cate a basis for the dual effect of calcium. For example, Holman has demonstrated that calcium has a stabilizing effect on membrane of smooth muscle (*8*). Reduction of calcium chloride concentration to 5 percent of normal caused an initial increase in excitation in a taenia coli preparation, with action potential frequencies remaining at a high rate. When the same preparation was exposed to high concentrations of calcium there was a transient cessation of action potentials.

On the other hand, Axelsson and Bulbring have demonstrated that calcium is essential for the coupling of membrane excitation with tension development by the contractile protein (*9*). In the taenia coli, when calcium chloride was removed from the bath, the action potentials persisted but there was a failure of tension development. The importance of calcium in the coupling reaction is emphasized when the permeability of the membrane to this cation is increased by depolarization with potassium sulfate (*3*). In this case the magnitude of the response is related directly to the calcium concentration in the environment.

Considering these established effects of calcium on two different processes involved in the overall contractile response (that is, membrane excitation and coupling), a hypothesis can be developed to describe the mechanisms by which an increase in calcium produces the depression of the F-component and the potentiation of the S-component observed in the current study. One must postulate that the excitability of the membrane governs the F-component (see Fig. 2). When the membrane is stabilized by an increase in concentration of calcium, its threshold for excitability is raised, and the magnitude of the F-component is decreased. Conversely, when calcium concentration is reduced below physiological levels the membrane is labilized, its excitability is increased, and a larger F-component results. Of the series of events that occurs between the initiating stimulus and ultimate tension development by the contractile protein, the rate-limiting process for the F-component seems to be membrane excitability. On the other hand, the rate-limiting factor for the S-component appears to be a process that varies directly with the calcium concentration. This factor could well be the availability of calcium for the coupling process. It would follow, then, that below a given concentration (about 0.3 m*M*) there is insufficient calcium available for this process to effect a S-component of an epinephrine response. Above this concentration, for a limited range (up to 1.0 m*M*), the magnitude of the S-component is a direct function of the amount of calcium available for coupling. These relationships between membrane excitability and the F-component on the one hand, and calcium coupling and the S-component on the other, are shown diagramatically in Fig. 2.

Relationships between extremes of calcium concentration and the two parts of this contractile response follow from the assumption that membrane excitation and excitation-contraction coupling constitute two consecutive processes in a single sequence of events leading to tension development by the contractile protein. If the available calcium for the coupling becomes extremely low, coupling will then become the rate-limiting factor of the F-component as well as of the S-component. This is illustrated in Fig. 1, where, after the aortic strip has been in a calcium-free environment for 28 minutes, the F-component, as well as the S-component, is decreased in magnitude. Conversely, extremely high concentrations of calcium depress the S-component as well as the F-component.

The unique feature of the response of the isolated aortic strip to epinephrine is that, in a given total response, opposing effects of an increased concentration of calcium on two separate processes, membrane excitation and coupling, can be identified. It is evident, therefore, that when only the total response is recorded, its magnitude will be depressed in situations where the F-component (membrane excitation) is the limiting factor, while it will be potentiated when the S-component (coupling) is the limiting factor. This situation forms an obvious basis for the conflicting results that have been described as effects of various calcium concentrations on vascular responsiveness (*10*).

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Beading Phenomena of Mammalian Myelinated Nerve Fibers

Abstract. *Fresh mammalian nerve, when subjected to a small stretch, shows a beading phenomenon. The larger fibers appear as a series of dilations and constrictions at intervals of 40 to 75 μ. Upon relaxation this beading is gone within a minute. The beading is not produced by the special technique of freeze-substitution used to show the phenomenon.*

Our previous studies of axoplasmic flow (*1*) raised the possibility that peristalsis, which was recently observed cinematographically in fibers of dorsal root explants (*2*), might be found in mature, living, and relatively intact mammalian nerve fibers. However, the usual techniques of histological preparation are inadequate for investigations where rapid changes in shape might be expected to occur during the slow penetration of fixatives (*3*). In order to "catch" and hold the shape of nerve fibers in their living form, the technique of quenching (quick-freezing) nerves at very low temperatures followed by freeze-substitution was used (*4*).

Sciatic nerves of anesthetized and just-killed rabbits and rats were gently freed from their surrounding limb muscles. They were kept straight (but not overly stretched) while they were quick-frozen with Freon-12. The temperature of the Freon was reduced toward −160°C with liquid nitrogen. The stick-like frozen piece of nerve was then kept in a 1 percent solution of osmium in acetone at −20°C for 7 days so that freeze-substitution could take place. During this time ice goes into solution from the tissue and acetone and osmium enter; the process starts at the surface and occurs successively at such fine increments that

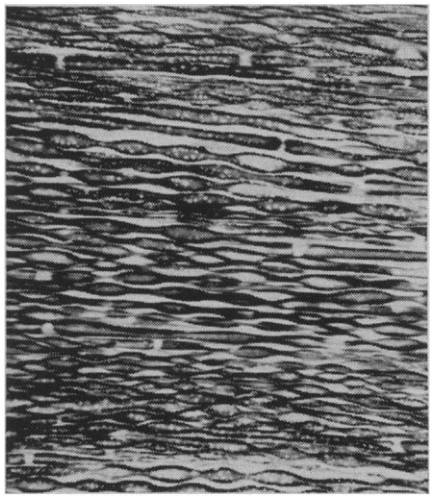

Fig. 1. Beading with slight stretch. Fresh rat sciatic nerve, with slight stretch applied, was quick-frozen *in situ*. Substitution-fixed with osmium-acetone.

fixation occurs with preservation of the original form. After the nerves had been fixed, they were imbedded in paraffin and sectioned at a thickness of 10 μ parallel to the long axis of the fibers.

Figure 1 shows a section of a nerve so prepared and the beaded appearance of many of the fibers. The myelin sheath takes up the osmium stain and clearly shows the form of the fiber by its darkly stained outline. As many as ten or more successive beads may be seen along a single fiber at intervals of 40 to 75 μ. In some fibers only a few beads may be seen, and the rest of the fiber is straight; in other parts of the nerve the fibers may show no beading at all. A similar degree of beading is seen in the fibers at the periphery of the nerve and within the depths. Under oil immersion microscopy, the osmium-stained myelin appeared to be of approximately equal thickness in both dilated and constricted regions of the beads. The nodes could be identified as clear gaps and usually did not have beads near them. Folds or intrusions of myelin are seen in many of the fibers near the nodes in the juxta-nodal regions. The more numerous, smaller, irregularly shaped clear areas scattered throughout the nerve fibers probably result from micro ice crystals which occur with low-temperature freezing (*5*).

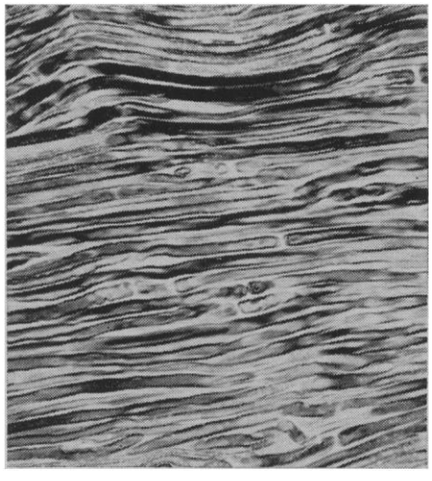

Fig. 2. Absence of beading in lax nerve. Rat sciatic nerve quick-frozen without stretching and substitution-fixed with osmium-acetone.

The beading is probably not produced by the process of freeze-substitution (a problem of earlier concern, *6*). Variations in the quick-freezing procedure caused little difference in the degree of beading. For example, the speed with which quick-freezing takes place is different with nerves quick-frozen with ethyl alcohol cooled by liquid nitrogen to the higher temperatures of $-80°$ to $-100°$C, but no difference was found in the degree of beading.

The random presence of ice crystals in the axons also suggests a generalized freezing effect rather than one acting at regular intervals along the fiber. Some regions in the nerve showed remarkably little ice crystallization, and the same degree of beading was found there as well as in other regions where a greater than usual degree of ice crystallization was present. The steps subsequent to quick-freezing seem still less likely to be responsible for beading. After fixation, instead of paraffin embedding, a set of nerves were hydrated and then cut with a freezing microtome. They showed the usual beading, indicating that the tissues are well fixed and stable in form after freeze-substitution has taken place.

A more persuasive argument that beading is not produced by the technique of freezing was the discovery that stretch is required for this degree of beading to appear. Sciatic nerves of rats were removed and kept lax before quick-freezing. These nerves are shown in Fig. 2. In both straight and curled portions of the nerve, the walls of the individual fibers appear to be unbeaded for the most part. A few fibers show some beading but with a smaller degree of dilation and constriction. The slight degree of stretch required to bring the nerve from a lax to a just-straight position is sufficient to cause beading. A more quantitative examination was made of the amount of stretch required. Rat sciatic nerves were stretched for 3 minutes with different degrees of tension and then quick-frozen at their stretched length. A tension as little as 2 g could excite a fair amount of beading; 5 to 10 g was sufficient to give good beading. More stretch gave rise to even more beading.

Retention of beading after a brief period of stretch was investigated. Nerves stretched with 8 g for a period of 1 minute and held at that length showed good beading. A series of nerves were then subjected to 8 g of stretch for 1 minute and allowed to relax for various periods of time before quick-freezing. Beading was retained for 0.5 to 1.0 minute after such a stretch. Fibers in the frog sciatic did not show beading when stretched.

The relation of the reversible beading phenomenon described in this report to the periodic changes in form observed in formalin-fixed nerve and possibly also to the incisures of Schmidt-Lantermann and its varied modifications (Golgi-Rezzonnico apparatus, infundibular membrane, and so forth) seen under varied conditions of staining (*7*) invites further study.

That this phenomenon is not of functional significance and only an expression of some physical change produced by stretch is an evident possibility. However, the possibility also remains that the beading excited by stretch is an exaggerated *en masse* activation of a mechanism which on a lesser scale could be responsible for axoplasmic flow (*8*).

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