natural product pyrethrin I, contain few of the functional groups of the original molecule. However, although their structures are greatly different, their mode of action appears to be the same. It is clear that combination with the active site in the insect nerve, to give a functional lesion, is not absolutely dependent on either an ester group or a cyclopropane ring. The configuration of the molecule, relative to appropriate size and shape to interact with the receptor site in the nerve, seems to be the critical factor for pyrethroid-like activity.

Since the change in excitability produced by allethrin is explicable in terms of conductance changes of the nerve membrane (*5*), the receptor can be visualized as the site in the nerve membrane that controls the conductance changes. Compounds A and C, and the chrysanthemumate analog (*2*) of compound C, must fit this receptor exceptionally well. However, the nature of the receptor and the manner in which it is affected by pyrethroids remain to be explored. One way toward explanation of these relations is further study of structure and activity in terms of both insecticidal activity and disturbance of conductance.

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Echinoderms: An Autoradiographic Study of Assimilation of Dissolved Organic Molecules

Abstract. *In a holothurian and an ophiuroid, tritiated glucose and glycine in great dilution are removed from seawater by uptake through the skin. Cells differ in their competence to metabolize specific nutrients, an indication that there are specialized cellular responses to exogenous organic molecules. Embryonic ophiuroid tissues have an exceptional capacity for assimilation.*

There is substantial evidence that many marine invertebrates, with the exception of the arthropods, can absorb and metabolize free organic molecules of low molecular weight dissolved in ambient seawater (*1*). The echinoderms have attracted much attention in this regard (*2*). These studies have usually used techniques of isotope counting and consequently provide little information on specific sites of cellular uptake. However, an autoradiographic study of three asteroid species (*3*) has reported that nutrients labeled with C^{14} are concentrated in the epidermis, particularly in the tube feet. To determine specific sites of assimilation and to gain information about their use in the cell, we have autoradiographically compared the uptake of tritium-labeled dissolved organic materials (DOM) in two echinoderms, the holothurian *Cucumaria lactea* and the ophiuroid *Amphipholis squamata*, collected from the Northumberland coast.

Experimental animals were immersed for 3 hours in 50-ml quantities of $6 \times 10^{-6}M$ glycine-2-H^3 (specific activity, 11.8 mc/mg) or $10^{-5}M$ D-glucose-6-H^3 (specific activity, 2.8 mc/mg) dissolved in filtered seawater (33 to 34 parts per thousand) at a temperature approximating that of local surface seawater. After being rinsed in clean seawater, some animals were fixed immediately (0-day animals); others were returned to holding tanks and fixed after intervals of 1, 3, and 7 days. Animals treated with glycine were fixed in Bouin's; those treated with glucose were fixed in Bouin's or Rossman's fluids. Autoradiographs were prepared from 8-μ paraffin sections by dipping them in liquid Ilford K2 nuclear emulsion; they were developed after 14 days, an optimum exposure determined empirically. The sections were stained in Mayer's hemalum through the emulsion. Rough estimates of assimilation were obtained by counting silver grains with the aid of a squared-graticule ocular. With a $\times$ 100 oil-immersion objective, each ocular square (field) represented 5.76 μ^2. The expression of number of grains per field for a specific tissue is based on the mean grain count for 60 representative fields, less a correction for background grains.

Since free glucose is washed out of tissues during histological processing, glucose labeling is attributable to glucose-6-H^3 incorporated metabolically into large, fixable molecules such as glycogen. When sections were incubated in 0.5 percent malt diastase (*p*H 6.0) before dipping, all labeling due to glucose-6-H^3 was abolished, showing that most, if not all, glucose labeling is associated with sites of synthesis or deposition of desmoglycogen.

Free glycine is also removed from tissues during processing. Therefore, visible labeling marks sites where this amino acid has been synthesized into a macromolecule, most probably a polypeptide. To test this, we added an inhibitor of protein synthesis to the labeled medium. An analog of transfer-RNA, chloramphenicol (4 mmole/liter), which interferes with the transfer of amino acids into peptide linkages at ribosomal sites (*4*), was added to the seawater together with $6 \times 10^{-6}M$ glycine-2-H^3. After a 3-hour exposure, the experimental animals were fixed and autoradiographs were prepared. The tissues appeared normal and healthy after this treatment. However, compared to controls lacking chloramphenicol, labeling was substantially reduced. A comparison of grain counts of identical tissues from experimental and control animals showed that protein synthesis was inhibited by 86.8 percent for *Cucumaria* and by 82.7 percent for *Amphipholis*. The focal position of glycine in metabolic pathways leaves the possibility that some was transformed and fixed into large, nonpeptide molecules. However, we conclude that most of the labeling is due to glycine

incorporated into newly synthesized polypeptides.

Table 1 shows the general distribution of assimilated DOM in the principal structures, tissues, and cells of *Cucumaria* and *Amphipholis*. The special sensitivity of the tube feet to glycine is conspicuous. Although all epidermal cells of the tube-foot stem in *Cucumaria* are strongly labeled, labeling is most dense on the sucker disk where the tall microvillar border of the sucker is completely blackened. Mucoprotein-secreting glands in the stem with ducts leading to the sucker surface are also labeled. Possibly most labeling of the sucker is due to glycine incorporated into newly synthesized and extruded mucoprotein. There is no counterpart to this situation in *Amphipholis*, in which a sucker and such glands are absent. A curious phenomenon in *Amphipholis* is the extraordinarily rich incorporation of glucose into the muscle cell bodies of the buccal tube feet and the jaw musculature. These muscles are so densely labeled that grain counting is scarcely possible. This is most striking in view of the negligible labeling of skeletal muscle in other parts of the body.

An outstanding feature of both glycine and glucose assimilation in *Amphipholis* is its richness in the bursal walls. Since the bursae are constantly flushed with seawater, presumably for respiratory purposes, this is not surprising. However, *Amphipholis* is a viviparous ophiuroid, brooding its embryos in the bursae from the time of fertilization until their liberation as well-developed juveniles (*5*). All of our experimental ophiuroids were carrying embryos in various developmental stages, and it is remarkable that incorporation of both glycine and glucose by embryonic tissues is even richer than that of the adults in which they lie. In early embryos labeling is restricted to the superficial, presumably ectodermal, cells. However, in advanced embryos with all adult structures in miniature, the distribution of label is similar to the adult. The richness of labeling in embryonic tissues suggests that assimilation via external tissues may be a very important source of nutrient to the postmetamorphic embryo after exhaustion of its endogenous yolk supply.

There are no significant differences in grain counts between tissues of either *Cucumaria* (Table 2) or *Amphipholis* held for various times after exposure to tritiated glycine. We conclude that there is no loss of glycine within 7 days of exposure nor a discernible movement of the amino acid to other tissues. On the other hand, the quantity of labeled glycogen diminishes rapidly in time after exposure. This is presumably only a reflection of glycogen usage in response to metabolic needs.

These observations provide further and direct evidence for the ability of echinoderms to take up dissolved nutrients at great dilution from seawater. More important, however, is the demonstration of differences in the competence of cells to imbibe and assimilate specific molecules. The diversity of labeling patterns shown by *Cucumaria* skin in response to exposure to glycine and glucose is an excellent example of this phenomenon. Evidently the ability to assimilate exogenous nutrients is not merely a concomitant activity of skin cells as a whole, but is a highly specific cellular activity involving the metabolic competence of specific cell types to assimilate specific exogenous nutrients, an aspect of the problem which has not previously been discussed. Another example of the same phenomenon is the

Table 1. Morphological distribution of labeled DOM in autoradiographs, indicating sites of assimilation in the major structures of *Cucumaria* and *Amphipholis*. Relative intensity of labeling is indicated as follows: ++++, very intense; +++, intense; ++, moderate; +, weak; 0, absent.

Tissue	Labeling intensity with	
	Glycine-2-H^3	Glucose-6-H^3
Cucumaria		
Epidermis	++	++
Dermal fibroblasts	0	0
Dermal cell strands (hemal?)	0	+++
Pharynx epithelium	+++	+++
Midgut epithelium	0	0
Cloacal epithelium	+++	+++
Respiratory tree epithelium	+++	+++
Circumoral ring neuropile	+	0
Radial cord neuropile	+	0
Peripheral nerves	0	0
Madreporite	0	0
Stone canal	0	0
Radial canals	0	0
Tube-foot ampullae	0	0
Tube-foot epidermis	+++	+
Tube-foot sucker surface	++++	0
Tube-foot connective tissue layer	++	++
Tube-foot muscular layer	0	+++
Tube-foot coelomic lining	++	+++
Tube-foot muco-protein glands	+++	0
Oral tentacles	+++	+
Coelomic peritoneum	0	+
Longitudinal muscle bands	0	+
Major hemal vessels	0	0
Coelomocytes	0	0
Gonads and gonoducts	0	0

Tissue	Labeling intensity with	
	Glycine-2-H^3	Glucose-6-H^3
Amphipholis		
Epidermis	+++	++
Dermis (fibroblasts)	0	+
Genital bursa epithelium	+++	+++
Gut epithelium	0	0
Neuropile of central nervous system	+	0
Peripheral nerves	0	0
Madreporite	0	0
Stone canal	0	0
Ring and radial canals	0	0
Tube-foot ampullae	+	0
Tube-foot epidermis	+++	++
Tube-foot connective tissue layer	++	+
Tube-foot muscle layer	0	++
Tube-foot coelomic lining	++	+
Buccal tube-foot epidermis	+++	++
Buccal tube-foot connective tissue layer	++	+
Buccal tube-foot muscle layer	0	++++
Buccal tube-foot coelomic lining	++	+
Coelomic peritoneum	0	0
Skeletal muscles (other than jaws)	0	+
Jaw muscles	0	++++
Axial complex and hemal vessels	0	0
Coelomocytes	0	0
Gonads and gonoducts	0	0

Table 2. Grain counts of *Cucumaria* tissues fixed at various times after a 3-hour exposure to glycine-2-H^3 and glucose-6-H^3. The numbers express the total number of silver grains observed in a microscopic field of 5.76 μ^2, and are mean figures for counts of 60 representative fields, less a correction for background grains.

Tissue	Time after exposure			
	0 day	1 day	3 days	7 days
	Glycine-2-H^3			
Epidermis cells	7.0	6.7	7.2	6.8
Tube-foot epidermis	16.7	17.3	16.7	16.2
Radial nerve cord	0.5	0.4	0.6	0.8
	Glucose-6-H^3			
Epidermal cells	8.8	6.9	4.7	3.8
Tube-foot muscle	10.8	8.5	6.9	4.4
Coelomic epithelium	4.2	2.1	1.0	0.5

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

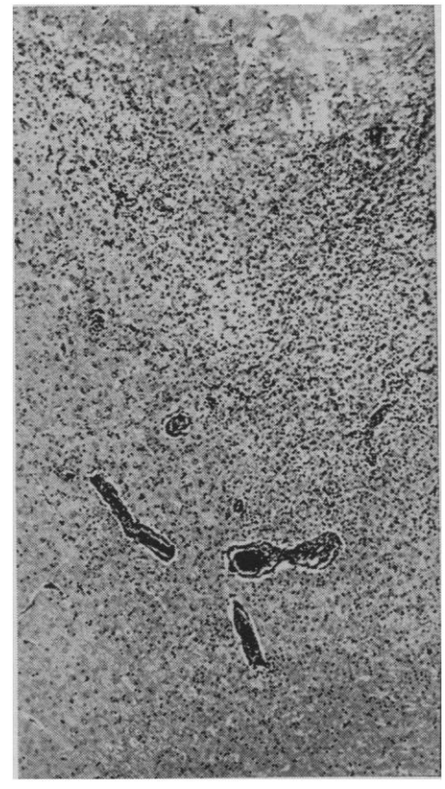

Fig. 1. Allergic encephalomyelitis localized in the vicinity of a thermal injury of the brain. Part of the zone of coagulation necrosis is visible at the top. Below this is a highly cellular area filled with phagocytic cells that have reacted to this nonspecific injury. The bottom half of the photograph 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.