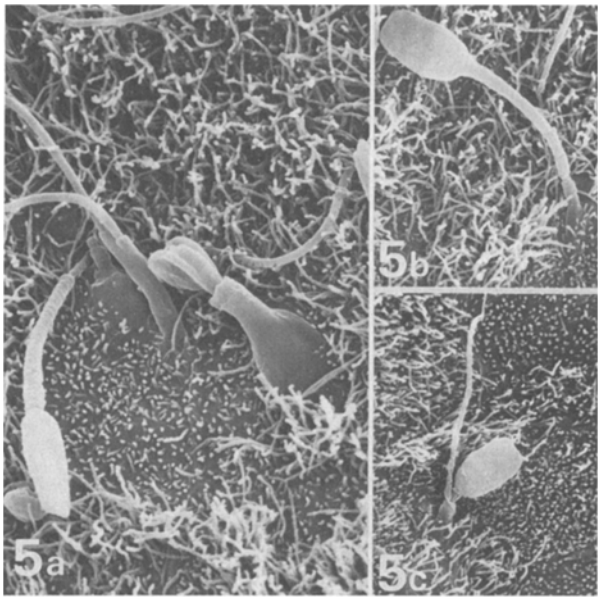

Figure 5. Scanning electron micrographs indicating various modes of spermiophagy by the epithelial cells. Many of the spermatozoa are taken up head-first(a), but others tail-first (b). Rarely they are taken up by the middle portion between head and tail (c). a, × 3600; b, × 3000; c, × 2800.

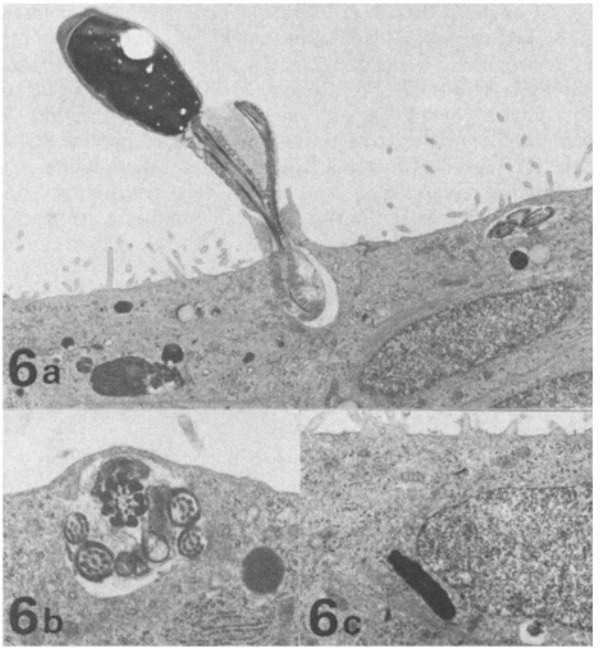

Figure 6. Transmission electron micrographs showing that spermatozoa taken up by phagocytosis are in various stages of digestion in the cytoplasm of the epithelial cells (for detailed explanation see text). a, × 4900; b, × 11,300; c, × 8200.

phagocytotic vacuoles probably by the action of lytic enzymes (fig. 6b) to become condensed masses finally (fig. 6c). In the cat, phagocytosis of spermatozoa by the epithelial cells is observed only in the terminal region of the vas deferens, while spermiophagy by luminal macrophages occurs more or less throughout the length of the vas deferens.

From the present study, the reason why there is epithelial spermiophagy in the terminal vas deferens is not clear, and the problem as to whether the epithelial cells ingest only damaged or abnormal spermatozoa or whether they can ingest even living ones also remaines unsolved. But it seems unlikely that the epithelial cells are merely engaged in selective removal of damaged or surplus spermatozoa[1], because our unpublished observations indicate that the epithelial cells, like luminal macrophages, are also capable of taking up latex beads injected into the cat vas deferens.

The epithelial cells may act to remove several kinds of foreign matter including degenerative spermatozoa in order to scavenge the lumen, though it is not obvious in what way they can discriminate foreign matter from non-foreign matter.

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Observations on the dynamics of argyrophilic nucleolar material in the nuclei of mice spermatids[1]

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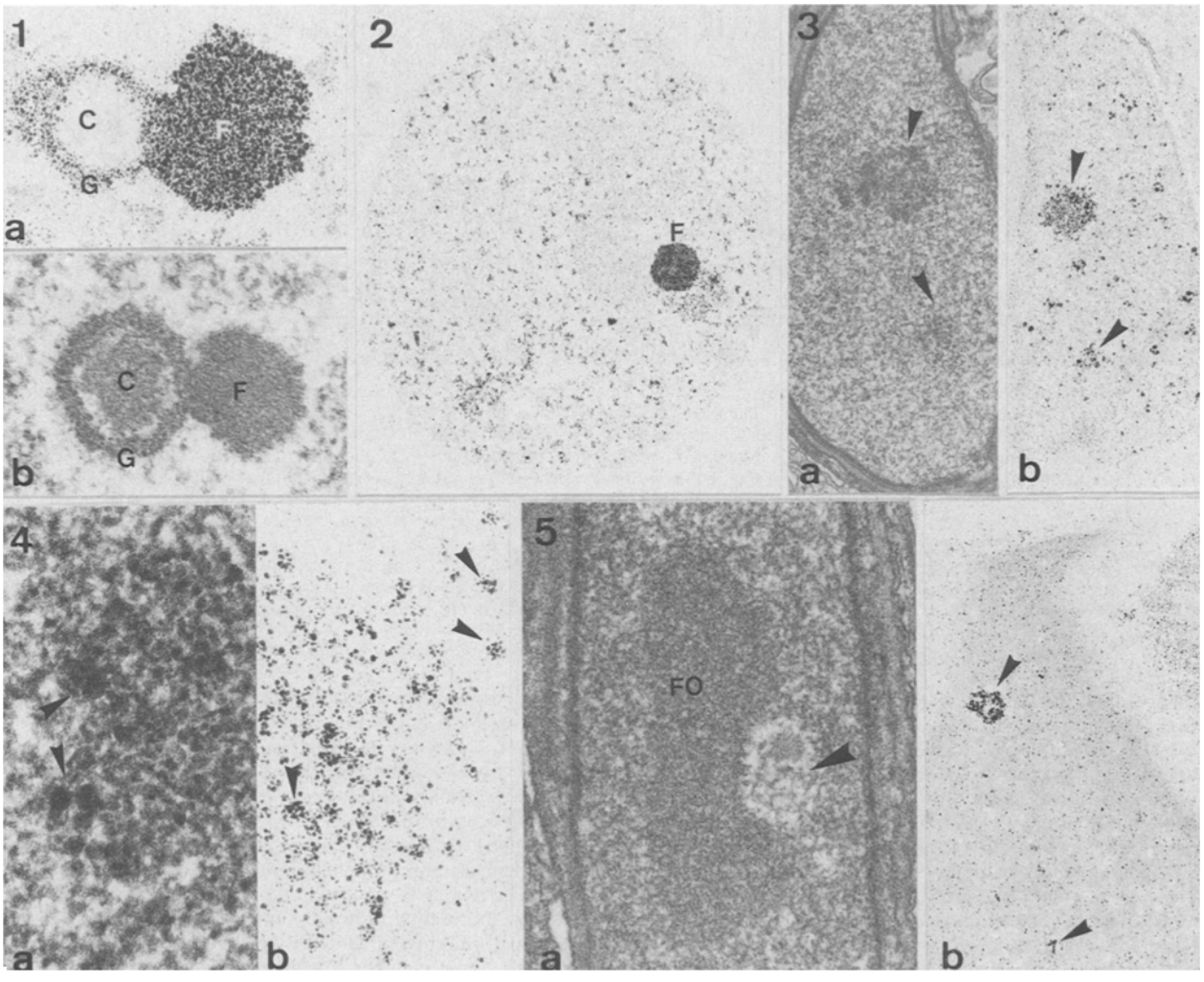
Summary. Applying a new silver staining technique it could be shown that in very early spermatids strong argyrophilia in nucleoli is confined to their granular and fibrillar components; fibrillar centers are devoid of silver. During subsequent developmental stages remnants of these nucleolar components are present in the form of intensively silver stained clusters of coiled fibers. As chromatin condensation proceeds, these fibrous structures decrease in size and density and are finally completely absent. The nucleus of the mature sperm contains only the space in which they formerly existed, now silver negative, as the so-called 'nuclear vacuole'.

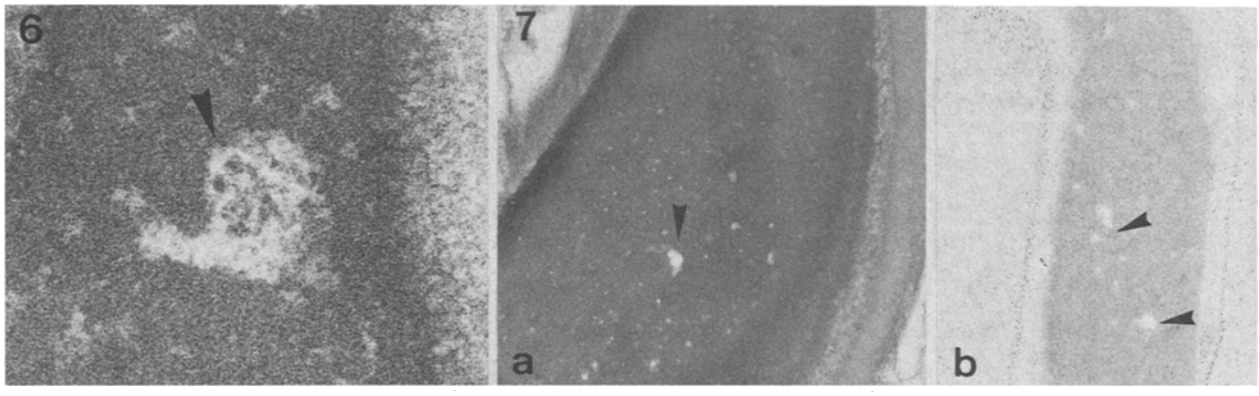
Key words. Mouse spermatids; nucleoli; silver staining technique.



Nuclear vacuoles; small, non-membrane bound cavities of irregular outline distributed at random through the condensed chromatin of mature spermatozoa, have been observed in various mammalian species including man[2–8]. In human spermatozoa they were interpreted as providing a means for eliminating gaseous metabolic waste from the nucleus[2]. Later on it was suggested that they are regulators against external osmotic gradients or are associated with the articulation mechanism[4,5]. At present, nuclear vacuoles are considered to be simply local defects left behind in the process of condensation of the chromatin[3,6–8]. More recently, Ohtomo[9] reported in guinea pig spermatids a connexion between the degeneration of nucleoli and the formation of nuclear vacuoles. Cytochemical proof for this is, however, lacking up to now. For several years silver staining techniques[10–14] have been used to identify specifically nucleolar organizer regions (NORs) in metaphase chromosomes and interphase nucleoli. It has been shown that these silver methods reveal specific nucleolar non-histone proteins[15–17]. Therefore, an examination of whether a relationship exists between nucleolar changes and the formation of nuclear vacuoles using an electronmicroscopic silver staining technique seemed to be indicated.

Material and method. The testes of 5 sexually mature mice were used. After killing by cervical dislocation they were removed and the seminiferous tubules were dissected and immediately fixed in 3% glutaraldehyde buffered with 0.1 M phosphate buffer pH 7.3 for 30 min at room temperature. Thereafter the pieces were rinsed for 20 min in 0.1 M phosphate buffer with 5% sucrose at pH 7.3 and dehydrated in a graded series up to 50% ethyl alcohol. The samples were then incubated in an ethanolic acriflavine PTA complex according to Chan-Curtis et al.[18] for 2 h at 4 °C. After dehydration in a graded series of acetones the pieces were embedded in Epon. The thin sections were then divided into 2 groups: one was contrasted with ethanolic uranyl acetate which produces a common staining pattern, the other was stained with silver according to the 1-step method of Howell and Black[19], whereby the staining procedure was modified for ultrathin sections as follows: the sections were stained floating on the mixture of colloidal developer and aqueous $AgNO_3$ in small glass dishes standing on a slide warmer which had been stabilized at 70 °C. After 30–60 sec, when the sections looked silvery, they were placed in deionized water and then mounted on copper grids. The silver sections were examined always uncontrasted. All sections were cut on a Reichert Om-U_2 and examined in a Jeol JEM 100S at 80 kV. The positive silver reaction was registered qualitatively by judging its relative strength. Since the staining procedure cannot be standardized, no attempts at quantitative evaluation were made.

Results and discussion. At the beginning of the Golgi-phase the spermatid nucleus displays the so-called 'padlock-like' nucleolus[20], where the 3 main nucleolar components are arranged in 2 intimately associated spheres; one represents exclusively the fibrillar component, but the other contains peripherally the granular component and in its innermost part the fibrillar centre (fig. 1b). Both the granular and the fibrillar component



Figures 1–7. Nucleolar aspects during spermatid development. Fig. 1a. Golgi phase showing a silver-impregnated 'padlock like' nucleolus with its three components: fibrillar component (F), granular component (G) and the silver negative fibrillar centre (C). Fig. 1b. Uranyl acetate staining. Both × 40,000. Fig. 2. In a more advanced stage the nucleus contains only the fibrillar component (F), which appears heavy silver stained. Besides, a few silver deposits are also seen on small aggregates distributed throughout the nucleoplasm. × 11,200. Fig. 3a. Elongation phase showing clusters of coiled fibers (arrowheads). Uranyl acetate staining. Fig. 3b. These fibrous structures are strongly argyrophilic (arrowheads). Both 11,200. Fig. 4a. Higher magnification to illustrate the structure of these clusters. The sometimes granular appearance of these structures seems to derive from the coiling of the fibrils (arrowheads). 4b. When stained with silver, the granular (arrowheads) as well as the fibrous structures are intensely covered with silver grains. Both × 84,000. Fig. 5a. A later stage shows the intimate connexion of the fibrous structures (arrowhead) to the centrally occurring focus (FO) of condensing chromatin. × 40,000. Fig. 5b. Silver staining reveals the strong argyrophilia of these structures (arrowheads). × 20,000. Fig. 6. Shows only few fibers in a cavity embedded in the now nearly fully condensed chromatin. Uranyl acetate. × 84,000. Fig. 7a. The mature sperm contains only the cavity in its homogeneous nucleus, now devoid of any fibrous material (arrowhead). Uranyl acetate. × 20,000. Fig. 7b. Silver staining confirms that these cavities (arrowheads) are indeed silver-negative. × 14,000.

show distinct covering with silver precipitates when the new silver staining method is applied (fig. 1a). This is in agreement with the findings of Krimer and Esponda[20], who interpreted the special arrangement of the nucleolar parts as nucleolar segregation, indicating a decrease of RNA synthesis. Light microscopic silver staining also confirmed that among the vertebrates examined so far (they range from fish to mammals) there exists a similar staining pattern of NORs in the various stages of spermatogenesis[21–26]. Thus silver-stained NORs are present in early spermatids, but they disappear definitively during the elongation of the spermatid. The argyrophilia of the NORs in this developmental period is considered as an expression of postmeiotic transcriptional activity of the rRNA genes[21–25].

In a slightly later phase, that sphere representing both the granular component and the fibrillar centre disappears, so that the nucleus contains in the early cap-phase only the fibrillar component, unaltered in its structure and silver staining reaction (fig. 2). Among the nucleolar silver-stainable structures, the fibrillar component is supposed to contain transcriptionally active ribosomal cistrons, while the fibrillar center itself seems to be transcriptionally inactive[27–30].

During nuclear elongation the remaining silver-positive nucleolar part begins to disintegrate. Only clusters of densely-coiled fibers arranged in more-or-less compactly convoluted spiremes appear now in the nucleoplasm. Silver staining shows them to be intensively argyrophilic (figs 3a, b; 4a, b). As chromatin condensation proceeds these fibrous structures, intimately attached to the centrally-occurring focus of condensing chromatin[31], decrease more and more in size and density, but still appear to be intensively covered with silver precipitates (figs 5a, b). At last only a few loosely arranged fibers remain in small cavities which are embedded in the nearly fully condensed chromatin (fig. 6), revealing a decreasing argyrophilia. As spermatid development continues, the silver-positive fibers will disappear completely, so that the mature sperm only contains the space in which they formerly existed and which is now called the 'nuclear vacuole' (figs. 7a, b).

These data demonstrate that from the beginning of nuclear elongation until late maturation steps there indeed exists a fibrous material, which, on the basis of its strong argyrophilia, very probably originates from the nucleolus of earlier stages, as was proposed previously by Ohtomo[9]. Several studies in recent years have shown that acidic proteins are responsible for the silver staining of nucleolar components[11, 15–17]. The silver-positive fibrillar component has been imagined as containing proteins which are in some way connected with the transcriptional activity of the NORs[32].

Since, however, fibrillar centres have also been reported to be connected to transcriptionally inactive NORs[27–30], a direct correlation between silver staining and nucleolar transcriptional activity has recently been questioned, and the nucleolar silver staining is interpreted as detecting those proteins that are responsible for decondensation of nucleolar chromatin, a state which can but must not indicate NOR activity[33]. This could explain the high argyrophilia of the fibrous residual nucleolar structures in the results presented here, which remain in a decondensed form up to the latest stages. The total elimination of this material occurs with the period of complete chromatin condensation, when it has become tightly packed by the formation of disulfide bonds[34], a state which is apparently responsible for the persistence of these cavities.

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The bisphosphonates HEBP and AHPrBP but not AHBP inhibit mineral mobilization and lysosomal enzyme release from mouse calvarial bones in tissue culture[1]

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Summary. Mice injected with DSP-4 [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine], a selective noradrenergic neurotoxin, had marked depletions of central noradrenaline and an attenuated post-decapitation reflex. DSP-4-treated mice exhibited an increased sensitivity to the α_2-adrenoceptor agonist clonidine as measured by inhibition of the pinna reflex, but normal sensitivity as measured by hypothermia. This differential sensitivity may reflect the presence of supersensitive postsynaptic α_2-adrenoceptors in some, but not all, CNS regions after DSP-4 treatment.
Key words. DSP-4; noradrenaline depletion; α_2-adrenoceptor; clonidine; pinna reflex; oesophageal temperature; mouse.

A peripheral injection of DSP-4 [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine] causes depletion of central noradrenaline (NA) in rodents[1]. This depletion has been shown by recent neurochemical and behavioral experiments to induce central α_2-adrenoceptor supersensitivity[2,3].

In the present study, we investigated whether or not the α_2-adrenoceptor influence on 2 physiologic variables, pinna reflex and body temperature, would be altered in mice by DSP-4. Both variables are sensitive to α_2-adrenoceptor activation by the α_2-agonist clonidine: the pinna reflex is inhibited[4] and body temperature is decreased[5,6]. The post-decapitation reflex (PDR) was also assessed in DSP-4 mice since recent reports indicate that this reflex is abolished in DSP-4 rats[2,7-9].

Materials and methods. Male mice (CD-1, Charles River, Manston, Kent, England), weighing 20–24 g, were injected i.p. with DSP-4 hydrochloride (75 mg/kg) or the control solution (0.9% saline) in a volume of 0.01 ml/g. They were then maintained under standard laboratory conditions (20 ± 1 °C, 12-h light-dark cycles, free access to food and water) for 10 days. At 10 days, the control and DSP-4 mice were divided into 2 groups and used in experiments.

The first group of mice was used to assess the PDR and the magnitude of NA depletion in several CNS regions (e.g., neocortex, hippocampal formation, hypothalamus, cerebellum, and spinal cord). For determining changes in the PDR, the latency to convulse and duration of the tonic-clonic convulsions were measured. The NA concentrations were measured using a fluorometric method[10]. Results were analyzed using the t-statistic for group means. The minimal level of significance was $p \leq 0.01$ (2-tail criterion).

The second group of mice was administered clonidine hydrochloride to determine possible alterations in the sensitivity of the pinna reflex and oesophageal temperature. For assessment of the pinna reflex, groups of 10 mice were injected with different doses of clonidine. The presence or absence of the reflex was determined 20 min later by stimulating each auditory meatus with a fine wire. If stimulation of either ear elicited a rapid twitch of the head, the pinna reflex was deemed present. The percentage inhibition of this reflex, based on the ratio of the number of animals showing reflex inhibition to the number of animals tested, was calculated for each dose of clonidine; ED_{50} values and 95% confidence intervals (C.I.) were subsequently obtained by probit analysis[11], and lines and ED_{50} values were compared[11]. Control mice were also tested with p-aminoclonidine, a peripherally-acting α_2-agonist[3]. For measurement of oesophageal temperature, groups of 5 mice were placed in observation cages mounted on a raised grid which facilitated air circulation (ambient temperature = 22 ± 1 °C). Saline or clonidine was injected, and oesophageal temperature was taken after 30 min using a digital thermometer (Type 3009 Ni-Cr/Ni-Al, Comark Electronics Ltd., Rustington, Sussex, England). (Preliminary experiments indicated that the hypothermic effect of clonidine was maximal at the 30-min timepoint.) Results were analyzed using 2-way analysis of variance followed by Dunnett's multiple comparison statistic (2-tail criterion, $p \leq 0.01$). Control mice were also tested with p-aminoclonidine.

Results. DSP-4 treatment produced marked reductions of NA ($\leq 25\%$ of control NA remaining) in all CNS regions examined (table), the exception being the hypothalamus in which there was only a trend towards NA depletion (77% of control NA remaining). The assessment of the PDR indicated significant differences ($p \leq 0.01$) between control and DSP-4 mice as regards both latency ($\bar{X} \pm SE$, $n = 8$: 6.1 ± 0.7 and 14.5 ± 1.7 sec, respectively) and duration (15.8 ± 0.9 and 9.0 ± 0.7 sec, respectively).

Clonidine inhibited the pinna reflex in both control and DSP-4 mice (fig. 1); however, the ED_{50} for DSP-4 animals was ~2