must grow preferentially in hamster cells but not in mouse cells. The finding that 5 million hamster embryo cells infected 4-7 days with 3049 virus usually contained only 10^4 plaque-forming units of infectious virus indicated that even the immunogenic virus itself did not replicate effectively in hamster cells.

If the phenomenon reported here does represent the influence of host cell on a functional manifestation of the virus particle, the work of Habel and Axelrod(11) is of particular interest. They have shown that DNA extracted from PV propagated in mouse cells demonstrated greater homology with normal mouse cell DNA than with DNA from either normal or PV-induced neoplastic hamster cells. If the increase in immunogenicity described here can be shown to involve alterations of PV DNA by either selection or host-controlled modification, DNA homology studies will need to be interpreted from the viewpoint of the host cell in which the virus has replicated.

Further studies to correlate oncogenic and immunogenic potency of PV strains with the antigenic composition of hamster tumors induced by these strains should provide much better insight into the genetic and biochemical basis of oncogenesis.

Summary. The capacity of a polyoma virus strain to stimulate transplantation immunity in adult hamsters against tumor cells induced by the same strain was significantly increased by allowing the virus to proliferate in hamster embryo cells for 4-7 days. The high immunogenic capacity was lost by a further growth cycle in mouse cells, suggesting that host modification of some viral character may have taken place.

The author wishes to thank Mrs. Judith Vande and Miss Ruth Jennings for their excellent technical assistance.

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Received July 15, 1964. P.S.E.B.M., 1964, v117.

7S Versus 19S Anamnestic Response in Rabbits. (29649)

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While a number of soluble antigens have been shown to induce immunologic tolerance in newborn rabbits, it has been more difficult to induce tolerance with corpuscular antigens (1,2). As yet no tolerance has been achieved with heterologous erythrocytes in newborn rabbits. Rather, small doses of heterologous erythrocytes produced immunization(3).

In pursuing this problem we have found an unexpected pattern in the anamnestic response of newborn rabbits injected with large amounts of antigen. These animals, after having produced a preferentially 7S immune response, were shown to exhibit later in the course of the experiment a 19S anamnestic response. Such a pattern has not been described previously. This observation led us to investigate the pattern of immune response in newborn rabbits in relation to the dose of antigen and the route of its administration.

Material and method. Newborn rabbits from outbred white females were used

throughout this study. Fresh human A red cells obtained under sterile conditions were used as antigen. The red cells were washed 3 times with physiologic saline and finally brought to an 80% suspension with saline [1 ml of this suspension = 0.8 ml of packed red blood cells(PRBC)] for injection into the rabbits. The WBC count was less than 4000/mm^3 and the platelet count less than 100,000/mm^3.

Injection—Schedule. 29 newborn animals from 5 litters were distributed into 3 groups matched according to litters. Nine newborn rabbits were immunized with a total large dose consisting of 60 ml of PRBC/kg BW (LD group). One group of 10 newborn animals was immunized with a total small dose of 3 ml of PRBC/kg BW (SD group). Ten newborn animals from the 5 litters were not immunized in order to follow the development of "natural antibodies" against human A red cells. Each antigen dose was divided into 4 equal parts and injected at weekly intervals starting at day of birth. To study the anamnestic response 3 booster injections of 0.4 ml of red cells were given intravenously to all animals at 6, 16 and 25 weeks. The animals were bled on the 12th day by cardiac puncture; all the other bleedings were done from the marginal ear vein at 4 and 8 weeks, and 5 days after each booster. One additional bleeding was done on the day prior to the last booster.

To study the influence of the route of administration 3 litter mates were injected IP and 3 intradermally (ID) with the small dose of antigen (3 ml PRBC/kg BW). The immunization and the bleeding were done according to the previous schedule, but only one booster injection was administered at 6 weeks.

Treatment of the serum with 2-mercaptoethanol. The sera were diluted in an equal amount of physiologic saline and treated with 2-mercaptoethanol (ME) in a final concentration of 0.1 M for 2 hours at room temperature. After the incubation the sera were dialyzed overnight in the cold against saline containing 0.02 M iodoacetamide.

Hemagglutination-test. Serial dilutions of 0.1 ml of the untreated and ME treated sera were made in physiologic saline. To each dilution an equal volume of a 2% suspension of A red cells was added. The tubes were incubated in a water-bath at 37°C for 30′. The hemagglutinations were read microscopically. Hemagglutination of some sera with ME sensitive Ab was also performed at 4°C.

The titer of the ME resistant Ab is the reciprocal titer obtained with ME treated serum. ME sensitive Ab activity is expressed as the difference of the titer of the untreated serum and that of the ME treated serum.

Density gradient ultracentrifugation(4). 0.3 ml of the serum was layered over a gradient formed from 21%, 14% and 7% saline and centrifuged in a Spinco model L ultracentrifuge with an SW 39 swinging-bucket rotor at 24,000 rpm for 15 to 16 hours. The fractions were collected through a small perforation made in the bottom of the centrifuge tube. The fractions were dialyzed against 0.15 M saline prior to assay for Ab activity. Protein concentrations were determined by the Folin and Ciocalteu method(5).

Absorption of the immune sera with guinea pig kidney. Some sera which demonstrated an antibody activity in mercaptoethanol sensitive antibody, or ME resistant activity, were absorbed with an equal volume of guinea pig kidney antigen suspension (Bacto, Difco Laboratories, Detroit, Mich.). The absorptions were made at 37°C for one hour and at 4°C for 48 hours. A human O serum which has a high titer of 19S anti-A antibody was absorbed as a control under the same conditions.

Results. Four newborn rabbits of the LD group and 5 newborn rabbits of the SD group were bled at 12 days of age. Only one animal in each group had hemagglutinins against A red cells. The titers were 1:40 for the animal of the LD group and 1:2 for the rabbit of the SD group. These 2 hemagglutinins were ME sensitive.

The results at 4 weeks are recorded in Table I. The results of the anamnestic responses of both groups are represented graphically in Fig. 1.

At 8 weeks, 4 of 6 animals of the small

dose group still had ME sensitive antibody activity. ME resistant Ab activity was present in all animals. In contrast, in the large dose group, only one of 5 animals exhibited ME sensitive Ab activity. The saline gradient ultracentrifugation of one serum containing only ME resistant hemagglutinins did not show heavy molecular Ab activity. In this experiment rheumatoid factor was included in the sample as a marker for 19S globulins.

In the period after 16 weeks of age no animal of the small dose (SD) group had ME sensitive Ab in the anamnestic response, while all the animals of the large dose (LD) group exhibited ME-sensitive hemagglutinin

TABLE I. 4 Weeks Bleeding—1 Week After End of Immunization.
Hemagglutination's titers against A RBC.

ME sensitive Ab (19S)			ME resistant Ab (7S)					
Controls			Large dose IP			Small dose IP		
No. of animals	Titer 19S	Titer 7S	No. of animals	Titer 19S	Titer 7S	No. of animals	Titer 19S	Titer 7S
20	—	—	1	190	10	10	50	—
21	—	—	2	390	10	11	—	—
22	—	—	3	90	10	12	25	—
23	—	—	4	750	50	13	45	
24	10	—	5	190	10	14	—	200
25	20	—	6	92	8	15	20	—
26	5	—	7	95	5	16	—	—
27	10	—	8	50	—	17	—	—
28	5	—	9	190	10	18	320	—
29	20	—				19	320	—

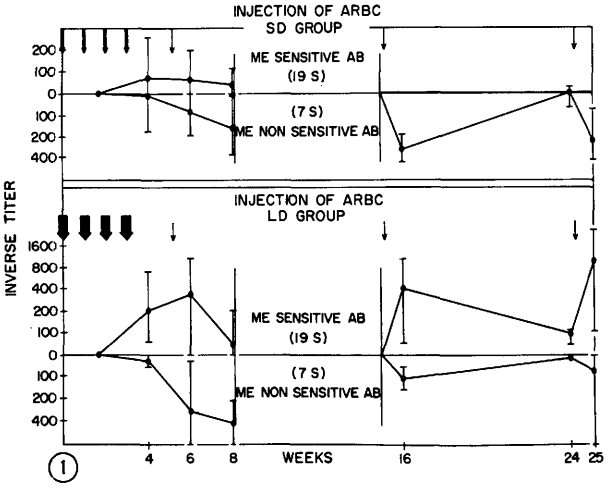

FIG. 1. Effect of the dose of antigen. In each group the arrows in bold type represent immunizing doses and the 3 small arrows the booster injection. Solid lines are anti-A hemagglutination titer and each point represents average titer of the group. In each group, ME sensitive Ab (19S) are above the 0 line and ME resistant Ab (7S) below the 0 line.

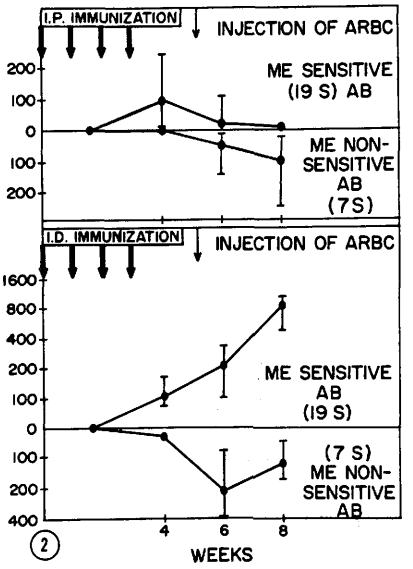

FIG. 2. Effect of route of administration. In each group arrows in bold type represent immunizing doses and small arrow the booster injection. Solid lines are anti-A hemagglutination titer and each point represents average titer of the group. In each group ME sensitive Ab (19S) are above the 0 line and ME resistant Ab (7S) below the 0 line.

TABLE II. Anamnestic Responses.

Small dose titer				Large dose titer	
No. of animals	19S	7S	No. of animals	19S	7S
			16 weeks		
			1	840	160
			2	480	160
			3	560	80
18	—	200	4	350	50
19	—	400	5	60	40
			25 weeks		
15	—	200	1	100	—
16	—	100	2	200	200
17	—	400	3	1200	800
18	—	200	4	1800	200
19	—	200	5	1600	400

activity in the second and third anamnestic response (Table II).

Although at 16 weeks we have only 2 animals in the small dose group, we still consider these results as significant since these 2 animals as well as 3 others produced only 7S antibodies at 25 weeks.

At the age of 24 weeks, 4 of 5 animals in the (SD) group had low titers of ME sensitive hemagglutinin activity (titer 1/15-1/25). This activity disappeared in all 5 animals after I.V. injection of 0.4 ml of human A red cells. At the same time the 5 animals of the (LD) group which had only slightly more ME sensitive activity (titer 1/60-1/20) before injection of the booster dose, developed a typical anamnestic response in ME sensitive Ab (titer 1/100-1/1800).

The results obtained with mercaptoethanol at 25 weeks were further evaluated in 4 sera (3 of the LD group, 1 of the SD group) by means of saline gradient ultracentrifugation. The ME sensitive activity was found in the heavy molecular fractions, the ME resistant activity in the fractions corresponding to 7S globulins.

The ME sensitive activity of 3 sera obtained at 16 weeks in the (LD) group was substantially decreased after absorption with the Forssman antigen of the guinea pig kidney. This absorption was equally effective for the ME resistant Ab of 2 sera obtained at the same time in the (SD) group. The anti-A hemagglutin of the untreated and ME treated control human O serum was also readily absorbed under the same conditions.

Five sera which exhibited ME sensitive Ab had the same hemagglutination titer at 4°C.

The influence of route of administration upon the class of antibody produced in the anamnestic response is represented in Fig. 2.

There was an earlier appearance of 7S antibodies in all animals immunized intradermally than in those immunized with the same dose of antigen IP.

In the anamnestic response those immunized IP produced small amounts of both classes of antibodies but 7S antibody only at 8 weeks. In contrast all animals immunized intradermally produced large amounts of both classes of Ab, but preferentially 19S Ab at 8 weeks.

Discussion. In our study extremely large amounts of heterologous erythrocytes injected into newborn rabbits failed to induce immunologic tolerance. On the contrary, the titer of hemagglutinins was higher with the large immunizing dose than with the small dose. These results corroborate the findings of Sterzl with bacterial antigens(6). In the discussion, we will use the term of 7S for ME resistant antibody and 19S for ME sensitive antibody, bearing in mind that ME sensitive Ab could also represent Y-1A antibody(7). 7S antibodies appeared earlier in animals immunized with the large dose of antigen. Similar results were recently obtained by Svehag(8).

The first anamnestic response at 6 weeks was qualitatively similar in the 2 groups, again high titers were found in the animals immunized with the large dose.

The second and third anamnestic responses differed qualitatively in the 2 groups. Whereas the animals immunized with the small dose of antigen exhibited a pure 7S anamnestic response, the animals immunized with the large dose produced a preferential 19S anamnestic response at 16 and 25 weeks of age. This is rather unexpected since these animals preferentially produced 7S Ab at 8 weeks of age. Only one animal had 19S Ab activity at this time. Such pattern has not been described previously. It was found that the 'immunologic memory" for 19S Ab is short

in contrast to the long-lasting 7S "immunologic memory"(9,10). A persistent 19S Ab response was found only with a few polysaccharide antigens which do not easily lead to 7S Ab formation(11,12).

Since we have used a complex antigen, the unexpected pattern of the anamnestic response may reflect an immune reaction to a different antigenic determinant(13). For instance, the 7S response may be directed against a protein, while the 19S response may be directed against a polysaccharide. However, the result of the absorption study with a Forssman antigen indicates that the sera with 7S and 19S Ab activity in the 2 groups behave similarly. Since the Forssman antigen is a polysaccharide which cross reacts with antisera against polysaccharide A, it may be concluded from this absorption experiment that the difference in anamnestic response cannot be explained by a different antigenic determinant involved in the 2 groups.

Very recently it has been found in rabbits immunized with mouse erythrocytes that the spleen is responsible for the formation of 7S Ab in the anamnestic response(14). In Davidsohn's experiment, splenectomized rabbits do not form 7S Ab in the anamnestic response against mouse erythrocytes as do control animals. Although this finding is in contradiction with the work of Stelos(12) using sheep erythrocytes it could have some bearing on our results. In both groups of the present experiment, the spleen may be the site of formation of 7S Ab in the anamnestic response. In addition in the animals of the large dose group the excess of antigen may stimulate the extra-splenic lymphoid tissue. When the antigen is injected intradermally at a site from which the antigen does not easily reach the spleen, the extra splenic lymphatic system might be stimulated first, a fact which may account for these results. The animals immunized I.D. behaved like the animals immunized with large doses of red cells. They exhibited a prolonged 19S anamnestic response. Thus it is possible that, in this system, the different pattern of the late anamnestic response obtained in animals immunized with small and large amounts of human A erythrocytes may be explained by differing sites of Ab formation. The fact that intradermal immunization with small amounts of antigen results in a pattern of antibody formation different from that obtained by I.P. injection of an identical amount of antigen, furthermore indicates that the route of antigen administration is also of importance in the type of antibody produced in the anamnestic response.

Summary. Large amounts of human erythrocytes injected in newborn rabbits failed to induce immunological tolerance. Newborn rabbits immunized with a large dose of antigen produced a persistent anamnestic response in 19S Ab (immunological memory). In contrast, newborn animals immunized with a small dose of antigen produced a 7S anamnestic response only.

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Received July 14, 1964. P.S.E.B.M., 1964, v117.