HYPERSENSITIVITY IN NEWBORN GUINEA PIGS

By S. B. SALVIN, Ph.D., M. B. GREGG, M.D., and R. F. SMITH, Ph.D.

(From the United States Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana)

(Received for publication, November 27, 1961)

Delayed hypersensitivity to soluble purified protein develops after injection of antigen into the foot-pads of adult guinea pigs.

This antigen may be administered (*a*) in a specific precipitate in antibody excess (1), (*b*) in minute quantities (2), or (*c*) as part of a conjugated protein (3–5). Ultimately, the delayed hypersensitivity may be followed by the appearance of detectable quantities of circulating antibody and the presence of Arthus type hypersensitivity. Formation of circulating antibody has been studied in newborn animals and found deficient when compared to adults (6–10). The presence of delayed hypersensitivity, on the other hand, has not been investigated extensively in newborns.

Neonatal guinea pigs sensitized subcutaneously with live tubercle bacilli showed slight, if any, skin reaction to the injection of a watery extract of *Mycobacterium tuberculosis* despite the presence of gross tuberculous lesions in the regional lymph nodes and spleen (11, 12). The mother pigs under the same conditions showed at the skin-testing site marked erythema, edema, and necrosis. These neonatal animals were approximately as sensitive to the systemic toxic action of old tuberculin injected into the peritoneal cavity as were adult tuberculous guinea pigs. Similar results were described (13) after intraperitoneal sensitization with old tuberculin and skin testing subsequently with the same antigen. Uhr (14), however, demonstrated delayed allergy in newborn guinea pigs 7 to 10 days after foot-pad injection of ovalbumin in complete Freund's adjuvant. The passive transfer of cells from sensitized adults failed to induce delayed hypersensitivity in neonatal guinea pigs, rabbits, and humans (15–17). In contrast, one report (18) describes the passive transfer of tuberculin hypersensitivity after exchange transfusion of infants with blood from tuberculin-positive adults. Contact hypersensitivity, a probable variant of delayed hypersensitivity, has been produced in neonatal infants (19, 20). In view of the evidence that newborn animals respond less readily than adults to antigenic stimuli, studies were initiated to determine the ability of neonatal guinea pigs to manifest delayed hypersensitivity under a variety of test conditions.

In this paper, results are presented indicating that newborn guinea pigs can develop delayed hypersensitivity, but do not manifest it in the skin after intradermal injection of antigen.

Materials and Methods

Animals.—Albino guinea pigs of the Hartley strain were used, with adult animals weighing between 350 and 450 gm and their weights recorded at the start of each experiment. Newborn animals were used within 24 hours after birth. In some of the experiments on passive transfer of hypersensitivity, inbred guinea pigs (strain 13) were employed.

Antigens.—Guinea pigs were sensitized with a highly purified diphtheria toxoid obtained through the courtesy of Dr. C. G. Pope, Wellcome Research Laboratories, Beckanham, Kent, England. A purified diphtheria toxoid, KP59A, obtained through the courtesy of Dr. James A. McComb, Biologic Laboratories, Massachusetts Department of Health, was employed for skin testing. Diphtheria toxin was obtained from Eli Lilly and Company, Indianapolis. A 5 times recrystallized hen egg albumin (HEA) (K & K Laboratories, Jamaica, New York) and purified bovine gamma globulin (BGG) (Armour Laboratories) were also used for sensitization and skin testing. 1-fluoro, 2,4-dinitrobenzene (DFB) was obtained from Eastman Kodak Laboratories. *Para*-aminobenzoic acid was conjugated to proteins by methods previously described (21, 22, 4). Antigens were diluted in physiologic saline (0.85 per cent) containing 1 per cent normal guinea pig serum (serum-saline).

Sensitization.—The antigen in serum-saline was introduced either directly or in Freund's adjuvant (Difco Laboratories) in a volume of 0.5 ml distributed in the 4 foot-pads.

Skin Tests.—Guinea pigs were tested (*a*) intradermally with 0.1 ml of purified toxoid (10 Lf/ml) or of HEA 50 μg/ml), or (*b*) by contact with 0.05 ml of 0.5 per cent hapten dissolved in 4 parts acetone–1 part corn oil. No animal was tested more than once. Reactions were observed, and diameters of areas of edema or induration recorded at 4 hours, 18 to 24 hours, and sometimes at 48 hours.

Antibody Determination.—Sera from guinea pigs were assayed for antibody by the rabbit intracutaneous test (23), which can detect 0.001 units diphtheria antitoxin/ml, or by passive cutaneous anaphylaxis (PCA) (24), which can detect as low as 0.002 μg antibody nitrogen (AbN)/ml.

Passive Transfer.—Newborn guinea pigs were sensitized in the foot-pads within 12 hours after birth (*a*) with 2 doses of 1 Lf diphtheria toxoid–rabbit antitoxin precipitate in antibody excess administered 7 days apart, the 1st in Freund's adjuvant plus mycobacteria and the 2nd in physiologic saline, or (*b*) with a single injection of 1 Lf diphtheria toxoid in Freund's adjuvant plus mycobacteria. Popliteal, axillary, and inguinal lymph nodes were excised and suspended in cold Tyrode's solution. The nodes were minced, and the cells washed by centrifugation 2 to 3 times in cold Tyrode's solution. The cells were then resuspended, counted with the aid of a Levy hemocytometer, and injected intraperitoneally into normal adult guinea pigs. 48 hours later, the recipients were tested intradermally with 1 Lf toxoid.

RESULTS

Delayed and Arthus Reactions to Proteins.—

89 guinea pigs were inoculated in the foot-pads within 12 hours after birth with 1 Lf purified diphtheria toxoid in Freund's adjuvant without mycobacteria (incomplete adjuvant), 33 with 1 Lf toxoid in Freund's adjuvant with mycobacteria (complete adjuvant), 17 with bovine gamma globulin in Freund's adjuvant without mycobacteria, and 10 with 5 μg HEA in incomplete Freund's adjuvant. Equal numbers of adult controls were inoculated simultaneously.

Of 149 newborn animals studied, not one showed a definite delayed response to intradermal injection of 1 Lf toxoid, 5 μg BGG, or 5 μg HEA from the 5th to the 21st day after sensitization (Fig. 1).

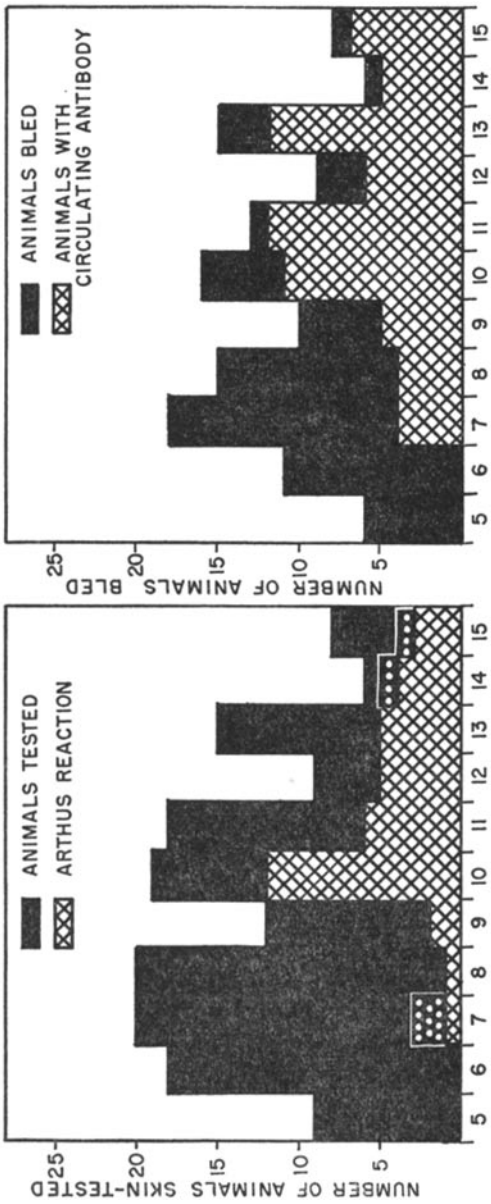

FIG. 1. Response of neonatal guinea pigs to injection of 5 μg hen egg albumin, 5 μg bovine gamma globulin, or 1 Lf diphtheria toxoid in *Freund's adjuvant in foot-pads*. 50 animals with 1 to 2 mg mycobacterium, the rest without. White circles refer to 4 guinea pigs which had questionable delayed hypersensitivity (induration about 10 mm in diameter), no Arthus reactions, but did have circulating antibody (by PCA test).

TABLE I

Passive Arthus Reactions in Adult versus Neonatal Guinea Pigs after Sensitization with Varying Amounts of Homologous Antibody Nitrogen and Intracutaneous Testing 24 Hours Later with 5 μg Homologous Antigen (HEA)

Amount of antibody nitrogen injected	Diameter of induration, *mm*	
	Neonatal	Adult
μg		
200	0	(No animals tested)
	0	
	10 × 10	
	10 × 11	
	14 × 14	
	15 × 15	
100	0	(No animals tested)
	0	
	13 × 14	
	14 × 14	
	14 × 15	
	15 × 15	
50	0	18 × 18
	0	18 × 20
	0	21 × 22
	0	22 × 23
	0	24 × 25
	10 × 10	
	10 × 10	
	18 × 18	
25	0	4 × 4
	0	17 × 19
	0	17 × 17
	20 × 20	22 × 23
		23 × 28
		25 × 27
		27 × 25
		28 × 27
15	(No animals tested)	0
		0
		13 × 14
		20 × 22
		24 × 24
		24 × 25
10	0	0
	0	0
		0
		0
		0
		15 × 15
		23 × 24
		27 × 27
5	0	0
	0	0

After the 7th to 8th day postsensitization, circulating antibody could be detected by either the rabbit test or PCA test. The ability to show Arthus reactions, however, did not appear at the same time as detectable circulating antibody, since of 66 animals that had circulating antibody from the 7th to the 16th day postsensitization, only 39 developed Arthus reactions on intradermal injection of 1 Lf toxoid. These results are in striking contrast to the course in adults in which after sensitization with 1 Lf toxoid in Freund's adjuvant (with or without mycobacteria), delayed hypersensitivity can be detected on the 4th day, and both Arthus reactions and circulating antibody simultaneously on the 11th to 14th day (2). Neonatal guinea pigs thus seemed less reactive than adults during both the delayed and Arthus phases.

Adult and newborn guinea pigs were then injected intravenously with varying amounts of guinea pig antibody to hen egg albumin, and skin-tested 24 hours later with the homologous antigen, HEA. A definite difference appeared between adult and neonatal guinea pigs in their ability to react after passive sensitization with a given amount of guinea pig anti-HEA antibody (Table I). Adult guinea pigs, weighing 350 gm, developed distinct Arthus responses to 10 to 25 μg AbN. Neonatal guinea pigs, on the other hand, did not consistently develop Arthus responses after administration of as much as 200 μg AbN.

To determine the approximate time when the skin of neonatal guinea pigs matured to immunologic adulthood, animals were sensitized in the foot-pads with 1 Lf toxoid in complete Freund's adjuvant at varying times after birth and tested intradermally at intervals thereafter with the specific antigen. 18 guinea pigs sensitized 7 days after birth failed to show delayed responses to intradermal injection of 1 Lf toxoid but beginning with the 10th day postsensitization detectable amounts of circulating antibody did appear. Of 10 animals sensitized 9 days after birth, 1 had a delayed reaction on the 7th day after sensitization and 1 had antibody on the 9th day; the rest showed no hypersensitivity or circulating antibody from day 5 to 12. Of 29 animals sensitized 11 and 12 days after birth, 16 had no skin reactions from days 5 to 10 after sensitization, 5 had delayed reactions on the 5th and 6th days, and 8 showed Arthus reactions from the 10th day on. 30 guinea pigs sensitized in the foot-pads 14 days after birth showed a typical transition through delayed hypersensitivity (from 5th to 9th day) to antibody formation (on about 11th day). Thus, guinea pigs, during the first 14 days after birth, do not demonstrate the same degree of skin reactivity as adults.

Contact Hypersensitivity.—

Guinea pigs sensitized with simple chemicals or haptens develop a hypersensitivity which is similar to delayed allergy (25). Accordingly, 90 1-day-old guinea pigs and 42 adults were sensitized by injection into the foot-pads with 50 μg DFB in Freund's adjuvant (without mycobacteria).

When tested on the surface of the skin with 0.05 ml of 0.5 per cent hapten, from days 5 to 20 postsensitization, all the adults showed contact allergy. 51 of the newborns (57 per cent) developed detectable contact hypersensitivity, 13 were doubtful, and 26 did not respond to surface application of the hapten (Table II). Normal guinea pigs were simultaneously tested on the sides with hapten, in order to serve as controls with which to compare experimental animals with regard to pinkness, elevation, and vesiculation of the sites of hapten application. Although the percentage of newborns developing contact reactions was less than the percentage of adults, neonatal guinea pigs did

TABLE II

Reaction of Neonatal Guinea Pigs to Sensitization in the Foot-Pads with 50 μg DFB in Freund's Adjuvant

Day after sensitization	No. of guinea pigs	No. definitely positive
5	4	3/4
6	10	4/10
7	11	3/11
8	10	6/10
9	5	4/6
10	6	1/6
11	6	6/6
12	10	2/10
13	2	2/2
14	11	9/11
15	2	1/2
16	3	3/5
17	3	3/3
18	2	2/2
20	2	2/2

develop contact allergy after sensitization with a hapten but did not indicate the presence of delayed allergy after sensitization with a complete protein.

Corneal Reactions.—

Allergic opacity has been produced in the cornea of hypersensitive adult guinea pigs after sensitization in the foot-pads with 5 μg HEA in complete Freund's adjuvant and subsequent intracorneal testing with 5 μg of homologous antigen (26, 27). Similar experiments were conducted in neonatal guinea pigs. 56 1-day-old guinea pigs were sensitized as above, 22 with *para*-aminobenzoic acid diazotized to hen egg albumin (PABA·HEA) and 34 with HEA, and tested intradermally and intracorneally with homologous antigen from day 5 to day 25.

Corneal opacification during this period could be detected in adults but not in neonatal guinea pigs.

The Influence of X-irradiation.—Total body x-irradiation of adult guinea pigs with 200 r 18 to 24 hours before sensitization did not noticeably inhibit

the development of delayed hypersensitivity (28, 29), although the appearance of circulating antibody was delayed. The possibility, therefore, arises that x-irradiation of newborn guinea pigs might defer the development of antibody sufficiently so that delayed reactions might be observed.

TABLE III

Influence of 200 r X-Irradiation on Hypersensitivity and Antibody Formation of Adult versus Newborn Guinea Pigs Sensitized with 1 Lf Diphtheria Toxoid in Incomplete Freund's Adjuvant as Antigen

Day after sensitization	X-ray, (newborns)			No X-ray, (newborns)			X-ray, (adult)			No X-ray, (Adult)		
	Antibody	Arthus*	Delayed*	Antibody	Arthus	Delayed	Antibody	Arthus	Delayed	Antibody	Arthus	Delayed
5	—	—	—	—	—	—	0/2	0/2	2/2	0/2	0/2	2/2
6	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	2/2	0/2	0/2	2/2
7	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3
8	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	2/2
9	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	3/3	0/3	0/3	2/3
10	0/2	0/2	0/2	2/2	0/2	0/2	0/2	0/2	2/2	0/2	0/2	2/2
11	0/2	0/2	0/2	2/2	0/2	0/2	0/2	0/2	1/2	2/2	2/2	—
12	0/3	0/3	0/3	3/3	0/3	0/3	0/3	0/3	3/3	3/3	3/3	—
13	0/2	0/2	0/2	2/2	0/2	0/2	0/2	0/2	2/2	2/2	2/2	—
14	0/2	0/2	0/2	1/2	0/2	0/2	0/1	0/1	1/1	2/2	2/2	—
15	—	—	—	—	—	—	1/2	1/2	1/2	2/2	2/2	—
16	2/2	0/2	0/2	2/2	0/2	0/2	2/2	2/2	—	2/2	2/2	—
20	3/3	3/3	—	3/3	3/3	—	3/3	3/3	—	3/3	3/3	—
21	1/1	1/1	—	3/3	3/3	—	—	—	—	2/2	2/2	—

* Positive reactions have a minimum diameter of edema or induration of 10 × 10 mm.

Accordingly, guinea pigs were divided into 4 groups and treated as follows: (*a*) newborn animals exposed to 200 r total body irradiation, then sensitized in the foot-pads with 1 Lf highly purified diphtheria toxoid in Freund's (incomplete) adjuvant; (*b*) newborns not irradiated, but sensitized, as above; (*c*) adult guinea pigs irradiated and sensitized, as above; and (*d*) adult guinea pigs not irradiated, but sensitized, as above (Table III).

Non-irradiated adult guinea pigs developed delayed hypersensitivity by the 5th day with Arthus type responses and circulating antibody being superimposed on the 11th day. Irradiated adult animals, on the other hand, although indicating the presence of delayed hypersensitivity at the usual time, did not develop detectable amounts of antibody until the 15th to 16th day. Newborn animals, both irradiated and non-irradiated, did not manifest any delayed or Arthus hypersensitivity in the skin until the 20th day after sensitization, at which time both irradiated and non-irradiated guinea pigs developed the capacity to form Arthus reactions. This absence of skin reactions occurred in spite of the fact that antibody was present in the circulation from the 10th day

on. In the irradiated newborns, although the appearance of circulating antibody was inhibited until the 16th day, delayed reactions did not develop in the skin. Arthus reactions eventually became discernible on the 20th day. Thus, the ability of skin of sensitized guinea pigs to react to specific antigen seems suppressed or undeveloped, although other immunologic functions such as development of circulating antibody progress normally.

Anamnestic Response.—An anamnestic response can be produced in guinea pigs by primary injection of antigen sufficient to produce delayed hypersensitivity but no detectable antibody and by a subsequent secondary injection sufficient to produce detectable circulating antibody (30).

To determine whether the immunologic mechanism associated with the anamnestic response was functioning normally in newborn animals, 1-day-old guinea pigs were injected with a primary dose of antigen sufficiently low to produce delayed hypersensitivity but not discernible antibody, and then exposed to a 2nd antibody-inducing dosage. The anamnestic response in the neonatal guinea pigs was then compared with that in adults.

Newborn guinea pigs developed an anamnestic response at the same rate as adults to three different antigens, namely, diphtheria toxoid, hen egg albumin, and hen egg albumin diazotized to *p*-aminobenzoic acid (Table IV). Furthermore, the rate of antibody production to PABA·HEA after a primary injection of HEA was the same in newborns as in adults.

Antigen Elimination.—The rate of elimination of an I^{131}-labeled antigen from the circulating blood has been employed as an indicator of the immune response (31). The logarithm of the antigen concentration in the blood decreases as a linear function of time, if the antigen is a native protein. If, however, the antigen is foreign to the animal, the elimination rate increases after a few days, presumably owing to the formation of circulating antibody and its reaction with antigen. The measurement of elimination of I^{131}-labeled antigen was applied to newborn and adult guinea pigs to determine further possible differences in the animals' responses to sensitization.

Purified bovine gamma globulin (BGG) was labeled with iodine^{131} by the "jet" method of Francis *et al.* (32) at a level of less than 2 atoms of iodine per molecule of protein. Groups of newborn and adult guinea pigs were sensitized in the foot-pads with 15 μg of BGG, PABA·BGG, or PABA·HEA in Freund's adjuvant (without mycobacteria) (4). Intravenous injections of 0.6 to 1.0 mg of the radioisotope-labeled protein, I^{131} BGG, were then administered to 3 animals from each group at each of 3 time intervals after initial sensitization, namely, 0, 7, and 13 days. Radioactivity of blood aliquots was determined at irregular intervals, and the average values for each group of animals calculated (Fig. 2).

When I^{131} BGG was injected intravenously immediately after sensitization in the foot-pads, the elimination rate was the same for the 3 groups of adult or newborn animals and resembled that for native proteins. When 7 days elapsed before the injection of I^{131} BGG, the animals sensitized with BGG eliminated

TABLE IV

Anamnestic Response in Neonatal and Adult Guinea Pigs, with 10 Days between Primary and Secondary Injections of Antigen

Day bled after 2nd injection	Antigen		Antibody	
	Primary	Secondary	Newborn	Adult
	0.1 Lf toxoid in saline	1.0 Lf toxoid in adjuvant (without mycobacteria)		
4			0/3*	0/3
5			0/2	0/2
6			0/3	0/3
7			0/2	0/2
8			2/3	2/3
10			2/2	2/2
12			3/3	2/2
	—	1.0 Lf toxin adjuvant		
9				0/2
10				0/2
12				1/2
13				2/2
	1.0 μg HEA in saline	5.0 μg HEA in adjuvant		
3			0/2	
4			3/4	1/2
5			3/4	2/2
6			4/4	2/2
7			2/2	
	—	5.0 μg HEA in adjuvant		
6			0/4	0/2
7			0/4	0/2
8			2/4	1/2
9			4/4	2/2
10			4/4	2/2
	1.0 μg HEA in saline	5.0 μg PABA·HEA in adjuvant		
7			0/7	0/6
8			4/7	4/6
9			6/7	4/6
10			6/7	5/6
	—	5.0 μg PABA·HEA in adjuvant		
7			0/3	
8			0/5	
9			0/5	
10			1/3	
11			3/3	

* Numerator indicates number of guinea pigs with antibody; denominator, number of animals tested.

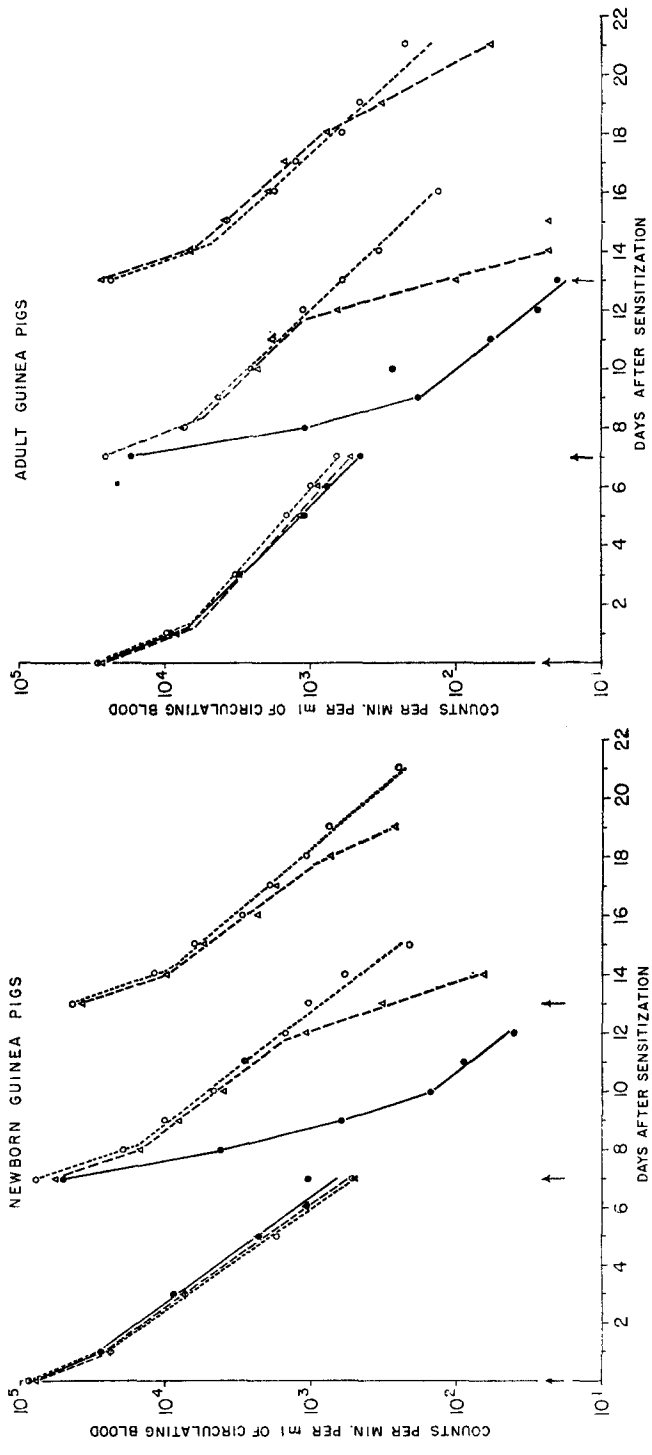

FIG. 2. Elimination rates of I^{131} BGG from blood of newborn and adult guinea pigs after sensitization in foot-pads with 15 μg BGG, PABA·BGG, or PABA·HEA in Freund's adjuvant without mycobacteria. ↑ indicates time of intravenous injection of I^{131} BGG; . ——— . indicates animals sensitized with BGG; △- - - -△ indicates animals sensitized with PABA·BGG; O——O indicates animals sensitized with PABA·HEA.

the antigen very rapidly or died of anaphylactic shock. Those guinea pigs with delayed hypersensitivity to BGG because of sensitization with the conjugate PABA·BGG eliminated I^{131} BGG at the same rate as controls for 4 days, but on the 5th day developed an immune elimination rate. Control animals sensitized with a heterologous conjugate, PABA·HEA, eliminated the antigen at a steady non-immune rate. Of interest is the similarity in the elimination rates between the control animals and those with delayed hypersensitivity but no detectable circulating antibody to BGG.

When I^{131} BGG was administered 13 days after sensitization, none of the guinea pigs sensitized with BGG survived the anaphylactic shock, although 2 mg phenergan were injected intraperitoneally 1 to 2 hours before the intravenous injection. The animals with delayed hypersensitivity to BGG because of sensitization with PABA·BGG and the controls showed responses at 13 days similar to the ones at 7 days after sensitization.

Newborn and adult guinea pigs responded in similar fashion. Both groups developed systemic anaphylaxis or rapid elimination of antigen, when they were sensitized with the protein BGG and later administered I^{131} BGG. Both newborn and adult groups that had been sensitized with the conjugate PABA·BGG showed similar accelerated elimination rates 4 to 5 days after intravenous inoculation of the labeled antigen. Thus, the newborn and adult guinea pigs were indistinguishable from each other in their elimination of BGG from the circulation during the stages of pre-hypersensitivity, delayed hypersensitivity, or antibody production.

Experimental Allergic Encephalomyelitis.—

The induction of this "auto-allergic" disease may be cellular in origin and therefore presumably is related to delayed hypersensitivity (33, 34). On this basis, 35 newborn and 35 adult guinea pigs were inoculated in the foot-pads with 100 mg (wet weight) ground adult guinea pig brain in Freund's adjuvant with 2 mg tubercle bacilli. The rate of onset of symptoms, the severity of response, and the percentage of diseased animals were compared between neonatal and adult guinea pigs.

18 of 35 newborns showed signs of paralysis, with 16 eventually dying. Signs of paralysis developed in 24 of 35 adults; and death occurred in 18. 17 of the 18 affected newborns developed their first gross signs from the 2nd to 11th day after sensitization, whereas 22 of the 24 adults developed their first signs from the 11th to the 18th day. Thus, newborns do have an "auto-allergic" system which in the case of allergic encephalomyelitis is as active as in adults.

Determination of Complement.—Since complement may play a role in passive cutaneous anaphylaxis (35, 36), participation of this serum constituent in the expression of delayed allergy in the skin may be necessary. To determine whether a deficiency of complement in the newborn animal occurred, therefore, 20 newborn and 15 adult guinea pigs were bled, and the complement determined as 50 per cent hemolytic units (37). A striking increase in the serum complement

levels did not appear in animals as they matured immunologically from 0 to 21 days of age. Also, the newborn animals had an average of 144, 50 per cent lytic units, and the adult 175. This difference seems slight, especially since some assays in newborns were higher than some in adults. Complement therefore does not seem to provide the basis for the difference in skin reactivity between newborn and adult.

Passive Transfer of Leukocytes.—Further information on the nature of delayed hypersensitivity in newborns may be provided in experiments on passive transfer of leukocytes. Newborn rabbits (16) and humans (17) have been reported not to react to intradermal testing with specific antigen after they had been injected with cells from hypersensitive adult donors. Presumably, not even adult sensitized leukocytes can overcome the deficiency inherent to

TABLE V

Development of Delayed Hypersensitivity after Passive Transfer of Leukocytes from Newborn to Adult Guinea Pigs

Sensitizing material	Number of cells $\times 10^6$	Strain of animal	Adult recipient skin reaction (mm induration)	
			4 hrs.	24 hrs.
1 Lf toxoid antitoxin precipitate (in complete adjuvant)	690	Hartley	0	18 × 23
	570	Hartley	0	20 × 21
	570	Hartley	0	12 × 12
	1000	Hartley	0	13 × 14
1 Lf toxoid (in complete adjuvant)	540	13	0	11 × 12

newborns that is associated with expression of hypersensitivity in the skin. Transfer of leukocytes from sensitized newborns to normal adults was attempted, in order to determine whether delayed hypersensitivity was present in the newborns but could not be expressed by intradermal testing. Results of these experiments (Table V) indicate that normal adult recipients when sensitized with leukocytes from newborn guinea pigs can manifest delayed hypersensitivity as indicated by intradermal testing. Of a total of 19 experiments with toxoid as the sensitizing antigen, it may be added, only these 5 transfers were considered positive in that diameters of induration were greater than 10 mm.

DISCUSSION

Delayed hypersensitivity is usually considered to be a condition that manifests itself systemically. Its detection, nevertheless, is usually associated with the response of a given tissue or organ, particularly the skin. Thus, to detect

the hypersensitive condition of a whole animal, the reaction of the skin is measured, although the response of other tissues or organs has been studied, such as the cornea (26, 27) and nervous tissue (38). Systemic tuberculin shock is supposedly associated with delayed hypersensitivity and has been used as an expression of delayed allergy (12). Fatal tuberculin shock still has not been described with highly purified soluble proteins, although minor changes such as fever and lymphopenia do occur in guinea pigs with delayed hypersensitivity (39). Thus, in the determination of delayed hypersensitivity, the presence of a potentially reactive skin is assumed.

Newborn guinea pigs of the Hartley strain do not have detectable delayed skin reactions after sensitization in the foot-pads with such purified proteins as HEA and diphtheria toxoid in Freund's adjuvant with or without mycobacteria. Delayed reactions cannot be discerned during the period when adult controls develop striking reactions of this type. Similarly, in the newborn, Arthus reactions may not appear, although circulating antibody may be present and although both circulating antibody and Arthus responses occur in adult controls. Further studies indicate that some basic immunologic processes, in addition to production of antibody to a primary stimulus, are functioning in the newborn. Thus, contact sensitization to the hapten DFB could be detected. Experimental allergic encephalomyelitis was readily produced in newborns. Also, an anamnestic effect occurred in newborns at the same rate as in adults. I^{131}-labeled antigen was eliminated from the blood of immune or hypersensitive newborns at the same rate as from the blood of similarly sensitized adults. The elimination of antigen in animals with delayed hypersensitivity induced by injection of conjugate was the same as in controls. Furthermore, successful passive transfer of delayed hypersensitivity from sensitized newborns to normal adult guinea pigs strongly suggests the presence of immunologically competent leukocytes.

The failure to produce allergic responses in the skin of newborns seems to be centered in a deficiency associated with the skin. This was emphasized by the inability to produce consistent Arthus reactions passively in newborns with as much as 200 μg AbN, whereas adults produced Arthus responses after passive sensitization with as little as 10 μg AbN. The inability to manifest hypersensitivity does not seem related to tolerance, since the guinea pigs do start reacting at about 14 days of age. The exact basis of the failure of the newborn skin to elicit a normal immunologic response is still in doubt, although the possible role of hormones and other pharmacologically active substances is being investigated.

SUMMARY

Neonatal guinea pigs during the first 2 weeks of life did not indicate the presence of delayed hypersensitivity intradermally, after sensitization with

purified soluble antigens in dose levels that induced detectable delayed hypersensitivity in the skin of adults. Although Arthus type allergy was detectable in newborns, circulating antibody frequently preceded its appearance by several days. Passive Arthus reactions were not produced in newborns as readily as in adults. Contact hypersensitivity and allergic encephalomyelitis were induced in newborns, but corneal reactions were not. Total body irradiation with 200 r inhibited antibody formation in newborns, as in adults. In addition, the induction period for anamnestic responses in newborns and the antigen elimination rate were the same as in adults. Passive transfer of delayed hypersensitivity from sensitized newborns to normal adults was accomplished.

BIBLIOGRAPHY

1. Uhr, J. W., Salvin, S. B., and Pappenheimer, A. M., Jr., Delayed hypersensitivity in guinea pigs by means of antigen-antibody complexes, *J. Exp. Med.*, 1957, **105,** 11.
2. Salvin, S. B., Occurrence of delayed hypersensitivity during the development of Arthus type hypersensitivity, *J. Exp. Med.*, 1958, **107,** 109.
3. Salvin, S. B., and Smith, R. F., The specificity of allergic reactions. I. Delayed *versus* Arthus hypersensitivity, *J. Exp. Med.*, 1960, **111,** 465.
4. Salvin, S. B., and Smith, R. F., Specificity of allergic reactions. II. Azoproteins in the anamnestic response, *Proc. Soc. Exp. Biol. and Med.*, 1960, **104,** 584.
5. Benacerraf, B., and Gell, P. G. H., Studies on hypersensitivity. I. Delayed and Arthus-type skin reactivity to protein conjugates in guinea pigs, *Immunology*, 1959, **2,** 53.
6. Freund, J., Influence of age upon antibody formation, *J. Immunol.*, 1930, **18,** 315.
7. Osborn, J. J., Dancis, J., and Julia, J. J., Studies on the immunology of the newborn infant. I. Age and antibody production, *Pediatrics*, 1952, **9,** 736.
8. Di Sant'Agnese, P. A., Simultaneous immunization of newborn infants against diphtheria, tetanus and pertussis, *Am. J. Pub. Health*, 1950, **40,** 674.
9. Dixon, F. J., and Weigle, W. O., The nature of the immunologic inadequacy of neonatal rabbits as revealed by cell transfer studies, *J. Exp. Med.*, 1957, **105,** 75.
10. Bishop, D. W., and Gump, D., Production of circulating antibody against defined antigen (BGG) by neonatal guinea pigs, *Proc. Soc. Exp. Biol. and Med.*, 1961, **106,** 24.
11. Freund, J., The influence of age on the skin sensitiveness of tuberculous guinea pigs, *J. Immunol.*, 1927, **13,** 285.
12. Freund, J., The sensitiveness of tuberculous guinea pigs one month old to the toxicity of tuberculin, *J. Immunol.*, 1929, **17,** 465.
13. Weiss, D. W., Inhibition of tuberculin skin hypersensitivity in guinea pigs by injection of tuberculin and intact tubercle bacilli during fetal life, *J. Exp. Med.*, 1958, **108,** 83.
14. Uhr, J. W., Development of delayed-type hypersensitivity in guinea pig embryos, *Nature*, 1960, **187,** 957.
15. Waksman, B. H., and Matoltsy, M., Quantitative study of local passive transfer of tuberculin sensitivity with peritoneal exudate cells in the guinea pig, *J. Immunol.*, 1958, **81,** 235.

16. Warwick, W. J., Archer, O., and Good, R. A., Passive transfer of delayed sensitivity to the newborn rabbit, *Proc. Soc. Exp. Biol. and Med.*, 1960, **105,** 459.
17. Warwick, W. J., Good, R. A., and Smith, R. T., Failure of passive transfer of delayed hypersensitivity in the newborn human infant, *J. Lab. and Clin. Med.*, 1960, **56,** 139.
18. Schlange, H., Die passive Uberträgung der Tuberkulinhautempfindlichkeit durch Blutaustauschtransfusionen und die Uberträgbarkeit erworbener Tuberkulinnegativitat, *Arch. Kinderheilk.*, 1954, **148,** 12.
19. Straus, H. W., Artificial sensitization of infants to poison ivy, *J. Allergy*, 1931, 2, 137.
20. Uhr, J. W., Dancis, J., and Neumann, C. G., Delayed-type hypersensitivity in premature neonatal humans, *Nature*, 1960, **187,** 1130.
21. Landsteiner, K., and van der Scheer, J., On the influence of acid groups on the serological specificity of azoproteins, *J. Exp. Med.*, 1927, **45,** 1045.
22. Goebel, W. F., and Avery, O. T., Chemo-immunological studies on conjugated carbohydrate-proteins. IV. The synthesis of the *p*-aminobenzyl ether of the soluble specific substance of type III pneumococcus and its coupling with protein, *J. Exp. Med.*, 1931, **54,** 431.
23. Fraser, D. T., The technique of a method for the quantitative determination of diphtheria antitoxin by a skin test in rabbits, *Tr. Roy. Soc. Can.*, 1931, **25,** 175.
24. Ovary, Z., Quantitative studies in passive cutaneous anaphylaxis of the guinea pig, *Internat. Arch. Allergy*, 1952, **3,** 162.
25. Eisen, H., Hypersensitivity to simple chemicals, *in* Cellular and Humoral Aspects of Hypersensitive States, (H. S. Lawrence, editor) New York, Hoeber-Harper, 1959, 89.
26. Holley, S. W., Corneal reactions of normal and of tuberculous guinea pigs to tuberculo-protein and tuberculo-phosphatide, *Am. J. Path.*, 1935, **11,** 937.
27. Salvin, S. B., and Gregg, M. B., The specificity of allergic reactions. IV. The cornea, *Proc. Soc. Exp. Biol. and Med.*, 1961, **107,** 478.
28. Salvin, S. B., and Smith, R. F., Delayed hypersensitivity in the development of circulating antibody. The effect of x-irradiation, *J. Exp. Med.*, 1959, **109,** 325.
29. Uhr, J. W., and Scharff, M., Delayed hypersensitivity. V. The effect of x-irradiation on the development of delayed hypersensitivity and antibody formation, *J. Exp. Med.*, 1960, **112,** 65.
30. Salvin, S. B., and Smith, R. F., Delayed hypersensitivity and the anamnestic response, *J. Immunol.*, 1960, **84,** 449.
31. Talmage, D. W., Dixon, F. J., Bukantz, S. C., and Dammin, G. J., Antigen elimination from the blood as an early manifestation of the immune response, *J. Immunol.*, 1951, **67,** 243.
32. Francis, G. E., Mulligan, W., and Wormall, A., Isotopic Tracers, London, Athlone Press, 2nd edition 1959.
33. Waksman, B. H., Experimental allergic encephalomyelitis and the "auto-allergic" diseases, *Internat. Arch. Allergy and Appl. Immunol.*, suppl. 14, 1959, 1.
34. Paterson, P. Y., Transfer of allergic encephalomyelitis in rats by means of lymph node cells, *J. Exp. Med.*, 1960, **111,** 119.
35. Osler, A. G., Hawrisiak, M. M., Ovary, Z., Siqueira, M., and Bier, O. G., Studies on the mechanism of hypersensitivity phenomena. II. The participation of

complement in passive cutaneous anaphylaxis of the albino rat, *J. Exp. Med.*, 1957, **106,** 811.

36. Osler, A. G., Randall, H. G., Hill, B. M., and Ovary, Z., *in* Mechanisms of Hypersensitivity. International Symposium, Boston, Little Brown and Co., 1959, 281.
37. Kabat, E. A., and Mayer, M. M., Experimental Immunochemistry, Springfield, Illinois, C. C. Thomas, 1948.
38. Swithinbank, J., Smith, H. V., and Vollum, R. L., The intrathecal tuberculin reaction, *J. Path. and Bact.*, 1953, **65,** 565.
39. Uhr, J. W., and Brandriss, M. W., Delayed hypersensitivity. IV. Systemic reactivity of guinea pigs sensitized to protein antigens, *J. Exp. Med.*, 1958, **108,** 905.