IMMUNOLOGIC UNRESPONSIVENESS INDUCED BY PROTEIN ANTIGENS*, ‡, §

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Felton's observation that large injections of pneumococcal polysaccharides rendered mice incapable of subsequent immunization with the same antigen (1) has been variously interpreted. The fact that significant amounts of the pneumococcal polysaccharide persisted in the mouse throughout the remainder of its life has made analysis of the phenomenon difficult. Felton postulated, as implied in his term "immunologic paralysis," that the persisting antigen was adversely affecting cells capable of antibody production. Others have suggested that persisting antigen might be neutralizing antibody as it was formed thereby accounting for the absence of circulating antibody (2). In a recent report (3), the authors presented evidence showing that pneumococcal polysaccharide antigen fixed in the tissues of the mouse was capable of neutralizing large amounts of passively transferred specific rabbit antibody, an observation consistent with the second postulated mechanism.

A somewhat similar suppression of the immune response appears to result from the administration of excessive amounts of foreign tissue antigens. Observations in several laboratories recently reviewed by Snell (4 *a*) indicate that pretreatment of recipient animals with large amounts of non-viable tumor tissue or even normal tissue from donor animals would result in enhanced growth of subsequently transferred living tumor. This enhanced tumor growth presumably results from a suppression of the immune response of the recipient. Billingham, Brent, and Medawar (4 *b*) working with skin grafts in mice and chickens found that injection of the tissue antigen into embryos near term induced a lasting unresponsiveness to later skin grafts in these animals after their birth.

In an effort to gain further insight into the mechanism of this immunologic alteration resulting from an excess of antigen, the present experiments were

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undertaken using as antigens heterologous serum proteins which are readily catabolized by the host even in the absence of an immune response (5). The use of a readily catabolized antigen makes it possible to compare the results of an overdose of antigen during the period the antigen is present in the host in considerable quantity and after it is no longer detectable. Our results indicate that prolonged suppression of the ability to respond to a serum protein antigen is extremely difficult, if not impossible, to achieve in normal adult rabbits in spite of administration of tremendous amounts of the antigen. However, in rabbits exposed to the foreign serum protein from the time of birth, to be referred to as "newborn" rabbits, and perhaps also in x-radiated adult rabbits, a prolonged unresponsiveness can be achieved after large foreign protein infusions. A similar observation in "newborn" rabbits exposed to bovine albumin has recently been reported (6). It is significant that in the "newborn" and x-radiated rabbits the unresponsiveness persists long after the time antigen can be demonstrated in the host. Since the protein antigen in this situation did not seem to produce paralysis of the immune mechanisms by virtue of its continued presence, but rather to produce a lasting inability to respond to the specific antigen in susceptible subjects, the term "immunologic unresponsiveness" was chosen to describe this phenomenon. The exact relationship between the change produced by an overdose of protein antigens and that produced by an overdose of polysaccharide antigens is not yet clear.

Experimental Procedure

A. In an attempt to overwhelm, fatigue, or make unresponsive, the immune mechanisms of the rabbit we arbitrarily selected a dose of foreign serum protein which would approximate the amount of corresponding autologous protein synthesized by the host. Either sterile pooled human plasma (HP) or a 5 per cent saline solution of Zeitz-filtered crystalline bovine serum albumin (BA), Armour and Company, Lot 29633, was given by combined intravenous and subcutaneous routes, in a ratio of 1:2, daily, six times per week in doses of 10 cc. per kilo to six groups of rabbits as follows:

1. Five adult male albino rabbits weighing from 2.2 to 2.4 kilos were infused with human plasma for a period of 53 days (HPA).
2. Six adult male albino rabbits weighing from 2.2 to 2.4 kilos were given 400 r-220 kv. whole body x-radiation as previously described (7) and 2 days later infusions of human plasma were started and continued for 58 days (HPX).
3. A litter of 5 albino rabbits was infused with human plasma for a period of 112 days beginning the day after birth (HPN). Injections were made subcutaneously for the first 4 weeks and after that as described above.
4. Six adult male albino rabbits weighing from 2.2 to 2.4 kilos were infused with bovine serum albumin for a period of 43 days (BAA).
5. Seven adult male albino rabbits weighing from 2.2 to 2.4 kilos were given 400 r-220 kv. whole body x-radiation (7) and 2 days later were started on infusions of bovine serum albumin which lasted for a period of 49 days (BAX).
6. A litter of 3 albino rabbits was infused with bovine serum albumin for a period of 98 days beginning the day after birth (BAN). Injections were made subcutaneously for the first 4 weeks and after that as described above.

TABLE I

Disappearance of Foreign Proteins from the Serum after Cessation of Infusions

Figures represent milligrams protein/milliliter serum.

Group	Rabbit No.	Foreign protein*	Concentration at termination of infusions	Wks. after infusions						
				1	2	3	3½	4	5	6
HPA	10-75	HA	6.9	1.8	—	0.6	—	—	0.06	—
		HGG	1.7	0.4	—	0.2	—	—	0.03	—
	10-77	HA	7.2	2.6	—	0.7	—	—	0.25	—
		HGG	1.4	0.5	—	0.2	—	—	0.06	—
HPX	10-33	HA	6.6	2.5	—	1.3	—	—	—	0.2
		HGG	1.4	0.6	—	0.2	—	—	—	0.03
	10-67	HA	5.9	1.9	Died	—	—	—	—	—
		HGG	1.0	0.4		—	—	—	—	—
	10-69	HA	9.8	4.1	Died	—	—	—	—	—
		HGG	1.6	0.5		—	—	—	—	—
	10-70	HA	7.9	4.2	—	1.9	—	Died	—	—
		HGG	2.0	0.7	—	0.2	—		—	—
	10-72	HA	7.9	3.2	Died	—	—	—	—	—
		HGG	2.7	0.6		—	—	—	—	—
HPN	10-62	HA	6.5	4.4	—	0.4	—	—	0.04	—
		HGG	1.8	1.0	—	0.1	—	—	0.04	—
	10-63	HA	2.9	3.1	—	0.5	—	—	0.07	—
		HGG	0.9	0.3	—	0.1	—	—	0.01	—
	10-64	HA	6.5	4.9	—	1.0	—	—	—	—
		HGG	1.3	0.5	—	0.2	—	—	—	—
	10-65	HA	6.9	3.8	—	0.7	—	—	0.1	—
		HGG	1.3	0.4	—	0.3	—	—	0.07	—
	10-66	HA	5.3	1.6	—	0.2	—	—	0.04	—
		HGG	2.1	0.3	—	0.1	—	—	0.05	—
BAA	11-39	BA	14.9	—	3.2	—	1.0	—	—	0.10
	11-40	BA	14.2	—	4.7	—	1.5	—	—	0.16
	11-41	BA	13.6	—	3.9	—	0.7	—	—	0.01
	11-42	BA	11.5	—	4.3	—	1.1	—	—	0.12
	11-43	BA	15.5	—	4.8	—	0.6	—	—	—
	11-44	BA	14.7	—	4.1	—	0.6	—	—	0.02
BAN	11-14	BA	7.1	4.1	—	—	—	0.4	—	—
	11-15	BA	15.6	5.3	—	—	—	0.4	—	—
	11-16	BA	14.6	5.7	—	—	—	0.6	—	—

* Human albumin, HA; human gamma globulin, HGG; bovine albumin, BA.

The metabolic consequences of the infusions were studied by determining concentrations and half-lives of both autologous and heterologous serum proteins during the infusion period and is reported in the accompanying paper (8). The immunological observations presented here include:—

1. Rate of loss of heterologous proteins from the serum following the period of infusions. Concentrations of the heterolgous proteins in the sera were periodically determined by quantitative immunochemical techniques (9) using calibrated rabbit antibovine albumin, rabbit

TABLE II

Duration of Immunologic Unresponsiveness

Protein used for testing is given in each instance. * indicates antigens not previously used in infusions. Fractions indicate number animals eliminating antigen at immune rate (10)/ number animals tested.

	No. rabbits at start of infusions	Period of infusions	Mos. after infusions										
			0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12
		days												
HPA	5	52	—	HA 0/2	HGG 1/2	—	HA 1/2	BA* 2/2	HGG 2/2	—	—	HA 2/2	—	—
HPX	6	58	—	HA 0/1	HGG 0/1	—	HA 0/1	BA* 1/1	HGG 0/1	—	—	—	—	—
HPN	5	109	—	HA 0/3	HGG 0/3	—	HA 0/3	BA* 3/3	HGG 0/3	—	—	HA 0/2	HGG 0/2	—
BAA	6	43	HGG* 5/5	BA 1/5	—	BA 4/5	—	—	—	BA 4/5	—	—	—	BA 4/5
BAX	7	49	—	—	BA 0/3	—	—	—	—	—	BA 0/1	BGG* 1/1	—	—
BAN	3	91	HGG* 3/3	BA 0/3	—	BA 0/3	—	—	—	—	BA 0/2	BA 0/2	—	—

antihuman albumin, and rabbit antihuman gamma globulin, fraction II sera[1] (Table I). The last traces of heterologous proteins in the serum were followed by qualitative ring tests.

2. Response of rabbits to small doses of the infused heterologous proteins given in the postinfusion period (Table II). In order to detect an ability to respond to previously infused proteins, doses of approximately 5 mg. of I^{131}-labelled bovine albumin (I*BA) were given intravenously to rabbits of the BAA, BAX, and BAN groups and I^{131}-labelled human albumin (I*HA) and human gamma globulin (I*HGG) to rabbits of the HPA, HPX, and HPN groups

[1] The human protein fractions used in preparation of the antisera were kindly made by Armour and Company, Chicago.

at various times after cessation of infusions. Iodination of proteins was carried out as previously described with final preparations containing approximately 1 atom of iodine per molecule of protein (10). Rapid elimination of these proteins indicated an immune response while elimination at rates comparable to the rate of loss of homologous protein indicated absence of an immune response (10). In the event of rapid elimination of antigen, analysis of serum taken 3 days after disappearance of antigen was made for antibody by a quantitative antigen precipitation technique (11).

3. Determination of antibody responses made during the period of infusions to trace constitutents of the infusion material. Because of apparent antibody responses of the HPA rabbits to minor constituents of the plasma, as indicated by development of serum sickness during infusion period in spite of an absence of antibody response to HA or HGG, qualitative ring tests using plasma fractions I to V were made with sera from the 3 HP groups. Similar tests were made using BGG, a < 0.01 per cent contaminant of the BA preparation, as antigen with sera from the BAA and BAN groups. These tests were carried out in 3 mm. tubes in which a 1 to 10 dilution of antigen was carefully layered over decreasing dilutions of serum. The

TABLE III

Disappearance of Passively Transferred Antibody from Serum of Unresponsive Rabbits

Group	Rabbit No.	Antibody transferred	μg. Antibody N/ml. serum			Antibody half-life days	
			3 days	8 days	13 days	Day 3–8	Day 3–13
HPN	10-62	Anti-HA	106.7	43.3	27.8	3.9	5.2
	10-63	Anti-HA	94.3	51.6	33.6	5.8	6.7
BAN	11-15	Anti-BA	90.8	46.7	29.6	5.2	6.2
	11-16	Anti-BA	83.6	42.4	30.1	5.1	6.8

tests were read at 24 hours and recorded as strongly positive, positive, weakly positive, or negative.

4. Response of rabbits to serum protein antigens not used in infusions. After infusions, I^{131}-labelled serum protein antigens not used in infusions were injected intravenously into rabbits and the rate of antigen elimination and magnitude of antibody response was determined as described above. I*BA was used as antigen for rabbits of the three human plasma groups and either I*HGG or I^{131}-labelled bovine gamma globulin (I*BGG) was used as antigen for the three bovine albumin groups (Table II).

5. Fate of rabbit anti-BA passively transferred to BAN rabbits and rabbit anti-HA transferred to HPN rabbits after cessation of infusions. Since the "immunologic paralysis" resulting from overdose of pneumococcal polysaccharide is associated with retention of antigen in the tissues which is capable of taking up considerable amounts of passively administered antibody (3) we determined the fate of passively administered antibody in the present unresponsive rabbits. 249 days after infusions rabbit serum containing 32.5 mg. anti-BA nitrogen was injected intravenously into the two remaining BAN rabbits and 284 days after infusions rabbit serum containing 33.0 mg. anti-HA nitrogen was injected into the two remaining HPN rabbits 3, 8, and 13 days after injection the concentration of antibody in the serum was determined by quantitative immunochemical precipitation (9) (Table III).

B. To observe any possible effect of induced immunologic unresponsiveness of rabbits on their offspring, an unresponsive female BAN rabbit (11–15) was mated with a temporarily

unresponsive male BAA rabbit (11–39) approximately 3 months after period of infusion and the offspring were tested for their ability to respond to BA. At 1 month of age the litter of 4 was given I*BA 15 mg. per kilo and rate of antigen elimination determined. At 4 months the amount of antibody made to a second injection of BA was measured.

C. To observe any histologic alterations which might be associated with an overload of foreign protein, 16 adult male albino rabbits were given 10 cc. of a 5 per cent BA solution per kilo daily six times a week for 3 weeks and 25 were given 10 cc. pooled human plasma per kilo daily six times per week for 7 weeks. Of the BA rabbits 5 were sacrificed after 2 weeks of injection, 5 after 3 weeks of injections and 6, 2 weeks after termination of 3 week injection period. Of the HP rabbits three were sacrificed at each of the following intervals after beginning infusions: 2 days, 9 days, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, and 7 weeks. All tissues were fixed in 10 per cent formalin, sectioned at 5μ and stained with hematoxylin and eosin.

RESULTS

As is evident from Table I, the rate of fall of the concentration of heterologous proteins in the serum of rabbits following the period of infusions is similar to the normal rate of catabolism of the corresponding autologous proteins (12). The disappearance of heterologous proteins is most rapid during the first post-infusion week while the animals are adapting to or recovering from the overload of protein (8) and slowest during the 2 to 3 week period. It is of considerable significance that the rate of disappearance of these foreign proteins does not increase near the end of the period of observation. At 5 and 6 weeks postinfusion the amounts of remaining foreign protein are small and even small amounts of antibody production at this time would be expected to increase the rate of antigen elimination (10).

Capillary precipitin ring tests were used to follow the presence of heterologous proteins in the sera of the three HP groups and the BAN group after the quantitative determinations listed in Table I were discontinued. On the basis of the ring tests HA was detectable for 81 days and HGG for 68 days after infusions were stopped in the serum of the HPA rabbits: HA and HGG were detectable for 75 days in the HPN rabbits, HA and HGG for 102 days in the HPX rabbit. BA was detectable in the sera of the BAN rabbits for at least 87 days.

Responses of the various groups of rabbits to I^{131}-labelled foreign proteins in terms of rates of antigen elimination (rapid elimination equals immune response and slow removal equals non-immune response) (10) are shown in Table II. Both groups of normal adult rabbits, HPA and BAA, recovered their ability to make an immune response to the previously infused proteins. At least 2 of 7 recovered within 2 to 3 months after infusions and 6 of 7 recovered within 4 to 6 months. Of the 2 HPA and 5 BAA animals, only 1 BAA animal failed to make an immune response to the infused proteins within 6 months after the infusions and this animal remained unresponsive throughout the 12 month observation period. It should be mentioned that about 10 to 20 per cent of the rabbits of the strain employed in our laboratory will fail to make a significant response to BA after the first exposure but after numerous exposures a response is always

elicited. Of the two x-ray groups and two "newborn" groups totalling 10 rabbits observed from 3 to 4 months after infusions, or 7 rabbits observed from 7 to 9 months after infusions not one rabbit regained the ability to make an immune response to previously infused proteins. Yet, even though unresponsive to previously infused proteins, all rabbits made immune responses to serum protein antigens not used in the infusions. 6 months after infusions all the HPX and HPN rabbits responded to I*BA, 1 month after infusions all BAN rabbits responded to I*HGG, and 10 months after infusion the single BAX rabbit responed to I*BGG.

In several instances when rapid elimination rates indicated immune responses quantitative antibody determinations were made on sera taken 3 days after elimination of antigen, when antibody concentration is usually maximum. The antibody concentrations varied depending upon whether the immune response was primary or secondary but they were either within the range of, or slightly lower than, what might have been expected from previously untreated normal animals.

Capillary ring tests using sera taken during the period of infusions showed antibody responses on the part of all rabbits to the minor constituents of the infused preparations. During the infusions, sera from the HPA animals were markedly positive when tested with a five per cent solution of human plasma fractions I, II, III, and IV and weakly positive with fraction V. Since considerable amounts of human albumin and human gamma globulin and probably other major plasma protein constituents were present continually in the sera during this period (8) it is apparent that the antibody detected by these tests was to the many different antigens present in small concentrations in the human plasma. Similar tests revealed antibodies to human plasma fractions I to IV but not V in the sera of the HPX and HPN rabbits during the infusion period. The sera of the BAA and BAN rabbits taken during the infusion period were positive when tested with BGG. BGG was known to make up less than 0.01 per cent of the crystalline BSA preparation used for the infusions.

Passively administered rabbit anti-HA in the unresponsive HPN rabbits and rabbit anti-BA in the unresponsive BAN rabbits was diluted in the plasma protein pool as would be expected in the absence of an antibody-antigen reaction and disappeared from the sera with half-lives comparable to the normal half-life of homologous gamma globulin (12 *a*) as shown in Table III. This is in marked contrast to the rapid disappearance of passively administered rabbit antipneumococcal polysaccharide antibody in mice immunologically paralyzed with large amounts of the corresponding pneumococcal polysaccharide (3), or to the rapid disappearance of passively administered homologous antiprotein antibodies in rabbits known to contain the specific protein antigen (13).

The four rabbits of the litter resulting from the mating of a permanently unresponsive BAN female and temporarily unresponsive BAA male all made a

primary type immune antigen elimination when challenged with I*BA 15 mg. per kilo at the age of 1 month. At 4 months of age in response to a similar injection all showed anamnestic type responses with antibody levels of 25.5 to 60.5 μg/antibody N/ml. serum.

Histologic study of the rabbits of the six regular infused groups which died during the experiment plus the additional 16 adult male rabbits given BA infusions and 25 adult male rabbits given HP infusions for the purpose of histologic study revealed several pertinent facts:—

1. Adult normal rabbits receiving HP infusions developed severe serum sickness (arteritis, myocarditis, and endocarditis) within 1 to 2 weeks after beginning of infusions. The active development of inflammatory lesions continued until at least 4 to 5 weeks after beginning of infusion. These lesions compared favorably in magnitude and frequency with those reported following a single large injection of foreign serum protein (14). Since there was no demonstrable antibody response to HA and HGG in these rabbits, it is likely that the serum sickness was related to an immune response to other constituents of the human plasma.

2. In addition to serum sickness, the adult normal rabbits receiving HP infusions developed a severe anemia (one to three million RBC) which became maximum within a month after initiation of infusion, persisted throughout the remainder of the infusion period, and then gradually disappeared during the first postinfusion month. This anemia may have been related to the hemagglutinating effect of HP on rabbit erythrocytes. There were considerable enlargement of the spleens and initial hyperplasia of the bone marrow followed by hypoplasia in these rabbits. There were no consistent changes in the number of circulating leukocytes associated with the HP infusions. It is of interest that an anemia of such severity did not develop in the HPX and HPN rabbits. In these animals red blood cell counts dropped to about three to four million in the animals receiving 400 r whole body x-ray and to four to five million in the HPN group. In the HPN group a definite leukocytosis with increases in both mononuclear and granulocytic cells occurred during the infusion period. The anemia and serum sickness probably accounted for the deaths of three of five of the HPA rabbits late in the course of the infusions.

3. In the animals receiving bovine albumin the histologic picture is much less complicated by manifestations of antibody responses to trace constituents in the infusions. The only morphologic manifestation of an antibody response found in the BA rabbits was the presence of granulomatous lesions in spleen and lymph nodes which are commonly associated with antibody production (14 *b*). This change was most likely associated with the antibody response made by these animals to the BGG contaminant in the BA. It is particularly interesting that rabbits receiving daily infusions of BA developed none of the necrotic or inflammatory lesions of serum sickness. It has been well demonstrated

that a single injection of BA comparable in size to one of our daily infusions will produce within about 2 weeks typical serum sickness in from 60 to 89 per cent of rabbits injected (14). The absence of serum sickness in our rabbits which were sacrificed at the end of a 2 week course of daily BA injections, at the end of a 3 week course, and 2 weeks after the termination of a 3 week course lends further support to the thesis that these animals were making little or no antibody to the BA. The BA infusions did not cause an anemia and produced no mortality.

4. In none of the HP or BA rabbits were there any morphologic alterations which could be correlated with the immunologic unresponsiveness resulting from the infusions.

DISCUSSION

On the basis of the rates of disappearance of infused proteins after the infusions were discontinued, it appears that the rabbits of all groups were unable, at least temporarily, to make detectable antibody responses to the major constituents of the infusions. In the three HP groups the rates of disappearance of HA and HGG and for the BAA and BAN groups the rate of disappearance of BA as seen in Table I are about the same as the normal rate of catabolism of autologous protein even when the amounts of the foreign proteins were very small. It is true that during and shortly after the infusions while large amounts of foreign protein were present in the rabbits, significant amounts of antibody production might not have been evident. However, a month or more after the infusions when the serum concentrations of foreign proteins were small, relatively little antibody synthesis would have resulted in rapid foreign protein elimination. For example, when the foreign protein concentration dropped to 0.1 mg./cc. serum or less, as little as 10 mg. antibody N synthesized by the rabbit (equivalent of approximately 50 μg. antibody N per cc. serum) would have neutralized and caused elimination of the remaining foreign protein (5). It is apparent that antibody to HA and HGG, if any, did not approach this level in the first few postinjection months because the infused HA and HGG were detectable in the serum for at least 2 to 3 months. It might be questioned whether the HPA and BAA rabbits which later showed antibody responses to the infused proteins were truly unresponsive for the first few postinfusion months. The fact that they eliminated the foreign proteins during this period at virtually the same rates as did the permanently unresponsive HPX, HPN, BAX, and BAN rabbits would indicate that they were unresponsive during this time.

The phenomenon of unresponsiveness was both specific and dependent upon doses of antigens as is evident from the fact that all the rabbits while becoming either temporarily or permanently unresponsive to the antigens present in large amounts in the infusions, at the same time made antibody responses to

the lesser constituents. Thus, the BAA and BAN rabbits were making detectable amounts of anti-BGG while they were unresponsive to BA; and the HPA, HPN, and HPX rabbits were making antibodies to some of the constituents of human plasma fractions I, II, III, and IV while unresponsive to HA and HGG. The ability to respond to entirely new antigens while still unresponsive to those infused was also demonstrated by these rabbits. Thus, whatever changes take place during the infusions and whatever immunologic stigmata remain in the permanently unresponsive rabbits, they are specific with regard to the antigen involved. Even antigens closely related to and partially cross-reacting with (15) those to which the animal cannot respond will call forth an antibody response, albeit somewhat less than normal in some cases. This was also observed in the case of pneumococcal antigens (1).

The dose of the antigen is also critical in the production of unresponsiveness as would be expected. Our infusions no doubt greatly exceeded the minimum dose for producing unresponsiveness. Temporary unresponsiveness in adult rabbits has been observed following doses of these same antigens much smaller than those employed here (16). In a recent report (6) as little as 1 mg. of alum-precipitated BA injected three times a week induced unresponsiveness in rabbits injected from the time of birth. However, in the present experiments daily injections of antigens amounting to a fraction of 1 per cent of the total infusion did not produce unresponsiveness as is evident from the response of all rabbits to either the BGG contaminant in the BA infusions or to the minor constituents in the HP infusions. Even in "newborn" or x-rayed rabbits the trace constituents in the infusions immunized and did not produce unresponsiveness. Since our BAN rabbits which received somewhat less than 0.1 mg. BGG per week shortly after birth and about 0.6 mg. BGG per week at the cessation of infusions made antibody responses to BGG, while rabbits receiving 3 mg. BA per week from the time of birth failed to respond to BA (6) the minimum suppressing dose for "newborn" rabbits is probably somewhere between these two values.

The duration of the unresponsive state appears to be at least in part related to the characteristics of the rabbit treated. The fact that a lasting unresponsiveness could not be induced in 6 of 7 normal adult rabbits would suggest that whatever the effect of the overdose of the antigen, it was transient and may have been dependent upon the continued presence of antigen in the tissues. The foreign protein persisting in these HPA and BAA rabbits after the infusions was not eliminated at an accelerated rate indicating that the unresponsiveness existed during the period the antigen was being followed in the sera. The majority of these rabbits regained their ability to respond 4 to 6 months after the infusions at a time when virtually all of the foreign protein should have been catabolized by a non-immune host. (Assuming the usual half-lives of foreign serum proteins in non-immune rabbits only a small fraction of a μg. of

the infused proteins would be expected in the entire rabbit 4 to 6 months after the infusions.)

In all the rabbits whose injections started shortly after birth, or after whole body x-radiation, the immunologic unresponsiveness persisted throughout the duration of the experiment (7 to 10 months). In spite of limited numbers, only 10 of these animals were observed for 3 to 4 months or 7 for 7 to 9 months, the persistence of unresponsiveness in all of them in contrast to the recovery of 6 of 7 normal adult rabbits in the same time interval would appear to be significant. Whether the immunological situations in "newborn" and x-radiated animals are in any way comparable cannot be determined from this study. From observations on the effects of radiation on the immune response it appeared that radiation interfered with the initial step or steps in the antibody response; *i.e.*, the recognition or initial fixation of antigen (7). In the case of the "newborn" animals in this study a somewhat similar set of circumstances may exist. The large infusions of foreign protein are begun before the rabbit acquires the ability to make an antibody response (rabbits exposed to serum protein antigens *in utero* or during the first days of life are incapable of making an antibody response (17)). Therefore, whenever the immunologic mechanisms become operative the foreign material is present in considerable quantity and it may be difficult for the rabbit to treat this material as an antigen. In such a situation it is even possible that the foreign protein might be accepted as non-antigenic along with the host's own constituents. A comparable situation exists in the case of the blood groups of fraternal twin cattle (18) and human beings (19) which have a common placental circulation. In the twin cattle there can be found a mixture of two distinct types of red blood cells which persist indefinitely in the circulation. Yet, all the offspring of such a twin parent will inherit only one of the red blood cell types indicating that one of the red blood cell types found in the parent does not belong to its genotype but in a true sense is foreign. This phenomenon is probably caused by the exchange of primitive red blood cells between the twin cattle *in utero via* the common placental circulation. The foreign, primitive cells become established in the new host and continue indefinitely to produce red blood cells of a type different from that produced by the host's own cells. In this situation the foreign cells apparently are not so recognized and there is no demonstrable antibody response on the part of the host to the foreign red blood cells which are present continuously in the host's circulation from the time of intrauterine existence. In the case of the foreign protein injections in "newborn" rabbits, on the other hand, the unresponsiveness may well persist after the disappearance of the foreign protein.

As far as demonstrating whether or not the unresponsiveness persisted after the disappearance of foreign protein, no direct measurements for the last traces of foreign protein in the tissues are feasible. On the basis of the rates of

catabolism of the foreign proteins observed during the postinjection period less than 1 μg. of foreign protein would be expected in each rabbit 3 to 4 months after the injections and virtually none 9 to 10 months after injections. The observations made with passively administered anti-BA in the BAN rabbits and anti-HA in the HAN rabbits indicated the absence of significant amounts of the infused proteins at a time when the rabbits were still unresponsive. An infused protein which might have been held intact by the tissues would have probably combined with and caused rapid elimination of the antibody as did occur in mice immunologically fatigued with pneumococcal polysaccharides (3). This would further support the possibility that the unresponsiveness in the "newborn" and x-rayed animals may well outlast the persistence of the foreign protein in their tissues.

Whatever the changes associated with the development of immunologic unresponsiveness, they were not transmitted to offspring. The litter of four rabbits resulting from the mating of a permanently unresponsive BAN female with a temporarily unresponsive BAA male responded to BA at 1 and 3 months of age in a normal fashion.

The fact that rabbits receiving a 3 week course of daily injections of BA did not develop the lesions of serum sickness during or after the injections was further evidence of a lack of antibody response in these rabbits. Had any near normal antibody response occurred in these rabbits some of the inflammatory lesions of serum sickness would have been expected. The histologic alterations found in the animals receiving human plasma were consistent with the antibody responses made by these rabbits to the lesser constituents of the plasma. It is of interest that extensive antibody production, sufficient to produce marked serum sickness, was carried out in rabbits simultaneously unresponsive to at least two of the major components of the human plasma. The anemia appearing in the HPA rabbits might be expected on the basis of the hemagglutinating effect of human plasma on rabbit erythrocytes. Why a more marked anemia did not develop in the HPX and HPN rabbits, however, is unexplained.

SUMMARY

1. Repeated large infusions of heterologous plasma proteins can induce a state of specific immunologic unresponsiveness in rabbits. In normal adult rabbits this unresponsiveness in most instances lasts only about as long as the heterologous proteins are detectable in the host (3 to 4 months). In rabbits infused from the time of birth and perhaps x-radiated adult rabbits the induced immunologic unresponsiveness lasted throughout the period of observation (10 to 11 months), long after disappearance of all detectable foreign proteins.

2. This unresponsiveness appears to be specific for the antigens administered in excess and does not prevent antibody responses to even closely related antigens.

3. The unresponsiveness was not transmitted to first generation offspring.

4. The mechanism of the temporary unresponsiveness which occurs in normal adult rabbits may be dependent upon the actual presence of the antigen in the host. However, the unresponsiveness does not result from a simple neutralization of antibody, as it is formed, by the antigen, as has been suggested in the case of pneumococcal polysaccharide induced immunologic paralysis.

5. On the other hand, the mechanism of the lasting immunologic unresponsiveness developing in the "newborn" and perhaps the x-rayed rabbits may depend upon the acceptance of the foreign protein as essentially non-antigenic by the host. A similar situation is seen in the naturally occurring placental transfer of dissimilar red blood cell types between fraternal twins.

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