This is the result of the convection term dominating the energy budget for small leaf sizes. Leaves larger than 1 by 1 cm may have temperatures very much above air temperature, particularly if the leaf's internal resistance to moisture is large. It would seem that there may be a physiological advantage for plants in arid or semiarid regions to have small leaves. Apparently, *Opuntia*, having large blades and using little water, has evolved a protein structure which is stable at high temperatures.

Measurements of surface temperature were made with a new pistol-grip infrared radiometer designed by the staff of Barnes Engineering Corporation. The radiometer weighs 1.1 kg, has a field of view of 20°, a time constant of 2 seconds, and an aperture of 1.2 cm. A germanium filter cuts out reflected sunlight, but the radiometer should not be used at the angle of specular reflection to the sun. Surface temperatures from −10° to 60°C were measured and were accurate within ± .25°C. Surface temperatures of animals, plants, soil, and rocks, and sky and cloud temperatures were measured with the new radiometer.

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Adoptive Autoimmune Encephalomyelitis in Inbred Guinea Pigs: Immunological and Histological Aspects

Abstract. *Major variables which determine the induction and severity of adoptive autoimmune encephalomyelitis are the age and strain of the animal, and the amount of killed mycobacteria in the adjuvant. Control of these factors results in consistent production of this disease in high incidence and in severe form. The pathologic changes in the central nervous system can be correlated with the clinical disease. Maturity of the target tissues in the central nervous system of the newborn appears to be an important factor which distinguishes the response of the guinea pig from that of other species.*

Adoptive transfer (*1*) of autoimmune encephalomyelitis (AE) between histocompatible guinea pigs (strain 13) has been regularly used recently to elucidate the mechanisms of autoimmune diseases (*2*). Nevertheless, the ease of reproducibility and the high incidence and severity of the passive AE has not been duplicated with other autoimmune systems (*3*), even when the same strain of guinea pig was used. This discrepancy has given rise to questions about the nature of the AE produced by adoptive transfer of lymph node cells in inbred guinea pigs. Difficulties encountered by others in inducing passive AE have warranted a delineation of the experimental variables which determine the outcome of appropriate transfers of adoptive autoimmune disease.

Strains 2/N and 13/N guinea pigs were used; adults were used as donors of lymph node cells (*4*) and animals of various ages were used as recipients. Donors were immunized with either guinea pig or rabbit spinal cord in complete Freund's adjuvant. Each animal received 0.5 ml of a water-in-oil emulsion, incorporating 0.25 ml of 50 percent suspension of spinal cord in phenol water, 2.5 mg of killed *Mycobacterium tuberculosis*, and 0.25 ml of the oil phase (Arlacel A–Bayol 55) unless otherwise noted. A single immunizing dose was given intradermally into several sites in the nuchal region and the footpads. Most transfers were carried out 5 days after sensitization of donors. The procedure of lymph node transfer was essentially the same as previously reported, except that, instead of stainless steel mesh, squares of nylon stocking were used to obtain uniform single-cell suspensions, and that, instead of Hanks's balanced salt solution, Eagle's Spinner medium containing glutamine was used (*4*). Recipient animals were weighed daily and observed for signs of clinical disease. Brain, spinal cord, and other tissues were taken for histologic examination at the time of death or of killing when moribund. Death from adoptive AE occurred usually from 8 days to 2 weeks after transfer.

Table 1. Effect of strain and age of guinea pigs on incidence of adoptive AE. Donors and recipients were of the same strain. The results are expressed as the number of afflicted out of the total number tested. All donors were adults or old adults.

Recipient	Incidence of AE	
	Clinical	Death
	Strain 13	
Newborn	9/9	6/9
Young	23/27	16/27
Adult	14/14	13/14
Old adult	7/16	4/16
	Strain 2	
Young	0/9	0/9
Adult	0/3	0/3
Old adult	0/8	0/8

Guinea pig spinal cord has been shown to be a more potent antigen than rabbit spinal cord in producing disease by active immunization (*5*); however, no difference attributable to these antigens was noted in the recipient animals under the conditions of these adoptive transfer experiments. This may be due to the fact that the donor animals had attained a size in which maximum sensitization occurred when either antigen was used. Consequently, for each age and strain group, the findings based on guinea pig and rabbit spinal cord antigens were pooled.

Successful transfers are more difficult with older recipients (Table 1). This is consistent with Celada's report of a barrier to syngeneic transfer of immune cells which increased markedly with age (*6*). However, in the guinea pig this might result from a dilution factor associated with a greater net increment of tissue of the central nervous system (CNS) or the reticuloendothelial system in the larger recipients. The resistance of strain 2/N animals to active AE is marked (*7*) but not absolute; full-grown female strain 2/N animals immunized with homologous antigen regularly develop florid, acute AE (*5*). However, even under optimum conditions for age and antigen (as with full-grown adult donors to young recipients), transfers between strain 2/N guinea pigs resulted neither in overt clinical disease nor in histologic changes in the CNS without clinical disease (*8*).

As noted, 2.5 mg of mycobacteria per guinea pig were used routinely in the adjuvant for sensitization. Decreases below 1.0 mg caused a striking reduction in incidence of adoptive AE in recipients (8 of 9 had lethal AE at 1.0 mg; 0 of 9 had no signs of disease

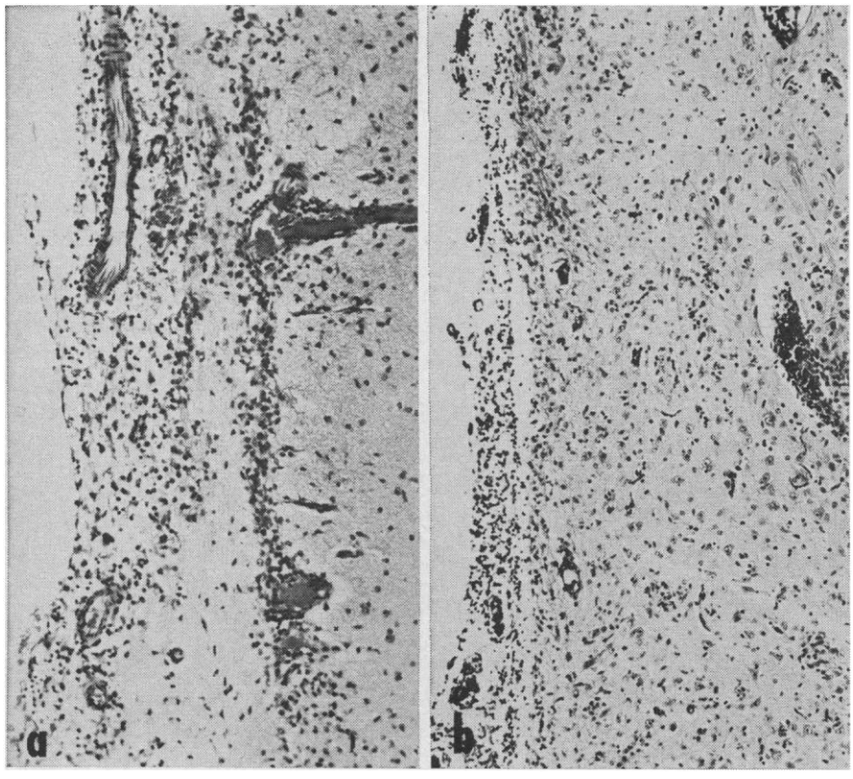

Fig. 1. Inflammatory changes in adoptive AE: brain of newborn (a) and of adult (b). Similar infiltrates in meninges and perivascular spaces of both. Hematoxylin and eosin; (a) × 94; (b) × 73.

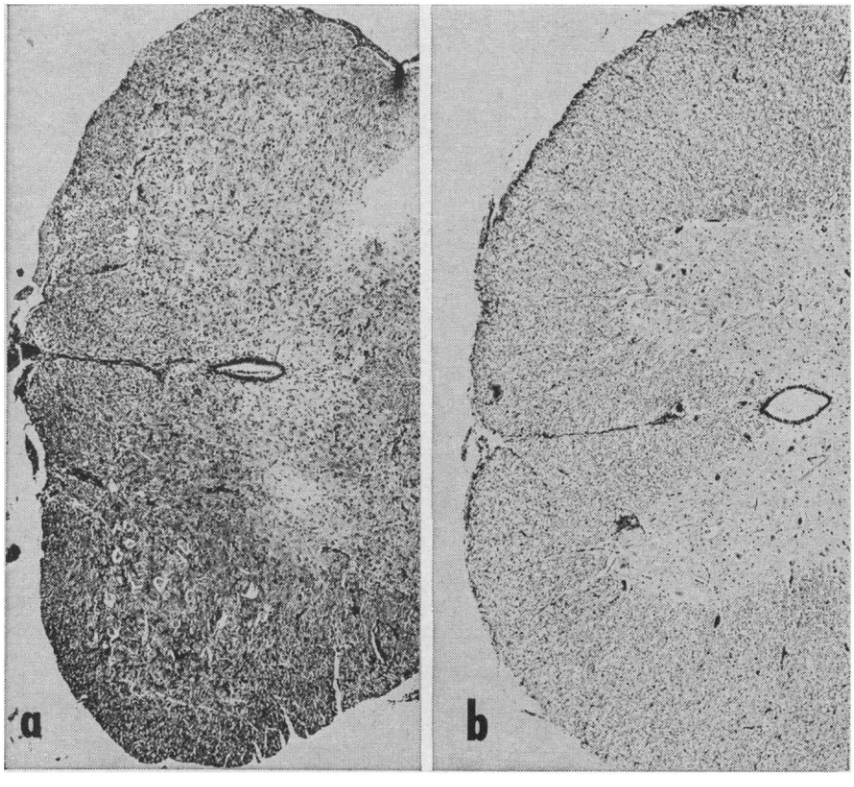

Fig. 2. Inflammatory changes in adoptive AE: spinal cord of newborn (a) and of adult (b). Infiltrate in newborn (a) is similar to Fig. 1. Infiltrate in adult (b) is minimal and in perivascular spaces only. Hematoxylin and eosin, × 40.

at 0.2 mg of killed *Mycobacterium tuberculosis*). Since 0.2 mg of killed *M. tuberculosis* is ample for induction of active AE (*9*), the high dose requirement for adoptive AE probably reflects the early transfer, with a concomitant short period of drainage of the mycobacterial adjuvant from the injection site to the draining lymph nodes harvested for transfer. As emphasized (*1*), an early time of transfer appears crucial for adoptive AE and probably for transfers of other autoimmune diseases (*3*).

Although the production of adoptive clinical disease has been documented (*1*), the pathologic changes in the CNS of adult guinea pigs that received transfers of lymph node cells have not been previously described. The pertinent histology for the adult recipient is essentially that reported for the newborn recipient (*2*), with the notable exception that the spinal cord in the former shows virtually no involvement. The characteristic histologic changes of acute active AE—namely a variable inflammatory cellular infiltrate throughout the brain, brainstem, meninges, and choroid plexus—have been described by Freund *et al.* (*10*) and others. The inflammatory cells are predominantly lymphocytes and histiocytes, and frequently show a perivascular distribution. The changes in the brain of the newborn guinea pig dying with adoptive AE are shown in Fig. 1a and in the adult in Fig. 1b. The same type of inflammatory change, involving meninges and perivascular space, is seen in the spinal cord of newborn animals (Fig. 2a), but is usually absent from the spinal cord of adult animals (Fig. 2b).

In the newborns of strain 13/N the low susceptibility which leads to an extremely chronic form of AE after active sensitization (*2*) contrasts sharply with the response of full-grown strain 13/N which manifested acute and lethal disease. It was therefore striking that the neonatal recipients not only developed disease, but that they regularly developed adult-type, acute lethal AE, since most passive diseases have been found less severe than their active counterparts. This provides strong evidence for the maturity of the target organ in neonatal strain 13/N guinea pigs. However, a predilection for spinal cord as well as brain in the neonates, as opposed to lack of spinal cord lesions in the adult, is suggestive of a relative degree of immaturity of

either myelination or blood-brain barrier (that is, more myelination or less barrier in spinal cord relative to brain in the newborn guinea pig).

The pattern of disease is significantly different in newborns of other species, where myelination of the CNS is less well developed at birth and in the neonatal period (*11*, *12*). In the neonatal monkey, the severe lesions of AE are found primarily in the hindbrain (paleoencephalon) and spinal cord (*13*), those regions which first become myelinated during embryonic development (*12*). This is in contrast to the high incidence of severe lesions in the cerebral hemispheres of adolescent and mature rhesus monkeys (*14*). The age-influenced susceptibility of a localized portion of the central nervous system to the induction of AE in the monkey is analogous to that described for the guinea pig (*11*, *12*). In the neonatal rat AE cannot be produced, and the CNS of neonatal rats appears devoid of encephalitogenic activity (*11*). These findings can also be correlated with a delay in myelination in the monkey and the rat, and thus provide further evidence for the concept of maturation of the CNS as a target organ being one of the necessary conditions for the induction of this disease. Whether a threshold amount of myelin alone, or whether maturity of the myelin already present as well, is required to provide the target organ is not known.

The role of maturity of the target organ has been considered above mainly in terms of degree of myelination; still to be examined are the maturation and general role of the blood-brain barrier in the mechanism of this disease. Thus, an age dependency might reflect differences in maturity of the route of sensitized cells, if not a humoral component, to the specific target organ.

Whatever mechanism causes the higher susceptibility of the paleoencephalon in neonatal animals, the greater success of transfer of AE with younger animals is not necessarily due to the radiosensitive barrier phenomenon (in mice) which apparently involves the receptivity of the environment of transplanted cells (*6*). The status (size and age) of the CNS as target organ may be equally important.

Thus, the failure to transfer AE in strain 2/N guinea pigs even when the adult donors are of an age and sex which regularly develop active disease (*8*), and when the recipients are as small as those strain 13/N guinea pigs which regularly show adoptive AE, would indicate that the young strain 2/N guinea pigs do not have a susceptible target in the CNS. This conclusion is especially tempting since such transfers of cells yield good adoptive tuberculin sensitivity in recipient strain 2/N animals. The test for tuberculin sensitivity determines that a given portion of transferred cells is indeed viable in the recipient. In addition, pools of cells are tested (*1*) for significant amounts of antigen by injection of portions from the pool into random-bred (Hartley strain) recipients. In that Hartley guinea pigs are as susceptible as, or more susceptible than, strain 13/N guinea pigs, they would show active AE if too much antigen were transferred with the cells. This controls for the possibility of active disease, since such histo-incompatible recipients cannot develop adoptive AE.

The foregoing experiments delineate the major variables now known to influence adoptive AE in guinea pigs; they furthermore serve to emphasize the simplicity and ease of reproducing the adoptive disease under the experimental conditions originally outlined (*1*).

The definition of the experimental variables operative in this autoimmune disease should be generally applicable in elucidating the role of cell-bound antibody in other diseases of immunologic etiology (*15*), and this procedure is suggested as a prototype for the induction of adoptive disease with other experimental models.

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15. In contrast to the ease of transfer of AE, the difficulties encountered in attempts to transfer other autoimmune diseases (*3*) may be due to the chronicity and reversibility of a disease such as autoimmune thyroiditis contrasted to the acute and irreversible nature of AE.

1 December 1967

Mitochondrial Malate Dehydrogenase: Reversible Denaturation Studies

Abstract. *The malate dehydrogenase isoenzymes from the mitochondria of chicken hearts have been partially resolved into separate pools. Reversible denaturation, in concentrated guanidine hydrochloride, does not change the isoenzyme distribution in each pool. This result suggests that the electrophoretic differences among the isoenzymes are not solely conformational in origin.*

There is now considerable discussion of those enzymes whose multiple molecular forms are thought to be due to conformational variations of covalently identical proteins, and the term "conformers" has been suggested for these groups of isoenzymes (*1*). This conformer hypothesis has been applied to explain the existence of the multiple forms of chicken heart mitochondrial malate dehydrogenase (*1*), human placental and leukocyte mitochondrial malate dehydrogenase (*2*), *Neurospora* malate dehydrogenase (*3*), and chicken brain creatine kinase (*4*). Conformers may be considered to be special cases of the group of proteins that includes beef heart cytochrome *c* (*5*), human hemoglobin H (*6*), and mouse hemoglobins (*7*), for each of which there is evidence of two conformational states in equilibrium with each other.

We have recently suggested that reversible denaturation of nonequilibrating isoenzymes, after their partial or complete resolution, will provide information about the applicability of the conformer hypothesis (*8*). Our argu-