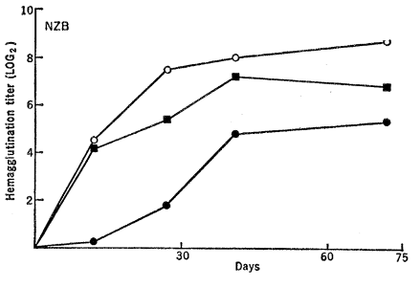

Fig. 1. Hemagglutination titers (log_2) for weanling and young adult NZB mice given either BGG or egg albumin. Open circles, 10.5 mg of egg albumin given at 18 or 43 days of age; solid squares, 10.5 or 24.5 mg of BGG given at 43 days of age; solid circles, 10.5 or 24.5 mg of BGG given at 18 to 25 days of age.

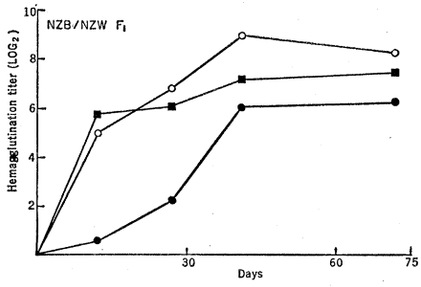

Fig. 2. Hemagglutination titers (log_2) for weanling and young adult NZB/NZW F_1 mice given either BGG or egg albumin. Open circles, 10.5 mg of egg albumin given at 18 to 20 days of age; solid squares, 10.5 or 24.5 mg of BGG given at 30 days of age; solid circles, 10.5 or 24.5 mg of BGG given at 18 to 20 days of age.

of 18 to 25 days are capable of becoming tolerant to large systemic doses of ultracentrifuged BGG, but that, once again, somewhat older mice of these same strains show a marked resistance to the development of immunological tolerance (*2*). A striking observation was the rapid loss of tolerance in the younger mice not seen with the age-matched C3H animals. By the end of this experiment (72 days), all NZB and B/W F_1 mice previously tolerant now had antibody titers approaching those of control animals.

This escape from tolerance implies that the immune system of the NZB mouse, as it develops, loses its capacity to remain immunologically unresponsive. This could be due either to the generation of an entirely new cell line capable of responding to the antigen in question, or to the recovery of a previously tolerant or repressed population of antigen-reactive lymphocytes. An accelerated recruitment of immunologically competent stem cells, or the action of a thymic trophic factor, or both, might be responsible for either of these cellular events. Claman and McDonald have shown that the thymus plays a role in regulating recovery from immunologic unresponsiveness (*3*), and others have shown the importance of the thymus in establishing immunologic competence (*4*).

In the NZB and B/W strains, there is a remarkable correlation between relative resistance to tolerance induction in young adult mice and rapid loss of tolerance previously induced in weanlings. This correlation between abnormalities of tolerance induction and duration suggests a common pathologic mechanism.

The rapid loss of tolerance in weanling mice has certain implications for the pathogenesis of serologic autoreactivity. Our results with BGG provide an experimental instance whereby the NZB and B/W F_1 mice lose tolerance to an antigen which, in a nonautoimmune strain, leads to long-term and profound suppression of antibody formation. If the same relation pertains to autoantigens, then initial tolerance to the more immunogenic determinants of these antigens might likewise be lost over a period of time.

Finally, although in our study there was a significant difference between weanling NZB, B/W F_1, and comparable C3H mice, exposure of NZB or B/W F_1 animals to self or to foreign antigens at still earlier times of life might render them tolerant for longer periods of time. The timing and extent of exposure to antigen will obviously be very critical in any attempt to restore or maintain self-tolerance in these mice.

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Thymus-Dependent Lymphocytes: Destruction by Lymphocytic Choriomeningitis Virus

Abstract. *Selective destruction of small lymphocytes in the thymus-dependent areas of lymph nodes and thymocytes was observed in mice infected with lymphocytic choriomeningitis virus. These changes were clearly evident in lymphoid and splenic tissue 3 days after infection and in the thymus by day 7. The destructive changes paralleled growth of the virus in these organs. The findings show that infection with lymphocytic choriomeningitis virus can temporarily cause the equivalent effect of neonatal thymectomy, that is, a "viral thymectomy," which appears to be related to the ability of this virus to cause persistent infection.*

The means whereby certain viruses sometimes cause disease, but at other times produce inapparent persistent infection of the adult animal, is not completely understood. It now appears from the work reported here that one such agent, lymphocytic choriomeningitis (LCM) virus, may do this by damaging, in the first few days after infection, those immune cells that normally exterminate the virus.

The pathogenesis of LCM virus infection of the mouse has long been known to involve a lymphocytic infiltration of the tissues (*1*). Tissue damage, illness, and recovery from the virus infection are believed to be due to a cellular response (*2*) involving a delayed hypersensitivity reaction with rejection of infected tissue similar in mechanism (*3*) to the homograft response. Agents known to reduce the cellular immune response ameliorate or abolish both acute clinical disease, mortality, and histopathology but not growth of the virus; these include cortisone (*4*), x-irradiation (*5*), amethopterin (*3, 6*), thymectomy (*7–9*), and antiserum to lymphocytes (*10, 11*). Inoculation of viscerotropic LCM virus close to or before the time of birth induces immunological tolerance to the virus and a persistent infection without the normal LCM disease (*4, 12*); inoculation of the adult animal with large doses of this virus induces a similar but less permanent state (*13*) which we refer to as high dose immune paralysis (HDIP). Conversely, grafting the tolerant animal with mature isologous lymphocytes has been shown to

cause gradual suppression of the virus and abolition of tolerance (*14*). All of these observations point to the key role played by the lymphoid organs in the pathogenesis of LCM disease and its persistent tolerated infection (*15*). Careful attention was therefore paid to the spleen, lymph nodes, and thymus during a study of HDIP mice. Spleens and thymuses of these animals showed marked diminution in size, the thymuses being reduced in weight by a factor of almost 4 in the virus-infected group compared with uninoculated controls. A histological study was therefore made of spleen, lymph nodes, and thymus from mice with acute infections caused by neurotropic or viscerotropic strains of LCM virus.

Female Nylar-A mice weighing 14 to 16 g were inoculated intracerebrally with approximately 1000 MID_{50} (mouse infective dose, 50 percent effective) of viscerotropic (M/B_6L_{11}) or neurotropic (M/B_8) strains of the W.E. strain of LCM virus (*13*). On days 1, 2, 3, 4, 5, 7, and 10, three mice infected with each virus were killed and fixed in formalin after the spleen, thymus, and mesenteric lymph node were weighed. Histologic examinations were made on sections, stained with hematoxylin and eosin, of mesenteric and deep axillary lymph nodes, spleen, thymus, and intestine (Peyer's patches). In addition 20 percent (weight/volume) organ suspensions were made (with the use of separate glass homogenizers) of spleen, thymus, and mesenteric lymph node (separately pooled) from three additional mice from each virus group. Titration of the virus in the organs and serum was made by inoculating mice intracerebrally with the suspensions and estimating the MID_{50} by the method of Reed and Muench. Mice inoculated with normal mouse brain or liver and uninoculated mice served as controls.

Two days after virus inoculation the earliest histological change was visible as a small amount of cell debris in the cortex of lymph nodes and in the white pulp of the spleen around the central artery. The small lesions were visible as pale areas of reticulum cell mesh with a relative absence of small lymphocytes.

In lymph nodes at 3 to 5 days after inoculation small pale-appearing lesions consisting of the mesh of reticulum cells were clearly evident in the cortex, especially near the marginal cortex (peripheral area of the cortex along the marginal sinus) (*16*) or medullary cord (Fig. 1a). In these areas only a few scattered small lymphocytes could be seen and there was some cell debris. Secondary nodules became ill-defined or disappeared in most lymph nodes. The marginal cortex became hyperplastic and was composed mainly of large basophilic and medium-sized lymphocytes. Reticulum cells with a prominent nucleolus were slightly increased, and there were occasional macrophages phagocytosing the cell debris. In the area around the postcapillary venules similar but less prominent findings were observed. Little or no change was seen in the medullary cord or sinus. By the 7th day, destruction of remaining small lymphocytes was prominent in the whole lymph node with extensive cell debris and phagocytosis (Fig. 1b). At the same time, the marginal cortex showed a complex pattern of proliferation of reticulum cells and large basophilic and medium-sized lymphocytes mixed with areas of extensive destruction of small lymphocytes. The sinus showed a profusion of lymphocytes, corresponding to the absence of changes in the Peyer's patches. By 10 days, small lymphocytes were almost completely absent from the cortex and medullary cord; proliferative changes of reticulum cell and large basophilic lymphocytes continued in the marginal cortex.

Changes in the white pulp of the spleen were parallel to those found in the lymph nodes. After 3 days, the small lesions enlarged around the arterioles in the white pulp (thymus-dependent area) (Fig. 1c). Increased mitoses were observed in the germinal centers. By the 4th to 7th days, suppression of small lymphocytes was marked in the whole lymphoid sheath and follicles. In contrast, reticulum cells increased, especially in the perifollicular zone. After 7 days, small lymphocytes had almost completely disappeared from the white pulp; reticulum cell proliferation continued.

No changes were detected in the thymus before the 5th day after infection. By day 7, destruction of cortical thymic lymphocytes became obvious with the production of much cell de-

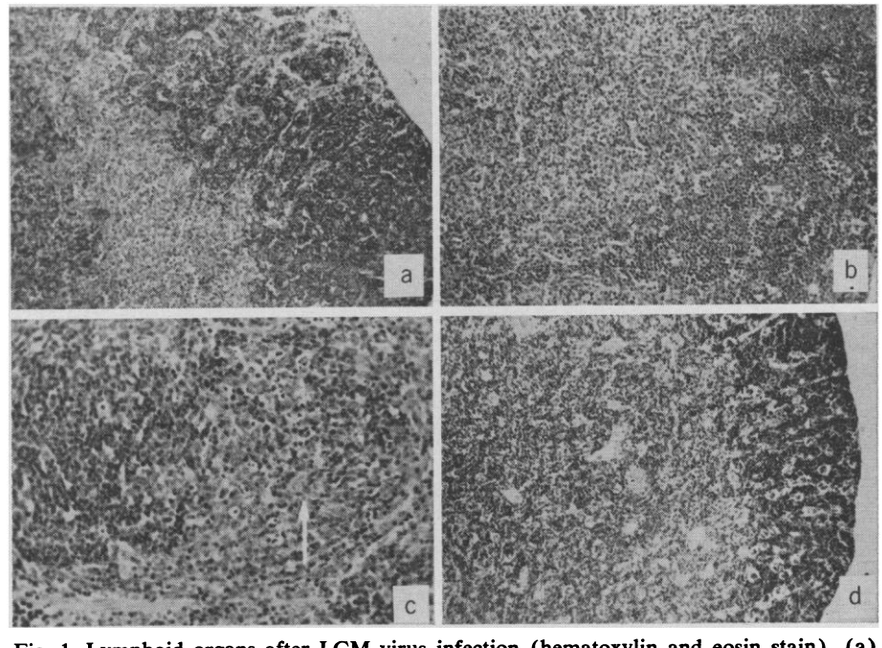

Fig. 1. Lymphoid organs after LCM virus infection (hematoxylin and eosin stain). (a) Lymph node 4 days after infection showing focal appearance of lesion in the cortex. There is no prominent change in the central (extreme left) and marginal (lower right) area of the cortex and medullary cord (upper right) (× 62). (b) Lymph node 7 days after infection showing almost complete disappearance of small lymphocytes from the thymus-dependent area of the cortex (central and upper left area). In the marginal area (right side) destruction of small lymphocytes is beginning and proliferation of large basophilic and medium-sized lymphocytes can be seen (× 80). (c) Spleen 3 days after infection showing destruction and disappearance of small lymphocytes from the thymus-dependent area around the central artery (arrow) (× 124). (d) Thymus 7 days after infection showing prominent destruction of thymic lymphocytes in the cortex which is thinner than normal. There is no prominent change in the medulla (× 63).

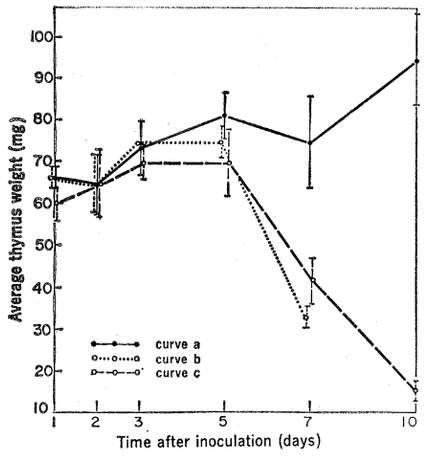

Fig. 2. Change in weight of thymus during LCM virus infection. Curve a, uninoculated control; curve b, neurotropic virus; and curve c, viscerotropic virus. Vertical bars represent standard deviations.

bris (Fig. 1d). This process continued until day 10 and thymic lymphocytes had almost completely disappeared from the cortex, but moderate numbers of small lymphocytes were present in the medulla.

No significant difference in lymphoid organs could be observed between mice infected with viscerotropic or neurotropic strains of the virus.

Change in the weight of the thymus during infection with LCM virus is shown in Fig. 2. At 7 and 10 days a marked depression of thymus weight was observed, reflecting an atrophic change to almost one-fourth of the control weight.

Similar graphs of the weights of the spleen and lymph nodes showed no significant difference from control values, since they overlapped within one standard deviation from the mean. Even so, the weight of lymph nodes

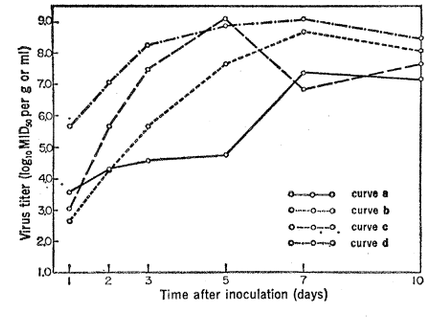

Fig. 3. Titers of viscerotropic LCM virus in lymphoid organs and serum. Curve a, serum; curve b, thymus; curve c, mesenteric lymph node; and curve d, spleen.

from mice infected with the viscerotropic virus was consistently lower. Between the 7th and 10th days the animals showed sickness, such as ruffled fur, hunched posture, and diarrhea, and all of the mice infected with neurotropic virus died, whereas mortality in the viscerotropic virus–infected group was only 30 percent by day 10.

As shown in Fig. 3, the titers of viscerotropic virus in spleen, lymph nodes, and thymus increased steeply to reach a peak at approximately 10^9 MID_{50} on day 5 or 7. The peak titer occurred first in lymph node on day 5, then in thymus on day 7, corresponding to the sequence of the formation of lesions. Virus titer in the spleen showed an early rise, reaching a peak on day 7. On the other hand, the virus titer of the serum rose much more slowly during the early phase of infection.

The growth of neurotropic virus in lymphoid organs was almost the same as that of viscerotropic virus. However, virus titers were usually lower by a factor of 10- to 100-fold, particularly in the serum.

The primary lesion due to LCM virus was in the thymus-dependent area of lymph nodes and spleen and is similar to the lesion in thymectomized mice (*17*). However, an additional finding in LCM virus infection is the proliferation of reticulum cells and large basophilic lymphocytes in the marginal cortex. The fact that very high doses (10^4 to 10^6 MID_{50}) of LCM virus can lead to persistent infection (*18*), while smaller doses kill, has puzzled virologists. The primary lesion could cause HDIP by damaging the cellular immunity for which small lymphocytes are responsible (*19*). This "allergic" cellular response is now generally accepted (*3, 7, 10, 11, 20*) as the cause of virus suppression, the histological changes of LCM virus infection, and also for the clinical symptoms and death of the animal. Thus, any agent, even large amounts of LCM virus itself, which can reduce the intensity of the cellular immune response will tend to cause LCM infection to become a persistent rather than a lethal infection in the mouse. In this light, the HDIP effect is similar to a low dose of virus plus neonatal thymectomy (*7–9*) or to a low dose plus antiserum to lymphocytes or thymocytes (*10, 11*). The more extensive viremia found with the viscerotropic virus may explain the greater survival associated with this strain (*20*), if, as would seem likely, higher doses of virus and viscerotropism cause greater destruction of small lymphocytes in the thymus-dependent area. A quantitative study might reveal differences in the severity of the histological lesions between the two virus strains used.

There appears to be some parallel between the action of LCM virus upon thymus-dependent lymphocytes and that of Friend virus upon antigen-sensitive cells (*21*) that exhibit a marked decrease in antibody formation after infection. However, Friend virus infection appears to damage the precursors of plasma cells and reticulum cells rather than the small lymphocytes in the thymus-dependent areas which are the target of LCM virus. Production of antibody in acute infection with LCM virus has been found in our laboratory (*22*) to be even more rapid after large doses of LCM virus leading to HDIP than after lethal or small immunizing doses. This correlates with the impression that the main lesion of LCM virus infection concerns cellular rather than humoral immunity (*23*) and with the observations of other workers (*18, 24*) that cellular and humoral immune responses are quite separate in LCM virus infection, allowing split tolerance to occur (*11*).

The results with this virus suggest that the induction of early lesions of the lymphoid system is one of the mechanisms whereby partial tolerance toward viruses can be induced. By this means the host-parasite balance is disturbed in favor of persistence of the parasite.

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12 November 1968

Free-Energy Transfer in Plants

Abstract. *Free-energy transfer was used to study water transport through the soil-plant system. Resistances to free-energy transfer are proportional to resistances to water transfer. Under certain conditions, the proportionality factor is 1. For a sunflower plant in moist soil, plant resistance to free-energy transfer was 30 times the soil resistance, and root-stem-leaf resistances were in a ratio of about 2 : 1 : 1, respectively. However, root and leaf resistances were equal when considered for a unit pathlength.*

The movement of water in living organisms has two distinct aspects: water entry into single cells and water transport among arrays of cells. Little is known about the second aspect, particularly with regard to transport through whole plants.

The liquid pathway from the soil through the plant is composed of a set of resistances in series. Studies have been devoted to water movement through the soil (*1*), root (*2, 3*), and the vascular system and leaf (*3, 4*), but comparative data for the entire pathway are unavailable. I now describe a way of measuring resistance, which is based on the transfer of free energy through the plant, and this concept is used to compare resistances in various portions of the soil-plant transport system.

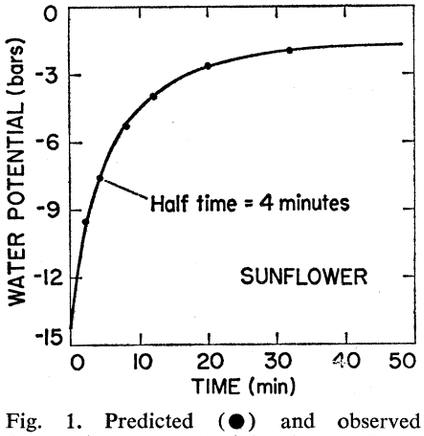

Fig. 1. Predicted (●) and observed (———) water potentials of a sunflower leaf recovering from a water deficit. Resistance to the transfer of free energy is determined from the half-time for recovery.

I have reported simultaneous recovery curves for water uptake and water potential in nontranspiring leaves recovering from water deficits (*5*). Most leaves had different and apparently unique kinetics of water uptake with time; however, the kinetics of changes in water potential appeared similar. Because a simple phenomenon seemed to govern changes in water potential, I have studied the water potential data, with the hope that the results could be applied to the flow of water through the plant.

The rise in water potential that occurs as a leaf recovers from a water deficit represents a transfer of Gibbs free energy to the leaf. Knowing the volume of water in the leaf and the change in potential with time permits an estimate of this transfer. Changes in potential at constant temperature are related to the energy dissipation (dS/dt) of irreversible thermodynamic processes by:

$$V(d\psi/dt) = dG/dt = -T(dS/dt) \quad (1)$$

where V is the average volume of water in the leaf (cm^3), ψ is the water potential of the leaf (bars), t is the time (seconds), G is the Gibbs free energy of water in the leaf (bar cm^3; 1 bar $cm^3 = 10^6$ erg), T is the Kelvin temperature, and S is the entropy (bar cm^3 deg^{-1}). The mathematical treatments of energy dissipation in heat and diffusive transfers are similar, and the diffusion of deuterated water follows these laws in some tissues (*6*). Thus, similar principles may be applicable to the free-energy transfer observed in leaves, particularly in view of the proportionality between changes in water potential and energy dissipation (Eq. 1).

Leaves are usually thin, largely two-dimensional arrays of reasonably homogeneous cells. They may be idealized to two infinite plane sheets of cells, one above a plane representing the vascular system and one below this plane. Since the vascular plane is generally located midway between the upper and lower epidermis, the pathlength (l, cm) for free-energy transfer to either epidermis would be half the leaf thickness. According to these boundary conditions, a leaf with a low and initially uniform water potential should follow the heat or diffusive transfer equation for a plane sheet (*7*) and should permit calculation of the resistance to free-energy transfer (r):

$$r = t_{1/2}/0.195l \quad (2)$$

where $t_{1/2}$ is the half-time for recovery (seconds).

To determine whether leaves behave as plane sheets during free-energy transfer, the appropriate transfer equation (*7*) was used to predict leaf-water potentials at various times during recovery from water deficits. Water potential was measured by sealing the blade of a slightly wilted sunflower leaf in a thermocouple psychrometer that estimates potentials of intact leaves (*5*). After the first measurement, the petiole of the leaf was cut under degassed water, and leaf water potential was followed during the subsequent water uptake. There was excellent agreement between the observed water potentials and those predicted from the transfer equation (Fig. 1). The same results were obtained with leaves of soybean, garden bean, papaya, abutilon, cottonwood, sugar maple, and tomato, as well as sunflower. Thus, free-energy transfer through planar leaves approxi-