TABLE III. Antibody N Levels in Rabbits Immunized with P-HRC and Treated or Not Treated with Cortisone.

Group	Animal #	Anti-PBSA γ Ab N/ml	Anti-PEA γ Ab N/ml	Anti-PRC*	Anti-PBSA / Anti-PEA
Cortisone	167	40	27	18	1.5
	168	42	31	lost	1.4
	169	80	55	31	1.5
	170	16	10	17	1.6
	171	4	0	17	
	172	10	3	20	
	Avg	32	21	21	1.5
Control	173	11	7	14	1.6
	174	12	lost	9	
	175	78	48	27	1.6
	182	38	23	18	1.7
	183	81	44	28	1.8
	184	30	23	12	1.3
	Avg	42	27	18	1.6

* γ Ab N obtained with one absorption of 1 ml of serum.

cortisone. Antibody was measured with a p-bromophenylureido-protein other than the one used for immunization. Cortisone reduced antibody production to the conjugated BSA but affected antibody produced to the conjugated stroma but little, if at all.

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Stimulatory Effect of BCG on Isoantibody Production in the Rabbit.* (20886)

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Although considerable work has been reported on the study of isoantibodies in the rabbit(1-7), there is little reference to the difficulty in inducing isoantibody production. In our study of the mode of inheritance of blood group antigens in the rabbit, the probability of isoantibody being produced in a particular recipient has been very small, even when tests indicate that differences exist between the cellular antigens of the donor and the recipient. Preliminary observations made while using the test of native resistance to tuberculosis devised by Lurie *et al.*(8) suggested that BCG injection might increase the probability of isoantibody production. There is considerable evidence to support the view that in rabbits and guinea pigs infection with tubercle bacilli leads to a nonspecific increase in the ability of these animals to produce antibody. Lewis and Loomis(9) found that hemolytic antibody titre could be increased more than tenfold following the injection of sheep erythrocytes into tuberculous animals. Lurie (10) attributes this increased productivity to the generally increased activity of the reticulo-

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endothelial system in animals exposed to tubercle bacilli or their products.

It was with the purpose of testing the effects of the intradermal inoculation of BCG on the ability of rabbits to produce isoantibody that this study was carried out.

Materials and methods. Rabbits of various races and strains which are maintained at this colony were used as donors and recipients. No regard was paid to weight or sex of the animals; the age in all cases was from early maturity to one year. In the first series of immunizations the donors and recipients were chosen at random, since we did not have any typing serum available. In later series, immunizations were based on known antigenic differences so that the recipient did not possess one or more antigens known to be present on the donor's cells. The recipients received 2 ml of donor's whole blood by the intravenous route 3 times a week for 3 or more weeks. The recipients were bled, and the serum obtained was inactivated at 56°C for ½ hour. The presence of antibody was measured by the agglutination test, which was carried out as follows. Using capillary pipettes which dropped approximately .025 ml per drop, 2 drops of the serum dilution were mixed with 2 drops of 2% washed red cell suspension and allowed to incubate in a 37°C water bath for 3 hours. At the end of this time the tubes were centrifuged at 1000 RPM for 2 minutes and then read with the aid of a concave mirror. Reaction was measured as 4+, 3+, 2+, +, or 0, depending upon the degree of clumping. All sera were tested against a panel of rabbit bloods and those which did not show agglutinating ability were further examined for the presence of incomplete antibody by a modification of the Coombs test in which anti-rabbit globulin made in the guinea pig was used. In only one case was incomplete antibody found. The BCG suspension was obtained at first from the Henry Phipps Institute. Later the BCG was grown in our laboratory on potato and glycerol water and then transferred to Sauton's medium. After one week's growth on this medium the surface growth was harvested and dispersed by rotation with glass beads. The suspension was centrifuged at 2000 RPM for 5 minutes and the supernatant diluted so that 85% of light of 540 Å was transmitted through the suspension. Injections of 0.2 ml of this vaccine into the rabbit by the intradermal route gave rise to a lesion that reached its maximum size in about 3 weeks and then regressed until, after about 8-12 weeks, it completely healed. The first injection of the rabbit cells followed the BCG injection by 5 days.

TABLE I. Attempts at Isoimmunization.

Series	No. of animals	No. producing antibody	P*
1	12	1	No P
2	7	4	BCG P
3a	5	1	" P
3b	6	0	No P
4a	6	0	BCG P†
4b	8	6	BCG P
5	12	9	" P
6a	8	6	" P
6b	9	2	No P‡

* P = Pretreatment.

† Viability of injected organisms doubtful.

‡ Non-producers in this group were later treated with BCG, and immunization was continued. Four of these 7 produced antibody.

Results. Table I shows the results of 6 series of immunizations involved in a test of the BCG technic. Of the series shown, No. 6 was a deliberate attempt to test the efficacy of the BCG injection as a stimulus to antibody production. All the other series were incidental to the study of resistance to tuberculosis, and the groupings into series were based upon factors which are involved in that study and which do not affect isoimmunization. Since handling and treatment of the animals were identical in all series and since statistical analysis indicates homogeneity of these series, it is felt that, for the final analysis, the various series can be treated as one homogeneous group.

In series No. 6, 17 animals were chosen at random from the population and divided into 2 groups. Group 6a, consisting of 8 animals, received a preliminary BCG injection, followed by the standard injection of rabbit red blood cells; Group 6b, consisting of 9 animals, received the standard injection of rabbit erythrocytes without the BCG pretreatment. By the end of the period in which a

total of 12 injections of cells was carried out, 6 of the 8 BCG-treated animals showed antibody production, while only 2 of the 9 which received no pretreatment showed evidence of antibody production. One week later the 7 animals in No. 6b which had not produced antibody were injected with BCG. Three weeks later, after 6 more injections of red blood cells, 4 of these 7 produced isoantibody. A total of 10 out of 15 animals produced antibody after BCG pretreatment as compared with 2 out of 9 without pretreatment.

In series No. 4a the BCG administered to the animals being immunized was also used for a test of resistance to tuberculosis by the Lurie method. On the whole this test was a failure, since the lesions which developed were very small and regressed rapidly. Furthermore, a sample of the vaccine spread on Lowenstein's medium for a count of viable organisms gave negative results at a 1/1000 dilution. It can be seen that none of the 6 animals in this series produced isoantibody. At the same time series No. 4b was started with BCG vaccine which was prepared as a different batch in our laboratory. It can be seen that here 6 of 8 animals produced antibody.

Altogether 3 out of 27 rabbits produced isoantibody with no BCG pretreatment and 26 out of 46 rabbits produced isoantibody following BCG injection. Statistical tests using the χ^2 method indicate that these results are significant at the .01 level.

Discussion. The results indicate quite clearly that the pretreatment with BCG greatly increases the probability of isohemagglutinin being produced. The role of BCG in relation to specificity must, of course, be considered. In our work on tuberculosis, more than 80 rabbits have been injected with BCG but not with red blood cells and their sera tested for isoantibodies. No isoantibodies have been found in these 80 rabbits.

The influence of the BCG inoculation on the specificity of antibody produced in the rabbits after stimulation by red blood cells was tested by comparing specificities of sera produced by animals which received BCG pretreatment plus red blood cells with the specificities of sera produced by animals which received no pretreatment. Seven specific antigens of the rabbit erythrocytes have been studied, and we have found that animals produce specific antibodies in response to the stimulation of known antigenic configurations without reference to pretreatment with BCG.

Through the use of this pretreatment with BCG we have greatly increased the probability of obtaining isoantibodies. The increased number of recipients producing antibody has facilitated the study of the inheritance and immunologic characteristics of isoantigens and their specific antibodies.

Summary. Iso-antibody production in the rabbit is greatly enhanced by pretreatment of the recipients with an intradermal injection of BCG. Of 46 animals given the BCG pretreatment 26 produced isohemagglutinin when stimulated with donor red blood cells, whereas only 3 out of 27 produced isohemagglutinin when the BCG pretreatment was omitted. Antisera to 7 different antigens in the rabbit have been prepared by the use of BCG pretreatment.

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