J. Anat. (1968), **102**, 3, pp. 501–509
With 11 figures
Printed in Great Britain

Sertoli cells as phagocytes: an electron microscopic study

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INTRODUCTION

When certain dyes or foreign particles are injected through the rete testis into the seminiferous tubules, they may be seen with the light microscope 24 h later in the cytoplasm of the Sertoli cells (Clegg & Macmillan, 1965). This observation is consistent with the ability of Sertoli cells to phagocytose germ cells under various abnormal circumstances (Lacy, 1962).

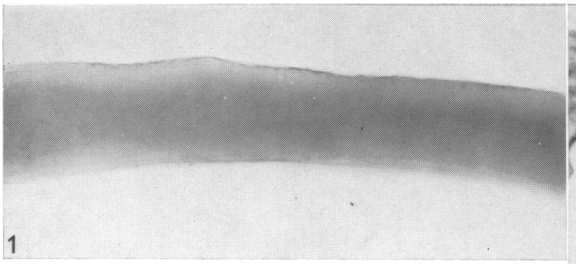
The present report describes an investigation into the phagocytic properties of the Sertoli cell, using the electron microscope. At first carbon suspensions were injected into the tubules, but it proved difficult to identify the earliest stages of ingestion. A proprietary preparation of saccharated iron oxide ('Ferrivenin'–Benger) was found to contain more suitable particles. The term Ferrivenin will be used for the sake of brevity.

MATERIALS AND METHODS

Young adult male albino rats of the Wistar strain were used throughout the experiment. Two were used as controls; in six others Ferrivenin was injected through the rete testis into the seminiferous tubules by the technique previously described by Clegg & Macmillan (1965). In three animals the ductuli efferentes were ligated immediately after injection. In four animals carbon particles (indian ink, Gunther Wagner 1438/A) were injected as above. Animals were killed 2, 4 and 24 h after injection. After the testis was removed, the fibrous capsule was slit and the whole testis was fixed in 3 % glutaraldehyde in phosphate buffer for 3 h. Thereafter it was washed in buffer (containing 10 % sucrose), and dissected under a binocular microscope. Only a few seminiferous tubules were injected in any one specimen. Those were identified by the presence of brown ferrivenin in the lumen. Some injected tubules were embedded in paraffin, and sections were stained by Perl's Prussian blue technique. Others were post-fixed for 2 h in 2 % osmium tetroxide, dehydrated in graded alcohols, and embedded in Araldite. Thin sections were cut on a Huxley ultramicrotome, and were then examined in an AEI EM 6B, or a Philips EM200 electron microscope.

During the course of the study changes were observed in Sertoli cell mitochondria, which could have been ascribed to focal accumulation of calcium. It was therefore necessary to attempt to identify calcium histochemically, and specimens for light microscopy were stained by the von Kossa method for calcium ions. Other specimens for electron microscopy were fixed in glutaraldehyde, and then decalcified for 24 h in 5 % formic acid. Some were then post-fixed in osmium tetroxide as above, while others were embedded in Araldite without post-osmication.

It also became necessary to exclude the possibility that changes seen were due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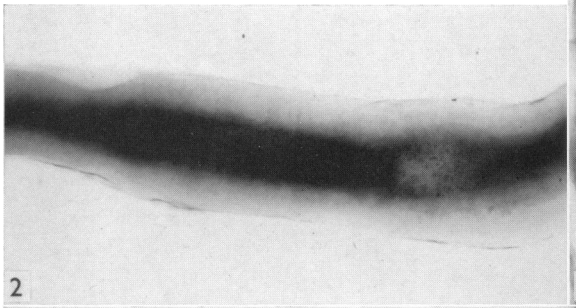
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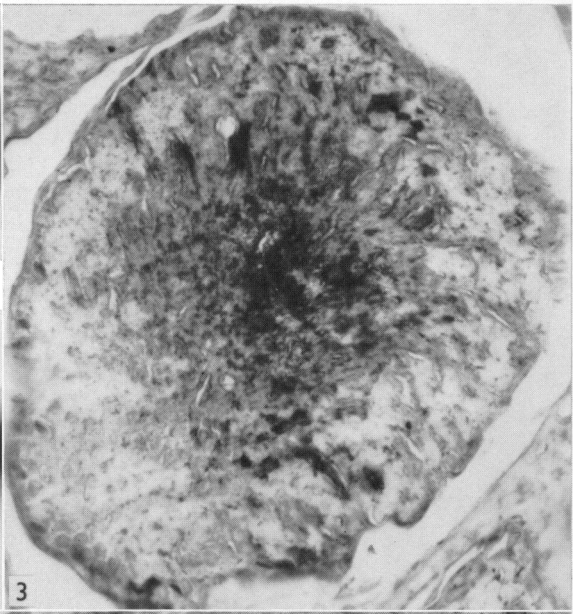
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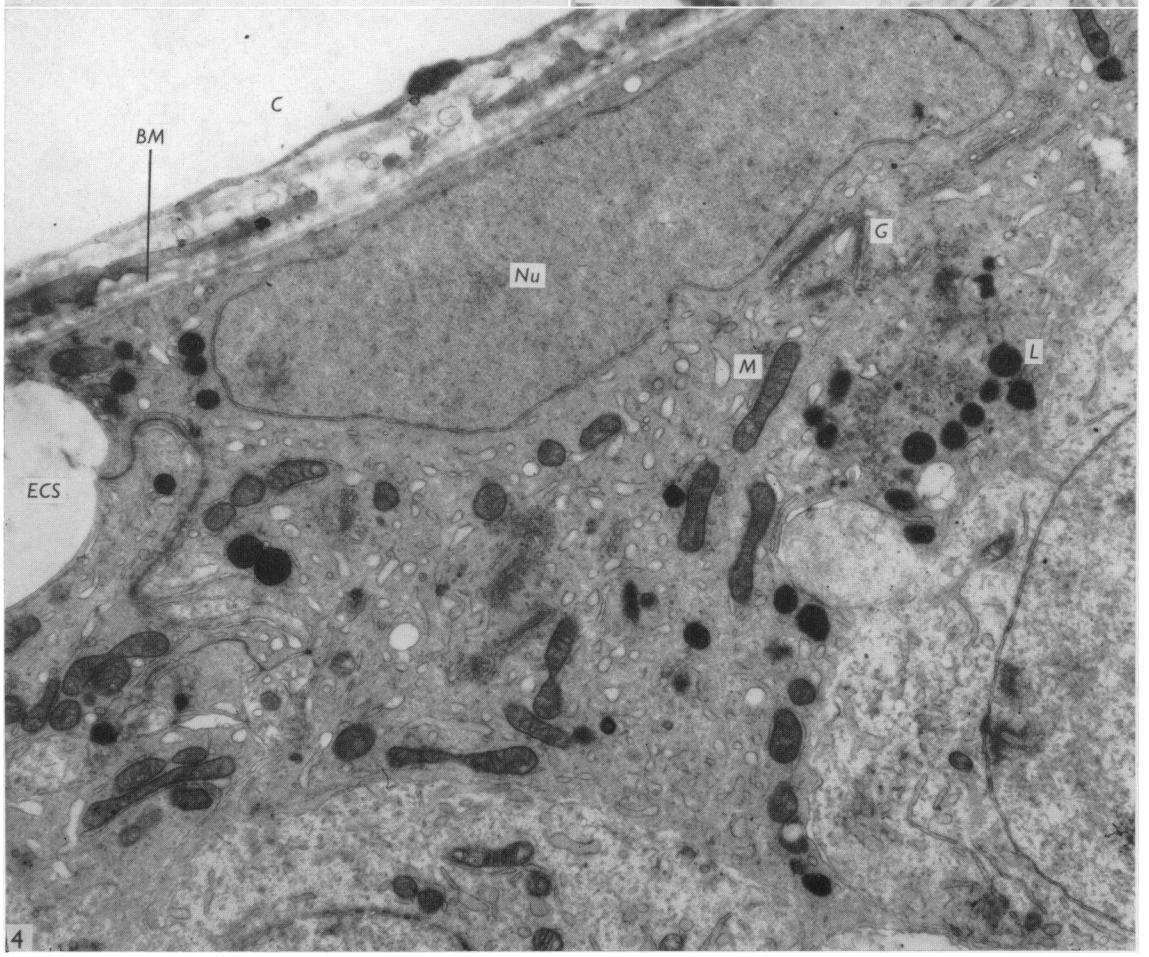

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entirely to non-specific degeneration, and a biological situation was sought in which for some reason Sertoli cells were degenerating. Extensive degeneration of Sertoli cells had been noted during an independent study of the effects of the administration of cadmium chloride on the testis (Clegg & Carr, 1967). The specimens used were taken from animals 48 h after a subcutaneous dose of 0·5 mg/100 g body weight of calcium chloride.

RESULTS

Ligation of the efferent ducts did not materially affect the results; the results of experiments with and without ligation will therefore be described together.

Seminiferous tubules injected with Ferrivenin were brown in colour when viewed with the dissecting microscope (Figs. 1 and 2) and gave a positive Prussian blue reaction (Fig. 3).

A typical Sertoli cell from a control animal is illustrated in Fig. 4. The cell was commonly pyramidal in shape, with a thin process extending down to the lumen of the tubule. The mitochondria were rod-like and contained no notable inclusion. Intramitochondrial dense bodies were not prominent. The cytoplasmic matrix was notably more electron dense than that of adjacent spermatogonia. Descriptions of the Sertoli cell have been published by Bawa (1963); Gardner & Holyoke (1963). Sertoli cell cytoplasm did not separate adjacent germ cells at all points as Vilar, Perez del Cerro and Mancini (1962) believe to be the case in the rabbit.

When spread on a formvar film Ferrivenin was seen to be composed of particles about 30 Å in diameter, with a considerable number of filaments of about the same diameter, but 200–300 Å long (Fig. 6).

Two hours after injection Ferrivenin was seen in the lumina of seminiferous tubules, and in the cytoplasm of Sertoli cells. Particles were seen aggregated along the luminal surface of the cells, and in large vesicles, where again they often lay against the bounding membrane (Fig. 5). In both of these sites the particles were very similar to those seen when Ferrivenin was examined on a film. The diameter of both filaments and particles tended to be slightly greater (about 38 Å), but this difference is within the range of experimental error. It could also be related to the possible presence of an additional coat of protein or polysaccharide. The distal border of the Sertoli cell was irregular, and showed flaps and projections (Fig. 5).

Four hours after injection, many cells contained Ferrivenin in phagocytic vesicles, and superficially similar though not necessarily identical particles in mitochondria (Fig. 7). Other Sertoli cells showed considerable swelling of intracellular membrane

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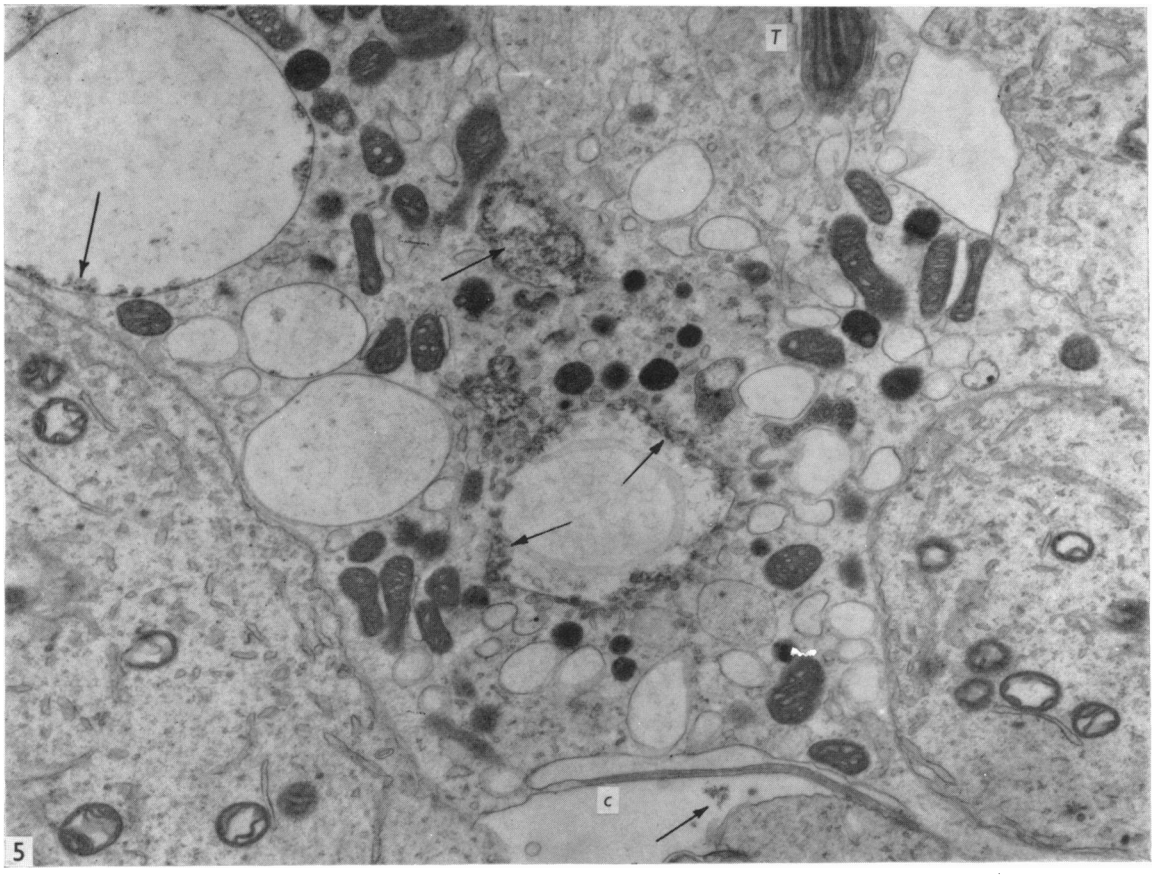
Fig. 1. Control seminiferous tubule, separated from testis; unstained whole mount. × 12.

Fig. 2. Seminiferous tubule 4 h after intratubular injection of Ferrivenin. The dense material is injected Ferrivenin. × 12.

Fig. 3. Cross-section of seminiferous tubule, 4 h after injection with Ferrivenin; the lumen contains much iron, stained by the Prussian blue reaction. × 40.

Fig. 4. Electron micrograph (× 8000) of the outer border of a seminiferous tubule from a control preparation. The outer capsule *C* is composed of several layers of fibroblast cytoplasm with collagen and basement membrane material (*BM*). The nucleus *Nu* of a Sertoli cell is surrounded by cytoplasm containing numerous aggregates of Golgi membranes (*G*), mitochondria (*M*) and dense bodies thought to be lysosomes (*L*). Extracellular spaces (*ECS*) are frequently seen between adjacent Sertoli cells and the basement membrane of the capsule.



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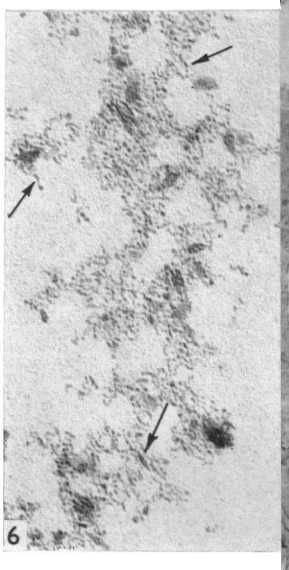

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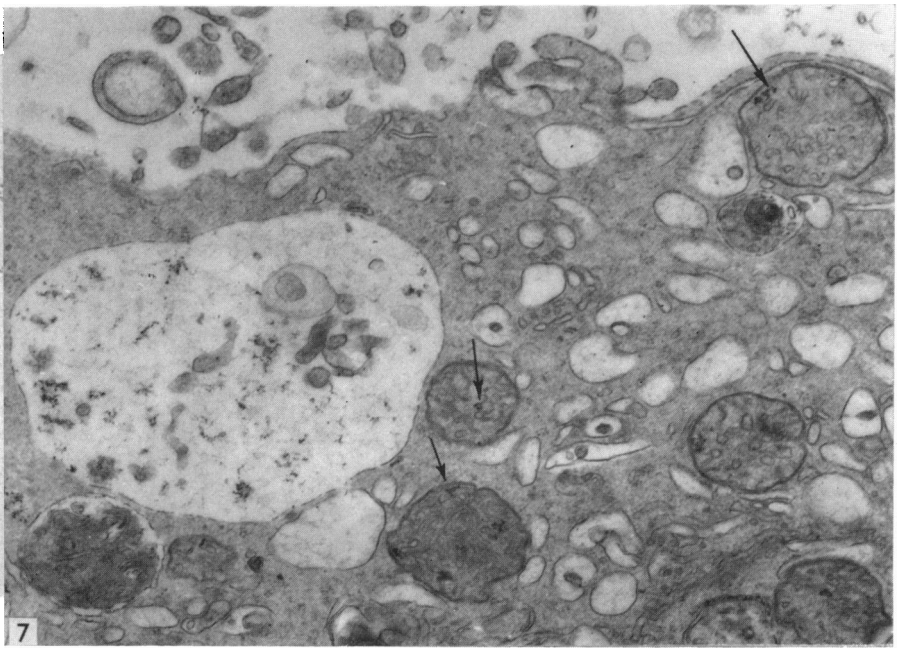

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systems, probably degenerative in nature. Such cells contained clumps of electron dense granules in their mitochondria (Fig. 8). Some such clumps were found below the surface membrane of mitochondria (Fig. 9). Sometimes clumps were seen within a vesicular structure which probably represented a cross-section of a tubular mitochondrial crista (Fig. 10) (i.e. between the membranes of a mitochondrial crista). These clumps measured 0·1 to 0·2 μm in overall diameter, and in favourable specimens were seen to be composed of particles about 40 Å (Fig. 11) in diameter. A few particles with the characteristic tetrad structure of ferritin (Farrant, 1954) and about 60 Å in diameter were seen in the cytoplasm.

Twenty-four hours after injection of Ferrivenin there was extensive necrosis of all components of the seminiferous tubule. Von Kossa's technique did not demonstrate calcium in specimens 4 and 24 h after injection, nor did decalcification for 24 h remove the intramitochondrial dense bodies from two tubules which 4 h previously had been injected with Ferrivenin. No intramitochondrial dense bodies of the type described were seen in Sertoli cells from animals treated with cadmium chloride, though these cells showed gross degeneration and mitochondrial swelling. No intramitochondrial dense bodies were seen in tubules which had been injected with carbon.

DISCUSSION

These findings confirm the previous report (Clegg & Macmillan, 1965) that Sertoli cells phagocytose foreign particles injected into the seminiferous tubules; this process occurs more rapidly than was demonstrated with the light microscope by these authors. The Sertoli cells ingest particles in a membranous vacuole, just as pulmonary macrophages do (Karrer, 1960).

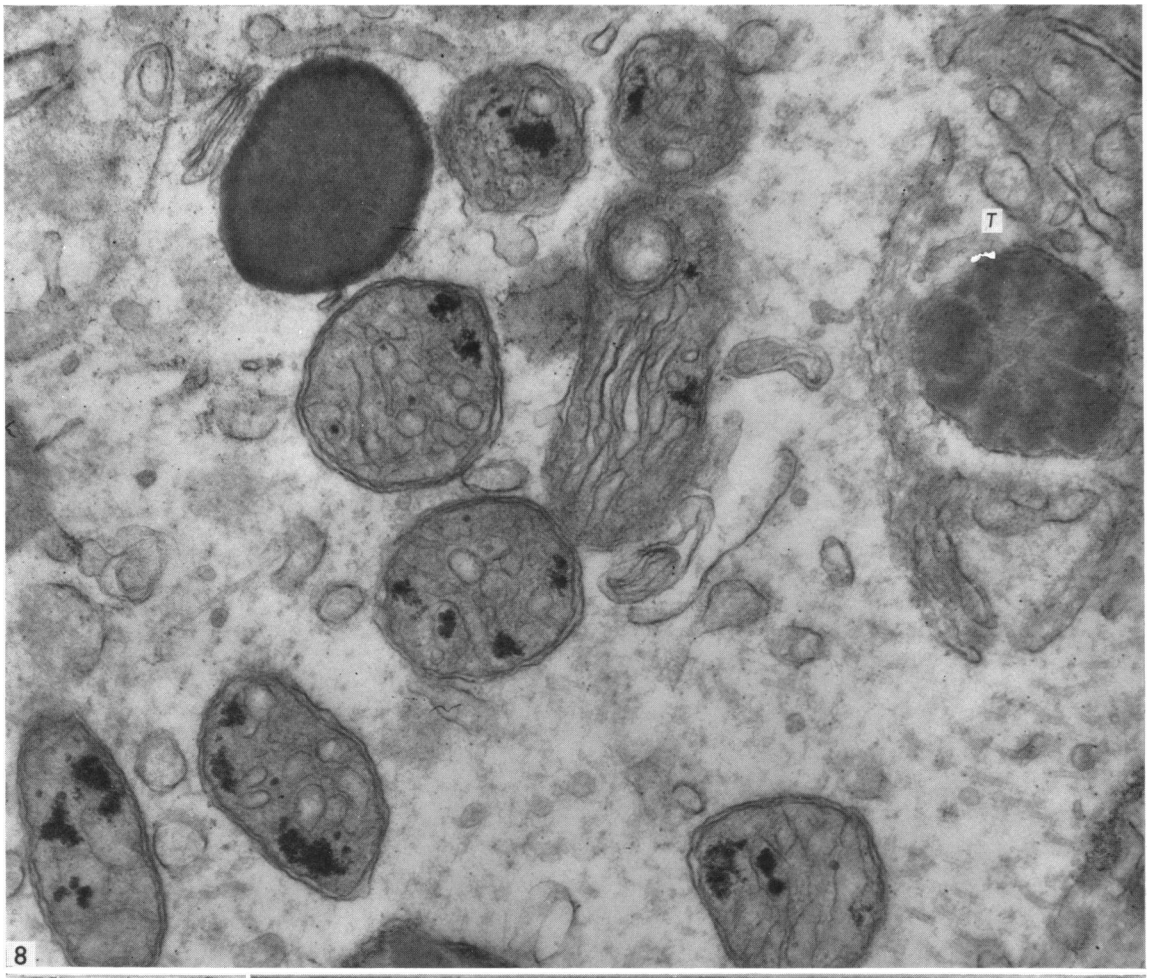
It is notable that as compared with the adjacent Sertoli cells, very few particles indeed were present in germ cells. Thus the Sertoli cell seems to have a specific phagocytic function, just as do true reticulo-endothelial cells elsewhere; it seems pertinent therefore to consider the relationship between the Sertoli cell and the reticulo-endothelial system. The topic has been briefly discussed previously (Oettle & Harrison, 1952; Clegg & Macmillan, 1965). Like the cells of the reticulo-endothelial system, the Sertoli cell is morphologically plastic (Rolshoven, 1940; Leblond & Clermont, 1952) and ingests foreign material avidly. It also possesses a high content of acid hydrolytic enzymes (Niemi, Harkoven & Kokko, 1962; Niemi & Kormano, 1965).

The original definition of the reticulo-endothelial system (Aschoff, 1924) included only cells which avidly took up foreign material introduced directly into the blood stream. This definition is based on a slightly artificial experimental procedure and is perhaps rather rigid, in that it excludes cells which may have rather similar powers, but which by topographical accident may not be in sufficiently close relation to the

Fig. 5. Sertoli cell 2 h after Ferrivenin injection. Note sperm tail (*T*), spermatids at the right and left of the figure, cytoplasmic flap (*c*), Ferrivenin at edge of cell and within it. (Arrowed.) × 10000.

Fig. 6. Ferrivenin on a formvar film, stained with lead citrate. Note the fine granules, and the filaments. (Arrowed.) × 200000.

Fig. 7. Sertoli cell cytoplasm 4 h after injection of Ferrivenin, showing aggregates of Ferrivenin in large vacuole, or invagination. Small dense bodies are present in adjacent mitochondria. (Arrowed.) × 25000.



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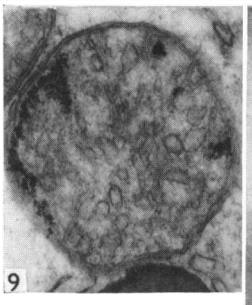

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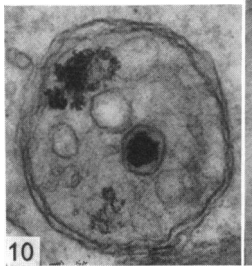

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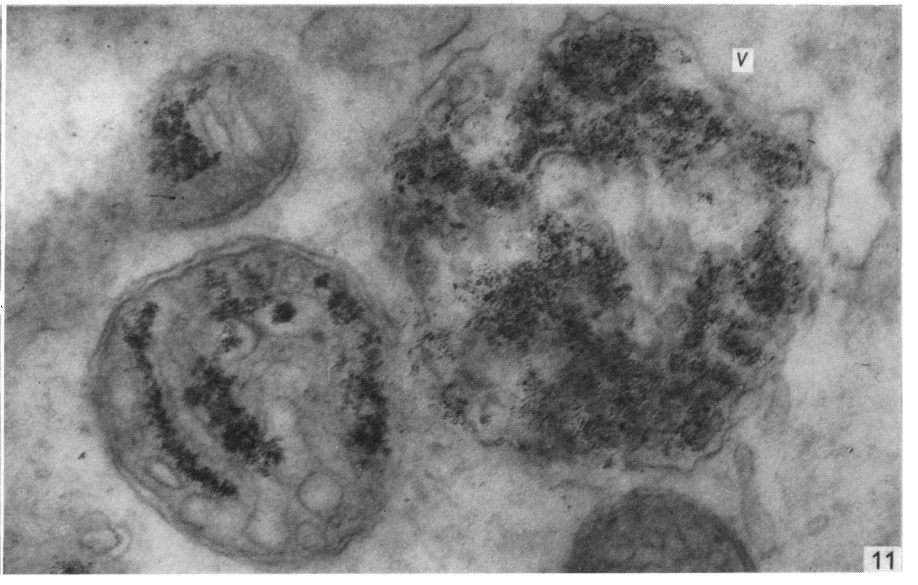

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circulation to manifest their properties. From the functional point of view, the Sertoli cells appear to have a similar function in the germinal epithelium, as reticulo-endothelial cells do in a less restricted milieu; they remove dead cells and foreign materials from their surroundings.

The significance of the striking intramitochondrial particles which appear after injection of ferrivenin is not certain. They do not appear after injections of carbon, nor when the Sertoli cells are degenerating after systemic cadmium administration. Two possibilities will be considered: that they are formed of calcium salts; or that they are formed of iron salts. In either case, the metal ions concerned may be deposited in the normal intramitochondrial granules.

Electron dense granules are commonly found in the mitochondria of many animal cells. When isolated mitochondria are exposed *in vitro* to high concentrations of divalent ions (Peachey, 1964) dense masses appear which were believed to represent enlargements of the normal electron dense granules, due to loading with divalent ions. This evidence has been adduced to support the hypothesis that these granules play a part in the normal intracellular metabolism of divalent ions. Rather similar bodies which develop into apatite-like crystals have been shown to occur in the mitochondria of renal tubular cells of animals subjected to calcium loading or treated with parathormone (Caulfield & Schrag, 1964). Similar bodies occur in myocardial cells poisoned in various ways (D'Agostino, 1963, 1964). These again were thought to contain an excess of calcium. It seems likely then that dense bodies containing calcium appear in the mitochondria of cells loaded with divalent ions, or suffering from severe ionic imbalance in advanced cellular degeneration.

Such calcium-containing bodies however are characteristically composed of small granules forming an apparently hollow shell. Rather similar dense bodies appear in autolytic liver cells (Trump, Goldblatt & Stowell, 1965), but in this instance the normal intramitochondrial dense bodies had previously disappeared. The dense bodies seen in the present experiments were probably due not to calcium loading because (1) histochemical tests for calcium were negative, (2) they could not be removed by formic acid, (3) they did not appear when the cells were degenerating after cadmium treatment.

The second possibility is that the intramitochondrial bodies in the present experiment represent an aggregation within the mitochondria of an iron compound derived from the injected Ferrivenin. The particles are similar in size and electron density to those of Ferrivenin. The electron density of the Ferrivenin (saccharated iron oxide)

Fig. 8. Sertoli cell cytoplasm 4 h after Ferrivenin injection. Note sperm tail (*T*). Dense bodies are present in the mitochondria. × 63000.

Fig. 9. As Fig. 8. Dense bodies arranged round the edge of a mitochondrion below the surface membranes. Considered in three dimensions this must represent a partial shell of material. × 63000.

Fig. 10. As Fig. 8. There is a dense body within a mitochondrial crista. × 63000.

Fig. 11. Sertoli cell 4 h after Ferrivenin injection. Ferrivenin particles are present in a large phagocytic vacuole (*V*). Dense bodies are present in the adjacent mitochondrion, arranged in one area between the cristae. Filaments were identified in the vacuole, but not in the mitochondrion; but otherwise the particles are of similar size and electron density. A few ferritin particles are present in the cytoplasm. × 75000.

particles had been ascribed (Richter, 1959) to the presence of a molecule of hydrated ferric oxide whose diameter is about 30 Å. Such material could presumably diffuse from the phagocytic vacuole to the mitochondria. Numerous particles were on occasion found just below the surface membrane of the mitochondria but no morphological evidence of the mode of ingress of the particles was found. The intramitochondrial dense bodies do resemble in structure and in the size of their subunits the intramitochondrial granules seen by Bessis & Breton-Gorius (1957) in erythroblasts. These they considered to be deposits of non-ferritin iron compounds. The intramitochondrial deposits which appear in Sertoli cell mitochondria after Ferrivenin injection may thus be composed of either calcium compounds, resulting from non-specific cell degeneration or of iron compounds, derived from the injected material. The evidence for either speculation is somewhat tenuous but on the whole the latter appears more likely.

The few ferritin particles seen may be a feature of the normal Sertoli cell, though no such particles were identified in control cells. Alternatively, Sertoli cells, like macrophages (Muir & Golberg, 1961) may be able to form ferritin from administered iron compounds.

SUMMARY

Ferrivenin (saccharated iron oxide) has been injected into the seminiferous tubules of rats, and its uptake by the Sertoli cells studied with the electron microscope. It is ingested by the Sertoli cells, and appears in intracellular vacuoles. Dense particles appear in Sertoli cell mitochondria. The possibility is discussed that these may contain ingested iron compounds.

This work was made possible by grants to the Department from the S.R.C., M.R.C., Nuffield Foundation and Unilever Ltd. We thank Professor R. Barer for his criticism, and Messrs P. Garlick and L. Murgatroyd for technical assistance.

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