

Presence of ribonucleoproteins and basic proteins in the nuage and intermitochondrial bars of human spermatogonia *

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(Accepted 24 April 1985)

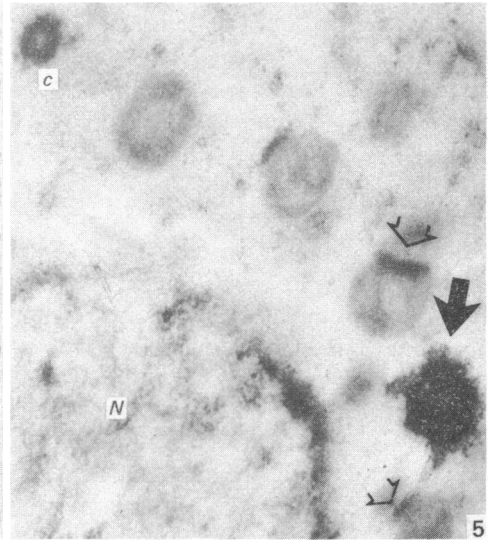
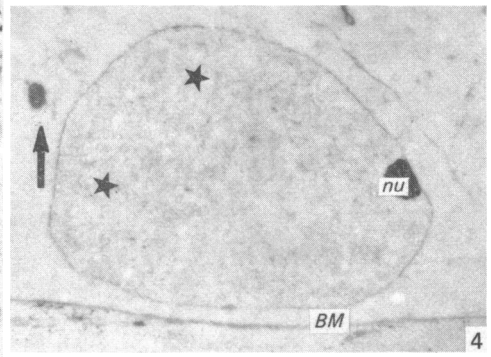
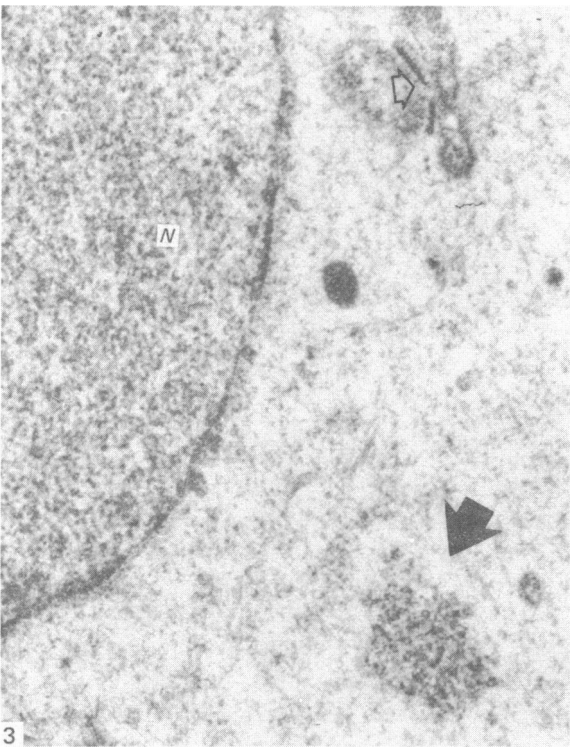
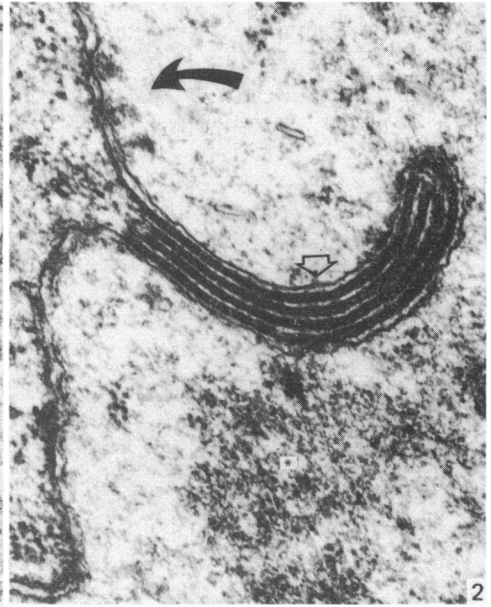
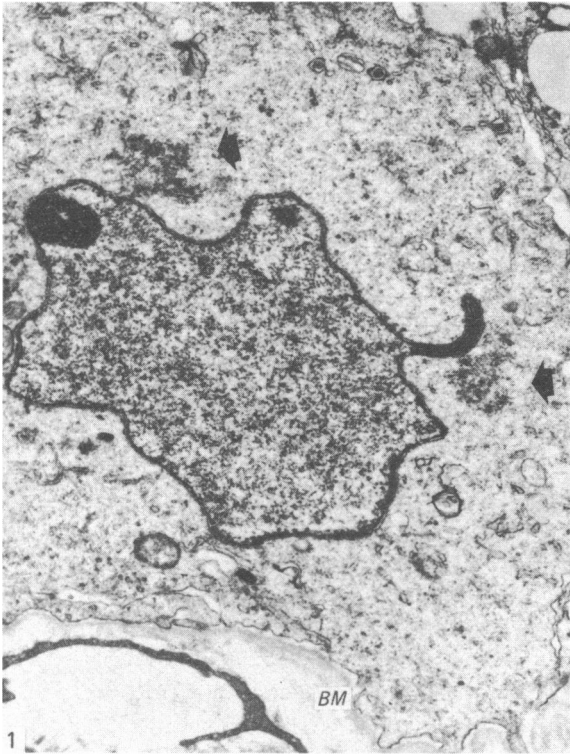
INTRODUCTION

The 'nuage' is a peculiar cytoplasmic structure of male germ cell in some vertebrate species. It has been reported in the spermatogonia of the rabbit (Nicander & Ploen, 1969), monkey (Gondos & Zemjanis, 1970), man (Burgos, Vitale-Calpe & Aoki, 1970) and *Xenopus laevis* (Kerr & Dixon, 1974), as well as in the spermatocytes of the rat (André, 1962; Susi & Clermont, 1970; Eddy, 1974; Russell & Frank, 1978), mouse (Fawcett, Eddy & Phillips, 1970), rabbit (Nicander & Ploen, 1969), guinea-pig, chinchilla, chinese hamster and monkey (Fawcett *et al.* 1970). The nuage consists of a fine fibrillar material which can be seen lying free in the cytoplasm or associated with mitochondria. In *Xenopus*, the nuage has also been observed in contact with the nucleus (Kerr & Dixon, 1974). The origin, fate and chemical composition of the nuage are not clearly understood, though the nuage has been related to nuclear material (Kerr & Dixon, 1974) as well as to dense interstitial material that accumulates between the mitochondria of spermatogonia or spermatocytes (Eddy, 1974). The present paper reports ultrastructural cytochemical observations of the nuage in human spermatogonia in an attempt to furnish new information concerning its cytochemical composition, as well as its probable origin and fate.

MATERIALS AND METHODS

Testicular biopsies were obtained shortly after death from 26 adult men who died in traffic accidents or from causes other than testicular or related pathological causes. The specimens were cut into 1 mm³ blocks and fixed in 4% glutaraldehyde in phosphate buffer for two hours. Thereafter, some blocks of each specimen were postfixed in 1% phosphate buffered osmium tetroxide, dehydrated in ethanol and embedded in Epon-812. Ultrathin sections were double stained with uranyl acetate and lead citrate. Other blocks were dehydrated after glutaraldehyde fixation and stained with 2% phosphotungstic acid (PTA) in absolute ethanol, using the Sheridan & Barrnett (1969) method, and embedded in Epon. Other blocks were dehydrated

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after glutaraldehyde fixation and embedded in Epon; ultrathin sections were stained either (a) with 2% aqueous phosphotungstic acid for 20 minutes at room temperature following the method of Stockert, Colman, Gimenez-Martin & Esponda (1975), or (b) with 5% uranyl acetate in distilled water for 15 minutes; after washing the sections were floated on 0.2 M ethylene diamine tetra-acetic acid (EDTA), pH 7.2, for 20 minutes at 40 °C, according to the method of Bernhard (1969).

RESULTS

Ultrastructural examination of glutaraldehyde and osmium fixed specimens showed one or two electron-dense masses (nuages), which recalled the chromatoid body of spermatids, in all the types of spermatogonium in the human adult testes (Fig. 1). These masses consisted of fine fibrils that were not surrounded by membrane (Fig. 2). They appeared either close to the nuclear membrane, sometimes associated with nuclear pores, or, distant from it, sometimes associated with the mitochondria. Of 198 nuages observed, 62 appeared close to the nuclear membrane and 45 were associated with mitochondria.

The nuages could be stained with ethanolic PTA (Fig. 3) and EDTA (Figs. 4, 5). The intermitochondrial bars also showed affinity for these two stains (Figs. 3, 5). Aqueous PTA did not stain either the nuages or the intermitochondrial bars.

The nuage was also observed in many spermatocytes but not in spermatids. These cells showed a true chromatoid body which was also stained with both ethanolic PTA (Fig. 6) and EDTA (Fig. 7).

DISCUSSION

The results of the present study suggest the presence of basic proteins (stained with ethanolic PTA) and ribonucleoproteins (stained with EDTA) but not polysaccharides (no staining with aqueous PTA) in the nuage of human spermatogonia and spermatocytes. Whereas many nuclear (chromatin, nucleolus) and cytoplasmic (mitochondria, intermitochondrial bars, Lubarsch crystals, centrioles and nuages) structures appeared stained with ethanolic PTA, only nucleoli, ribosomes and centrioles (whose RNA content is well known), besides the nuage and intermitochondrial bars, showed great affinity for EDTA stain. These cytochemical findings suggest a relationship between ribonucleoproteins, the nuage and the intermitochondrial bars; such a notion is further supported by morphological observations.

Fig. 1. Human spermatogonium showing two cytoplasmic nuages (arrows) in contact with the nuclear membrane. *BM*, basal lamina. Glutaraldehyde and osmium. $\times 7450$.

Fig. 2. Higher magnification of a nuage of Figure 1 showing a mass of fine electron-dense fibrils (asterisk) in contact with a nuclear fold (open arrow). Similar material is seen associated with nuclear pores (arrow). Glutaraldehyde and osmium. $\times 30500$.

Fig. 3. Part of a spermatogonium stained with ethanolic PTA showing the nuage (arrow). The nuclear chromatin (*N*), mitochondria and intermitochondrial bars (open arrow) are also stained. $\times 18400$.

Fig. 4. Spermatogonium stained with EDTA. The nuage (arrow) and nucleolus (*nu*) appear intensely stained, contrasting with the unstained nuclear chromatin (stars). *BM*, basement membrane. $\times 3500$.

Fig. 5. Part of a spermatogonium stained with EDTA. Besides the nuage (arrow), the intermitochondrial bars (open arrow) and a centriole (*c*) are stained. *N*, nucleus. $\times 18500$.

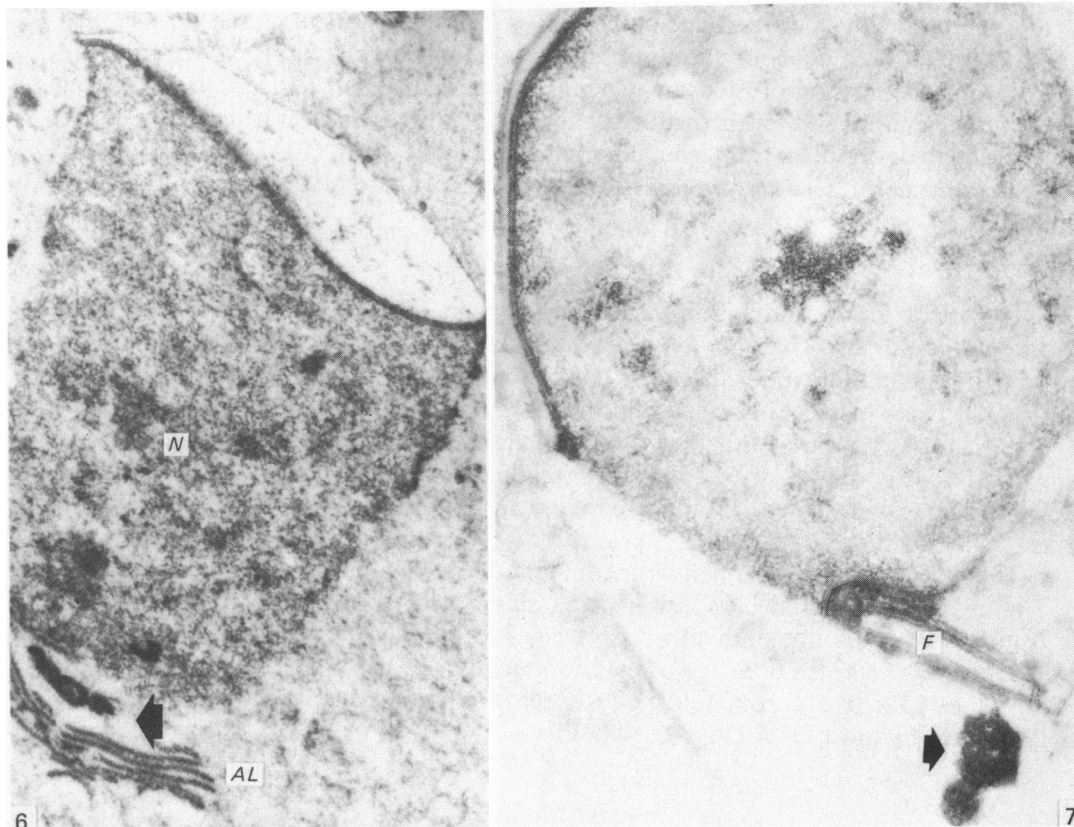


Fig. 6. Spermatid stained with ethanolic PTA showing a chromatoid body (arrow) between the nucleus (*N*) and annulate lamellae (*AL*). $\times 18\,500$.

Fig. 7. Spermatid stained with EDTA showing a chromatoid body (arrow) near the flagellum (*F*). $\times 18\,500$.

The nuage often appeared closely apposed to the nuclear membrane. The frequency of this apposition is so high that it does not seem to be fortuitous. Moreover, nuage material associated with nuclear pores was sometimes observed. This suggests that the nuage originates from the nucleus and migrates to the cytoplasm through the nuclear pores.

The nuage has also been associated with mitochondria in several other species (André, 1962; Nicander & Ploen, 1969; Fawcett *et al.* 1970; Eddy, 1974; Kerr & Dixon, 1974). It is of interest that all species showing nuages in spermatogonia or spermatocytes also exhibit intermitochondrial cement substance in these cells. Since the intermitochondrial bars also contain ribonucleoproteins, it is probable that the bars may originate from the nuage.

On the other hand, the chromatoid body of spermatids exhibits similar morphological (Fawcett *et al.* 1970; Phillips, 1974; Holstein & Roosen-Runge, 1981) and cytochemical (Sud, 1961; Krimer & Esponda, 1980) features to those of the nuage. This also suggests a relationship between the chromatoid body and the nuage. Although most authors (Comings & Okada, 1972; Söderström & Parvinen, 1976; Parvinen & Parvinen, 1979; Krimer & Esponda, 1980) have postulated a nuclear origin for the chromatoid body, Fawcett *et al.* (1970) have suggested that the chromatoid body of the mouse originates from the intermitochondrial cement

substance of spermatocytes. This opinion is compatible with the disappearance of both the nuage and the intermitochondrial bars and the appearance of a true chromatoid body in the spermatids (Russell & Frank, 1978). In addition, the presence of ribonucleoproteins in both intermitochondrial bars and chromatoid bodies seems to support this hypothesis. Therefore, in the human testis we consider that the nuage gives origin to the spermatogonium intermitochondrial bars which, in turn, give rise to the spermatid chromatoid body, whose final fate, according to the belief of several authors (Fawcett *et al.* 1970; Phillips, 1974; Holstein & Roosen-Runge, 1981) seems to be the formation of the annulus in the spermatozoon tail.

SUMMARY

Ultrastructural cytochemical study of the nuage in the human adult testis revealed that this structure was a cytoplasmic fine fibrillar electron-dense mass, similar to the chromatoid body of spermatids, in all spermatogonial types and spermatocytes. The nuage was often observed in relation with the nucleus or mitochondria. Cytochemical techniques showed staining affinity of the nuage for both ethanolic phosphotungstic acid and ethylene diamine tetra-acetic acid. The intermitochondrial bars were also stained with the two procedures. The results suggest that the nuage originates from the nucleus and migrates to the cytoplasm through nuclear pores, giving rise to the intermitochondrial bars.

This work was supported by a grant from the 'Comisión Asesora de Investigación Científica y Técnica', Madrid, Spain.

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