ROLE OF THE THYMUS IN IMMUNE REACTIONS IN RATS

I. THE IMMUNOLOGIC RESPONSE TO BOVINE SERUM ALBUMIN (ANTIBODY FORMATION, ARTHUS REACTIVITY, AND DELAYED HYPERSENSITIVITY) IN RATS THYMECTOMIZED OR SPLENECTOMIZED AT VARIOUS TIMES AFTER BIRTH*

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Studies on the immunologic function of lymphatic organs have concerned themselves successively with the role of the spleen, lymph nodes, marrow, gastrointestinal lymphatic tissue, thymus, and bursa of Fabricius (an analogue of the thymus found in birds). The formation of antibody in spleen, lymph nodes, marrow, and gastrointestinal lymphatic tissue (*e.g.* appendix) has been demonstrated either directly, by the use of the immunofluorescent technique, indirectly, by the use of cells derived from these organs to transfer antibody-forming capacity passively to tissue cultures or to unimmunized recipients, or by extirpation experiments (reviewed in references 1 and 2). Similarly the role of these organs, lymph nodes in particular, as a source of the "sensitized cells" responsible for delayed sensitivity has been elucidated by extirpation or by passive transfer (reviewed in references 3 and 4). With regard to the thymus and the bursa of Fabricius, the available evidence (reviewed in reference 5) suggests that they are not *directly* concerned in the manufacture of antibody or sensitized cells but may play some indirect role. Few extirpation experiments have thus far been reported.

The mammalian thymus is derived from the entoderm of the third pharyngeal pouch. It contains, at first, reticular cells of epithelial origin and, later, cells resembling small lymphocytes which have been shown to be derived from these epithelial precursors (5*a*). Thymectomy

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ot rabbits aged 3 weeks or more does not alter their capacity to make antibody against protein antigens (6-8). Fichtelius *et al.* (9) have presented data to show that thymectomy of young adult guinea pigs may lead to some impairment of ability to give a primary antibody response but that it does not affect the secondary response. Archer and Pierce have shown that rabbits thymectomized at birth may have, thereafter, a diminished capacity to make antibody (10). No experiments on delayed sensitization to protein antigens in thymectomized animals have been reported. However, several recent publications (11-13) have indicated that homograft rejection is markedly delayed in animals thymectomized at or shortly after birth. Thymectomy in adult life has not been shown to affect this process.

The bursa of Fabricius in birds resembles the mammalian thymus closely, both anatomically (14) and in histochemical properties (15). It also produces small lymphocytes derived from epithelial precursors (14). Birds have a thymus in addition to the bursa. Surgical extirpation of the bursa during the 1st week of life results in permanently impaired capacity to make antibody (16). Bursectomy 5 weeks after hatching results in only slight decrease of antibody-forming capacity and bursectomy after 5 weeks does not affect it. Recently Wolfe and his coworkers have found that bursectomy does not influence homograft rejection (17). Thymectomy of newly hatched chickens, on the other hand, may slow homograft rejection (17). Testosterone and certain modified testosterone, such as 19-nor-testosterone, when applied to 5 day eggs suppress the development of the bursa of Fabricius. Chemical bursectomy with such agents markedly inhibits antibody-forming capacity (16, 18) and is said to reduce the ability to reject homografts (19). Unfortunately this drastic treatment causes a reduction in spleen and thymus size, in addition to suppression of the bursa of Fabricius, and yields underweight birds suffering from chronic diarrhea, which seldom reach adulthood (16).

The experiments to be reported in the present paper show that both antibody formation (and Arthus reactivity) and delayed sensitization are impaired in thymectomized rats immunized with bovine serum albumin in a standard manner.

Materials and Methods

Surgical Technique.—1 to 5 day old Sprague-Dawley rats¹ were immobilized in filter paper and placed in a glass container immersed in ice water. In 15 to 20 minutes, deep hypothermic anesthesia was attained: spontaneous respirations ceased and the heart rate slowed to about 10 beats per minute. At this juncture, animals were taken from their container and placed supine on a small animal board. With the use of clean, but not sterile, technique the chest was opened by a sternal splitting incision. Retraction of the overlying muscle exposed the thymus filling the anterior mediastinum. The thymus was freed from its attachments to the mediastinal pleura, pericardium, and underlying great vessels by blunt dissection and delivered, generally intact but occasionally piecemeal. After washing the mediastinum with sterile Hanks' solution the manubrium was approximated with one or two 6-0 silk sutures, and the skin incision closed with three or four interrupted sutures. Bleeding was generally minimal. With practice, we were able to reduce operative mortality to 10 to 20 per cent. Sham-operated litter mates were handled in an identical fashion except that the thymus was left *in situ*. Initially, non-operated litter mates were subjected to the anesthetic procedure but this was found to be an unnecessary precaution.

In some experiments, rats were thymectomized at 2 to 3 weeks of age. The procedure was identical to that indicated above. Thymectomy of older rats, for technical reasons, has not been possible. Thymectomy of adult guinea pigs is, by contrast, a simple matter because the

¹ Obtained from the Charles River Breeding Laboratory, Brookline, Massachusetts.

thymus in this species is wholly cervical in location. Young adult, Hartley strain guinea pigs were operated upon under ether anesthesia. A midline incision was made from the angle of the mandible to the sternal notch, to reveal the thymus occupying its superficial position in the lower neck. The organ was dissected free and removed intact, and the wound closed with Michel clips. No attempt was made to locate and remove the accessory thymuses commonly found in the guinea pig (20). Sham-operated animals were handled in an identical fashion, except that the thymus was left *in situ*. There was no mortality.

Splenectomy was also carried out on newborn Sprague-Dawley rats under hypothermic anesthesia. The abdomen was entered by a transverse left flank incision and the spleen, after being freed from the stomach, was removed intact. Bleeding was minimal. The wound was closed with interrupted silk sutures.

Postoperative Care.—Animals were kept at room temperature until respirations and activity had returned to normal. They were then returned to their mothers. Rats were weaned and the sexes separated at 21 to 25 days. They were handled in our routine fashion, no special precautions with regard to infection being taken. They were fed Purina laboratory chow and water *ad libitum*. All animals, both thymectomized and controls, were weighed from time to time on a standard small animal scale.

Sensitization and Testing of Rats with Bovine Serum Albumin.—8 week old thymectomized rats and litter mate controls were sensitized to bovine serum albumin (BSA) by a single injection of 0.1 ml of antigen in complete adjuvant into the left hind foot-pad. The adjuvant mixture was made up of 10.0 volumes of saline containing BSA,² 1.5 volumes of arlacel A,³ 8.5 volumes of bayol F,⁴ and killed tubercle bacilli⁵ at a final concentration of 3 mg/ml, emulsified to give a relatively stable water-in-oil emulsion. The 0.1 ml dose contained 0.5 mg BSA. Only thymectomized rats which had shown a consistently good weight gain were sensitized. All animals were skin-tested at approximately 10 and again at 20 days with 30 μ g crystalline BSA in 0.1 ml saline, injected intradermally in the shaved flank. Arthus reactions were read at 4 hours. The diameter and degree of gross edema and hemorrhage were recorded; and, in some instances, the lesions were biopsied. Delayed reactions (21) were read at 24, 48, and 72 hours, and the diameter and degree of induration (graded from 0 to ++++) recorded.

Sensitization and Testing of Adult Guinea Pigs with Egg Albumin.—2 weeks following thymectomy or sham operation, adult guinea pigs were sensitized (22) to egg albumin (Ea) by injecting 0.1 ml of specific precipitate in complete adjuvant intradermally over the sternum. The specific precipitate was prepared from crystalline Ea,⁶ precipitated at two times equivalence with rabbit anti-Ea and washed three times. The 0.1 ml dose contained 3.0 μ g of antigen protein. All animals, 9 days after sensitization, were depilated with nair, a commercial depilatory, and skin-tested by intradermal injection of 3.0 and 0.3 μ g doses of Ea in 0.1 ml saline. Delayed reactions were read at 24 hours, and the diameter of erythema and degree of induration recorded. There was no hemorrhage or necrosis. No reaction of the Arthus type preceded the delayed reaction.

Bleedings.—Blood for antibody determinations and electrophoresis was obtained by cardiac puncture before sensitization and 3 and 4 weeks after. After clotting and centrifugation, the serum obtained was stored at -20° for subsequent study.

Antibody Determinations.—For *hemagglutination*, all rat sera were heated at 56°C for 25 minutes and absorbed overnight at 4°C with thoroughly washed sheep red blood cells. Sheep

² Crystalline bovine serum albumin, Pentex Inc., Kankakee, Illinois.

³ Mannide monooleate, obtained from Atlas Powder Co., Wilmington, Delaware.

⁴ A light mineral oil, obtained from Esso Standard Oil Co., Linden, New Jersey.

⁵ Kindly provided by Dr. I. S. Danielson, Lederle Laboratories, Pearl River, New York.

⁶ Crystalline egg albumin, Armour.

erythrocytes were formalinized according to the method of Csizmas (23) and tanned by the method of Stavitsky (24). Twice washed cells were sensitized for 1 hour at room temperature with BSA (0.5 mg BSA per ml of $\frac{1}{40}$ tanned cell suspension) before dilution to make up a 1 per cent suspension. Titration was performed by a modified Takátsy microtitration technique. The plastic plates were kept overnight at 4°C prior to final reading. Preliminary testing of the system with known rat anti-BSA sera assured reproducible titers. Unknown sera were tested in duplicate to ensure accurate end-point determinations.

For determination of *heteroagglutinins*, human erythrocytes of blood group O, as well as rabbit, sheep, mouse, and guinea pig blood samples were collected in Alsever's solution, the red cells washed twice, and 1 per cent suspensions prepared. Agglutination was carried out in 7×45 mm tubes by mixing each serum, in serial doubling dilutions, with equal volumes of washed erythrocytes of each type and allowing the tubes to stand for 3 hours at room temperature. Readings were performed microscopically by gentle transfer of each tube's content to a microscope slide and examination under low power. Agglutination was scored as 0 to + + + +, + being taken as the end-point of antibody activity.

For *gel diffusion*, the basin technique of Ouchterlony was used (25). Wells were cut in a 2 to 3 mm layer of "purified" agar (Difco) 1 per cent on a glass plate. BSA (50 mg protein/ml) was placed in the center well. Plates were kept at room temperature in a moist chamber and read, and in some instances photographed, at 24 hours.

Electrophoresis.—Paper electrophoresis was carried out on 2.5×12 cm cellulose acetate strips in veronal buffer, pH 8.6 and 0.1 ionic strength. Strips were run for 2 hours at 175 volts and 10 ma, then dried and stained with Ponceau S red. The distribution of protein fractions was measured on a Beckman-Spinco densitometer, model R.B., and the percentage represented by each major protein fraction calculated by standard methods.

Sera from two rats with high anti-BSA antibody titers were pooled. Anti-BSA was precipitated from a 1.0 ml aliquot of pooled serum by successive daily additions of BSA, followed each time by standing overnight at 4°C and centrifugation, continued until equivalence had been closely approached (three additions of BSA, total 100 μ g). The control serum, after dilution with a volume of saline equal to that added to the antibody-depleted serum, was compared with the latter by moving boundary electrophoresis in veronal buffer pH 8.6 at an ionic strength of 0.1.

Injection of Tissue Homogenates.—In one experiment with rats thymectomized at birth, thymuses and spleens from normal 75 to 150 gm Sprague-Dawley rats were homogenized in a Potter-Elvehjem homogenizer and the homogenates suspended in saline. 1.5 ml of a freshly prepared 10 per cent suspension (150 mg thymic or splenic tissue) was administered intraperitoneally to recipient thymectomized or control rats 2 days before sensitization to BSA and again 1, 4, and 7 days after sensitization.

Banal Inflammation and Spread of Dye through the Tissues.—Four thymectomized and five sham-operated rats received an intradermal injection in one flank of 0.05 ml turpentine (1:20 in olive oil). The resultant inflammatory response (induration, erythema, and central necrosis) was read at 20 minutes, 2, 6, and 24 hours. The same animals also received 0.1 ml 0.5 per cent Evans blue injected intradermally in the opposite flank, and the spread of dye (diameter of the blue spot) was measured at 20 minutes, 2, 6, and 24 hours.

Postmortem.—All rats were autopsied at the end of experimentation. Lymph nodes and spleen were taken in every animal for histologic examination. The mediastinum was inspected for residual thymus, and all tissue which could conceivably be thymic was removed and examined microscopically. The great majority of thymectomized animals had no residual thymus. However, thymectomy was considered satisfactory if less than 100 mg residual thymus was found.

RESULTS

Growth and Development.—Save for the exceptions noted below, the health of thymectomized rats was excellent. Text-fig. 1 illustrates weight-gain curves of representative thymectomized, sham-operated, and non-operated rats of both sexes. Thymectomized and sham-operated animals gained weight steadily and equally, although, in some litters, they tended to be slightly lighter than non-operated controls. Only animals which followed this course were used for subsequent experimentation.

"Wasting Disease."—In about 25 per cent of litters, thymectomized rats after an initial period of normal growth began to fail. There was a failure to gain weight for several days, followed by a gradual, but accelerating weight loss leading to cachexia and death in from 10 to 20 days. The onset of this illness occurred as early as 20 days in some litters, as late as 60 days in others (Text-fig. 2). Rats afflicted with wasting disease became lethargic, pallid, and flabby to the touch and their hair became coarse and matted. In some litters this illness eventually killed every thymectomized member, even while sham-operated and non-operated litter mates continued to gain weight in the usual fashion (center group in Text-fig. 2). However, in some afflicted litters, certain rats proven at postmortem to have been without thymus, escaped this illness. Wasting disease was not observed in rats thymectomized at 2 to 3 weeks of age.

Arthus Reactions to BSA and Antibody Determinations.—Rats, sensitized by foot-pad injection of BSA in adjuvant and skin-tested with 30 μ g BSA, were found to develop Arthus reactions closely comparable to the Arthus response in other species. In the gross, there were pale, edematous lesions, often with a small central area of hemorrhage, maximal at 3 to 4 hours. Microscopically, there was massive subcutaneous edema with extensive infiltration of polymorphonuclear leukocytes and some hemorrhage in the deep dermis, muscle, and subcutaneous connective tissue (Fig. 1).

Arthus reactions were sharply reduced in animals thymectomized shortly after birth and sensitized at 8 weeks (Table I). At 9 to 12 days, none of nineteen thymectomized rats had positive Arthus reactions while controls gave responses of maximal size. By 19 to 21 days, the thymectomized animals could be divided into two groups: one, in which Arthus reactivity had still not appeared, and a second, in which Arthus reactivity approached that of control animals. Hemagglutinating antibody titers were low or insignificant at 21 days in the first group, and were unchanged a week later when the experiment was terminated. No precipitating antibody could be found by gel diffusion in the sera of these animals. In the Arthus-positive group, hemagglutinating antibody titers were comparable to those of control animals and precipitating antibody was present by 3 weeks (Text-fig. 3). Thymus or spleen homogenates were given

TEXT-FIG. 1. Growth curves of rats from representative litters showing failure of thymectomy to affect weight gain.

TEXT-FIG. 2. Growth curves of rats from individual litters in which wasting disease (cachexia) developed at different times (left and right). Not all the thymectomized rats were affected. Curves in center are from two litters of identical age, pooled at the time of weaning: here all thymectomized rats of both litters were affected.

TABLE I
Relation of Arthus Reaction to Humoral Antibody in Thymectomized and Control Animals

Category*	No. of rats	Average diameter of Arthus reaction		No. of sera with hemagglutination titer at 3-4 weeks†								No. with positive precipitin
		9-12 days	19-21 days	≤4	8	16	32	64	128	≥256		
		mm	mm									
Thymectomized:												
Arthus-negative	8	1.2	1.1	5	0	4	0	0	0	0	0/8	
Arthus-positive§.	11	2.6	11	0	0	0	3	1	6	4	9/9	
Sham-operated	18	13	13	0	0	0	4	3	9	3	10/10	
Non-operated	18	12	13	0	0	2	6	2	4	4	—	

* About half the rats in each category were injected with thymus or spleen extract during sensitization.

† Baseline titer was 0 in all of thirty-two preimmunization sera tested.

§ Reactions ≥8 mm.

before and during the sensitization period to about half the animals, both thymectomized and controls. This procedure had no apparent effect on antibody formation and Arthus reactivity; the data therefore have been combined in the table with data obtained in rats not so treated. "Natural" heteroagglutinins were not reduced in thymectomized rats (Table II).

TEXT-FIG. 3. Precipitation in gel of antibody to BSA; six different control sera (top) and six sera of thymectomized rats (bottom) after 24 hour diffusion against BSA (50 mg/ml) in center wells. Only the control sera give precipitation, each showing a single line of precipitate.

TABLE II
Natural Heteroagglutinins in Unsensitized Thymectomized and Control Rats

Type of erythrocyte	Average agglutinin titer	
	Thymectomized (8 sera)	Sham-operated (10 sera)
Human, type O.....	0.8	0.8
Rabbit.....	2.2	1.4
Mouse.....	0.5	0.6
Guinea pig.....	1.0	0.8
Sheep.....	0.2	0.8

Delayed Reactions to BSA.—Rats sensitized with BSA plus adjuvant develop typical delayed reactions with deep induration and a variable degree of central necrosis, maximal at 24 hours, when skin-tested with BSA. However, rats thymectomized during the 1st week of life showed an appreciable diminution of the delayed response when sensitized at 8 weeks of age. Inspection of Table III shows that three out of twenty-one thymectomized rats had 24 hour delayed reactions greater than 10 mm in diameter, when tested 9 to 12 days after sensitization; sixteen out of eighteen controls had delayed reactions greater than 10 mm at this time. The degree of induration (not shown in the table)

was also much reduced in thymectomized rats. Sham-operated and non-operated litter mates showed reactions, identical in diameter and in degree of induration to those of randomly selected non-litter mate controls of like age. By 19 to 21 days 9 out of 21 thymectomized animals had developed significant delayed sensitivity to BSA, but even at this late date the remaining rats failed to do so. This would seem to establish that, at least in some animals delayed reactive capacity, while compromised, is not totally abolished. It is of interest to note that sensitivity of control animals had passed its acme and begun to wane by 21 days, even while that of thymectomized animals was increasing. Treatment with homogenates of thymus or spleen, injected before and

TABLE III
Effect of Thymectomy on Delayed Skin Reaction to BSA

Category	24 hr. reactions to 30 μ g BSA	
	9-12 days	19-21 days
	mm	mm
Thymectomized		
Untreated.....	0, 0, 0, 0, 0, 0, 2, 2, 13, 16	0, 0, 0, 0, 0, 4, 10, 12, 12, 13
Thymus extract.....	0, 0, 2, 3, 8	0, 4, 7, 10, 12
Spleen extract.....	0, 0, 7, 8, 9, 11	0, 5, 7, 10, 11, 13
Sham-operated.....	11, 11, 13, 15, 16, 17	8, 9, 9, 9, 11
Non-operated.....	0, 12, 15, 16, 16	7, 7, 11, 12, 14
Non-litter mate control.....	4, 12, 14, 15, 15, 18, 18	6, 11, 11, 12, 12, 12, 14

during the sensitization period, did not enhance to any appreciable extent the impaired delayed reactive capacity of thymectomized animals.

Effect of Thymectomy at 2 to 3 Weeks.—The Arthus and delayed reactions of rats thymectomized at 2 to 3 weeks of age are shown in Table IV. By 9 to 10 days eight out of twelve of the thymectomized rats had positive Arthus reactions of intensity comparable to those in control rats. The remaining animals remained Arthus-negative through the 16th day. All thymectomized rats developed some degree of delayed reactivity. It was considerably reduced in comparison with the reactivity of control rats at 9 to 10 days, but less strikingly so at 16 days.

Effect of Splenectomy.—Splenectomy shortly after birth in a group of six rats failed to affect the later development of either Arthus or delayed reactivity to BSA as compared with fourteen sham-operated controls.

Delayed Reactions to Ea in Guinea Pigs Thymectomized as Adults.—There was no inhibition of delayed sensitization in adult guinea pigs sensitized with Ea-anti-Ea specific precipitate 2 weeks after thymectomy (Table V).

Electrophoresis and Characterization of Anti-BSA Antibody in Rats.—In

the sera studied thus far, hemagglutinating and precipitating antibody activities were closely parallel. In all sera examined, the antibody gave a single line in Ouchterlony plates (Text-fig. 3). An attempt to identify the precipitating

TABLE IV
Effect of Thymectomy at 2-3 Weeks of Age on Arthus and Delayed Reactions to BSA*

Category	No. of rats	Average diameter of Arthus reaction		Average diameter† of delayed reaction	
		9-10 days	16 days	9-10 days	16 days
Thymectomized					
Arthus-negative	4	3	3	6	9
Arthus-positive§	8	10	12	11	11
Sham-operated	8	10	14	15	12
Non-operated	15	13	14	17	12

* Nine rats thymectomized at 2 weeks and five at 3 weeks.

† Diameter of induration at 24 hours.

§ Reactions ≥ 8 mm in diameter.

TABLE V
*Failure of Thymectomy in Adult Guinea Pigs to Affect Delayed Sensitization with Specific Precipitates of Ea**

Thymectomized			Sham-operated		
Guinea pig	Reaction‡ to tests with		Guinea pig	Reaction‡ to tests with	
	3.0 μ g	0.3 μ g		3.0 μ g	0.3 μ g
3	20	18	1	19	15
4	18	14	2	17	13
5	22	15	9	22	17
6	25	17	10	19	16
7	17	14			
8	20	17			
Average	21.9	15.6		19.3	15.0

* Adult guinea pigs, thymectomized and sensitized 2 weeks later by injection of Ea-anti Ea specific precipitate in Freund's adjuvant.

‡ 24 hour reactions to skin tests with 3.0 and 0.3 μ g Ea.

antibody by moving boundary electrophoresis of serum, from which antibody had been removed by serial absorption with BSA, was unsuccessful (Text-fig. 4). Average values for serum albumin and for α -, β -, and γ -globulins determined by paper electrophoresis are shown in Table VI. No significant difference could be found among thymectomized, partially thymectomized, and sham-operated rats with regard to any of the major plasma protein components measured.

TEXT-FIG. 4. Ascending electrophoretic pattern of pooled antiserum from two control rats with high titer of anti-BSA, before (left) and after removal of antibody by precipitation with 50 μ g of BSA. The percentages calculated from these patterns were:

	Albumin	α	β	γ
Before.....	51	27	14	8
After.....	47	31	15	7

TABLE VI
Serum Protein Values in Rats Thymectomized at Birth*

Category	No. of rats	Per cent of total protein			
		Albumin	α	β	γ
Thymectomized†.....	14	48.3	27.1	15.3	9.3
Thymectomized, but residual thymus or reacted.....	10	50.6	25.4	14.2	9.2
Sham-operated.....	15	47.1	28.1	12.7	12.1
Non-operated.....	10	47.6	26.8	14.1	11.5

* All sera taken before administration of antigenic stimulus.

† Less than 100 mg residual thymus.

Chemically Induced Inflammation.—Turpentine in olive oil, injected intradermally in both thymectomized and control rats, produced erythema and induration, which reached 11 to 14 mm diameter by 24 hours, and minimal necrosis. Evans blue, injected intradermally, gave a blue spot which increased in diameter to reach 20 to 25 mm at 24 hours. With neither of these indices did the thymectomized animals show a difference from the controls. As will be shown in our next paper, the local passive transfer of tuberculin sensitivity was accepted equally well by experimental and control rats.

DISCUSSION

The normal weight gain and general good health of most of our thymectomized rats are in accordance with earlier observations (26, 27). The wasting disease which afflicted these animals in certain cages appears comparable to the "thymic cachexia" which has plagued investigators of thymic function over the years (28, 29, and many others). In our experiments, most litters escaped this illness; but in a few, every thymectomized member eventually succumbed, individual animals being affected *seriatim*. The onset varied from as early as 20 days to as late as 60 days. These observations suggest that thymic cachexia may be a chronic infectious process of undetermined type. Thymectomized rats which escaped cachectic disease in infected cages may have had ectopic thymic tissue or residual immunologic competence on some other basis.

The rat has been widely regarded as unsuitable for the study of conventional immunological reactions. The studies of Freund and Stone have implied that special techniques were required to induce Arthus reactions in this species (30), and delayed reactivity of the tuberculin type has been generally supposed not to occur (see review in reference 31). We have recently shown that intense delayed sensitivity to protein antigens is readily induced by the use of suitable adjuvant mixtures and the foot-pad route of inoculation (21). The present study shows that the same technique is highly effective in inducing antibody formation and Arthus reactivity. The Arthus reactions in the rat were closely similar, in time course and in gross and histological character, to Arthus reactions described in other species and, as in other species (32), were exactly correlated with the presence of precipitating antibody.

It is clear, from the data we have presented, that rats, thymectomized during the 1st week of life, have a markedly impaired capacity to develop Arthus sensitivity to BSA and have a low titer of precipitating and hemagglutinating antibody against this antigen. Those rats which gave an immune response still had lymphocytes in their lymphoid tissues (33); *i.e.*, were not effectively depleted. Yet even in these animals there was a marked delay in the appearance of sensitization. Our data thus agree with the finding of Archer and Pierce (10)

that rabbits, thymectomized at birth and presumably healthy thereafter, show an impaired ability to make antibody against BSA.

It would be premature, however, from studies on a single antigen-antibody system, to infer that all types of antibody formation are suppressed in thymectomized animals. "Natural" heteroagglutinin titers, though very low, in agreement with the findings of others (34), were found to be identical in thymectomized and control rats. Our attempt to characterize the anti-BSA antibody, formed in control but not in thymectomized animals, was unsuccessful. Mice injected with BSA in complete adjuvant form an antibody of β -mobility (35), and there is now evidence that the rat antibody is of the same type (35 *a*). However, both β - and γ -globulins were present in the sera of our thymectomized rats in undiminished titer. Absorption of antiserum by removal of precipitating antibody gave no measurable change in any of the protein peaks. Finally the lymphoid tissues of the thymectomized rats contained normal complements of plasma cells and germinal center cells (33), both of which have been clearly shown to produce γ -globulin antibody (36, 37) and perhaps antibody of other types as well (38). It is possible that lymphocytes or cells of the lymphocyte line are capable of making certain antibodies. There is evidence that 19S macroglobulin (β 2M) antibodies are formed in cells resembling large or small lymphocytes (39), and the macroglobulin of Waldenström's macroglobulinemia is apparently made by a "lymphoid plasma cell," which may in truth belong to the lymphocyte series (40). The deficiency in the antibody-forming mechanism of the thymectomized rat may conceivably be concerned with such 19S antibodies or with some other as yet uncharacterized antibody system. It is apparent that a full evaluation of the effect of early thymectomy on antibody formation will only be possible when a variety of antigen-antibody systems have been studied in thymectomized animals with a complete evaluation, not only of the amount, but also of the molecular character of each antibody studied.

The striking loss of ability of thymectomized rats to develop delayed reactivity to BSA is in agreement with evidence showing a similar diminution in other types of delayed response (11, 12, 41). We cannot review here evidence implicating the lymphocyte as the essential cell in the initiation and expression of delayed hypersensitivity (see reference 42 for review). It is now well established that animals depleted of lymphocytes, whether by non-specific or specific means, have diminished delayed hypersensitive responses (43, 44). The thymectomized rat is greatly depleted of small lymphocytes, both in the blood (41) and in the spleen and lymph nodes (33). We relate the compromised delayed responsiveness of thymectomized rats to this deficiency in potentially sensitizable or reactive cells. Those thymectomized animals whose reactive capacity was least showed the greatest depletion of lymphocytes, both in blood and lymphoid organs, whereas those whose reactive capacities were less affected showed a less

striking depletion of these cells (33). The failure of splenectomy to influence reactions to BSA agrees with earlier experiments (45) showing the failure of splenectomy to influence tuberculin sensitivity adversely, and points up the fact that the effect of thymic extirpation does not depend simply on the removal of a large amount of lymphoid tissue.

The small lymphocyte responsible for delayed reactive capacity would seem to originate primarily in the thymus (5). The failure of thymus or spleen homogenates, in large doses, to restore the ability of thymectomized rats to develop delayed sensitivity argues strongly against any significant role for lymphopoietic hormonal factors of thymic origin such as the thymic lymphocytosis-stimulating factor described by Metcalf (46). In experiments complementary to ours, Miller (11) has shown that replacement by grafting of intact, viable thymus, in mice thymectomized at birth, restores their reactive capacity against homograft antigens.

Rats thymectomized at 2 to 3 weeks of age showed a less striking loss of delayed reactivity than rats thymectomized at birth; guinea pigs thymectomized as adults showed none at all. Miller (11) and Martinez *et al.* (12) have shown, similarly, that thymectomy of adult mice fails to affect their ability to reject homografts, though thymectomy as late as 30 days was minimally effective (12). A possible explanation for this finding might be that the responsibility for making and delivering small lymphocytes is, in early life, an exclusive property of the thymus but that, as the animal matures, this responsibility is passed on to an increasing extent to other organs such as lymph nodes and spleen. An alternative explanation might be that the thymus retains its function as a source organ of small lymphocytes throughout life but that, after its extirpation, a considerable interval of time must elapse before the body is depleted of the now irreplaceable supply of small lymphocytes. The long life span of the lymphocyte seems to be clearly established (47). In animals, past the peak of their growth curves, in which significant dilution of a finite cell population by growth alone is excluded, lymphocyte depletion sufficient to impair delayed reactive capacity significantly could conceivably require several months. This possibility is at present being investigated.

SUMMARY AND CONCLUSIONS

Rats thymectomized at birth gained weight and otherwise developed normally, but were found to be very susceptible to intercurrent infections. Both Arthus reactivity and delayed hypersensitivity to BSA were markedly impaired in rats thymectomized during the first week of life and significantly impaired in rats thymectomized as late as 3 weeks after birth. The inhibition of Arthus reactivity in thymectomized rats was well correlated with their failure to develop significant titers of precipitating or hemagglutinating antibody. However, natural heteroagglutinin titers were not altered in these animals, and no ab-

normality of serum proteins, including γ -globulin, could be detected by paper electrophoresis. The loss of immunologic activity could not be corrected by injecting homogenates of spleen or thymus before and during the sensitization period. Splenectomy at birth did not influence Arthus or delayed reactivity.

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EXPLANATION OF PLATE 12

FIGS. 1 *a* to 1 *c*. Arthus reaction at 4 hours in sham-operated rat, sensitized with BSA and adjuvant and skin-tested at 20 days with 30 μ g BSA. Fig. 1 *a* shows extensive edema and cellular infiltration in deep dermis, muscle, and subcutaneous connective tissue. Figs. 1 *b* and 1 *c* show vascular occlusion by leukocytic thrombi and massive infiltration of polymorphonuclear leukocytes. All hematoxylin and eosin. Fig. 1 *a*, $\times$ 32, Fig. 1 *b*, $\times$ 120, Fig. 1 *c*, $\times$ 260.

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