Stades	Nombre d'opérations	Animaux survivants	Animaux élevés jusqu'à la métamorphose	Animaux présentant 1 membre normal	Animaux présentant 1 membre déformé	Animaux présentant 1 membre déformé et affecté d'une polydactylie post-axiale
I	44	10	10	10 (100%)	0 –	0 –
II	48	32	26 (6)*	26 (100%)	0 –	0 –
III	160	63	34 (29)*	32 (94%)	1 (3%)	1 (3%)
IV	65	29	20 (9)*	14 (70%)	2 (10%)	4 (20%)
Va	64	29	19 (10)*	6 (31,5%)	7 (37%)	6 (31,5%)
Vb	70	35	16 (19)*	2 (12,5%)	4 (25%)	10 (62,5%)

* Nombre des animaux survivants fixés en vue de l'étude du membre opéré, en cours de morphogenèse.

sous l'épiderme de remplacement était plus développé (tableau).

Nous n'avons jamais observé de déficience de la séquence proximo-distale du membre. Le problème se pose quant au rôle joué par l'épiderme au cours du développement du membre postérieur du pleurodèle, aux stades considérés. Si l'épiderme du bourgeon de membre joue un rôle inducteur semblable à celui de la crête apicale épidermique des amniotes, cela implique que l'épiderme de remplacement est resté suffisamment indifférencié pour prendre le relais de l'épiderme du membre dans le jeu des interactions inductrices épiderme-mésoderme; si, par contre, l'épiderme ne joue pas un tel rôle morphogénétique, le mésoderme du bourgeon doit posséder toutes les potentialités pour former un membre dont la séquence proximo-distale est complète, quelle que soit la nature de l'épiderme qui le recouvre. A cet égard, nos expériences ne sont pas définitives; seul l'épiderme des nageoires dorsale et ventrale a été éprouvé dans les conditions particulières décrites. Des recherches expérimentales ultérieures seront consacrées à l'étude de l'importance, dans le développement du membre postérieur, d'épidermes de remplacement de nature différente, et à celle du rôle du composant mésodermique.

1 J.W. Saunders, J. exp. Zool. *108*, 363 (1948).
2 M. Lauthier, Wilhelm Roux'Archiv *175*, 185 (1974).
3 M. Lauthier, J.Embryol. exp. Morph. *38*, 1 (1977).
4 M. Steinberg, Carnegie Inst. Wash. Yb. *56*, 347 (1957).

Cytoskeletal filaments of heart conducting system localized by antibody against a 55,000 dalton protein

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Summary. Cow heart conducting cells characteristically contain cytoplasmic intermediate-sized filaments. We report here the preparation of a specific antibody to a 55,000 dalton protein of isolated cow Purkinje fibres. Confirmation has been obtained that these filaments consist of the 55,000 dalton protein, using the indirect immunofluorescence technique. Cross-reaction is seen with vascular endothelium and smooth muscle cells of various origin, suggesting close identity of different types of intermediate-sized filaments.

Cytoplasmic filaments (100-Å filaments, intermediate filaments) distinct from myofilaments and microtubules have been demonstrated in a wide variety of cell types[1-4]. Tonofilaments and neurofilaments belong morphologically to the same class of filaments. Several proposed functions of these cytoplasmic filaments have been suggested, of which the cytoskeletal one seems to be the best justified[5-7]. Several authors have reported the mol. wt of the filament protein subunit to be about 55,000 daltons[4-10]. However, few have presented data on direct biochemical[4,5] and immunohistochemical[1,8] comparison of these morphologically related filaments.

Cow heart Purkinje fibres contain large numbers of intermediate-sized filaments which can be concentrated using detergents and actomyosin extractants[6]. To investigate further the antigenic relationship between filaments of various cell types and under various conditions, we have prepared an antibody to the 55,000 dalton protein from isolated Purkinje fibre strands of cow heart. We report here the staining of Purkinje fibre cytoplasm, as well as smooth muscle and vascular endothelial cells, by the indirect fluorescein-labeled technique using anti-serum against the 55,000 dalton protein.

Purkinje fibre columns were isolated mechanically from the surrounding connective tissue sheath yielding a pure fraction of conducting cells[11]. Isolated cells and cell columns were extracted with Triton X-100 and alternating high and low ionic-strength solutions in order to remove membranes and myofibrillar proteins. This procedure gives a fraction of cytoplasmic filaments, Z disk material and desmosomes. In spite of this extensive extraction of structural components, the 3-dimensional appearance of the cell columns remained intact (figure 1). This finding strongly supports the idea that cytoplasmic filaments perform a cytoskeletal role. The extensive cytoskeleton of these cells is perhaps related to the considerable tension forces to which the conducting cells are exposed during heart work.

SDS gel electrophoresis of extracted material revealed a major 55,000 dalton protein believed to constitute the filaments, and a 110,000 dalton component, probably an α-actinin, believed to correspond to the remaining Z disk and desmosome material[6]. After extraction, the concentrated fraction was dissolved in SDS buffer. The 55,000 dalton protein was highly purified by chromatography on an AcA-44 gel filtration column. Amino acid analysis (unpublished work) showed a close similarity to the reported composition of brain neurofilaments[10], astrocyte filaments[12] and smooth muscle 100-Å filaments[5].

Antiserum to the 55,000 dalton protein was produced by injecting rabbits s.c. with 500 µg of the purified protein in

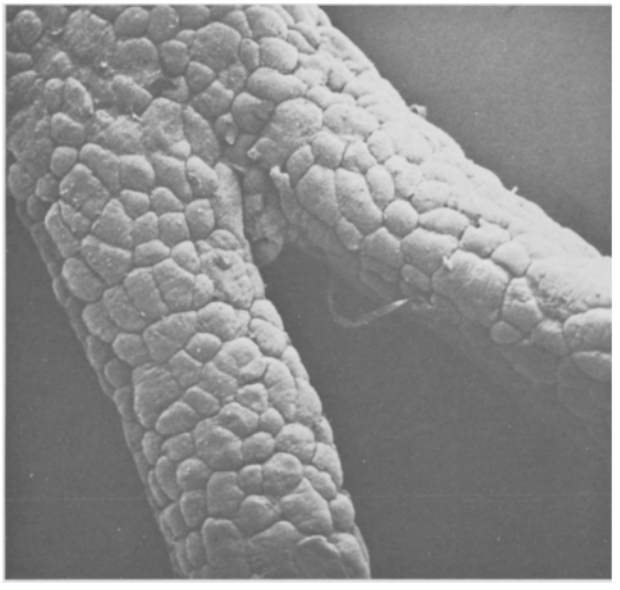

Fig. 1. Scanning electron microscopic (SEM) survey of branching Purkinje fibre bundle isolated from its connective tissue sheath and subsequently extracted with Triton X-100 and high ionic-strength solutions. The bundle was then fixed in glutaraldehyde and osmium tetroxide and further processed for SEM by the critical point drying method. The 3-dimensional appearance of the Purkinje fibres is intact. × 100.

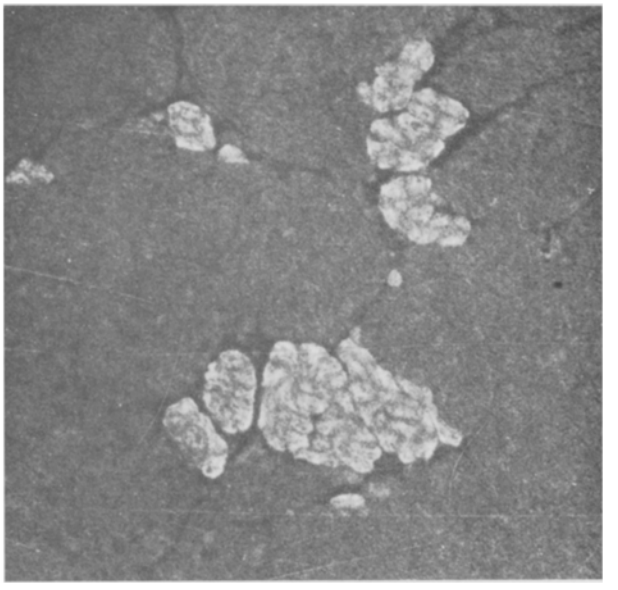

Fig. 3. The localization of cytoplasmic filament protein in Purkinje fibres of cow heart. Cryostat sections of moderator bands were treated with antiserum to cytoplasmic filament protein with indirect immunofluorescence and viewed with epifluorescent optics in a Leitz Orthoplane microscope. Conducting cells are distinct from surrounding ordinary myocardium. × 40.

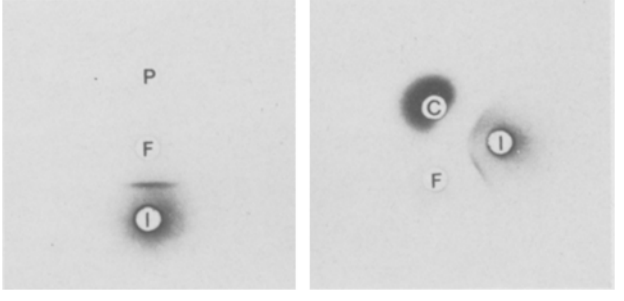

Fig. 2. *a* Double diffusion test of the pure 55,000 dalton protein and its antiserum. Immune serum was applied at bottom (I) and preimmune serum on top (P). Pure filament solution in 0.1 M sodium phosphate buffer supplemented with 0.1% SDS in the middle (F). *b* Double diffusion test with the pure filament protein (bottom left; F) and the crude heart Purkinje fibre preparation (top left; C). To the right, antiserum was applied (I). Complete fusion reaction can be seen.

incomplete Freund's adjuvant. 2 weeks later an additional 250 µg in incomplete adjuvant was administered, and 10 days after that the rabbits were bled from the ear artery. Control serum was obtained from rabbits before immunization. The antisera were analyzed in double diffusion test with 1% agarose in 0.1 M sodium phosphate buffer pH 7.4 (figure 2, a and b). It should be stressed that the antigen preparations had to be dissolved in 0.1% SDS buffer prior to application in the double diffusion test, in order to keep the filament protein in solution. This discontinuous property of the diffusion system was not found to interfere with the precipitation pattern.

Transverse cryostat sections, 4–6 µm thick, were cut from moderator bands (trabeculae septomarginales) from fresh cow hearts. Sections of hog stomach were used for comparison. The sections were immersed in chilled absolute acetone, air dried, and incubated for 1 h at 37 °C with rabbit antiserum in a moist chamber. Sections were then washed in phosphate buffered saline (PBS: 0.01 M sodium phosphate, 0.15 M sodium chloride, pH 7.4) for 3× 10 min, reacted for 1 h with fluorescein-conjugated goat antirabbit globulin and again washed in PBS. Sections were finally mounted in a 1:4 PBS:glycerol solution. Serial sections were treated for demonstration of myofibrillar ATPase[13].

With this indirect immunofluorescence technique, it was possible to identify with ease conducting cells, even when situated intramyocardially (figure 3). At higher resolution, the staining pattern was seen to correspond to the distribution of the Purkinje fibre cytoplasmic filaments as demonstrated by electron microscopy[9]. Other cell organelles such as nuclei and myofibrils were not fluorescent (figure 4a), cf the inverse staining properties of sections treated for demonstration of myofibrillar ATPase (figure 4b). Also, reactivity of vascular smooth muscle and endothelial cells could be demonstrated in the moderator band sections. In sections of hog stomach, strong fluorescence was seen in the smooth muscle cells. No staining was seen in sections treated with preimmune serum.

The results confirm our previous hypothesis that the 55,000 dalton protein of cow heart conducting cells is the main constituent of the cytoskeletal filaments of these cells[6]. Further verification of this statement could be obtained by immunoelectron microscopical demonstration of specific binding of labeled antibody to the filaments (work in progress).

The fact that smooth muscle cells also showed a reaction, strongly indicates an antigenic relationship between the cytoplasmic filament protein of Purkinje fibres and smooth muscle 100-Å filament protein. Others have shown cross-reaction between intermediate filaments of other cells. Localization of neurofilament protein antibody has been demonstrated in neuroblastoma cells[3], as well as in vascular endothelium and heart muscle cells[1]. Smooth muscle 100-Å filament antibody has reacted with skeletal and cardiac myocytes[8].

Very little is known of the embryogenesis of the heart conducting system. With the use of specific antiserum, it may now be possible to investigate further the differentia-

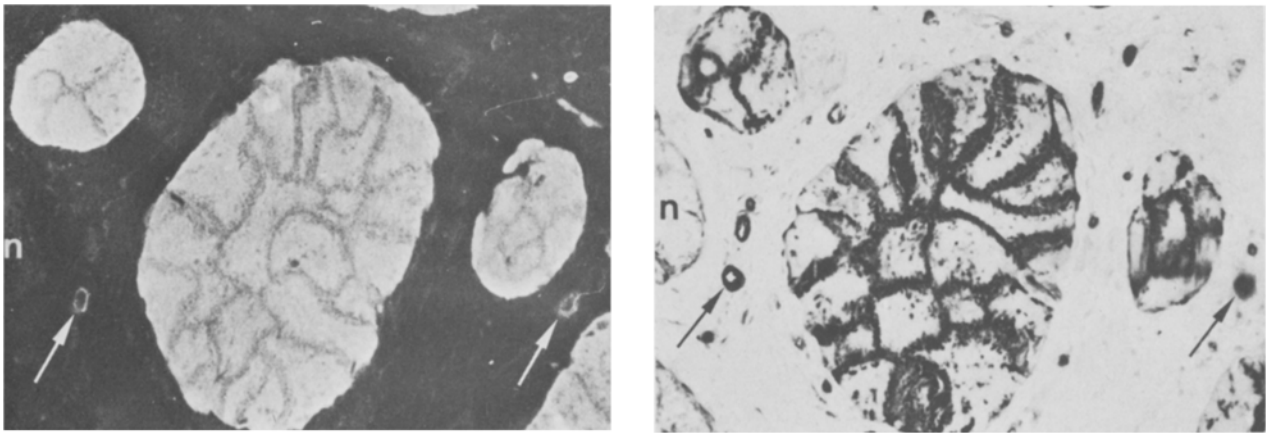

Fig. 4. *a* Staining pattern observed in Purkinje fibre bundles treated with anticytoplasmic filament globulin using the indirect immunofluorescence technique. The cytoplasm of individual Purkinje fibres shows fluorescence, while cell boundary regions towards other Purkinje fibres do not. The latter 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.

1 S.H. Blose, M.L. Shelanski and S. Chacko, Proc. natl Acad. Sci. USA *74*, 662 (1977).
2 K. Jimbow and T.B. Fitzpatrick, J. Cell Biol. *65*, 481 (1975).
3 A.O. Jorgensen, L. Subrahmanyan, C. Turnbull and V.I. Kalnins, Proc. natl Acad. Sci. USA *73*, 3192 (1976).
4 S-H. Yen, D. Dahl, M. Schachner and M.L. Shelanski, Proc. natl Acad. Sci. USA *73*, 529 (1976).
5 J.V. Small and A. Sobieszek, J. Cell Sci. *23*, 243 (1977).
6 A. Eriksson, L-E. Thornell and T. Stigbrand, J. Ultrastruct. Res. *57*, 225 (1976).
7 P. Cooke, J. Cell Biol. *68*, 539 (1976).
8 E. Lazarides and B.D. Hubbard, Proc. natl Acad. Sci. USA *73*, 4344 (1976).
9 L-E. Thornell, A. Eriksson, T. Stigbrand and M. Sjöström, J. molec. cell. Cardiol., in press (1978).
10 P.F. Davison and B. Winslow, J. Neurobiol. *5*, 119 (1974).
11 L-E. Thornell, J. molec. cell. Cardiol. *7*, 191 (1975).
12 L.F. Eng, J.J. Vanderhaeghen, A. Bignami and B. Gerstl, Brain Res. *28*, 351 (1971).
13 H.A. Padykula and E. Herman, J. Histochem. Cytochem. *3*, 170 (1955).

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