invaded by matrix and are extremely delicate. The tooth corresponds exactly in shape and size with an anterior tooth of the contemporary carnivore *Allosaurus*.

Strong evidence that this fossil mass is the contents of a stomach is the fact that it was found among the skeletal remains of a sauropod dinosaur. The bones had been exposed and were broken, scattered and weathered, as is usual in such instances. A few of the larger pieces were collected by Shrum and examined by me. Included are the distal end of a large fibula and the centrum of a cervical vertebra at least 45 cm long with the articulating surfaces preserved. These pertain to one of the large sauropods, but the exact species probably cannot be determined.

The diet and food preferences of the sauropods have been debated. The teeth provide no diagnosis; they are confined to the front part of the mouth, are blunt and relatively small, and in some cases are separated by gaps. The continuous replacement process evidently did not provide an even, regular tooth row, and the whole dental array would appear to be unequal to the task of food gathering for such massive animals. Although the sauropods are usually considered to have been herbivores, a few writers believe they had a mixed or even carnivorous diet. The present specimen strongly hints that the sauropods may have been indiscriminate in their food gathering to the point of being omnivorous.

Although most of the fossil fragments are woody vegetal material, there are enough pieces of broken or digested bone to show that a considerable amount of flesh must have been eaten. The amorphous material may be partly mud scooped up in the process of gathering other material, or it may represent organic-rich sediment or sapropel with enough food value to have been purposefully sought out. Only by utilizing abundant readily available food sources could the sauropods survive. They were apparently among the most effective harvesters of river and lake food resources ever evolved. After the Jurassic they were mostly replaced by the duckbill dinosaurs with teeth that were better suited for grinding and chewing tough, woody material.

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Electrochemical Coupling in Potentiation of Muscular Contraction

Abstract. *Diverse potentiators of contraction have basically identical, active-state mechanical effects, but act by different membrane-mediated electromechanical coupling mechanisms. The falling phase of the action potential is greatly prolonged by Zn^{2+} (0.1 mM) and UO_2^{2+} (0.5 to 1 μM), neither of which affects the mechanical threshold. Caffeine (1 mM), like the lyotropic anions, acts conversely. Thus changes in the duration and mechanical threshold of the action potential determine independent electromechanical coupling processes which can act individually, or conjointly in the action of other potentiators, in determining the duration of the active state and thus the potentiation of twitch tension.*

Characteristically, potentiation of contraction is a great increase in twitch tension without change in tetanus tension. Such potentiation may be produced in skeletal muscle fibers by many substances remarkably diverse, both in chemical nature and in the concentrations at which they are most effective (*1*). For example, caffeine, an uncharged molecule, is very effective at a 1 m*M* concentration (*2*); lyotropic anions are most effective at a 0.1*M* concentration (*3, 4*); and certain heavy metal anions are most effective at smaller concentrations—Zn^{2+} at 0.05 m*M* (*5*) and UO_2^{2+} at 0.5 μ*M* (*6*). Potentiators modify still other mechanical features of contraction, but the basic alteration inducing all these effects is prolongation of the active state of the contractile component (*1, 3, 4*). The anions, however, and evidently all potentiators, do not act directly on this component but act at relatively superficial sites of the plasma membrane and perhaps the sarcoplasmic reticulum (*1; 3–5*). Accordingly, potentiators, after exerting their primary effects at these membrane sites, may cause potentiation by altering processes of excitation-contraction coupling that link reactions of the membrane to activation of contraction (*1, 7*).

It has been postulated (*4; 8–10*) that the potentiating alterations of excitation-contraction coupling are essentially electromechanical and are mediated by some combination of (i) prolonging the action potential, and (ii) lowering the mechanical threshold, which, as determined in potassium-depolarization contractures of muscle fibers (*4*), is the membrane potential at which the minimum mechanical response appears. While many potentiators prolong the action potential (*9, 10*), only lyotropic anions have been reported to reduce the mechanical threshold (*4, 11*). We therefore tested the effects of a number of potentiators on both action potential and mechanical threshold. We report here a general account of our results, which increase our knowledge of the mechanisms of potentiation, and which relate to the chemical differences of some of the potentiators.

We recorded action potentials of single fibers of frog sartorius muscles by standard techniques, using internal glass microelectrodes filled with 3*M* KCl. The excised muscle was mounted at about 1.2 times its rest length in a small Lucite chamber. Action potentials were obtained after successive periods of equilibration in Ringer's solution, test solution, and then, either in the Ringer's solution again, or some modification of it which would cause reversal of potentiator effects. We used square-wave shocks of 0.3 msec duration and of sufficiently low strength that, when applied to the surface of the muscle, they excited only a few fibers and thus reduced contractile movement and minimized loss of electrode impalement. Each action potential was simultaneously recorded on a fast and a slow sweep to facilitate temporal analysis of all phases of the potential. No simultaneous records were made of the mechanical responses, but average effects of the potentiators used in these experiments are well known from other work in our laboratory.

As shown in Fig. 1, 0.1 m*M* Zn^{2+} and 0.5 μ*M* UO_2^{2+}, which after 24 minutes and 60 minutes, respectively, produce maximum twitch potentiations of about 100 to 150 percent (*5, 6*), cause a 200 to 300 percent prolongation of the entire falling phase of the spike. The rising phase and other features of the response were somewhat modified,

but these minor alterations are not discussed here. Similar results for zinc have been reported by others (*12*). Reversal of the potentiation caused by these ions was very slow in pure Ringer's solution (*5, 6*), but was rapid for the zinc-treated muscles when calcium ethylenediamine tetraacetic acid (Ca-EDTA), but not phosphate, was added to the reversal medium and for the uranyl-treated muscles when phosphate (but not Ca-EDTA) was added (see *13*).

Analogous reversal effects were obtained for the alterations in action potentials. Phosphate and Ca-EDTA penetrate very slowly, if at all, into muscle fibers, and they form very tightly-binding complexes with Zn^{2+} and UO_2^{2+}, respectively. Therefore, the rapidity with which they cause reversal indicates that the metal ions were readily accessible—that is, they were at excitatory membrane sites—and that reversal was accomplished by the ability of the reversing agents to form complexes with the metal ions and thus remove them from these sites. We infer that the direct consequence of the primary action of the metal ions at the fiber surface is to prolong the action potential, and that this change [as suggested for the action of other potentiators (for example, see *8–10*)] then modifies excitation-contraction coupling so as to prolong the active state and potentiate the twitch.

Caffeine, in a 1 m*M* concentration, causes an average twitch potentiation of 90 percent (*2*) but, as shown in Fig. 1, it does not significantly affect the action potential (*14*). Careful measurement shows, however, that the duration of the repolarization phase of the action potential is, in general, prolonged by about 10 percent of its normal value. But in relation to causing potentiation, we consider such a change insignificant in comparison with the 20 to 30 times greater relative prolongation of the action potential caused by the heavy metal ions. Etzensperger (*15*), by using 3.6 m*M* caffeine, has obtained much greater prolongation of the action potential than we have, but our result is especially noteworthy because it shows that great potentiation occurs in twitch responses that have very small alterations in the action potential.

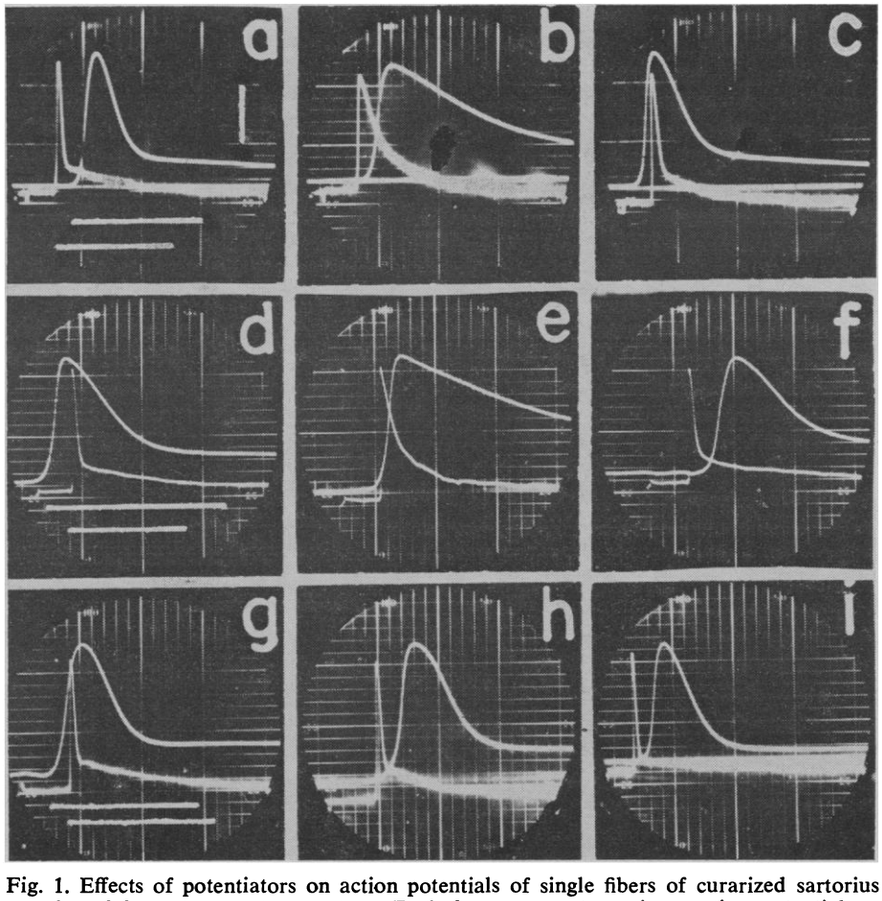

Fig. 1. Effects of potentiators on action potentials of single fibers of curarized sartorius muscles of frog at room temperature. Each frame presents a given action potential on both a fast (upper) and a slow (lower) sweep. The corresponding time calibration lines indicate: upper in all instances, 5 msec; lower in *a*, 25 msec; in *d* and *g*, 50 msec; and each pair of such calibrations applies to all three records of its row. Voltage calibration for all records: vertical line in $a = 50$ mV. Zero potential level for all fastsweep records is given by uppermost heavy horizontal line. Zinc series: *a*, normal; *b*, after 24 minutes in 0.1 m*M* Zn^{2+} (0.1 m*M* $ZnCl_2$ in Ringer's solution); after *b* the muscle was replaced in pure Ringer's for 30 minutes but the action potential remained as in *b*; *c*, after 3 minutes in Ringer's solution containing 0.1 m*M* Ca-EDTA. Uranyl series: *d*, normal; *e*, after 60 minutes in 0.5 μM UO_2^{2+} (0.5 μM uranyl acetate in Ringer's solution); after *e*, 60 minutes in pure Ringer's solution did not change the action potential; *f*, after 7 minutes in 2 m*M* phosphate (added as a buffer mixture, *p*H 7.0, of sodium phosphates). Caffeine series: *g*, normal; *h*, after 20 minutes in 1 m*M* caffeine base in Ringer's solution; *i*, 15 minutes after return to normal Ringer's.

Since the heavy metal ions and 1 m*M* caffeine differ so much in their effect on the action potential, the question arises whether they also act differently on the mechanical threshold. We therefore determined the effects of the potentiators on the generation of contractures caused by potassium, using a technique essentially like that of Hodgkin and Horowicz (*4*), but in place of single fibers we used the toe muscle (IV) of the frog.

Figure 2 shows that 1 m*M* caffeine alters potassium contractures, in the same manner as NO_3^- and SCN^- (*4*), and Br^- (*11*), so that the depolarization of the membrane required to activate a given output of contracture tension is about 15 mv less in the presence of the caffeine than it is under normal conditions. Thus, caffeine acts essentially like the anions by shifting the mechanical threshold from a membrane potential of about −58 to −72 mv. Accordingly, we infer that as the action potential runs its course in a twitch, the mechanical threshold and increasing mechanical activation are generally reached, under the action of caffeine, at a more negative series of membrane potentials than hold for the normal response, and that it is this overall electromechanical alteration that predominantly determines potentiation by 1 m*M* caffeine. In a series of comparable experiments, we find that, in contrast to the action of caffeine, 0.1 m*M* Zn^{2+} and 1 μM UO_2^{2+} cause no change in either the mechanical threshold or the general curve of tension output as a function of K^+ concentration (*16*).

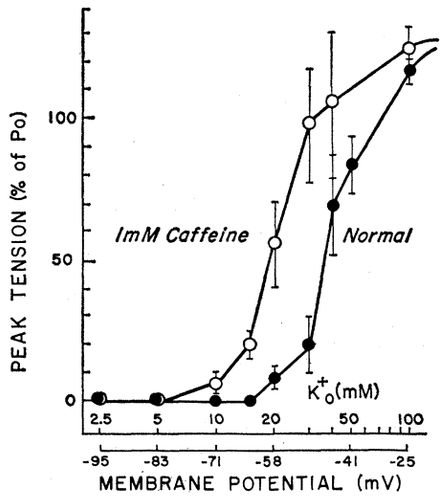

Fig. 2. Effect of 1 m*M* caffeine base on peak contracture tension output of extensor toe (IV) muscles of frog, suddenly exposed to different concentrations of K^+. All the contracture test solutions contained the same cation concentration made up of 1.8 m*M* Ca^{2+} and an appropriate mixture of K^+ and choline. Choline replaced Na^+ to avoid transient excitation of twitches due to high concentrations of K^+. Contracture tensions are given relative to maximum tetanus tensions (P_o) which were separately activated by massive stimulation of the muscles in ordinary Na-Ringer's solution. The points give average tensions (and associated standard errors) from at least four, and in most instances seven to nine tests.

There are evidently two different electromechanical mechanisms for potentiating contraction. In the first, which is characteristic of the heavy metal ions, the repolarization phase of the action potential is in general greatly prolonged, but there is no change in mechanical threshold. In the second mechanism, exemplified by our results with 1 m*M* caffeine, there is a barely appreciable change in the action potential, but the mechanical threshold is considerably lowered. The anions NO_3^- and Br^- (about 120 m*M*) and SCN^- (12 m*M*) act in a way similar to 1 m*M* caffeine, since they lower the mechanical threshold (*4, 11*), but have negligible action potential effects (*9, 17, 18*), and especially in the sense that the changes that do occur do not shift the membrane potential beyond even the lowered threshold and therefore do not have mechanical consequences. The two basic mechanisms depend on the production of changes in systems concerning membrane-potential behavior —the one by directly increasing the duration of the action potential, the other by lowering the mechanical threshold. Hence, this, in addition to the evidence previously discussed, indicates that potentiators have their primary action at certain sites on the membrane.

Among the attempts to explain the role of the action potential in activating contraction (*9, 10, 18*), Etzensperger (*10*) has proposed that, in potentiated contractions, the most important effect is a prolongation of the course of the action potential, which then prolongs the active state by increasing the time during which the membrane potential is shifted beyond the mechanical threshold. This must be the basis of the first type of potentiating mechanism, since the only evident change which is electromechanically pertinent is prolongation of the action potential. Even so, it may not be the time alone, but the integral of the relevant voltage over time that is significant.

The purely temporal feature of the action potential is not so significant in the second type of potentiating mechanism. In their discussion of the electromechanical basis of anionic potentiation, Hodgkin and Horowicz (*4*) suggest that lowering the mechanical threshold "is likely to be at least as important" as various augmentations of the action potential. Our foregoing discussion indicates, however, that the lowering of the threshold is the predominant, if not the sole, change engendering potentiation by the anions. With 1 m*M* caffeine, the lowered threshold is the dominating factor because, as our results indicate, the prolongation of the mechanically effective duration of the action potential (indirectly ascribable to reducing the threshold) is small in comparison with that caused by the heavy metal ions. Thus, we infer that, in general, lowering the mechanical threshold causes potentiation not merely by indirectly extending the mechanically effective period of the action potential, but by acting as an independent and in some cases a sole factor. Some potentiators may act by causing pronounced changes in both threshold and action potential. This should be true for caffeine, at the higher concentrations which prolong the action potential considerably (*15*), and also for quinine, which has a powerful potentiating effect (*1*), prolongs the action potential (*9, 15*) and, in 0.1 m*M* concentration, reduces the mechanical threshold as effectively as caffeine or NO_3^- (*19*). Much further work is required to determine the electrochemical nature of these electromechanical processes and to establish whether they are the only processes which cause potentiation (*20*).

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16. The mechanical thresholds have been determined in media with the Na replaced by choline and, therefore, the values of the membrane potentials relevant to the action of caffeine are about 5 mv more negative than would appear in media containing sodium (*4*). The presence of choline, however, is not the cause of the constancy of the mechanical threshold in the presence of the heavy metals, because the same behavior occurs in Na-containing media. In reference to the action of caffeine, it is interesting that J. Axelsson and S. Thesleff [*Acta Physiol. Scand.* **44**, 55 (1958)] find that the ability of the drug to produce contracture (in concentrations greater than about 2 m*M*) is sensitized by a prior K depolarization: this, of course, is the reverse of our finding that 1 m*M* caffeine sensitizes the fiber to the contracture-producing effect of K depolarization.
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20. We presented this work as a paper at the meeting of the Society of General Physiologists, Woods Hole, Mass., 4–7 Sept. 1963. After this meeting we received a communication from J. Etzensperger, informing us that he has obtained effects of caffeine on potassium contractures which are quite similar to ours.
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