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The Metabolism of Different Immunoglobulin Classes in Irradiated Mice

I. CATABOLISM

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Summary. The catabolism of the different classes of immunoglobulins (Ig) of normal and irradiated mice was studied. A lethal dose of X-rays brought about no significant alterations in the catabolic rates of IgM and IgA, which kept to a time of about 12 hours. Contrariwise, the IgG (IgG_1, IgG_{2a} and IgG_{2b}) displayed a distinctly accelerated catabolism in the irradiated mice, the half-life of these Ig's being reduced by approximately one-half. We propose an interpretation of this phenomenon, based, on one hand, on the great radiosensitivity of the intestinal epithelium and, on the other hand, on the mode of catabolism of IgM and IgA which appears to be different from that of IgG.

INTRODUCTION

It is well established that a drop occurs in the serum concentration of γ-globulins of mice in the days following whole-body irradiation (Grabar, Kashkin and Courcon, 1963; Sassen, Kennes and Maisin, 1966). The different classes of immunoglobulins (Ig): IgM, IgA and IgG, seem to differ with respect to their sensitivities to irradiation (Bazin and Micklem, 1967; Bazin, 1968). This phenomenon may be due to a number of causes. Irradiation is likely to damage the Ig-producing cells, but it is unlikely that irradiation at a dose that can bring about the haemopoietic death of an animal could diminish, at the cell stage, an Ig synthesis already begun. Reuter, Sassen and Kennes (1967b) have shown that mice are still capable of synthesizing γ-globulins 7 days after irradiation at a dose of 700 or 1000 r. Likewise, one of us has shown that mice still synthesize IgM, IgA, IgG_1 and IgG_{2a} between the 24th and 48th hour following a lethal X-ray dose (Bazin, 1968). Lastly, a study of the immunological response of spleen cells of mice immunized against sheep red blood cells has demonstrated that mature cells engaged in the synthesis of specific antibodies are highly radio-resistant (Makinodan, Nettesheim, Morita and Chadwick, 1967). Quite different factors may, however, be operative. Thus Friedberg (1960) found changes in the overall water content of the organism after irradiation. Possible modifications in the distribution of fluid between the intra- and extravascular compartments may be related to this phenomenon (Reuter, Gerber, Kennes and Remy-Defraigne, 1967a). The catabolism of γ-globulins, too, seems subject to modification by irradiation (Reuter *et al.*, 1967b). Hollingsworth (1950), however, has shown that, in rabbits, a dose of 300 r does not alter the rate of decay of transferred antibodies. Furthermore, Perkins and Marcus (1957) found that an irradiation at doses higher than LD_{100}/30dH does not reduce the titre of previously produced antibodies. In these two publications there is no mention of the

classes of Ig involved. As a consequence, these various results still leave room for doubt, since the different Ig classes were not studied separately. Considering that the different Igs have very dissimilar metabolisms (Bazin, 1967b), it is possible that the disturbances set up by irradiation may affect the catabolism of each Ig in an individual manner.

In this work we have studied the catabolism of the five classes or sub-classes of mouse Ig (Fahey, Wunderlich and Mishell, 1964a, b).

MATERIALS AND METHODS

Animals

Mice from pure XVII, C3H, C57/BL and BALB/c strains were employed. The XVII animals were used for the catabolism studies and the C3H and BALB/c animals as recipients for mouse myeloma proteins. The C57/BL mice furnished the serum needed for the isolation of normal IgG.

Irradiation

Certain XVII mice were subjected to whole-body irradiation with an X-ray dose of 950 r (225 kV, 12 mA, filtration 0·3 mm Cu and 2 mm Al, cathode-to-target distance 40 cm), which normally produces a 100 per cent animal mortality rate 9–12 days after irradiation.

Nomenclature of immunoglobulins

In continuity with our preceding publication (Bazin, 1966), we here employ the following nomenclature for mouse immunoglobulins, slightly modified from that of Fahey *et al.* (1964a, b): IgM (or β_{2M}), IgA (or β_{2A}), IgG_1 (or 7S γ_1), IgG_{2a} (or 7S γ_{2a}), IgG_{2b} (or 7S γ_{2b}). Synonyms for these names have been listed elsewhere (Bazin, 1967b).

Titration of immunoglobulins

Control mice were bled at the end of each experiment. Each Ig was titrated by the method of Mancini, Carbonara and Heremans (1965), with specific antisera described in an earlier publication (Bazin, 1966). The only modification consisted in the use of pure IgM, and myeloma proteins for the IgA, IgG_1, IgG_{2a} and IgG_{2b}. Purified solutions of each Ig were assayed by the biuret method (Gornall, Bardawill and David, 1949) against a solution of bovine serum albumin titrated by Kjeldahl, and were used as references. In the case of the IgG classes, analyses of the purified solutions always revealed some contaminants belonging to other Ig classes or sub-classes. Because of this, the values obtained were slightly overestimated. The IgG_{2b} was not assayed separately.

Preparation of Ig labelled with iodine-131

(a) *Extraction of Ig.* The IgM was isolated from a mixture of sera from normal mice of XVII or C3H strains by a method already described (Bazin, Savin and Micklem, 1968).

The three IgA preparations used in this study were obtained from serum of BALB/c mice with E III 8 myeloma, given to us by Dr G. Lespinats and Dr G. Hermann. Fifteen millilitres of this serum were applied to a Sephadex G-200 (Pharmacia) column (5×100 cm, Tris–HCl buffer, pH 8·0, 0·1 M+1 M NaCl) and, using the Ouchterlony method, we recovered the myeloma concentrate from the eluate, reconcentrated it and passed it a second time over an identical column. The central portion of the myeloma IgA elution peak was then chromatographed on ion-exchange cellulose. For experiments C4 and C5

we used a DEAE-cellulose (DE-52, Whatman) column (2·5×40 cm equilibrated with a sodium phosphate buffer, pH 5·5, 0·2 M). The protein solution was dialysed against the buffer, applied to the column, and eluted with the same buffer. Next, the peak eluted with a sodium phosphate buffer (pH 4·8, 0·3 M) was precipitated with half-saturated cold ammonium sulphate. For experiment C6 we used a CM-cellulose (CM-52, Whatman) column, and the peak employed was collected with an 0·05M sodium phosphate buffer of 6·5, the column having been washed beforehand with an 0·175 M phosphate buffer of pH 5·5. This peak was also precipitated three times with half-saturated ammonium sulphate.

Isolation of normal IgG. Normal IgG was isolated from serum of C57/BL mice immunized by two intraperitoneal injections, 8 days apart, of 600×10^6 killed *Bordetella pertussis* organisms (Institut Pasteur) suspended in 0·10 ml of Propidon (Specia). The mice were bled 15 days after the second injection. Ten millilitres of the pooled sera were applied to a Sephadex G-25 S (Pharmacia) column by a method already described (Bazin *et al.*, 1968). The proteins were eluted with a sodium phosphate buffer (pH 7·4, 0·1 M) with 0·15 M NaCl added. The second portion of the second eluate peak was concentrated against Aquacid (Calbiochem), and applied to a Sephadex G-200 column (100×2·5 cm). The middle of the second elution peak was considered to represent a purified solution of normal IgG.

Isolation of IgG_1. The IgG_1 solutions for experiments C8 and C10 were obtained from serum of BALB/c mice with myeloma VII A2, given to us by Dr G. Lespinats and Dr G. Hermann. Fifteen millilitres of mouse serum were dialysed against 0·0175 M sodium phosphate buffer of pH 8·0, and applied to a DEAE-cellulose (DE-52, Whatman) column equilibrated with the same buffer. The breakthrough fraction collected with the same buffer was discarded. The proteins eluted with a similar buffer of molarity 0·05 M were precipitated three times with cold half-saturated ammonium sulphate, and chromatographed on a Sephadex G-200 (Pharmacia) column (100×2·5 cm) with an 0·1 M Tris–HCl buffer of pH 8·0 containing 1 M NaCl. The central portion of the second peak was taken as a purified solution of IgG_1 after concentration by ultrafiltration. The solution used for experiment C9 was obtained in the same way, but from 10 ml of serum from mice with MOPC/70A myeloma, originating from Dr M. Potter's laboratory. This serum was given to us by Dr J. F. Doré.

Isolation of IgG_{2a}. The purified solutions of IgG_{2a} were prepared by the method of Askonas (1961) from serum of C3H mice with myeloma 5563, given to us by Dr M. Fougereau.

Isolation of IgG_{2b}. The IgG_{2b} was prepared from serum of BALB/c mice with tumour S188, given to us by Dr M. Cohn. Ten millilitres of serum dialysed against an 0·0175 M sodium phosphate buffer of pH 6·5 were applied on a 40×2·4 cm column packed with DEAE-cellulose (DE-52, Whatman) equilibrated with the same buffer. The breakthrough fraction was discarded. The proteins eluted with an 0·035 M phosphate buffer of the same pH were precipitated three times with cold half-saturated ammonium sulphate. After a short dialysis against a Tris–HCl buffer (pH 8·0, 0·1 M, +1 M NaCl) these proteins were applied on a 100×4 cm Sephadex G-200 (Pharmacia) column. The eluting buffer was the same as already described for the dialysis preceding this filtration on gel. The middle fraction of the second peak of this chromatography was considered to be pure IgG_{2b}. The two samples used in experiments C14 and C15 were prepared separately.

(b) *Labelling with iodine*-131. The various Ig preparations were labelled by the McFarlane (1958) method, modified only to the extent of removing the free iodine on a Sephadex G-50 column (30×2 cm), followed by overnight dialysis at +4° against saline.

(c) *Control of purity of proteins and labelling.* All the ^{131}I-labelled Ig preparations were analysed by immunoelectrophoresis or Ouchterlony analysis. The labelling was verified by radioautography of these immunodiffusion plates, at protein concentrations of the different samples varying from 3 to 10 mg/ml, as already described (Bazin, 1966, 1967a). Exposure times of radioautographs varied from 2 to 30 days. The samples were taken either before injection or at the middle or end of the experiments. All the injected Igs underwent checks at least twice during each study.

Experimental procedures

All the animals were kept in cages containing wood shavings which were changed very frequently. The water, freely available, contained 0·45 per cent of NaCl and 0·01 per cent of KI. Food was provided *ad libitum*. The mice received an intravenous injection of 1–5 μCi of a radioactive solution in a volume of 0·2–0·5 ml. The amount of immunoglobulin injected into each mouse was 0·50–0·75, 0·4–0·6 and 1–2 mg of IgM, IgA and IgG, respectively.

The total radioactivities of the experimental animals were measured by means of a scintillation spectrometer. The mice were confined in a Plexiglass tube which greatly restricted their movements, and were placed at a constant distance from the scintillation crystal, so as to obtain satisfactory and reproducible geometry. The radioactivity was determined 30 minutes after the inoculation and daily thereafter. In the other experiments, in order to evaluate the serum radioactivity, samples of about 60 μl of blood were tapped from the orbital sinus (Pettit, 1913), 30 minutes after the injection and thereafter every 3 hours for the IgM and IgA, or every 24 hours for the IgG. The microhaematocrit tube used for the sampling was centrifuged and then 20 μl were placed, by means of a precision micropipette, in a small tube which was hermetically sealed. The sera were kept at $-20°$ and counted together at the end of each experiment, in a crystal well connected to a spectrometer.

Calculations

(a) *Irradiation due to the injection of ^{131}I-labelled Ig.* The maximum limit of the dose absorbed by the organs of the test animals during the first biological half-life period were evaluated as a function of the activity injected with each Ig preparation.

This maximum limit can be expressed as:

$$D_t = D_\infty \left(1 - 0{\cdot}5\, e^{-0{\cdot}693 \frac{t}{T}}\right),$$

with

$$D_\infty = \frac{A_0\, m_0\, E\, G}{100\, M} \times \frac{1}{0{\cdot}693 \left(\frac{1}{T} + \frac{1}{t}\right)},$$

where $A_0\, m_0$ is the initial activity;

M is the average weight of the mice, in grams;

E is the average energy (of the β radiation) in ergs;

G is the geometrical factor;

T is the radioactive half-life of iodine-131, in seconds; and

t is the biological half-life of the Ig under consideration, in seconds.

(b) *Rate of catabolism.* The rate of catabolism of each Ig was estimated from its biological half-life ($T_{\frac{1}{2}}$), which was determined graphically from the data from each experiment. The radioactivity values were expressed as percentages of the inoculated dose. They were corrected for physical decrease of the isotope in the cases where the determinations were based on the whole body radioactivity counts. The radioactive half-life of iodine-131 was taken to be 8·075 days.

RESULTS

IgM

It proved difficult to determine the catabolism of this protein by measuring the decay of the overall radioactivity of the normal and irradiated mice inoculated with ^{131}I-labelled IgM. Thirty-six hours after the injection a large part of the radioisotope still present was concentrated in the liver. On the other hand, there was only slight radioactivity left in the serum, as determined by direct counts. Nevertheless, by radioautography of Ouchterlony plates made with this serum, it was demonstrated that this radioactivity did in fact correspond to IgM, but the amounts were very small.

Furthermore, the shoulder in the whole-body radioactivity decay curve for these animals suggested that the direct counting method was probably not correct for proteins with short half-lives. In such a case, indeed, it appears that one cannot disregard the fate of the catabolized molecules and their decay products which, by their own half-lives, significantly contribute to the whole-body radioactivity count.

Hence the results here described were obtained by measurement of the serum radioactivity of the mice inoculated with ^{131}I-labelled IgM.

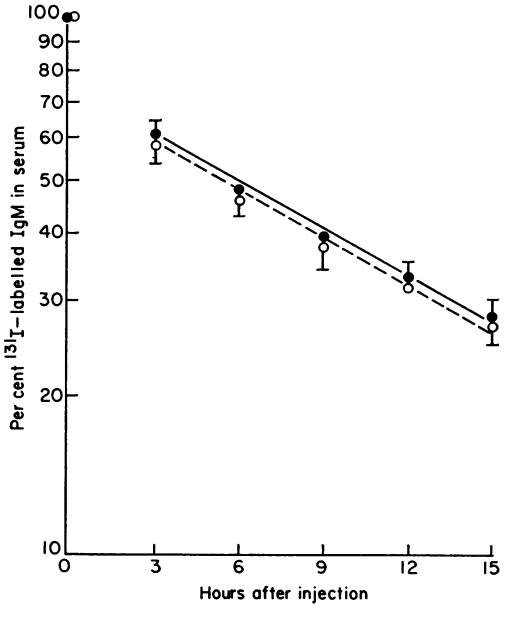

FIG. 1. Catabolism of an ^{131}I-labelled IgM solution in normal (●) and irradiated (○) mice (experiment No. 2). The vertical bars represent the ranges of the data observed.

TABLE 1

HALF LIFE OF ^{131}I-LABELLED IgM IN NORMAL OR IRRADIATED MICE

Experiment	Age of mice (days)	Sex	No. of mice	Irradiation (r)	Mean serum Ig levels (mg/ml)				Time between irradiation and ^{131}I-labelled Ig	Half-life of ^{131}I-labelled IgM (hours)
					IgM	IgA	IgG_1	IgG_2		
C1	200	M	4	0	1·07	0·20	0·34	14·40	–	12½
			4	950	–	–	–	–	–0·5 hours	12
C2	220	M	4	0	2·63	0·19	0·34	21·00	–	10½
			4	950	–	–	–	–	+ 5 days	10
C3	110	F	4	0	0·98	0·09	0·31	22·50	–	12
			4	950	–	–	–	–	+ 5 days	12

TABLE 2

HALF-LIFE OF ^{131}I-LABELLED IgA IN NORMAL OR IRRADIATED MICE

Experiment	Age of mice (days)	Sex	No. of mice	Irradiation (r)	Mean serum Ig levels (mg/ml)				Time between irradiation and injection of IgA	Half-life of ^{131}I-labelled IgA (hours)
					IgM	IgA	IgG_1	IgG_2		
C4	230*	F	4	0	1·28	0·66	0·49	18·00	–	13
			4	950	–	–	–	–	–0·5 hours	13
C5	300	F	4	0	1·46	0·26	1·24	28·40	–	11
			4	950	–	–	–	–	+ 5 days	11
C6	130	M	4	0	2·48	0·18	1·50	21·50	–	10
			4	950	–	–	–	–	+ 5 days	10

* 0·2 ml *Bordetella pertussis* vaccine (6×10^8 organisms) given intraperitoneally at age 60 days and 90 days.

Three different solutions of IgM from normal mice were studied in the XVII mice. The biological half-lives found during these experiments are given in Table 1. Fig. 1 shows the serum radioactivity decay, expressed as a percentage of the inoculated dose, in the normal or irradiated animals in experiment 2. The verification of the degree of purity and the labelling of the IgM sample used in that experiment is illustrated in Fig. 2. As in experiment C2, the controls for experiment C1 showed no other labelled proteins. The protein solution used in experiment C3, however, showed traces of α-lipoprotein. The half-life of the IgM was found to approximate 12 hours in both the normal animals and those irradiated either 5 days before or 30 minutes after the injection of labelled IgM.

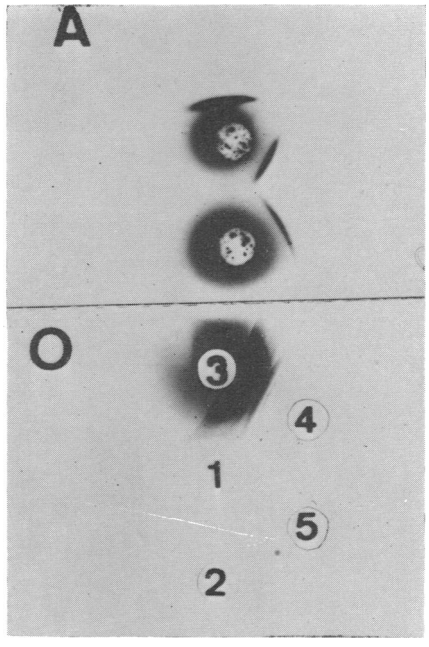

FIG. 2. Ouchterlony test (O), and its radioautography (A), of the ^{131}I-labelled IgM solution used for experiment No. 2. (1) ^{131}I-labelled IgM solution (2 mg/ml); (2) serum taken from an irradiated mouse 3 hours after injection of the labelled solution; (3) rabbit antiserum to whole mouse serum; (4) normal mouse serum; (5) rabbit anti-mouse IgM.

IgA

As with the IgM, the determination of the IgA catabolism was calculated from the decay of the serum radioactivity after intravenous injection of a preparation of ^{131}I-labelled IgA. Three samples of myeloma IgA from plasma-cell tumour E III 8 were studied separately. Table 2 gives the main details of these tests, and Figs. 3 and 4 show the details of experiment C5. In all three cases, the radioautography checks of the protein solutions studied showed that these contained only labelled IgA. In experiment C4, the protein was injected 30 minutes before irradiation, and in experiments C5 and C6, five days after it. No difference in the rate of catabolism could be detected in these three tests. The data show that the IgA half-life is about 12 hours in both the normal and the irradiated mice.

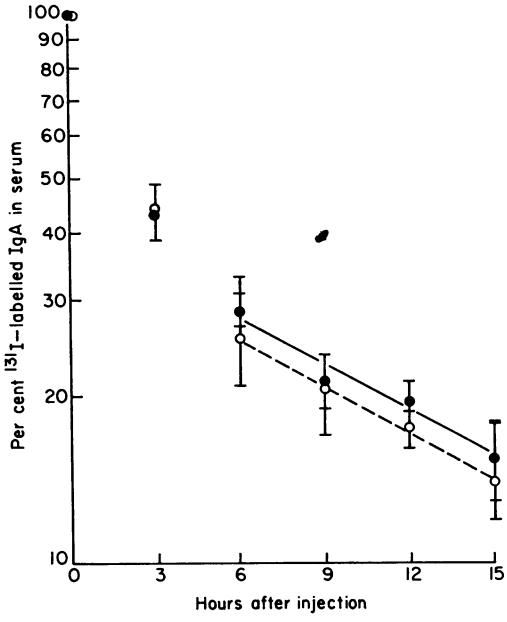

FIG. 3. Catabolism of an ^{131}I-labelled IgA solution in normal (●) and irradiated (○) mice (experiment No. 5). The vertical bars represent the ranges of the data observed.

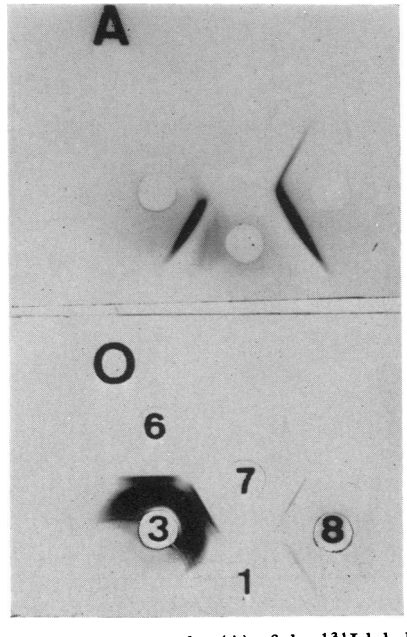

FIG. 4. Ouchterlony test (O) and radioautography (A) of the ^{131}I-labelled IgA solution used for experiment No. 5. (1) ^{131}I-labelled IgA solution (1 mg/ml); (3) rabbit antiserum to whole mouse serum; (6) serum of normal mouse under experiment (15 hours after inoculation); (7) serum of irradiated mouse under experiment (6 hours after inoculation); (8) rabbit antiserum to IgA mouse serum.

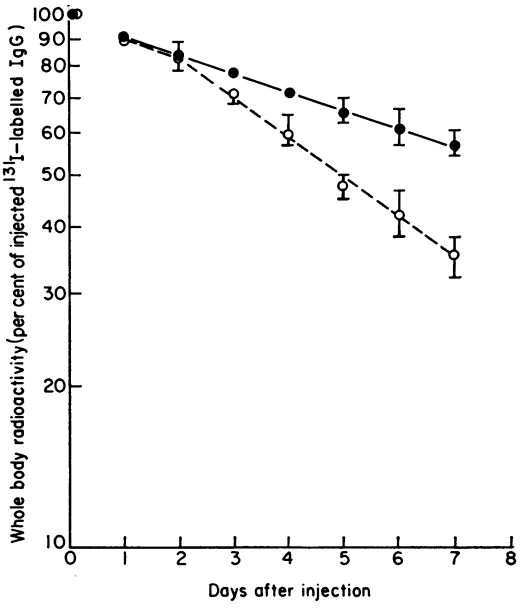

FIG. 5. Catabolism of ^{131}I-labelled IgG in normal (●) and irradiated (○) mice (experiment No. 7). The vertical bars represent the ranges of the data observed.

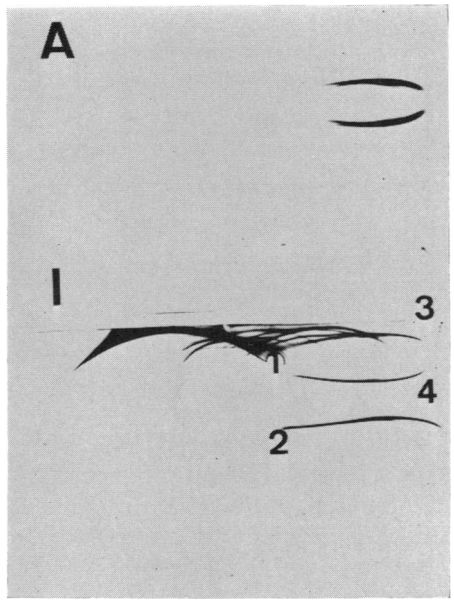

FIG. 6. Immunoelectrophoretic analysis (I) and radioautography (A) of the ^{131}I-labelled IgG solution used in experiment No. 7. (1) 'Biologically screened' mouse serum used to inoculate the experimental mice; (2) normal mouse serum; (3) rabbit antiserum to whole mouse serum; (4) rabbit antiserum to mouse serum IgG.

IgG

A mixture of IgG from normal mice, labelled with iodine-131, was injected into XVII mice. Four days later these mice were bled and their serum was inoculated into the four normal and four irradiated (950 r) mice used in experiment C7. This biological screening process enabled the denatured molecules to be eliminated. It certainly also reduced the percentage of IgG_{2b} in the preparation. Fig. 6 shows the immunoelectrophoretic analysis and the radioautography check of this preparation which is a mixture of IgG_1, IgG_{2a} and IgG_{2b}. The half-life of this mixture of normal IgGs in the recipients, as measured from total-body radioactivity (Fig. 5) was 8·5–9 days and 4 days for the normal and irradiated animals, respectively.

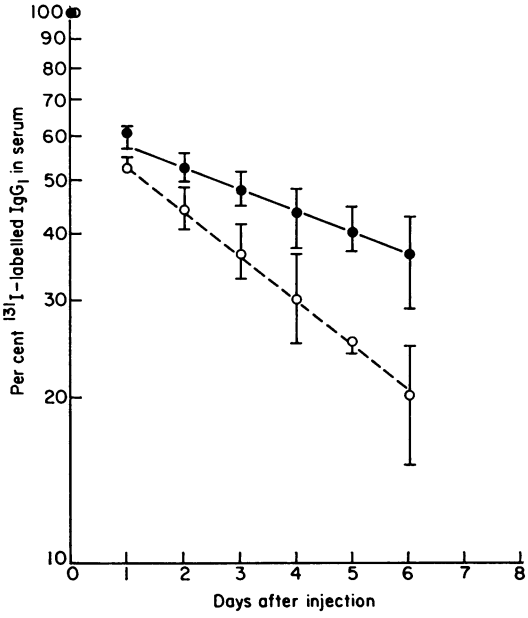

FIG. 7. Catabolism of an ^{131}I-labelled IgG solution in normal (●) and irradiated (○) mice (experiment No. 7). The vertical bars represent the ranges of the data observed.

IgG_1

Two preparations of IgG_1 from the VIIA2 and MOPC/70A myelomas were used in this study (Table 3). Experiments C8 and C9 were effected by whole-body radioactivity counts, and experiment C10 by counts of the serum radioactivity of the inoculated mice. Figs. 7 and 8 show the details of experiment C10. In particular, the ^{131}I-labelled protein appeared to be practically pure, as in the checks effected for experiments C8 and C9. The two first experiments indicated a half-life of about 10 days and 6 days for the normal and irradiated animals, respectively. In the case of the serum radioactivity study, the half-life emerged as 8 and 4 days for the normal and irradiated animals, respectively. This test was performed with the same IgG_1 preparation as was used in experiment C8.

TABLE 3

HALF-LIFE OF ^{131}I-LABELLED IgG_1 IN NORMAL OR IRRADIATED MICE

Experiment	Age of mice (days)	Sex	Method of determination	No. of mice	Irradiation (r)	Mean serum Ig levels (mg/ml)				Half-life of ^{131}I-labelled IgG_1 (days)
						IgM	IgA	IgG_1	IgG_2	
C8	120	M	Whole body	4	0	1·46	0·18	0·49	10·80	11
			radioactivity	4	950	–	–	–	–	6·5
C9	150	F	Whole body	4	0	2·36	0·18	0·37	8·20	10
			radioactivity	4	950	–	–	–	–	6
C10	200	M	Serum	4	0	1·34	0·21	0·39	16·00	8
			radioactivity	4	950	–	–	–	–	4

TABLE 4

HALF-LIFE OF ^{131}I-LABELLED IgG_{2a} IN NORMAL OR IRRADIATED MICE

Experiment	Age of mice (days)	Sex	Method of determination	No. of mice	Irradiation (r)	Mean serum levels (mg/ml)				Half-life of ^{131}I-labelled IgG_{2a} (days)
						IgM	IgA	IgG_1	IgG_{2a}	
C11	230	M	Whole body	3	0	0·84	0·19	0·31	35·00	12
			radioactivity	3	950	–	–	–	–	7
C12	180	F	Serum	3	0	1·67	0·56	0·48	11·00	6
			radioactivity	3	950	–	–	–	–	4
C13	130	M	Serum	4	0	1·16	0·14	0·49	35·00	7
			radioactivity	4 and 4	950	–	–	–	–	4 and 4·5

IgG_{2a}

In the IgG_{2a} catabolism study three samples of myeloma proteins from plasma-cell tumour 5563 were used. Fig. 10 indicates the degree of purity of the preparation employed in the experiment in C12. Besides IgG_{2a}, the sample contained traces of IgG_1 and probably IgG_{2b}. In no case, however, did we detect in these preparations any trace of impurities belonging to serum proteins other than the three IgG categories. The protein solutions in tests C11 and C12 went through an 8-day 'biological screening' before being inoculated into the experimental mice. The tests concerning IgG_{2a} were carried out with the two techniques employed previously. Test C11 was effected by study of the whole-body radioactivity, and C12 and C13 by counting of the serum radioactivity. The main results of these three experiments are summarized in Table 4. Fig. 9 relates to test C13 in which two

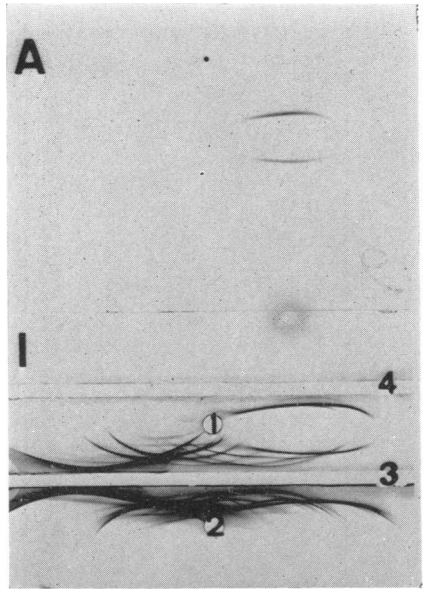

FIG. 8. Immunoelectrophoretic analysis (I) and radioautography (A) of the ^{131}I-labelled IgG_1 solution used in experiment No. 10. (1) Serum of normal mice under experiment (48 hours after inoculation); (2) normal mouse serum; (3) rabbit antiserum to whole mouse serum; (4) rabbit antiserum to mouse serum IgG.

groups of animals underwent irradiation either 1 hour or 3 days after injection of the labelled IgG_{2a}. The half-life of the preparation used in experiment C11 was found to be 12 days in the controls and 7 days in the irradiated mice. As to experiments C12 and C13, the half-life fell from 6 or 7 days in the controls to about 4 days in the irradiated mice. As IgG_{2a} displays the longest half-life of the different Igs we looked among these groups of animals to find the maximum limit of the irradiation dose due to the ^{131}I inoculation. The highest reading was found in experiment C11, with about 5 rad.

IgG_{2b}

Two myeloma S188 preparations were used in this work. As can be seen from Fig. 12, the experimental preparation was relatively pure, although slightly contaminated with the

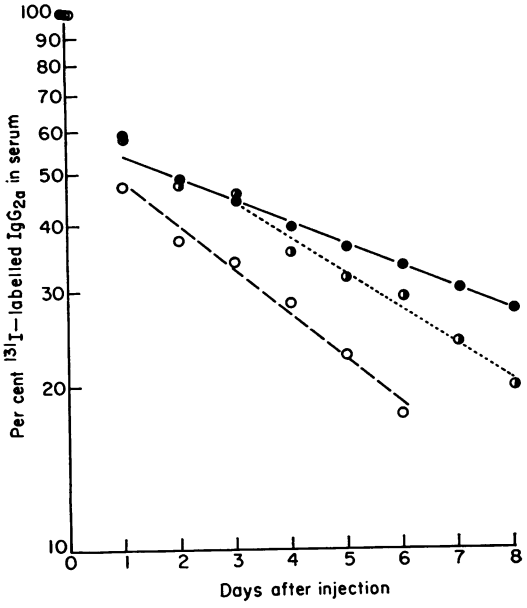

FIG. 9. Catabolism of an ^{131}I-labelled IgG_{2a} solution in normal (●) and irradiated (○) mice at the time of inoculation of the labelled Ig, and 3 days after injection (◑).

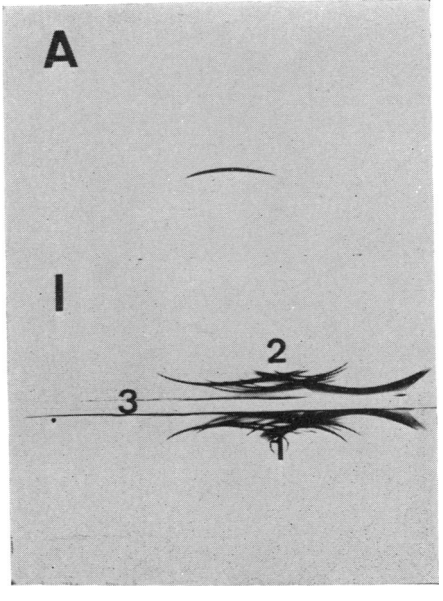

FIG. 10. Immunoelectrophoretic analysis (I) and radioautography (A) of the ^{131}I-labelled IgG_{2a} solution used for experiment No. 13. (1) Mouse serum used, after 'biological screening', to inoculate the experimental mice; (2) normal mouse serum; (3) rabbit antiserum to whole mouse serum.

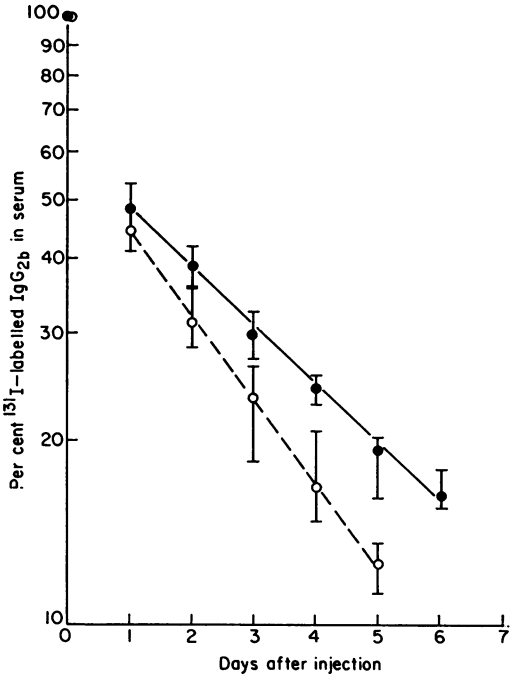

FIG. 11. Catabolism of an ^{131}I-labelled IgG_{2b} solution in normal (●) and irradiated (○) mice. The vertical bars represent the ranges of the experimental data.

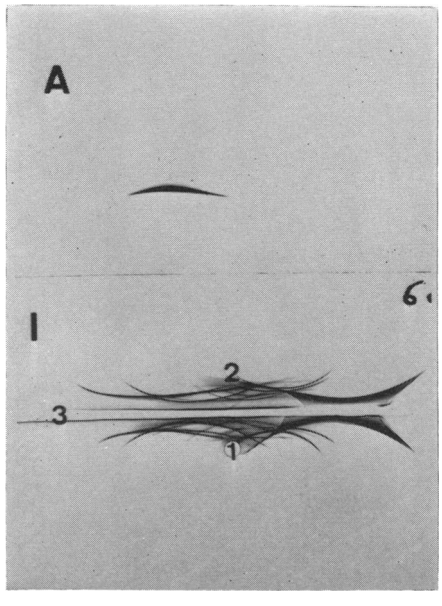

FIG. 12. Immunoelectrophoretic analysis (I) and radioautography (A) of serum of the normal mice in experiment No. 15, taken 3 days after inoculation of the ^{131}I-labelled IgG_{2b}. (1) Serum of the experimental mice; (2) normal mouse serum; (3) rabbit antiserum to whole mouse serum.

other two types of IgG. As the half-life of this IgG_2 sub-class proved to be relatively short, we studied it only by counting the plasma radioactivity after inoculation of the labelled protein solution. Table 5 gives the results of experiments C14 and C15 and Fig. 11 gives the results of experiment C15 in particular. The half-life of the myeloma IgG_{2b} studied was found to range from 3 to 3½ days in the controls and from 2 to 2½ days in the irradiated animals.

DISCUSSION

The experiments described above show that the catabolism of IgM and IgA is only very slightly influenced in the irradiated animals. In no case did we find any difference that could be regarded as significant. On the other hand, the catabolism of IgG_1, IgG_{2a} and IgG_{2b}, as studied by means of myeloma proteins, was found to be distinctly accelerated in the irradiated mice. A mixture of normal IgGs gave a similar result.

The earliest data on Ig catabolism in mice concern the serum 'γ-globulins' without mention of the classes of Ig involved. Dixon, Talmage, Maurer and Deichmiller (1952) found a half-life of 1·9 day, Humphrey and Fahey (1961) 4·6 days and Tee, Watkins and Wang (1965) 4·2 days. Sell (1964) attributed a half-life of 3·2 days to these same proteins, with extremes ranging from 2·5 to 6·2 days. Sell and Fahey (1964) later reported a half-life of 5·4 days with maximum and minimum values of 13·2 and 2·3 days. Lastly Fahey and Sell (1965) published a study of the catabolism of each class of Ig. According to these authors IgM had a half-life of 0·5 day with, in reality, two distinct components corresponding to 0·2 and 0·6 day, respectively. IgA was found to have a half-life of 1·3 day (range: 0·7–1·3 day). Mouse IgG appeared to have a half-life which varied widely with their serum concentrations (Fahey and Robinson, 1963). Depending on whether the experimental animal was exposed to a very weak or very strong microbial environment, or had a paraprotein tumour, these serum concentrations were found to differ greatly. In the conventional animal the half-lives of IgG_1, IgG_{2a} and IgG_{2b} were 4·0, 5·4 and 2·5 days, respectively, with extreme values of 1·8–14, 1·5–11·8 and 0·7–5·5 days. Except in the case of IgM where the values were obtained by the study of haemolysin disappearance (19S), the other results stemmed from measurements of body radioactivity following the injection of ^{131}I-labelled Ig.

In the present study of Ig catabolism, two methods were employed, namely whole-body radioactivity or serum radioactivity. We found constantly, and more especially in tests C8 and C10 performed with the same protein solution, that the IgG half-life value calculated from the whole-body radioactivity was greater than the value obtained by study of the serum radioactivity. The difference is probably due to the time taken by the body to excrete the free iodine arising from the breakdown of the labelled molecules (Wetterfors, Liljedahl, Plantin and Birke, 1965). Fahey and Sell (1965) advanced this hypothesis in the case of mouse IgA. It is also possible, as Moretti (1967) observed, that in mice some iodine is eliminated through the skin, and this would presumably distort the results. On the other hand, having given the experimental animals potassium iodide in their drinking water, we did not observe any considerable concentration of iodine-131 in their thyroid gland.

TABLE 5

HALF-LIFE OF ^{131}I-LABELLED IgG_{2b} IN NORMAL OR IRRADIATED MICE

Experiment	Age of mice (days)	Sex	Method of determination	No. of mice	Irradiation (r)	Mean serum Ig levels (mg/ml)				Half-life of ^{131}I-labelled IgG_{2b} (days)
						IgM	IgA	IgG_1	IgG_2	
C14	210	M	Serum	4	0	3·91	0·29	0·51	8·90	3·5
			radioactivity	4	950	–	–	–	–	2·5
C15	100	M	Serum	4	0	1·55	0·21	0·43	21·00	3
			radioactivity	4	950	–	–	–	–	2

When studying the influence of X-ray irradiation on the catabolism of Ig, an important point to note is the dose absorbed by the experimental animals which is due to the inoculation of radioactive iodine. Considering only the energy released by the β-rays of the ^{131}I, we calculated a maximum dose of 5 rad in the case of experiment C11. Such a dose, spread over 8 days, does not seem to be capable of significantly disturbing the control animals or the animals irradiated with X-rays.

A problem to be borne in mind is the possibility of denaturation of the injected proteins. Although the magnitude of this factor was not assessed by studies of the excretion of radioactive iodine in the urine, there are reasons to believe that it has not played a significant rôle in our results. Firstly, McFarlane's (1958) method was chosen for the iodination because it has been stated not to denature the proteins (Freeman, 1967). Furthermore, we worked with labelling rates well below 0·5 iodine atom per molecule of IgM or IgA and below one iodine atom per IgG molecule (Freeman, 1967). Whenever possible the injected samples had been submitted to prior 'biological screening' by passage through a primary recipient mouse. Each metabolic experiment was carried out with a separate preparation, except in the case of C8 and C10. This diversity of origin and treatment of the samples employed did not perceptibly affect the reproducibility of the results. Finally, in every case, extrapolation of the straight terminal segment of the plasma decay curve to the vertical axis gave intercepts suggesting extravascular–intravascular distribution ratios compatible with values for IgM, IgA and IgG published in the literature (Schultze and Heremans, 1966). The size of the extravascular compartment would, on the other hand, greatly have been overestimated by this method if large amounts of swiftly catabolized material had been present in the preparations.

It must be noted that the injected amounts of IgM or IgA of each sort were fairly considerable and may have raised the serum level of these proteins. The catabolism of IgA or IgM, however, is independent of their concentration in the blood (Fahey and Sell, 1965). As to the amounts of IgG injected, although relatively high, they are, in our opinion, negligible. One must consider, first, that as regards the IgG_{2a}, its level is high in the serum of our experimental animals. More significantly, the distribution of these proteins between the intra- and extravascular compartments is roughly half-and-half (Schultze and Heremans, 1966), a fact which indicates a very large total pool. Lastly, the findings of Fahey and Robinson (1963) show that a quantity of 1–2 mg given in a single injection should not significantly disturb the catabolism of these proteins.

Slight denaturation due to the isolation procedure might have caused slight and uniform acceleration of the catabolism of the proteins studied. In most cases, however, a denatured preparation will show greatly accelerated initial catabolism, indicating the rapid elimination of grossly damaged molecules, followed by normal catabolism for the remaining intact molecules (Freeman, 1967). In the present study, however, the same half-life was found for ^{131}I-labelled IgG_{2a} which had or had not undergone 'biological screening'.

Another important problem is that of the purity of the normal and myeloma proteins injected. On one occasion the IgM was found to be contaminated with traces of α-lipoprotein. The control tests of the IgA showed it to be pure. The IgG_1, IgG_{2a} and IgG_{2b} solutions, however, were definitely not. These preparations are only mixtures in which one constituent is predominant, owing to the relative proportions of these different Igs in the initial myeloma serum. In no case, however, did we detect any serum constituents other than IgG. The accuracy of immunodiffusion techniques depends on numerous factors,

and the designation 'pure' means little in itself because there are no set standards permitting comparison. On the other hand, radioautography of Ouchterlony or immunoelectrophoresis plates seems to be a far more conclusive test because lines invisible after staining can be revealed by this method.

Each protein sample inoculated was catabolized at a nearly identical rate in all test animals, but appreciable variation was encountered from one sample to the next. For this reason no importance can be attached to comparisons based on samples of different origin. The variations imputable either to the different protein preparations or to the animal groups greatly exceed the experimental errors detected within each test.

In order to try to explain the variations in the catabolism of IgG, a number of factors have to be considered. Firstly one must take into account possible shifts of the fluid distribution ratio between the extra- and intravascular compartments and of the total plasma volume. As already mentioned, changes of this kind do occur in irradiated animals (Friedberg, 1960; Wetterfors *et al.*, 1965). Reuter *et al.* (1967a) considered that the relative increase of the extravascular compartment in relation to the total plasma volume appeared to account for the enhanced catabolism of γ-globulins in irradiated rats. This, however, could not be the cause of the increased IgG catabolism here observed, for this phenomenon occurred both in the whole-body and the serum radioactivity measurements.

Fahey and Robinson (1963) showed that the catabolism of mouse IgGs is linked to the concentration of these proteins in the serum. There seems to be only a single regulating mechanism, dependent on components of the Fc fragment of the molecules. Within certain limits, the rate of catabolism increases when the overall level of these Igs rises and vice-versa. Hence the paradoxical observation of an increased rate of IgG catabolism together with a decreased IgG serum level must signify, not compensation only, but actual overrunning of this regulating mechanism.

Several other, secondary, explanations can be advanced. For instance, it is known that changes in the reticulo-endothelial system may cause acceleration of the catabolism of plasma proteins (Schultze and Heremans, 1966). Hormonal influences on protein catabolism are known to exist and may perhaps have played a rôle in our experiments, at least as regards the adrenocortical hormones (Rothschild, Oratz and Schreiber, 1967). These two explanations are not very satisfactory, however, for they presumably apply to all the serum proteins.

Yet the different immunoglobulins do not seem to be degraded indentically at the various catabolic sites. The fast-catabolizing IgM and IgA seem to be essentially sensitive to an endogenous catabolism process. Conversely, the IgGs, partly protected against that process (Brambell, Hemmings and Morris, 1964), are far more susceptible to intestinal catabolism. There is no doubt as to the reality of the intestinal lesions that follow irradiation (Regaud, Nogier and Lacassagne, 1912). They could significantly increase the IgG catabolism in a selective manner, by increasing the intestinal losses, which constitute the main pathway of degradation for this class of immunoglobulins (Schultze and Heremans, 1966). On the other hand, in the case of the IgM and IgA, the increase of intestinal losses could be considered negligible by comparison with the endogenous catabolism, which would be very little altered. The albumin and IgG seem to be the two kinds of serum proteins most affected by this intestinal catabolism (Schultze and Heremans, 1966). There have been numerous studies on the catabolic sites of albumin. Wetterfors *et al.* (1965) and Lefaure, Cier and Cier (1966) showed that there is a shortening of the albumin half-life in irradiated rabbits. Furthermore, Palmer and Sullivan (1959) demonstrated that the

albumin level could be lowered in rats simply by irradiating the intestine, and Sullivan (1960) as well as Hornsey and Vatistas (1968) showed that polyvinylpyrrolidone (PVP), a substitute for plasma proteins, was massively excreted from the plasma into the lumen of the intestinal tract, after irradiation of the rat and mouse. Hence it is probable that the enhanced IgG catabolism in irradiated mice is indeed due to intestinal lesions: the more so, since in the case of exudative gastroenteropathies it has been shown that the serum IgM level remained remarkably normal, whereas the IgA and IgG levels fell off owing to the intestinal losses (Birke, 1967). This compares well with our results where we found a slight IgM, medium IgG and pronounced IgA decrease in irradiated mice (Bazin and Micklem, 1967; Bazin, 1968). We can, therefore, note that the physiological state of irradiated mice is comparable, as regards the serum Ig levels, to that of an exudative gastroenteropathy in which intestinal protein losses are a confirmed fact. There is still some uncertainty, however, concerning the relative chronology of these intestinal lesions and the increase of catabolism. The return to normal albumin catabolism, in rabbits irradiated with 300 r, occurs between the 9th and the 16th day. At lethal doses the half-life of this protein shows no tendency to normalize before the death of the animals (Lefaure *et al.*, 1966). Our findings with mouse IgG are exactly the same.

It has not been possible, even with the refined methods of quantitation employed, to assign a precise onset to the enhancement of IgG catabolism after irradiation. Using ^{131}I-labelled PVP, however, Hornsey and Vatistas (1968) clearly demonstrated that in mice given a dose of 1000 r, the intestinal losses of the colloid tracer began on the 4th day and remained elevated until about the 9th to 10th day. This type of chronology does not correspond to that of the intestinal lesions, which are apparent already at the 6th hour and have disappeared by the 6th day after irradiation (Duplan, 1966).

It is, however, not certain that the metabolism of the intestinal epithelium is restored to normal at that date (Maisin, 1966). After a lethal irradiation the metabolic disturbances of the intestinal mucous membrane are considerable and independent of its histological aspect.

The present investigation has provided new details on the catabolism of mouse immunoglobulins. It demonstrates that X-ray irradiation very significantly increases the catabolism of the IgG, whilst leaving the IgM and IgA catabolism unchanged. These findings account at least partially for the fact that, in mice, after a lethal irradiation, the serum IgG level shows a greater drop than the IgM level. On the other hand, this work has not thrown any light on the mechanism governing the sharp drop of the serum IgA level which has been observed after irradiation.

Furthermore, it is certain that the different effects of irradiation on the catabolism of the various Ig classes, more especially with regard to the 19S–7S distinction, make for additional difficulty in the study of immunological reactions of irradiated animals. Whether the type of immunity be active, passive or adoptive, the IgM and IgA catabolism will be comparable to that found in the control animals, whereas the IgG catabolism will be increased. In consequence, with one and the same production of haemolysin for instance, the serum concentration of IgM and IgA antibodies will be the same in the irradiated and the control animals, whilst an identical synthesis of precipitating antibodies of IgG type will give a lower serum level in the irradiated animals than in the controls.

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