These results draw attention to the value of the delayed test for the screening of potential chemotherapeutic agents.

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Effect of Splenectomy on Primary and Secondary Response to Sheep Erythrocytes in Rats. (31794)

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Rowley(1) has demonstrated that hemolysin production in the splenectomized rat is a function of the dose and route of administration of red cell antigens. A small dose of sheep red blood cells (SRBC) injected intravenously led to the production of minimal amounts of antibody. He also found that the time of antigen administration after splenectomy did not change these results. However, splenectomy of *S. typhi* immunized rats showed somewhat different results. Rats splenectomized before *S. typhi* injection showed the same antibody depression as seen in rats splenectomized before SRBC injection. But when the spleen was removed 14 days *after S. typhi* injection, there was little effect on the agglutinin response(2,3).

It has recently been shown that antibody-containing and antibody-producing cells migrate from the spleen into the blood during late stages of the primary immune response (4,5). These cells will naturally reenter lymphoid organs in the course of their migration. It seems reasonable to think that if the cells which are found in the circulation are immunologically competent, removal of the spleen 7 or 14 days after antigen should not inhibit the response to that antigen. It also seems possible that if splenectomy were several days after antigen, some antigen might be 'processed' and might prepare the animal for an anamnestic response even if the spleen has been removed before the second injection of antigen.

Therefore the purpose of the experiments reported here was 2-fold: (1) To see if splenectomy 7 or 14 days after SRBC antigen would cause decreased production of hemolysins, and (2) To see if splenectomy 7 or 14 days after SRBC antigen would render rats unable to respond to a subsequent injection of the same antigen.

Materials and methods. Male albino Sprague-Dawley rats weighing 200-300 g were used. They were maintained on Purina rat pellets and water *ad libitum*.

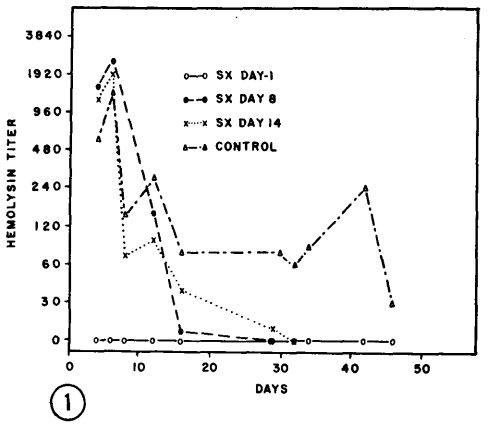
A 1% suspension of sheep red blood cells (SRBC) was prepared by washing whole sheep blood 3 times and diluting in physiologic saline so that 1 ml of SRBC lysed with 4 ml of water gave a reading of 150 in a Klett-Summerson colorimeter with a 540 mμ filter. One ml of antigen was injected intravenously (i.v.) into the lateral tail vein of each rat while the animal was under light

ether anesthesia. Blood for antibody titration was collected from a cut into the lateral tail vein. The same animals were used for each bleeding throughout the experiments and titers were run on individual sera. Sera were stored at −20°C. As described previously(6), the amount of hemolytic antibody was determined on sera inactivated at 56°C for 30 minutes using 2% sheep erythrocytes as the antigen. Titers were recorded as the reciprocal of the highest dilution of antiserum which resulted in complete hemolysis of the sheep red cells.

Results. Although only 2 of the experiments are discussed below, the results given are supported by other similar experiments in this series.

The first experiment examined the effect of splenectomy at various intervals after antigen. All rats were injected intravenously with 1.0 ml 1% SRBC. The animals were divided randomly into 5 groups and were splenectomized one day before, 8 or 14 days after antigen administration. Sham-splenectomized controls were injected in the same manner. As seen in Fig. 1, the rats splenectomized one day prior to antigen administration did not produce detectable amounts of hemolytic antibody. The sham-splenectomized rats and those not splenectomized until days 8 or 14, produced essentially the same peak titers of antibody as the control rats at 6 days after antigen. However, the antibody titer in splenectomized animals fell rapidly below detectable levels following splenectomy, while sham-splenectomized rats continued to produce small amounts of hemolysin after the initial decrease in titer. No titer is recorded on day 8 for the rats splenectomized on that day, hence the slight rise in titer seen on day 12 in all other groups cannot be seen in this group.

The second experiment examined the effects of splenectomy on the response to a second intravenous injection of antigen. Four groups of rats were used. All groups received antigen on day 1. Group A was not treated further. Group B was splenectomized 21 days later and reinjected on day 30. Group C was splenectomized on day 21. Group D received a second injection of antigen on



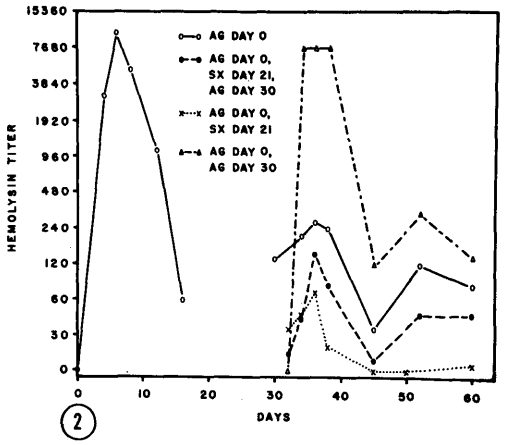


FIG. 1. Hemolysin titers of rats bled on days indicated. Each point is the average titer of at least 3 animals. SX indicates splenectomy on day shown.

FIG. 2. Hemolysin titers of rats bled on days indicated. Each point is the average titer of at least 3 animals. SX indicates splenectomy on day shown, AG indicates antigen injection on day shown.

day 30. The results of this experiment are shown in Fig. 2. Normal and splenectomized rats, whether reinjected or not, show some variation in titer between day 30 and the end of the experiment. This variation is not statistically significant. In the non-splenectomized control group, Group A, which received only the initial injection of antigen, antibody production followed the typical primary response pattern with a peak titer at 6 days followed by return to low antibody levels by day 60. The rats splenectomized before the second injection of antigen, Group B, had slightly lower hemo-

lysin titers than did the control group, which had only received one injection. In fact, the response was lower than the normal primary response. These observations confirm Rowley's data(1) showing that the spleen is necessary for a response to a second injection of low doses of SRBC. The group splenectomized after the initial dose of antigen and not reinjected, Group C, produced the lowest level of circulating antibody, as expected. The non-splenectomized control rats given 2 injections of antigen showed a typical secondary rise in titer. These data indicate that the spleen is necessary for maintenance of normal low level antibody production and is also necessary for a response to a second i.v. injection of SRBC.

Discussion. In the experiments reported here, we showed that (1) splenectomy of rats after a single intravenous injection of SRBC inhibits continued production of antibody, and (2) splenectomy of rats after a first but before a second intravenous injection of small amounts of antigen results in much lower levels of antibody in response to the second injection of antigen. These data confirm Rowley's(1) data and extend his experiments as follows: Our data demonstrate that splenectomy abolishes hemolysin production completely when the antigen is given in small doses intravenously. The normal response of a rat to a single injection of SRBC is a rise in antibody titer until day 6, followed by a decrease in titer to a baseline level of antibody production. Splenectomy after the peak is reached causes the antibody titer to drop to zero rather than to maintain baseline levels. This drop is not immediate, but occurs 2-3 weeks after splenectomy. We also found that splenectomy before an initial injection of antigen results in no antibody production, while splenectomy between a first and second injection of antigen results in only small amounts of antibody in response to the second injection.

Thus it seems that splenectomy, whether after a primary injection of antigen or before a secondary injection of antigen, removes the cells capable of producing hemolytic antibody in response to intravenous injections of small amounts of SRBC antigen.

Summary. Splenectomy one day before antigen will inhibit completely the response to i.v. SRBC, but will depress the response below normal levels when performed 8 or 14 days after antigen. Secondary response is inhibited by splenectomy prior to the second injection of antigen. This appears to be different from results obtained with *S. typhi* flagellar antigen, in which no effect of splenectomy on secondary response can be demonstrated. This suggests that the anamnestic response to i.v. injections of SRBC depends on the presence of the spleen.

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Cellular Respiration in Intermittent Magnetic Fields.* (31795)

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The possibility that man may be exposed to high magnetic fields has arisen from the proposed use of magnetic shielding against cosmic radiation and of magnetohydrodynamic propulsion in space travel(1). Reports on the inhibition of tumor growth *in vivo*(2) and *in vitro*(3) have conceivable clinical implications. In seeking a more

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