tiveness of irradiation (*1*, *10*). Nevertheless, it is conceivable that a general mechanism of cellular sensitization to the alkylating agents is involved which is common to all of the effective antimetabolites. One possibility that would be consistent with all the effective drug combinations implies a simultaneous action on DNA; uracil mustard would alkylate the polynucleotide molecules and the various antimetabolites each would limit repair processes by producing different enzymic blockades that decrease the supply of essential metabolites (*11*).

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2 August 1962

Maintenance of Globulin Levels in X-irradiated Rabbits after Immunization

Abstract. Rabbits were injected with antigen 24 hours after x-irradiation. Antibody titers were correlated with relative changes in gamma-beta globulin levels determined electrophoretically. Irradiated, immunized rabbits did not form detectable antibodies but had significantly greater globulin levels than non-immunized, irradiated controls. This relative difference occurred at the time nonirradiated, immunized rabbits were producing primary antibody.

A primary antibody response can be prevented or delayed by x-rays (*1*). Prevention or partial alleviation of the radiation effect has been achieved by injecting nucleic acid derivatives from a variety of sources at the time of antigen injection (*2*). It was suggested that such substances provide replacements for radiation-damaged materials which normally mediate the antigenic stimulus.

The effect of radiation on antibody formation might result from elimination of antibody-forming cells by direct destruction or from prevention of cell division through interference with nucleic acid synthesis.

Alternatively, should antibody synthesis derive from a process of information transfer by nucleoprotein with or without antigenic fragments (*3*) the effect of radiation could stem from x-ray-induced nucleic acid disorganization leading to aberrant intermediates capable of participation in cellular metabolism and, possibly, to faulty transfer or utilization of the antigenic information. Protein produced in response to such aberrant stimuli might not be identifiable by reaction with the antigen stimulating its production. Antibody formation would appear to have been prevented; yet globulin synthesized in response to antigen would be present.

Male New Zealand rabbits were given a single intravenous injection of bovine albumin (BSA, 10 or 15 mg) (*4*), in rabbit hemoglobin particle adjuvant (*5*). Antigen was injected 24 hours after the rabbits were x-irradiated. The whole body was exposed to 400 r generated at 250 kv and 15 ma by a Picker x-ray machine. The vertical beam was filtered by 1.0 mm of aluminum and 0.5 mm of copper. The half value layer of the beam was 3.0 mm of copper. The dose was measured in air to the center of the box.

Blood samples were collected periodically. Serum proteins were separated with the Spinco model R paper electrophoresis system. Paper strips were analyzed in the Spinco Analytrol. Relative concentrations of individual serum fractions were obtained by drawing verticals and relating the area under each section of the photometrically obtained protein curve to the total curve (*6*). Total serum protein was determined by the biuret method (*7*).

Occurrence of an immune response was judged in two ways. The immune catabolism technique (*8*) was employed with enough I^{131}-labeled bovine serum albumin (*9*) to contain approximately 3×10^6 count/min. Antibodies were also detected by the Farr ammonium sulfate procedure (*10*). The antigen binding capacity was taken as the percentage of 0.05 μg of BSA-I^{131} bound by a 1:5 dilution of serum. With these procedures primary antibody was detected in normal animals between 7 and 10 days after injection.

Similar experiments were performed on nonirradiated, immunized and on irradiated, nonimmunized rabbits. Ten normal rabbits were included as bleeding controls. Comparisons were made between these groups and the irradiated, immunized group.

Figure 1 shows the average primary response to antigen by immune elimination and by Farr techniques. The irradiated, immunized group did not produce antibodies. They showed a constant rate of antigen elimination and an unchanged antigen binding capacity. The nonirradiated rabbits showed an increased rate of elimination between the 7th and 8th days and an increased binding capacity 1 or 2 days later. Eleven of the immunized, irradiated rabbits were bled again 20 days after injection.

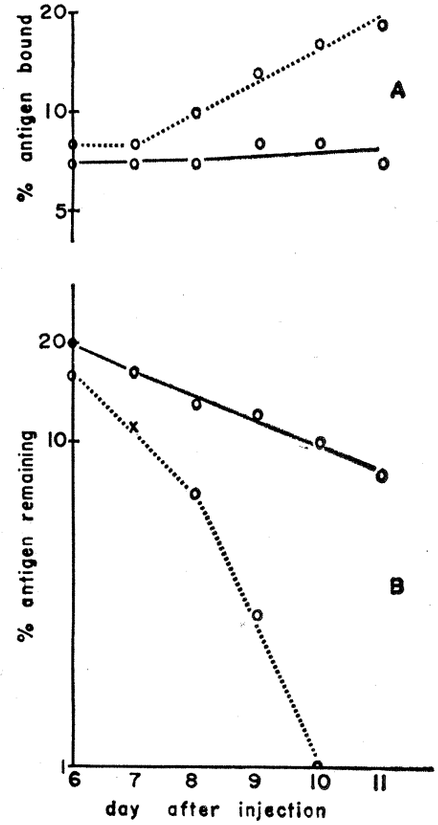

Fig. 1. Semilogarithmic plot of response to bovine albumin by irradiated, immunized rabbits (solid line) and non-irradiated, immunized rabbits (dotted line). *A*, Mean antigen binding capacity expressed as percentage of antigen bound by a 1:5 serum dilution. *B*, Mean disappearance of circulating antigen expressed as percentage of initial activity. Minimum of ten rabbits per point (*X* represents nine rabbits).

The average binding capacity of these samples was 8 percent. There was no evidence of a delayed response.

In order to show further that irradiation had prevented a primary antibody response, six rabbits from the irradiated, immunized group were challenged with antigen 26 days after primary injection. No secondary response occurred in these animals.

Based on the relative percentage of each fraction of the total protein, the globulin fractions of sera from irradiated, immunized or nonirradiated, immunized rabbits showed little change. It was only when these groups were compared with the nonimmunized, irradiated control that differences became apparent. Relative percentages of the combined gamma and beta globulin fractions are plotted in Fig. 2. A difference between irradiated controls and irradiated, immunized animals was evident the 8th day after irradiation. The average difference between these groups on the 10th and 11th days was statistically significant ($P<.001$). Globulin levels in the irradiated, immunized animals paralleled those of normal, immunized rabbits during the period when sera from the latter contained antibodies to bovine serum albumin.

We conclude, therefore, that immunization was responsible for maintaining globulin levels in the irradiated animals. Since antibody was not detected we assume that the antigen-adjuvant preparation injected (although containing 0.6 mg of DNA and 4 mg of rabbit hemoglobin) did not restore antibody formation as Taliaferro and Jaroslow (*2*) reported for depolymerized but not for polymerized DNA as used here. The very weak antigenicity of the adjuvant materials should eliminate these when the cause of the relative differences between the immunized and nonimmunized, irradiated rabbits is considered. The difference between the irradiated groups also indicates that permeability changes induced by radiation were not a factor.

The observed serum protein changes were slight since only a single injection of a small amount of antigen was employed. Nevertheless, antigen administration resulted in reversal of the expected loss in gamma-beta fraction and may be said to have exerted a protective effect on globulin levels. It is hoped that additional experiments with labeled amino acids will enable us to determine whether the synthesis of new (but nonantibody) globulin follows the immunization of irradiated rabbits as is suggested here.

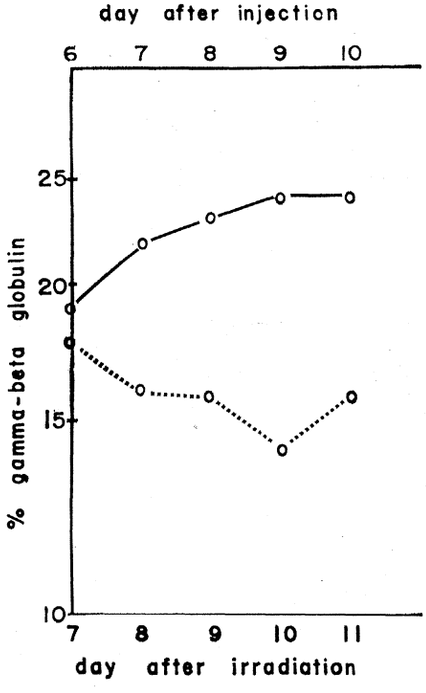

Fig. 2. Semilogarithmic plot of mean gamma-beta globulin levels in irradiated, immunized rabbits (solid line) and in irradiated, nonimmunized rabbits (dotted line). The values represent percentage of total serum protein. Minimum of ten rabbits per point. The immunized rabbits were injected 24 hours after x-irradiation.

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Four-Electrode Method for Measuring the Direct-Current Resistivity of Ice

Abstract. The improved system here described has been used successfully in systematic measurements of direct-current conductivity of ice doped with hydrofluoric and hydrochloric acids. The use of four electrodes allows continuous control of measurement reliability.

Direct-current-conductivity measurements are the basis for many other experimental techniques, such as studies of activation energy and the Hall effect. In the case of ice, however, such measurement is complicated by exceedingly pronounced electrode polarization effects.

It was thought possible that sandwich electrodes of a suitable type could be developed, in analogy to semiconductor techniques. Attempts to develop such electrodes were made by Mary Gourley of this laboratory as early as 1956 (*1*). The first truly usable sandwich electrode systems for ice were described and applied by Gränicher and his associates (*2*) and by C. Jaccard (*3*) of the ice research group at the Swiss Federal Institute of Technology. Jaccard's system consisted of polyvinyl chloride filter foils coated with gold under high vacuum and saturated with a dilute hydrofluoric acid solution. Some measurements on "pure" ice and ice samples made of a dilute hydrofluoric acid solution were published. No consistent results could be obtained with ice prepared from dilute hydrochloric acid (*4*) because an oxide film forms on a gold anode at the ice-electrode interface.

It was apparent that a broad experimental study of direct-current conductivities and activation energies of ice doped with different types of ionic impurities over a wide range of concentrations would fill a definite gap in ice research.

I have developed an improved four-electrode method that will work satisfactorily even with ice doped with hydrochloric acid. Separate circuits for measuring current and potential are used. Several hundred samples of ice doped with hydrofluoric and hydrochloric acids have been studied by means of this method. Concentrations ranged from about 10^{-6} to $3 \times 10^{-3} M$.

The samples are of cylindrical shape, with a diameter of 30 to 35 mm and height of 8 to 10 mm. On each end surface is placed a circular disk of acid-resistant Whatman filter paper No. 40