

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 organelles³. 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 epithelium⁵, 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.

- 1 This study was supported by a grant (75/907) of the Fundação de Amparo à Pesquisa do Estado de São Paulo. The authors are indebted to Dr E. O. Rocha-e-Silva for generously providing them with the insect specimens.
- 2 V. B. Wigglesworth and M. M. Salpeter, *J. Insect Physiol.* 8, 299 (1962).
- 3 M. L. S. Mello and H. Dolder, Fine Structure of the Malpighian tubes in the Blood-sucking Insect, *Triatoma infestans* Klug. *Protoplasma*, 93, 275 (1977).
- 4 H. Dolder and M. L. S. Mello, *Revta Saúde publ.* 12, (1978).
- 5 H. S. Johnson, *Z. Zellforsch.* 57, 385 (1963).

Unusual high volume of sarcoplasmic reticulum in a wasp leg muscle

J. N. Weaving and M. J. Cullen

Muscular Dystrophy Research Laboratories, Regional Neurological Centre, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne (Great Britain), 28 October 1977

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 = $21.4 \mu\text{m}^2/\mu\text{m}^3$). 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 muscle⁶. This communication reports an unusually high volume of SR in a leg muscle of the wasp *Vespa 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 mm³), washed in phosphate buffer and postfixed in 1% OsO₄. After dehydration the blocks were embedded in Spurr resin. Sections were cut on a Reichert OM U2 ultramicrotome, stained with uranyl ace-

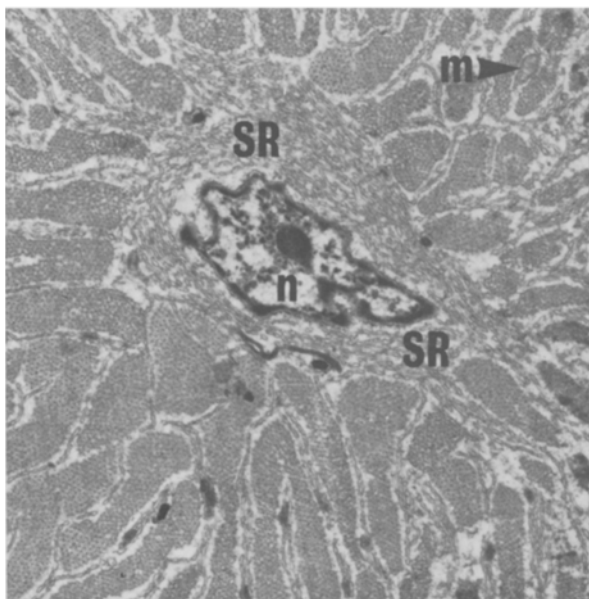


Fig. 1. Transverse section through the centre of a fibre of the retractor unguis muscle of *Vespa* showing the large accumulation of sarcoplasmic reticulum (SR) around the nucleus (n). Note the single mitochondrion (m). $\times 15,000$.

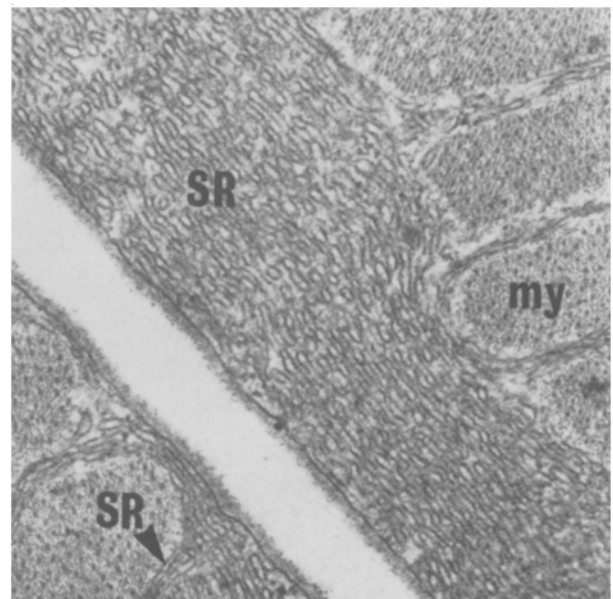


Fig. 2. Transverse section through the edge of 2 fibres of the retractor unguis muscle of *Vespa* showing the large accumulations of sarcoplasmic reticulum (SR) inside the sarcolemma. my, myofibril. $\times 40,000$.

The volume fractions of the structural constituents (expressed as a percentage of the fibre volume) and the surface density of the SR (expressed as $\mu\text{m}^2/\mu\text{m}^3$ of fibre volume) of the retractor unguis muscle and extensor tibiae muscle of *Vespula vulgaris* (L)

		Retractor unguis	Extensor tibiae
Volume fractions	Myofibrils	47.4	64.2
	SR*	34.5	22.1
	Sarcoplasm	16.3	10.6
	Nuclei	1.8	2.2
	Mitochondria	-	0.9
Surface density	SR*	21.4	14.7

* The figures for the SR also indicate those for the T system which, except at the dyads, is very difficult to distinguish from the SR. The volume occupied by the T system is, however, extremely small (<1%).

tate and lead citrate and examined in a Siemens 102 electron microscope.

Morphometric analysis of the components of the myofibres was made using a multipurpose test system⁷ in which the sampling grid contained 84 lines and 168 points. The analysis was carried out on random selected prints taken from transverse sections at a final magnification of 20,000. For each muscle 2500–3000 points were counted. The volume fractions of each of the components and the surface density of the SR were calculated from the standard formulae^{5,7}.

Results. The arrangement of the components of the myofibres in the wasp leg muscles conforms to that of the tubular pattern common in arthropod muscle⁸. The nuclei are arranged in a central core passing down the middle of the fibres. The myofibrils are mostly strap-like and radiate out from the central core. The SR is arranged in single, double or triple layers between the myofibrils. Part of the SR is differentiated into electron-dense plaques which form dyads with the T system at the A band-I band junction region.

An unusual feature of the wasp retractor unguis muscle described here is the large accumulation of SR, surrounding the central nuclei, and immediately inside the sarcolemma and distal to the myofibrils (figures 1 and 2). The elements of the SR found in these large accumulations are continuous with the elements running between the myofibrils. This unusual large development of the SR in the retractor unguis muscle was not seen in the adjacent extensor tibiae muscle where the fine structure was that of

normal tubular muscle. The results of the morphometric analyses of these 2 muscles are shown in the table. Another unusual feature of the retractor unguis is that the mitochondrial content is extremely low. Mitochondria are present (figure 1) but extremely rare and did not feature in the morphometric analysis of the muscle.

Discussion. The values obtained for the retractor unguis for the relative volume and surface areas of the SR are considerably higher than other published figures for insect leg muscles. In a careful analysis of tonic and phasic fibres in the locust leg, Cochrane et al.⁵ obtained volume fractions of 1.1%, 6.8% and 19% and surface densities of $1.0 \mu\text{m}^2/\mu\text{m}^3$, $2.9 \mu\text{m}^2/\mu\text{m}^3$ and $11.9 \mu\text{m}^2/\mu\text{m}^3$ for the tonic extensor tibiae, phasic extensor tibiae and phasic retractor unguis respectively. Measurement of these features in the jumping muscle of the flea gave figures of 18% and $16.0 \mu\text{m}^2/\mu\text{m}^3$ respectively⁴. Why the figures for the wasp leg reported here should be so much higher and why the SR is organized in such an unusual way is unclear. One might attempt to look for an answer in the behaviour or locomotory habits found in wasps but not in other insects examined to date. One specialized behavioural feature of wasps is that they are able to carry relatively heavy prey such as caterpillars when flying. It is likely that the retractor unguis which operates the tarsal claw is involved in holding the load during flight. However, that would be a tonic function and the fine structure is not that of a typical tonic muscle where the SR content is normally low. A characteristic of phasic muscles is that they have a relatively high mitochondrial content^{2,3} so on this criterion this muscle does not have a typical phasic structure either. An explanation of the unusual structure of the muscle clearly awaits more knowledge of its normal function in the wasp. In the meantime it remains an interesting but unexplained observation.

- 1 W. Hasselbach, in: *The Enzymes*, vol. 10, p. 432. Ed. P.D. Boyer. Academic Press, New York 1974.
- 2 H.Y. Elder, *Biol. Bull.* 141, 434 (1971).
- 3 M.J. Cullen, Thesis, Oxford University (1971).
- 4 M.J. Cullen, *Phil. trans. r. Soc. Lond. B* 271 (1975).
- 5 D.G. Cochrane, H.Y. Elder and P.N.R. Usherwood, *J. Cell Sci.* 10, 419 (1972).
- 6 J. Rosenbluth, *J. Cell Biol.* 42, 534 (1969).
- 7 E.R. Weibel, G.S. Kistler and W.F. Scherle, *J. Cell Biol.* 30, 23 (1966).
- 8 J.W.S. Pringle, in: *The Structure and Function of Muscle*, vol. 1, p. 433. Ed. G.H. Bourne. Academic Press, New York 1972.

Possible presence of autoantibodies to zona pellucida in infertile women¹

T. Mori, T. Nishimoto, M. Kitagawa, Y. Noda, T. Nishimura and T. Oikawa

Department of Obstetrics and Gynecology, School of Medicine Kyoto University, Sakyo-ku, Kyoto 606, and Department of Biology, Faculty of Science, Yamagata University, Yamagata 990 (Japan), 12 December 1977

Summary. Of 52 serum samples from infertile women tested against isolated porcine oocytes by immunofluorescence, 8 produced intense reactions in zonae pellucidae, while only one of 52 sera from control subjects showed the reaction. Autoantibodies to the zona may be present in these women.

Following the discovery of an ovary-specific antigen(s) located in the zona pellucida in hamsters² and in rabbits³, many attempts have been made to investigate the immunobiological effects of antibodies to the zona on the reproductive process, for the purpose of immunological regulation of fertility⁴. It has been reported that the human zona pellucida contains at least one antigen specific to the human ovary⁵, and that a common antigen is shared by human and porcine zonae⁶. These facts provide the theoret-

ical basis for detection of antibodies to the human zona using porcine zonae as a target. In the present communication, an attempt was undertaken to examine whether or not the antibodies to porcine zonae are present in sera of infertile women as a potential etiological factor in the infertility.

A total of 104 blood samples was taken from 52 infertile patients, including 29 with unexplained sterility, 17 with ovulatory failure, 5 with known etiology of tubal or sperm