Encephalitogenic Potencies of Nervous System Tissues.* (28632)

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Myelin is probably the component of nervous tissue that contains the antigen responsible for development of experimental allergic encephalomyelitis (EAE). However, several observations are not completely consonant with this view. We have surveyed a number of tissues of neuroectodermal origin for encephalitogenic properties in the rat. The results add to the body of evidence that favors myelin as the source of encephalitogenic antigen, and demonstrate a more widespread distribution of this antigen among vertebrate classes than has been recognized heretofore.

Methods. Female 180-230 g Hemlock Hollow Wistar rats, a strain known to be susceptible to EAE(1), were fed Purina Laboratory Chow and tap water *ad libitum*. Encephalitogenic emulsion was prepared by cycling 40% w/v tissue homogenate and an equal volume of Freund's complete adjuvant between 2 syringes connected by a double-hubbed needle. The adjuvant contained 4 mg/ml killed dried tubercle bacilli suspended in 8.5 parts mineral oil (Bayol F) and 1.5 parts emulsifying agent (Arlacel A). EAE was produced by injecting 0.05 ml emulsion (which contained 10 mg tissue wet weight) into the right foot pad of rats under light ether anesthesia. Clinical signs of EAE appeared after 9 or more days and consisted of paresis or paralysis and, occasionally, urinary incontinence. Dragging or limpness of the tail was a common but not completely reliable sign and was not scored. Rats were exsanguinated 21 days after challenge. Entire spinal cord and hindbrain in all cases, and forebrain in some cases, were fixed in Bouin's solution. Sciatic and brachial plexus nerves were fixed in neutral formalin. Paraffin sections were stained by hematoxylin-eosin or cresyl violet. Histologic evidences of EAE were graded by number of lesions: 1—up to 4 or 5 lesions on the entire slide, 2—a few lesions in each of several different low-power fields, 3—lesions in many different fields, 4—many lesions in all or almost all fields. Slides were read without knowledge of the group from which tissues were derived.

Tissues. All tissues were stored in frozen state and were prepared as 40% aqueous homogenates containing 0.5% phenol. Central nervous system (CNS) tissues from rat, guinea pig, human, chicken, turtle *(Chrysemys picta)*, frog *(Rana pipiens)* and fish (carp, *Cyprinus carpio*) were homogenized by cycling between 2 syringes, after removal of meninges. Gray matter from human, guinea pig or rat cerebral cortex was isolated by shaving it from the surface of frozen brains. Human white matter consisted of corpus callosum and centrum semiovale, isolated by blunt dissection before freezing. Frog brain was made poor in white matter by removing medulla and optic chiasm. Frog "cord" included cord and lower medulla but not filum terminale. Human lumbosacral plexus, guinea pig sciatic and brachial nerves, rat sciatic nerves, lamb pineals and mouse glioma were freed of excess connective tissue and were cut twice on a freezing microtome before homogenization. Mouse glioma, a brain tumor (ependymoblastoma) maintained in subcutaneous transplants, was obtained in frozen state from Dr. L. C. Scheinberg(2).

Results. Table I presents pooled results of 5 experiments on rats. Each experiment included a strongly encephalitogenic mammalian tissue (rat cord or human white matter) as positive control. Chicken and turtle spinal cords were encephalitogenic; the lesions of EAE in cord, brain, meninges, roots and nerves of the recipient rats did not differ in morphology or distribution from those observed in EAE produced with mammalian CNS tissue. Even frog cord was weakly encephalitogenic; among 20 rats, histologic lesions of EAE occurred in 5, with definite clinical signs in 1 of these and equivocal signs in 2. The (weakly) encephalitogenic quality

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TABLE I. Encephalitogenic Potency in Rats of Various Tissues Emulsified in Freund's Complete Adjuvant.

No. of rats	Tissue antigen*	EAE Clinical, incidence	EAE Histologic, severity†
34	Cord, rat	74%	3.5
10	" chicken	30	1.7
10	" turtle	40	2.1
20	" frog	5	.6
10	" carp	0	0
8	" neonatal rat	0	0
9	Brain, white matter, human	56	3.3
9	Brain, cortex, guinea pig	11	1.7
10	" " rat	10	.5
12	" " human	0	.1
10	" frog	0	0
10	Glioma, mouse	0	0
9	Pineal, lamb	0	0
10	Nerve, guinea pig	‡	.8
9	" rat	‡	.3
10	" human	‡	.1
9	Kidney, neonatal rat	0	0

* Single injection, 10 mg dose, in right foot pad.
† Group avg, graded individually from 0 to 4 according to number of EAE lesions.
‡ Clinical signs not recorded because they were due to neuritis, confirmed histologically.

of frog cord was confirmed in guinea pigs: 0.1 ml emulsion was injected intradermally over the sternum in 10 male, 430 g Hartley guinea pigs (May Rabbitry, San Antonio). At time of sacrifice 21 days later, one of them had slight weakness; this animal and one additional animal had mild but typical histologic lesions of EAE. Five additional guinea pigs injected with rat cord emulsion (as positive control) had many EAE lesions.

Carp spinal cord was not encephalitogenic for the rat. Unmyelinated spinal cord from neonatal rats (less than 24 hours old) was also inactive, as previously reported by Waksman(3). Unlike spinal cord or cerebral white matter from adult mammals, gray matter from cerebral cortex of adult guinea pigs, rats or human was only moderately, slightly or minimally encephalitogenic. These EAE lesions had the usual distribution and did not localize in cerebral cortex of the recipients. Frog brain (which consists mostly of gray matter), mouse glioma and lamb pineal were not encephalitogenic. Myelinated peripheral nerve produced allergic neuritis(4,5), but also a few CNS lesions that were indistinguishable from EAE.

Discussion. Evidence that favors myelin as the source of encephalitogenic antigen includes the following. Lesions of EAE are more numerous in white matter than in gray (6). Fluorescein-labelled globulins from EAE serums localize on myelin *in vitro*(7). Immature CNS tissue, prior to myelination, is non-encephalitogenic(3,8,9). Fractions of CNS obtained by ultracentrifugation are encephalitogenic in proportion to their content of myelin(10). White matter is more encephalitogenic than gray matter(11); in our experiments, human cortex gave even less EAE than rat or guinea pig cortex, perhaps because its greater thickness permitted higher concentration of the myelin-poor upper layers. Spinal cord has greater activity than brain, presumably due to higher content of white matter(12, also our results with frog cord and brain).

Data not in favor of myelin as the encephalitogenic agent include the following. Lesions of EAE occur in choroid plexuses and meninges, where there is no myelin. Early lesions in CNS involve meninges and vessel walls(13). The earliest damage may be an alteration of vascular permeability(14) rather than a direct attack on myelin. Serum globulin from EAE animals binds to glial membranes and cytoplasm as well as to myelin in tissue culture, and the earliest recognizable damage is in glial cytoplasm(15). Mouse glioma tissue produces EAE in mice even though this tumor tissue does not contain myelin(2). However, it is difficult to interpret this last observation in view of our negative results with mouse glioma injected in rats†, and the fact that the glial tissue present in adult gray matter or immature spinal cord is not encephalitogenic. In this connection we tested lamb pineal, another tissue of neuroectodermal origin but with low myelin content, and found it inactive (although lamb brain is encephalitogenic).

† Also, Dr. Marian W. Kies (personal communication) has found no clinical or histological EAE in any of 5 guinea pigs given 1 mg lyophilized mouse glioma tissue emulsified in complete adjuvant. As a control, 1 mg mouse brain produced clinical signs in one and histological lesions in all of 5 guinea pigs.

Our data are in accord with evidence that points to myelin as the source of encephalitogenic antigen. However, various myelins differ in their properties. Nerve myelin causes neuritis but little EAE. CNS myelin is not species-specific, inasmuch as we produced EAE with spinal cord from mammalian, avian, reptilian, and even amphibian species. However, spinal cord of fish, representing a class further removed from mammals than the foregoing, was inactive. Failure of previous investigators to detect encephalitogenicity of turtle or frog CNS(8,16) may have been due to their use of different recipient species, different route of inoculation, late sacrifice of recipients (after mild lesions have disappeared), or the use of brain or brain-cord mixtures that contain little white matter.

Summary. Tests of spinal cord, brain, white matter, gray matter, glioma, pineal and peripheral nerve tissue homogenates emulsified in Freund's complete adjuvant indicate that, in the rat, encephalitogenicity is proportional to content of CNS myelin. The distribution of the organ-specific encephalitogenic antigen in CNS myelin extends further down the evolutionary tree than has been recognized heretofore, as turtle or even frog spinal cord can produce EAE in rats.

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Dietary Fats and Plasma Lipids in Chicks.* (28633)

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In studies with growing chicks fed cholesterol and fats, Adamson *et al.*(1) observed that unsaturated fat caused greater increase in serum and liver cholesterol than saturated fat. We have previously reported that unsaturated fats like sesame oil and mustard

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