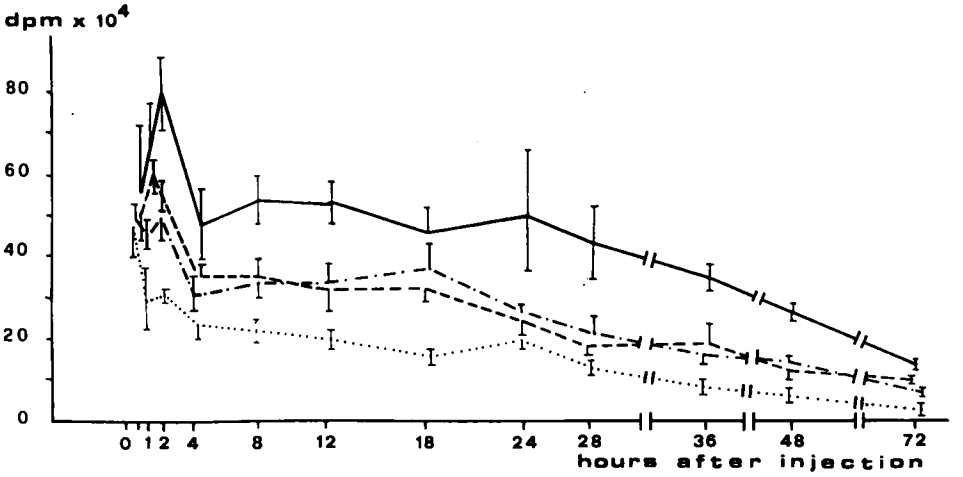

Fig. 3. Soluble and insoluble TCA. Activity in the whole small intestine in irradiated and control animals, total dpm (symbols as in Fig. 2).

1960) in a liquid scintillator. The TCA insoluble fraction was assayed in the sediment, washed twice with cold TCA and dissolved with NaOH 1N. The assays were performed in duplicate. The activity at different times was expressed as dpm/g tissue and as dpm/ml for plasma. Student's t-test was used to evaluate statistical significance.

Results

The preliminary experiments demonstrated that the uptake of ^{14}C leucine in the TCA insoluble fraction increased from 10 a.m. to 8 p.m. (Fig. 1) in all tissues examined. The TCA soluble fraction showed a similar behaviour with a maximum at 3 p.m., even if the TCA insoluble/TCA soluble ratio was different in the tissues.

Plasma activity. The highest levels of tracer bound to low molecular weight compounds appeared 30 min after injection while at the 1 h interval they were reduced to about 30 per cent (Fig. 2).

At subsequent times the values gradually decreased reaching very low levels at the end of the experiment. The TCA insoluble fraction increased sharply within 1 h after injection; after that the activity gradually decreased. At 72 hours the values appeared to be about 60 per cent of the maximum level.

In irradiated animals the TCA insoluble fraction reached the highest level 2 h after injection and was reduced at 4 hours. After a new increase the curves of control and irradiated animals overlapped although most control levels were generally higher.

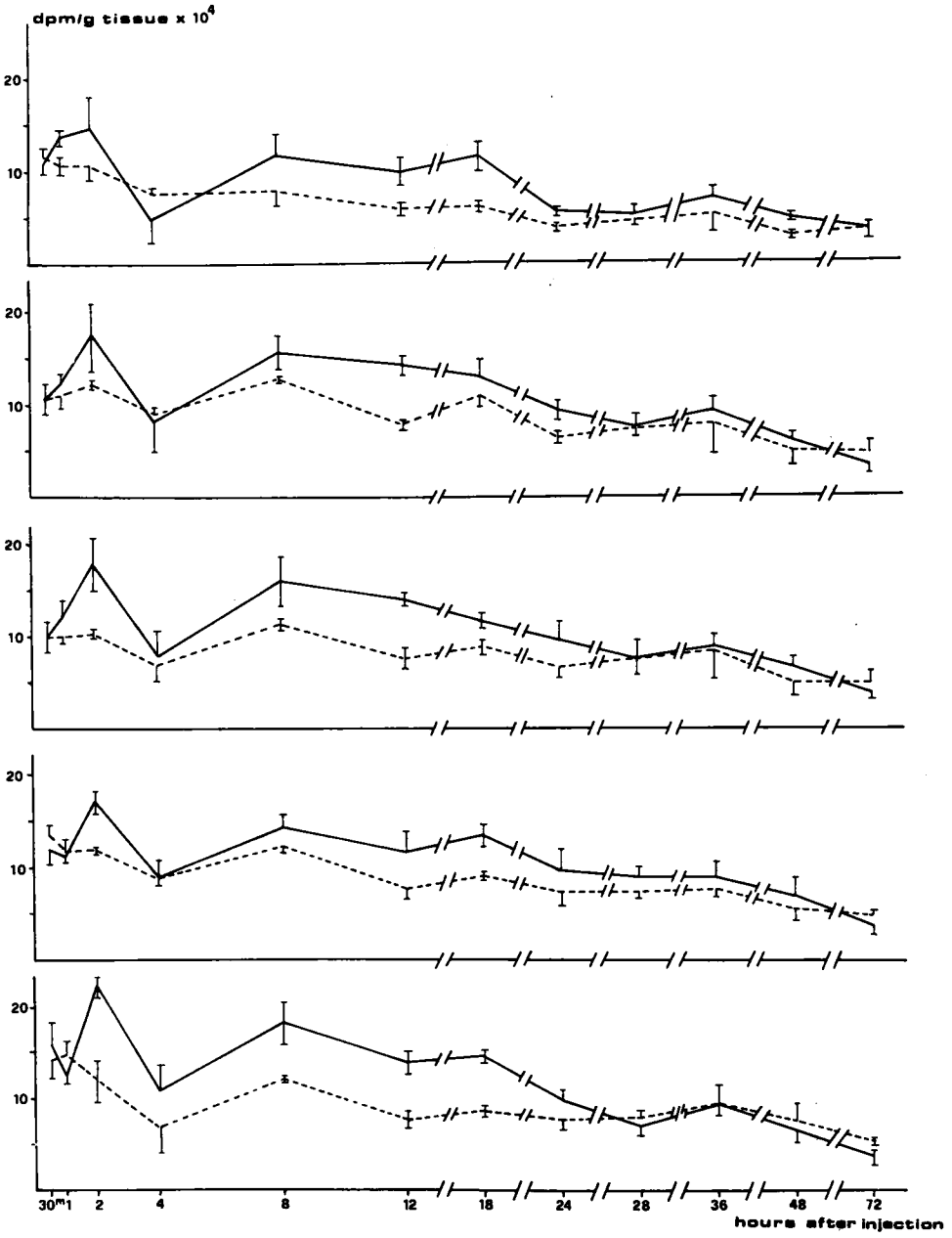

Fig. 4. TCA insoluble fraction as dpm/g tissue in each of the 5 segments of the small intestines (duodenum at the top) in control (——) and irradiated (---) animals.

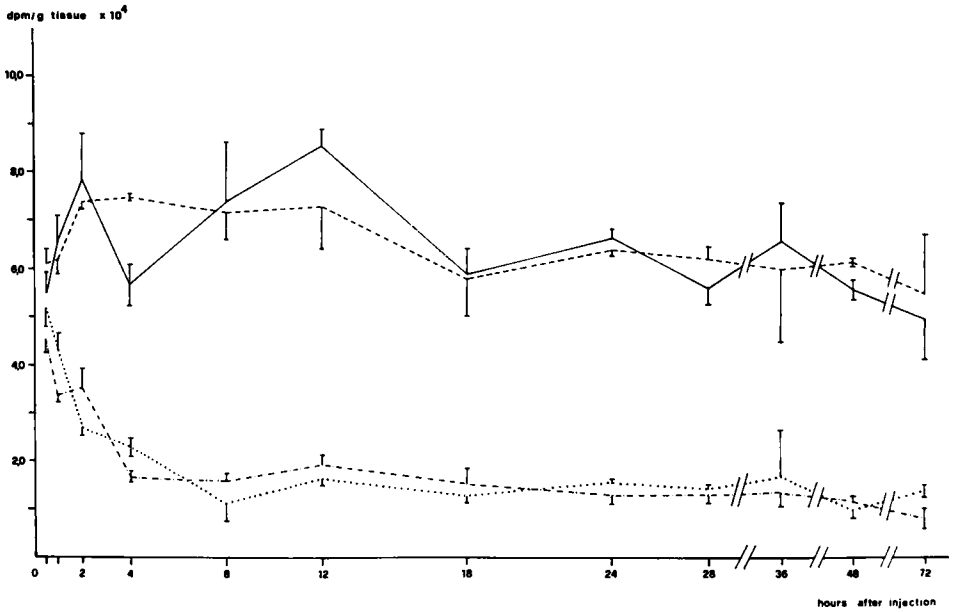

Fig. 5. TCA, soluble and insoluble fraction. Activity in the kidneys in control and irradiated animals, dpm/g tissue (symbols as in Fig. 2).

Small intestine. The total activity, expressed as dpm × weight of the whole small intestine, is given in Fig. 3. The TCA soluble fraction appeared to be higher than in other tissues. In the controls the activity reached a peak within 2 h, then decreased rapidly at 4 h and slowly after 18 hours. In the irradiated animals the TCA soluble fraction was significantly lower than in the controls and decreased progressively after the first interval.

The uptake of labelled aminoacid in the TCA insoluble fraction was very fast in the controls and reached the highest level 2 h after injection. After a reduction at 4 h, the levels appeared constant until 28 h and then decreased progressively. In irradiated animals the behaviour was similar but the highest level of activity appeared 1 h after injection.

The activity after irradiation was always lower than in the controls and this decrease appeared to be statistically significant at some intervals. The differences were more evident at longer intervals.

Fig. 4 shows the TCA insoluble activity expressed as dpm/g of tissue in each of the five segments of the small intestine. In this way, weight variations observed at longer intervals after irradiation were excluded. The curves appear similar to those reported in Fig. 3.

The differences between the five segments may be summarized as follows: (a) the value at 1 h after irradiation was higher than at 30 min in the first segment,

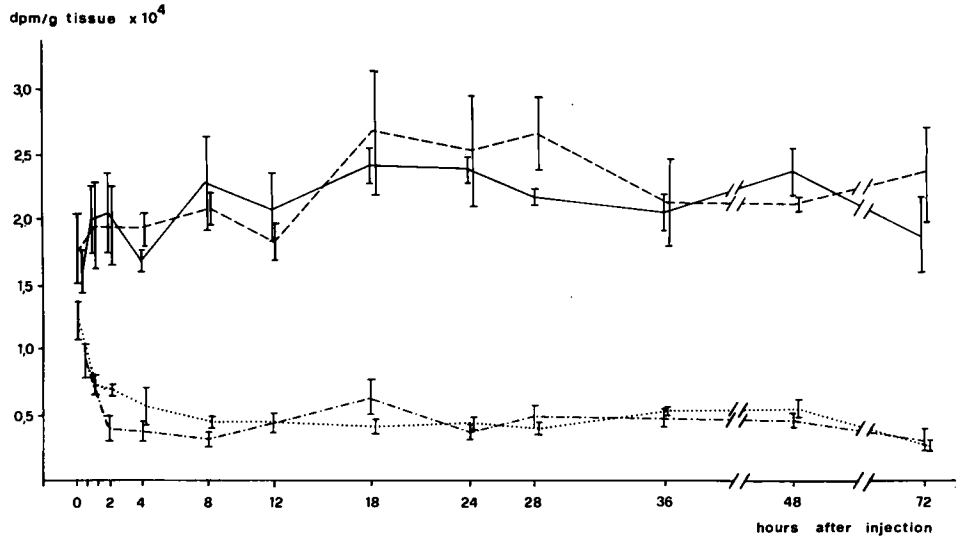

Fig. 6. Soluble and insoluble TCA. Activity in the dorsal skin in control and irradiated animals, dpm/g tissue (symbols as in Fig. 2).

gradually decreasing from the duodenum to the terminal ileum, and (b) activity levels in the first segment were lower than in the fifth.

In irradiated animals the first increase of the TCA insoluble fraction was not evident. The curves appeared flattened and gradually became smoothed from the first to the last time of killing. This general behaviour was evident and most constant in the first segment while in others some fluctuations were observed. The comparison of activity levels between control and irradiated animals showed a significant reduction at some intervals; the highest differences were observed in the last segment. Control and irradiated animals varied moderately from 24 h onwards.

Kidney. The TCA soluble activity in the kidneys (Fig. 5) was markedly reduced from 30 min to 4 h after injection, with a gradual reduction down to about 25 per cent of the maximum level at the last recording. The TCA soluble activity in irradiated animals fluctuated slightly but in a similar way. The TCA insoluble fraction gradually increased until 2 h after injection and after reaching a minimum at 4 h increased again at 12 hours. After that it decreased gradually.

In the irradiated animals fluctuations at 4 and 12 h after injection were not observed; at these intervals the activity reached the highest level; after that, a moderate reduction occurred similar to that observed in the controls.

Skin. The behaviour of the TCA soluble activity was similar to that in the kidneys (Fig. 6). Control levels sharply decreased within 2 h and then remained constant

until the end of the experiment. The behaviour of the irradiated animals overlapped that of the controls.

The TCA insoluble activity in the controls had a maximum at 2 h and a minimum at 4 h, like other tissues. Later the activity increased and remained constant until the end of the experiment. The behaviour of the irradiated animals overlapped that of the controls.

Discussion

The uptake of a labelled aminoacid and elimination of the tracer after injection in control and irradiated animals were compared in tissues with different degrees of cellular proliferation and protein synthesis. The tissues behaved in different ways. Highest levels of TCA insoluble activity were reached in the small intestine (where protein synthesis and proliferation are high) and in parotid glands (where protein synthesis is high even if proliferation is poor) (CAMERON & THRASHER 1971); kidneys, skin and plasma showed lower levels of activity. Plasma labelled molecules reach all cellular compartments although plasma proteins are continuously synthetized in the liver. In every tissue part of the tracer may be used for the synthesis of new molecules. This phenomenon is particularly evident in the small intestine. Protein synthesis in the epithelial cells occurs (a) in the proliferative compartment (crypts) to produce macromolecules for new cells, (b) in the differentiating compartment (upper part of the crypt) (ALPERS & KINZIE 1973) to synthetize—among the other molecules—brush border enzymes, and (c) in the differentiated compartment (villus) for the turnover of brush border enzymes and other proteins. These molecules seem to be replaced more than once before reaching the lumen. Besides, it must be noted that the turnover of the epithelial cells in rats is about 36 hours (LEBLOND & WALKER 1956).

When cells are extruded in the lumen, their proteins and other macromolecules are hydrolized by pancreatic enzymes and absorbed as aminoacids or other monomeric forms in the distal tracts. This process may explain the highest levels of activity observed in the terminal ileum.

The increase of TCA insoluble fraction observed in all tissues until 2 hours after injection may be explained by the high initial level of TCA soluble activity and by its rapid reduction. In the kidney and skin no marked differences occurred between the controls and the animals irradiated 2 h before injection, when the tracer is available for cells where biochemical injury is appearing. In irradiated intestine, on the other hand, activity appears generally reduced in a significant way.

These results were confirmed by autoradiographic observations. In kidney and skin no differences appeared in the distribution of activity in the irradiated and control animals, and the morphologic alterations appeared moderate. In the small intestine of the controls the activity appeared uniformly distributed in the whole crypt-villus system at all intervals. Heavy cellular alterations such as inhibition of mitotic activity, pyknosis, karyolysis and karyorrhexis phenomena were observed

in sections of irradiated animals, beginning at the first interval. Autoradiographic observations confirmed a reduced uptake of the tracer in the crypts while in the villus the distribution of activity appeared normal. At subsequent intervals the whole epithelium appeared injured and formed by cells reduced in number and with altered morphology. These alterations might explain the reduction of tracer uptake, assayed in the homogenate.

When the activity is expressed as dpm/g of tissue or dpm/g of protein, levels between control and irradiated animals appear similar: these results may indicate a still active protein synthesis in irradiated cells of the small intestine.

The results indicate that a dose of 8 Gy administered 2 h before injection of ^{14}C leucine does not induce differences in uptake and elimination of tracer in tissues with low proliferative activity and turnover.

In the present experimental conditions, in accordance with those of LIPKIN et coll., the protein synthesis in highly differentiated tissues showed low sensitivity to radiation, contrary to tissues with high proliferative activity. In the latter tissues, the sensitivity is higher, which might be a consequence of the irradiation effect on the proliferative compartment.

SUMMARY

The uptake and elimination of ^{14}C leucine were analysed in controls and in rats irradiated 2 h before injection with 8 Gy whole-body irradiation. Plasma, small intestine, kidney and skin were assayed after homogenization for TCA soluble and insoluble activity curves. In highly differentiated tissues with poor proliferative activity and low protein turnover, the uptake and elimination of the tracer did not appear to be affected by irradiation. In the small intestine differences between control and irradiated animals seemed significant.

ZUSAMMENFASSUNG

Die Aufnahme und die Ausscheidung von ^{14}C Leucin bei Kontrollen und Ratten, die 2 Stunden vor der Injektion Ganzkörperbestrahlung mit 8 Gy erhielten, wurden analysiert. Plasma, Dünndarm, Niere und Haut wurden nach Homogenisierung hinsichtlich TCA löslicher und unlöslicher Aktivitätskurven analysiert. In hoch differenzierten Geweben mit niedriger Proliferationsaktivität und niedrigem Proteinumsatz, war die Aufnahme und die Ausscheidung des Tracers nicht durch Bestrahlung beeinflusst. Im Dünndarm waren die Unterschiede zwischen Kontrollen und bestrahlten Tieren signifikant.

RÉSUMÉ

Les auteurs ont étudié la fixation et l'élimination de la ^{14}C leucine chez des rats témoins et des rats irradiés par une irradiation corporelle totale avec 8 Gy deux heures avant l'injection. Les courbes d'activité de TCA solubles et insolubles ont été établies après homogénéisation pour le plasma, l'intestin grêle, le rein et la peau. Dans les tissus hautement différenciés qui ont une activité proliférative faible et un métabolisme protéique faible, la fixation et l'élimination du traceur ne semblent pas modifiées par l'irradiation. Sur l'intestin grêle les différences entre les rats témoins et les rats irradiés ont paru significatives.

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