The cuticle is impermeable to water despite the variety of chain length of the lipid molecules. The main barrier to water loss is believed to be a lipid monolayer in which the molecules are tightly packed (*9*). This crystalline degree of order would be difficult to achieve with the lipids of variable chain length which occur in insect cuticles. On the other hand, such mixtures might readily form liquid crystals and liquid monolayers. Critical temperatures for water loss could then be explained as phase changes in liquid-crystalline systems.

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Immune Reactivity in Mice Thymectomized Soon after Birth: Normal Response after Pregnancy

Abstract. *Female CBA mice that had been thymectomized soon after birth were mated with normal male T6 mice. After delivery of at least one litter, the parous females exhibited normal immune reactions to sheep erythrocytes and skin homografts. The transplacental passage of a humoral substance from the thymus glands of the developing fetuses is suggested as the mechanism responsible for the restoration of immunological responsiveness in neonatally thymectomized parous female mice.*

Mice thymectomized soon after birth characteristically exhibit diminished immune reactivity to certain particulate antigens and to skin homografts (*1, 2*). This diminished reactivity can be restored to normal in a variety of ways, such as (i) by intravenously injected isologous adult lymph node and spleen cells (*3*) and by relatively large numbers of isologous thymus cells (*4*), (ii) by subcutaneous grafts of intact isologus (*1*) or homologous (*5*) thymus tissue during the first week of life, and (iii) by intraperitoneal implants of intact isologous (*6, 7*) neonatal or embryonic thymus tissue in cell-tight Millipore diffusion chambers.

The foregoing restorative methods are also effective in preventing the wasting disease which is a common feature that follows neonatal thymectomy in some strains of mice. In the experiment reported here, pregnancy induced a restoration of immunological reactivity in neonatally thymectomized female mice.

Mice of the CBA strain were either thymectomized or given a sham operation within 16 hours of birth. At weaning, the females and males were separated, and only the females were used in subsequent procedures. When the females were 5 to 8 weeks old the absolute number of lymphocytes in the peripheral blood was determined for each mouse. At 8 weeks of age the neonatally thymectomized females were nonselectively divided into two groups. One group was mated with normal, healthy T6 males, whereas the other group was not allowed to mate and served as a control group. The female mice that received the sham operation comprised another control group and they also were not allowed to mate. After each female in the mated group had delivered one or two litters, another count of the absolute number of lymphocytes in the peripheral blood was performed on all the mice in the mated group, and the neonatally thymectomized control group. By this time all the mice were 13 to 17 weeks of age. Each mouse in all three groups was then challenged intraperitoneally with washed sheep erythrocytes (0.2 ml of a 20 percent suspension in saline). Ten days after challenge the titer of the serum antibody to sheep erythrocytes was determined, the blood having been obtained from the orbital sinus. Finally, each parous female was given a graft of full-thickness male T6 skin and Ak skin. The skin grafts were performed from 1 day to 1 month postpartum. Mice in the two control groups were grafted only with full-thickness Ak skin. After removal of the protective dressings on the eighth postoperative day, each graft was examined daily for visual evidence of rejection. From the time of weaning throughout the experimental period, each mouse was weighed twice weekly. Mice dying of wasting disease or those killed at the conclusion of the experiment were autopsied, and the retrosternal area was examined microscopically for thymus remnants. Only data from completely thymectomized female mice are included in the results.

Nine of the 17 mice which were mated with T6 males became pregnant, and each delivered at least one litter of mice prior to the 13th week of age. The other eight mice died of wasting disease before becoming pregnant or delivering any litters. Although the lymphocyte counts prior to mating of these eight mice are included in the results below, these mice were neither given skin grafts nor injected with sheep erythrocytes. Similarly, four of the 16 unmated control mice thymectomized at birth died of wasting disease before hemagglutination titers or skin homografts could be performed, and seven died before another count of the lymphocytes could be made.

The numbers of circulating lymphocytes were similar (Fig. 1) in the two groups of the thymectomized mice before they were nonselectively separated into a mated experimental group (17 mice) and an unmated control group (16 mice). In each group the lymphocyte ranged from 800 to 5100 per cubic millimeter, with a count of less than 4000 in most mice. The lymphocyte counts in ten 6- to 8-week-old sham-thymectomized mice ranged from 4400 to 10,400 per cubic millimeter, with all but one of the mice having a count of more than 6600. The number of lymphocytes after birth ranged from 2300 to 4800, whereas at a corresponding age in the neonatally thymectomized control group the count ranged from 1100 to

4600. Thus there was no significant change in the absolute number of lymphocytes postpartum in the mated mice as compared with either the number present prior to pregnancy or the number found in neonatally thymectomized control mice of a similar age.

The neonatally thymectomized unmated mice displayed a markedly impaired ability to reject Ak skin homografts, 11 of 12 such mice retaining the grafts for more than 15 days. All of the parous females rejected both the Ak and T6 skin homografts within 15 days (Table 1). The parous mice thus rejected skin homografts within the same time interval as did the group of ten mice that received the sham operation.

Seven of the 12 thymectomized controls tested failed to show any detectable hemagglutinins, and the other five showed a markedly diminished response. However, the hemagglutinin titers in eight of the nine parous females were normal and of the same order as those seen in the controls that received the sham operation. One parous female showed a markedly diminished hemagglutinin response.

In all, nine of the 16 neonatally thymectomized unmated female mice died of wasting disease before the 15th week of age, whereas the nine parous females were all still healthy at more than 17 weeks of age.

Thus the immunological reactivity of neonatally thymectomized CBA mice is restored to normal by one or more of the processes accompanying pregnancy. Moreover, this restoration is accomplished without a significant change in the numbers of circulating lymphocytes.

At least two possible mechanisms, one or both of which may play a role in the restoration by pregnancy of neonatally thymectomized mice, can be postulated. The developing fetus may influence, by way of the placental circulation, the maternal immunological system by either a humoral or a cellular contribution. That a humoral factor from the thymus can induce the development of normal immune reactivity in neonatally thymectomized animals has been shown by the intraperitoneal implantation of intact embryonic and neonatal thymus tissue in cell-tight Millipore diffusion chambers (*6, 7*). Experiments with subcutaneous homologous thymus grafts have also led to a similar conclusion (*5*). On the other hand, fetal erythrocytes can enter the maternal circulation (*8*), and also maternal white cells, platelets (*9*), and leukemic cells (*10*) can sometimes be found in the circulation of a newborn infant. However, in view of the design of this experiment, the passage of fetal immunologically competent cells is not a likely explanation for the observed results. In this experiment the mating of a neonatally thymectomized CBA female with a T6 male results in (CBA × T6)F_1 offspring. If significant numbers of (CBA × T6)F_1 lymphoid cells had entered the maternal circulation, it is unlikely that there would be immunological rejection of these cells because of the maternal immunological unresponsiveness. Thus, a lymphoid chimera would have been created bearing both CBA and T6 characteristics, and subsequent acceptance of T6 skin homografts would be expected. However, T6 homografts were rejected just as well as grafts from a third source (Ak) were rejected. In addition, had fetal lymphoid cells entered the maternal circulation, it is unlikely that such cells would have attained immunological maturity in a host whose own cells had not developed the capacity to take part normally in immune reactions. Thus, the observed results favor the possibility that a humoral substance from the thymus glands of developing fetuses induced a change in the maternal lymphoid tissues, leading to a restoration of normal reactivity. That this restoration was accomplished without an appreciable change in the number of circulating lymphocytes before mating supports similar observations in neonatally thymectomized mice bearing thymus in diffusion chambers (*6*).

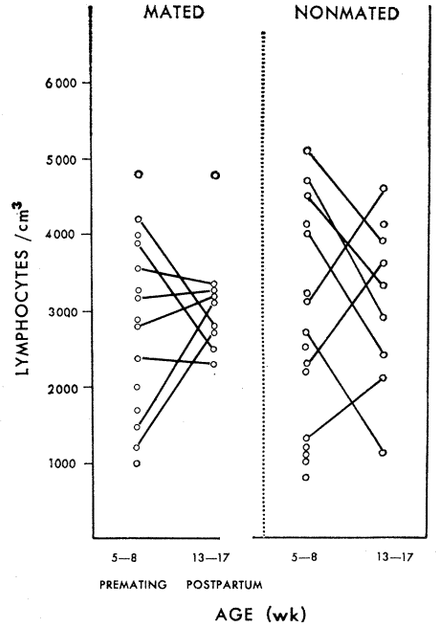

Fig. 1. The absolute number of blood lymphocytes in two groups of neonatally thymectomized female CBA mice. (Left) The number during the premating period and postpartum in mice that were mated with T6 males. (Right) The number in the nonmated control group. The lines connect the two values obtained in each mouse. Open circles not connected by lines represent single determinations in mice which did not survive long enough to have a subsequent determination.

Table 1. Survival of skin homografts in neonatally thymectomized female CBA mice. In the mice mated with T6 males the grafts were placed postpartum.

Graft	No. of mice accepting grafts		
	<15 days	15 to 40 days	>40 days
Sham thymectomy			
Ak skin	10	0	**0**
Thymectomy			
Ak skin	1	4	**7**
Thymectomy, mated with T6 males			
Ak and T6 skin	9	0	0

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