isotonic contractions resumed. If the blood was first mixed with CT and allowed to stand for 20 minutes before the acetylcholine chloride was added (the CT being at a concentration of about 50 μg/ml), contractions of greater amplitude occurred, as shown in Fig. 2*A* (right). The hydrolytic effect of cholinesterase in blood upon acetylcholine was inhibited by the presence of CT. If the rabbit intestine was first treated with CT (50 μg/ml) for 2 minutes, the contractions caused by the additional acetylcholine chloride (0.25 μg/ml) were much greater than those elicited by acetylcholine chloride alone (Fig. 2*B*); the CT alone (50 μg/ml) caused no contractions.

When erythrocytes of human blood were treated with CT extract from shark liver, the cholinesterase enzyme activity was inhibited by 26 percent ($p < .001$) as determined by the electrometric method of Michel (*6*). In these experiments, 1 ml of buffer or of toxin solution (320 μg/ml or an estimated 0.42 μg/ml of pure toxin) was added to 1 ml of erythrocyte solution (0.02 ml of red blood cells) and 18 ml of buffer solution. Bovine erythrocyte cholinesterase (*7*) was then tested according to Ellman's method (*8*) with four different concentrations of CT (estimated as pure toxin) arranged logarithmically from 0.25 to 2 μg/ml. The cholinesterase activity was inhibited by 48 percent with CT at a concentration of 2 μg/ml. The data showed a linear relationship between the logarithmic concentration and the percentage of inhibition within the range studied. Our results are comparable with those of Winter (*9*) who used an automatic analyzer to demonstrate the effects of organic phosphate insecticides on bovine erythrocyte cholinesterase.

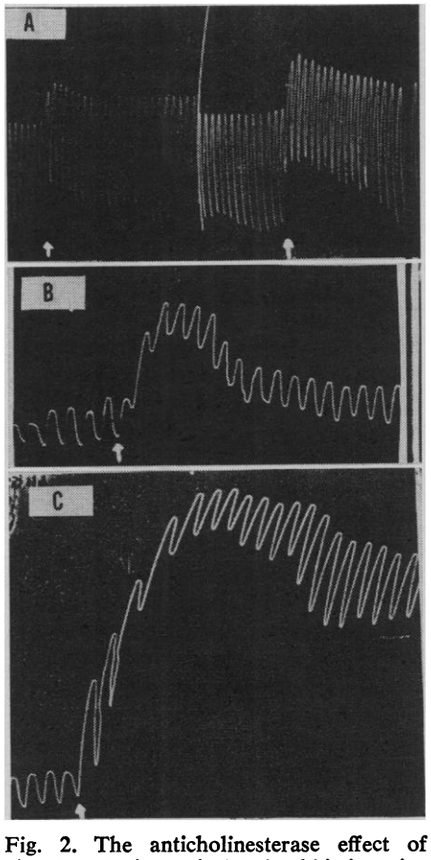

Fig. 2. The anticholinesterase effect of ciguatera toxin on isolated rabbit intestine in 25 ml Tyrode's solution bath. Acetylcholine chloride or mixed solution added at arrow. *A* (left), Acetylcholine chloride (0.1 μg/ml) and blood; (right), acetylcholine chloride and blood premixed with ciguatera toxin (50 μg/ml). *B*, Acetylcholine chloride (0.25 μg/ml). *C*, Intestine treated with ciguatera toxin (50 μg/ml) before the addition of acetylcholine chloride.

Various drugs were tested for their prophylactic and therapeutic action. Atropine (up to 5 mg/kg) injected before or after an injection of CT was effective in counteracting the muscarine-like action of CT. Atropine with magnesium sulphate (200 mg/kg) had the additional effect of abolishing the muscular paralysis and tremors due to the nicotine-like action of CT. The time that mice or rats given lethal doses of CT survived could be prolonged by giving successive doses of atropine, but death could not be prevented.

Physostigmine (*10*) injected with or without atropine before the administration of CT had little protective action on mice or rats (10 to 20 percent survived). Protopam chloride (2-formyl-1-methyl pyridinium chloride oxime), when administered by various routes with atropine, prevented death of both rats and mice injected with CT. Greater effectiveness was obtained by giving up to six successive doses of Protopam chloride (20 μg/g intravenously and 120 μg/g intramuscularly or intraperitoneally for the initial dose, reduced by 50 percent in successive doses) at 45-minute intervals, the number of doses given depending on the amount of CT injected. Although the effects of CT resembled those of the lipid-soluble organophosphorous compounds, the antagonistic action of Protopam chloride was not as great as against diisopropyl fluorophosphate and Paraoxon (*11*). It is not certain whether the mode of antagonistic action of Protopam to CT is comparable to that of the phosphate esters. Reactivation of cholinesterase inhibited by phosphate esters has been described by Nachmansohn (*11*) and O'Brien (*12*).

Since cyanosis was observed consistently before cessation of respiration, and since artificial respiration prevented death, the cause of death from ciguatera toxin is believed to be asphyxia.

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Glycerinated Skeletal and Smooth Muscle: Calcium and Magnesium Dependence

Abstract. *Contractions of glycerinated skeletal (striated) and vascular (smooth) muscles are similar in their calcium dependence but differ in their magnesium dependence. The threshold concentration of free Ca^{++} for contraction of either muscle was 1.8 × 10^{-7}M; maximum tension developed when the concentration of free Ca^{++} was slightly greater than 10^{-6}M. The Mg concentration required for contraction of smooth muscle was at least ten times as great as that for skeletal muscle.*

It is generally accepted that contraction and relaxation in striated muscle depend on the concentration of Ca^{++} in the sarcoplasm surrounding the myo-

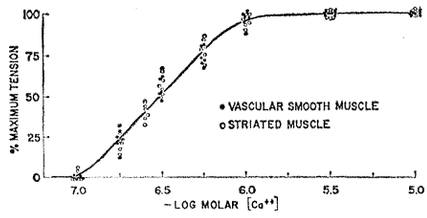

Fig. 1. Effect of concentration of free Ca^{++} on tension developed by individual psoas (striated) and vascular (smooth) muscle fibers. Conditions: temperature, 20°C; 150 m*M* KCl; 20 m*M* histidine buffer (*p*H 6.6); 5 m*M* ATP and 5 m*M* Mg; 4 m*M* $EGTA_{total}$. Free $[Ca^{++}]$ was varied by changing the ratio $Ca_{total}/EGTA_{total}$ appropriately. Calcium requirements for contraction of these two preparations are virtually identical.

fibrils. The threshold concentration of free Ca^{++} for superprecipitation and adenosine triphosphatase activity of skeletal muscle myofibrils and actomyosin was shown by Weber and Winicur (*1*) and Ebashi (*2*) to be about $10^{-7}M$. Seidel and Gergely (*3*) demonstrated that with glycerinated psoas fibers, at least $10^{-6}M$ *total* Ca must be present in the bath containing adenosine triphosphate (ATP) to increase the rate of contraction. However, threshold concentrations of *free* Ca^{++}

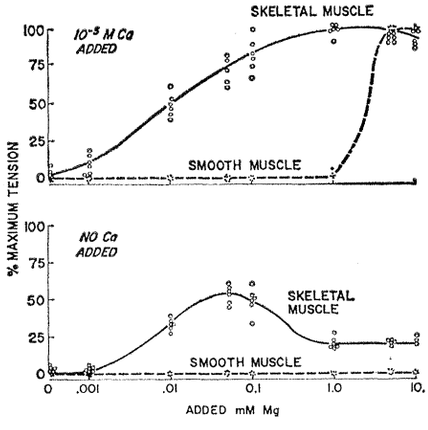

Fig. 2. Comparison of tension developed by striated and vascular smooth muscle with increased [Mg]. Upper graph: in presence of $10^{-5}M$ Ca based on the Ca/EGTA ratio (actual $[Ca^{++}]$ would be lower than this because of its chelation by ATP; free Ca^{++} was calculated to be adequate to produce a maximum response). Lower graph: in the absence of added Ca. Conditions: temperature, 20°C; 150 m*M* KCl; 20 m*M* histidine buffer (*p*H 6.6); 5 m*M* ATP; and 0.5 m*M* EGTA. The Mg requirement for contraction of smooth muscle is much greater than that for contraction of skeletal muscle.

for activity of glycerinated fibers of striated or smooth muscle have not been reported. We have studied the dependence on free Ca^{++} concentration of development of tension and rate of contraction by glycerinated psoas and vascular smooth muscle fibers.

Fibers were extracted in 50 percent glycerol for 1 to 3 months. Psoas fibers were teased to a diameter of 50 to 100 μ; the uncoiled fibers of the media of hog carotid artery (*4*) were 150 to 300 μ thick. All fibers were soaked for 15 minutes in 10 m*M* EGTA [ethyleneglycol bis (β amino-ethylether)-*N*,*N*^{1}-tetraacetic acid] (*p*H 7) before they were mounted in the bath. Isometric tension developed by 5-mm lengths of these fibers was measured by a Grass FTO3 Force Transducer system which was displaced less than 200 μ per gram load. Psoas fibers were used at equilibrium length and the carotid media fibers were stretched 5 to 10 percent to give a resting tension of 60 mg or about 30 g/cm^2. Solutions in the bath were agitated continuously by a stream of air bubbles.

The various concentrations of free Ca^{++} were obtained by using an EGTA-CaEGTA buffer system similar to that of Weber and Winicur (*1*). The concentration of free Ca^{++} was derived from the data presented by Portzehl, Caldwell, and Ruegg (*5*), which show the relation of this concentration to the ratio of total Ca to total EGTA in the presence of Mg at *p*H 6.6, with a binding constant of 1.3×10^{-6} being used. Since changes in *p*H are known to affect the binding constant of the ligant, each test solution was adjusted to *p*H 6.6 just before the fiber was exposed to it. The *p*H was rechecked after contraction and found to be unchanged.

No tension developed in either striated or smooth muscle fibers when 5 m*M* ATP was added to a bath containing 4 m*M* EGTA, 20 m*M* histidine (*p*H 6.6), 150 m*M* KCl, and 5 m*M* Mg. It can be estimated that under these conditions there is less than $10^{-8}M$ free Ca^{++} in the bath. For studies of the dependence of tension development on the concentration of free Ca^{++}, a muscle was exposed to a series of solutions of successively higher Ca^{++} concentrations, each buffered to *p*H 6.6 (Fig. 1). With free Ca^{++} concentrations less than $1.8 \times 10^{-7}M$ in the bath there was no tension development, but in solutions containing this concentration or greater concentrations, contraction nearly always occurred. There was no significant difference between the psoas fibers and vascular smooth muscle fibers in this calcium threshold for contraction (Fig. 1).

Increasing the free Ca^{++} concentration by increments from 10^{-7} to $10^{-5}M$ caused an increase in both the rate of tension development and amount of tension developed by striated and smooth muscle fibers; in concentrations of Ca^{++} above $10^{-5}M$, however, no further increase occurred. As shown in Fig. 1, the relation between tension and the negative logarithm of the Ca^{++} concentration appears linear over a wide range. The maximum amounts of tension developed were 1000 g/cm^2 and 100 g/cm^2 for skeletal and vascular smooth muscle, respectively. The maximum rate of contraction of skeletal muscle (approximately 100 mg/sec) was about 50 times as great as that of vascular smooth muscle (approximately 2 mg/sec). The Ca-initiated contraction could be completely reversed by removal of the Ca with EGTA.

The Ca^{++} requirements for contraction of these striated and smooth muscle preparations appear to be quite similar; their Mg requirements, however, show distinct differences. Figure 2 shows that at concentrations of Mg far below those present intracellularly (that is, less than 0.1 m*M* Mg in the presence of 0.5 m*M* EGTA), psoas fibers developed considerable tension even in the absence of effective concentrations of Ca^{++} (below $10^{-7}M$), whereas vascular smooth muscle fibers did not develop tension at any concentration of Mg in the virtual absence of Ca^{++}. At Mg concentrations greater than 0.1 m*M*, tension development by psoas fibers was markedly inhibited in the absence of Ca. This inhibition (relaxation) was not complete, though it might have been expected to be since the psoas preparation failed to contract in the absence of Ca when 5 m*M* Mg was present (Fig. 1). Though Ca was omitted in the bath solution used for this study (Fig. 2, lower curve), sufficient free Ca^{++} must have been present as contaminants of Mg or ATP to cause this partial contraction. The apparent conflict in the results presented in Figs. 1 and 2 can be attributed to the difference in the concentrations of EGTA in the two sets of experiments:

4.0 mM in the experiments illustrated in Fig. 1, 0.5 mM in those illustrated in Fig. 2.

In studies designed to test this action of EGTA, an increase in EGTA from 0.5 to 4 mM in the presence of Mg at a concentration greater than 1 mM caused nearly complete relaxation. The inhibition of contraction by concentrations of Mg greater than 0.1 mM is completely reversed by the addition of $10^{-5}M$ free Ca^{++}. Maruyama and Watanabe (*6*) have reported similar biphasic effects of Mg on the superprecipitation and adenosine triphosphatase activity of myosin B from skeletal muscle, and recently the same has been reported for glycerinated psoas muscle (*7*).

Vascular smooth muscle does not develop tension unless the Mg concentration of the bath closely approximates the ATP concentration, even though free Ca^{++} is present. With 5 mM ATP and free Ca^{++} in the bath (that is, under conditions approximating those in active muscle), the Mg requirement of vascular smooth muscle is much greater (at least tenfold) than that of skeletal muscle. This contraction of glycerinated vascular smooth muscle in high Mg concentrations is not produced by release of chelated Ca since in these studies (Fig. 2, upper curve) the concentration of Ca^{++} was sufficient to give a maximum contraction.

In its dependence on Ca and Mg, glycerinated skeletal muscle behaves as one might predict from studies of adenosine triphosphatase activity and superprecipitation. Similar values for Ca^{++} concentrations for threshold and maximal activity have been found for the glycerinated muscle model, adenosine triphosphatase activity, and superprecipitation. The dual effect of Mg, activation or inhibition depending on its concentration, also is qualitatively and quantitatively similar in all three. Thus a substantial link is added in the chain of evidence suggesting that the properties of actomysin which are rate-limiting for its adenosine triphosphatase activity and superprecipitation are, indeed, the same as those that are rate-limiting, *in situ*, for the development of tension.

The glycerinated smooth muscle model exhibited a striking similarity to skeletal muscle in the Ca^{++} requirement for contraction. On the other hand, the Mg^{++} requirements of these two muscles were distinctly different. The high concentration of this cation required for contraction of smooth muscle raises the possibility that it may have a regulatory role in the contraction of this tissue.

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Noradrenaline Stores in Nerve Terminals of the Spleen: Changes during Hemorrhagic Shock

Abstract. *In dogs subjected to hemorrhagic shock, a marked decrease in the noradrenaline content of the sympathetic nerve terminals in the normally innervated spleen is revealed by means of a histochemical fluorescence method. Deprivation of the sympathetic impulse-flow to the tissue immediately before the animals are subjected to shock prevents this depletion. The results support the hypothesis that the vasoconstriction which occurs during shock is due to the effect of noradrenaline released locally in the tissues, and not to circulating noradrenaline.*

We have reported that the tissue content of noradrenaline markedly decreases during hemorrhagic and endotoxic shock in dogs and in rabbits (*1*). It has also been reported that sympathetic denervation or blockade of the sympathetic nerves protects the tissue from otherwise irreversible damage in shock (*2, 3*). It was suggested that the abundant release of noradrenaline from the sympathetic nerve terminals in the tissue during shock, rather than circulating catecholamine, causes vasoconstriction and impairment of blood flow and tissue oxygenation.

We have studied the noradrenaline content of the nerve terminals in the spleen by the fluorescence method of Falck and Hillarp (*4*) which is highly specific for monoamines in, for example, the cell bodies, axons, and terminals of adrenergic neurons. The tissue, taken from different parts of the spleen at different times, was freeze-dried, treated with formaldehyde gas, embedded in paraffin, sectioned, and mounted for fluorescence microscopy. By this treatment noradrenaline is converted to an intensely green fluorescent substance, easily recognized in the fluorescence microscope used.

Dogs weighing 20 to 25 kg were subjected to irreversible hemorrhagic shock by a standardized procedure (*1*) according to which the blood was collected in an open reservoir by means of a catheter inserted in a femoral artery. The aortic pressure was kept constant at 35 mm-Hg by adjusting the height of the reservoir. When the animals had taken back 40 percent of the maximum amount of blood removed they were given back the remaining amount of blood in the reservoir. With this technique most dogs die in irreversible shock 2 to 4 hours after transfusion. The spleen was examined by laparotomy. Either 4 weeks before or immediately before the dogs were subjected

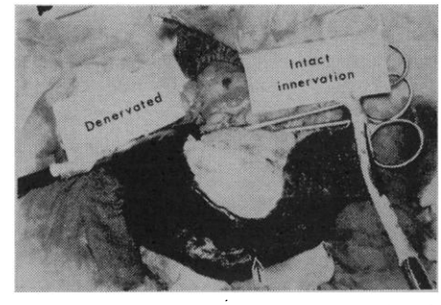

Fig. 1. The spleen of a dog bled for 3 hours. The contracted half of the spleen with intact innervation is on the right; on the left is the denervated half, unchanged in size. Before bleeding, these two parts were about the same size. The borderline between the two parts is indicated by the arrow.