# STIMULATION OF BIOSYNTHESIS OF γM- AND γG-ANTIBODIES IN IRRADIATED RABBITS

L. G. Prokopenko

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Injection of homologous serum γ-globulin into irradiated and immunized rabbits stimulates the production of γM-antibodies and accelerates the appearance of γG-antibodies. An increase in the immunizing dose of antigen stimulates the formation of γM-antibodies in irradiated rabbits but has no effect on the production of γM-antibodies.

The change over from biosynthesis of γM-antibodies to the formation of γG-immunoglobulins in irradiated animals takes place later than in healthy animals [2, 6, 13]. This is perhaps due to disturbance of mitotic activity and of differentiation of the lymphoid cells.

The writer has shown that injection of 5 mg homologous serum γ-globulin into irradiated rabbits increases antigen fixation in the regional lymph glands [3] and accelerates antibody production [4]. Stimulation of antibody formation in irradiated animals can also be obtained by increasing the immunizing dose of antigen (from 500 million to 3 billion bacterial cells of an Escherichia coli vaccine) [5]. These conclusions are based on the results of determination of the total antibody content in the blood without regard to the types of immunoglobulins concerned.

The object of the present investigation was to study the effect of homologous γ-globulin and of large doses of antigen on the formation of γM- and γG-antibodies in irradiated animals.

## EXPERIMENTAL METHOD

Experiments were carried out on 24 rabbits weighing 2-2.5 kg. All the animals were irradiated with x rays in a dose of 1000 R, and 24 h later they were immunized with a single dose of heated E. coli vaccine. Group 1 consisted of irradiated rabbits immunized with 500 million bacterial cells; group 2 of irradiated animals immunized with 500 bacterial cells and also receiving 5 mg homologous γ-globulin; group 3 consisted of irradiated rabbits immunized with 3 billion bacterial cells.

γ-globulin was isolated from the serum of 3 donor rabbits by Gubenko's rivanol-ethanol method [1]. The titer of normal antibodies against E. coli in a 5% solution of γ-globulin was 1 : 120. The protein preparation and antigen were injected into the right leg muscles at intervals of 1-1.5 h.

X-ray irradiation was given with a type RUM-3 apparatus (180 kV, 10 mA, skin-focus distance 40 cm, filter 0.5 mm Cu + 1 mm Al, dose rate in air 30±1 R/min). γM- and γG-antibodies were differentiated by means of 2-mercaptoethanol [8, 16], which selectively breaks down γM-immunoglobulins. The antibody content was determined by the bacterial agglutination test on the 5th, 10th, and 15th days of the experiment. The analysis was made before and after treatment of the sera with 2-mercaptoethanol.

## EXPERIMENTAL RESULTS

After treatment of the sera from the rabbits of group 1 with 2-mercaptoethanol, antibodies were completely destroyed on the 5th, 10th, and 15th days of the experiment (Table 1). In the sera of rabbits of group 2, 2-mercaptoethanol destroyed the antibodies on the 5th and 10th days after immunization. Addition of 2-

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TABLE 1. Effect of 2-mercaptoethanol (2-ME) on Antibody Titer in Sera of Irradiated Rabbits

| Group of rabbits | 5th day | | 10th day | | 15th day | |
|---|---|---|---|---|---|---|
| | before 2-ME | after 2-ME | before 2-ME | after 2-ME | before 2-ME | after 2-ME |
| 1 | 1:20 | <1:5 | 1:40 | <1:5 | 1:100 | <1:5 |
| | 1:5 | <1:5 | 1:20 | <1:5 | Died | — |
| | 1:5 | <1:5 | 1:30 | <1:5 | 1:60 | <1:5 |
| | 1:15 | <1:5 | 1:30 | <1:5 | 1:75 | <1:5 |
| | 1:20 | <1:5 | 1:60 | <1:5 | 1:80 | <1:5 |
| | 1:5 | <1:5 | Died | — | — | — |
| | 1:10 | <1:5 | 1:25 | <1:5 | Died | — |
| | 1:15 | <1:5 | 1:50 | <1:5 | 1:100 | <1:5 |
| 2 | 1:80 | <1:5 | 1:240 | <1:5 | 1:80 | 1:30 |
| | 1:60 | <1:5 | 1:160 | <1:5 | 1:50 | 1:20 |
| | 1:120 | <1:5 | 1:240 | <1:5 | 1:160 | 1:40 |
| | 1:75 | <1:5 | 1:160 | 1:5 | 1:80 | 1:30 |
| 2 | 1:160 | <1:5 | 1:400 | <1:5 | 1:100 | 1:40 |
| | 1:80 | <1:5 | 1:160 | 1:5 | Died | — |
| | 1:100 | <1:5 | 1:240 | <1:5 | 1:120 | 1:40 |
| | 1:100 | <1:5 | 1:320 | <1:5 | 1:80 | 1:20 |
| 3 | 1:30 | <1:5 | 1:200 | <1:5 | Died | — |
| | 1:40 | <1:5 | 1:240 | <1:5 | 1:200 | <1:5 |
| | 1:40 | <1:5 | 1:320 | <1:5 | 1:240 | <1:5 |
| | 1:30 | <1:5 | 1:160 | <1:5 | 1:160 | <1:5 |
| | 1:60 | <1:5 | 1:240 | <1:5 | 1:160 | <1:5 |
| | 1:20 | <1:5 | Died | — | — | — |
| | 1:40 | <1:5 | 1:400 | <1:5 | 1:320 | <1:5 |
| | 1:20 | <1:5 | 1:320 | <1:5 | 1:320 | <1:5 |

mercaptoethanol to the sera on the 15th day of the experiment, however, simply reduced the titers of antibodies but did not destroy them completely. The action of 2-mercaptoethanol on the sera of the rabbits of group 3 was the same as on those of the animals in group 1 (Table 1).

These experiments show that an increase in the immunizing dose of antigen stimulates production of γM-antibodies only, whereas homologous γ-globulin stimulates the formation of γM-antibodies in irradiated rabbits and also accelerates the appearance of γG-antibodies. It is interesting to note that the beginning of production of γG-antibodies is accompanied by a marked decrease in the total antibody titers. This is in agreement with data in the literature concerning inhibition of the biosynthesis of γM-immunoglobulins after the beginning of production of γG-antibodies [11].

According to some investigators [9], synthesis of γM- and γG-antibodies is effected by the same cell, but at different stages of differentiation. Meanwhile data have been obtained indicating that these types of immunoglobulins are produced by different populations of cells [12]. It has been postulated that γM-antibody-forming cells start to synthesize antibodies without preliminary mitoses, while the precursors of γM-antibody-forming cells, as they differentiate into plasma cells, pass through a series of successive mitotic divisions [7, 14].

Injection of homologous γ-globulin in the present experiments apparently stimulated the conversion of lymphoid cells which had not been destroyed by ionizing radiation into γG-antibody-producing cells. The mechanism of this stimulation cannot be attributed completely to the activating effect of γ-globulin on the ingestive activity of lymphoid cells and the accumulation of antigen in them. Evidence of this is given by the fact that an increase in the immunizing dose of antigen, likewise leading to its accumulation in the regional lymph gland [5], did not accelerate the production of γG-antibodies.

The increase in fixation of antigen in the lymphoid tissue of irradiated animals evidently brings about an increase in number only of the γM-antibody-producing cells, without accelerating the appearance of cells synthesizing γG-immunoglobulins.

Considering that normal antibodies against E. coli were present in the specimen of γ-globulin used in these experiments, it may be supposed that the stimulation of antibody biosynthesis as a result of its injection is explained by the formation of an antigen – antibody complex (with antigen in excess) in the regional lymph gland, and that this complex possesses higher immunogenicity than the free antigen [10, 15].

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