unusual affinity for these nutrients shown by the oral structures, especially the water-vascular organs. That cells of these structures (the jaw muscles of *Amphipholis* being a notable instance), which are morphologically indistinguishable from similar cells in other parts of the body, should show such special sensitivity argues strongly for the existence of specialized cellular responses in relation to uptake of free nutrients. Ferguson's suggestion (*2*) that the superficial tissues of echinoderms may receive at least some of their nutritional requirements via uptake through the skin seems reasonable and is reinforced by these observations. The rich labeling of embryonic *Amphipholis* tissues also suggests that the question of nutrient uptake in larvae, both brooded and planktonic, deserves attention. However, the question of "skin digestion" in echinoderms (*6*) needs reinvestigation before its significance can be evaluated; work in this laboratory (*7*) has failed to confirm its existence. Although the relative importance of exogenous free organic molecules in the whole nutritional economy of echinoderms is debatable, the demonstration of its occurrence in three echinoderm classes, as well as in other marine invertebrates, emphasizes the ubiquity of the phenomenon and its potential importance. Granted its doubtful nutritional significance in the organisms investigated so far, it is not difficult to envisage other taxa and other ecological conditions where the existence of appropriate cellular mechanisms for nutrient uptake through the skin may be of great adaptive value.

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Allergic Encephalomyelitis: Passive Transfer Prevented by Encephalitogen

Abstract. *Allergic encephalomyelitis was produced in rats by passive transfer of lymph node cells from donors immunized intradermally with neural tissue or an encephalitogenic basic protein plus adjuvants. The same basic protein, injected intravenously into the recipients before or after transfer of lymph node cells, prevented the disease. Even established lesions were reversed. Inhibition by basic protein was specific for encephalomyelitis; it had no effect on passive transfer of allergic adrenalitis.*

Experimental allergic encephalomyelitis (EAE) is one of the best characterized models for studying delayed hypersensitivity and autoimmune disease in animals. Depending on the manner and timing of presentation, a basic protein antigen from tissue from the central nervous system can either produce, prevent, or suppress this condition (*1*). Previous studies have been concerned with the effects of the basic protein on actively immunized animals. In spontaneous autoimmune diseases, however, the event of active immunization either does not occur or has occurred at an unknown interval before the onset of symptoms. The later parts of the pathogenetic sequence (steps occurring during development of overt manifestations of autoimmune diseases) may involve the attack of specifically immunized lymphoid cells on target tissues. It is important, therefore, to record here the prophylactic and therapeutic effects of the basic protein on the form of EAE produced by passive transfer of such specifically immunized lymphoid cells. These results may help us to achieve greater insight into the interaction between lymphoid cell and target tissue in delayed hypersensitivities, and to develop immunologically specific therapeutic measures.

In a typical experiment, 32 adult male or female Lewis rats were immunized with guinea pig spinal cord antigen and adjuvants (*2*, *3*). Seven days later, all or most of these donor rats had symptoms or lesions of EAE or both. At this time, the lymph nodes draining the sites of inoculation were removed and processed into a cell suspension (*2*, *4*). The suspension, containing 1.5 to 3.2 $\times 10^8$ nucleated cells per dose, was injected intravenously into each of 32 male Lewis rats. (Lewis rats are isogenic and accept grafts of lymphoid cells.) The recipients were prepared, and their threshold for EAE was lowered, by inflicting a nonspecific thermal injury to the brain 2 to 4 days beforehand (*2*, *5*). The basic protein or other materials were injected intravenously into the recipients 1 hour after the cell suspension, except as specified otherwise. The rats were killed 24 hours later. Their brains were removed, fixed, sectioned, and stained. Lesions produced by EAE were found only around the zone of thermal coagulation necrosis because of the short survival time (*2*).

Each of the 17 experiments reported here included a control group of three

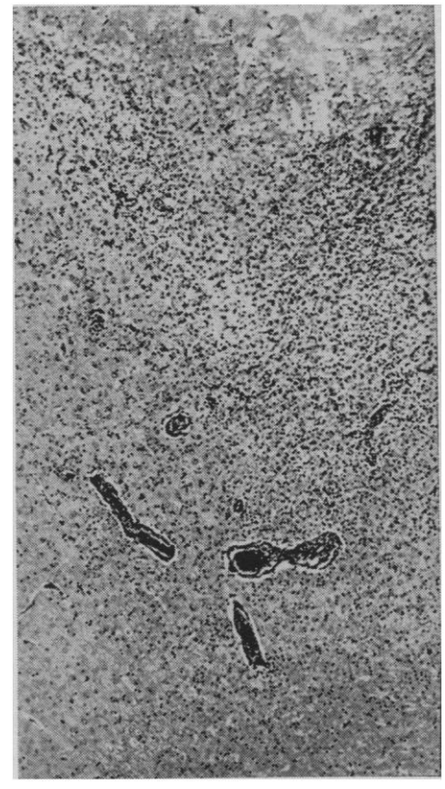

Fig. 1. Allergic encephalomyelitis localized in the vicinity of a thermal injury of the brain. Part of the zone of coagulation necrosis is visible at the top. Below this is a highly cellular area filled with phagocytic cells that have reacted to this nonspecific injury. The bottom half of the photograph shows adjacent, intact brain tissue with three large and several small venules surrounded by dense perivascular infiltrates of mononuclear cells. These perivascular infiltrates, typical of allergic encephalomyelitis, were absent in rats treated with basic protein. Hematoxylin and eosin, ×60.

or four recipients that received only saline, 1 hour after cells. All these controls had many perivascular infiltrates of mononuclear inflammatory cells, typical of EAE (Fig. 1). The same was true of 12 rats that were given 2 mg of calf thymus histone and four rats that were given basic protein of guinea pig spleen 1 hour after cells. Each of these 17 experiments also had a group of three or four recipients each of which received, 1 hour after the cell suspension, 0.2 or 2.0 mg of basic protein derived from guinea pig brain myelin (*6*) dissolved in 1 ml of saline. Most of their brains had no EAE lesions and were indistinguishable from brains of rats that had received thermal injuries but no cell transfer. A few of these rats had one to five minor lesions with a few vessels incompletely surrounded by relatively scanty inflammatory cells ("trace" lesions). In each experiment, slides were randomized and read without knowledge of origin, and none of these brains was graded higher than "trace."

Basic protein was fully effective when injected a few minutes before the cell suspension, but the inhibition decreased progressively as the interval between basic protein and the injection of cell suspension was extended (Table 1). Six hours after cell transfer, a time when perivascular lesions begin to form (*2*), injection of basic protein inhibited EAE completely. Most remarkable was the effect of basic protein injected the day after transfer, when the EAE lesions were fully developed in parallel controls. There was partial but definite amelioration of the EAE lesions in rats killed 1 day after basic protein (2 days after cell transfer), and an early therapeutic effect was evident, by comparison with controls, in rats killed a mere 8 hours after basic protein injection (28 hours after cell transfer). The inhibitory effect of basic protein was not transitory; its injection 1 hour after cells inhibited EAE in rats killed 1, 2, or 3 days later. However, only the intravenous route of administering basic protein was effective; injection of basic protein into the peritoneal cavity or foot pad yielded only very slight inhibition of EAE.

The inhibitory effect of basic protein proved quite resistant to heat. A solution of basic protein which had been heated 30 minutes in a boiling water bath inhibited EAE completely. A similar test on basic protein autoclaved for 15 minutes revealed its ability to inhibit passive transfer of EAE almost completely.

Table 1. Inhibition of allergic encephalomyelitis (EAE) by brain basic protein injected intravenously before (−) or after (+) passive transfer with immunized lymph node cells. The results are a composite of four experiments, each of which included a control group given saline in place of basic protein. The EAE scores are based on the number and intensity of lesions, all of which were localized adjacent to a previously inflicted thermal injury of the brain.

Time of injection	Number of rats with EAE scores		
	0 or ±	1+	2+ or 3+
Dose: 2.0 mg protein			
−6 days			3
−2 days		3	3
−1 day	3	1	
+1 hour	3		
+6 hours			
Dose: 0.2 mg protein			
−1 day		1	2
−4 hours	1	2	
−1 hour	2	1	
0 hour	3		
+1 hour	8		
+16 hours	1	3	
Dose: saline			
+1 hour			14

The inhibitory effect of basic protein was not restricted to the particular conditions of donor rat immunization outlined above. The EAE was produced readily by passive transfer of lymph node cells from donor rats that had been immunized with rat spinal cord tissue or purified basic protein from guinea pig brain in place of the usual guinea pig spinal tissue. In addition, passive transfer was successful despite elimination of either the pertussis vaccine or the killed tubercle bacilli from the adjuvant combination given to donors. In all these experiments, intravenous injection of basic protein inhibited EAE completely in the recipients. On the other hand, basic protein had no inhibitory effect on the passive transfer of allergic adrenalitis. [The two experiments on adrenalitis were similar to those on EAE except for the use of rat adrenal antigen in place of guinea pig spinal cord for donor immunization, and the presence of thermal injury in the recipient's adrenal instead of brain (*2*).]

Certain modifications of the recipient were made without altering the inhibitory effects of basic protein. Adrenalectomy of recipients enhances passive transfer of EAE (*7*); nevertheless, 0.2 mg of basic protein inhibited EAE completely in adrenalectomized recipients. In another experiment, cyanide instead of heat was used to induce localization of EAE in the forebrain (*2*); again, basic protein produced complete inhibition. In two experiments, neither cyanide nor heat injuries were inflicted, and the recipients were observed for 1 week after transfer of cells and injection of basic protein. All of the control recipients developed clinical signs and lesions of EAE. The rats treated with basic protein had far fewer, much milder clinical signs and histologic lesions, and the inhibition was proportional to the dose of basic protein.

Thus, the inhibitory effect of basic protein from guinea pig brain was specific for EAE but not for any particular encephalitogenic antigen or adjuvant used in the donors, or for any particular method of detecting the disease in the recipients. The data preclude the possibilities that the inhibition was mediated by the adrenals or by an antibody response to basic protein in the recipients. Basic protein probably interacted with the specifically immunized lymphoid cells, but it is not known if the neutralization of their encephalitogenic capacity involved cytotoxicity, sequestration, blockade, or some other mechanism. Nevertheless, the evidence for this action on lymphoid cells, either before or after lesions have developed in the target organ, offers hope for development of specific immunotherapies as well as new methods for studying cellular dynamics in autoimmune diseases.

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3. A 40 percent homogenate of spinal cord emulsified in equal volume of Freund's complete adjuvant; 0.05 ml injected in the right foot pad of Lewis rats (Microbiological Associates, Inc.). Immediately thereafter, 0.1 ml of concentrated pertussis vaccine injected in dorsum of same foot.
4. Right popliteal, inguinal, sacral, lumbar, renal, and axillary nodes were cleaned, minced, pressed through an 80-mesh screen, washed three times, and resuspended in saline, all at 1° to 8°C.
5. The scalp was incised under ether anesthesia; preheated 37½-watt, pyramid-tip soldering iron

applied for 7 seconds to the right parietal bone; skin closed with clips.

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Ruthenium Tetroxide for Fixing and Staining Cytoplasmic Membranes

Abstract. *Rat liver and kidney were fixed for electron microscopy with ruthenium tetroxide. When compared with tissues fixed with osmium tetroxide, the membranes showed well without additional staining. The trilaminar structure of the cytoplasmic membranes was clearly seen. In width the nuclear, mitochondrial, and cytoplasmic membranes investigated resembled the cell membrane.*

Ruthenium tetroxide was first used as a stain in histology in 1887 by Ranvier (*1*); its use as a fixative also has been investigated (*2*), but its use in electron microscopy as a fixative or stain has never been reported.

Tissues of rat kidney and liver were fixed in cacodylate-buffered glutaraldehyde overnight, rinsed three times (10 minutes for each change) in isotonic sodium acetate buffer (*p*H 7.1 ± 0.1), and refixed in ruthenium tetroxide made up in sodium acetate–acetic acid buffer (*p*H 7.1 ± 0.1) adjusted to be isotonic with plasma. The optimum concentration of ruthenium tetroxide proved to be between 0.1 and 0.05 percent (weight to volume) when the tissue was fixed for 1 hour at 4°C. The tissue was rinsed in sodium acetate buffer, dehydrated in methanol, and embedded in Araldite. Sections were cut on a Huxley microtome and viewed in a Siemens Elmiskop-1B electron microscope.

A striking difference between tissues fixed with osmium tetroxide and with ruthenium tetroxide is the appearance of the cytoplasmic and nuclear membranes (Figs. 1–6). Whereas these are single, rather diffuse lines when fixed with osmium tetroxide, they appear as trilaminar structures similar in appearance to those normally seen in plasma membranes in preparations fixed with osmium tetroxide.

The widths of the membranes were measured only at the points at which both electron-dense lines appeared to be sharp and uniform. The widths reported (Table 1) are the means of at least 20 measurements taken from no less than four different cells; they were calculated by use of the magnification figures provided by the electron microscope. No external standards were used for calibration of the instrument, and all pictures were taken at the same magnification. The membrane widths of these tissues fixed with ruthenium tetroxide showed a remarkable degree of uniformity, suggesting that all have similar structure.

Table 1. Membrane widths of tissues fixed with ruthenium tetroxide.

Membrane	Width (A°)	Mean deviation (A)
Plasma, of red blood cell	81.0	10.7
Inner nuclear	77.2	5.7
Outer nuclear	78.0	8.0
Mitochondrial cristae	157.8	4.6
Of mitochondrial cristae (half-width of cristae)	78.9	
Endoplasmic reticulum (granular)	75.9	5.2
Plasma, of glomerular epithelial cell	79.2	10.4

The plasma membrane may join the endoplasmic reticulum membrane, and this in turn may fuse with the outer nuclear membrane which is continuous with the inner nuclear membrane in the region of the nuclear pore (Fig. 3). These observations agree with the suggestion that all these types of membrane are similar in structure. In one instance the plasma membrane was seen to be continuous with the outer nuclear membrane, leaving the inner nuclear membrane apparently exposed to the extracellular space.

The plasma membrane of many types of cell shows a more complex outer structure (Fig. 2) presenting an electron-dense coating in addition to the electron-dense layers normally seen in preparations fixed with osmium tetroxide and potassium permanganate. In some types of cell, this additional layer

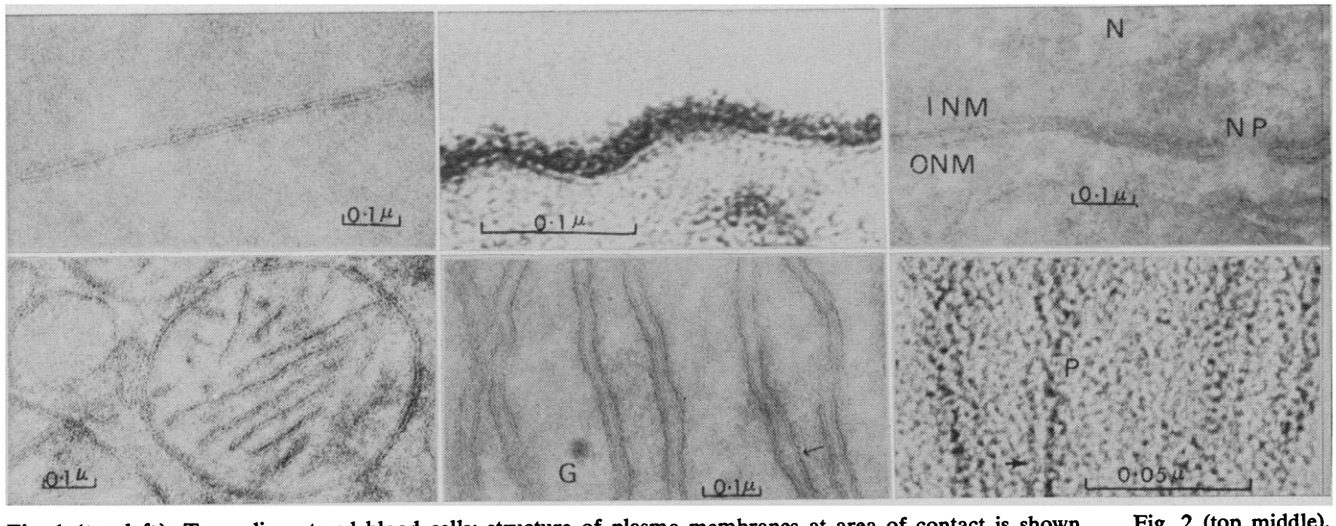

Fig. 1 (top left). Two adjacent red blood cells; structure of plasma membranes at area of contact is shown. Fig. 2 (top middle). Outer plasma membrane of glomerular epithelial cell; outer coating is shown. Fig. 3 (top right). Section through edge of nucleus of epithelial cell of glomerulus; *N*, nucleus; *INM*, inner nuclear membrane; *ONM*, outer nuclear membrane; *NP*, nuclear pore. Fig. 4 (bottom left). Mitochondria of liver cell. Fig. 5 (bottom middle). Endoplasmic reticulum of liver cell; *G*, densely stained glycogen particle. Arrow indicates membrane pore. Fig. 6 (bottom right). Enlargement from Fig. 5; *P*, membrane pore (Fig. 5). Arrow shows region in one of the electron-dense lines resolved into trilaminar structure.