streptokinase, with or without ε-aminocaproic acid) showed evidence of degradation, except for 1 sample where the IgG_3 protein was incubated with 0.1 M ε-aminocaproic acid (figure 2). It thus seems that this compound can have a protective effect when small amounts of plasmin are present in the sample, as is probably the case for most purified IgG_3 preparations.

In conclusion, our results show that plasmin induces the same type of denaturatives changes in monoclonal IgG proteins as seen during storage, or after incubation with reducing agents[8], confirming the postulated role of this enzyme. The speed of the reaction, in our observations, was intermediate between that reported by Connell and Painter[7] and the very slow degradation described by Skvaril et al.[3]. The amount of enzyme, its state of activation, and the presence of activating or inhibiting substances as contaminants of the IgG preparations may be of relevance in determining differences in the activity of the enzyme, beyond the individual degrees of susceptibility of each IgG subclass.

The early response of ^{59}Fe incorporation in the bone marrow of irradiated rats[1]

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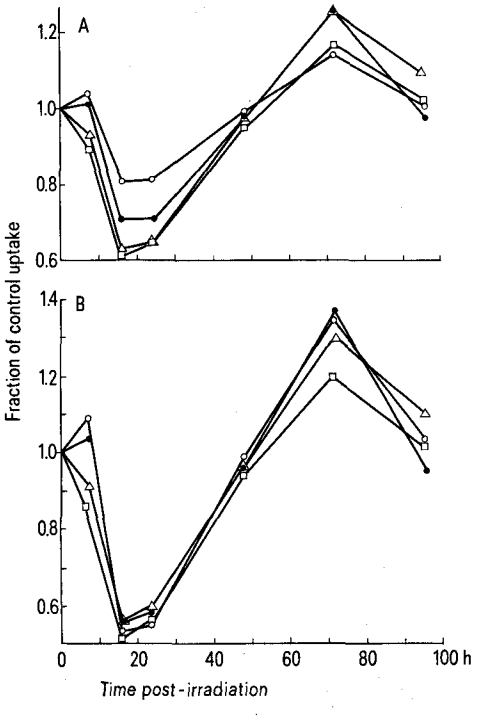
Summary. The change in radioiron uptake in selected bone marrow samples in irradiated rats is representative of the total skeletal uptake only if the baseline or radioresistant uptake has been subtracted.

Holá et al.[2] have stated that for a given radiation exposure to mice, the degree of reduced ^{59}Fe uptake in various bone marrow compartments depended upon the particular bone under study. This conflicts with the common practice of using the uptake of radioiron in an individual bone, e.g., the femur or the tibia, as being representative of the erythropoietic activity of the total skeleton[3]. The study reported here was designed to examine this conflict and to seek a method for circumventing it.

Method. Female Sprague-Dawley rats, weighing between 170 and 200 g were used. The animals were anesthetized with sodium pentobarbitol and irradiated through their ventral surfaces, 4 at a time, on a rotating table. A 1000 kVp General Electric Industrial X-ray unit was used under the following conditions: 1000 kVp, no additional filtration, HVL of 3.0 mm Pb, and a target to midline distance of 66.0 cm. The exposure rate was 15.8 R/min. and a total whole-body exposure of 50 R was given. At varying times post-exposure and under light halothane anesthesia, the animals were injected via the femoral vein with 5 μCi of ^{59}Fe labeled ferrous citrate. 6 h later, the the rats were sacrificed via cervical separation. Approximately 1 h prior to sacrifice, ^{51}Cr labeled red cells were injected via the femoral vein. Just prior to sacrifice, a 25 μl blood sample was drawn from the tail vein. This blood sample was used to subtract any bone activity which was due to radioiron in the circulating blood. The eviscerated animals were ashed at 595 °C and cleansed bone samples were counted individually in an autogamma counter. The data were taken for 16 control animals and 4 irradiated rats at each point. An additional 24 rats were irradiated with exposures of 700, 850 and 1000 R and the ^{59}Fe uptake measured at 24 h post-exposure. It has been shown that with exposures of these levels, the uptake



Fraction of control uptake vs time in h post-irradiation. A. Ratio of total uptakes; B. Ratio of net uptakes. ○, Scapula; □, Femur; ▲, Cervical spinal segements 1–5; △, Sternum.

1 This paper is based on work performed under contract with the US Energy Research and Development Administration at the University of Rochester Biomedical and Environmental Research Project, and has been assigned Report No. UR-3490-949.

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Net ^{59}Fe uptake in control and baseline rats (% of injected ^{59}Fe)

Bone sample	Control	Baseline
Scapula	0.617 ± 0.20*	0.371 ± 0.022
Femur	5.43 ± 0.28	1.14 ± 0.06
Cervical Vertebrae (1–5)	0.913 ± 0.034	0.301 ± 0.016
Sternum	1.05 ± 0.04	0.172 ± 0.011

*Mean ± 1 SE.

of ^{59}Fe in bone falls below the control value but shows no additional reduction with increasing exposure[4]. It is assumed that this represents ^{59}Fe taken up in storage or other radioresistant sites, and these uptakes will be referred to as 'baseline' uptakes. Therefore, the net uptake will be the total uptake minus the baseline uptake. The results will be presented as two factions: the ratio of the total uptakes of irradiated to control and the same ratio of the net uptakes.

Results. Rat bones may be divided into 2 classes; those which show a reduction in ^{59}Fe uptake following exposure and those insensitive bones which show no reduction following irradiation[4]. All of the sensitive bones will be presented here. The control iron uptakes and the baseline uptakes for these samples are presented in the table in terms of percentages of the injected ^{59}Fe. A plot of the fraction of total control uptake with time post-exposures is presented in figure A. It may be noted that among the bones considered, the femur and sternum show greater reductions in uptake than the scapula or cervical spinal segments. This is similar to the results reported by Holá[2]. However, when the ratios of the net uptakes are plotted, as in figure B, these differences in response of the minima are reduced.

Also, it may be noted that the minimum in uptake for each bone occurs between 16 and 24 h post-exposure. This type of response was noted for all radiosensitive bones even with differing dose rates (8.2 R/min. and 116 R/min.). Whole-body exposures of 100 R and 300 R gave similar results. The only notable difference was a more pronounced minimum at 24 h post-irradiation, and a delayed return toward normal uptake values.

Discussion. The uptake of ^{59}Fe in the femur or tibia may be considered as being representative of the erythropoietic activity of the total skeleton in irradiated rats as long as the baseline activity has been subtracted. The minima in uptake in this study occur earlier than those reported by Holá et al. However, others have reported maximum responses at 24 h postirradiation[5,6]. This difference may be due to the mode of injection. Holá used an intraperitoneal injection route while an intravenous route was used in this study.

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Immunostimulation by a formula-defined diet[1]

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Summary. A formula-defined diet (diet 3, table 1) acted as an adjuvant in the response of the immune system to SRBC in the rat. Similar minimum and maximum antibody levels were measured in both males and females. In the males, the maximum was reached with diet 3 alone while females required the complementary action of diet 3 and LPS mR 595.

A sex-dependent enhancement of the uptake of tritiated thymidine (^{3}H-TdR) by hematopoietic tissues has been reported when rats were fed certain formula-defined diets[2]. Those results suggested that such formula-defined diets could also affect the response of the immune system in a sex-related manner.

We now report the effect of elemental diet 3[3] on the response of the rat immune system to sheep erythrocytes (SRBC). The composition of the diet has already been described elsewhere[4]. Anti-SRBC antibody levels were measured in the serum of male and virgin female Sprague-Dawley rats 6–7 weeks old, fed either the elemental diet or Purina laboratory chow. In addition, we investigated to what extent diet and sex modify the adjuvant effect of lipopolysaccharide (LPS) mR 595[5].

Randomized groups of 6 animals were fed as previously described[4]. SRBC[6] were resuspended at a 1:10 dilution in phosphate buffered saline (PBS) and injected i.p. (1 ml, approximately 10^8 cells). LPS was prepared in PBS and 40 µg injected ip simultaneously to SRBC. Anti-SRBC antibody content of serum was determined by passive hemagglutination 7 days later.

Results are presented in table 2. In controls fed laboratory chow, both males and females had comparable anti-SRBC antibody levels. Remarkably higher levels (by a factor of 23 on the average in the males and 7 in the females) were found in animals fed diet 3. By contrast, LPS was found

Table 1. Detailed composition of diet 3

	Grams per 100 g
Casein hydrolysate	12.560
Sucrose	46.839
Corn syrup solids (glucose oligosaccharides)	16.812
Long-chain triglycerides	11.982
Medium-chain triglycerides	2.997
Tapioca starch	5.234
Minerals	2.611
Free amino acids	0.384
Choline chloride	0.028
Water	0.553
Sodium citrate hydrous	0.656
Potassium chloride (from casein)	0.248

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