Forest virus in mice, has been found to have demonstrable chemoprophylactic effects in 12 g mice infected peripherally with a special hamster passaged line of MEF1 poliomyelitis virus. 2. Experiments are under way to find out (A) if stronger chemoprophylaxis can be obtained, (B) if more prolonged action can be obtained, and (C) if oral activity can be attained by 8450 against poliomyelitis virus.

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A Quantitative Comparison of Antibody Produced by X-Radiated and Normal Rabbits.* (20297)

PAUL H. MAURER, FRANK J. DIXON, AND DAVID W. TALMAGE.†

From the Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pa.‡

The antibody response can be divided into a brief initial radiosensitive phase and a second radioresistant phase which occupies the remainder of the period of response(1a,b,c,d). Once initiated, antibody production is little affected by large doses of whole body radiation in spite of the fact that lymphatic tissues, bone marrow, and spleen—all thought to be important in the production of antibody—are extremely radiosensitive. Several explanations of this observation have been given: first, the radiosensitive tissues might function only in the brief, initial phase of the antibody response and that the actual synthesis of antibody might take place in radioresistant tissues(1c); second, since even large doses of radiation do not destroy all of the radiosensitive cells in the body it is possible that the small number of these cells remaining might carry on the full antibody response; and third, that there are radioresistant reserve tissues capable of carrying out antibody formation once it has begun in spite of damage to radiosensitive tissues(2).

In view of the above, it seemed worthy to investigate whether the character of antibody molecules might be altered in radiated rabbits. The only readily demonstrable differences between antibodies are immunochemical in nature (3a,b,c,d) and these differences are based on the average behavior and reactivity of all the different kinds of antibody to the same antigen. The present report deals with a comparison of the immunochemical characteristics, as evaluated by the quantitative precipitin reaction and the initial combining ratio of antibody to antigen, of antibodies to bovine gamma globulin§ (BGG) and bovine serum albumin‖ (BSA) formed in X-radiated and normal rabbits.

Materials and methods. Male albino rabbits weighing from 2 to 2.3 kg were immunized and radiated as indicated in Table I. A single intravenous injection of either I^{131} labelled BGG (I*BGG), 30 mg/kg, or I^{131} labelled BSA (I*BSA), 15 mg/kg, was given to each animal. Preparation of the iodinated antigens has been previously described(4a,b). Two hundred and twenty KV whole body X-radiation was given as previously described(1c) in either 400 r (LD_{5-30}) or 800 r (LD_{75-30}) doses. Antigen concentrations in the blood were determined by protein bound radioactivity(4b) and bleedings for antibody analyses were made 3 days after elimination of all antigen at which time serum antibody concentrations were usually at a maximum. In order to have sufficient serum for quantita-

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† Present address: Department of Medicine, University of Chicago, Ill.

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§ Armour and Co. Fraction II bovine serum.

‖ Armour and Co. crystalline bovine serum albumin.

TABLE I. Relation of Immunization, Radiation, and Antibody Response.

Animal No.	Immunization and radiation	Antibody bleeding days after inj.	Serum antibody conc., μg antibody N/ml
381, 382	I*BGG 5 hr after 400 r	13	7.5
266–269	" 48 " " " *	14	25.1
295	" 3 days before 800 r	13	72.1
423, 301	" " " " "	13	34.2
408	" 1 hr " "	13	54.0
725, 726, 728, 734	I*BSA 48 hr " 400 r	12–16	25.8
707, 709, 711, 712, 713	" simultaneous with 400 r	17–19	6.1
717, 720, 721, 722, 724	" 6 hr after 400 r	17–19	5.0
472, 481, 484	I*BGG—no X-ray	10	81.0
478, 490	" " "	10	48.0
698, 704	I*BSA— " "	14	38.0

* Seven injections egg albumin and edestin prior to X-ray to "protect" immune response.

tive analysis, sera from similarly treated animals with antibody concentrations at about the same level were pooled as shown in Table I. The quantitative immunochemical procedures used were those described by Heidelberger and coworkers(5). In some experiments I^{131} labelled antigen was used and its concentration was measured as previously described (6). Nitrogen was measured by the Markham modification of the micro-Kjeldahl procedure (7). The methods used for decomplementing low titer rabbit antisera with an egg albumin-anti-egg albumin (rabbit) specific precipitate has been presented(6). Gross and microscopic study of the tissues of some of the animals listed in Table I was made following the antibody bleeding. In addition the tissues of other animals receiving 400 r and 800 r whole body radiation and sacrificed 3, 5, 8, and 11 days post radiation have been studied to afford a complete picture of the post radiation tissue changes. Since the radiation changes are more clear-cut with the 800 r dose only these histologic observations will be presented.

Results. A convenient method for comparing anti-protein antibodies is to plot the ratio of antibody N/antigen N in the precipitate against the square root of the antigen nitrogen in the precipitate. The intercept of the straight line gives the initial combining ratio (2R) of antibody N/antigen N. A complete discussion of this can be found in(8). As the value for the initial combining ratio (2R) is known to change upon repeated immunization of the same animal(9), its usefulness as an absolute method in comparing antibodies produced against the same antigen is somewhat limited. Major differences in reactivity of antibodies, however, can be detected. Analysis of the decomplemented antisera from rabbits immunized with I*BGG, 5 and 48 hours re-

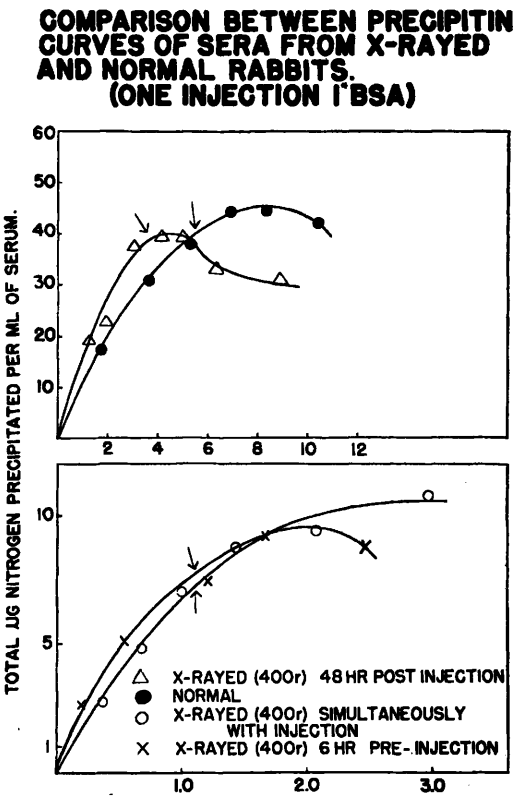

FIG. 1. The arrows indicate the equivalence point.

TABLE II. Initial Combining Ratios (2R) of Anti BSA Sera.

Exp. group	Antibody N/ antigen N = 2R
I*BSA 48 hr before 400 r	18.0
" 6 " after "	17.2
Control	16.1

spectively after 400 r whole body radiation showed precipitin curves and initial combining ratios entirely comparable with those of antisera produced by nonradiated rabbits (Reference 6, Fig. 7 and 8). As was pointed out in earlier studies(6) it is extremely important to decomplement rabbit sera before immunochemical analysis especially when the concentration of antibody is low. Failure to do this may result in erroneous values for both antibody nitrogen and initial combining ratios.

These experiments were repeated with crystalline BSA using 400 r as indicated in Table I. The results summarized in Fig. 1 indicate that the immunochemical behavior of the anti-BSA sera from radiated rabbits is essentially the same as the control. Considering that the quantitative analyses were performed on sera having a low level of antibody N the agreement is very good. Inhibition of precipitation occurs in the region of antigen excess as is to be expected. The initial combining ratios (Table II) of the above mentioned sera are the same, within the experimental errors involved.

In order to observe the effects of larger amounts of radiation 800 r was given to one group of rabbits and sera obtained during the second post radiation week when there was little morphologic evidence of recovery from the radiation injury as described below. Although these sera were analyzed without decomplementation it is obvious that their initial combining ratios would be the same as found in nonradiated controls (Fig. 2). In addition, the immunochemical behavior of the antibodies from the 800 r animals as shown by the precipitin curves closely parallels that of the controls (Fig. 2). In the animals receiving 800 r whole body radiation there was severe damage to the radiosensitive tissues. Injury to the bone marrow resulted in loss of nearly all of the hematopoietic cells leaving behind fat cells, scattered reticuloendothelial cells, and a peculiar gelatinous intercellular matrix. This destruction was well developed within 5 days and significant recovery did not begin for 2 weeks. The spleens of these animals were considerably reduced in size within a few days largely at the expense of the lymphoid tissue. Small lymphocytes were almost entirely destroyed and only small foci of large lymphoid cells and plasma cells persisted about the splenic arterioles. A moderate number of macrophages and fibroblasts appeared in and about the splenic white pulp during early post radiation period. There was a diffuse regeneration of lymphoid tissue which began during the second week post radiation but development of lymphoid follicles and appearance of small lymphocytes was not seen for several weeks. The lymph nodes at the hilus of the intestinal mesentery showed marked damage which reduced the total number of cells in the node to 10-20% of the normal. Lymphoid follicles and small lymphocytes were completely destroyed leaving a loose network of reticuloendothelial cells and fibers in which were scattered large lymphocytes, plasma cells, macrophages and fibroblasts. There was little evidence of regeneration before the middle of the second post radiation week and normal structure was not regained for a month or more. The lymphoid tissue of the appendix showed degenerative changes comparable to those seen in the nodes. The lymphoid follicles were replaced by

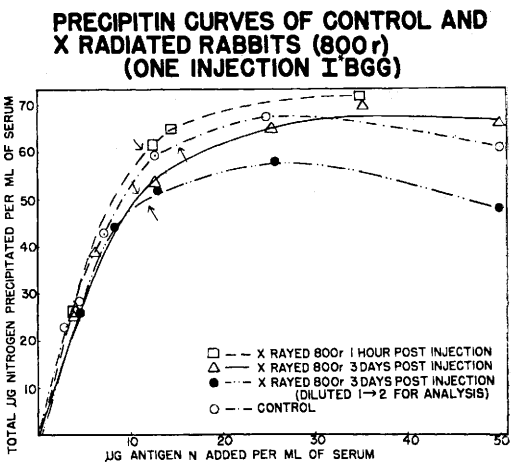

FIG. 2. The arrows indicate the equivalence point.

clumps of foamy macrophages and the amount of lymphoid tissue remaining one week after radiation could not have been more than 10% of normal. There was little evidence of recovery before the third post radiation week.

Discussion. The radiation dose of 400 r prior to or near the time of immunization was used to interfere with the early radiosensitive phase of antibody production.¶ Eight hundred r was given after the injection of antigen to allow us to study antibody formed in animals showing maximal radiation injury. With neither 400 r nor 800 r was there demonstrable immunochemical alteration in the character of the serum antibody although the concentration of the antibody was greatly reduced in those animals radiated prior to or at the time of immunization (Table I).

The observations in the 800 r group are the most dramatic. Here animals with the greatest part of their lymphoid and hematopoietic tissues destroyed, and the remainder of these tissues certainly far from normal, synthesized normal amounts of antibody which did not differ immunochemically from antibody produced by nonradiated animals. This observation is consistent with the view that the synthesis of antibody (second phase of the antibody response) may occur in radioresistant cells or tissues(1c).

Conclusions. The antibody formed by rabbits radiated with 400 r whole body radiation prior to, simultaneously with, or after immunization and by rabbits radiated with 800 r after immunization although often reduced in amount was indistinguishable from that formed by nonradiated rabbits.

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¶ The second group of animals in Table I (266-269) had several injections of egg albumin and edestin the week before radiation to increase nonspecifically the radioresistance of immune response. Antibody concentration of these sera is definitely higher than that obtained from animals not treated prior to radiation indicating some protective effect from this procedure.

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