However, with tris-disrupted brush borders incubated at 37°C in the presence of these labeled sugars and under these same conditions, there was almost a 50 percent increase in the DPM ratio of D-mannose-1-H^3 to D-glucose-C^{14} and no change in the DPM ratio of D-mannose-1-H^3 to L-glucose-1-C^{14}. This, of course, indicated a stereospecificity for this binding, since the actively transported sugar, D-glucose, was being preferentially bound to the preparation of tris-disrupted brush border membranes. Incidentally, when the tris–brush border suspension was kept on ice for a period longer or shorter than 45 minutes, less binding of D-glucose occurred.

The percentage change in the DPM ratio of D-mannose-1-H^3 to D-glucose-C^{14} in the presence of tris-disrupted brush borders under various experimental conditions is shown in Fig. 1. It can be seen that binding of D-glucose in plain distilled water is similar to that in Na^+ (105.8 m*M*) with K^+ (30.0 m*M*), Mg^{2+} (6 m*M*) and ATP (6 m*M*), which Faust and Wu (*9*) have shown to stimulate mucosal adenosine triphosphatase activity. Additional experiments also have indicated that the preferential binding of D-glucose under these conditions is unaffected by 0.1 m*M* ouabain, an inhibitor of adenosine triphosphatase activity stimulated by Na^+ and K^+ in hamster mucosal homogenates (*9*). These data suggest that neither added Na^+ ions nor adenosine triphosphatase activity stimulated by Na^+ with K^+ and Mg^{2+} are required for this binding. However, the concentration of Na^+ in the experiments in which Na^+ is not added is still greater than the concentration of uniformly labeled D-glucose-C^{14} (Fig. 1). Some of these Na^+ ions may be closely associated with glucose binding sites. Therefore, it is impossible to test the Na^+-dependency hypothesis for D-glucose binding in these experiments, although it is obvious that an increase in the Na^+ concentration does not produce an increase in D-glucose binding. However 0.1 m*M* phlorizin completely inhibits the binding of D-glucose, presumably by competing with D-glucose for binding sites within the tris-disrupted membranes (Fig. 1). The complete inhibition of D-glucose binding in the presence of 1 m*M* $HgCl_2$ suggests that perhaps protein and sulfhydryl groups (*10*) within the disrupted microvilli preparation are involved in this process. Binding of D-glucose at 23°C instead of 37°C in plain distilled water is reduced by approximately 70 percent, indicating a temperature dependency (temperature coefficient, Q_{10}, of 2.4) for this phenomenon (Fig. 1).

We find that D-glucose is preferentially bound to isolated and disrupted brush borders of epithelial cells from hamster jejunum. The properties of this binding are compatible with the initial step in the proposed mechanism of active sugar transport by the small intestine.

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Crayfish Muscle: Permeability to Sodium Induced by Calcium Depletion

Abstract. *Membrane of crayfish muscle fibers becomes selectively permeable to sodium when the calcium concentration of the bathing medium is reduced. Removal of calcium or its reduction below 1 or 2 millimole per liter causes large transient depolarizations up to 70 millivolts in amplitude. They resemble prolonged action potentials and occur only in the presence of sodium. The responses are abolished when tris(hydroxymethyl)aminomethane or lithium is substituted for sodium, and are blocked by tetrodotoxin even in the presence of sodium.*

In general the gradedly responsive arthropod muscle fibers derive their depolarizing electrogenesis from the inward electrochemical gradient for Ca (*1*) or Mg (*2, 3*). The fibers can develop spikes in Na-free media with an increase in the concentration of alkaline earth cations or by treatment with procaine or tetraethylammonium ion (TEA). Tetrodotoxin (TTX) does not affect these spikes (*3, 4*), whereas it abolishes spikes that require the presence of Na (*5, 6*). We have observed a depolarizing response of crayfish muscle fibers that is induced by removal of Ca, is dependent on the presence of Na, and is blocked by TTX.

Single fibers were isolated (*7*) from the flexor in the meropodite of the walking legs of crayfish of the genus *Orconectes* sp. They were equilibrated in various media, the standard saline being that of Van Harreveld (*8*). The basic experimental variable was a change in the concentration of Ca; in the experiments illustrated, the muscles were challenged with a Ca-free medium. In some (Fig. 1), all the Na was substituted stoichiometrically with Li or tris(hydroxymethyl)aminomethane (tris HCl). In others, the control medium was free of K (Fig. 2). In still another series the medium for equilibration was free of Cl, the latter being substituted stoichiometrically by propionate, to which the muscle fibers are effectively impermeable (*9*); in these experiments (Fig. 3) the medium was also free of calcium, and ethylenediaminetetraacetate (EDTA) was added in amounts between 1.0 and 0.01 mmole per liter. In certain experiments of each series, TTX (5×10^{-7} g/ml) was added. The muscle fiber was held in a lucite chamber which had a volume of 1.5 ml. The solution was changed at a rate of 3 to 4 ml/sec, and the flushing was continued for at least 2 seconds.

The changes in membrane potential when the bathing medium was changed were recorded with an intracellular microelectrode. In some experiments a second microelectrode was also introduced into the fiber to apply polarizing currents for resistance measurements (Fig. 1C). The electrophysiological equipment was standard for the

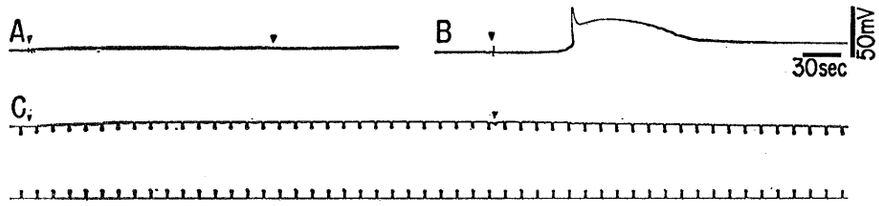

Fig. 1. Sodium-dependent depolarization induced by removal of Ca. All records from one muscle fiber. (A) The preparation had been equilibrated in a Na-free (tris-Cl) medium which contained 13.5 mmole of $CaCl_2$ per liter. During the 3-minute period indicated by the arrows, the preparation was exposed to a Ca-free medium. (B) About 30 minutes later. The fiber was equilibrated in the standard (NaCl) medium containing the same amount of $CaCl_2$ as (A). The $CaCl_2$ was removed at the arrow. The record shows the response to a challenge with the Ca-free medium. During a previous such challenge the fiber depolarized by 70 mv at the peak of the spike-like response, but a vigorous contracture expelled the recording microelectrode. (C) The fiber was again equilibrated in the standard saline, but with TTX (5×10^{-7} g/ml) added. When the Ca was removed, there was no large depolarization despite the presence of Na. In this recording a second electrode applied pulses of constant inward current, indicated by the upward deflections on the lower trace. The changes in potential induced in the fiber by the applied currents decreased during the depolarization evoked by removal of Ca.

laboratory. The records shown in Figs. 1 to 3 were made with ink registration, but the membrane potential was also monitored on a cathode ray oscillograph.

In one series of experiments the fibers were initially equilibrated in a Na-free tris-Cl or LiCl saline, with a normal $CaCl_2$ concentration (13.5 mmole/liter). The effects of substituting tris or Li for Na were identical. In the experiments of Fig. 1 the resting potential of the fiber was −84 mv. On changing to a Ca-free tris-Cl medium (A, first arrow), the fiber depolarized by about 3 mv, the membrane potential remaining nearly constant thereafter until the Ca was restored (second arrow). The fiber was then equilibrated for about 20 minutes in the standard (NaCl) medium (B). The resting potential was −85 mv. On changing to a Ca-free medium (arrow), there was no perceptible change in potential for almost the first 30 seconds. During the next 30 seconds, however, a small depolarization developed which accelerated in rate during the last 10 seconds of that period. When the fiber had depolarized by about 10 mv, a spike-like change in potential occurred which reached a peak depolarization of some 50 mv. The peak could be as large as 70 mv. After subsiding somewhat, the depolarization slowly increased again to an elevation of some 40 mv from which it gradually declined to the resting potential.

The depolarization was accompanied by a strong contracture which was largest during the first cycle of repetitions of the experiment. Attempts to obtain accurate measurements of the changes in resistance during the depolarization were unsuccessful, since the contractions tended to expel one or both of the impaling microelectrodes. A large decrease in effective resistance, as much as tenfold, was always observed early during depolarization before the contracture had disrupted the measurements. A decrease in effective resistance of 25 percent due to removal of Ca was determined in one experiment after the fiber had repolarized to the steady state.

In the presence of TTX (Fig. 1C), there was only a small depolarization (about 4 mv), similar to that which had been produced in the Na-free medium (A), and there was no mechanical response. The small depolarization was associated with a decrease of the effective resistance by about 25 to 30 percent, or like the steady-state change produced on removal of Ca.

The Na-dependent depolarizations initiated by Ca removal were altered somewhat when K was absent from the medium (Fig. 2). The resting potential was −110 mv (Fig. 2A). Removal of the Ca caused an immediate depolarization which ranged between 10 and 15 mv in different experiments and which continued to increase, however, at the rate of about 15 mv/min. When the membrane had depolarized by about 25 mv, the change became more rapid, and the depolarization rose to a peak of about 50 mv from which it declined slowly. In this experiment, the normal Ca level was restored while the membrane was still depolarized by about 25 mv. The introduction of Ca caused rapid repolarization by only about 7.5 mv. Thereafter, the repolarization resumed its slow rate. The large depolarizations required the presence of Na.

The Ca was again removed, but in the presence of TTX (Fig. 2B). The initial depolarization was somewhat slower and the maximum change was a steady depolarization of only 15 mv. When the TTX was removed (second arrow), the fiber immediately underwent further depolarization at the rate of about 15 mv/min, and there followed a second, faster depolarization resembling that of record A.

These data indicate that the depolarization induced by removal of Ca may be separated into three components. The first, a more or less steady, small depolarization, develops immediately on removing Ca, is independent of the presence or absence of Na, and is not blocked by TTX. A second requires the presence of Na, increases gradually, and is blocked by TTX. In the absence of K from the medium (Fig. 2), this Na-dependent depolarization develops immediately upon removing Ca, and is superimposed on the Na-independent component. When K is present (Fig. 1), this initial Na-dependent and TTX-sensitive depolarization is markedly slowed. It also may be smaller in amplitude when it gives rise to a third component which is also Na-dependent and causes a large prolonged spike-like depolarizing sequence. Presumably the two Na-dependent depolarizing processes are related in the same way that the local response and the spike of conductile activity are related, the smaller depolarization initiating the larger.

The spike-like depolarizations subsided while the fiber was maintained in a low-Ca or a Ca-free medium. A new response could be evoked by another challenge after the preparation had been equilibrated again in a medium containing Ca. These procedures could be repeated indefinitely. Lowering the Ca to 1 or 2 mmole/liter elicited a response only if the fiber had been equilibrated again in 13.5 m*M* $CaCl_2$. If the challenge was complete removal of Ca, prior equilibration in 1 or 2 mmole of $CaCl_2$ per liter was adequate. In every case the experimental challenge evoked only a single transient depolarization. However, after a fiber had repolarized in a Ca-free medium, repeated washing in fresh solution lowered the membrane potential.

Furthermore, a second response could be elicited by adding EDTA (1 mmole/liter or less) to the bath. The depolarizations produced in this way were similar in form and magnitude to the initial responses.

While each reduction of Ca caused only a single spike-like transient depolarization when the bathing medium contained Cl as the anion, repetitive spike-like responses could be elicited (Fig. 3) when the Cl had been substituted with an impermeant anion (propionate). In the sequence of spike-like oscillations that followed (Fig. 3F), the durations of the "spikes" became progressively shorter, and the oscillations subsided with the membrane only slightly depolarized from the initial level.

A different pattern of oscillatory changes was induced by another experimental procedure (Fig. 3, A–C). This fiber was in the quiescent state represented by the end of record F. In Fig. 3A, the membrane potential was −50 mv. When a medium which contained Cl, but was otherwise unchanged, was introduced, the fiber repolarized by nearly 30 mv. After about 30 seconds the fiber underwent an abrupt spike-like depolarization, with a peak some 55 mv positive to the repolarized level. Less than 10 seconds later, there was a second spontaneous depolarization which settled from its peak to a steady value somewhat positive to the initial resting potential. In Fig. 3B, the resting potential became −55 mv. When Cl was introduced, the fiber again repolarized. After about 25 seconds a train of spike-like depolarizations developed, each followed by a hyperpolarizing undershoot. The durations of successive oscillations were prolonged, and each developed a plateau resembling that of the prolonged spikes of crayfish muscle fibers (*4*). However, the base line from which the successive spikes began drifted gradually toward more depolarization, so that the successive oscillations became smaller and eventually disappeared.

In Fig. 3C, the resting potential was −67 mv. The spike-like oscillations developed more slowly in the presence of Cl, and the total number was less before the membrane potential had reached a steady value about 5 mv positive to the initial resting potential. This spike-like oscillatory activity was never seen in the absence of EDTA. The repolarization elicited by introducing the Cl in the presence of TTX

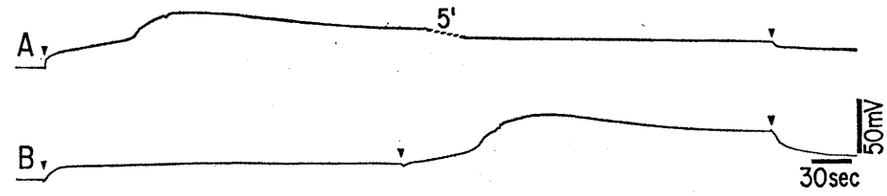

Fig. 2. Changes induced by removal of $CaCl_2$ in a fiber equilibrated in a K-free saline containing 13.5 mmole of $CaCl_2$ per liter. (A) Removal of Ca causing depolarization. (B) The same fiber was equilibrated in the K-free saline for about 20 minutes, but with TTX (5×10^{-7} g/ml) added. The $CaCl_2$ was removed (first arrow); TTX was removed (second arrow); then $CaCl_2$ was reintroduced (third arrow).

(Fig. 3D) was only slightly smaller than in Fig. 3C, but there were no oscillatory changes during a period of some 15 minutes, and the membrane potential remained at its repolarized level. However, when the TTX was removed, an immediate slow depolarization developed (Fig. 3E) which gave rise to a brief spike-like response and a prolonged plateau of depolarization. Except for the differences in amplitude, these changes in membrane potential resembled those seen in Fig. 1A. Thus, the oscillations appear to result from the competitive effects of the Na-dependent depolarizing electrogenesis and of a repolarizing electrogenesis. In the experiment of Fig. 3F, the repolarization must have been due to efflux of K, but in the presence of Cl (Fig. 3, A–C), the contribution of Cl movement cannot be discounted.

In the presence of 13.5 m*M* Ca, crayfish muscle fibers are effectively impermeable to Na (*9*). The data indicate that the membrane of the fibers becomes transiently permeable to Na when the Ca of the medium is greatly reduced. In the presence of Na the fibers depolarize, developing a response which resembles that of Na-dependent spike electrogenesis (*10*). The electrogenic processes normally present in crayfish muscle fibers are insensitive to TTX (*4, 11*). The Na-dependent depolarization, however, is blocked by TTX. Thus, the Na channels which become available in a medium with reduced Ca appear to resemble in their chemical structure Na channels involved in spike electrogenesis of axons (*5*) and electroplaques (*6*).

The Na channels that become available in the crayfish muscle fibers on removal of Ca must differ from those of axons and electroplaques in the following respects. (i) Whereas Li can substitute for Na in the spike electrogenesis of many cells (*12*), the Na channels of the crayfish muscle fiber membrane are impermeable to Li. (ii) The block of the Na channels by TTX in crayfish muscle fibers is rapidly reversible even after the fiber is exposed to the agent for more than 15 minutes (Fig. 3, D and E). In the spike-generating membranes, irreversible block of

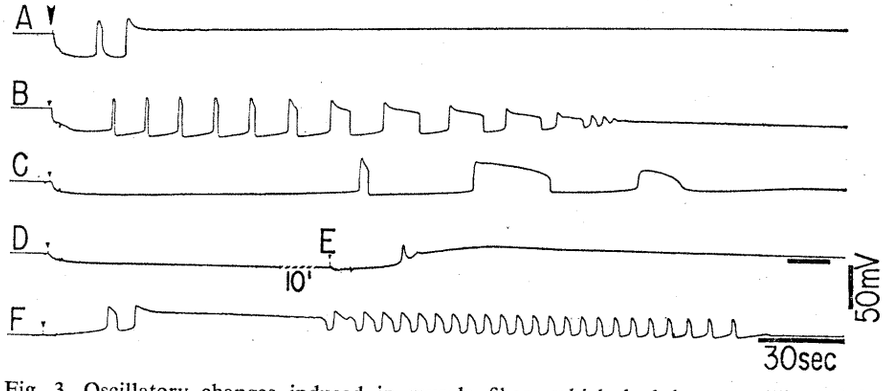

Fig. 3. Oscillatory changes induced in muscle fibers which had been equilibrated in Ca-free, Cl-free media. (A–E) In the equilibration medium, EDTA was also present. The concentrations were 1 (A), 0.1 (B), and 0.01 mmole/liter (C–E) Cl was substituted for propionate at the arrow in A–D. Absence of oscillatory activity in D was correlated with the presence of TTX (5×10^{-7} g/ml). After about 12 minutes (arrow in E) TTX was removed, and the fiber promptly responded with depolarization. (F) This fiber was initially equilibrated in a propionate medium free of Ca; EDTA (0.01 mmole/liter) was added at the arrow.

Na channels may develop after a brief exposure to TTX. (iii) The depolarizations induced in the crayfish muscle fiber are long-lasting, whereas Na activation of spike electrogenesis is usually short-lived, being reversed by depolarizing Na inactivation (*10*). However, under abnormal ionic conditions even squid giant axons develop prolonged spikes which may reflect marked slowing of Na inactivation (*13*).

The increase in Na permeability which is indicated by an increase in membrane conductance as well as by the depolarizing electrogenesis is only transient. The muscle fiber repolarizes even in a Ca-free medium. The intracellular concentration of Ca in crayfish muscle fibers is rather high (about 20 mmole/liter) (*14*), much of it presumably in bound form. The disturbance of the Ca gradient across the membrane, caused by removal of Ca from the medium, probably initiates an outward movement of this cation. Thus, it seems likely that the repolarization which signals a decrease in Na permeability is linked with the replenishment of Ca in the membrane and its vicinity. This conclusion is supported by the finding that a fiber repolarized in a Ca-free medium undergoes a second depolarization on addition of EDTA, or merely on changing to a fresh Ca-free medium. The replenishment of Ca should be depressed more by larger quantities of EDTA. This expectation is also fulfilled (Fig. 3).

No adequate morphological studies are available for correlation with the physiological data. However, an alteration in the Na : Ca ratio of the bathing medium causes transient changes in the membrane resistance and in the thickness of the unit membrane of the amoeba (*15*).

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Gametophytes of Four Tropical Fern Genera Reproducing Independently of Their Sporophytes in the Southern Appalachians

Abstract. *Vegetative reproduction and dispersal by way of gemmae are known to occur in four types of fern gametophytes. Although they belong to basically tropical rain-forest genera, all four types have now been discovered growing naturally in the vicinity of Highlands, North Carolina, as clones on shady, damp rocks. Their sporophytes were rare or absent. The gametophytes are now identified as* Grammitis nimbata *(Jenm.) Proctor, Grammitidaceae;* Hymenophyllum tunbridgense *(L.) J. Sm., Hymenophyllaceae;* Vittaria lineata *(L.) J. Sm., Vittariaceae; and* Trichomanes, *probably representing several species, Hymenophyllaceae. Identification of the* Grammitis *was facilitated by the presence of some juvenile or dwarfed sporophytes; this constitutes the first report of this species, genus, and family for temperate North America. Growth and spread of fern gametophytes independently of, and distant from, the corresponding sporophytes is a novel phenomenon which should be investigated in other parts of the world.*

The gametophytes or sexual plants of most fern families and genera lack any method of reproducing themselves. New thalli may occasionally be formed from branches or by fragmentation due to senescence of older parts, but usually fertilization and the formation of a sporophytic plant prevent further growth of the original gametophyte. However, in a few primarily tropical fern genera, single gametophytes can form large, long-lived populations. These gametophytes have specialized vegetative structures of dispersal, gemmae, which are tiny bodies usually made up of two to ten cells. The gametophytes are thus able to spread by air and form new colonies, generally resembling patches of liverworts or algae. Such gemmaebearing gametophytes may occur independently of their sporophytes, in some cases separated from them by hundreds of miles (*1, 2*).

The clonal fern gametophytes that reproduce by gemmae fall into four distinct types: (i) an elongate-cordate type with "string-of-beads" type of gemmae; (ii) a branching ribbon-like type with marginal rhizoids and small, plate-like gemmae; (iii) a branching ribbon-like type with diffuse rhizoids and spindle-shaped gemmae; and (iv) a filamentous type with spindle-shaped gemmae. These are found respectively in the Grammitidaceae (*3*); the Hymenophyllaceae, genus *Hymenophyllum* (*4*); the Vittariaceae (*1*); and the Hymenophyllaceae, genus *Trichomanes* (*4*). The new concept of pteridophyte gametophytes existing and reproducing as self-dispersing clones, often far from their sporophytes, is of considerable botanical interest.

We now describe an unexpectedly rich display of this phenomenon in the vicinity of Highlands Biological Station, Macon County, North Carolina. The region was originally chosen for study of clonal gametophytes because of earlier reports that, in nearby Pickens County, South Carolina, there was a single colony of sporophytes of *Hymenophyllum tunbridgense* (L.) J. Sm. (*5*) unknown elsewhere in the United States, and its other occurrences being in the mild climates of Europe and the West Indies. All the basic types of gemma-reproducing gametophytes were encountered at Highlands, including the first record of a new family of ferns for temperate North America. Highlands is located in the southern Appalachian