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

of studies, comparative morphology, for example, high-magnification pictures (× 500 and over) are not too useful. Often the species being studied is represented by only a few specimens and coating is not desirable. Pease *et al.* (*1*) noted that living or freshly killed specimens often contain enough moisture to prevent surface-charging, presumably because of the loss of ionized water. The term "charging" refers to the uneven buildup of electrons usually on prominent parts of the surface, resulting in an uneven discharge of secondary electrons (which are "collected" to produce the picture). Charging produces highlights or "hot spots" which often obscure features or produce streaks in the picture. Unfortunately, however, it is often not possible to utilize fresh material.

A modified SEM (JEOL JSM-U3) was used in the study reported here. Modifications included a specially adapted supplementary deflection coil (Pseudo-Kikuchi) inserted in the top of the specimen chamber in order to maintain beam efficiency down to ×4, an additional high-voltage power supply (0 to 5 kv) (Fluke model 408B), and a modification of the objective lens circuitry allowing beam focusing for accelerating voltages as low as 1.5 kv. Minor modifications were made in the specimen holders and the substages to facilitate the positioning of specimens and to compensate for the increased working distance required by the deflection coil. These changes make it

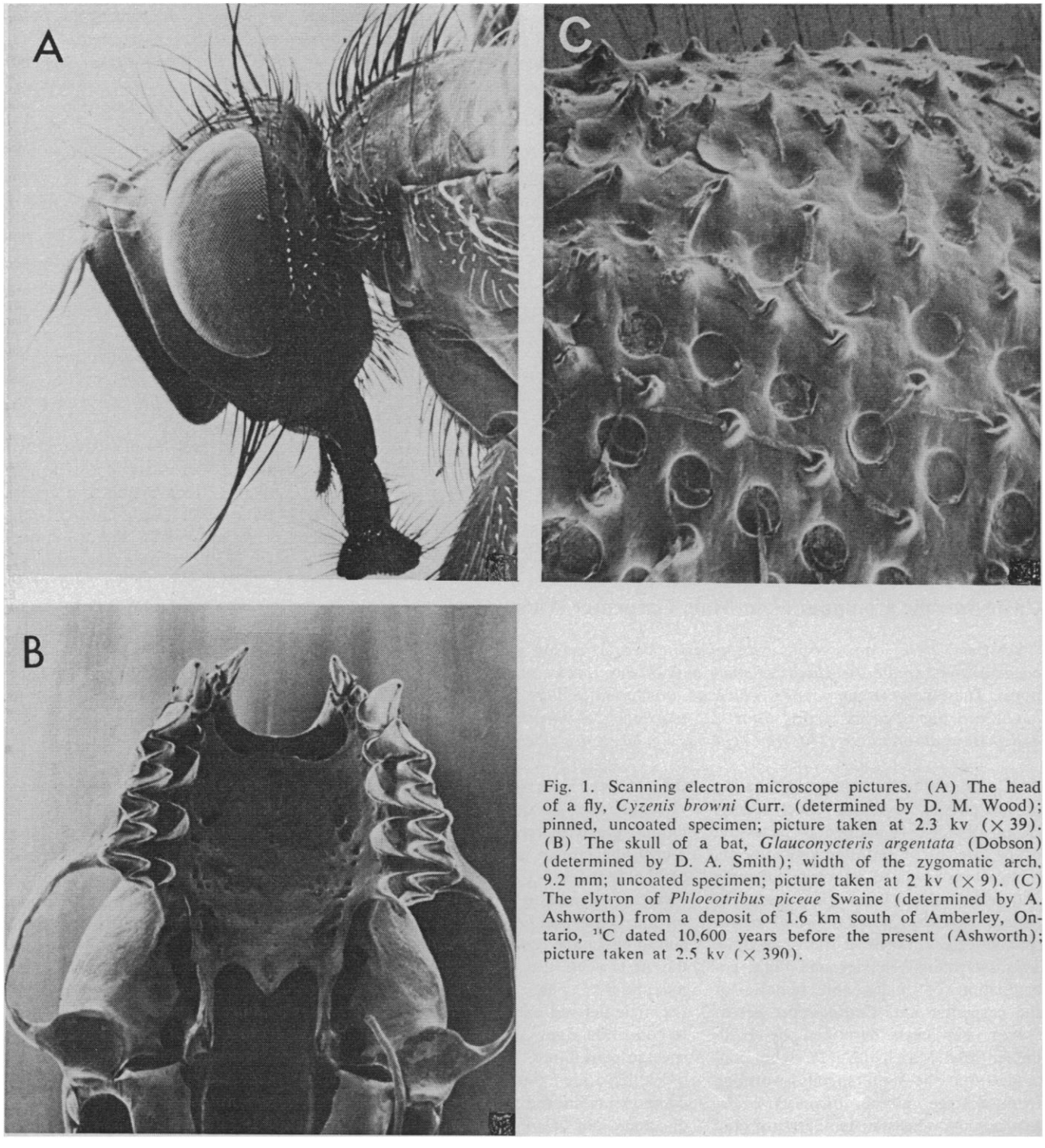

Fig. 1. Scanning electron microscope pictures. (A) The head of a fly, *Cyzenis browni* Curr. (determined by D. M. Wood); pinned, uncoated specimen; picture taken at 2.3 kv (× 39). (B) The skull of a bat, *Glauconycteris argentata* (Dobson) (determined by D. A. Smith); width of the zygomatic arch, 9.2 mm; uncoated specimen; picture taken at 2 kv (× 9). (C) The elytron of *Phloeotribus piceae* Swaine (determined by A. Ashworth) from a deposit of 1.6 km south of Amberley, Ontario, ^{14}C dated 10,600 years before the present (Ashworth); picture taken at 2.5 kv (× 390).

possible to study samples up to 25 mm in length and width. Samples consisted of ultrasonically cleaned, dried, pinned insects (Fig. 1A), mammal bones (Fig. 1B), and fragments of insects from late Pleistocene deposits (Fig. 1C).

Satisfactory to very good results were obtained with such specimens, but a number of problems were encountered in the process. It is difficult to maintain the integrity of the beam with a low accelerating voltage (1.5 to 2.5 kv) without an unacceptable loss of resolution or a large amount of distortion. The type of specimen, the angle of the surface scanned, and contaminants in the chamber all produce varying effects on resolution and distortion. With nonconductive samples low accelerating voltages are essential if surface-charging is to be held at an acceptable level (*2*). The use of low accelerating voltages slows the buildup of "hot spots" but does not eliminate them. Moreover, the longer a specimen is subject to the scanning beam before the picture is taken, the greater the possibility of charging. The rapidity with which a specimen can be positioned in the viewing chamber, therefore, is as important a factor as the low accelerating voltage. If charging occurs during the time necessary for focusing, it is often possible to "bleed off" the charge by reducing the intensity of the beam or shutting it down for 5 or 10 minutes.

Some dried insects, even with rapid handling and a low accelerating voltage, charge rapidly. In many cases, if the specimens are placed in a humidor for 24 hours or if the surface is made wet, some of the charging may be eliminated. Brody and Wharton (*3*) found a mixture (by weight) of glycerol (96.6 percent), potassium chloride (0.05 percent), and water (3.35 percent) useful for reducing charging in mites. In a few cases (for example, very convex, smooth, leaf-feeding beetles, Chrysomelidae) we found a conductive coating, such as gold or gold-palladium, essential. According to Echlin (*4*), SEM pictures of uncoated botanical specimens can be obtained in a similar manner, particularly if fresh specimens are used.

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Caste-Specific Compounds in Male Carpenter Ants

Abstract. *Three caste-specific substances new to arthropod glandular secretions occur in the mandibular glands of male ants of five species in the genus* Camponotus. *These volatile compounds, which are not found in alate females or workers, have been identified as methyl 6-methyl salicylate, 2,4-dimethyl-2-hexenoic acid, and methyl anthranilate. The free acid has not been described previously.*

Coordinating the pairing and mating of the two sexes of an insect species is an obvious fundamental necessity. Many species of termites and ants exhibit a synchronized swarming of male and female alates from many nests, ensuring that large populations of reproductives are airborne at the same time. This swarming behavior and flight coordination of males and females of the carpenter ant, *Camponotus herculeanus*, has been described by Hölldobler and Maschwitz (*1*), who concluded that the simultaneous swarming from a large number of nests is dependent upon season, temperature, and time of day. However, it was also convincingly demonstrated that the mandibular gland secretion of the males is a critical factor in initiating the activity of the females before swarming. This secretion is used to scent the area immediately surrounding the nest entrance and to entice the females to swarm from the nest when the male flight is at a maximum (*1*). This releaser activity of the male-derived exudate would appear to constitute a new function for a caste-specific secretion.

We have identified the major volatile components in the mandibular glands of males of *Camponotus novebora-censis, C. pennsylvanicus, C. nearcticus, C. rasilis*, and *C. subbarbatus* as the initial part of a program investigating factors governing the swarming behavior of certain species of ants. Three new arthropod natural products have been chemically characterized from among these species and found to be methyl 6-methyl salicylate, 2,4-dimethyl-2-hexenoic acid, and methyl anthranilate. The free acid has not been described.

Heads of male *C. nearcticus* were crushed in *n*-pentane; the resulting pentane extract was subjected to gas chromatographic–mass spectrometric analysis (*2*). Of the three peaks detected, the first and major showed a molecular ion (M) at *m/e* 166. This compound appeared to be aromatic (*m/e* 77 and 78), containing both a $COOCH_3$ group (loss of CH_3OH and $HCOOCH_3$ from *m/e* 166) and a phenolic hydroxyl (conversion to an *O*-acetate, M^+ 208; and a slow reaction with CH_2N_2 forming a methyl ether, M^+ 180). Both the retention time and the mass spectrum of methyl 6-methyl salicylate (*3*) correspond to those of this peak in the male heads, whereas the retention times of methyl 5-methyl salicylate and methyl 3-methyl salicylate were distinctly different.

The second peak from this extract is an unsaturated acid with a molecular ion at *m/e* 142 (conversion to a methyl ester, M^+ 156). Reduction of the methyl ester provided a dihydro derivative (M^+ 158) whose fragmentation pattern showed it to be an ester of an α-methyl substituted acid (intense peaks at *m/e* 88 and 101). Ozonolysis of the acid yielded 2-methylbutanal, suggesting the structure 2,4-dimethyl-2-hexenoic acid. This acid was synthesized by slow hypochlorite oxidation of the known 3,5-dimethyl-3-hepten-2-one (*4*) and also from hydrolysis of the product of the Wittig reaction between 2-methylbutanal and the ylid derived from triphenylphosphine and methyl 2-bromopropionate (*5*). The resulting acids had retention times and mass spectra identical to those of the natural product. The geometry of the double bond and the configuration of the asymmetric center in the natural substance are unknown. This relatively simple compound has not heretofore been described.

The third peak exhibits a molecular ion at *m/e* 151, suggesting that it contains one nitrogen atom. It appeared to be aromatic (doubly charged ions, in-