preincubation with native RNAse. The preincubation of liver homogenates with less completely inactivated RNAse involves more complicated phenomena. The results are interpreted to mean that the inactivation of MVA utilization observed with native RNAse is due to enzymatic activity of RNAse rather than to the basic properties of the protein that are not significantly altered on mild denaturation. Additional evidence is thus provided for the involvement of RNA (or a ribonucleoprotein) in the complete system essential for conversion of mevalonic acid to nonsaponifiable material by liver homogenates. Evidence has been presented previously that the RNA is essential for maintenance of ATP levels required in a number of steps involved in utilization of mevalonic acid.

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Production of Circulating Antibody against Defined Antigen (BGG) by Neonatal Guinea Pigs. (26225)

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The immunologic capacity of young guinea pigs is generally acknowledged as being considerably less than that of adults(1-5). Investigations leading to this conclusion employed chemically undefined antigens, such as sheep serum(1), diphtheria toxin(2), tubercle bacillus(3), typhus vaccine(4), and thyroid tissue(5), and the demonstrations principally concerned sensitization rather than antibody detection. On the other hand, studies of acquired tolerance, naturally induced *in utero,* toward postnatally implanted maternal skin homografts(6,7), and demonstration of plasma cells in lymph nodes of newborn guinea pigs(8) constitute indirect evidence that guinea pigs may already possess the capacity to form antibodies in neonatal stages.

The present study was undertaken to ascertain whether guinea pigs can form humoral antibody against defined antigen, bovine gamma globulin (BGG), immediately after birth.

Methods. Guinea pigs of outbred stock were inoculated 6 to 30 hours after delivery. Sensitization was accomplished by single intramuscular injections of BGG* (25 mg/100 g body weight) combined with Freund adjuvant.† Animals were bled by intracardiac puncture or challenged for anaphylaxis by intracardiac or intravenous injection. Sera were stored at −19°C.

The passive cutaneous anaphylactic (PCA) procedure, as developed by Ovary (10), was employed for detection of circulating antibody; the method is capable of demonstrating precipitable and nonprecipitable antibody and is sensitive down to the order of 0.003 μg antibody nitrogen(9,10). Intracutaneous injection of 0.1 ml undiluted antiserum was followed, 5 to 6 hours later, by

* Bovine gamma globulin, fraction II, from Nutritional Biochemicals Corp., Cleveland, O.

† Freund adjuvant, complete, from Difco Laboratories, Detroit, Mich.

TABLE I. PCA Reactions of Sera of Guinea Pigs Injected with BGG and Adjuvant 6 to 30 Hr after Birth.

No.	Body wt, g	Post-inj. age of serum (days)* 7	9	11	15
661	86	+++			
662	77	0			
663	90	0			
676	94	0			
677	91	0			
678	95	0			
703	84	0			
704	74	0			
705	79	0			
706	67	0			
710	91	0		++++	
711	88	0		++++	
712	95	0		0	++
716	106	0		++++	
717	105	0		++	++++
868	101		++		
869	78		++		
870	79		0		
871	84		0		
872	85		0		
873	79		++		
874	107		+		
734	99		++		+++
735	106		±		++
736	95		++		+++
793	78		++	++	
794	92		++	+++	
795	85		+++	+++	
796	70		++	++++	
797	70		+		
798	91			++++	
799	73			++++	
800	90			+++	
802	130			++++	
803	118			++++	
791	114			++++	++++
792	124			++++	++++
723	84				++
718	107				+++
719	96				++++
720	84				+++
729	—				+++
730	—				++++
732	—				+++
726	—				++
727	—				++
728	—				+++

* Quantitatively scored according to diameter (mm) of cutaneous reaction: 0, negative; ±, 1-5; +, 6-10; ++, 11-15; +++, 16-20; ++++, >20.

intravenous injection of 0.1 ml (10 mg) BGG in saline plus 0.5 ml of 0.5% Evans Blue solution. Cutaneous reaction appeared within 5 minutes and persisted; scoring index (Table I) was only slightly modified from that of Ovary(10).

Agar-diffusion tests were performed on neonatal and adult sera according to the Ouchterlony procedure(11). Systemic anaphylaxis, using 13 to 25 mg BGG in saline as challenging dose, was induced in a small series of animals. Plasma-cell studies were carried out on lymph nodes and spleen, treated in accordance with the triple stain technic of Trevan and Sharrock(12).

Results. Injection of newborn guinea pigs with BGG and adjuvant on the day of, or immediately following, birth induced formation of specific antibody detectable infrequently as early as the seventh day (1/15) and generally by the ninth day (12/15) (Table I). Titers increased with age to the eleventh and fifteenth day after inoculation. No cross-reactivity occurred when bovine serum albumin (BSA) was employed for antigenic challenge or when normal (control) guinea-pig serum or anti-BSA serum was tested against BGG. Percentages of positive PCA reactions with sera of different postinjection intervals are shown in Fig. 1.

Agar-diffusion tests on these neonatal sera gave negative results. Ouchterlony tests on adult sera, on the other hand, gave positive reactions 9 days after single intramuscular injections of BGG and adjuvant.

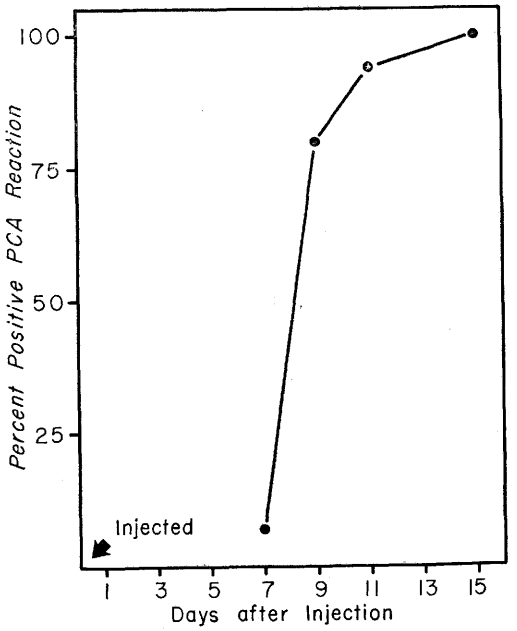

FIG. 1. Positive PCA reactions of neonatal guinea-pig sera after inj. with BGG and adjuvant at birth.

TABLE II. Systemic Anaphylaxis in Newborn Guinea Pigs Sensitized toward BGG Injected with Adjuvant.

Animal No.	Inj. age (days)	Sensitization dose (mg/100 g b.w.)	Challenge		Reaction*
			Age (days)	Dose (mg)	
661	2	29	12	25	+++
662	2	32	9	13	+
663	2	28	9	25	+
798	1	25	12	20	+
799	1	25	12	20	++
802	1	25	12	20	+++
803	1	25	12	20	+++
829	2	25	9	20	+
830	2	25	9	20	+

* Reaction: +, mild; ++, severe; +++, lethal.

Neonatal guinea pigs, inoculated at 1 or 2 days of age, suffered systemic anaphylaxis when challenged at 9 or 12 days of age (Table II). At day 12, sensitization was sufficient to cause fatal reactions. No unfavorable response occurred when BGG-sensitized animals were challenged with nonspecific antigen (BSA).

Plasma cells and plasma-cell precursors were observed in methyl green-pyronin-orange G preparations of lymph nodes from normal late prenatal, neonatal, and adult animals. The presence of these cells in newborn guinea pigs confirms the findings of Gyllensten(8). No attempt was made to determine the effect of inoculation on plasma-cell population.

Discussion. Detection of induced anti-BGG antibody in newborn guinea pigs by PCA but not by the agar-diffusion procedure may be attributed to extreme sensitivity of the former method in demonstrating low concentrations of precipitable antibody, without necessarily invoking the possibility that the detectable antibody is of the nonprecipitable type. Since even the adult guinea pig is a relatively poor producer of precipitable antibody, compared, for example, with the rabbit(9), low antibody titers would be expected in neonatal guinea pigs.

Until antibody production by fetal guinea pigs is unequivocally demonstrated, some question will exist as to whether this capacity is limited to postnatal stages or is established at a reduced rate in the late prenatal period, prior to parturition. In the above series (Table I), approximately 9 days were required to develop a fair titer of antibody and 11 days to produce a relatively high titer. In 2 groups of neonatal guinea pigs injected on the fourth and sixth days of age, antibody concentration was moderate after 7 days and high after 9 days. In adults, strong PCA reactions have indicated high-titer sera 7 to 8 days from time of injection. It would appear then that antibody-production capacity is increasing during the first week and may be present to a degree before birth.

The results indicate that attempts to induce effective tolerance in the guinea pig toward skin homotransplantation, or other forms of immune reaction, must be initiated in the fetus. They also clarify the immune status of neonatal guinea pigs subject to induced aspermatogenesis, as discussed elsewhere(13).

Summary. Neonatal guinea pigs, injected with BGG and adjuvant immediately after birth produce circulating antibody, detectable by PCA but not by agar-diffusion tests. Sera collected 7, 9, 11, and 15 days after intramuscular injection gave 7, 80, 94, and 100% positive PCA reactions, respectively. Rate of antibody production during the first week after birth is lower than that of adults. Capacity for humoral antibody formation probably exists at a reduced level in the fetus.

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Observations on Mechanism of Potassium Excretion due to Administration of Hydrochlorothiazide. (26226)

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Administration of thiazide diuretics to normal subjects and to patients with compensated congestive heart failure produced only moderate urinary excretion of potassium (K)(1,2). On the other hand, these agents induced much greater kaliuresis in patients with edema and ascites(3-6). This difference suggested to us that aldosterone may influence K loss due to the thiazide diuretics inasmuch as most patients with significant edema and ascites excrete abnormally large quantities of aldosterone in the urine(7). This hormone is believed to promote renal excretion of K only when there is an adequate sodium (Na) transport to the distal tubular site for Na and K exchange (8-10). The present studies in laboratory animals represent an attempt to clarify the influence of aldosterone-like steroids on K excretion with thiazide diuretics.

Method. Holtzman rats, 150-200 g, were housed in a constant environment and fed Purina chow biscuits and tap water for 4 days prior to study. Adrenalectomy was performed through a dorsal incision under ether anesthesia and the animals were then maintained on sugar and tap water postoperatively. Approximately 24 hours after adrenalectomy the animals were injected subcutaneously with 2.5 ml of 0.9% saline and with desoxycorticosterone (DOC) as the acetate form in 0.25 ml of ethanol-isotonic saline solvent (28/72 by volume). DOC was used as a standard aldosterone-like steroid(13) because of its availability. Hydrochlorothiazide (H-C) was given subcutaneously in 0.25 to 0.5 ml of ethanol-saline, either alone or in combination with DOC at the time of the saline load. Control injections of solvent were used where indicated. Animals were placed in metabolism cages (4 rats/cage) for collection of a 4-hour sample of urine. Bladders were emptied by suprapubic pressure before and at the end of collection. Urinary sodium and potassium were determined by flame photometric analysis. Statistical comparisons were done by Students "t" test.

Results. Table I summarizes the effects of DOC and H-C on Na and K excretion in adrenalectomized rats. DOC alone at doses of 3, 6, and 24 μg induced a strong retention of Na with only slight insignificant changes in urinary excretion of K. H-C significantly increased urinary output of both Na and K at doses of 0.6 and 1.2 mg/kg ($P<0.05$). There was no significant increase in potassium excretion with the 0.3 mg/kg dose when

TABLE I. Urinary Excretion of Na and K Following Treatment with Desoxycorticosterone and Hydrochlorothiazide in Adrenalectomized Rats.

Treatment			Mean response	
DOC, μg	H-C, mg/kg	No. of measurements	Na	K
—	—	8	.69 ±.23	.37 ±.09
3	—	11	.36 ±.19*	.46 ±.15
6	—	9	.18 ±.06*	.42 ±.14
24	—	4	.12 ±.04*	.38 ±.03
—	.3	4	.93 ±.15	.47 ±.07
—	.6	5	1.21 ±.25*	.61 ±.15*
—	1.2	5	1.28 ±.30*	.62 ±.09*
3	.3	3	.78 ±.07	.76 ±.07†
6	.3	4	.56 ±.13	.75 ±.15†
3	.6	6	1.08 ±.17	.72 ±.03
6	.6	4	.75 ±.19	.70 ±.11
24	.6	4	.66 ±.11	.94 ±.09†

* $P<0.05$ relative to untreated controls.

† $P<0.05$ relative to corresponding treatment with hydrochlorothiazide (H-C) above.

Na and K = mean meq output/4 hr ± stand. dev. Each measurement = 4 animals.