Structure and function of the nematosome

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Summary

The ultrastructural morphology of human placental and mouse placental nematosomes has been investigated. The description includes a three-dimensional analysis of the shape of the organelles based on serial sectioning, measurements of the repeat distance of the subunit fibre of the organelle derived by optical diffraction analysis and the results of an ultrastructural cytochemical study designed to test whether the organelle contains nucleic acid.

Key words: nematosome, placenta, organelle ultrastructure.

Introduction

General morphology

The nematosome is a non-membrane-bound, cytoplasmic organelle of unknown function. It is spherical or ovoid, about 1 μm in mean cross-sectional diameter and consisting of fibres approximately 40–60 nm in diameter. Each fibre appears to be made up of smaller filaments 2.5–6.0 nm in diameter. The whole structure seems to be embedded in a homogeneous ground substance of lower density.

Distribution

This inclusion was first described as a 'peculiar fibrous structure', when found in rat and mouse placentae by Enders (1965) during a comparative study of various haemomonochorial placenta. He described the area in which the organelle was found as the 'middle layer' of the trophoblast.

Since this time, the nematosome has been reported again in mouse placenta (Toro & Rohlich, 1966; Bjorkman, 1970; Hernandez-Verdun, 1971, 1972; Hernandez-Verdun & Bouteille, 1976; King & Hastings, 1977) and rat placenta (Toro & Rohlich, 1966). It has also been observed in baboon placenta (Wynn et al. 1971) and human placenta (Martin & Spicer, 1973; Szvaras, 1977; Jones & Ockl ford, 1985), while dragonfly oocytes (Halkka & Halkka, 1975, 1977), rat embryo primitive ectodermal and yolk sac endodermal cells (Takeuchi, 1980) and even pollen of campanulae (Dunbar, 1973) also contain nematosomes.

In addition, the organelle has been found in rat sympathetic neurones (Grillo, 1970), paracervical ganglia (Kanerva & Teravainen, 1972), trigeminal ganglia (Peach, 1972), cerebellum (Chan-Palay, 1973), cerebral cortex (Routtenberg & Tarrant, 1974; Knox et al. 1980), dorsal root ganglia (Jacobs et al. 1975), spinal ganglia (Volk, 1980; Van den Bosch de Agui lar & Vanneste, 1981), superior cervical ganglia (Heym & Addicks, 1982), medial accessory olive (Bourrat & Sotelo, 1983), reeler mutant mouse cerebellum (Mariani et al. 1977), pcd mutant mouse Purkinje cells (Landis & Mullen, 1978) and dog spinal ganglion neurons (Fercacova & Marsela, 1983).

Study of these published locations may be useful in giving some clue to the function of the nematosome if it is assumed that this is an accurate reflection of the true distribution. The nematosome is found in two main classes of tissue: embryonic and neural. The embryonic localization may be of trophic origin, as placental tissue, which may be syncytiotrophoblastic (rat and mouse) or cytotrophoblastic (baboon and human), or from tissue developed from the inner cell mass (primitive ectodermal and yolk sac endodermal cells). The tissue involved may be of male germinal origin (pollen) or from the female germ line (oocytes).
The neural distribution is autonomic, both sympathetic (e.g. superior cervical ganglia) and parasympathetic (e.g. paracervical ganglia), and somatic, both peripheral (e.g. trigeminal and dorsal root ganglia) and central (spinal, cerebellar and cerebral).

The actual position within the cell seems to be variable, although certain workers claim to have observed preferential localizations close to the following structures: smooth endoplasmic reticulum and associated vesicles (Toro & Rohlich, 1966; Grillo, 1970), nuclear envelope (Toro & Rohlich, 1966; Grillo, 1970; Hernandez-Verdun, 1971), plasma membrane (Toro & Rohlich, 1966; Jones & Ockleford, 1985), coated vesicles (Grillo, 1970), groups of intracytoplasmic fibres (Hernandez-Verdun, 1971), free ribosomes (Hernandez-Verdun, 1971) and rough endoplasmic reticulum (Dunbar, 1973; Jacobs et al. 1975).

Chemical characteristics
A preliminary investigation of the histochemical nature of the nematosome was attempted at the light-microscope level by Grillo (1970). She identified structures in the cytoplasm that resembled nematosomes in shape, size, incidence and location and performed various staining and digestion experiments in order to observe their reactions. They were stained a purple colour by Azure B and Toluidine Blue O and this colouration was sensitive to RNase digestion and acid hydrolysis. Grillo's experiments indicate that these structures contain some ribonucleic acid.

Hernandez-Verdun (1972), however, attempted an investigation of the chemical nature of these organelles in mouse at the electron-microscope level. He surmised initially that glycogen cannot be a major component of the inclusion since embedding in glycol methacrylate produces no visible ultrastructural modification. He also suggested that the nematosome consists of a basic protein component, as the organelle stained well with ethanolic phosphotungstic acid in the absence of osmium fixation. RNA is believed by him to be present since staining using Bernhard's technique is positive in Epon-embedded sections. Furthermore, he thought there was no polysaccharide component present, since phosphotungstic acid at a low pH did not stain the inclusion. Using enzyme digestion techniques to verify his assumptions, Hernandez-Verdun found that Pronase digestion left a fine precipitate in place of the filaments normally present in the nematosome, and that, although RNAse alone did not modify the nematosome structure (despite the fact that cytoplasmic ribosomes were digested), RNAse treatment when preceded by a brief Pronase digestion caused the nematosome to be effectively digested.

The aim of this study has been to elucidate further the structure and composition of the organelle as a basis for understanding its function.

Materials and methods

Electron microscopy
Human trophoblast was obtained by collecting placenta from Leicester Royal Infirmary immediately after postpartum inspection. Mouse tissue (TR11 syncytiotrophoblast) and human tissue was fixed as previously described (Jones & Ockleford, 1985; Nevard, 1985), then dehydrated for periods of 10 min in a series of aqueous ethanol solutions with concentrations ranging from 50% to 100%. Dehydration was followed by propylene oxide replacement and then embedding in Araldite or L. R. White resin. Ultrathin sections, approximately 60 nm thick, were cut and stained using 10% uranyl acetate in methanol for 1 min followed by 2% aqueous lead citrate for 6 min.

A JEOL 100CX electron microscope operated by an accelerating voltage of 80 kV was used to find and photograph nematosomes until serial sections of a nematosome sectioned from end to end were obtained and complete sequences could be analysed.

Features on electron micrographs were measured using a Projectina measuring microscope, and an indication of the average distance from one side of one fibre to the same side of the next was calculated. The overall diameter of organelle profiles was also measured using this device, provided the organelle was not too large.

The average profile diameter of a cytotrophoblast cell section, and the average nematosome profile diameter were calculated from measurements made on several electron micrographs using the graphics tablet of a Kontron MOP electronic planimeter.

Optical diffraction
Negatives were contrast-reversed and reduced in magnification onto Agfa Rapidoline Ortho R.A. 710p camera film using a Durst M800 enlarger fitted with a Dutub 2 image reducing extension. The camera film was processed using an

\[ \text{Spacing (um) = 4887.6} \times \frac{1}{\text{diffraction spacing (mm)}} - 17 \]

Fig. 1. Calibration of the optical diffractometer was performed by obtaining diffraction patterns from electron microscope grids with known spacings (400, 300, 200 bars/in). The regression line of these points (O) was calculated and spacings were estimated either graphically, e.g. points A and B, or using the equation:

\[ \text{Spacing (um) = 4887.6} \times \frac{1}{\text{diffraction spacing (mm)}} - 17 \]
Agfa Rapidoprint DD3700. Contrast reversals were made by contact printing these images onto the same camera film and processing them in an identical manner. The camera film was mounted in a Rank Taylor Hobson image analyser 3000. This system (Gibbs & Rowe, 1977) incorporates a laser optical diffractometer producing optical

Fig. 2. Transmission electron micrograph (TEM) of a human placental nematosome (arrow). The organelle is situated in a cytotrophoblast cell (identifiable by its characteristically electron-lucent cytoplasm). The coiled subunit threads of the organelle appear to be relaxed into rather straighter regions that are separated by kinks. The optical diffraction pattern (inset) has a spot density increased at a spacing equivalent to 72 nm and suggests a distorted hexagonal outline, which may also be seen in the direct image. X37335.

Fig. 3. In this TEM of a human placental nematosome, the plane of section has cut through a portion of the organelle in which the fibres are running parallel to each other, are hexagonally packed and have their long axes approximately perpendicular to the plane of section. The optical diffraction patterns show hexagonal symmetry. Using circular masking various groups of the fibres (insets A–D) were studied in this way. Inset A is a natural pattern whereas insets B–D show patterns image-enhanced using the squaring facility of the image enhancer. With averaging measurements of 15 pairs of spots, an interfibre spacing of 74 nm was calculated. X171000.

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transforms. These or direct images were recorded using a vidicon tube camera. Output from this camera was channelled to a signal processor (Electronic Visuals Ltd, Surrey) with low cut filter and squaring facilities. Processed images, optical diffraction patterns and power spectra were displayed on monochromatic monitors (Hitachi VM 906E/K, Rank Taylor Hobson Display Unit EV8060).

Images from the monitors were photographed using a Polaroid CR9 Land Oscilloscope camera loaded with Polaroid type 667 Coaterless film (3000 ASA).

Optical diffraction patterns were obtained for squared and unsquared images. Spots were marked on tracing paper overlays and measured in millimetres. The displacements of spots from the central beam are proportional to real spacings in the specimen. The diffraction spacings were calibrated using a series of electron-microscope grids with known mesh sizes. A regression line was fitted to the observed and known values and the appropriate equation was deduced (Fig. 1).

**Ultrastructural cytochemistry**

Term placental tissue was fixed and processed for ultrastructural localization of RNA containing organelles using the method of Bernhard (1969). Briefly, this procedure utilized fixation in a low concentration of aldehyde without osmium fixation. Following ultrathin sectioning from Epon blocks the tissue was stained using 5% uranyl acetate for 1 min. The uranyl stain was then selectively chelated by carefully timed (7 min) treatment with 0.2M-ethylene diamine-tetraacetic acid (EDTA).

Indium trichloride staining was done using the method of Watson & Aldridge (1961) at pH 4.5 and in the presence of acetone.

**Fig. 4.** Using the squaring facility of the image enhancer and parts of the electron micrograph shown in Fig. 3 it was possible to represent the information in the images in considerably simplified fashion (A–F) to reveal fibres and the filamentous bridges (arrows) that may be responsible for maintaining the regularity of spacing between the fibres.
Fig. 5. This TEM of an exceptionally regular nematosome from human placenta shows a clearer example of the overall hexagonal structure (small arrowheads) indicated feebly by Fig. 2. With the squaring facility set to capture those very strong near axial spots in the diffraction pattern of the organelle this sixfold symmetry is clearly demonstrable (inset). ×126 400.

Results

Transmission electron microscopy of human placental nematosomes

A total of 81 sections through 33 different nematosomes were obtained during this study. In all cases the organelle was situated in the cytoplasm of a cytotrophoblast cell. No particular localization seemed evident; the inclusion was situated in some cases near the nucleus (Fig. 2), in others near polysome containing cytoplasm (Figs 3, 4), the cell membrane or basement lamina (see Figs 6, 16), or infrequently in an area populated by many mitochondria. In several cases where nematosomes were found associated with a membrane, their shape seemed to be modified so as to appose the plasma membrane more closely (see Fig. 16). None of the organelles appeared to be membrane-bounded.

The profiles seen tended to be oval or nearly circular with diameters ranging from 0.85 to 0.15 μm and averaging 0.5 μm × 0.4 μm (n = 81). Each profile consisted of a number of fibres ranging from 25 to 50 nm in diameter, with an average diameter of 35 nm. These threads tended to be helically disposed in the more circular profiles (Figs 2, 5, 10) and in these cases regular thinnings of the fibres could be seen (Fig. 10). In the more oval figures fewer fibres could be seen in general and these tended to be arranged in more nearly parallel, straight lines (Figs 6, 16). The maximum recorded fibre length was 1.9 μm (Fig. 10). Real length may be much greater and the possibility that the organelle is composed of a single folded strand has not been ruled out.

Projecting from and possibly coalescing to form the fibres are fine filaments. Measured using the projection microscope, these have diameters in the range 6–7 nm (Figs 4–7).

The number of consecutive sections obtained through organelles ranged from one to eight. Two whole nematosomes were sectioned. The first consisted of four sections with nematosome material extending through the full depth of the section and two sections into which it extended partly (Fig. 11). The second contained five full-depth sections and two partial sections (Fig. 12). Assuming each section had a thickness of 60 nm, this would indicate the nematosomes in question were at least 0.24 μm and 0.30 μm long, respectively. The maximum diameters measured for each organelle were 0.3 μm and 0.8 μm, respectively, indicating an almost spherical three-dimensional
shape for the first organelle, and a more ovoid shape for the second.

In one case, seven complete sections were obtained through part of an organelle (data not shown). Here the maximum diameter was 0.8 μm, and there were six sections from one end to the profile of largest diameter. If it is assumed that this organelle is symmetrical, as in the cases completely sectioned, the total end-to-end distance would be 0.72 μm.

In general, only one section of a nematosome was found in any cytotrophoblast cell profile; however, in three cases two such organelles were found in a single cytotrophoblast cell profile (Fig. 13).

On average, one profile was found per seven cytotrophoblast cell profiles examined, but the range of incidence was great. Sometimes nematosomes were observed in cytotrophoblast cells examined consecutively, yet in one case 33 consecutive cytotrophoblast cell profiles were examined without a nematosome section being observed.

Transmission electron microscopy of mouse placental nematosomes

Nematosomes were found in the TRII layer of the mouse placenta. The fibres making up to the organelle appeared similar to those present in the nematosomes from human placenta, except that cross-sections through the organelle occupied a larger area and showed profiles of a greater number of fibres (Figs 8, 9). Overall, the fibres in mouse nematosomes were less well packed than human ones, but in some regions (e.g. Fig. 9) closer packing of fibres revealed the hexagonal arrays found in some human placental nematosomes.

Within the fibres, filaments could occasionally be detected exhibiting a shallow helical multi-stranded organization (Fig. 9). From these sub-fibres, filaments extended to neighbouring fibres (Fig. 8). It is these projecting filaments that make the position of the true edge of the fibre hard to measure except by averaging methods such as optical diffraction. It is possible that the regular spacing of fibres in close-
packed regions of the organelles is maintained by these filamentous cross-bridges acting as spacers.

Ultrastructural cytochemistry of human placental tissue

The Bernhard (1969) technique revealed isolated electron-dense areas within the tissue, which generally had low electron density. Particularly prominent were nucleoli and ribosomes (Fig. 14), observations that suggest there is some selectivity of the technique.

A number of nematosomes were identified. These stained with an electron density similar to or less than that of the nucleoli and clearly less than the ribosomes (Figs 13A, 14A).

A similar pattern of staining to that produced by the Bernhard method was produced using indium trichloride staining (Fig. 15). Nucleoli, nuclear heterochromatin, cytoplasmic ribosomes and nematosomes were more heavily stained than other cellular and extracellular components.

Optical diffraction analysis

Analysis using optical diffraction has revealed new information about the structure of the nematosome. Whereas fibres may appear straight over extended distances, they may be coiled into more closely packed arrays. In these latter situations they may exhibit a tendency to lose even curvature and relax into straighter segments separated by kinks. Thus organelles of approximately spherical outline reveal a rounded hexagonal appearance that yields a diffraction pattern with sixfold rotational symmetry (Fig. 5). This behaviour indicates a degree of stiffness in the fibre.

Fibres close-packed and cut in transverse section show hexagonal symmetry on a much smaller scale (e.g. Figs 3, 4). This close packing includes a spacer
Figs 8, 9. For legends see p. 36

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Fig. 10. For legend see p. 36

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region 25–30 nm broad around each fibre that is bridged only by fine filaments. These elements are clearly demonstrated by image analysis methods (Fig. 4). The diffraction pattern here includes major spots representative of repeating structure in the organelle at 70–75 nm. This is presumably the average distance from one point on a fibre to the equivalent point on its neighbouring fibre.

Fibres sectioned longitudinally reveal pairs of spots, one of which, at 70–75 nm, probably represents the same inter-fibre repeat.

Sample measurements of inter-fibre repeat for two nematosomes comparing optical diffraction and direct measurement using the projection microscope are:

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Optical diffraction</th>
<th>Projection microscope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nematosome 1 inter-fibre</td>
<td>72·6 nm</td>
<td>72·3 nm</td>
</tr>
<tr>
<td>repeat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nematosome 2 inter-fibre</td>
<td>74·5 nm</td>
<td>74·1 nm</td>
</tr>
<tr>
<td>repeat</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Diffraction analysis revealed great similarity between the size and packing of fibres and the dimensions of inter-fibre spacings of mouse and human placental nematosomes (Figs 2–9).

Discussion

Size and shape

Serial sectioning has shown that the organelle may be ovoid or nearly spherical. This is consistent with the views of other workers, who came to this conclusion on the basis of the oval and circular profiles they obtained in sections. It will be of interest to trace the 35 nm diameter fibres in order to find out whether one or more separate strands are present in one organelle.

Figs 8, 9. The same nematosome in both Figs, derived from negatives of different magnification. The nematosome shown at low power in Fig. 9 is partly shown at high power in Fig. 8. The optical diffraction patterns (insets) were produced by the circular masking of a group of six close-packed fibres cut in transverse section. The spacings revealed are 51 and 85 nm for Fig. 8, and 52 and 80 nm for Fig. 9, respectively. These are similar to those obtained from equivalent diffraction patterns from human placental nematosomes. In Fig. 9 there is evidence of shallow pitch spiralling of filaments around the long axis of the fibre (arrows). In Fig. 8 and Fig. 9 filamentous cross-bridges span the electron-lucent gaps between the fibres. As with human fibres, these are also disposed diagonally to the long axis of the fibres. Fig. 8, ×173 700; Fig. 9, ×928 900.

Fig. 10. A. This TEM of a term human placental nematosome reveals the longest continuous fibre measured in our series. ×141 000. B. This lower-power survey micrograph shows the cytoplasmic location of the organelle. ×8370.

Knowledge of the number of threads comprising a nematosome at present eludes us owing to superposition effects in sections of the same order of thickness as organelle diameters.

Particularly at high magnifications, the edges of the fibres appear to blend with the ground substance. This phenomenon is in part due to the fact that the fibres appear to be made up of apparently disorganized smaller filaments (approximately 6 nm in diameter), producing a diffuse edge to the large fibres. Also these thin filaments tend to cross between fibres, making it difficult to ascribe the origin of a bridging filament to a particular fibre.

The original distance measured by optical diffraction is very nearly the same as the directly measured distance for the fibre repeat (the distance from one side of one fibre to the same side of the adjacent fibre). We assume that this is the actual distance being measured by the diffraction apparatus, since these spots will be closer together than the spots produced by the repeating filaments, fibres and inter-fibre spaces (as these repeats are smaller), and it is probable that these other spots will be lost in the general noise that develops as the squaring level is reduced to permit viewing of an area further from the centre.

The fact that the fibre repeat distances measured by both methods are so similar indicates that the direct measurements of fibre diameter and inter-fibre space are reasonably accurate.

Most of our measurements agree reasonably well with those made by earlier workers (e.g. see Grillo, 1970). However, organelle diameter data have to be considered with caution. This study has produced a value of 0.45 μm for the average nematosome profile diameter. This value is not the average maximum organelle diameter, but (assuming the nematosome is spherical) half of this value. However, it appears from the measurement of published micrographs and a comparison of these with the values cited by the authors for ‘average diameter’ that in all of the papers cited the average profile diameter and not the average maximum diameter is being referred to. This is to be expected, since it is difficult to obtain a value for maximum organelle diameter without using serial-sectioning techniques, and in none of the studies concerning nematosomes has this method been used.

If inaccurate, measurements are likely to be over-estimates rather than underestimates of the average profile diameter, since larger profiles will be more easily, and hence more frequently, recognized than small ones. Considering this fact it is relevant to note that, in this study, two nematosomes with a largest profile of less than 0.30 μm were observed. There is no indication, in any of the papers cited concerning the nematosome, of the number and size of profiles observed.
An interesting pattern emerges if the values in the literature for average organelle profile diameter are themselves averaged in groups of the same type of tissue:

<table>
<thead>
<tr>
<th>Type of tissue</th>
<th>Average profile diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neural tissue</td>
<td>1.1 μm</td>
</tr>
<tr>
<td>Embryonic tissue</td>
<td>1.0 μm</td>
</tr>
<tr>
<td>Rat and mouse Synctiotrophoblast</td>
<td>0.74 μm</td>
</tr>
<tr>
<td>Miscellaneous embryonic tissue</td>
<td>0.64 μm</td>
</tr>
<tr>
<td>Human and Baboon</td>
<td>0.58 μm</td>
</tr>
</tbody>
</table>

These results suggest that nematosomes in human placenta tend to be smaller than those in other tissues, particularly rat and mouse placentae. This is an unexpected result.

**Frequency**

There appears to be a small difference in frequency of organelles observed per cell according to the type of tissue studied. This work has indicated that an average of five to six nematosomes are present per cytrotrophoblast cell. Our value is in reasonable agreement with the previously suggested frequency for the organelle in this context and contrasts with findings using neural tissue, where the suggested frequency is one to two organelles per cell. All estimates concerning embryonic tissue have been made using placental tissue. Estimates of 10–15 per cell in mouse placenta, and up to 10 per cell and one to two per cell in human placenta have been proposed. Considering also the estimate from this study (5–6 per cell), it would appear that there is a greater frequency of nematosomes in placental than neural tissue, when frequency is measured as number of organelles per cell.

**Ultrastructural cytochemistry**

The staining of the nematosomes with uranium (Bernhard, 1969) and indium trichloride in tissue where nucleoli, chromatin and ribosomes stained indicates the presence of nucleic acid in the nematosome. In this respect our results with placental nematosomes are similar to those of Grillo (1970), who studied the nematosomes in neurones. The data are compatible with the presence of RNA in nematosomes. Were this shown to be the case by further more refined cytochemical study, it would be of particular interest in cytrotrophoblast, because these cells contain relatively few organelles and especially little rough endoplasmic reticulum. They divide and produce syncytiotrophoblast that is distinctive by virtue of the abundance of ribosomes and other cell organelles. The presence of a stored form of ribosomal RNA in the cytrotrophoblast cells would be a convenient way of rapidly producing the vast numbers of ribosomes required.

It is also consistent with the presence of the organelle in neurones. In another model the nematosome may represent a form in which mRNA is transported from the cell body to the synapse. Szarvas (1977) believes that in this context the nematosome may be responsible for the control of neurosecretion by regulating the activity of the ribosomes, and hence the amount of transmitter substance available.

At this stage it is worth emphasizing that the Bernard and indium techniques, although demonstrably exhibiting the expected pattern of selectivity of staining of nucleic acids, are not exceptionally specific probes in chemical terms and we recognize a need to apply more refined probes with precise molecular specificity.

**Proposed functions**

*Storage form of RNA.* Apart from the concept of Grillo (1970), that the nematosome is a cytoplasmic nucleolus, several workers have suggested that the...
nematosome may have some kind of organizational or storage role in the cell. These are reasonable suppositions, since RNA has the ability to encode other than ribosome-specific information, and this may be stored under some circumstances.

Halkka & Halkka (1975) suggested that the nematosome may be a form of RNA concerned with storage rather than any dynamic function, considering the structural changes undergone by the organelle during the development of the oocyte. They proposed that such a function would be feasible and provide sufficient information for early glycogen synthesis, synthesis of microtubule protein and other similar basic operations. This hypothesis was supported by Dunbar (1973), who suggested that the nematosome may represent a storage material as noted earlier.

Inhibitor of mitosis. Hernandez-Verdun (1971, 1972) suggested that the nematosome is present in cells with no well-organized centriolar material, since both neural cells and syncytial cells do not undergo mitosis. His hypothesis suggested that this may be because of the presence of the nematosome, and he cited the work of Steinman (1970), who cultured cells with colchicine and observed the accumulation of bodies that Hernandez-Verdun describes as resembling nematosomes. On inspection, however, these bodies are more granular than fibrous in form, and have no particularly well-defined overall structure, although the comparison can be understood. Evidence tending to refute this hypothesis includes the fact that Steinman's experiments did not produce true nematosomes, and that nematosomes have been observed in the cytotrophoblast cells of the baboon and human placentae, which do undergo mitosis. However, considering this last point, no cytotrophoblast cell has been observed simultaneously undergoing division and containing a nematosome. It is possible that nematosomes are found only in certain cytotrophoblast cells (explaining the low frequency), and that it prevents mitosis in these cells. This may be one reason for the increase in frequency with the age of the placenta (Jones & Ockleford, 1985). The fact that the nematosome has also been observed in developing dragonfly oocytes is a potential problem in the acceptance of this hypothesis, although the nematosome is formed prior to the inactive phase of development, and it subsequently changes form and finally breaks up as the active part of the cycle is begun. In Dunbar's work (1973) the presence of the nematosome is less easily explained as the nematosomes appear to be intimately bound up in the developmental processes occurring in Campanulae pollen, being last seen in intimate contact with the Golgi apparatus of the cell. Furthermore, it is unlikely that this hypothesis is consistent with the finding of nematosomes in rat embryo primitive ectodermal and yolk sac endodermal cells (Takeuchi, 1980), as these are also particularly active mitotically.

Inactive microtubule-organizing centres. Jones & Ockleford (1985) particularly disputed the suggestion that the nematosome is always related to the absence of a centriole, having themselves observed the two organelles in close proximity, and suggested that the nematosome may be an inactive collection of microtubule-organizing centres that redistribute when the cytotrophoblast develops into syncytium. They cite the evidence of Stubblefield & Brinkley (1967) and Dippel (1968, 1976), who demonstrated an RNase-sensitive component in basal bodies and centrioles, the axosome complex, and the electron-dense element at the foot of the A tubule. Jacobs et al. (1975) supported this theory with their observation that nematosomes were frequently associated with electron-dense aggregates, and very occasionally an 'undoubted microtubule-organizing centre'.

The occurrence of nematosomes in neurones is quite consistent with this theory. A nerve cell has a
particularly extensive cytoskeleton in order to maintain the shape of the axon and the synapse. The presence of microtubule-organizing centres is therefore to be expected. Furthermore, the finding of nematosomes in mitotically active cells such as pollen, oocytes and primitive ectodermal and yolk sac endodermal cells is reasonable on the basis of this hypothesis, since reproduction events call for extensive use of the cytoskeleton as, for example, in the separation of chromosomes.

Fig. 13. TEM of a human placental cytotrophoblast cell apparently containing at least two nematosomes. A, ×29200, Bernard stained; B ×38600, conventional stain; C, ×56000, conventional stain.
Fig. 14. A similarly photographically processed pair of TEMs of sections showing a nematosome. They are Bernard stained (A) and conventionally stained (B), respectively. The staining of the nematosome (arrow) is similar to that of nucleic acids. Note ribosomal, nucleolar (n) and nuclear heterochromatin staining. A, ×18 900; B, ×16 400.
The presence of organizing centres in the syncytium is also quite reasonable on this basis, since this tissue has an extensive cytoskeleton (Ockleford et al. 1984), particularly noticeable towards the apical membrane. There is at least one puzzling point, however, and that is the question of why nematosomes should be present in human and baboon in the cytotrophoblast only, and in rat and mouse in the syncytiotrophoblast only. It

Fig. 15. TEM of indium trichloride-stained human trophoblast. There are two heavily stained nematosomes and heavily stained ribosomes in the portion of the cytotrophoblast cell showing in the lower right of the field. The syncytium above exhibits intense staining of the peripheral regions of nuclear heterochromatin and in the cytoplasm the numerous ribosomes. ×23 000.
is possible that in the cases of the human and the baboon, the organelle, when passing from the cytotrophoblast cell to the syncytium, separates into active microtubule-organizing centres that are not of the same form as the nematosome.

Pathological entity? One possibility that has been discussed is that the nematosome is a pathological feature (Jones & Ockleford, 1985). No evidence was found of any relationship between the presence of nematosomes in human placentae and overt gestational pathologies. However, an ultrastructural pathological atlas (Ghadially, 1982) shows an ECHO virus-infected cultured cell. In this micrograph three inclusions can be identified, two of which (the granular and particulate inclusions) are, structurally, likely to represent elements or stored products of the icosahedral virus particle 'factory'. The third, identified as a 'vermicellar inclusion', is similar to the nematosome as described here in mouse and human cells and by others in a variety of species as cited. The thread of the vermicellar inclusion has a diameter much larger than that of the mature virus particles. Were the nematosome threads in reality composed of chains of superimposed viral particles, evidence for this would have probably been seen during the optical diffraction analysis. In none of our research have we seen any association of nematosomes with similar elements of viral nature, nor have we found any record in any of the extensive literature that has been reviewed (Nevard, 1985). The name ECHO is an acronym for enteric cytoplasmic human orphan virus. By definition these picornaviruses are capable of infecting the human species only. The observed range of the nematosome is much greater than this, as demonstrated in the literature cited in the Introduction and, for example, in Figs 8 and 9 of this article showing the structures in mice. We therefore believe that the published image of the vermicellar inclusion does not imply that the nematosome is viral. If there is some non-random connection between the three inclusions, it may be an example of viral derepression of the synthesis of this organelle and so in some way similar to the increased incidence observed in neurones after chronic administration of alcohol (Volk, 1980). If the inclusions are precursors for any virus type (an unlikely possibility in

Fig. 16. A. A nematosome closely associated with the plasma membrane has an unusual shape, which maximizes contact. ×120 000. B. This nematosome lies with fibres parallel to the membrane at a spacing similar to the inter-fibre spacing; this raises the suggestion that cross-bridges similar to the inter-fibre cross-bridges mediate the contact. The membrane itself has an unusually electron-dense appearance at the point of contact. ×130 500. C. An example of a nematosome contacting the membrane at the basal surface of a cytotrophoblast cell adjacent to the basal lamina. ×119 800.
our view, since we have detected neither mature viral particles nor any evidence of budding) then we would imagine that the shape of the nematosome thread would best fit it for inclusion in an anisometric type, for example, one of the filoviridae.

Various proposals have been made concerning a possible function for the nematosome, none of which completely explains the distribution of nematosomes, and which we have attempted to summarize. It is quite possible, because of its comparatively low incidence on a per cell basis, that the full extent of the distribution of the organelle is not yet appreciated. As we have indicated, even the 'true nematosomes' with which this work is directly concerned, differ between tissues and it may be found that different organelles are being studied. Alternatively, the similar looking organelles designated by names different from 'the nematosome' (Nevard, 1985), may well be related to it, for example as different forms of the same structure at different stages of development, as suggested by Halkka & Halkka (1975).

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References


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