Immunization to Homologous Milk in the Rabbit.*† (26775)

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The production of experimental "auto-allergic" lesions involving many tissues(1-9) has led to the anticipation that "auto-allergies" affecting other tissues, which contain parenchymal elements or secretions separated from the blood stream, will be discovered. The breast is such a tissue, since it is an exocrine gland whose main products are secreted externally and are not found in the circulation under normal conditions(10). That animals can make an immune response to homologous breast secretions was shown by Lewis(11) who demonstrated antibody to homologous casein in goats after multiple intravenous injections of goat casein. The present paper describes an attempt to produce anti-milk antibodies and "auto-allergic" breast disease in rabbits by immunization with homologous milk or casein or autologous milk in Freund's adjuvant(12).

Materials and methods. Rabbit milk was pooled, defatted by centrifugation, and frozen until use. Casein was precipitated by adjusting the pH of a 1:4 milk dilution in saline to 4.5 with 0.5 N HCl(13). After centrifugation the supernate was decanted and used as the whey fraction. The precipitated casein was washed 2 times in ether and solubilized by the dropwise addition of 0.05 N NaOH. The pH was not allowed to go higher than 9.8 and was finally adjusted to 7.4. The solutions of casein had a purity of 86% by paper electrophoresis(14). Protein nitrogen was determined by a modification of the micro Kjeldahl technic(15). "Milk-adjuvant" was prepared by addition of 10 parts defatted milk (50% in saline) to 1 part Arlacel C and 9 parts Bayol F containing 2.0 mg killed tubercle bacilli per ml of final volume and homogenizing to a high viscosity in a Waring Blendor. "Casein-adjuvant" was prepared in the same way with casein solution containing 8.9 mg casein N/ml. The rabbits received weekly injections of the given adjuvant for 2 to 10 months, first in the rear foot pads (1 ml), next in the front foot pads (1 ml), and subsequently intradermally in the back (½ ml). All injection sites were remote from the breast area. Animals were bled prior to the first injection and at monthly intervals just prior to an injection. Two male rabbits were immunized with milk-adjuvant and tested for antibody to homologous milk, casein and whey by the precipitin technic(16), double diffusion in agar(17), and hemagglutination. The precipitin technic yielded only qualitative information because of the incomplete precipita-

TABLE I. Total N (μg/ml) Precipitated by Homologous Casein, Whey and Milk per ml of Sera from the Sera of Male Rabbits Immunized with Weekly Injections of Homologous Milk Adjuvants.

Rabbit No.	Bleeding	Antigen added/ml of sera: Casein (μg N) 14	30	60	120	180	20	Whey (μg N) 40	80	100	160	Milk (dil.) 1-100	1-40	1-20	Hemagglut. titers†
7212	1/30/59*	8.4	0.6	5.2	2.4	4.8	—	4.4	11.2	13.2	7.2	13.2	21.6	22.0	2
"	6/24/59	36.4	57.6	41.2	41.6	33.6	8.4	—	3.6	14.4	6.0	68.8	235.6	180.8	64
7209	1/30/59	9.2	18.4	—	12.4	—	3.6	6.8	5.6	—	8.6	—	—	—	4
"	10/15/59	37.2	44.8	45.6	56.8	38.4	40.4	28.4	52.0	—	49.6	104.2	286.8	244.4	128

* First inj. given 1/31/59.
† Reciprocal of highest dilution of sera giving complete hemagglutination. Erythrocytes sensitized with homologous milk.

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TABLE II. Hemagglutinin Titers* of Sera from 5 Virgin Female Rabbits Immunized with Weekly Injections of Homologous Milk Adjuvant.

Rabbit No.	8/13/59†	8/14	9/2	9/23	10/12	11/25	1/12/60	2/25	5/16	6/21	7/1	7/14	7/21	7/30	Date of delivery
7681	2	8	8	4	32	8	128	32	64			16	16	8	7/15/60
7682	4	4	32	16	8	16	4	4							
7683	4	4	128	16	8	32	32	64	16						
7685	4	4	8	16	32	8	8	16	64						
7686	2	8	8	16	8	8	128	8	8	4	4		2		6/25/60

* Reciprocal of highest dilution giving complete hemagglutination. Erythrocytes sensitized with homologous milk.
† First inj. given 7/22/59.

tion of the antigens even in excess antibody. Hemagglutinins were measured by a modification of the Takatsy plate technic(18), employing a 1% solution of formalinized, tanned erythrocytes(19,20) sensitized with defatted rabbit milk (1:100 in saline). Five adult virgin female rabbits were injected with homologous milk-adjuvant and another 5 with homologous casein-adjuvant. The sera of both groups were tested for antibody by hemagglutination and precipitin tests at monthly intervals. Two of the female rabbits receiving milk and 4 receiving casein were then bred. Bleedings and breast biopsies were taken approximately 1 day, 1 week, 3 weeks and 5 weeks post partum. The tissue was fixed in 10% formalin and embedded in paraffin. Sections were stained with H & E and examined microscopically. Three does were injected with autologous milk-adjuvant. Two of these 3 were bled, bred and biopsied as described above. The sera of the pregnant animals were tested for antibody to whole milk by the hemagglutination technic. Control breast biopsies were also taken from 8 non-immunized does 1 to 22 days post partum.

Results. Homologous rabbit anti-milk antibody was demonstrated by precipitation and by hemagglutination in the sera of the 2 male rabbits immunized with milk-adjuvant (Table I). Precipitin tests were positive to casein, whey and defatted milk with serum from rabbit 7209 but positive only to casein and defatted milk with serum from rabbit 7212. Post immunization serum from rabbit 7209 formed 2 precipitin bands with whole milk in agar, while the serum from rabbit 7212 formed only one band with whole milk. Preimmunization sera failed to form any bands with whole milk in agar or to agglutinate erythrocytes sensitized with milk. Homologous anti-milk antibody was also demonstrated by hemagglutination and precipitation in the sera of the female rabbits immunized with either homologous milk in adjuvant (Table II) or homologous casein in adjuvant (Table III). Following delivery, 3 of the 6 rabbits immunized with homologous material showed what appeared to be a secondary rise in antibody titer. No circu-

TABLE III. Hemagglutinin Titers (HA)* and Precipitation (P)† of Sera from 5 Virgin Female Rabbits Immunized with Weekly Injections of Homologous Casein Adjuvant.

Rabbit No.	Date of bleeding‡ 8/13/59 (HA)	9/9/59 (HA)	9/23/59 (P)	9/23/59 (HA)	10/12/59 (P)	10/12/59 (HA)	11/20/59 (P)	11/20/59 (HA)	12/1/59 (HA)	12/15/59 (HA)	Date of delivery
7758	4	16	11.6	128	13.6	16	26.6	4	32	32	11/21/59
7759	4	64	8.8	8	11.2	128	7.6	32	4	32	11/24/59
7760	4	32	1.6	8	12.8	64	10.4	2	16	8	11/23/59
7762	4	64	2.0	16	13.2	16	29.6	16	32	8	11/23/59
7763	2	4	—	4	8.0	32	11.2	Died			—

* Reciprocal of highest dilution giving complete hemagglutination. Erythrocytes sensitized with homologous casein.

† Total μg N precipitated/ml of sera by addition of 22 μg casein N. The μg N precipitated by prebleeding sera (8/13/59) were subtracted from each value.

‡ First inj. given 8/14/59.

lating antibody could be demonstrated in the sera of the 3 rabbits immunized with autologous milk. Post partum breast biopsies of rabbits injected with either homologous or autologous material revealed no lesions that could be classified as "auto-allergic"(21). Some infiltration of plasma cells was observed between acini, but this infiltration was seen with equal frequency in control animals post partum.

Discussion. Following injection of 2 male rabbits with homologous milk incorporated in Freund's complete adjuvant, antibody to milk and casein appeared in the sera. The serum of one of the male rabbits also contained antibody to homologous milk whey. Female rabbits injected with homologous casein also formed circulating antibodies reacting with both whole defatted milk and casein. On the other hand, female rabbits immunized with autologous milk did not produce circulating antibody to autologous milk. Breast biopsies taken from lactating rabbits which had been previously injected with either homologous or autologous milk revealed no significant, consistent histologic abnormalities. This finding does not rule out the possibility that some forms of mastitis in humans might be "auto-allergic" in etiology; however, attempts to demonstrate circulating antibody in humans with plasma cell and chronic cystic mastitis also have been unrewarding(22). A suggestive case has been reported of a lactating mother who suffered from allergic reactions while weaning her child and had sever cutaneous reactions to her own mlik(23). This sensitivity could be passively transferred with her sera.

Summary. Injection of both male and female rabbits with homologous milk or casein in adjuvant resulted in formation of homologous anti-milk antibody demonstrated by hemagglutinin and precipitin technics. Injection of female rabbits with autologous milk did not result in formation of antibody to autologous milk. "Auto-allergic" breast lesions did not occur during lactation in females injected with either homologous or autologous milk.

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A Study of Lipoprotein Lipase Produced by Perfusion of Isolated Rabbit Heart with Heparin.* (26776)

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Intravenous administration of heparin increases a clearing factor in human and animal plasma. This clearance has been shown to be a result of the appearance in the serum of lipoprotein lipase (LPL), which catalyzes the hydrolysis of triglyceride moiety of chylomicron and low density lipoprotein(1). Using an isolated rat heart, Crass demonstrated the appearance of LPL in the perfusing solution, which consisted of 5% serum and 3 to 10 mg % heparin in Ringer buffer (2). In this paper we are interested in the metabolism of transport of lipides relating to the problem of atherosclerosis. The mechanism of appearance of LPL in circulating solution in presence of heparin and serum was studied.

Method. The rabbits used were 8-month-old males weighing 1.5 to 2.0 kg with hearts of 3.5 to 5.0 g. Sodium heparin contains 1,000 U of heparin per ml with a concentration of about 10 mg/ml. A rabbit was anesthetized with ether and exsanguinated. Its heart was immediately isolated and washed well with bicarbonate buffer of pH 7.4 (Krebs-Ringer). Care was taken to avoid introduction of air into the circulating system. The aorta was connected to a glass tube and then by rubber tube to a pump (Sigma motor T-6). Rubber tubing was used to connect the pump to the polyethylene tube which ran to the accepting flask. Rubber contamination appearing during contact with the pump was filtered off through a glass tube filled with gauze. O_2 gas was bubbled through the liquid in the flask at a rate of 2 to 3 bubbles per second. Except in the pump, temperature of the circulating liquid was maintained at 30 to 31.5°C by keeping the entire perfusion system in a water bath of 33°C. With the pump set to deliver 10 to 12 ml/min, the isolated heart contracted well for 2 to 3 hours. The circulating liquid consisted of varying concentrations of heparin and serum (described in Table I) in bicarbonate buffer of Krebs-Ringer (pH 7.4). At the start of the experiment total volume of circulating solution was 120 ml. Eight ml of perfusing solution was taken from the flask at intervals of 20 min and immediately stored at 0°C until determination of LPL activity.

Determination of enzyme activity. Three ml of perfusion solution were incubated with 0.5 ml of 3% oil emulsion (coconut oil : glucose : Tween 60 = 15 : 5 : 0.5), 0.5 ml of

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