THE IMMUNE RESPONSE OF RABBITS TOLERANT TO BOVINE SERUM ALBUMIN TO THE INJECTION OF OTHER HETEROLOGOUS SERUM ALBUMINS*· ‡

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A total immunological unresponsiveness (tolerance) to serum protein antigens can be induced in rabbits by neonatal injection of the protein (1–13). This tolerance lasts for a considerable period of time after the rabbit reaches maturity (8) and is specific (1, 7). A similar total tolerance of shorter duration has been induced in mice (14) and a partial tolerance has been induced in chickens (15–19). The ability of specifically tolerant animals to produce antibodies against antigens related to the antigen used to induce tolerance has been studied previously (6, 7, 12). The present study was designed to observe the effect of the injection of tolerant rabbits with antigens related to the inducers of tolerance on the tolerant state itself.

Materials and Methods

Antigens.—Commercial preparations of serum albumins were obtained from the following sources: bovine, Armour and Co., Lot V68802; human, Squibb, Lot 591R; dog, pentex, Lot 33F01; sheep, pentex, Lot 140G01; horse, pentex, Lot 39F02; and pig, pentex, Lot 570F01. Serum albumin from mouse, dog, and hamster were prepared by fractionation of whole serum (20) and purified by the method described by Schwert (21). The serum albumins prepared in this manner showed a purity of 97 per cent as determined by paper electrophoresis. Bovine gamma globulin (BGG) was purchased from Armour and Co. (Lot C-904). Bovine serum albumin (BSA) was trace labeled with I^{131} (I*) by the method previously described (11). Protein-bound I* activity was determined in either well-type gamma counters or NaI crystal scintillation counters. The specific activity (counts/μg N) of the I* BSA was determined so that I* activity could be converted to μg of BSA N.

Antisera.—Anti BSA and anti BGG were obtained from hyperimmunized albino rabbits prepared by a series of injections of either BSA or BGG given over a 2 to 4 month period, totaling 250 to 300 mg. The last injection was given 7 days before bleeding. The sera obtained from 15 rabbits were pooled.

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Induction of Immunological Tolerance to BSA.—Rabbits were made tolerant to BSA by the method previously described (9, 10). Newborn rabbits were given several injections of BSA during the first 5 days after birth, totaling 500 mg. Prior to each experiment, each rabbit (at age of 3 to 4 months) was injected with 20 mg of I* BSA, and the failure to show an immune elimination of the I* BSA over a 13 day period was used as an index of a tolerant state. Under these conditions, 100 per cent of the rabbits were tolerant.

Injection of Heterologous Albumins.—BSA-tolerant rabbits were given four daily subcutaneous injections of 20 mg of human, pig, sheep or bovine albumin. On the 5th day they received an intravenous injection of 30 mg/kg. body weight of the same albumin. The series of injections was repeated 2 weeks later. BSA-tolerant rabbits were also injected in a similar manner with BGG. Five days after the last injection, all of the rabbits were bled and the sera tested for anti-BSA and antibody against the heterologous albumin used for immunization.

Elimination of I BSA from the Circulation.*—Immediately following the antibody bleeding, the tolerant rabbits were injected intravenously with 20 mg I* BSA and bled periodically. The sera were analyzed for both I* BSA and for I* BSA-anti BSA complexes. I* BSA complexes were measured by determining the amount of I* BSA bound to the globulins precipitated at 50 per cent ammonium sulfate saturation (22). The immune elimination of I* BSA was used as evidence of the loss of the tolerant state.

Antibody Determinations.—Precipitating antibody was measured by the quantitative immunochemical procedure described by Heidelberger and coworkers (23). In situations where the levels of precipitating antibody were low and could not be accurately measured with this procedure, a quantitative technique (24), employing I* antigen, was utilized. Other tests for antibody were performed by: (*a*) a modification of the Takatsy hemagglutination technique (25), employing formalized rabbit erythrocytes (26); (*b*) the ammonium sulfate technique of Farr (27); and (*c*) the double diffusion in agar technique. The ammonium sulfate technique was performed with 1.0 μg I* BSA N and increasing dilutions of antisera, and the results are reported as the μg of I* BSA N bound to the globulin in 1 ml of serum. Double diffusion in agar was performed by a modification of the technique described by Ouchterlony (28). An 0.7 per cent solution of Ionagar 2, purchased from Consolidated Laboratories, Inc., Chicago Heights, was prepared and pipetted on glass plates over a 40 x 40 mm area. The borders of the area were contained by placing microscope slides on the glass plates. Three to 4 ml of agar were used to fill the 40 x 40 mm area to a depth of 2.0 mm. The antigen and antibody wells were cut in the cooled agar. Plates prepared in this manner could be washed free of the unbound protein, dehydrated, and then stained with azocarmine G or amido Schwartz. A permanent record of the reactions was then available. Double diffusion in agar was performed with concentrated sera and varying concentrations of the antigens.

RESULTS

BSA-Tolerant Rabbits Injected with Human Serum Albumin (HSA).—Five BSA-tolerant rabbits and five normal rabbits of approximately the same weight and age were given a series of injections of HSA, and both the antibody produced and the result of a subsequent injection of I* BSA were studied (Table I). Both groups of animals produced high levels of antibody which precipitated with HSA, although more anti-HSA was produced in the normal group than in the tolerant group. Sera from the normal group also precipitated with BSA, while sera from the BSA-tolerant group did not. Four of the five tolerant rabbits, however, lost their tolerance to BSA following the injection

of HSA, since they showed an immune elimination of a subsequent injection of I* BSA, and their sera contained anti-BSA when analyzed by the Farr technique. The sera from the normal rabbits cross-reacted with all four of the heterologous albumins tested, while sera from the tolerant rabbits showed much less diversified cross-reactivity with heterologous albumins. Five BSA-tolerant rabbits not injected with HSA showed no antibody to BSA or HSA and no immune elimination of I* BSA. Table II shows results obtained in

TABLE I

Serological Changes in BSA-Tolerant Rabbits Following Injections of HSA

Rabbit No.	Antibody N precipitated		Cross-reaction	BSA-Binding capacity‡	Immune elimination of I* BSA§	Reaction in agar with various albumins				
	with HSA	with BSA				Bovine	Sheep	Pig	Mouse	Dog
	μg	μg	*per cent*							
Tolerant										
79-44	410.0	0	0	7.2	+	−	−	+	+	+
79-45	406.0	0	0	7.3	+	−	−	−	−	+
79-46	288.0	0	0	14.5	+	−	−	−	−	+
79-48	132.8	0	0	0.0	−	−	−	−	−	+
79-49	334.0	0	0	4.0	+	−	−	−	−	+
Normal										
80-37	824.0	40.0	5	51.5		+	+	+	+	+
80-39	502.0	20.1	4	8.2		+	+	+	+	+
80-40	804.0	77.6	9	104.3		+	+	+	+	+
80-41	544.0	52.8	10	87.1		+	+	+	+	+
80-42	242.8	11.2	5	7.9		+	+	+	+	+

‡ The μg I* BSA N bound by the globulin fraction (precipitated with 50 per cent $(NH_4)_2SO_4$) of 1 ml sera.

§ 20 mg I* given intravenously following antibody bleeding.

another experiment with nine BSA-tolerant rabbits injected with HSA. At least eight of these rabbits rapidly eliminated a subsequent injection of I* BSA (Fig. 1) at a rate consistent with that of a secondary response. All of the nine tolerant rabbits produced anti-BSA hemagglutinins and anti-BSA as detected by the Farr technique. The one tolerant rabbit which did not give an immune elimination of I* BSA did have a small amount of non-precipitating anti-BSA in its serum. Whether this response was the result of temporary loss of tolerance, or a production of anti-BSA insufficient to eliminate the I* BSA from the circulation, cannot be evaluated. Five of the BSA-tolerant rabbits in this latter experiment produced small amounts of precipitating anti-BSA as detected by precipitation in both fluid media and agar. Again, the antibody

produced in the tolerant rabbits with heterologous albumins did not cross-react as widely as did the antibody produced in the normal rabbits. The sera of nine BSA-tolerant rabbits injected with BSA and five BSA-tolerant rabbits not injected showed no precipitation with HSA or BSA, no non-precipitating

TABLE II

Serological Changes in BSA-Tolerant Rabbits Following Injections of HSA

Rabbit No.	Antibody N precipitated		Cross-reaction	Hemagglutination (BSA)‡	BSA-binding capacity§	Immune elimination of I* BSA‖	Reaction in agar with various albumins						
	with HSA	with BSA					Bovine	Sheep	Pig	Mouse	Dog	Horse	Hamster
	μg	μg	per cent										
BSA-Tolerant Rabbits Injected with HSA													
83-15	389.1	2.3	0.6	256	13.9	+	+	+	−	+	+	+	+
83-16	332.6	2.1	0.6	4096	11.6	+	+	−	−	+	+	+	+
83-17	811.7	2.6	0.3	1024	18.2	+	+	−	−	−	+	+	+
83-18	231.7	0	0	1024	6.5	+	−	−	−	−	+	−	−
83-19	471.1	3.1	0.7	1024	15.8	+	+	−	−	−	+	+	+
83-20	130.6	0	0	2048	11.2	+	−	−	−	−	−	−	−
83-21	23.1	0	0	64	4.0	−	−	−	−	−	+	−	−
83-22	174.7	0	0	1024	11.2	+	−	−	−	−	+	−	−
83-23	637.7	3.7	0.6	>4096	15.8	+	+	+	+	+	+	+	+
BSA-Tolerant Rabbits Injected with BSA													
9 rabbits¶	0	0	0		0	−	−	−	−	−	−	−	−
BSA-Tolerant Rabbits—Not Injected													
5 rabbits	0	0	0		0	−	−	−	−	−	−	−	−

‡ Results given as the reciprocal of the highest dilution showing agglutination of BSA-sensitized erythrocytes.

§ The μg I* BSA N bound by the globulin fraction (precipitated with 50 per cent $(NH_4)_2SO_4$) of 1 ml sera.

‖ 20 mg I* BSA given intravenously following antibody bleeding.

¶ At the time of antibody bleeding, all rabbits contained circulating BSA in their sera (tested for by double diffusion in agar with anti-BSA).

antibody to HSA or BSA, and no reaction with other heterologous albumins. The control rabbits, also, showed no immune elimination of an injection of I* BSA

Following the injection of I* BSA into BSA-tolerant rabbits, previously injected with HSA, soluble I* BSA-anti BSA complexes appeared in the sera just prior to, and during, the immune elimination of I* BSA. I* BSA–anti-BSA complexes were not detected in the sera of these rabbits when they were injected with I* BSA prior to injection of HSA. In addition, soluble complexes

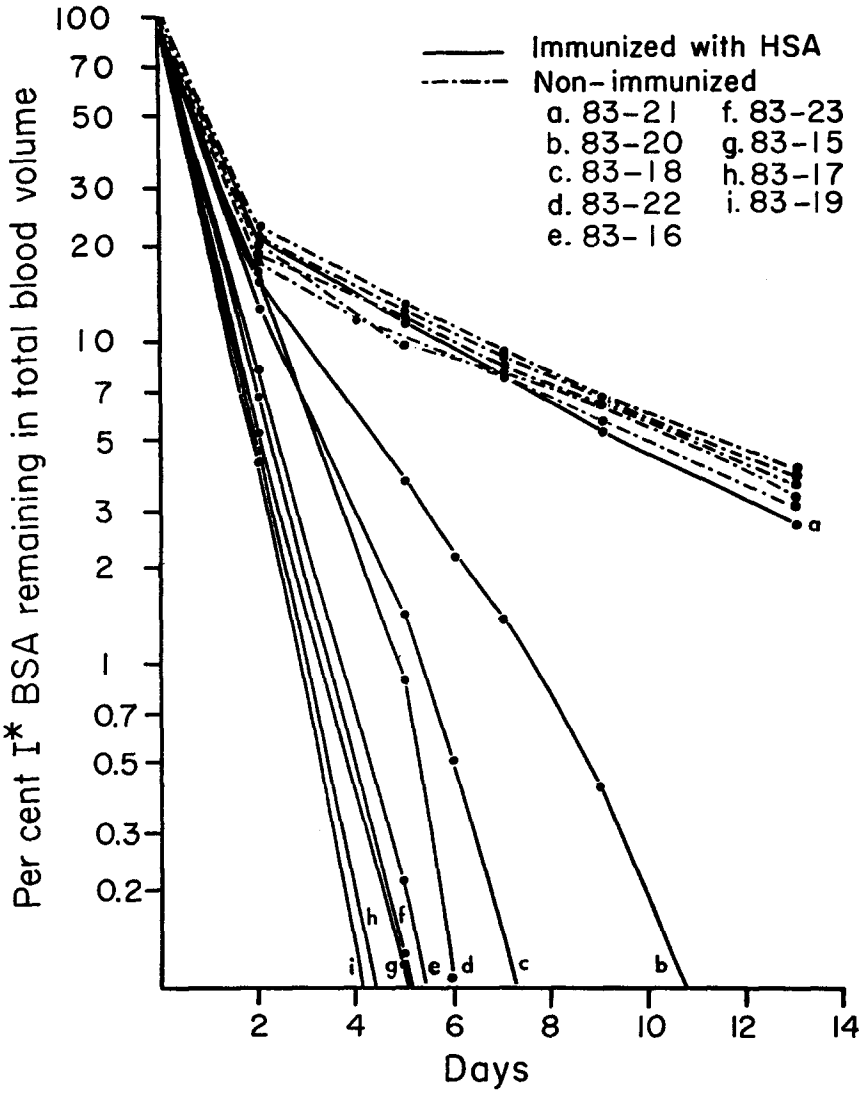

FIG. 1. Elimination of I* BSA from the blood of BSA-tolerant rabbits immunized with HSA.

did not appear in the sera of five control tolerant rabbits at any time during the 13 days following injection of I* BSA.

BSA-Tolerant Rabbits Injected with Pig Serum Albumin (PSA).—Five BSA-tolerant rabbits and five normal rabbits of approximately the same weight and age were given a series of injections of PSA, and both the antibody produced and the effect of a subsequent injection of I* BSA were studied (Table III).

All of the normal rabbits produced antibody which precipitated with both PSA and BSA. Two of the BSA-tolerant rabbits produced considerable levels of antibody precipitating with PSA, and another tolerant rabbit produced only a small amount of anti-PSA. The two remaining tolerant rabbits produced no anti-PSA. The two rabbits (79-42 and 79-43), which produced large amounts of anti PSA, lost their tolerance to BSA, since they showed an immune elimination of a subsequent injection of I* BSA, and their sera contained hemagglutinins to BSA and antibody capable of binding I* BSA. The serum of one of these two rabbits (79-42) precipitated in agar with BSA and all of the heterologous albumins tested. The remaining four tolerant rabbits did not form precipitin bands in agar with either BSA or the heterologous albumens tested. All of the sera from the normal rabbits, including one serum which had only 44 μg of anti-PSA N, formed precipitin bands in agar with BSA and all of the heterologous albumins tested. The sera of five BSA-tolerant rabbits not injected with PSA contained no antibody to either PSA or BSA. These five rabbits also showed no immune elimination of an injection of I* BSA.

TABLE III

Serological Changes in BSA-Tolerant Rabbits Following Injections of Pig Serum Albumin

Rabbit No.	Antibody N precipitated		Cross-reaction	Hemagglutination (BSA)‡	BSA-binding capacity§	Immune elimination of I* BSA‖	Reaction in agar with various albumins				
	with PSA	with BSA					Bovine	Sheep	Human	Dog	Hamster
	μg	μg	*per cent*								
Tolerant											
79-39	35.2	0	0	0	0	−	−	−	−	−	−
79-40	0	0	0	0	0	−	−	−	−	−	−
79-41	0	0	0	0	0	−	−	−	−	−	−
79-42	944.0	0	0	64	23.4	+	+	+	+	+	+
79-43	678.0	0	0	8	7.9	+	−	−	−	−	−
Normal											
79-56	1163.0	372.8	32	1024	297.0		+	+	+	+	+
79-57	432.0	96.0	22	1024	178.0		+	+	+	+	+
79-58	44.0	38.6	87	128	89.1		+	+	+	+	+
79-59	396.0	86.8	22	1024	83.8		+	+	+	+	−
79-60	1206.0	278.8	23	4096	363.0		+	+	+	+	+

‡ Results given as reciprocal of the highest dilution showing agglutination of BSA-sensitized erythrocytes.

§ The μg I* BSA N bound by the globulin fraction (precipitated with 50 per cent $(NH_4)_2SO_4$) of 1 ml sera.

‖ 20 mg I* BSA given intravenously following antibody bleeding.

BSA-Tolerant Rabbits Injected with Sheep Serum Albumin (SSA).—Seven

BSA-tolerant rabbits and five normal rabbits of approximately the same weight and age were given a series of injections of SSA, and both the antibody produced and the effect of a subsequent injection of I* BSA were studied (Table IV). All of the normal rabbits produced antibody which precipitated with SSA

TABLE IV

Serological Changes in BSA-Tolerant Rabbits Following Injections of Sheep Serum Albumin

Rabbit No.	Antibody N precipitated		Cross-reaction	Hemagglutination (BSA)§	BSA-binding capacity‖	Immune elimination of I* BSA¶	Reaction in agar with various albumins						
	with SSA‡	with BSA					Bovine	Sheep	Pig	Mouse	Human	Dog	Hamster
	μg	*μg*	*per cent*										
Tolerant													
79-50	0	0	0	0	0	—	—	—	—	—	—	—	—
79-51	0	0	0	0	0	—	—	—	—	—	—	—	—
79-52	0	0	0	0	0	—	—	—	—	—	—	—	—
81-42	0	0	0	0	0	—	—	—	—	—	—	—	—
81-43	0	0	0	0	0	—	—	—	—	—	—	—	—
81-44	0	0	0	0	0	—	—	—	—	—	—	—	—
81-45	0	0	0	0	0	—	—	—	—	—	—	—	—
Normal													
81-48	254.8	86.8	34		59.4		+	+	+	+	—	+	+
81-49	736.0	442.0	60		145.2		+	+	+	+	+	+	+
81-50	50.4	29.2	57		16.8		+	+	—	+	—	+	+
81-51	1248.0	654.0	62		198.0		+	+	+	+	+	+	+
81-52	930.0	500.0	54		156.8		+	+	+	+	+	+	+

‡ At the time of antibody bleeding, all BSA-tolerant rabbits contained circulating SSA in their sera (tested for by double diffusion in agar with rabbit anti-sheep serum albumin).

§ Results given as the reciprocal of the highest dilution showing agglutination of BSA-sensitized erythrocytes.

‖ The *μg* I* BSA N bound by the globulin fraction (precipitated with 50 per cent $(NH_4)_2SO_4$) of 1 ml sera.

¶ 20 mg I* BSA given intravenously following antibody bleeding.

and BSA. The sera of the normal animals also formed bands in agar with most of the six heterologous albumins tested. In contrast, the BSA-tolerant rabbits produced no antibody capable of reacting with either SSA or BSA, nor did sera from these animals form precipitin bands in agar with any of the heterologous albumins tested. In addition, the BSA-tolerant rabbits did not show an immune elimination of a subsequent injection of I* BSA. At no time did the tolerant animals contain circulating I* BSA–anti-BSA complexes following the injection of I* BSA. Thus, the BSA-tolerant animals neither produced anti SSA, nor lost their tolerance to BSA.

BSA-Tolerant Rabbits Injected with Bovine Gamma Globulin (BGG).—In order to test the specificity of the ability of cross-reacting serum albumins to cause loss of tolerance in BSA-tolerant rabbits, five BSA-tolerant rabbits were given a series of injections of BGG. All of these animals produced antibody which precipitated with BGG (Table V), but there was no antibody in their sera which reacted with BSA, nor did these rabbits show an immune elimination of a subsequent injection of I* BSA.

TABLE V

Serological Changes in BSA-Tolerant Rabbits Following Injections of BGG

Rabbit No.	Antibody N precipitated		Hemagglutination (BSA)§	BSA-binding capacity‖	Immune elimination of I* BSA¶
	with BGG	with BSA‡			
	μg	μg.			
83-33	201.6	0	0	0	–
83-34	179.2	0	0	0	–
83-35	347.2	0	0	0	–
83-36	217.6	0	0	0	–
83-37	747.0	0	0	0	–

‡ Analyzed by both quantitative precipitin and double diffusion in agar techniques.

§ Results given as the reciprocal of the highest dilution showing agglutination of BSA-sensitized erythrocytes.

‖ The μg I* BSA N bound by the globulin fraction (precipitated with 50 per cent $(NH_4)_2SO_4$) of 1 ml sera.

¶ 20 mg I* BSA given intravenously following antibody bleeding.

DISCUSSION

Mature rabbits made tolerant to BSA by neonatal injections of BSA lost their tolerant state when injected with certain heterologous albumins which cross-react with anti-BSA. The ability of a heterologous albumin to cause loss of tolerance to BSA depended upon the degree of serological relationship between that heterologous albumin and BSA (Table VI). Heterologous albumins distantly related to BSA were more effective in producing a loss of tolerance than albumins more closely related. Twelve of fourteen BSA-tolerant rabbits lost their tolerance following a series of injections of HSA but none of the seven BSA-tolerant rabbits lost its tolerance following a series of injections of SSA. HSA and SSA cross-react 15 and 75 per cent, respectively, with rabbit anti-BSA (20). Two of five BSA-tolerant rabbits lost their tolerance to BSA following a series of injections of PSA which cross-reacts 32 per cent with rabbit anti-BSA. Downe (5), who made neonatal rabbits tolerant to chicken serum and injected them with turkey serum at maturity, observed antibody in their sera which precipitated with turkey serum but not with chicken serum. Similarly, Curtain

(12) injected rabbits made tolerant to Bence-Jones protein with an antigenically related myeloma globulin and found antibody in the sera which reacted with myeloma globulin but not with Bence-Jones protein. The amount of anti-BSA produced in BSA-tolerant rabbits following injections of either HSA or PSA was extremely small in comparison to the amount produced in normal rabbits injected with these antigens. Precipitating anti-BSA was observed in the sera of only six of the sixteen rabbits which lost their tolerance to BSA.

BSA-tolerant rabbits not only failed to lose their tolerance to BSA following injections of SSA, but they failed to make a detectable response to the portion of the SSA molecule not related to BSA. That SSA has determinant groups present on its surface which are serologically distinct from any of the determi-

TABLE VI

The Loss of the Tolerant State of BSA-Tolerant Rabbits Injected with Cross-Reacting Heterologous Serum Albumins

Source of albumin	Cross-reaction with anti-BSA‡	Fraction of rabbits losing tolerance
	per cent	
Human	15	12/14
Pig	32	2/5
Sheep	75	0/7
Bovine	100	0/9

‡ Pooled hyperimmune rabbit sera (reference 20).

nants present on BSA has been previously reported (20). Similarly, two BSA-tolerant rabbits failed to make antibodies to PSA following injections of PSA. In agreement with this observation are the findings of Cinader and Dubert (6) who showed that only two of six HSA-tolerant rabbits showed an immune response to injections of a diazo-*p*-benzenesulfonic acid derivative of human albumin (DHSA) which produces an extensive cross-reaction with rabbit anti-HSA. Thus, it appears that tolerance to serum protein antigens is not only directed to the individual determinant groups but also to the over-all antigenicity or chemical-physical properties of the antigen. It may be possible, however, to produce antibodies to the SSA determinants which are not related to BSA by injecting the BSA-tolerant rabbits with SSA incorporated in Freund's adjuvant. Curtain (12) reported that rabbits made tolerant to either normal or abnormal human serum globulins were capable of making antibody to closely related globulins injected after incorporation into Freund's adjuvant.

The mechanism by which injections of albumins cross-reacting with BSA into BSA-tolerant rabbits resulted in a loss of the tolerant state is not understood. There are, however, several possible mechanisms which could account for this

phenomenon. Tolerance may be directed to both the over-all antigenicity or general physical-chemical properties of the antigen and also the individual determinants on the antigen molecule. Thus, SSA may not be recognized as an antigen in BSA-tolerant rabbits because of its over-all antigenic or physical-chemical similarity to BSA, while HSA may be recognized as an antigen because of its over-all antigenic or physical-chemical dissimilarity to BSA. Once the HSA surmounts the first barrier and is recognized as being antigenic on the basis of its over-all properties, then it is confronted with a second barrier, which would be recognition of the individual determinants as being antigenic or non-antigenic. In a BSA-tolerant rabbit, an immune response would be made to the determinants on HSA not related to BSA, and possibly to some related determinants, if the second barrier is not a complete tolerance to these related determinants. The amount of antibody made to the related determinants may depend upon the degree of tolerance which is established toward the original determinants. Injections of SSA would not be expected to cause either BSA-tolerant rabbits to lose their tolerance or to produce anti-SSA, since the over-all antigenicity or physical-chemical properties of this albumin would not permit it to carry the related or unrelated determinants past the first barrier. The ability of a BSA-tolerant rabbit to accept BSA or any heterologous albumin as a non-antigenic substance would depend, therefore, on the ability of the albumin to be recognized as similar to BSA, primarily on its over-all antigenicity or physical-chemical composition, and secondarily on its individual determinant groups. The results obtained with BSA-tolerant rabbits injected with PSA are also consistent with this hypothesis. The two rabbits which made a good response to PSA went on to make antibody reacting with BSA, while the two rabbits which did not make a response to PSA failed to make antibody reacting with BSA. An alternate explanation for the ability of injection of certain heterologous albumins to cause BSA-tolerant rabbits to lose their tolerant state is the relative location of the related determinants on BSA and on the heterologous albumins. The BSA-tolerant rabbit may not need to be entirely tolerant to a partially hidden determinant on BSA. The tolerant rabbit, however, may make an antibody response to the same determinant if it is more prominently placed on HSA or PSA. The loss of the tolerant state could also be the result of a mutation of antibody producing cells which arise from cellular proliferation during the immune response to the heterologous albumins. This latter suggestion would be in agreement with the failure of injection of SSA into BSA-tolerant rabbits to result in a production of anti-SSA or in a loss of the tolerant state. If the loss of tolerance was the result of a mutation of cells arising from cellular proliferation, this mutation would have to be specific for cells which proliferate during the response to heterologous albumins, since injections of BGG into BSA-tolerant rabbits result in a good immune response but not in a loss of the tolerant state. BGG does not show a cross-reaction with rabbit

anti-BSA (Fig. 2), although Timourian and Schechtman (29) reported a 3 to 6 per cent cross-reaction between BSA and rabbit anti-BGG. These workers found that there was a 70 to 80 per cent depression in the capacity of BGG-tolerant rabbits to produce antibodies to BSA. However, both this depression in capacity to produce antibody to BSA and the 3 to 6 per cent cross-reaction between BSA and anti-BGG were probably the result of BSA present in commercially prepared BGG preparations.

The present results can be discussed in relationship to autoimmune diseases if it is accepted that the tolerance of neonatal rabbits to heterologous serum proteins is similar to the tolerance to their own body constituents. The ability

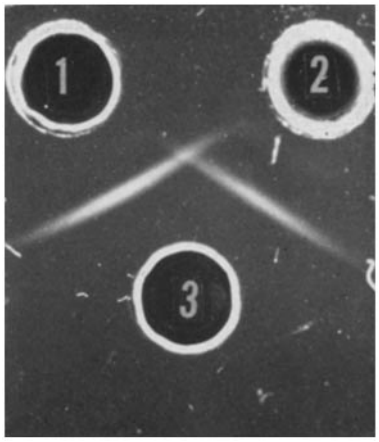

FIG. 2. The serological reactions of a mixture of BGG and BSA with rabbit anti-BGG and rabbit anti-BSA. *1*, anti BGG; *2*, anti BSA; and *3*, BGG and BSA.

of injections of cross-reacting albumins to cause BSA-tolerant rabbits to lose their tolerance suggests that it may also be possible to cause animals to lose tolerance to their own body constituents by exposing them to antigens which cross-react with their own constituents. Goodman (30) reported that rabbits injected with nucleoprotein extracted from human liver produced antibodies which would react with both human liver nucleoprotein and extracts from rabbit liver. Heller and Yakulis (31) showed that guinea pigs injected with connective tissue extracts of rabbits developed antibodies which would react both with rabbit and guinea pig connective tissues and also developed a disease manifested by stunted growth. Milgrom and Witebsky (32) also reported that rabbits developed antibodies to their own gamma globulin when they were injected with gamma globulin prepared by fractionation of whole serum with ammonium sulfate. The reaction of the rabbit sera was much stronger with gamma globulin of foreign species than with gamma globulin from the rabbit.

It was concluded that the antibody produced was stimulated by the animals' own gamma globulin which had undergone changes during the fractionation with ammonium sulfate. At the present, experiments in our laboratory are being performed to attempt to cause BSA-tolerant rabbits to lose their tolerance to BSA by injecting them with chemically, physically, and enzymatically altered BSA.

The relative ease with which rabbits become tolerant to heterologous serum proteins in comparison to bacterial antigens (8) may be explained on the basis of the antigenic and physical-chemical similarity of the heterologous proteins to the serum proteins of the rabbit. Considering the extensive and diversified cross-reactions among mammalian serum proteins (33–36, 20), the normal rabbit is probably partially tolerant to any given heterologous protein, since the serum proteins of rabbits are probably serologically related to most heterologous serum proteins. Differences in other properties, such as ability to circulate in the body and ability to reach all the tissues after injection, may also play an important role in determining the ability of heterologous serum proteins and bacterial antigens to induce tolerance in rabbits.

Rabbits injected with 500 mg. of BSA during neonatal life not only fail to show an immune elimination of a subsequent injection of I* BSA given 3 to 4 months later, but fail to make even small amounts of anti-BSA which could complex with the circulating I* BSA. Bussard (37) reported that rabbits made tolerant to yeast glucose-6-phosphate dehydrogenase by neonatal injection of 2 mg of the enzyme made small amounts of antibody which were capable of complexing with circulating antigen, but which were insufficient to cause an immune elimination of the antigen. Probably as a result of the small amount of antigen injected into the neonatal animals, however, the tolerance produced by Bussard was not total for all the animals and was readily lost.

SUMMARY

Immunological tolerance produced in rabbits by neonatal injections of BSA can be terminated by a series of injections of certain heterologous serum albumins which cross-react with BSA. Injections of albumins distantly related to BSA were more effective in terminating the tolerant state than injections of albumins closely related to BSA. It was concluded from results obtained with several heterologous albumins that immunological tolerance to BSA is directed to both the over-all antigenic or physical-chemical composition of the protein and the individual determinant groups present on the protein. Several possible mechanisms were given to explain the ability of cross-reacting albumins to terminate the tolerant state of BSA-tolerant rabbits.

A possible relationship between the termination of tolerance in BSA-tolerant rabbits injected with cross-reacting albumins and autoimmunity was discussed. It was also suggested that the relative ease with which tolerance could be estab-

lished to heterologous serum proteins in comparison to bacterial antigens is the result of the close serological and physical-chemical relationship of the heterologous serum proteins to the serum proteins of the rabbit.

Rabbits injected with 500 mg of BSA during the first 5 days of life failed to form antibody capable of either eliciting an immune elimination of an injection of I* BSA given 3 to 4 months later or complexing with the circulating I* BSA.

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