Dose dependence and cause of X-Irradiation induced protein loss in rats.

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Introduction

Whole body X-irradiation in the dose range between 1 to 10 Gy causes significant cell death in radiosensitive organs and degradation of the dead cells during the first 24 h after radiation in the rat. Protein loss occurs, however, until 3 days after irradiation. It was the purpose of a series of studies to elucidate the mechanism and the dose dependence of this phenomenon using rats as a model system. Nitrogen intake by food, nitrogen excretion in urin and feces, amino acid levels, protein synthesis and protein degradation by enzymes were investigated. The spleen and intestinal wall were studied as radiosensitive organs, the intestinal content to monitor protein loss into the intestinal cavity, the liver as radioresistant organ, the blood plasma as mirror of protein transfer within the body, the blood erythrocytes as indicators of the functional state of the hematopoietic system and the remaining carcass of the body in order to be able to add up all compartments of the body. The addition permits to compare the significance of the metabolic alterations in the individual organs after irradiation with regard to the whole body. Rats of 100±5 g body weight at the time of irradiation were taken for all experiments to have well standardized organ weights and biochemical parameters.

1. Results of experimental studies and discussion

The nitrogen liberation by cell death during the first 24 h in the radiosensitive organs as intestinal mucosa, hematopoietic system, gonad and skin is functionally important but quantitatively minor (2 to 3 % of whole body nitrogen) and provides no explanation for the 20 to 25 % nitrogen loss of the total body 3 days after 6 Gy. Nitrogen loss into the intestinal cavity of an unirradiated animal was approximately 1.5 % of total body nitrogen per day. The loss was not substantially increased during the first three days after irradiation up to doses of 10 Gy. The concentration of free amino acids was increased in the intestinal content, the blood plasma and the liver, but otherwise unaltered during the first days after irradiation. The protease activity between pH 2 and 8.5 was measured against hemoglobin as substrate, but also against the accompanying protein in the tissue extracts (autolytic activity) (1). The concentration of the protease activity in the surviving cells was increased on the first day after irradiation in the liver (2), but unaltered or decreased on days 2 and 3 in the liver and the other organs (3,4). The decrease of the protease activity in the erythrocytes coincided with the disappearance of reticulocytes and the reappearance of the activity with the production of a macrocytic erythrocyte population (5,6,7) which is similarly released in strongly bled animals (8,9). The esterase activity concentrations in spleen, liver and blood plasma between pH 5 and 8.5 were slightly (10 to 30 %) increased (10,11,12,13). The total cellular or organ activity of protease and esterase was, however, always diminished, i.e. the digestive capacity of the surviving cells was decreased. The diminution of protease activity was less than the decrease of body protein. This means that the body lost preferentially non-protease and non-esterase protein. Due to the decrease of the cellular protease activity, it is unlikely that the protein loss between days 1 to 3 after irradiation is explained by proteolytic degradation. Food intake was diminished

uring the first days after irradiation. his did not lead to decreased amino cid concentrations in the blood and rgans (2,3,4) indicating that also a ack of amino acids as supply for pro-ein synthesis was not the reason for rotein loss.

ince neither low food intake, lack of mino acids, increased protease or sterase activity nor increased nitro-en loss through feces or urin did eadily explain the protein loss, it was kely that a decrease of protein syn-hesis was the cause for the diminution f body proteins. Protein synthesis as indeed significantly depressed in pleen, intestinal wall, carcass, ery-hrocytes (disappearance of reticulo-ytes) during the first 6 to 14 days fter 6 Gy irradiation (Fig. 1a). An ncreased or a normal protein synthesis as observed in the liver (Fig. 1c). he protein synthesis in the intestinal all was also increased (Fig. 1c) after transient dip on days 1 and 2 after radiation. The protein transfer into ne blood plasma was significantly in-reased during days 1 to 30 after irra-iation (Fig. 1c). The influences of radiation on protein synthesis were ose dependent (Fig. 2a,c). Despite ne increased intestinal protein syn-nesis, the unaltered liver protein ynthesis and the increased protein ransfer through the blood plasma Fig. 2c), the total body protein ynthesis was very significantly epressed (Fig. 2a). This was not due o a lack of amino acids, since amino cid concentrations in all organs were ther unaltered or increased (Fig. o,d and Fig. 2b,d). The rat synthe-zes a great amount of protein per ay (Tab. 1). Most of this protein is hort lived and quickly degraded be-ause otherwise the total protein con-nt of the rat would quickly increase. he fast protein turnover can be ppreciated from the fast incorporation f the radioactive precursor amino acid ^{3}H-leucin into acid precipitable mate-al (Fig. 3) which is completed within ss than 10 min after intravenous in-ction of ^{3}H-leucin. The decrease of the fast turning protein metabolism leads over several days to the gradual decrease of total body protein. The decrease of total body protein to 70 % of the original values three days after irradiation with 10 Gy (Fig.4) is paralleled by a significantly greater reduction of protein synthesis to 45 % of the initial values (Fig. 2a). The quantitively most important contribution to the total body protein synthesis comes from the carcass (56.8 %) (Tab. 1). This explains why the decrease of protein synthesis in the carcass cannot be counterbalanced by the increase of protein synthesis in the liver and the intestinal wall. The latter organs account for 17.2 % and 21.0 % of the total protein synthesis in the unirradiated animal.

Conclusions

The conclusion from all experiments is that the major reason for protein depletion of the organism after irradiation is due to the reduction of protein synthesis. The reason for this decrease is not apparent, but there is no evidence for the lack of precursor amino acids.

If one asks which of the investigated parameters could be suitable for biological radiation dosimetry in humans, the answer is that all the parameters of this study are not well suited for dosimetry. Esterase and protease activities in serum are low and not dramatically altered after irradiation (except for the autolytic protease activity). Similar considerations apply to the protein and amino acid concentration in the blood plasma. The transfer of protein into the blood plasma is increased during the first days after irradiation in a dose dependant way but injection of radioactive or otherwise labeled amino acid precursor, and analysis of the circulating plasma protein after a lapse of time are necessary to detect the increased release of labeled protein. Provided this would be feasable in humans, the problem as to whether an

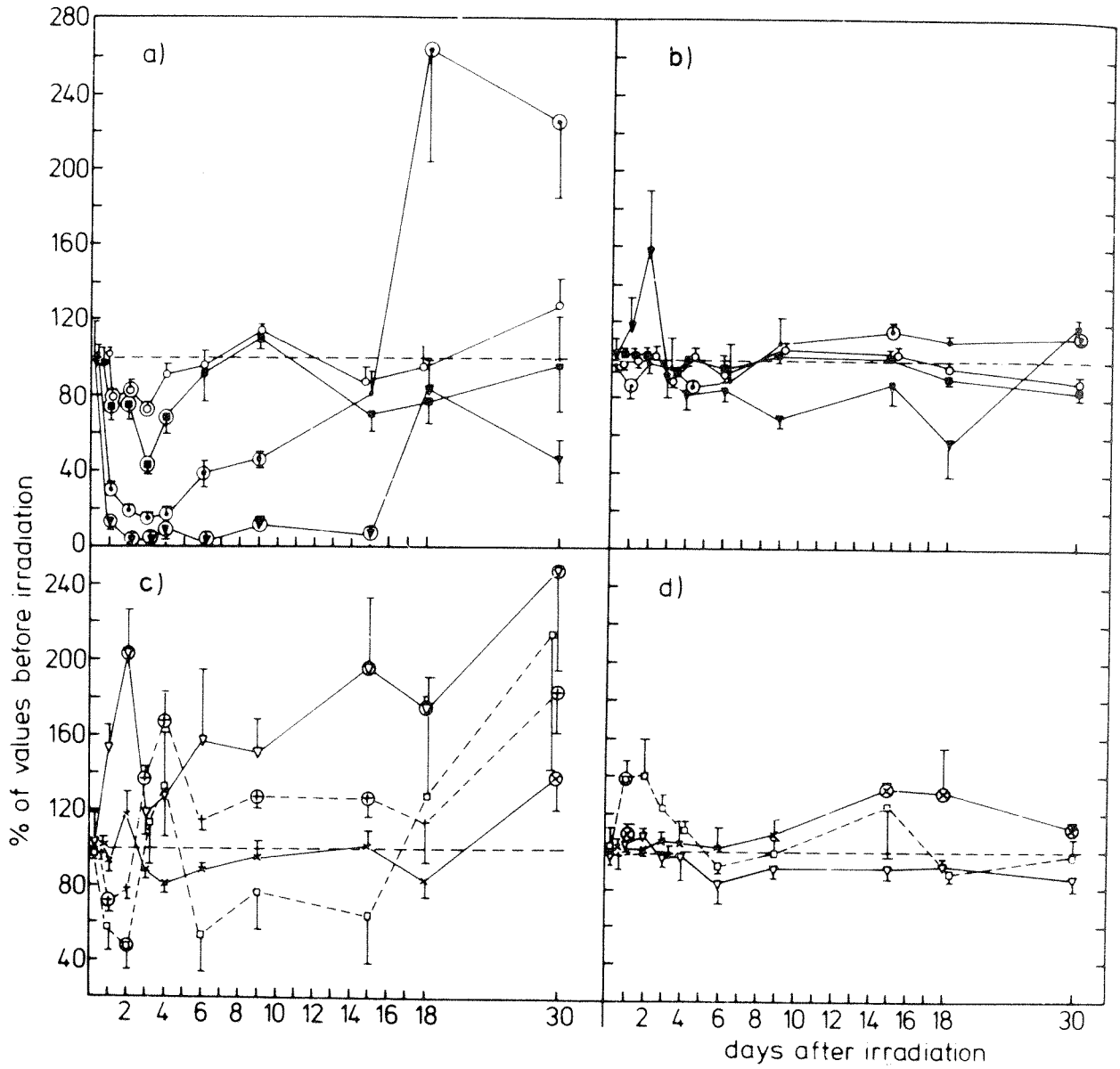


Fig. 1: Protein synthesis (a,c) and amino acid concentration (b,d) in the spleen (.), the erythrocytes (▼), the carcass (■), the total organism (o), the liver (x), the blood plasma (▽), the wall (+) and content (□) of stomach and intestins of 6 Gy (600 rd) whole body X-irradiated (280 kV, 1 mm Cu) female Sprague-Dawley rats. Each point represents the mean of 10 experiments on days 0, 1 and 3, and the mean of 5 experiments on the other days. One rat was used per experiment. Symbols which are encircled in this and the following graphs mean that the corresponding data points deviate significantly ($2p<0.05$) from the control values before irradiation.

increased protein release into the blood plasma is specific for radiation injury, remains still to be elucidated.

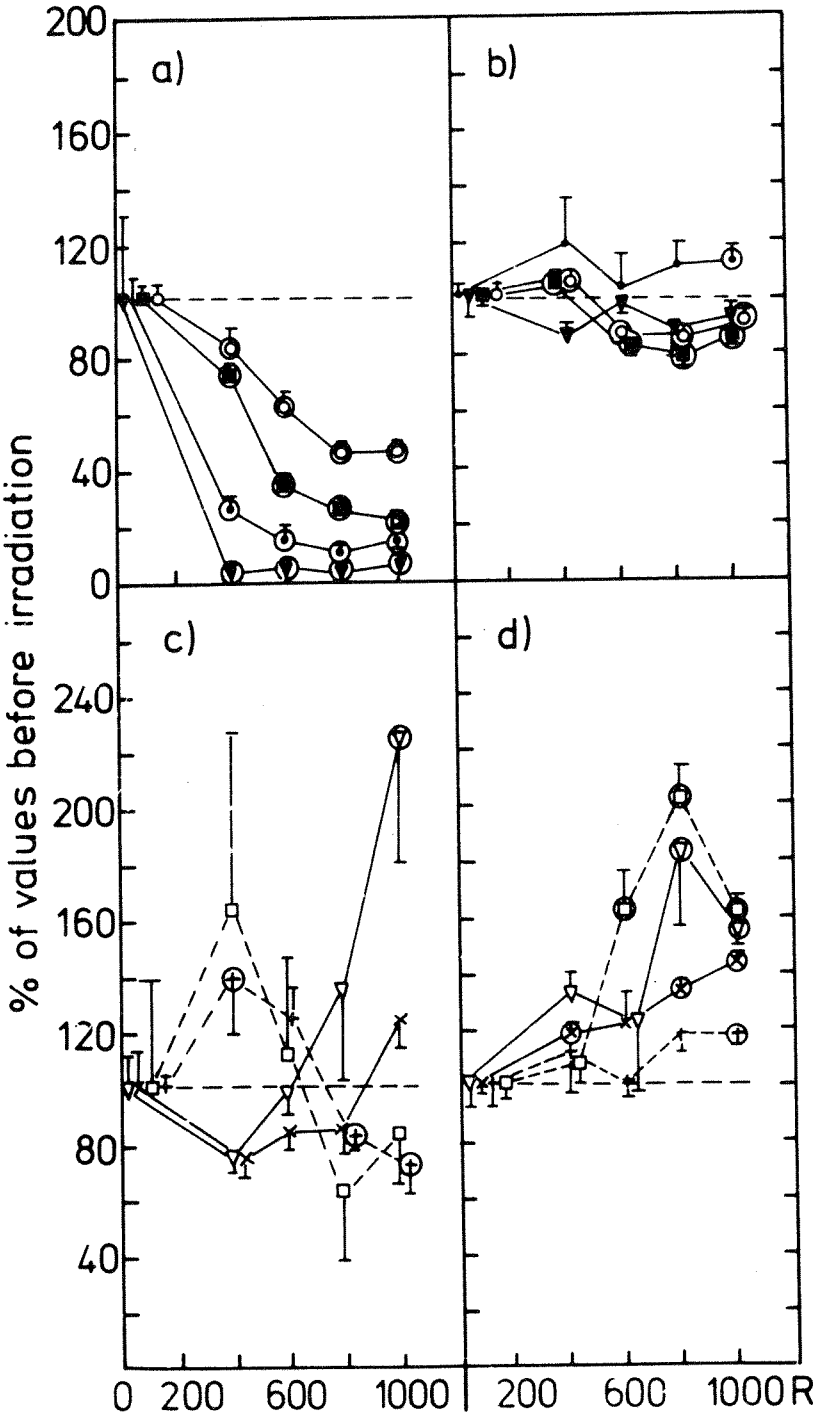


Fig. 2: Protein synthesis (a,c) and amino acid concentration (b,d) in different organs of the rat 3 days after whole body X-irradiation with 4 to 10 Gy (400 to 1000 rd). Symbols are as in Figure 1. Each point represents the mean of 5 experiments with one rat per experiment.

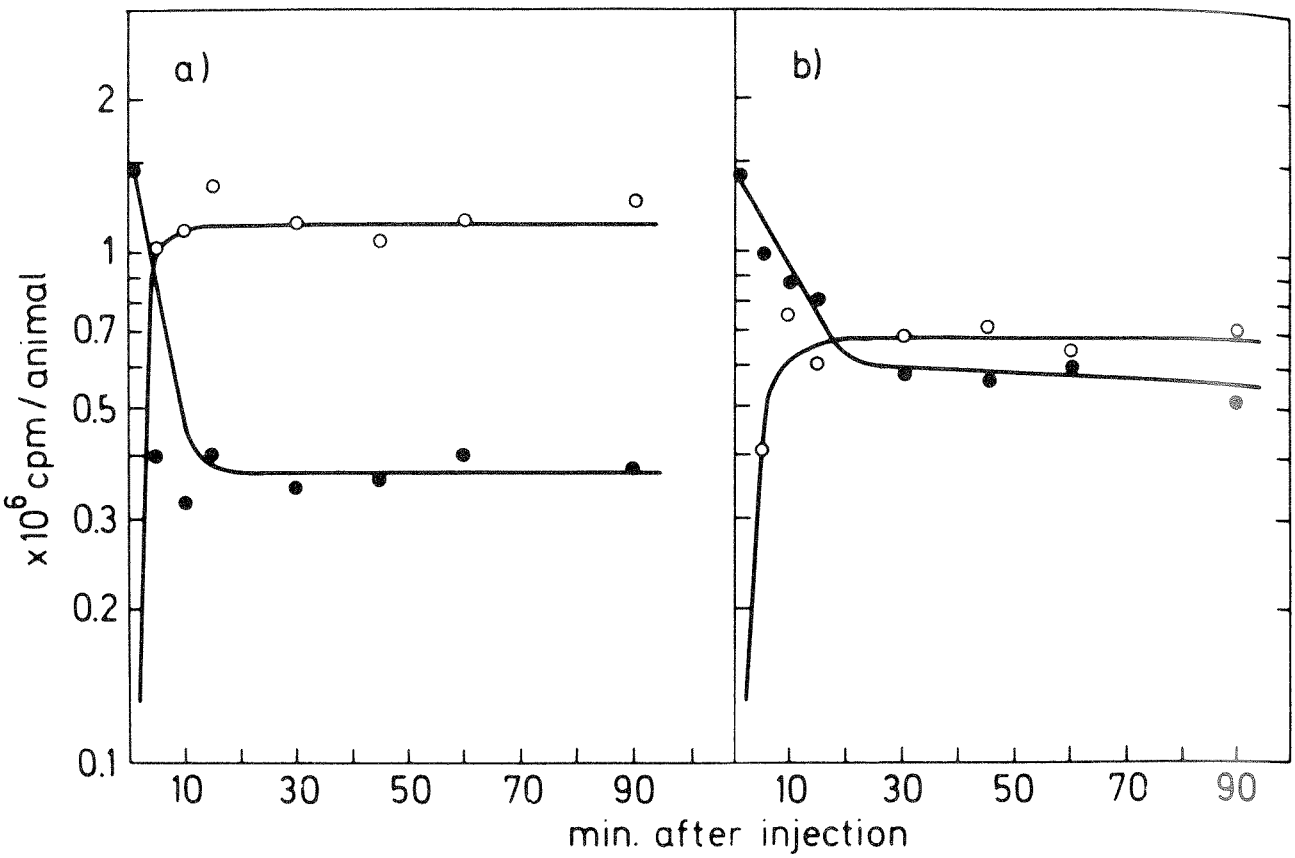
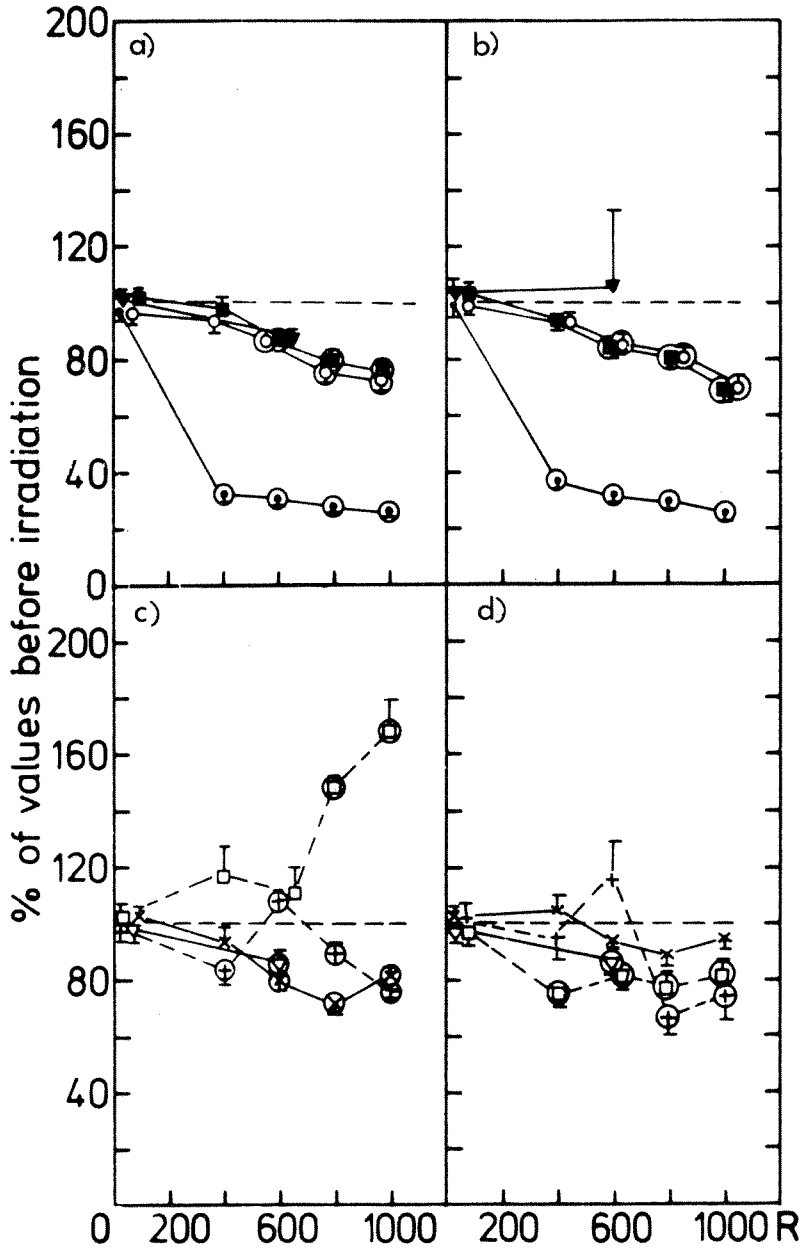


Fig. 3: Time-dependent incorporation of ^{3}H-l-leucine (148 kBq) into total body protein of rats before (a) and 3 days after whole body X-irradiation with 10 Gy (1000 rd) (b). The time course of incorporation of leucine into acid precipitable material (o) is complementary to the disappearance of free leucine radioactivity (●) in both conditions. The amount of incorporated ^{3}H-leucine is lower in the irradiated animal.



g. 4: Organ weight (a,c) and organ nitrogen (b,d) in different organs of the rat three days after whole body X-irradiation with 4 to 10 Gy (400 to 1000 rd). Symbols are as in Figure 1 and animals as in Figure 2.

Table 1: Weight, protein synthesis, nitrogen content, free amino acids and free ^{3}H-leucine in different organs of 100 g rats prior to X-irradiation

	n[4]	spleen	liver	blood plasma[3]	erythro cytes[3]	stomach and intestine wall	stomach and intestine content	carcass	total rat
weight (g) or volume (ml)[1]	10	0.32±0.02	4.86±0.38	2.23±0.15	1.11±0.15	5.87±0.59	6.89±1.30	76.0±2.9	100 ±2
^{3}H-l-leucine protein bound (cpmx10^3)[2]	10	9.88±1.97	206±62	54.5±11.6	6.09±3.48	246±37	14.3±8.1	678±143	1188±138
organ distribution of synthesized protein (% of total protein bound ^{3}H-l-leucine)	10	0.84±0.20	17.2±4.0	2.43±0.55	0.26±0.17	21.0±4.7	1.24±0.76	56.8±8.5	99.8±0.9
protein synthesized or secreted									
(mg/min)	10	0.11	2.34	0.33	0.04	2.86	0.17	7.73	13.6
(mg/h)		6.85	140	19.8	2.12	171	10.1	463	816
(mg/24h)		164	3371	476	50.9	4116	243	11132	19600
nitrogen content (mg)	10	9.01±0.75	142±11	15.7±1.3	44.7±3.7	158±29	75.2±19.0	1593±246	2008±259
free amino acids (µMol)	10	21.0±1.8	199±16	7.03±0.49	7.18±1.62	426±41	203±62	3479±568	4349±500
free ^{3}H-l-leucine (cpmx10^3)[2]	10	1.32±0.40	20.3±3.9	15.8±0.7	0.38±0.48	38.7±2.55	18.9±3.9	227±64	325±70

1 Blood plasma, erythrocytes, stomach and intestine content in ml/100 g rat

2 Means from the 15, 30, 45, 60 and 90 min experiment except for the blood plasma and intestine content where the protein bound radioactivity does not plateau. The mean of the 45, 60 and 90 min experiment was calculated in this case. cpm = count per minute

3 All data in these colums are given for one half of the total blood plasma and the total erythrocyte volume because only half of the total amounts can be removed by bleeding until respiratory arrest (4). The other half remains in the body and is accounted for in the other organs.

4 Number of experiments, one animal per experiment

ummary

. significant loss of protein (10-30 %) ccurs in rats between 1 and 3 days fter whole body X-irradiation in the ose range of 1 to 10 Gy although rotein loss due to death of radiosen- itive tissues amounts to only 2 or 3 % f total body protein. The spleen, ver, intestinal wall, intestinal con- ent, blood plasma, erythrocytes and ne remaining carcass of the body were nvestigated. The protein loss was ainly due to a significant depression f protein synthesis in the carcass. he unchanged protein synthesis of ne liver, the increased protein syn- nesis in the intestinal wall and the levated protein transfer into the blood lasma were not able to compensate nis decrease. The protein loss cannot e explained by decreased food intake r amino acid levels, increased nitro- en loss through feces and urine, or ncreased protease or esterase activity. one of the measured parameters eems suitable for fast radiation osimetry in humans.

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