turnover or loss of that substance must be greater at the upper extremity of bone so that the residual content is less than that of controls after 7 days. On the other hand, the lower extremity, with a much slower growth potential, seems to have accumulated more cation binder than that of the normal chick.

In previous studies, the hypertrophic epiphyseal plate of rickets(9,14) and fluorosis (13) has shown an accumulation of cation binder, while with impaired synthesis, the epiphyseal plate of magnesium deficient rats has shown a lower residual content(18).

Summary. In lathyric rats and chicks, the *in vivo* sulfate fixation per individual cell was equal or slightly higher than that of normal animals. Since there were more active cells in the hypertrophic plate of the lathyric rat, the overall sulfur upake by cartilage was apparently greater. In 7 day lathyric chicks, the plate was not hypertrophied but the sulfate uptake seemed slightly greater. *In vitro* Ca^{45} uptake by demineralized cartilage of the upper extremity of the tibia was considerably less than that of the lower extremity and also less than that of controls, indicating a more rapid utilization or turnover.†

† PROC. SOC. EXP. BIOL. AND MED. (v98, 318) contains a report by Castellani and Castellani-Bisi showing that in a mixture of epiphyseal plates from osteolathyric rats, hexosamine was significantly decreased (36%) while hydroxyproline remained practically unchanged.

1. Geiger, B. J., Steenbock, H., Parsons, H. T., *J. Nutrit.*, 1933, v6, 427.

2. Ponseti, I. V., Shepard, R. S., *J. Bone Joint Surg.*, 1954, v36A, 1031.

3. Selye, H., *Rev. Can. de Biol.*, 1957, v16, 1.

4. Selye, H., Bois, P., *Endocrinology*, 1957, v60, 507.

5. Bélanger, L. F., submitted to *J. Bone Joint Surg.*

6. Bauer, G. C. H., Carlsson, A., Lindquist, B., *Acta Path. Bact. Scand.*, 1955, v37, 407.

7. Ponseti, I. V., Wawzonek, S., Shepard, R. S., Evans, T. C., Stearns, G., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 366.

8. Menzies, D. W., Mills, K. W., *J. Path. Biol.*, 1957, v73, 223.

9. Bélanger, L. F., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 150.

10. Bélanger, L. F., Leblond, C. P., *Endocrinology*, 1946, v39, 8.

11. Bélanger, L. F., *Nature*, 1952, v170, 625.

12. Bélanger, L. F., *Can. J. Biochem. Physiol.*, 1954, v32, 161.

13. Bélanger, L. F., Visek, W. J., Lotz, W. C., Comar, C. L., *J. Biophys. Biochem. Cytol.*, 1957, v4, 559.

14. Bélanger, L. F., Migicovsky, B. B., *J. Exp. Med.*, 1958, v107, 821.

15. Neuman, W. F., Boyd, E. S., Feldman, A., 1952 Tr. 4th Conf. Metabolic Interrelat., Josiah Macy, N. Y.

16. Dziewiatkowski, D. D., *J. Biol. Chem.*, 1951, v189, 187.

17. Dziewiatkowski, D. D., di Ferranti, N., Bronner, F., Okinaka, G., *J. Exp. Med.*, 1957, v106, 509.

18. Bélanger, L. F., *J. Histochem. Cytochem.*, 1958, v6, 146.

Received September 4, 1958. P.S.E.B.M., 1958, v99.

Factors Influencing Isoimmunization in Rabbit. Effect of Early Postnatal Immunization on Specific Isoantibody Response.* (24435)

CARL COHEN (Introduced by Louis K. Diamond)
Battelle Memorial Inst., Columbus, O.

Although our knowledge of blood groups, incompatibility, and hemolytic disease has increased considerably in recent years, the fact that only about 5% of Rh-negative women become immunized during pregnancies in which the necessary preconditions for Rh incompatibility exist has not been fully explained(1). Mitchison, Brambell(1) and

* This study was carried out at Roscoe B. Jackson Memorial Lab., Bar Harbor, Me., and supported by grants from Nat. Inst. of Neurol. Dis. and Blindness, Bethesda, Md.

Owen(2) have suggested that the probability of isoimmunization may be dependent on a form of immunological tolerance which is predicted by the "self marker" theory of Burnet and Fenner(3). On this basis the Rh-negative daughter of an Rh-positive mother should be unable to produce Rh antibody if she is carrying an Rh-positive child. Booth *et al.* (4) could not corroborate this explanation by their data. However, Owen(2) has suggested that the explanation on the basis of "tolerance" is still useful, since the effect is represented by a difference in amount or kind of antibody produced even if an absolute form of tolerance does not exist. Since Billingham *et al.*(5) demonstrated *actively acquired tolerance* to skin grafts in mice and chicks, many attempts have been made to induce tolerance-like phenomena with all types of antigens in mice, chicks, rabbits, and rats(6,7,8,9). It has been implied in some of these studies that true acquired tolerance is most effective in cases where there is a close relationship between the origin of antigenic material and the recipient animal. A number of investigators have reported that injection of large amounts of defined soluble antigens into newborn rabbits leads to persistent specific inhibition of antibody production(10,11,12,13,14). In the present study we tried to influence the amount of isoantibody produced in adult rabbits by introducing incompatible blood into the newborn rabbit. This represents the first systematic testing of the hypothesis of Mitchison, Brambell, and Owen that inhibition of antibody production may result from exposure of the fetus or newborn to incompatible homologous blood.

Materials and methods. Rabbits were of mixed genetic stocks produced at Jackson Laboratory and were selected as donors and mates solely on the basis of blood type. Immune testing sera were prepared by isoimmunization(15), and all animals were blood typed for the 8 specific erythrocytic antigens which are controlled by 5 genetic loci(16). We were particularly concerned with the antigens belonging to the one genetic system for which we have accumulated data on the probability of isoantibody production. This blood group system has 3 alleles, each of which gives rise to a detectable antigen (A, D, or F). We were able, therefore, to determine the complete genotype of each animal used. Matings were made so that all offspring in a particular litter lacked the donor antigen (type A red cells). *Injection schedules*: Newborn animals were divided into 2 groups; one was injected intraperitoneally 15 times, starting at birth, with 0.5 ml of incompatible citrated blood, and the other (controls) received no early postnatal injections. After the first 15 injections, treated animals were separated into 3 subgroups. One subgroup received 2 intraperitoneal injections/week of incompatible blood until the age of 4 months. Another subgroup received one injection/week of incompatible blood until reaching approximately 3½ months age. A third subgroup received no injections following the first 15 injections. At age 4 to 5 months, all animals were subjected to our regular isoimmunization program, which consisted of injection of incompatible red blood cells in a modified Freund's adjuvant given intermuscularly on 2 occasions followed by a series of intravenous injections of glycerine-citrate-treated red cells. The animals were bled one week after the 5 intravenous injections, which were given over a 2-week period. They were then subjected to another course of 6 intravenous injections over a 2-week period and were bled again one week after last injections. *Antigens.* The initial 15 injections, as well as those given before beginning our regular immunization procedure, were of sterile rabbit blood in ACD solution; proportion of whole blood to ACD solution was 3:1. The antigen used for injection in our standard immunization procedure consisted of washed red cells preserved at −4°C in a glycerine-citrate mixture(17). The cell suspension was thawed and injected without removing the glycerine. At no time did we see any unusual reaction to the injection of the glycerine-citrate red cell mixture. *Titrations.* Trypsin-treated cells were used for titration against sera made up in serial doubling dilutions. The serum-cell mixtures were read for agglutination and strength of reactions was scaled according to a modification of scoring system suggested by Race and Sanger (1). The values given were 4+ = 10, 3+

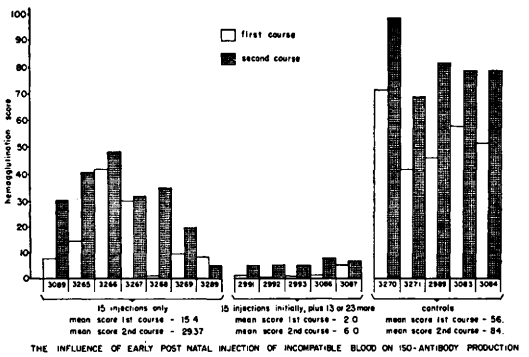

FIG. 1. Influence of early postnatal inj. of incompatible blood on isoantibody production.

$= 8$, $2+ = 5$, $1+ = 2$, $\pm = 1$, and $0 = 0$. Tiration of each serum against a standard cell was represented by a score, and the scores of the sera from animals in different treatment groups were compared.

Results. We were at first concerned with the effect of repeated injection of blood into very young rabbits on their general health and weight gain. Repeated introduction of blood brought no untoward effects; rather treatment appeared to improve the probability of an animal's surviving the critical postnatal period. It is not unusual in breeding of rabbits to suffer the largest loss of animals shortly after birth. There was some postnatal loss in control animals, but we had no loss in animals receiving the equivalent of repeated intraperitoneal transfusions, even though blood was isoantigenically foreign. All animals were bled at regular and frequent intervals from 2 months of age to just before start of our standard immunization procedure. In no case was there evidence of isoantibody production or of normally occurring isoantibody. In our treated animals repeated injections did not stimulate production of anti-A.

After subjection to standard isoimmunization procedure all animals in the non-treated control groups produced uniformly good titres of anti-A; treated animals reacted in a different manner. The treatment groups and schedules are shown in Table I. Fig. 1 illustrates the results graphically. It can be seen that the amount of antibody produced is dependent upon postnatal treatment. Comparison of mean scores, when animals are divided into 3 groups based upon treatments, clearly indicates that there is a marked difference. Mean scores of these groups are statistically highly significantly different from one another.

Discussion. In the present study, a single-red-cell incompatibility system was used, and the only known variable was the type of post-

TABLE I. Summary: Treatment to Induce Acquired Tolerance to Incompatible Rabbit Blood in Newborn Rabbits.

Treatment group	Initial, 15 inj.	No. of inj. after first 15	Hemagglutination score after standard immunization procedure as young adults 1st course	2nd course	
a	None	None	51	78+	Control
	+	23	1	5	Inj. 2 times/wk after first 15
	+	23	0	5	
	+	23	2	5	
b	None	None	58	80+	Control
	"	"	53	78+	"
	+	13	2	8	1 time/wk after first 15
	+	13	5	8	
	+	None	7	30	
c	+	None	14	40	
	+	"	42	48	
	+	"	28	31	
	+	"	1	35	
	+	"	9	18	
	None	"	73	100	Control
	"	"	43	70	"
d	+	"	7	5	

natal treatment. Our results clearly indicate that early postnatal introduction of incompatible blood is capable of markedly altering the antibody response. When only the initial 15 injections were given, productivity was decreased but there was still a substantial amount of antibody in all except one animal (3289). The continued stimulation, even with as little as 0.5 cc 'week, was capable of depressing antibody production to a point where the amount produced was negligible. The mean scores of 2 and 6 represent mean titres of less than 1:2: in the rabbit these titres are not considered significant of isoantibody production.

The marked inhibition of isoantibody production does not fit into the typical patterns when compared with the phenomena of acquired tolerance and specific inhibition of antibody production. The antigens we have used are found only on the erythrocyte. Extensive trials by us as well as others(18,19) have failed to disclose these antigens in other tissues such as liver or spleen or on sperm cells or leucocytes. The stimulus for inhibition thus can be attributed only to the antigen of the red cell, and the amount of effective isoantigen on this cell is thought to be relatively small. The inhibition which results from our treatment would therefore be difficult to explain on the basis of prolonged retention of relatively large amounts of antigen. Since this antigen has not been found on the leucocytes, an explanation relving on implantation and reproduction of living cells is not acceptable.

It is clear that no matter what mechanism is responsible for our results, we have been able to alter markedly the reaction of a rabbit to isoantigen. Whether a similar phenomenon can occur by prenatal treatment of the fetus is now being investigated.

Summary. Introduction of incompatible whole blood into newborn rabbits is capable of altering response of these animals to a later encounter with the same isoantigen. An initial 15 injections given from the day of birth decreased isoantibody production significantly, but not completely. Injections given in addition to the initial 15 effected complete inhibition of specific isoantibody production.

The author gratefully acknowledges the technical assistance of Robert Schuster.

1. Race, R. R., Sanger, R., *Blood Groups in Man,* 2nd Ed., 1954, Blackwell Scientific Publications, Oxford.

2. Owen, R. D., Wood, H. R., Foord, A. G., Sturgeon, P., Baldwin, L. G., *Proc. Nat. Acad. Sci.,* 1954, v40, 420.

3. Burnet, F. M., Fenner, F., *Production of Antibodies,* 2nd Ed., 1949, Macmillan and Co., London.

4. Booth, P. B., Dunsford, I., Grant, J., Murray, S., *Brit. Med. J.,* 1953, v2, 41.

5. Billingham, R. E., Brent, L., Medawar, P. B., *Nature,* 1953, v172, 603.

6. Owen, R. D., *Proc. Roy. Soc. (B),* 1956, v146, 8.

7. Hasek, M., *ibid.,* 1956, v146, 67.

8. Bauer, J. A., Peckham, P. E., Osler, A. G., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 714.

9. Smith, R. T., Bridges, R. A., *Trans. Bull.,* 1956, v3, 145.

10. Downe, A. E. R., *Nature,* 1955, 176, 740.

11. Hanan, R., Oyama, J., *J. Immunol.,* 1954, v73, 49.

12. Dixon, F., *J. Exp. Med.,* 1955, v101, 233, 245.

13. Smith, R. T., Bridges, R. A., Thomson, E., *J. Lab. and Clin. Med.,* 1956, v48, 944.

14. Cinader, B., Dubert, J. M., *Brit. J. Exp. Path.,* 1955, v36, 515.

15. Cohen, C., *J. Immunol.,* 1955, v74, 432.

16. ———, *Genetics,* 1955, v40, 770.

17. Chaplin, H., Mollison, P. L., *Lancet,* 1953, v1, 215.

18. Keeler, C. E., Castle, W. E., *Proc. Nat. Acad. Sci.,* 1933, v19, 403.

19. Kellner, A., Hedal, E. F., *J. Exp. Med.,* 1953, v97, 33, 51.

Received September 4, 1958. P.S.E.B.M., 1958, v99.