speculate that the whole pattern of dominance by blue-green algae is the result of a series of events allowing their kinetics of nutrient uptake and use to ensure their success. Thus, if we begin with a sparse mixed population of algae and a *p*H near neutrality, the productivity increases as nutrients are added to the point where free CO_2 disappears and bicarbonate CO_2 is utilized. At this point the *p*H rises. Their apparently advantageous CO_2 uptake kinetics allow the blue-green algae to begin to dominate. Furthermore, since, as I have recently found, the phosphate uptake kinetics favor blue-green algae over green algae, the phosphate supply of the blue-green algae is relatively assured (*4*). Because of this, the nitrogen-fixing blue-green algae are able to obtain nitrogen as well. Thus, one advantage leads to another, and so on.

Green algae are generally considered more desirable in that they do not form floating scums. Moreover, because green algae are more acceptable ecologically in that they are within the food chain, it would be reasonable to investigate the effect of the injection of CO_2 with or without air into lakes as a means of stimulating the growth of green algae at the expense of blue-green algae. I do not mean to imply that phosphate removal to control gross algal production should not be the ultimate goal, but, in view of the many lakes for which phosphates enter from diffuse sources rather than from controllable sources, alternatives are needed.

Finally, a disclaimer—this report should be taken to mean that I feel that the rate of supply of CO_2 is an important factor in regulating the qualitative nature of the phytoplankton. But I do not believe that the rate of supply of CO_2 from natural sources, in most cases, limits the gross production of phytoplankton.

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4. The as yet unpublished results of my experiments with algal cultures show that the half saturation constants for phosphate uptake by blue-green algae are significantly lower than those for green algae over a wide range of environmental conditions.
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Localization of Calcium Pump Activity in Smooth Muscle

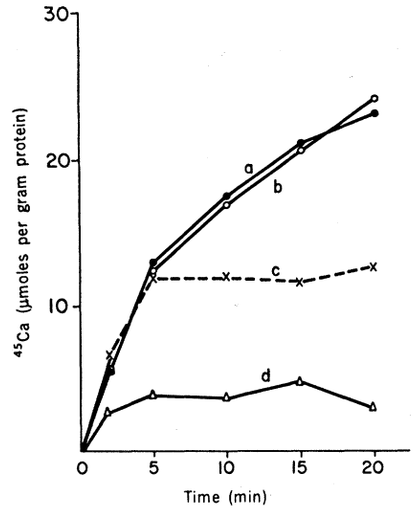
Abstract. *A microsomal fraction isolated from longitudinal smooth muscle of guinea pig ileum actively sequesters calcium ion in the presence of magnesium and adenosine triphosphate in a fashion previously described for microsomes of the rabbit aorta. This activity in guinea pig ileum appears to be associated primarily with the plasma membrane as is found in the red cell. By contrast the uptake of calcium in aortic smooth muscle appears to be associated to an appreciable extent with intracellular membranes, possibly analogous to the sarcoplasmic reticulum of skeletal muscle.*

Calcium ion is the agent that activates contractile elements in all types of muscle fibers. However, the triad (or dyad), a subcellular organelle that serves as both a source and a sink for the divalent ions in skeletal (*1–3*) and cardiac muscle (*2–4*), has not been found in smooth muscle. Based on evidence obtained from numerous physiological studies performed on various types of smooth muscle fibers, there is currently a consensus that the calcium ions associated with mechanical activity in smooth muscle may arise from two different sources (*5, 6*). One is the pool of calcium that is present in the extracellular fluid or that is loosely bound to superficial sites in the muscle fiber; the other is a tightly bound pool of calcium that is sequestered in some intracellular location (or locations) in the fiber. Recent histological and histochemical studies have pointed to two specific loci inside the cell where mobilizable calcium may be sequestered. These are the sarcoplasmic reticulum and the mitochondria of the muscle fiber (*7, 8*).

The contention that an intracellular as well as an extracellular (or superficial) site may store the activator ions needed for contraction does not imply that an intracellular store of calcium plays an equally important physiological role in all types of smooth muscle fibers. For example, the main pulmonary artery of the rabbit, immersed in a calcium-free medium, retains its capacity to contract long after extracellular and presumably loosely bound calcium ions have been removed (*8*). By contrast, the taenia coli of the rabbit, under the same conditions, retains its capacity to contract for a much shorter period of time (*8*). Estimates of the amount of sarcoplasmic reticulum present in each of the two smooth muscles correlate with these findings. The main pulmonary artery was assessed as having over twice as much sarcoplasmic reticulum by volume as does the taenia coli (*8*). In another study carried out in the longitudinal smooth muscle of the guinea pig ileum, it was found that the degree of contractility exhibited by the muscle in a calcium-free environment bears a precise relationship to the level of calcium that is present in a loosely bound form in the tissue (*9*). This fraction of the calcium escaped from the tissue with a half-time of 4 to 6 minutes. It would appear, therefore, that a firmly bound intracellular pool of calcium supports mechanical activity to a significant degree in the main pulmonary artery of the rabbit, is much less supportive in the taenia coli of the rabbit, and is of negligible significance in the longitudinal muscle of the guinea pig ileum.

As a reasonable extension of these findings, one may postulate that differences in the magnitude and functional importance of sequestered depots of calcium should correlate with differences in the cellular locations of metabolically dependent calcium pumps. A smooth muscle fiber that utilizes primarily external or superficially bound calcium for contraction might be expected to have a large part of its capacity to bind or extrude cytoplasmic calcium localized in the plasma membrane. A fiber that also mobilizes intracellular calcium might be expected to contain some part of its capacity to actively transport or bind calcium in the membranes of its intracellular organelles. This postulate regarding the possible correlation between the sources of mobilizable calcium and the cellular locations of active calcium pumps was examined by investigating the sites at which active calcium pumps exist in two different types of smooth muscles.

Fig. 1. A representative experiment depicting calcium uptake by microsomal vesicles prepared from longitudinal smooth muscle of the guinea pig ileum. Incubation at 37°C is described in the text. (Curve a) Sodium azide omitted from complete medium; (curve b) complete medium; (curve c) oxalate omitted from complete medium; (curve d) Mg-ATP omitted from complete medium.



One type was the smooth muscle from the aorta of the rabbit; the other was the longitudinal smooth muscle of the guinea pig ileum. The former appears to have a highly functional reservoir of sequestered calcium (*5*, *8*); the latter does not (*9*).

The initial step in our investigation consisted of isolating subcellular microsomal fractions from both these smooth muscle preparations. A microsomal fraction from the longitudinal muscle of guinea pig ileum was prepared in the following manner. Approximately 2 g of tissue were homogenized in 30 volumes of 0.25*M* sucrose. The homogenate was centrifuged at 1500*g* for 10 minutes. The supernatant obtained was centrifuged at 27,000*g* for 10 minutes, and the new supernatant was centrifuged at 105,000*g* for 60 minutes. The final pellet was resuspended in 4 ml of isotonic sucrose for immediate use. All operations were carried out at 0° to 2°C. Preparation of microsomes from rabbit aorta has been previously described (*10*).

Calcium uptake was studied at 37°C in a 3-ml incubation mixture containing tris(hydroxymethyl)aminomethane (tris-HCl, *p*H 7.4); 30 μmole; KCl, 300 μmole; the magnesium salt of adenosine triphosphate (Mg-ATP), 9 μmole; ammonium oxalate, 15 μmole; sodium azide, 15 μmole; $^{45}CaCl_2$, 0.4 μc; $CaCl_2$, 0.06 μmole; and 0.2 ml of the microsomal fraction. We determined the uptake of radioactive calcium by the vesicles by filtering and washing samples of the microsomal particles in the incubation medium on a Millipore filter and counting them in a liquid scintillation counter (*11*).

Density gradient studies were carried out in a continuous 20 to 40 percent sucrose gradient containing 0.005*M* tris-HCl, *p*H 7.8. Approximately 2 mg of microsomal protein were layered onto a 5-ml volume gradient that was then centrifuged at 130,000*g* for 16 hours at 0°C in a Beckman SW50L rotor using a Beckman L2-65 ultracentrifuge. Gradient fractions were obtained by puncturing the bottom of the tube and collecting 0.7-ml samples. The following enzyme assays were carried out on the various fractions that were separated out by the sucrose gradient procedure: Na,K-dependent adenosine triphosphatase (*12*), NADH oxidase (*13*), and 5′-nucleotidase (*14*). Protein was determined by the method of Sutherland *et al.* (*15*).

The microsomal fraction of longitudinal smooth muscle from the guinea pig ileum, examined with an electron microscope, contained smooth membranous vesicles. Previous work has demonstrated similar vesicles in a microsomal preparation from the rabbit aorta (*10*).

Uptake of calcium by the microsomal fraction of the longitudinal muscle from guinea pig ileum had not been characterized previously. Results obtained in

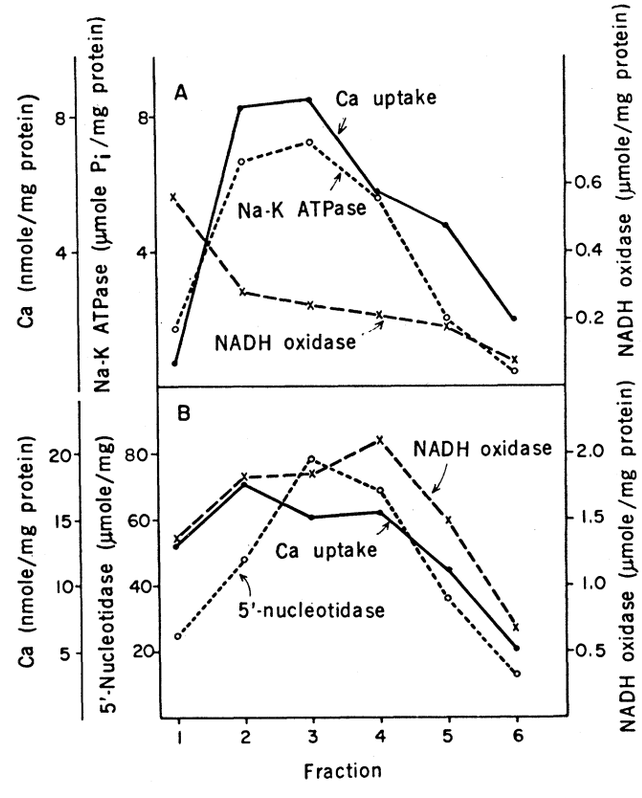


Fig. 2. Distribution of calcium uptake activity and enzyme markers in six fractions obtained by centrifuging microsomal vesicles in a sucrose density gradient. (A) Representative data from guinea pig intestinal smooth muscle. (B) Representative data from rabbit aorta. Specific activity of calcium uptake was measured from 5 to 15 minutes after start of incubation. Specific activity of Na,K-adenosine triphosphatase (*ATPase*) is in micromoles of inorganic phosphorus (P_i) per milligram of protein, per 15 minutes. Specific activity of 5′-nucleotidase is in micromoles of inorganic phosphorus per milligram of protein, per 30 minutes. NADH oxidase specific activity is in micromoles of NADH per milligram of protein, per minute.

our study are presented in Fig. 1. We found that calcium sequestration by the microsomes from the guinea pig required the presence of Mg-ATP. The magnesium salt of adenosine diphosphate (Mg-ADP) did not serve as a substitute. Moreover, the uptake was augmented by the addition of oxalate ion. This ion causes precipitation of the sequestered calcium (*16*) and permits continuous uptake of calcium with time, since outflow of calcium is negligible. The calcium uptake system was not affected by sodium azide, a substance which poisons the ATP-dependent calcium uptake in mitochondrial systems (*10*).

The microsomal fraction of guinea pig longitudinal smooth muscle was layered on a sucrose density gradient and the sucrose fractions were collected after appropriate centrifugation. The specific activities (activity per milligram of protein) of selected marker enzymes in each sucrose gradient fraction were subsequently determined. These markers were Na,K-dependent adenosine triphosphatase to denote the presence of the plasma membrane and NADH oxidase activity to denote the presence of endoplasmic reticulum (*13*). The specific activity of calcium uptake by each fraction was also measured. A representative distribution of the calcium binding and enzyme activities is shown in Fig. 2A. It is apparent that the specific activity of calcium uptake rises and falls in parallel with the specific activity of the plasma membrane marker. By contrast, the activity of NADH oxidase, the endoplasmic reticulum marker, starts relatively high and declines in succeeding fractions.

For comparison, a preparation of microsomes from the rabbit aorta (*10*) was also layered on a sucrose density gradient. With aorta, 5′-nucleotidase serves as marker for plasma membrane (*14*), since Na,K-dependent adenosine triphosphatase activity is not found in this tissue (*17*). The plasma membrane marker rises and falls in a manner similar to that seen with the guinea pig ileum. In this case, however, the specific activity of calcium uptake does not parallel the specific activity of the plasma membrane marker (Fig. 2B). It appears to be more closely related in distribution to the endoplasmic reticulum marker.

The gradient density studies would indicate that the calcium sequestration system of the longitudinal smooth muscle from the guinea pig ileum is associated primarily with the plasma membrane, whereas in the aortic smooth muscle from the rabbit, it is associated, in large part, with intracellular structures. This agrees with the physiological and histological evidence cited for the existence of a functional sarcoplasmic reticulum in the vascular muscle and for its relative insignificance in the intestinal smooth muscle of the guinea pig.

Recent physiological experiments suggest that the intracellular calcium pool may play a fairly specific role in the contraction process of vascular smooth muscle. In rabbit aorta the contractile response to norepinephrine can utilize intracellular calcium, whereas potassium-induced contraction is apparently dependent on loosely bound calcium ion associated with the extracellular pool (*18*). The phasic contraction of rat mesenteric artery induced by epinephrine appears to utilize intracellular calcium, whereas the tonic contraction is apparently dependent on the extracellular calcium pool (*19*). The response of the rabbit ear artery to norepinephrine appears to be bimodal. One component of the response can utilize intracellular calcium pools (*20*).

We have previously demonstrated calcium sequestration activity in rabbit aorta and now have obtained evidence for similar activity in a plasma membrane fraction of smooth muscle of guinea pig intestine. Active calcium extrusion by plasma membrane has also been uncovered in studies performed on the erythrocyte membrane (*21*). Calcium uptake by fragments of skeletal muscle plasma membrane appear to be as active as that of the sarcoplasmic reticulum (*22*).

Calcium uptake, which presumably serves to produce muscle relaxation, is analogous in both skeletal muscle and smooth muscle, but the order of magnitude of the activity is appropriately higher in skeletal muscle. In turn, the vascular muscle seems to possess a greater activity than that found in the intestinal muscle.

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Scanning Electron Microscopy: Low-Magnification Pictures of Uncoated Zoological Specimens

Abstract. *Good low-magnification (× 5 to × 500) scanning electron microscope pictures of dry, uncoated zoological specimens may be obtained with a low accelerating voltage (1.5 to 3 kilovolts) in conjunction with a short exposure to the scanning beam.*

Discussion of applications of the scanning electron microscope (SEM) has dealt largely with the usefulness of high magnification and resolution in the study of surface features. In these studies the object being scanned is usually either metallic or coated with a conductive substance such as gold.

Biological uses of the SEM have so far been rather limited. For many types