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Passive Transfer of Delayed Sensitivity to the Newborn Rabbit.* (26140)

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Recent studies of the immune capacity of the human newborn infant have demonstrated that the newborn infant is unable to accept or to manifest a passive transfer of delayed allergy(1). A similar unresponsiveness to passively transferred delayed allergy in the newborn guinea pig was demonstrated by Waksman and Matoltsy(2). They were unable to transfer delayed sensitivity to tuberculin to newborn guinea pigs with peritoneal exudate leukocytes, but were uniformly successful with such transfers to mature guinea pigs. Parallel limitations in capacity of the newborn human and newborn rabbit have been demonstrated by several workers(3,4,5, 6). Data presented here show that like newborn infants newborn rabbits are limited in this capacity to express or to accept passively transferred delayed allergy.

Methods and materials. The animals used in the experiment were New Zealand white rabbits, newborn and mature. The newborn rabbits were 7, 14, 21, and 28 days of age, and the mature rabbits weighed 5 to 6 lb.

Delayed sensitivity was induced by infecting adult animals with Dematco strain of streptococcus. The Dematco streptococcus is group A, type 12 strain of beta-hemolytic streptococcus which was originally obtained from a patient with acute glomerulonephritis. This strain produces large quantities of streptokinase and streptodornase B. One-tenth cc of an 18-hour broth culture of the Dematco strain streptococcus was injected into each hind foot pad of the 5-6 lb rabbits.

These rabbits were skin tested 7 to 10 days after infection to determine their degree of sensitivity. Skin testing was done with 0.01 cc of physiologic (8.5%) saline containing 100 units of streptokinase and 3.3 units of streptodornase‖ injected intradermally in the inner surface of the rabbit's ear. As a control, 0.01 cc of physiologic saline was injected in the opposite ear. The skin testing sites in the ears were examined 18-24 hours later for induration and erythema. The skin reactions were read on the basis of square millimeters of erythema and induration, as follows: 0-9 mm^2 – no transfer, 10-19 mm^2 – 1+, 20-39 mm^2 – 2+, 40-59 mm^2 – 3+, 60-79 mm^2 – 4+, over 80 mm^2 – 5+. The same rating system was used for grading the passive transfers.

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‖ Varidase (Lederle).

TABLE I. Results of Passive Transfer of Delayed Sensitivity to Newborn and Control Rabbits.

Exp.	Age of newborn (wk)	Intensity of transfer to	
		Newborn	Control
1	1	0	5+
2	1	0	2+
3	1	0	4+
4	1	0	0
5	1	0	1+
6	1	0	3+
7	1	0	3+
8	2	0	0
9	2	0	1+
10	2	0	1+
11	2	1+	2+
12	2	0	5+
13	2	0	2+
14	2	0	0
15	2	0	0
16	2	0	3+
17	2	0	2+
18	2	0	0
19	3	2+	0
20	3	0	2+
21	3	4+	1+
22	3	0	5+
23	3	0	0
24	3	0	1+
25	3	0	5+
26	3	2+	2+
27	3	3+	5+
28	4	5+	5+
29	4	5+	5+
30	4	5+	5+

Preparation of leukocytes for transfer. The rabbits giving a positive skin test were sacrificed. The spleen and the 2 popliteal nodes were removed. Leukocytes were separated from these organs by teasing with dissecting needles or by forcing the whole organ through a 40 mesh stainless steel sieve which had been coated with Arquad® to prevent agglutination of leukocytes. The resultant material was suspended in 40 cc of saline and permitted to stand for 10 minutes to allow settling of the coarse particles of tissue. The supernatant fluid was withdrawn and centrifuged for 12 minutes at 1500 rpm. The cell button containing a mixture of red and white cells was resuspended in a standard volume, usually 3 or 4 cc of saline. Aliquots of the leukocytes were injected by the same routes —intravenous, intraperitoneal, or subcutaneous—into a newborn and a mature control rabbit in each experiment.

Skin testing. After transfer of white blood cells, the animals were skin tested with 0.01 cc of physiologic saline containing 1000 units of streptokinase and 33 units of streptodornase. When the cellular transfer was done intravenously, the skin testing was done immediately after injection. When the transfer was done intraperitoneally or subcutaneously, the skin testing was carried out 24 hours after the transfer of cells.

Results. In Table I are the results of several experiments with rabbits aged 1-4 weeks. At the youngest age, no newborns accepted passive transfer of delayed sensitivity, but with increasing maturity, larger proportions were able to demonstrate this phenomenon.

Newborn rabbits aged 7 days are uniformly unable to accept passive transfer of delayed sensitivity. At the age of 14 days, one of 11 accepted the passive transfer and with increasing maturation, the acceptance rate rose to 4 of 9 at 21 days and 3 of 3 at 28 days (Fig. 1). Passive transfers of delayed sensitivity to mature control animals were successful in 24 of 30 attempts.

Discussion. These data indicate that the newborn rabbit is similar to the newborn human infant in its inability to accept the passive transfer of delayed sensitivity when compared with the immunologically mature rabbit. The data do not indicate the basis for

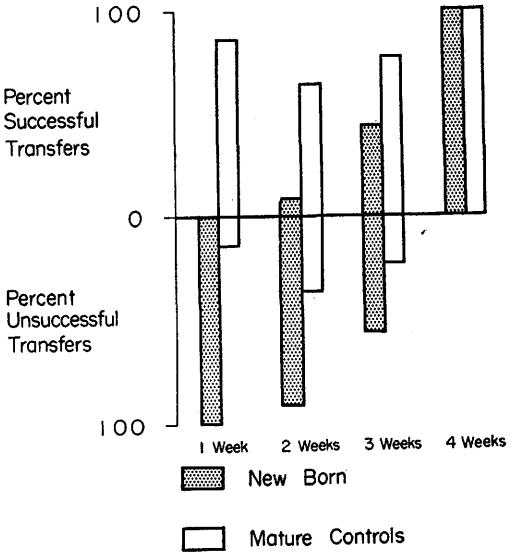

FIG. 1. Progressive maturation of newborn rabbit's capacity to sustain the passive transfer of delayed hypersensitivity.

the failure of the response. This could be the result of the newborn rabbit's inability to sustain the transferred cells, to coordinate the cellular and vascular elements responsible for expression of delayed hypersensitivity, or to perform an essential step in development of the hypersensitive state. The capacity to accept passively transferred delayed sensitivity appears to mature by 4 weeks of age, with gradual transition between 2 and 3 weeks of age. This phenomenon may be common to all animals expressing delayed sensitivity. It should be studied further, not only because of its possible significance in understanding the basis for delayed allergy, but because of important implications which this deficiency may have in understanding the relative inability of the newborn to defend himself against infection and toxic stimuli.

Summary. The newborn rabbit is limited in its capacity to accept the passive transfer of delayed hypersensitivity, as is the case in the human newborn. The ability to accept passively transferred delayed sensitivity develops by 4 weeks of age in newborn rabbits.

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Biosynthesis of Squalene by Rat Preputial Gland.* (26141)

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During a study of effects of hormones on the rat preputial gland, it was found that this gland appears to synthesize and accumulate squalene. Preputial glands are present in both sexes of several species of rodents as a pair of small, clubshaped organs lying along the urethra, and emptying their oily secretion into the preputial sac. The function of these glands is unknown, but they contain large amounts of β-glucuronidase and acid phosphatase. A mouse preputial gland tumor incorporated C^{14} from acetate into sterols when incubated *in vitro* while normal mouse preputial glands were less active in this regard(1). Administration of androgens to rats of either sex causes a marked increase in size of preputial glands, whereas, hypophysectomy leads to atrophy. The preputial gland is similar in microscopic structure to the cutaneous sebaceous glands, and in the rat, its responses to administration of hormones are thought to be similar to those of the sebaceous glands(2).

Methods. Preputial glands were obtained by excision immediately after death (by blow on head) from normal Sprague-Dawley male rats which were 6 to 7 weeks old and weighed 160-200 g. The glands, which weighed from 20 to 75 mg, were dissected free of fat and other tissue, and were incubated whole or split longitudinally in 1 ml of Krebs-Ringer phosphate buffer (pH 7.4) to which had been added sodium acetate 1-C^{14} (12.5 μc of C^{14} in 25 μmoles of sodium acetate). Incubation was carried out in a Dubnoff shaking incubator at 37°C under a flow of oxygen and shaking at 100 strokes per minute for 3 hours. The tissue and incubating fluid were then heated in a steam bath overnight with alcoholic potassium hydroxide solution in tubes sealed under nitrogen. The nonsaponifiable lipids were extracted with petroleum ether and separated into squalene and sterol fractions by column chromatography on alu-

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