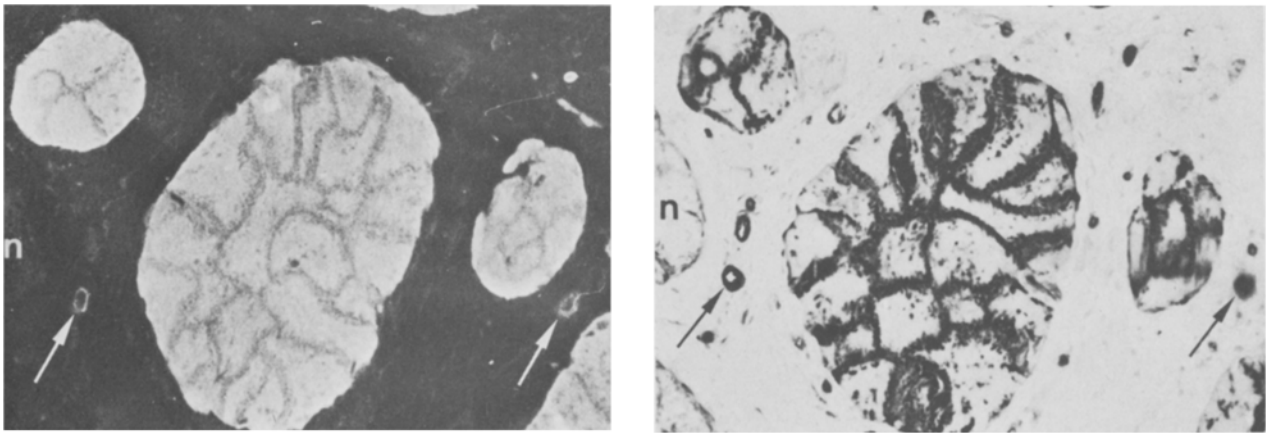

Fig. 4. *a* Staining pattern observed in Purkinje fibre bundles treated with anticytoplasmic filament globulin using the indirect immunofluorescence technique. The cytoplasm of individual Purkinje fibres shows fluorescence, while cell boundary regions towards other Purkinje fibres do not. The latter corresponds to the localization of Purkinje fibre myofibrils. Note fluorescence of capillary endothelium (arrows), but not of nerves (n). *b* Serial cryostat section of moderator band treated to show myofibrillar ATPase. The ATPase activity (corresponding to the myofibrils) is located at the cell borders towards other Purkinje fibres. Capillaries (arrows) and nerves (n). × 240.

tion of myocardium into the specialized conducting tissue even at embryonal stages (work in progress). Also it will be possible to investigate the relationship of cow Purkinje fibre cytoplasmic filaments to those of other species as well as the relationship of conducting tissue filaments to filamentous proteins of other muscle and non-muscle tissues. Preliminary studies indicate strong crossreaction to heart conducting cells of several species. This adds further evidence to the theory that intermediate filaments are of close identity irrespective of species or tissue origin.

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Vesicular inclusions in the nuclei of epithelial cells of Malpighian tubes of the hemipteran, *Panstrongylus megistus*

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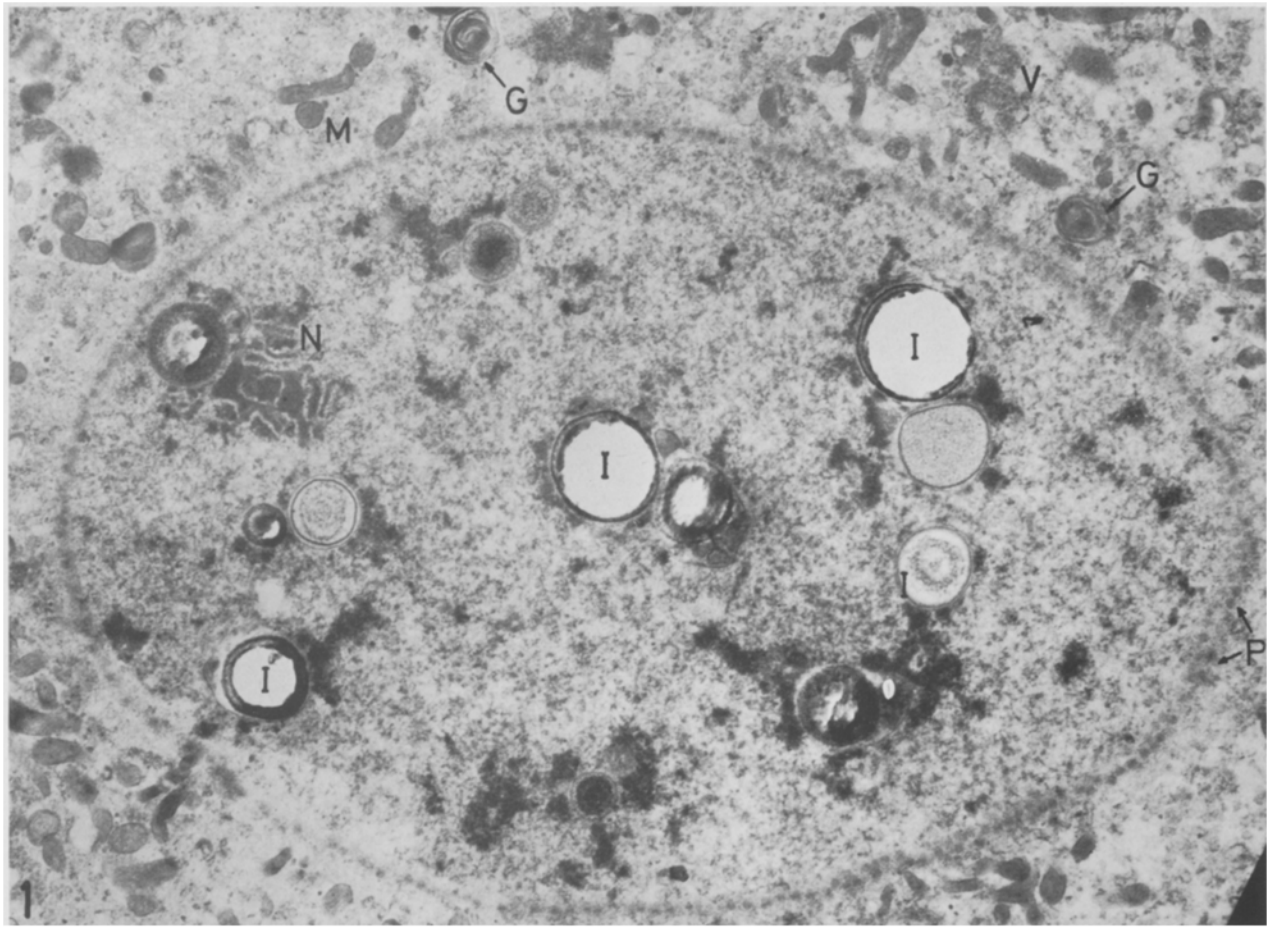
Summary. Vesicular inclusions observed in Malpighian tubes of hemipterans have been associated with a virus-like infection rather than a lysosomal-type activity, which is the case of the identical cytoplasmic structures.

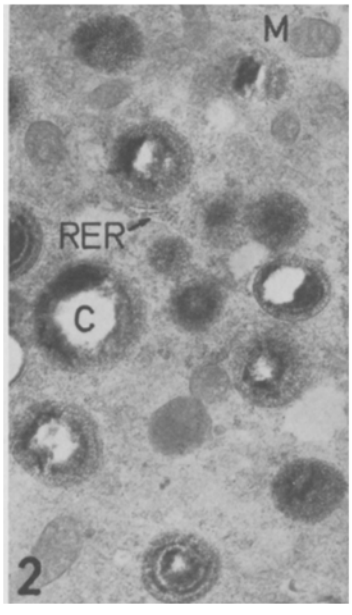
Large vesicular inclusions have been found in the nuclei of apparently healthy epithelial cells of the Malpighian tubes of *Panstrongylus megistus* Burmeister, fixed in 3% glutaraldehyde in phosphate buffer (pH 7.2) and 1% osmium tetroxide, dehydrated in ethanol and embedded in epon. These inclusions are identical with structures hitherto considered typical cytoplasmic organelles in hemipterans (figures 2 and 3). Designated by various names, such as globules, spherules or concretions, they appear regularly in the Malpighian tubes and in the midgut of various insects, including hemipterans[2,3].

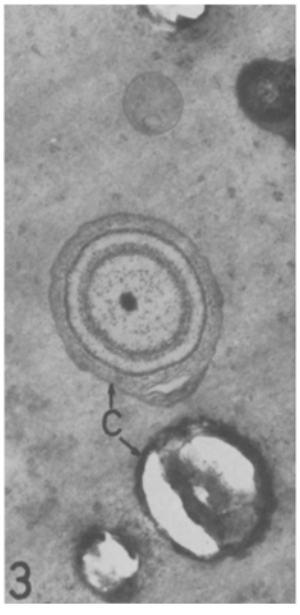
The nuclear inclusions were observed in different stages of development. Initially they contain a flocculent material and occasional spherical particles (30–40 nm), encased in an incompletely enveloping membrane (figure 1). Layers of alternating density are progressively added, which may lead to very dense structures. These are very fragile when submitted to the stresses of sectioning and the electron beam, often appearing uneven or broken (figures 1–3).

The nuclear inclusions are usually associated with nucleolar structures, which may be disorganized in more severely affected nuclei, or remain untouched in less modified ones. Small spherical particles are found near newly forming vesicles and within the surrounding nucleolar features (figure 4). Fibrous nuclear inclusions have been described in hemipterans, associated with a commonly observed virus-like particle[4]. However, the rare occurrence of intranuclear vesicular inclusions is suggestive of an uncommon form of infection caused by a virus-like agent.

Although a structural resemblance may be found between







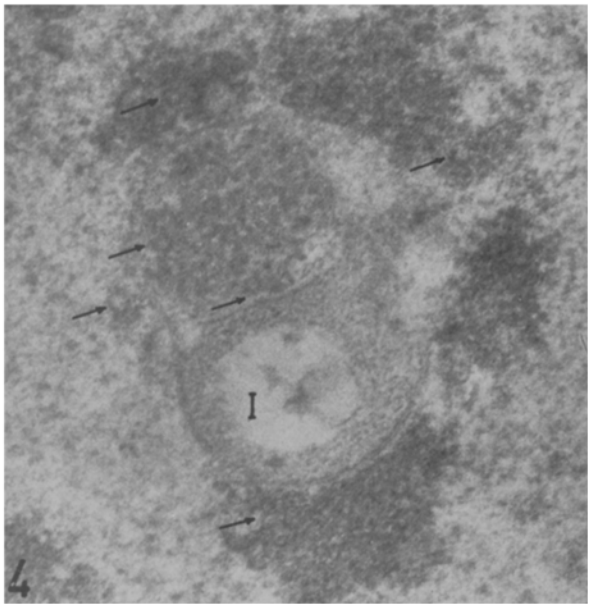


Fig. 1. Vesicular inclusions (I) in different stages of development occur within the nucleus of an epithelial cell of the Malpighian tubes. Nucleolar structures (N) may be observed near vesicular inclusions. P = nuclear pore complex; G = membranous globules; V = groupings of virus-like particles. × 9,400.

Figs. 2 and 3. Cytoplasmic concretions with a typical layered structure, frequently broken during sectioning. RER = rough endoplasmic reticulum; M = mitochondria. Fig. 2 × 15,500. Fig. 3 × 25,000.

Fig. 4. Intranuclear vesicular inclusion at an early stage of formation. Rounded virus-like particles (arrowheads) can be found within the vesicles and surrounding nucleoli. × 51,780.

cytoplasmic concretions and these nuclear inclusions, the former occur normally in all distal cells of the Malpighian tubes and are not dependent on the presence of virus-like particles. This is not the case in the intranuclear vesicles. The cytoplasmic structures have been assumed to be participating in the elimination of useless organelles[3]. However, it is difficult to imagine a lysosomal activity within the nucleus, in the case of vesicular inclusions, and a more passive role of segregation of waste products may be more acceptable.

An identical condition was described in hen oviduct epithelium[5], although a different interpretation was given as to the origin of the nuclear inclusions. Since the laboratory species of *Panstrongylus megistus* used is often fed hen's blood, this may be a case of virus transmission from the hen to the insect. In this case, the hemipteran may serve as a secondary host in the optimum laboratory conditions of temperature and humidity, but this may not be true of wild specimens living under different conditions.

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Unusual high volume of sarcoplasmic reticulum in a wasp leg muscle

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Summary. Ultrastructural and morphometric examination of a wasp leg muscle showed that it contained a high volume of sarcoplasmic reticulum (volume fraction = 0.35, surface density = 21.4 $\mu m^2/\mu m^3$). As well as being arranged in double or triple layers between the myofibrils, the SR was found in large multilayered accumulations around the nuclei and in the subsarcolemmal space. Fibres of adjacent muscles had the normal volume and arrangement of SR.

It is generally accepted that the sarcoplasmic reticulum (SR) is the main site of calcium storage in skeletal muscle and that there is a rough correlation between the amount of SR and the speed of the contraction-relaxation cycle of the muscle[1,2]. In insects the volume of SR ranges from < 1.0% in asynchronous (fibrillar) flight muscle to about 18–20% in fast-twitch phasic muscles[2-5]. The greatest volume of SR has been found in a fast acting lobster muscle where it occupies 75% of the muscle[6]. This communication reports an unusually high volume of SR in a leg muscle of the wasp *Vespula vulgaris* (L).

Materials and methods. As part of a study of the effects of storage at low temperature on the ultrastructure of skeletal muscle, a wasp was stunned and immersed in liquid nitrogen for 24 h. Its tissues were fixed by freeze substitution in cold (4 °C) 5% glutaraldehyde in 0.1 M phosphate buffer. The metathoracic leg muscles were exposed and fixed for a further 2 h in glutaraldehyde. The muscles were then cut into small blocks (< 1 mm^3), washed in phosphate buffer and postfixed in 1% OsO_4. After dehydration the blocks were embedded in Spurr resin. Sections were cut on a Reichert OM U2 ultramicrotome, stained with uranyl ace-

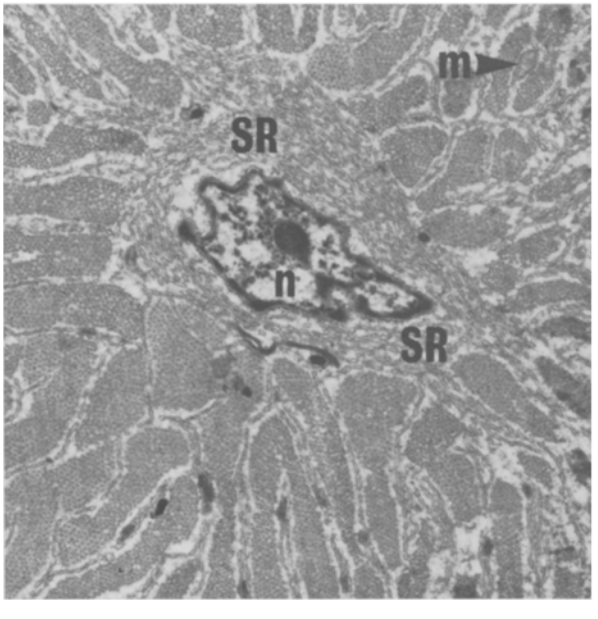

Fig. 1. Transverse section through the centre of a fibre of the retractor unguis muscle of *Vespula* showing the large accumulation of sarcoplasmic reticulum (SR) around the nucleus (n). Note the single mitochondrion (m). × 15,000.

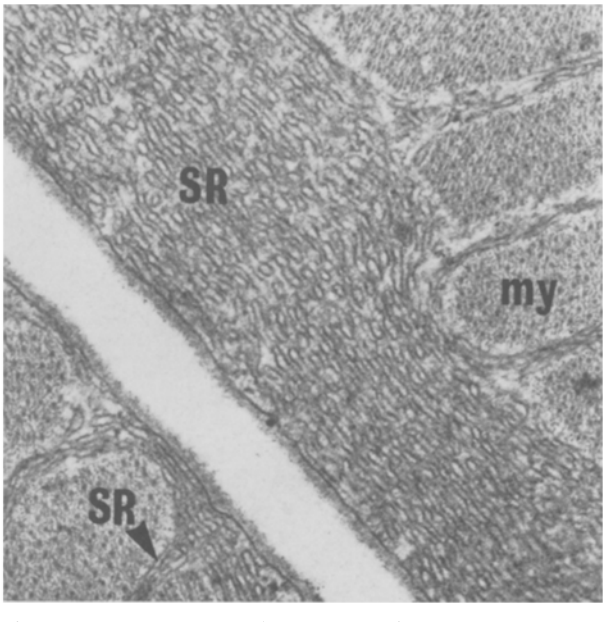

Fig. 2. Transverse section through the edge of 2 fibres of the retractor unguis muscle of *Vespula* showing the large accumulations of sarcoplasmic reticulum (SR) inside the sarcolemma. my, myofibril. × 40,000.