

convert cholesterol to pregnanolone. Histochemistry on human corpora lutea from cyclic stages has shown

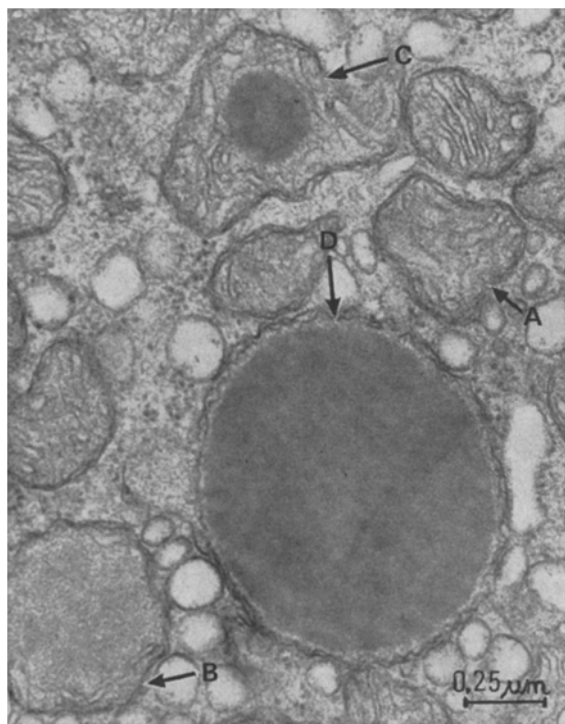


Fig. 2. A) Mitochondrion with obscured cristae. B) Mitochondrion where only few cristae are preserved. C) Mitochondrion with a round osmiophilic inclusion in a central position. D) A mitochondrion filled with an osmiophilic inclusion with the same appearance as a lipid inclusion. 135 days of pregnancy. Electron micrograph. $\times 40,000$.

NADPHase activity on the mitochondrial cristae¹². Mitochondria in guinea-pig ovaries have been seen in close relationship with rough endoplasmic reticulum¹³. Since the mitochondria contain NADPHase, the electron dense inclusions may be a condensation of some metabolic products from the steroid synthesis. So the inclusions in mitochondria from bovine granulosa cells of pregnancy may be a morphological feature closely related to steroid synthesis.

Zusammenfassung. Im Gelbkörper von Kühen wurden während der Periode von 80–240 Trächtigkeitstagen in den Granulosaluteinzellen Einschlüsse gefunden, die beinahe ganze Mitochondrien ausfüllten. Die Elektronendichte war der von Fett-Tröpfchen ähnlich. Die Einschlüsse könnten morphologisch dahin deuten, dass die Mitochondrien zur Steroidsynthese der Granulosazellen enge Beziehungen haben.

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Bikaner (India), 13 November 1972.

¹² P. LAFFARGUE, A. CHAMLIAN and L. ADECHY-BENKOËL, J. Microsc. 13, 235 (1972).

¹³ R. J. RUBY, R. F. DYER and R. C. SKALKO, Z. Zellforsch. 97, 30 (1969).

¹⁴ Supported by the F.A.O. Veterinary Faculty for F.A.O.-Fellows and Scholars, The Royal Veterinary and Agricultural University, Copenhagen, Denmark.

The Occurrence of Filamentous Banded Elements as Components of *Mytilus galloprovincialis* Byssus

In the course of an extensive research on the ultra-structure of the byssal apparatus of *Mytilus galloprovincialis*, the presence has been discovered of periodic filamentous elements within the byssus structure.

The presence of such elements had remained undetected in the previous ultrastructural observations of MERCER¹, RANDALL et al.², JACKSON et al.³ and BAIRATI⁴, and appears to be of some interest from the standpoint of the presence and location of the collagen protein in byssus.

Samples of *M. galloprovincialis* byssus obtained from live animals were fixed with a 3% glutaraldehyde solution buffered with s-collidine EM (TAAB) to a pH of 7.2 with the addition of 30 mg sucrose per ml (470 mOsm), then post-fixed in a 1% osmium tetroxide solution. They were embedded in Durcupan-ACM (Fluka) and sectioned, special care being devoted to the location of the cutting surfaces. The sections were contrasted with uranyl acetate and lead citrate, and were then examined with a Siemens Elmiskop 101 electron microscope.

Figure 1 shows what is the most frequent appearance of these periodic filamentous elements: prevalently anisodiametric bands approximately 0.2 μ m in diameter. While their thickness is fairly constant, their length within the sections varies considerably, probably in relation to their wavy course. As their boundaries with the material forming the byssus matrix are never sharp,

they look more like specific portions of the matrix itself than independent elements proper, this being the reason why 'filamentous banded elements' (FBE) would seem a more appropriate term to describe them rather than 'fibres' in the true meaning of the word, the definition being based more on their structure than on their shape.

From Figure 2 the FBE appear to consist of protofilaments approximately 75 Å in diameter, longitudinally arranged and clearly distinguishable in the less dense portion of the period.

The period itself is made up of a denser portion (A) and a markedly lighter area (B): 3 bands being at times recognizable in A, 2 of them (a, a) along the borders and one (b) forming the central, and lighter, zone. The whole period measures an average of 1000 Å, the extensions of the 2 portions being somewhat variable. As will be seen, the period is centrosymmetrical and non-polarized. As to the FBE, so far they have been identified mostly in the

¹ E. H. MERCER, Aust. J. mar. Freshwat. Res. 3, 199 (1952).

² J. T. RANDALL, R. D. B. FRASER, S. JACKSON, A. V. W. MARTIN and A. C. T. NORTH, Nature, Lond. 169, 1029 (1952).

³ S. F. JACKSON, F. C. KELLY, A. C. T. NORTH, J. T. RANDALL, W. E. SEEDS, M. WATSON and G. R. WILKINSON, in *Nature and Structure of Collagen* (Butterworths Scientific Publication, London 1953), p. 106.

⁴ A. BAIRATI JR., Boll. Zool. 39, 205 (1972).

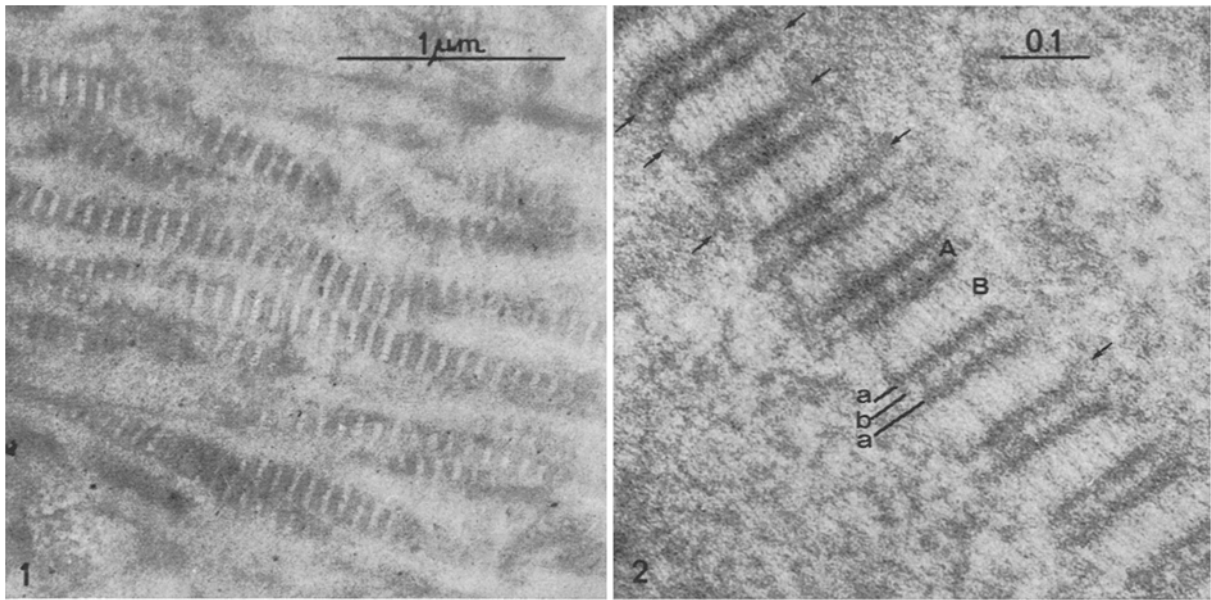


Fig. 1. Electron microscopic image of an ultrathin section cut tangentially to the surface of a byssus thread at its insertion within the stem: the figure shows the typical banded appearance of the FBE and their fusion with the surrounding byssus matrix. $\times 30,000$.

Fig. 2. High magnification picture of a FBE. A) dense portion; B) light portion; a, b) represent different zones (bands) of portion A. Note the filamentous pattern of portion B and the junctions between the FBE and the byssus matrix (arrows). $\times 120,000$.

areas where the threads branch out from the stem, but their presence has occasionally been observed in the stem laminae under formation, at root level.

No evidence is available as yet as to the nature of these FBE. As a work hypothesis, however, the suggestion is made that they might be of collagen material, considering: 1. that the presence of collagen in byssus, and particularly in the threads, has been demonstrated by biochemical analysis (JACKSON et al.³; PIKKARAINEN et al.⁵; PUJOL et al.⁶); 2. that tests performed with polarized light microscope, particularly in areas where FBE have been demonstrated (BAIRATI and VITELLARO⁷), are typical for the presence of collagen, and 3. that the ultrastructure of FBE presents a periodic paracrystalline stage of filamentous molecules, similar to other collagen structures.

The FBE period appears to be entirely different from the typical period of collagen fibrils in vertebrates. It should be remembered, however, that several instances have been observed in which tropocollagen molecules or protofilaments aggregate in peculiar ways (F.L.S., fibrohyaline tissue collagen or even form non-periodic entities (Annelida and Nematoda cuticles).

The closest approximations to the aspects exhibited by FBE are to be found in human acoustic neurilemmomas (LUSE⁸), in the experimentally constrained rat nervous fibers (PILLAI⁹), in many cases of acoustic nerve tumors both in vivo and in vitro (CRAVIOTO and LOCKWOOD¹⁰), and in lymphnodes undergoing fibrotic evolution from Freund's adjuvant (BAIRATI et al.¹¹); similar structures were observed in human Meissner corpuscles by CAUNA and ROSS¹², in the connective tissue of cutaneous nerves by CASTANO¹³, in lymphnodes of human subjects suffering from Hodgkin's disease by MOLLO et al.¹⁴ and by FRIEDMAN et al.¹⁵ in the membranous labyrinth of humans with Ménière's disease. All these cases have in common with byssus 1. a structure featuring banded, filamentous periodic elements, even though such elements are of variable sizes and structures, and 2. the fact of being located within a high-density, microfilamentous matrix.

Research is in progress aimed at identifying the nature of byssal FBE by enzymatic tests performed at submicroscopic level. Should experimental evidence be obtained of their collagenous character, the properties of these collagen molecules (PM, dimensions, etc.) and how they are aggregated within the FBE will still have to be investigated.

Résumé. L'examen au microscope électronique de sections ultra-minces de différentes portions du byssus de *Mytilus galloprovincialis* a révélé pour la première fois la présence de structures filamenteuses périodiques (FBE). Leur période a une longueur de 1000 Å en moyenne, elle est symétrique et comporte deux fractions: A et B. Il est possible que ces structures correspondent à une organisation particulière de la protéine collagène.

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⁵ J. PIKKARAINEN, J. RANTANEN, M. VASTAMÄKI, K. LAMPIAHO, A. KARI and F. KULONEN, *Eur. J. Biochem.* 4, 555 (1968).

⁶ P. PUJOL, M. ROLLAND, S. LASRY and S. VINET, *Comp. Biochem. Physiol.* 34, 193 (1970).

⁷ A. BAIRATI JR. and L. VITELLARO, *Proc. IX Int. Congr. Anat.*, Ed. D. A. J. DAMOV; Leningrad, p. 192. (1970).

⁸ S. A. LUSE, *Neurology* 10, 881 (1960).

⁹ A. P. PILLAI, *J. Ultrastruct. Res.* 11, 445 (1964).

¹⁰ H. CRAVIOTO and R. LOCKWOOD, *J. Ultrastruct. Res.* 12, 92 (1965).

¹¹ A. BAIRATI, M. G. PETRUCCIOLI and B. PERNIS, *Boll. Soc. ital. Biol. sper.* 43, 1443 (1967).

¹² N. CAUNA and L. ROSS, *J. biophys. biochem. Cytol.* 8, 467 (1960).

¹³ P. CASTANO, *Proc. 7th Italian Congr. Elect. Micr.*, Modena 1969.

¹⁴ F. MOLLO, G. MONGA and A. STRAMIGNONI, *J. Microscop.* 7, 451 (1968).

¹⁵ I. FRIEDMAN, T. CAWTHORNE and E. BIRD, *J. Ultrastruct. Res.* 12, 92 (1965).