

Immunocytomas represent a monoclonal proliferation of small lymphocytes which are predominantly of the B-cell type. They are associated with lymphoplasmacytoid or typical plasma cells but immunoblasts are rarely seen. Three subtypes are prescribed: lymphoplasmacytic, lymphoplasmacytoid and polymorphous (1). A high content of epithelioid cells was described in certain immunocytomas (2) and our patient had numerous granulomas in her spleen. Russell bodies can be found in the cytoplasm of the plasma cell and Dutcher bodies are PAS positive intranuclear inclusions found in the same cells. Immunological staining for light chains demonstrates their presence in 61% of cases (3). Heavy chains presence is more easily found (89%) with a high predominance of IgM often accompanied by IgD (70%) (1, 3) and 10% express IgD and IgA. However, IgE is present in exceptional cases (4). A monoclonal gammopathy is present in about one-third of the cases and is often of the IgM type, rarely IgG or IgA. Clinically, a massive splenomegaly without peripheral lymphadenopathy can be the mode of presentation in some cases (5, 6). To our knowledge, this is the first report in the literature of an immunocytoma with the exclusive expression of heavy chains of the IgD type and no light chain expression. The monoclonal gammopathy is also of the IgD type and is associated with a depression in the secretion of the other immunoglobulins. Unfortunately, our patient was lost to follow-up and the significance of such an exceptional case is difficult to assess. Long-term follow-up of similar cases may determine whether their prognosis is different from other patients with immunocytomas.

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## PROTEIN SYNTHESIS IN JEJUNUM OF SPRAGUE-DAWLEY RATS AFTER IRRADIATION

It is well known that irradiation causes morphological changes in tissue. However, the specific effect of radiation on protein synthesis in a tissue or organ is not completely understood. Previous studies (1-2) performed in rat skin showed an inhibition of protein synthesis with doses of 1500 R or more, while with levels near 750 R, a significant stimulatory effect was observed, leading to the conclusion that the effects of radiation on protein synthesis could be due to a mechanism which is independent of DNA damage.

The jejunum is considered particularly sensitive. Total abdominal irradiation is associated with a decline in the effective resistance of the gastrointestinal tract and a rise in the passive permeability properties of the small and large intestine (3-4). The aim of the present study was to determine protein synthesis in jejunal mucosa of rats after a single exposure to abdominal irradiation at various dose levels.

**Material and methods.** Sixty male Sprague-Dawley rats, (Bio-centre, S.A., Barcelona, Spain), were utilized for this study. Determination of the normal range of jejunal protein synthesis was done in a control group of 15 healthy animals (mean body weight: 191.1 ± 6.2 g). The irradiation experiments were performed on 45 rats (mean body weight: 169 ± 3.1 g), housed individually in metabolic cages and fed standard chow and water ad libitum until irradiation. The animals were divided into three groups (n = 15) to receive 3 dose levels of radiation 4 Gy, 6 Gy and 10 Gy.

The rats were anesthetized intraperitoneally with 5% sodium pentobarbitone at a dose of 0.1 ml per 100 g of body weight. An 11 × 10 cm field on the abdomen from the xiphoid process to the pubis received a single x-ray exposure (Stabilipan, Siemens 250 kV, 15 mA, 1 mm Cu-filter, SSD 43 cm). The dose was calculated at midline (total thickness 3.5 cm) to deliver 1.03 Gy/min. After irradiation, the rats were kept in individual metabolic cages, fed a clinical modular diet ad libitum (Table 1) for 4 days, and killed by decapitation on the fifth.

Protein synthesis was determined in each irradiated group and in the controls with the 'flooding dose' method (5). L-(1-<sup>14</sup>C) leucine was combined with unlabelled L-leucine to give c. 1 MBq and 135 μmol/mm. One ml of this solution per 100 g of animal body weight was administered through the lateral tail vein. In each irradiated group, five animals were killed 2 min after the radionuclide injection, and the remaining ten animals, 10 min after. In the control group, six animals were killed at 2 min, and nine at 10 min. After decapitation, a midline abdominal incision was made to remove the proximal small intestine, discarding the first 20 cm from the pylorus, and taking the following 20 cm after stretching with a 3.5 g weight. Jejunal tissues were obtained by slitting the intestine longitudinally and scraping the luminal side with a microscope slide. The samples were then transferred into

**Table 1**

*Composition of modular diet used to feed rats after total abdominal irradiation*

Protein (kcal)	14
Lipid (kcal)	49
Carbohydrates (kcal)	37
MCT (% kcal)	40
LCT (% kcal)	60
Total nitrogen (g)	3.2
Keltrol (g)	0.5
Total kilocalories	100

Composition for 100 ml of diet.

a polypropylene tube and stored at  $-30^{\circ}\text{C}$  until analysis. Samples were homogenized in a metallic mortar precooled with solid  $\text{CO}_2$  to obtain a fine powder. Portions of 0.1–0.5 g were then precipitated in cold 2%  $\text{HClO}_4$  and centrifuged 10 min at 3 000 G. Two ml supernatant was ultrafiltered by centrifugation in conical membranes (Centriflo CF25, Amicon) for 15 min at 500 G. This ultrafiltrate was used to determine the specific activity of free leucine. The precipitate was washed three times with 10 ml of 2% (w/v)  $\text{HClO}_4$ , resuspended in 10 ml of  $\text{NaOH}$  (0.3 M) and incubated at  $37^{\circ}\text{C}$  for 1 h. In this solution the specific radioactivity of the protein-bound leucine was determined. Another fraction was ultrafiltered by centrifugation in conical membrane at 1 550 rpm for 30 min and used for correcting contamination due to free leucine. Measurements of radioactivity were done with a beta scintillation counter (Intertechnique). Leucine was analyzed by single-column ion-exchange chromatography (Chromaspeck, Rank Hilger). Protein content was determined by Lowry's method (6). The rate of protein synthesis, expressed in percentages of the protein synthesized daily, was calculated from the equation:  $K_s = S_b \times 100 / S_a \times t$ , where  $S_b$  is the specific radioactivity of leucine in protein,  $S_a$  is the mean specific radioactivity of free leucine (groups of animals were killed at 2 and 10 min to assess the change in specific radioactivity over 10 min), and 't' is the time expressed in days.

The histologic changes occurring in the intestinal mucosa were observed in sections prepared from samples of 5 cm length removed after the 20 cm discarded for the protein synthesis study.

Statistical analysis of the data was made with Student's t-test and ANOVA. A  $p$ -value  $<0.05$  was considered statistically significant.

**Results.** Special care was taken to select animals having a similar initial body weight; there were no statistically significant differences between the groups. All groups exposed to radiation showed a decrease in body weight (4 Gy:  $-2.6\%$ ; 6 Gy:  $-17.4\%$ ; 10 Gy:  $-23.0\%$ ), while the control group, fed the same modular diet, showed a body weight increase by  $8.9\%$ .

Concerning the fractional synthesis rate ( $K_s$ ), expressed in percentages of protein synthesized daily, no statistically significant differences were observed in jejunal mucosa among the groups (ANOVA  $p=0.56$ ). However, a slight decrease in fractional synthesis was evident with the increasing radiation doses when compared with the control group, especially for doses above 6 Gy (Table 2).

Few histologic changes in the small intestine were observed after doses of 4 and 6 Gy. However, after 10 Gy, the villi were shortened and severe villi atrophy was evident, with megaloblastosis and megalocytosis of epithelial cells.

**Discussion.** Postirradiation protein synthesis depends on intake, absorption, and utilization of food, as well as direct effects of radiation at the biochemical, cellular, tissue, and organ level. While the effects of radiation in the gastrointestinal tract have been widely explored in terms of absorption, less attention has been focused on the study of protein synthesis. The first conceptual difficulty to explain measurements of total protein synthesis results is due to the

fact that total tissue protein consists of innumerable individual proteins, turning over at separate rates and interacting with one another. The second is that exposure to radiation causes biochemical transformations in the structure of each specific protein which lead to metabolic consequences.

Since the composition of diet administered may significantly affect the protein synthesis rate, the reference values reported by other authors are not precisely comparable (5).

The degree of injury to an organ depends on the number of cells damaged. Organ protein content is the result of synthesis and breakdown. However, since protein may be influenced by water content, the results we obtained were expressed as the fractional synthesis rate ( $K_s$ ), or the fraction synthesized daily and expressed in percentages rather than the absolute synthesis rate. The present data show no statistically significant differences in leucine incorporation in the jejunum following total abdominal irradiation with a single dose. However, with increasing doses of radiation the response varied considerably, showing a trend towards reduced leucine incorporation. Further studies should be performed in order to determine the possible effects of radiation injury on jejunal protein synthesis at different periods of time after radiation exposure.

In conclusion, the model used in this study gives every indication of being a practical and accurate method for observing the influence of radiation on the gastrointestinal tract. The fact that the global protein synthesis rate did not show an important decrease has led us to hypothesize that the study of specific protein synthesis (both short and long half-life) might produce more concrete information on gastrointestinal cell radiation damage.

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**Table 2**

Fractional synthesis rate of protein synthesis ( $K_s$ ) determined in jejunum of Sprague-Dawley rats

Groups	No. of animals	Jejunum $\pm$ SEM
4 Gy	10	162.5 $\pm$ 15.0
6 Gy	10	156.6 $\pm$ 25.0
10 Gy	10	137.3 $\pm$ 26.2
Control	9	179.9 $\pm$ 12.5