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The Metabolism of Different Immunoglobulin Classes in Irradiated Mice

V. CONTRIBUTION OF THE GUT TO SERUM IgA LEVELS IN NORMAL AND IRRADIATED MICE

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Summary. It was demonstrated in earlier publications that the acute and selective fall in serum IgA levels in irradiated mice was due to a specific failure to renew the plasma pool of this immunoglobulin. In the present work, it is shown that irradiation causes some reduction in the rate of biosynthesis of IgA, but that this phenomenon only partly accounts for the observed disturbance in the metabolism of IgA. This conclusion is based both on direct measurements on the rate of biosynthesis *in vitro* of IgA by the isolated small bowel wall of irradiated and normal C_3H mice, and on immunohistological studies and plasma cell counts on the gut, spleen and lymph nodes of these animals.

On the other hand, it is shown that irradiated mice continue to excrete approximately normal amounts of IgA into their intestinal contents. The conclusion is that irradiation of the gut causes a rechannelling in the output of IgA synthesized by the plasma cells of the lamina propria, the major part being diverted to the intestinal lumen instead of being fed into the circulating pool of serum IgA.

INTRODUCTION

It has previously been demonstrated that the very marked decrease in the concentration of immunoglobulin IgA in the serum of mice exposed to a whole-body dose of X-rays is not caused by excessive catabolic or intestinal losses of this protein. This was first inferred from the absence of changes in the metabolic half-life of ^{131}I-labelled myeloma IgA injected prior to irradiation (Bazin and Malet, 1969). The conclusion was strengthened by the recent demonstration (Bazin, Levi and Heremans, 1970) that lethal or supralethal X-irradiation was without significant effect on the fate of IgA synthesized by a grafted tumour, protected from the X-rays, or of IgA obtained from normal donor mice and infused into hypogammaglobulinaemic germ-free mice.

The logical implication of these negative findings was that the metabolic disturbance affecting IgA in irradiated animals resided in the mechanism responsible for the renewal of the circulating pool of this immunoglobulin. The present study is concerned with a search for positive arguments in favour of this view. The investigation was greatly facilitated by the previous demonstration (Bazin, Maldague and Heremans, 1970) that the gut was closely implicated in this metabolic lesion, since the selective fall in serum IgA did

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not occur when this organ was excluded from the action of X-rays, whereas the phenomenon occurred in full strength when the bowel alone was exposed to irradiation.

It will be demonstrated here that the rate of biosynthesis of IgA in the gut wall is only moderately affected by X-irradiation, and that the major cause of depletion of the serum IgA pool resides in the fact that much of the intestinal production of this immunoglobulin is diverted from its normal pathway of secretion into the serum to the benefit of abnormal excretion into the gut lumen.

MATERIALS AND METHODS

Animals

All experiments were carried out on C3H mice, aged 3 months, bred at the University of Louvain. Throughout the observation period the animals were allowed free access to standard food pellets and water.

Irradiation

A single dose of 700 R (Maxitron, General Electric, 250 KV, 1 Al+0·25 Cu filters, dose rate: 45 R/min) was administered to the mice while they were being constrained in Plexiglass boxes.

Antisera

The purification of mouse immunoglobulins and the preparation of the corresponding specific antisera in rabbits have been described elsewhere (Bazin 1966; Bazin and Malet, 1969). Each of these antisera produced a single precipitin line when tested against whole mouse serum in Ouchterlony plates or by immunoelectrophoretic assay.

Immunofluorescence

Antisera were labelled with fluorescein isothiocyanate according to published methods (Crabbé, Bazin, Eyssen and Heremans, 1968; Crabbé, Nash, Bazin, Eyssen and Heremans, 1969). These reagents were used without special absorption, as their specificity had been found to be satisfactory.

The animals were bled and their spleens, mesenteric, axillary and inguinal lymph nodes as well as a sample of the duodenum and ileum were snap-frozen with dry ice and processed to 3–5 μ sections. The further processing of the preparations was carried out as already described (Crabbé, Nash, Bazin, Eyssen and Heremans, 1970).

In counting fluorescent cells, an effort was made to reduce sampling errors by examining, for each class of immunoglobulins, at least four sections obtained from three different portions of each specimen. The specificity of the fluorescence was controlled by incubating some sections with fluorescent antisera previously absorbed with whole mouse serum until all precipitating antibodies had been removed, as judged by immunodiffusion assays.

Biosynthesis of IgA by mouse gut wall in vitro

(*a*) *Extracellular IgA in individual bowel cultures.* Immediately after a test bleeding, normal or irradiated mice were sacrificed by decapitation. The small bowel, including the duodenum and terminal ileum, was removed, rapidly flushed out with 20 ml of Eagle Minimum Medium (Serva, Heidelberg, Germany) containing 500 IU of penicillin and 500 mg of streptomycin per ml, and minced to minute fragments (of about 1 mm^3) by

means of scissors, all operations being carried out at room temperature. The mince from each bowel was suspended in 20 ml of Eagle Minimum Medium to which 350 IU of penicillin, 250 mg of streptomycin, and 1 mg of human IgA had been added. The inclusion of human IgA was intended as an internal control of proteolysis; the protein used for this purpose had been purified from a human myeloma serum and found to sediment as a single 7S peak in the analytical ultracentrifuge.

Each culture mixture was incubated at 37° in 250 ml Erlenmeyer flasks, under gentle rocking. After 1 hour, 5 ml of culture fluid free of gross tissue debris was withdrawn, and stored at −20° after clarification by centrifugation.

The cultures were continued under the same conditions for another 24 hours, after which the fluid was clarified by centrifugation and frozen at −20°.

The 1-hour and the 25-hour culture fluids were then lyophilized, reconstituted with distilled water to final volumes of 1 ml and 2 ml respectively, and used for the determination of mouse IgA and human IgA as described below.

(*b*) *Intra- and extracellular IgA in pooled cultures.* The conditions of tissue culture in this experiment were the same as in the preceding experiment, except that twelve minced intestines were suspended in 240 ml of culture medium, contained in a 1 litre Erlenmeyer flask.

After 1 hour of incubation, 120 ml of the culture mixture, including the equivalent amount of tissue, were removed and frozen at −20°. Another 30 ml of culture fluid, without tissue, were also removed and stored at −20° after clarification by centrifugation. The remaining 90 ml of culture mixture was centrifuged and the supernatant stored at −20°, whereas the sediment was added to the remaining mixture, which was frozen *in toto*, without separation of cells and culture medium.

The samples containing tissue in addition to culture medium were repeatedly frozen and thawed before clarification by centrifugation. Ultimately, all samples were lyophilized and reconstituted with distilled water to small volumes, prior to the quantitative determination of their mouse and human IgA contents.

Intestinal perfusion

One day after a test bleeding, mice were irradiated with a whole-body dose of 700 R. One to 6 days later, the irradiated animals and their sham-irradiated controls were anaesthetized by an intraperitoneal injection of 1 ml of 1 per cent Rectanol ® (Laboratoire Robert & Carriere, Paris) in saline, after another test bleeding. A rubber catheter (2 mm diameter) was inserted into the proximal part of the duodenum and sutured to the gut wall by means of silk. Another catheter (2 mm diameter) was affixed in similar fashion in the lumen of the terminal ileum. A total of 400 ml of phosphate buffered saline containing 100,000 IU of Trasylol ® (Bayer, Leverkusen, W. Germany) intended as a trypsin inhibitor, was perfused through the small bowel over a period of 8 hours. The effluent was collected into ice-cold 1 M acetate buffer of pH 3·5. For each mouse, four successive 2-hour perfusates were collected, concentrated in the cold by dialysis *in vacuo*, to a final volume of 1–2 ml, and used as such for the determination of IgA.

Density gradient ultracentrifugation

A density gradient of sucrose (10–35 per cent) in borate buffered saline (pH 8·4) was employed. The protein samples were applied on top of the gradient. The SW 39 rotor of the Spinco Model L ultracentrifuge was run for 18–20 hours at 35,000 rev/min at 4°,

after which the tubes were punctured and drained from the bottom in 30–40 ten-drop fractions. Estimates of molecular size of mouse IgA and human IgA from different origins were obtained, using samples of mouse serum, 19S α_2M, purified milk 11S IgA, purified human 7S IgG, purified human 7S myeloma serum IgA, human 5·5S transferrin and purified horse 2·6S immunoglobulin L chains as markers for the different molecular size regions. The sedimentation coefficients of the marker proteins were verified in the Spinco model E analytical ultracentrifuge.

Morphological studies

For electron microscopic studies, 2 cm-long segments of ileum or jejunum were carefully dissected and summarily fixed by gentle perfusion with 10 ml of a 2 per cent solution of glutaraldehyde in isotonic phosphate buffer of pH 7·4. Small fragments were post-fixed by immersion in the same glutaraldehyde solution for 24–48 hours, followed by additional fixation with osmium for 1 hour. Epon-embedded ultrathin sections were stained with uranyl acetate and lead citrate.

For optical microscopic studies, 1 mm thick transverse sections of ileal or jejunal tissue pre-fixed with glutaraldehyde and post-fixed with osmium as described above, were embedded in Epon and processed to 1 μ thickness by means of a Reichert Ultramicrotome. These sections were stained with a 1 per cent aqueous solution of toluidine blue for ½–1 hour.

Measurements of protein concentrations

Quantitative determinations of mouse immunoglobulins were performed by the Mancini technique (Mancini, Carbonara and Heremans, 1965) using antisera monospecific for mouse IgA, IgM, IgG_1 and IgG_{2a}, respectively (Bazin, 1966; Bazin and Malet, 1969).

The same technique was used for the quantitation of the proteins used as molecular size markers.

RESULTS

EFFECTS OF X-RAYS ON THE PLASMA CELLS OF DIFFERENT CLASSES IN VARIOUS TISSUES (TABLE 1)

In non-irradiated control mice the counts of plasma cells containing the different classes of mouse immunoglobulins, IgM, IgA, IgG_1 and IgG_{2a}, were in substantial agreement with previous findings (Crabbé *et al.*, 1970). In particular, the plasma cell population of the lamina propria of the duodenum and ileum was composed of an overwhelming majority of cells staining with anti-IgA (Table 1 and Fig. 1a).

After irradiation by 700 R, a moderate though significant reduction in plasma cell counts of all four immunoglobulin classes was observed in the intestinal lamina propria, but no measurable changes appeared to occur in the spleen and peripheral lymph nodes. The lymphoid tissue of the mesenteric lymph node shared some of the radiosensitivity of the lamina propria, although its overall response was more akin to that of the spleen. The decrease in plasma cell counts in the bowel wall became first noticeable towards the 2nd or 3rd day after irradiation and some tendency towards restoration was observed after 1 week.

In addition to these quantitative changes, the immunohistological appearance of the affected tissues conveyed the impression that the staining of the plasma cells with fluorescent antisera was less intense and more diffuse than usual (Fig. 1A and B).

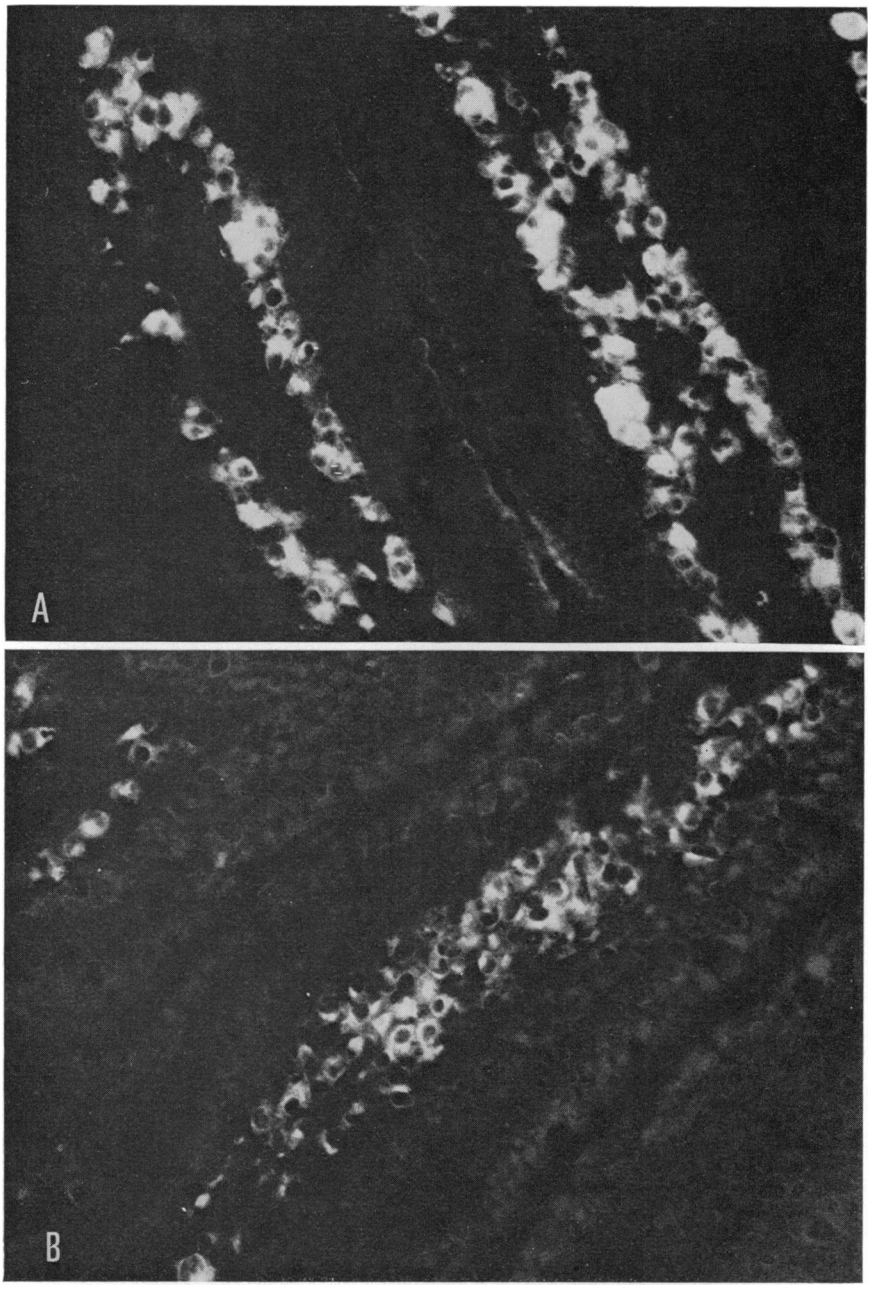

FIG. 1. IgA-containing plasma cells in normal and irradiated mouse intestine. A, Jejunal mucosa from a normal control. B, Jejunal mucosa from a mouse, 4 days after 700 R whole body irradiation.

TABLE 1
EFFECTS OF X-RAYS ON PLASMA CELL COUNTS

Time after irradiation		Controls	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
No. of mice		8	1	1	1	2	2	2	2	1	1
Intestine:											
Duodenum	IgM	+	+	+	+	+	+	+	+	+	+
	IgA	+++++	+++++	+++++	++++	+++ +++	+++ +++	+++ +++	++++ +++	+++	+++
	IgG_1	++	+	−	−	− −	− −	− −	+ −	+	+
	IgG_{2a}	+	++	−	−	+ −	+ −	NT NT	− −	−	−
Ileum	IgM	+	+	+	−	+ −	+ −	+ +	+ +	+	+
	IgA	++++	++++	++++	++++	+++ ++++	+++ +++	+++ ++	+++ +++	+++	+++
	IgG_1	++	+	−	−	− −	− −	− −	+ −	+	+
	IgG_{2a}	++	++	−	−	− −	− −	NT NT	− −	−	−
Mesenteric lymph nodes:											
	IgM	+++	+++	++	+++	+++ +++	++ ++	+++ ++	+++++ ++	++	+++
	IgA	+++	+++	++	++++	+++ ++++	++++ +++	+++ +++	+++ +++	+++	+++
	IgG_1	+++	+++	+++	+++	++++ +++ +++	+++ ++ ++	+++ + +	++++ +++ +++	++	NT
	IgG_{2a}	+++	+++	++	++	NT ++	+++ +++	NT NT	NT ++	++	NT

Peripheral lymphoid tissues:											
Spleen	IgM	++++	++++	+++	+++++	+++ ++++ +++	+++ +++ +++	++++ ++++ +++	+++++ ++++ ++++	+++	++++
	IgA	+++	+++	+++	+++	+++	+++	+	+++	++	+++
	IgG_1	++	+++	++	++	++ ++	++ +	NT +	++ ++	++	++
	IgG_{2a}	++	++	+	++	+	+	NT	+	+	+
Peripheral lymph nodes:											
	IgM	++	++	+	NT	NT NT	+ NT	NT NT	++ NT	NT	NT
	IgA	++	+	+	++	NT NT	++ NT	++ NT	++ NT	NT	NT
	IgG_1	++	+	+	−	− NT	− NT	++ NT	++ NT	NT	NT
	IgG_{2a}	++	+	++	NT	− NT	− NT	NT NT	+++ NT	NT	NT

No. of cells per tissue section: −, none; +, 1–10; ++, 10–100; +++, 100–500; ++++, 500–1500; +++++: 1500; NT, none tested.

BIOSYNTHESIS OF IgA BY TISSUE CULTURES OF NORMAL AND IRRADIATED GUT (TABLE 2)

As expected, the decline in serum immunoglobulin levels in this series of irradiated mice was more pronounced in the case of IgA than with the other classes of immunoglobulins.

The IgA-synthesizing capacity of the tissue cultures of the bowel wall fragments was estimated from the net gain in IgA in the culture medium between the 1st and the 25th hour of incubation. In the cultures of non-irradiated mouse gut, this increase averaged 6·55 mg over the 24-hour period, compared to a total amount of 2·80 mg of IgA found at the onset of the observation period, i.e. after 1 hour of incubation. Both the amount of IgA present after 1 hour and the net increase in IgA during the ensuing 24-hour culture period were significantly lower in the irradiated animals than in the controls, the former quantity being reduced to about one-half and the second to about $\frac{2}{3}$–$\frac{3}{4}$ of the values found in non-irradiated material.

To interpret the significance of net gain in IgA in such cultures, it is necessary to take into account a number of factors other than biosynthetic activity.

Firstly, one must reckon with the possibility that some of the IgA synthesized by the culture or present in the medium at the onset of the experiment may become degraded by proteolysis during the 24-hour period of incubation at 37°. Not only may proteolytic enzymes be expected to be produced by the intestinal mucosa, but one must also consider the possibility of proteolytic degradation by the cathepsins set free from cells which die during the observation period. The effects of proteolysis on the determination of the amount of IgA synthesized during the culture period may in turn be manifold. On one hand, any material sufficiently degraded to abolish its precipitation with the antiserum will escape detection; on the other hand, incomplete degradation might reduce molecules of mouse IgA to the size of their Fc fragments (Askonas and Fahey, 1962) which, on account of their fast diffusion may give rise in the Mancini assay to precipitin rings of excessive size (Heremans, 1970), leading to an overestimation of the amount of immunoglobulin actually synthesized.

Using human myeloma IgA as an internal control, it was indeed found in our cultures that about 60 per cent of this material became antigenically inactivated—and therefore presumably was destroyed—during the 24-hour incubation of the culture of normal mouse bowel. In irradiated animals, the amounts of human IgA lost under similar conditions were not significantly different from this normal control value, as judged by appropriate *t*-tests (Table 2).

An additional check on the possible incidence of proteolysis on the validity of the immunochemical quantitation of human or mouse IgA was provided by a direct evaluation of the molecular size distribution of these proteins in the supernatants of the cultures. In the density gradient, added human 7S IgA showed no evidence of degradation to antigenically active products smaller than 7S (Fig. 2, b–d). The IgA in normal mouse serum sedimented slightly slower than the human 11S marker, with little evidence of inhomogeneity (Fig. 2a).

After the 1st hour of incubation, much of the IgA present in the culture medium sedimented distinctly faster than 11S, with smaller amounts being present in the range characteristic of mouse serum IgA (Fig. 2b), and occasionally, also in the 7S range. There was no consistent difference between the IgA size distributions, after 1 hour, in the cultures from irradiated animals compared to those from the controls, except that in the

former the heavier forms of IgA tended to decrease in importance. The culture fluids analysed after 24 hours of incubation showed an entirely different distribution of mouse IgA, with the lighter forms predominating, both in the controls and in the irradiated animals (Fig. 2c, d).

TABLE 2
EFFECT OF X-RAYS ON SERUM IMMUNOGLOBULIN LEVELS AND IgA-SYNTHESIZING CAPACITY OF BOWEL WALL

Time after irradiation	Controls	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
No. of mice	12	5	5	5	5	5	5
	Serum immunoglobulin concentration						
IgM							
Pre-irradiation (mg/ml)	5·24±0·36*	5·59±0·11	5·82±0·63	5·78±0·32	5·73±0·23	5·35±0·25	5·47±0·19
Post-irradiation (percentage of pre-irradiation)	112±12	104±8	91±4	87±5	78±2	72±6	77±8
IgG_1							
Pre-irradiation (mg/ml)	0·99±0·07	1·19±0·15	1·11±0·10	0·78±0·11	1·13±0·07	0·93±0·13	1·03±0·04
Post-irradiation (percentage of pre-irradiation)	108±10	102±7	84±7	112±20	82±7	85±13	71±3
IgA							
Pre-irradiation (mg/ml)	1·72±0·23	1·75±0·39	2·48±0·23	1·74±0·30	2·79±0·27	1·70±0·25	1·78±0·22
Post-irradiation (percentage of pre-irradiation)	122±19	87±9	39±3	62±7	33±5	57±9	44±5
	IgA-Synthesizing capacity of bowel wall cultures						
Mouse IgA in culture medium							
After 1 hour (mg)	2·80±0·24	1·88±0·16	1·69±0·18	1·27±0·12	1·40±0·10	1·30±0·88	1·36±0·05
Net gain 24 hours later (mg)	6·55±0·75	4·95±0·86	3·74±0·36	4·17±0·27	3·64±0·20	5·20±1·05	4·00±0·15
Net gain in 24 hours as percentage of net gain in controls	100	75	57	64	56	79	61
Human IgA percentage control degraded by culture 24 hours	58±7	64±9	76±2	73±5	49±9	56±8	34±14

* Values are indicated as means±SD.

On the whole, these density gradient analyses indicate that no significant amounts of material the size of IgA-Fc fragments were formed during the incubation, and that therefore, the quantitative estimates of IgA obtained by the Mancini method were not in error by excess.

Another factor to be taken into account is that the net increase in IgA in the culture medium over the 24-hour period of incubation need not necessarily be attributed to *de novo* biosynthesis alone; part of this IgA might represent preformed intracellular protein released during the incubation period, either by a process of active secretion or through

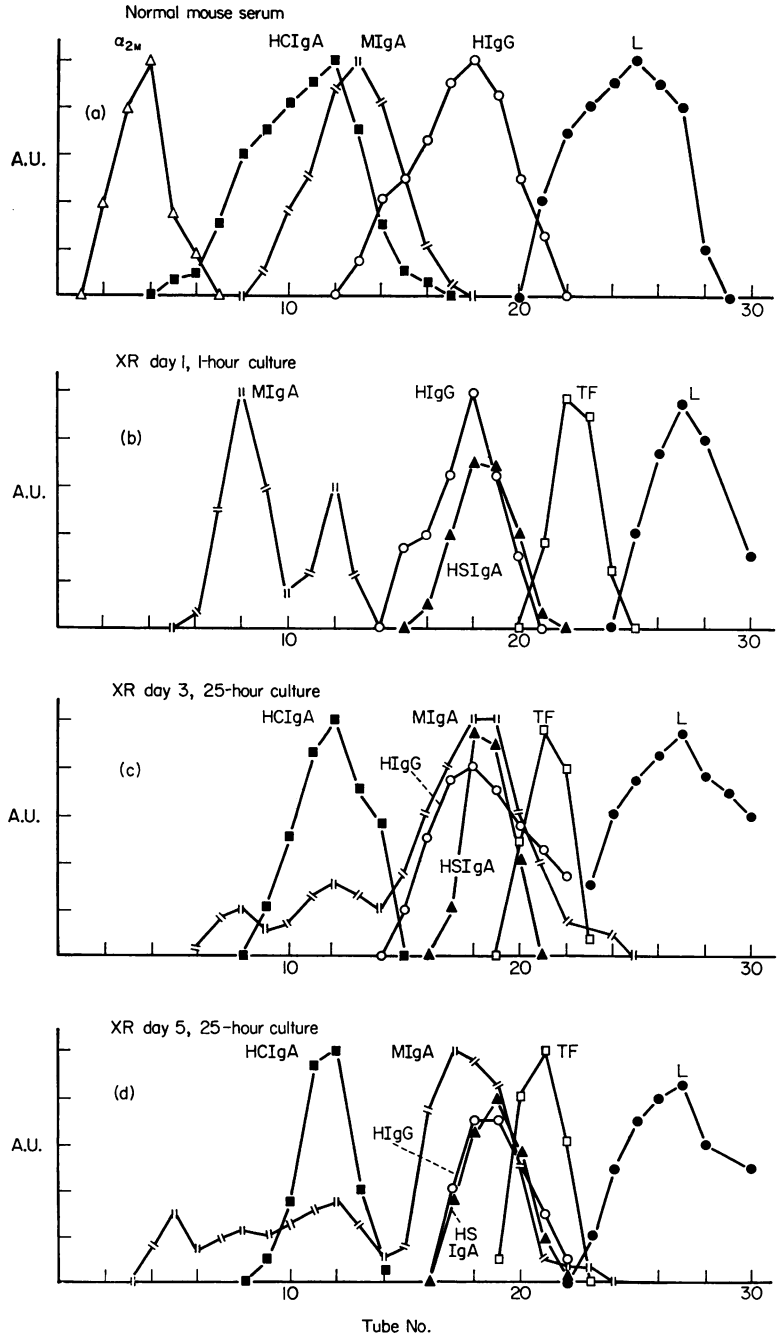

FIG. 2. Molecular size distribution of mouse IgA in tissue cultures of small bowel wall. Sucrose density gradient ultracentrifugation (sedimentation from right to left) of: (a) Normal mouse serum; (b–d) supernatants of cultures of intestine from irradiated mice. Abscissae, tube numbers; ordinates, arbitrary protein concentration units. Abbreviations: α_{2M}, mouse α_2-macroglobulin (19S); TF, human transferrin (5.5S); L, light chains from horse IgG (2.6S); HCIgA, purified IgA from human colostrum; HSIgA, human serum (myeloma) IgA (7S); MIgA, mouse IgA; HIgA, human IgA (7S); HIgG, human IgG (7S).

decay of the cells. To assess the participation of secretion or release of IgA, an experiment was devised in which intra- and extracellular IgA were titrated separately in the cultures, both after 1 hour and after an additional 24 hours of incubation. The results are set out in Table 3 and indicate that the net gain is practically entirely accounted for by newly synthesized IgA.

INTESTINAL EXCRETION OF IgA IN IRRADIATED MICE

In the series of animals submitted to intestinal perfusion following irradiation by 700 R, the usual pronounced fall in serum IgA levels was observed (Table 4). This phenomenon first became perceptible by the 3rd day and reached impressive proportions after 5 or 6 days.

In view of the broad physiological range of the serum IgA levels in normal mice (Table 4), the intestinal perfusion data are indicated both in absolute values and compared to the concentration of serum IgA in the individual groups prior to irradiation or perfusion.

TABLE 3
INTRA- AND EXTRACELLULAR IgA IN TISSUE CULTURES OF MOUSE GUT

Time of incubation	IgA in culture fluid = Extracellular IgA (mg)	IgA in culture fluid + cells (mg)	Difference = Intracellular IgA (mg)
1 hour	9·84	14·16	4·32
25 hours	18·00	21·84	3·84
Net gain	8·16	7·68	− 0·48

The results indicate that the cumulative amounts of IgA recovered over an 8-hour period of intestinal perfusion were not significantly different in irradiated animals compared to controls. Nor were the recoveries of intestinal IgA markedly different at different time intervals after irradiation, except perhaps for the last day when the animals were so debilitated as to succumb during the perfusion. In each experiment, it was noted that the amounts recovered decreased sharply during the first 2-hour period, to remain more or less constant thereafter.

When expressed by the ratio of intestinal IgA *vs* serum IgA prior to irradiation, the data of Table 4 confirm the absence of significant changes after exposure to 700 R, except in the very early or very late stages of the observation when this ratio was somewhat decreased.

In contrast, the amounts of IgA recovered from the intestinal contents grew disproportionately large in comparison to the declining serum IgA levels measured just prior to the perfusion, during the days following irradiation, and this discrepancy, compared to the normal intestinal: serum IgA ratio, also became increasingly significant.

As in the tissue culture experiment, it was necessary to check the validity of the immunochemical quantitation method by verifying the molecular size distribution of the IgA titrated in the intestinal perfusion fluids. Owing to the limited amounts of IgA recovered after the 2nd hour of perfusion, these analyses had to be restricted to the initial sample of each series. In control mice, intestinal IgA sedimented in a rather heterogeneous manner, the bulk of the material being distinctly heavier than the 11S human marker IgA

TABLE 4
INTESTINAL EXCRETION OF IgA IN IRRADIATED MICE

Time after irradiation	Controls	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
No. of mice	13	3	3	6	7	4	2
				Serum IgA levels			
Before irradiation (mg/ml)	—	4·45 ± 1·17*	2·35 ± 1·51	3·10 ± 0·55	2·79 ± 0·61	4·62 ± 0·23	4·09 ± 0·14
After irradiation just prior to intestinal perfusion (mg/ml)	2·74 ± 0·36	4·40 ± 1·50	2·75 ± 1·93	1·25 ± 0·22	1·14 ± 0·22	0·85 ± 0·28	0·51 ± 0·15
Difference (per cent of pre-irradiation level)	—	99	117	40	41	18	12
				Amounts of IgA recovered by perfusion of the gut			
Perfusion periods							
Hours 0–2 (mg)	0·44 ± 0·06	0·29 ± 0·06	0·29 ± 0·08	0·49 ± 0·25	0·44 ± 0·09	0·41 ± 0·07	0·18 ± 0·06
Hours 2–4 (mg)	0·16 ± 0·05	0·13 ± 0·02	0·03 ± 0·02	0·11 ± 0·03	0·11 ± 0·01	0·19 ± 0·02	0·06 ± 0·02
Hours 4–6 (mg)	0·09 ± 0·01	0·07 ± 0·00	0·01 ± 0·00	0·05 ± 0·00	0·11 ± 0·01	0·16 ± 0·01	0·09 ± 0·02
Hours 6–8 (mg)	0·09 ± 0·01	0·03 ± 0·00	0·04 ± 0·04	0·06 ± 0·00	0·09 ± 0·01	0·16 ± 0·03	0·09 ± 0·02
Total amount (mg)	0·78	0·52	0·37	0·71	0·75	0·92	0·42
		Ratio	Intestinal IgA recovered over 8-hour period / Amount of IgA in 1 ml of serum				
Referred to serum IgA level prior to irradiation	—	12·4 ± 1·7	43·0 ± 32·0	27·3 ± 9·3	45·1 ± 15·8	19·9 ± 1·6	10·3 ± 1·8
(Significance)	—	$P < 0·02$†	NS	NS	NS	NS	$< 0·05$†
Referred to serum IgA level after irradiation but just prior to perfusion	30·0 ± 3·0	13·6 ± 2·6	37·2 ± 26·0	66·6 ± 22·4	104·0 ± 37·3	145·4 ± 38·8	86·4 ± 13·6
(Significance)	—	$P < 0·05$†	NS	$P < 0·05$‡	$P < 0·02$‡	$P < 0·01$‡	$P < 0·01$‡

* Values are indicated as means ± SD.
† Significantly lower than in controls.
‡ Significantly higher than in controls.
NS, Not significant.

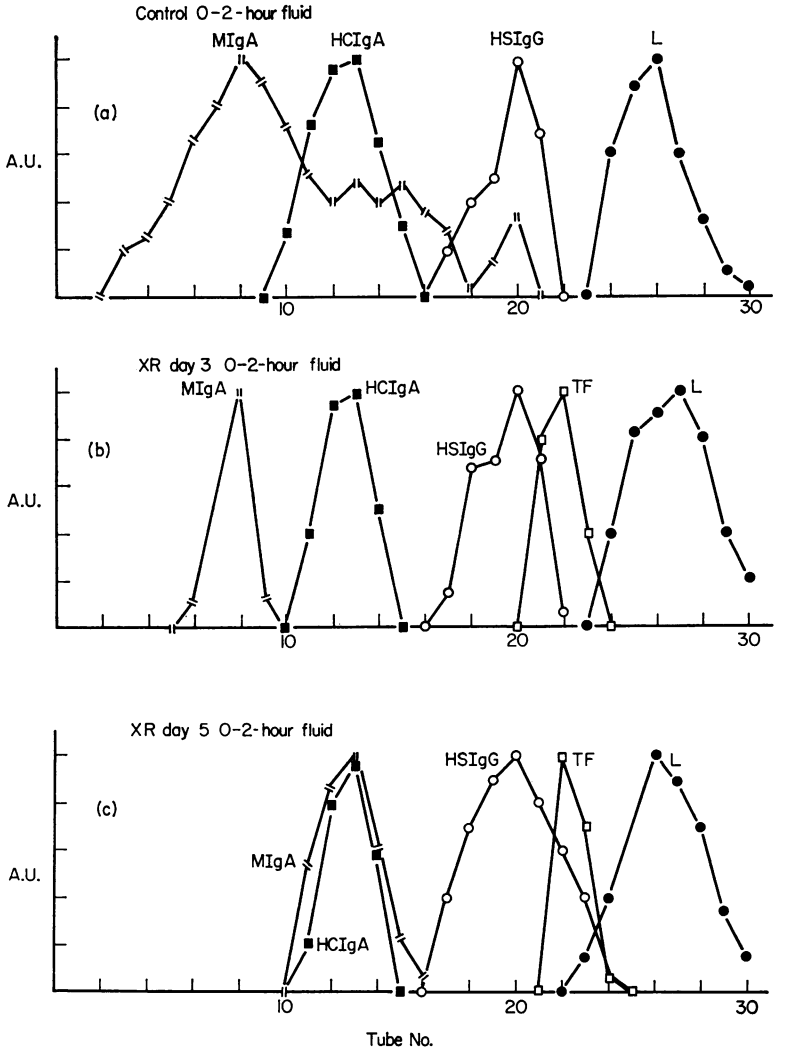

FIG. 3. Molecular size distribution of mouse IgA in perfusates of the bowel lumen; same conditions and indications as in Fig. 2.

(Fig. 3a). After irradiation, the major part of intestinal IgA continued to sediment together with or faster than the 11S markers, no material smaller than this size being present in significant amounts (Fig. 3b, c).

HISTOLOGICAL AND ELECTRON MICROSCOPIC STUDIES

A detailed description of the morphological lesions in the gut of irradiated mice will be presented elsewhere (Maldague, Baudon, Hamels, Bazin and Heremans, in prep.) but the following findings will be summarized here, as they have an immediate bearing upon the pathogenesis of the metabolic lesions of IgA following irradiation.

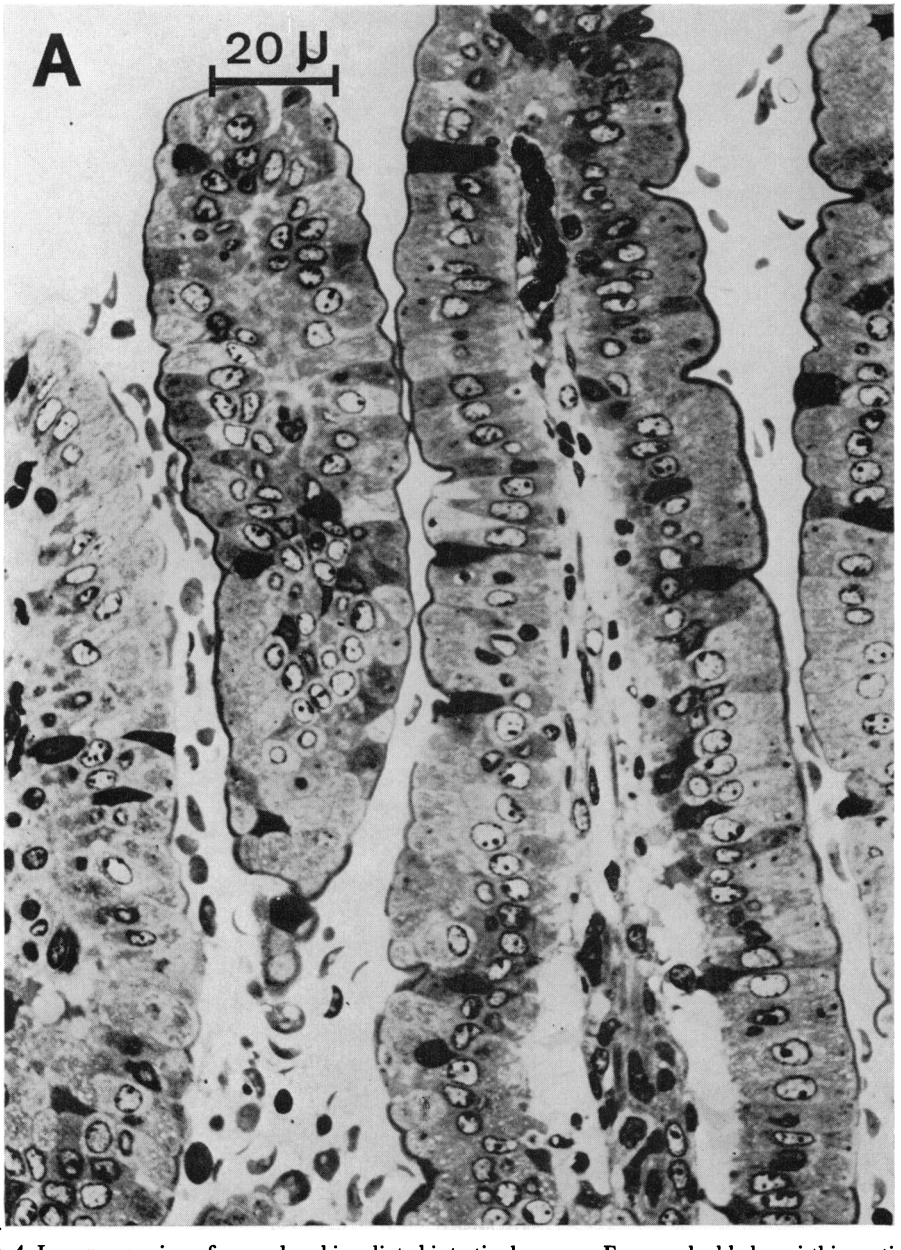

FIG. 4. Low-power view of normal and irradiated intestinal mucosa. Epon-embedded semi-thin sections (1 μ) stained with toluidine blue. A Non-irradiated jejunum; B Jejunum, 3rd day after 700 R; C Ileum, 3rd day after 700 R. Note shortening of villi and reduction in number of epithelial cells, without gross drastic discontinuities in the epithelial lining. Note also tendency of stroma to collapse.

X-ray damage resulted in a marked reduction in number and size of the epithelial cells (Fig. 4B and C), both factors causing a shortening of the villi. These abnormalities reached a maximum by the 3rd day after irradiation. They were associated with a great reduction in rough ergastoplasmic reticulum and a disappearance of the microvilli

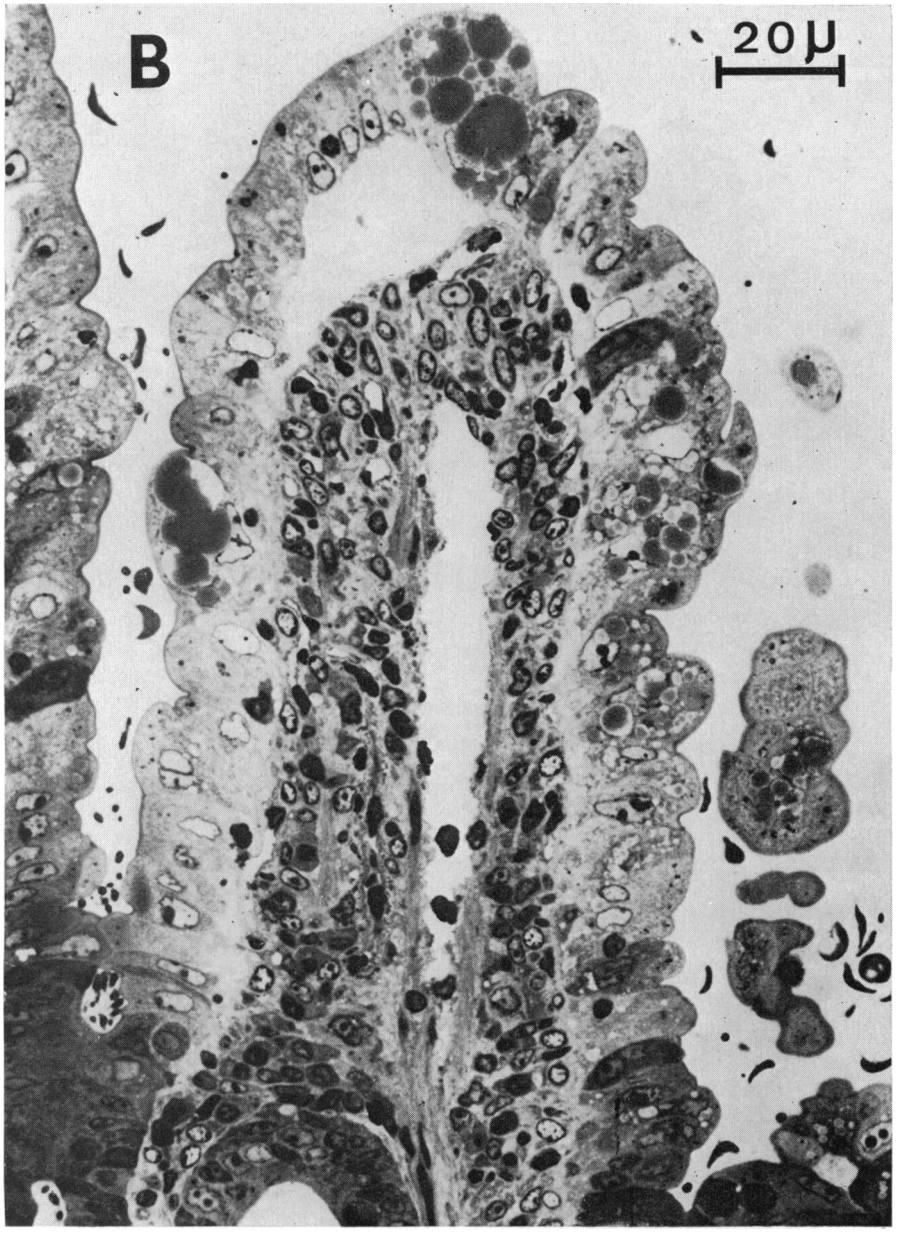

FIG. 4B.

(Fig. 7). In the jejunum (Fig. 4B) the cytoplasm of the epithelial cells was found to be loaded with fat droplets. Some regeneration, as judged by mitotic activity, already started by the second day irradiation, but even on day 4 most of the newly formed ribosomes still occurred as free clusters (Fig. 8), similar to those in immature cells.

The intercellular junctions between adjoining epithelial cells were studied with particular care on account of their possible bearing on the excretion of IgA into lumen of the

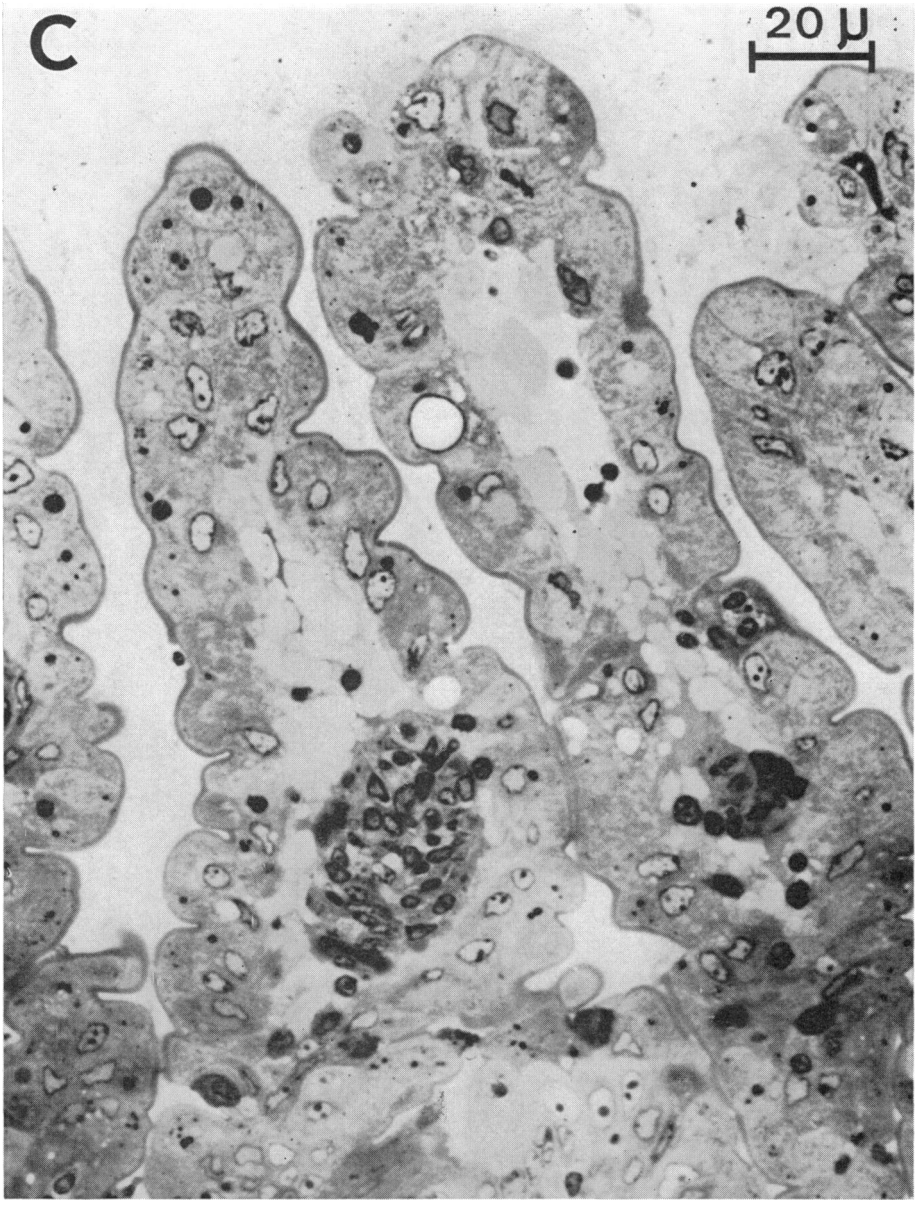

FIG. 4C.

gut. In this regard, the optical microscopic studies of the normal (Fig. 2A) and irradiated (Fig. 4B, C) epithelium provided no information, but the electron microscopic pictures disclosed an interesting finding. In the normal epithelium, the spaces separating adjoining cells were extremely narrow and of absolutely regular width, from the basement membrane up to the luminal border, being obliterated at places by desmosomes and closed, at the apical end, by a terminal bar (Fig. 5). 1–2 days after a lethal dose of X-rays, some widening of these intercellular spaces was noted, especially in the epithelium of the crypts

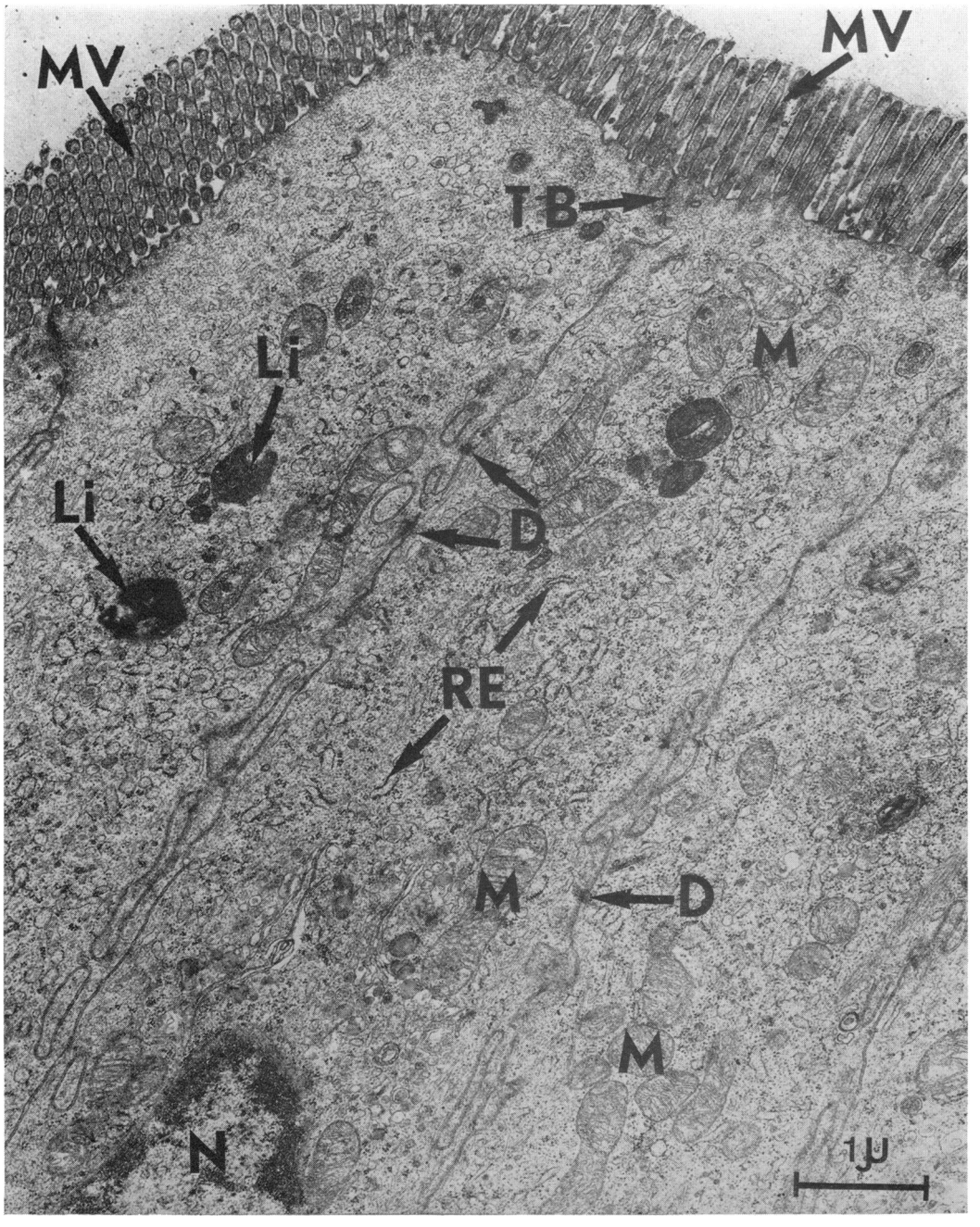

FIG. 5. Ultrastructure of cell junctions in normal jejunal epithelium. N, nucleus; M, mitochondria; Li, lipid droplets; MV, microvilli; RE, ribosome-studded endoplasmic reticulum. Note regularity and tightness of intricately folded intercellular spaces which are bridged by desmosomes (D) and terminal bars (TB).

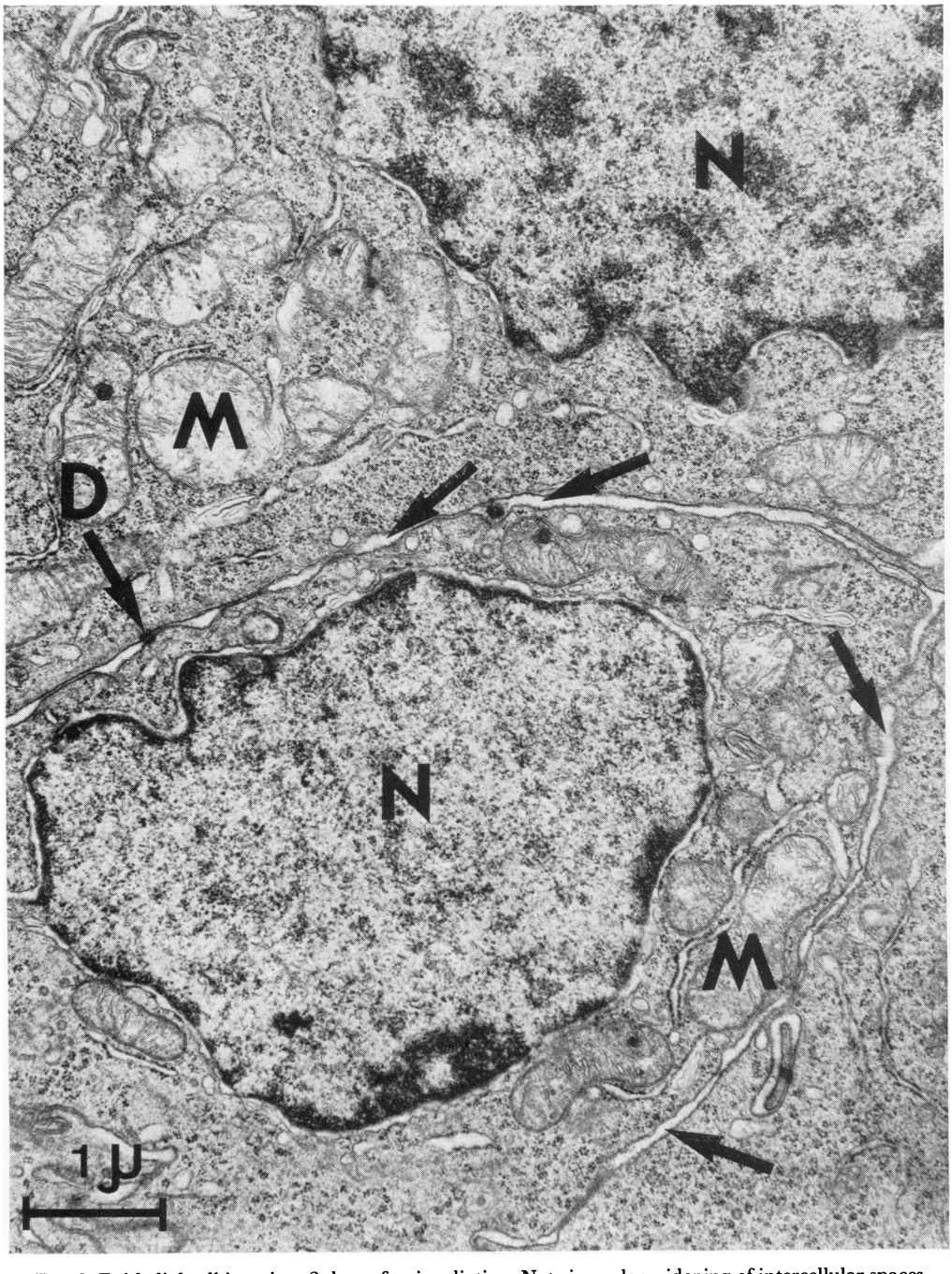

FIG. 6. Epithelial cell junctions 2 days after irradiation. Note irregular widening of intercellular spaces (arrows) with normal persistance of desmosomes (D).

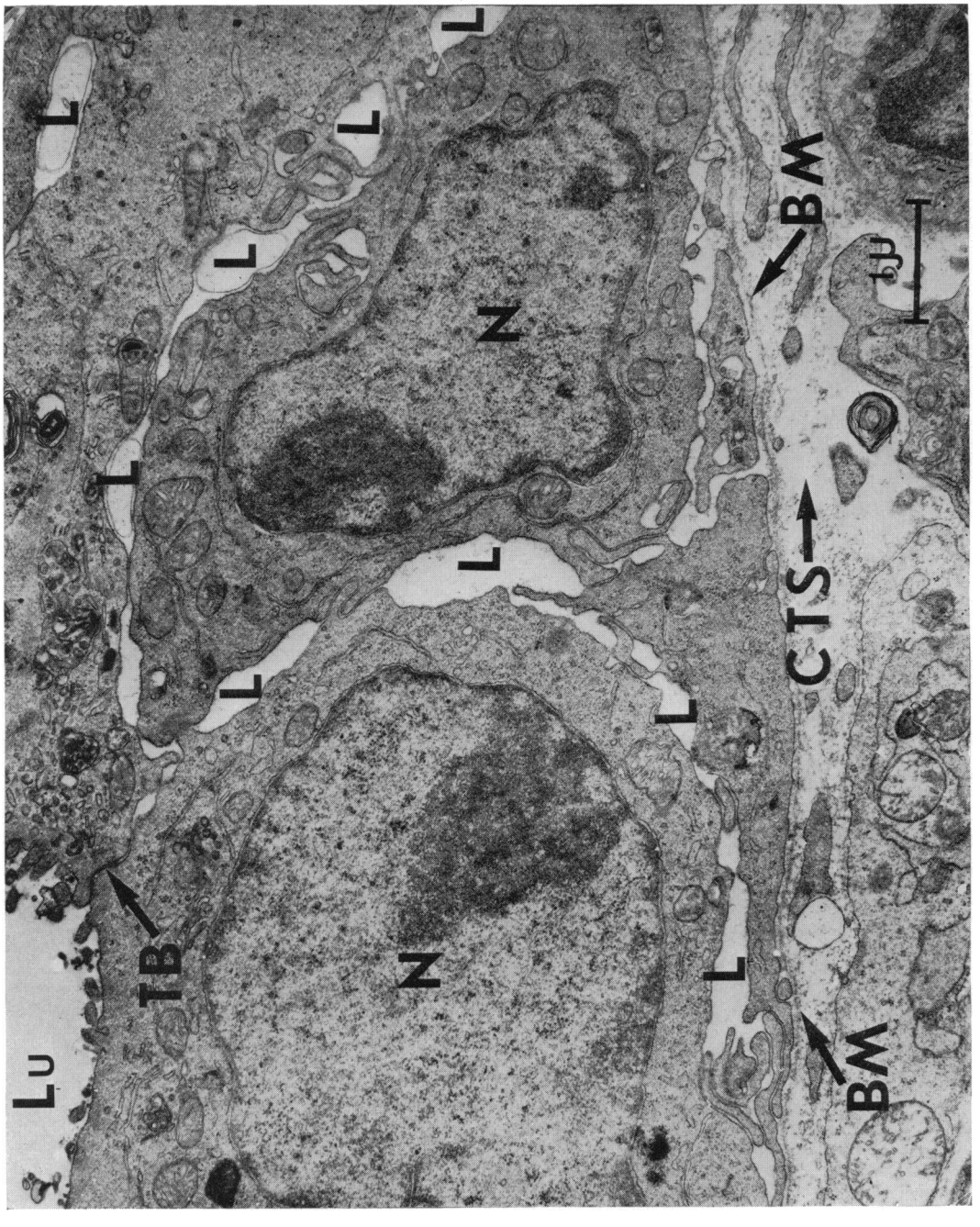

FIG. 7. Epithelial cell junctions 3 days after irradiation. Basement membrane (BM) and terminal bars (TB) remain intact but at many places the intercellular spaces have expanded to form lakes (L) providing almost patent communication between connective tissue space (CTS) and intestinal lumen (Lu).

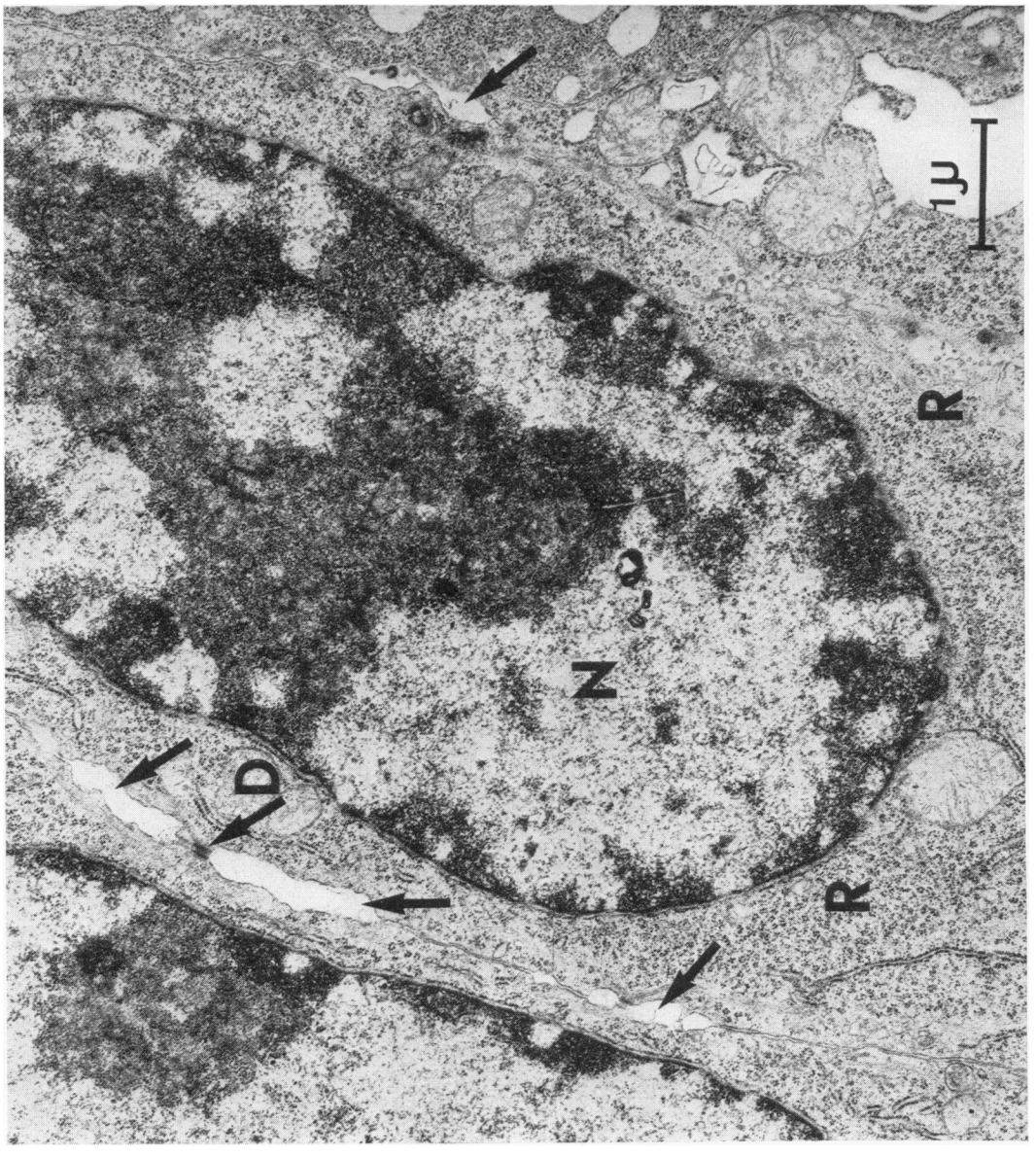

FIG. 8. Epithelial cell junctions 4 days after irradiation. Despite evident signs of repair (hypertrophic nuclei, N; numerous free ribosomes, R), cell junctions still show some patent spaces (arrows) and are not folded (compare Fig. 5).

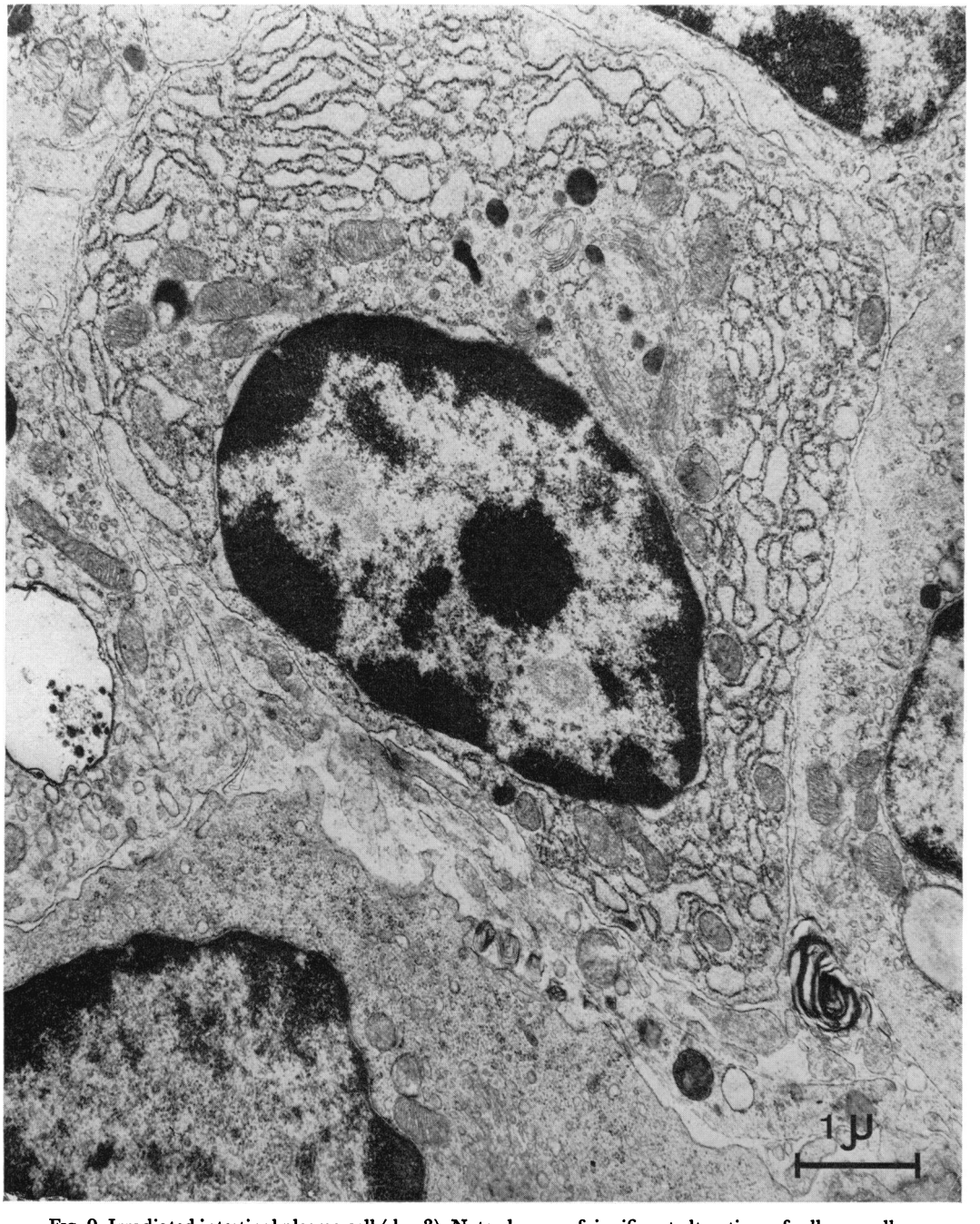

FIG. 9. Irradiated intestinal plasma cell (day 3). Note absence of significant alterations of cell organelles.

(Fig. 6). On the 3rd day after irradiation (Fig. 7), the intercellular spaces had become widely patent, creating a network of intercellular channels which freely abutted on the basement membrane. Desmosomes appeared to be relatively resistant to disjunction, and most of the bars were found to be intact. In no instance was gross sloughing of the epithelium observed and even the tips of the villi presented no discontinuities in excess of those observed in normal animals (Fig. 4). It was also noticed that during the repair phase the reestablishment of intercellular contacts progressed more slowly than the restoration of the cytoplasmic size and shape (Fig. 8). In many places desmosomes could be seen to interrupt areas of incomplete attachment of adjoining cells.

In confirmation of the immunohistological observations, it was found that the lamina propria tissue did not suffer a marked decrease in the numbers of its plasma cells. Many of the latter retained their normal ultrastructural appearance, even at the peak of radiation damage on the 3rd day (Fig. 9).

DISCUSSION

(1) *Effects of X-rays on the rate of biosynthesis of IgA*

As explained in the introduction, there exists a whole body of evidence pointing to a selective failure in the renewal of the pool of circulating IgA as being the mechanism responsible for the peculiar effects of X-rays on the serum levels of this class of immunoglobulins.

The most straightforward explanation would of course be that the biosynthesis of IgA is more sensitive to damage by X-rays than that of other immunoglobulin classes.

The protein-synthesizing machinery of cells in general is not reputed to be particularly affected by doses of ionizing radiation that would cause haematopoietic death if given on a whole-body level (Sarker, Devi and Hempelmann, 1961; Lipkin, Quastler and Muggia, 1963), and this seems true also in the special case of immunoglobulin-producing cells (Kennedy, Till, Siminovitch and McCulloch, 1965; Makinodan, Nettesheim, Morita and Chadwick, 1967; Reuter, Sassen and Kennes, 1967; Bazin, 1968; Sado, 1969). Indeed, whatever immunosuppressive effects may be credited to X-rays appear to rest chiefly on damage inflicted upon cell types involved in the earlier stages of the immune response (Kennedy *et al.*, 1965; Makinodan *et al.*, 1967). It must be stressed, however, that demonstrations of the relative radioresistance of immunoglobulin-synthesizing cells have been concerned with extraintestinal lymphoid tissue and with immunoglobulin classes other than IgA. That these reservations may be relevant is suggested by a report that the staining of the intestinal plasma cells of the guinea-pig with pyronin is decreased by whole-body X-irradiation with doses as low as 300 R (Ansari, Eder and Nägele, 1962). Similarly, ultrastructural lesions were described in the intestinal plasma cells of mice submitted to whole-body irradiation with lethal doses (Deby and Malkany, 1968), but neither in this nor in the preceding report was any comparative study on extraintestinal plasma cells included.

The work presented here (Table 1) indicates that a whole-body dose of 700 R caused a moderate reduction in numbers of the plasma cells in the small bowel wall, whereas no such effect was observed in the spleen or peripheral lymph nodes. It would also seem that this relative radiosensitivity of intestinal plasma cells is related to the particular environment to which they are exposed in the gut, rather than to their being specialized in the biosynthesis of the IgA class of immunoglobulins, since the rare cells producing IgM,

IgG_1 or IgG_{2a} appeared as much affected as those of the IgA type. One may venture the thought that toxic metabolites from damaged cells as well as massive admittance of bacterial endotoxins from the gut contents are involved in this increased vulnerability of plasma cells in an organ whose exquisite radiosensitivity first attracted attention 60 years ago (Regaud, Nogier and Lacassagne, 1912).

Since the majority of the intestinal plasma cells synthesize IgA (Crabbé *et al.*, 1968), and since in the dog, nearly 80 per cent of the circulating IgA is contributed by the gut (Vaerman and Heremans, 1970), it seemed tempting to ascribe the selective fall in serum IgA after irradiation to the relative radiosensitivity of the intestinal plasma cells. This hypothesis would also agree with the previous demonstration (Bazin *et al.*, 1970) that the gut is the site of the metabolic lesion affecting serum IgA in irradiated mice.

Nevertheless, the quantitative data provided by the biosynthesis experiments with isolated pieces of intestine indicate that the preceding explanation, though partially valid, cannot account for the major part of the fall in serum IgA levels following irradiation. The decrease in the rate of biosynthesis of IgA already suggested by the immunohistological studies, was confirmed, but the lowest activity observed (at day 4) still attained 56 per cent of the normal. On the average, the rate of synthesis of IgA by the gut was reduced by about one-third. This does not match the 50–90 per cent fall in serum IgA levels (Bazin and Micklem, 1967; Bazin, 1968; Bazin and Malet, 1969; Bazin *et al.*, 1970; Bazin and Doria, 1970).

2. *Effects of X-rays on the fate of IgA produced by the gut*

There is good evidence that the IgA synthesized by the plasma cells from the lamina propria is drained off in two opposite directions. Part of it reaches the blood stream *via* the mesenteric lymphatics, as demonstrated by comparative analyses of serum and mesenteric lymph in the dog (Vaerman and Heremans, 1970), as well as by the finding that X-ray damage to the gut caused a selective fall in serum IgA (Bazin *et al.*, 1970) without affecting the rate of disappearance of this protein from the serum (Bazin and Malet, 1970; Bazin *et al.*, 1970). On the other hand, the intestinal plasma cells of the mouse must be regarded as the major source of the IgA found in intestinal secretions, since only negligible amounts of serum IgA were found to pass into the intestinal contents (Crabbé *et al.*, 1968; Bazin *et al.*, 1970).

It was suggested in a preceding article (Bazin *et al.*, 1970) that the selective effects of X-rays on serum IgA might be explained by a shift in the distribution of IgA synthesized in the bowel wall, the proportion excreted increasing at the expense of the proportion supplied to the serum. This hypothesis seems to be substantiated by the findings reported here. It was found (Table 4) that the absolute amounts of IgA excreted into the lumen of the small bowel did not undergo any significant reduction following irradiation, although the total amounts synthesized (Table 2) did decrease by about one-third.

The lack of effects of X-rays on intestinal IgA is best brought out when the values found for the latter are compared to the serum IgA levels prior to irradiation, so as to cancel any variation due to individual differences in IgA-synthesizing capacities. The combined result of the invariance of the absolute intestinal IgA excretion rate and the fall in serum IgA following irradiation was a rise, peaking at day 5, of the *relative* amounts of intestinal IgA, as referred to the serum IgA level during the perfusion experiment (Table 4).

In conclusion, irradiation of the gut brings about a modest decrease in the IgA-synthesizing capacity of this organ, but the rate at which IgA is supplied to the intestinal

contents is not perceptibly affected. This can only mean that the contribution of the bowel wall to the pool of IgA in the blood stream must be affected very severely and the latter factor must in fact constitute the main reason for the observed selective fall in serum IgA levels.

The explanation for this shift in the distribution of IgA produced by the lamina propria is suggested by the morphological findings. The great radiosensitivity of the stem cells of the intestinal epithelium (Bloom and Bloom, 1954) is obviously responsible for the reduction in number of the cells coating the villi (Friedman, 1945; Knowlton and Hempelman, 1949; Weber, Craig and Friedman, 1951; Williams, De Long and Jarre, 1952; Williams, Toal, White and Carpenter, 1958), as well as for their flattening in shape and, indirectly, for the macroscopic shortening of the villi (Fig. 4B and C). The patency of the intercellular spaces after irradiation was cursorily mentioned by several authors (Quastler and Hampton, 1962; Detrick, Latta, Uphman and McCandles, 1963; Hugon, Maisin and Borgers, 1963, 1966), but its significance in the derangement of the function of the epithelial barrier does not seem to have been fully appreciated. The present work demonstrates that these communications between the interstitial spaces of the lamina propria and the intestinal lumen are a constant lesion after an X-ray dose of 700 R, and that leaks persist at a time when the more conspicuous cytoplasmic lesions are already largely repaired. There is little doubt that these abnormal communications provide the pathway by which the distribution of IgA synthesized in the lamina propria is rechannelled to the benefit of the intestinal contents and at the expense of the circulating pool of serum IgA.

An additional lesion which might play a role resides in the gross disorganization of the lacteal capillaries draining the interstitial lamina propria fluid (Fig. 4C), suggesting the possibility of obstruction along the pathway leading from this fluid to the bloodstream.

Light and electron microscopic studies of the irradiated mouse gut disclosed numerous patent intercellular junctions by which lamina propria fluid may escape into the intestinal lumen. It is suggested that this is the morphological substrate for the observed metabolic disturbance affecting IgA after irradiation.

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