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The Metabolism of Different Immunoglobulin Classes in Irradiated Mice

II. ROLE OF THE GUT

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Summary. Adult C_3H mice were irradiated by 700 r *in toto* or with the gut shielded. In similar experiments the gut alone was irradiated. The earlier observation of a selective and profound decrease of serum IgA, moderate decrease in IgG_1 and IgG_2, and little change in IgM, was confirmed for the animals irradiated without protection of the gut. Similar effects were found when the gut alone was irradiated, whereas whole-body irradiation with the gut shielded caused only little change in the different serum immunoglobulins. Hence the characteristic responses of the serum immunoglobulins appeared to result from X-ray damage to the intestine. From this and a preceding study it was hypothesized that such damage resulted in moderate losses of IgG from the blood stream into the intestinal lumen, but that the selective and severe depression of serum IgA was mostly due to the escape, through the damaged epithelium, of that portion of the secretion from intestinal plasma cells which normally would have joined the blood stream.

INTRODUCTION

It has been shown (Bazin and Micklem, 1967; Bazin, 1968) that the serum levels of the different immunoglobulin classes in mice respond differently to irradiation by X-rays. On the 9th or 10th day after a lethal dose of X-rays, the concentrations of IgM, IgA and IgG were found to have dropped to 65, 15–20 and 35–50 per cent, respectively, of their initial value.

Tracer studies undertaken to elucidate these phenomena (Bazin and Malet, 1969) have shown that the fractional catabolic rates of IgM and IgA remained unchanged after irradiation, whereas the fractional catabolic rates of the three subclasses of IgG (IgG_1, IgG_{2a} and IgG_{2b}) were significantly increased.

The digestive tract has been described as an important site of catabolism of albumin (Birke, Liljedahl, Olhagen, Plantin and Ahlinder, 1963) as well as of immunoglobulins (Andersen, Glenert and Wallerik, 1963). On the other hand, the gastro-intestinal mucosae of the mouse are known to harbour a vast population of plasma cells containing IgA

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(Crabbé, Bazin, Eyssen and Heremans, 1968), and are noteworthy for their exquisite radiosensitivity (Regaud, Nogier and Lacassagne, 1912).

It therefore seemed warranted to undertake an investigation on the possible role of the intestinal tract in the metabolic changes of immunoglobulins in irradiated mice. This was done by different procedures in which either the gut was shielded while the animal was being irradiated, or the gut alone was exposed to X-rays to the exclusion of the rest of the animal.

MATERIALS AND METHODS

Animals

All animals were 2–3-months-old male C3H mice from a stock bred at the University of Louvain.

Experimental techniques

Surgical preparation of the animals

The mice were anaesthetized by a 1 ml intraperitoneal dose of Rectanol® (Laboratoire Robert & Carrière, Paris), diluted to 1 per cent with saline.

The abdominal skin was incised along the midline over a length of about 3 cm, and a 2 cm long incision was made through the muscular and peritoneal wall. The small intestine, caecum and part of the colon were exteriorized. The animals were constrained lying on their side, with the exposed gut on a pad of gauze wetted with saline.

Irradiation

(a) *Irradiation restricted to the gut.* For this purpose the local irradiation technique described by Maldague (1966) was adapted to the gut. The cylindrical localizer of the X-ray tube (General Electric 'Maxitron', 250 kV, 1 Al+0·25 Cu filtration) was lowered so as to make contact with the mesenteric pedicle. The spleen and stomach, as well as the rectum and part of the colon, remained in the peritoneal cavity and were not exposed to irradiation. The mesenteric lymph node mass, however, was included among the irradiated tissues. The dose of X-rays given in this kind of experiment was 750 r.

(b) *Shielding of the gut in whole-body irradiated animals.* The exteriorized gut was shielded by introducing it into a specially devised lead chamber together with the pad of saline-wetted gauze by which it was protected. This chamber consisted of a flat square box with 2 cm thick walls and removable lid. On one side a 20×2 mm rectangular opening was left between bottom and lid, to allow passage of the mesenteric pedicle. This technique provided effective shielding of the greater part of the gut and the mesenteric lymph nodes. The duodenal loop, the rectum and the terminal portion of the large bowel were however irradiated. The dose of X-rays in this type of experiment was 700 r.

Post-operative care

After irradiation the abdominal wall was sutured at two levels: the muscular and peritoneal layer by means of No. 00 catgut and the skin by means of surgical silk.

The animals were housed together and allowed free access to water and food.

Titration of immunoglobulins

Blood samples were obtained from the retro-orbital sinus just prior to irradiation and on day 5 thereafter. The concentrations of IgM, IgA, IgG_1 and IgG_{2a} (hereafter called IgG_2) in the serum were measured by single radial immunodiffusion (Mancini, Carbonara and Heremans, 1965), using specific antisera as described in a preceding publication (Bazin, 1966).

RESULTS

IRRADIATION RESTRICTED TO THE GUT

Fourteen mice were anaesthetized and their intestinal tract exposed in the manner described. Of these, seven received a dose of 750 r on the exteriorized viscera, whereas the rest served as controls. All animals survived the experiment.

The results of this experiment are given in Tables 1 and 2. The statistical significance of the differences between irradiated and control animals is also indicated in Table 1. The manipulation exposing the intestinal tract, without irradiation, hardly affected the levels of the different immunoglobulins except for a slight fall in IgA. Irradiation of the exposed gut in contrast produced dramatic falls in three of the four immunoglobulins, the exception being IgM. The great decrease in the serum level of IgA was by far the most striking finding in this series of animals.

TABLE 1

SERUM IMMUNOGLOBULIN LEVELS IN MICE AT DAY 5 FOLLOWING LOCAL IRRADIATION OF THE GUT

	IgM	IgA	IgG_1	IgG_2
Gut exposed, no irradiation	92±6	82±11	102±7	110±12
Gut exposed and irradiated	81±5	33±6	63±6	50±3
P	Not significant	0·01	0·01	0·01

The figures are averages (±1 SD) from seven animals and represent the mean concentrations of the different immunoglobulins on day 5, expressed as percentages of the corresponding values determined in the same animals on the day before the experiment.

TABLE 2

DECREASE IN IMMUNOGLOBULIN LEVELS IN MICE AT DAY 5 FOLLOWING LOCAL IRRADIATION OF THE GUT

	IgM	IgA	IgG_1	IgG_2
Gut exposed, no irradiation (n = 7)				
Initial level	1·34±0·12	1·61±0·34	0·88±0·19	2·74±0·60
Decrease at day 5	−0·12±0·7	−0·35±0·20	−0·01±0·07	−0·25±0·35
Gut exposed and irradiated (n = 7)				
Initial level	1·38±0·10	2·04±0·28	1·30±0·24	2·99±0·38
Decrease at day 5	−0·27±0·07	−1·36±0·20	−0·47±0·09	−1·53±0·22

The figures are averages (±1 SD) from seven irradiated animals and seven non-irradiated controls, and are expressed in arbitrary units. One unit of each immunoglobulin is the concentration of that protein in a pool of normal mouse sera.

TOTAL BODY IRRADIATION WITH THE GUT SHIELDED

Two types of controls were used in this experiment. The first group were animals which received no irradiation, but whose gut was exposed for the same time as applied to the experimental animals. The other controls were mice which received the same dose of X-rays as the experimental animals, but with exposure of the gut. The dose of X-rays in the experimental animals and the second type of control was 700 r. Of the twenty-five animals employed, three died (one non-irradiated mouse and two non-shielded irradiated mice), on the 2nd day after the experiment.

TABLE 3

SERUM IMMUNOGLOBULIN LEVELS IN MICE AT DAY 5 FOLLOWING WHOLE-BODY IRRADIATION WITH THE GUT SHIELDED

	IgM	IgA	IgG_1	IgG_2
No irradiation, gut exposed (n = 7)	94±6	67±8	103±14	93±6
Whole-body irradiation; gut exposed, not shielded (n = 7)	63±2	12±2	66±7	67±3
Whole-body irradiation; gut exposed, shielded (n = 8)	84±6	71±7	84±9	88±4

The figures are averages (±1 SD) from the numbers of animals indicated in parentheses, and represent the mean concentrations of the different immunoglobulins on day 5, expressed as percentages of the corresponding values determined in the same animals on the day before the experiment.

TABLE 4

DECREASE IN IMMUNOGLOBULIN LEVELS IN MICE AT DAY 5 FOLLOWING WHOLE-BODY IRRADIATION WITH THE GUT SHIELDED

	IgM	IgA	IgG_1	IgG_2
No irradiation, gut exposed (n = 7)				
Initial level	1·38±0·11	1·89±0·41	0·48±0·06	1·35±0·07
Decrease at day 5	−0·06±0·08	−0·77±0·31	−0·06±0·04	−0·09±0·07
Whole-body irradiation, gut exposed, not shielded (n = 7)				
Initial level	1·32±0·07	1·65±0·28	0·59±0·14	1·35±0·13
Decrease at day 5	−0·48±0·02	−1·47±0·27	−0·21±0·08	−0·46±0·07
Whole-body irradiation, gut exposed, shielded (n = 8)				
Initial level	1·29±0·06	1·40±0·23	0·60±0·11	1·32±0·05
Decrease at day 5	−0·19±0·07	−0·45±0·14	−0·12±0·05	−0·15±0·06

The figures are averages (±1 SD) from the indicated number of experimental animals and the two types of controls, and are expressed in arbitrary units. One unit of each immunoglobulin is the concentration of the protein in a pool of normal mouse sera.

The results from this series are indicated in Tables 3 and 4. The findings in the non-irradiated series with the gut exposed were rather similar to those in the same type of control from the preceding series, except that the fall in IgA levels was somewhat more pronounced. Irradiation with exposure but shielding of the gut produced results which did not statistically differ from the findings in non-irradiated controls. The effect merely amounted to a moderate decrease in all immunoglobulins. The animals that received total body irradiation, without any shielding but with exposure of the gut, showed clearly decreased levels of IgM, IgG_1 and IgG_2, and a particularly impressive fall in the concentration of IgA.

DISCUSSION

It had previously been demonstrated (Bazin and Micklem, 1967; Bazin, 1968) that whole-body irradiation in mice is followed by a profound and selective decrease of the IgA level in the serum, and by similar changes of much smaller magnitude in the other serum immunoglobulins, least of all in IgM. These findings were confirmed in the totally irradiated unshielded animals used as controls in the second experiment here described.

The present investigation clearly indicates that the dramatic effect of total body irradiation on the concentration of serum IgA is essentially due to the radiation damage received by the gut. The fall in serum IgA was prevented by shielding the intestine while irradiation of the gut alone was sufficient to produce the typical decrease in serum IgA. These effects were significantly greater than the moderate decline in serum IgA levels caused by the mere extraperitoneal exposure of the gut in control animals.

The levels of IgG in the serum were more influenced by local irradiation of the gut and by total irradiation than by irradiation with the gut shielded. As already reported (Bazin, 1968), the serum level of IgG was clearly but not considerably decreased on day 5 after whole body irradiation. This could also be related to X-ray damage suffered by the intestine, since the effect was obtained with the gut alone irradiated but not in those irradiated *in toto* with the gut shielded. In the latter animals both IgM and IgG showed the same moderate decrease. The protective effect of shielding the intestine was, however, not so clear cut as in the case of IgA.

It must be emphasized that the technique employed did not allow irradiation of the intestinal tract over its entire length, and that the mesenteric lymph node was included among the tissues exposed to X-ray damage. On the other hand, in the experiments where the gut was shielded, such protection was extended to the mesenteric lymph node, and it was impossible to keep the distal and proximal extremities of the intestinal tract from being irradiated. It is possible, therefore, that with a better technique the differences between the results of the two types of experiment might become even more striking than here observed. One may also surmise that the shielded parts of the body suffered slight damage by back-scattering from the irradiation of other tissues. It must also be taken into account that blood was sampled only on day 5 after the experiment. This delay was chosen on the basis of previous findings which had indicated that the greatest differences between the different Ig classes would be observed at that time, though it did not coincide with the moment of maximal depression of the serum IgM and IgG levels.

The serum immunoglobulins clearly fell into three classes so far as the effects of irradiation were concerned. IgM was hardly affected and, as shown here, the gut was not implicated in whatever decline in IgM may be observed. This is not surprising if one recalls that the intestine is only a very minor site of IgM production, as judged from the scarcity of IgM plasma cells in this tissue (Crabbé *et al.*, 1968). It has been shown elsewhere (Bazin and Malet, 1969) that the biological survival of ^{131}I-labelled IgM is not shortened in irradiated mice, indicating that no important intestinal losses of this immunoglobulin occur through the damaged mucosa. The small decrease in IgM serum levels observed at day 5 may be due to radiation damage to the cells producing this immunoglobulin. Doses of the order of 700–750 r have been shown not to inhibit to any great extent those cells which are already secreting antibodies, though the renewal of this cell population is more seriously affected (Kennedy, Til, Siminovitch and McCulloch, 1965; Makinodan, Nettesheim, Morita and Chadwick, 1967).

IgG_1 and IgG_2 in irradiated mice were present at decreased concentrations. The experiments here described indicate that radiation damage to the gut was the major factor involved in this change. It has been shown in a previous study (Bazin and Malet, 1969) that these two immunoglobulins are abnormally rapidly removed from the blood stream after *in toto* irradiation. Taken together, all data suggest that the fall in serum IgG level after irradiation is to a large extent due to abnormal losses through the damaged intestinal mucosa. In this respect, IgG resembles albumin, which is also lost in great amounts during the exudative gastroenteropathy following irradiation (Palmer and Sullivan, 1959). Increased vascular permeability is known to prevail in the irradiated intestinal mucosa (Hornsey and Vatistas, 1968). However, diminished synthesis may also play a role in the fall of IgG_1 and IgG_2 serum levels. This is suggested by their moderate decrease in control animals having their gut shielded during whole body irradiation (Tables 3 and 4).

IgA is a protein whose concentration in the serum undergoes a very early and pronounced fall after whole-body irradiation (Bazin, 1968). The present experiments clearly indicate that the metabolic lesion responsible for this effect is localized in the gut. Several interpretations may be considered.

Firstly, the intestinal mucosa of the normal adult mouse possesses an extremely dense population of plasma cells containing IgA (Crabbé *et al.*, 1968), and has been shown to synthesize IgA very actively *in vitro* almost to the exclusion of other immunoglobulins (Mandel and Asofsky, 1968). Although much of this IgA is presumably excreted into the lumen of the gut, there is good reason to believe that part of it joins the blood stream to contribute to the pool of circulating IgA. This, at any rate, is what happens in the dog, whose mesenteric lymph (which drains the *lamina propria* of the gut) has been reported to contain IgA in great excess over the amount reaching the mucosa by way of the arterial blood supply (Vaerman and Heremans, 1970). Turnover studies with [^{131}I]IgA in mice have shown that the half-life of this protein is not significantly shortened after irradiation (Bazin and Malet, 1969). Although these investigations were carried out with a myeloma protein, and need confirmation with regard to normal serum IgA, they seem to indicate that the observed fall in concentration is due to a shutdown of the supply of IgA to the plasma rather than to losses from the blood stream. Escape of IgA from the plasma into the lumen of the damaged gut would at any rate not exceed in proportion that of IgG and hence should not be able to produce the precipitous selective fall in serum IgA which is observed. Hence, one may wonder whether the major metabolic lesion affecting IgA is not simply a suppression of its synthesis by the intestinal plasma cells. This idea is supported by reports on the damaging effect of irradiation on the intestinal plasma cells of the guinea-pig (Ansari, Eder and Nägele, 1962) and the mouse (Deby and Malkany, 1968). Again it may also be relevant to note that X-rays impair the capacity of immunologically competent cells to proliferate (Makinodan *et al.*, 1967).

However, this hypothesis would imply that cells synthesizing IgA—or at least those among them that are localized in the intestinal mucosa—are considerably more vulnerable to X-ray damage than are other varieties of immunoglobulin-producing cells. Such an *ad hoc* postulate lacks experimental support. Rather, immunofluorescence studies on the irradiated mouse gut do not seem to indicate any important decrease in the counts of IgA-type plasma cells (in preparation). Locating the selective sensitivity at the level of the lymphoid precursor cells is equally unsatisfactory, the more since the different types of haematopoietic stem cells are known to be of similar radiosensitivity (Silini, Pons and Pozzi, 1968). One may still argue that the location of IgA-producing cells in a site so

exquisitely radiosensitive as the gut may adversely affect their metabolic functions. However, biosynthesis of IgA in the mouse has been found to persist at the 24th hour following a total body dose (950 r), higher than that employed in the present study (700 r) (Bazin, 1968).

The alternative hypothesis is that the rate of synthesis of IgA by the irradiated intestinal plasma cells is not decreased any more profoundly than that of other immunoglobulins, but that in the irradiated animal the greater part of the IgA secreted in the *lamina propria* escapes into the lumen through the damaged epithelium instead of joining the lymphatics and hence the blood. This interpretation would be consistent with the already mentioned role of the gut as a major supplier of the pool of plasma IgA. Considering the magnitude of the effect observed, one even wonders whether the intestinal mucosa may not be the most important source of IgA in the body. This hypothesis finds support in the knowledge that X-ray doses of the order employed in the present study are able to damage intestinal epithelial cells (Hugon, Maisin and Borgers, 1963; Casarett, 1968). Such lesions have been reported to develop within 6 hours and to persist for 5 or 6 days. Electron-microscopic studies on the intestinal epithelium and immunochemical investigations on the gut content of irradiated mice, now being undertaken at this laboratory, will be reported elsewhere.

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