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RNA Synthetic Activities of Sertoli Cells in the Mouse Testis

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Electron microscope autoradiographic studies of RNA synthesis in Sertoli cells of the adult mouse, using [^{3}H]uridine as a precursor, show a random distribution of grains in the nucleoplasmic area and a localized product on the nucleolar fibrillar component at early labeling times (15 min). The condensed chromatin bodies associated with the nucleolus seem to have a nucleolar organizing function. At later labeling times (3 h) certain interchromatin granules occurring in clusters in the nucleoplasm become partially labeled. After 7–8 days, the disappearance of label from Sertoli cells, contrasting with the longer persistence in spermatocytes, suggests a rapid turnover of RNA in Sertoli cells.

Differential labeling patterns and structural changes in Sertoli cells cytoplasm are related to variations in functional activities presumably required for normal spermatogenesis and/or spermiogenesis.

Sertoli cells are found in the seminiferous epithelium of several mammalian species. Though various functions have been attributed to these cells, it is generally accepted that Sertoli cells provide mechanical support for germinal cells. Moreover, they participate in the release of sperm into the lumen of the seminiferous tubules (Vitale-Calpe and Burgos, 1970), dispose of cell debris by a phagocytic mechanism (Clegg and Macmillan, 1965; Kierszenbaum, 1970), and play an important role in the transfer of nutrients and metabolites between the intertubular space and the lumen of the tubules, acting as "bridge" cells (Vilar *et al.*, 1962). It is also known that Sertoli cells constitute a unique stable cell population in the seminiferous epithelium. Whereas spermatogonia, spermatocytes, and spermatids are involved in mitotic, meiotic, and differentiation processes, respectively, Sertoli cells remain in constant interphase.

Earlier autoradiographic studies at the light microscopy level have shown a high RNA synthetic rate in Sertoli cell nuclei (Monesi, 1965; Utakoji, 1966). A more recent study using electron microscope autoradiographic techniques (Kierszenbaum and Tres, 1974) has demonstrated a differential RNA synthesis in autosomal bivalents in spermatocytes in meiotic prophase as a consequence of a suggested regulatory mechanism. Since spermatocytes and spermatids are closely associated with cytoplasmic extensions of Sertoli cells and are separated from the tubular wall by these cells and by spermatogonia, it is possible to assume that, beside the transport of nutrients and metabolites, some kind of regulation may be mediated through Sertoli cells during spermatogenesis and spermiogenesis.

In this paper, the incorporation of tritiated uridine into mouse Sertoli cells at different labeling times has been studied

[1] A term introduced by James (1948) and used by Salpeter and Bachmann and Wisse and Tates to a process involving the treatment of an exposed emulsion with a solution of gold chloride. Metallic gold is thus deposited on the latent image before development and leads to an enhancement of the sensitivity and of the size of the final developed grain.

with electron microscope autoradiographic techniques. Features of DNA transcription at nucleolar and extranucleolar loci and differences in cytoplasmic labeling are linked to hormonal mechanisms required for normal gametogenesis.

MATERIALS AND METHODS

Adult male Swiss mice, 30–45 days old, were injected with [5,6-$^3H_2$]uridine (sp act 42.4 Ci/m*M*, New England Nuclear, Boston, Mass.) directly into both testes under the tunica albuginea at a dose of 10 μCi per testis, in 0.05 ml of sterile aqueous solution. Reasons for selecting this injection route are discussed elsewhere (Kierszenbaum and Tres, 1974). Testes were removed under anesthesia at the following postinjection intervals: 5, 15, 30, and 60 min; 3 and 24 h; 7, 8, 10, and 12 days. Control animals were injected with the solvent of the labeled precursor.

For *electron microscope autoradiography*, small pieces of testes were fixed in 2.5% glutaraldehyde in 0.1 *M* phosphate buffer (pH 6.9) for 2 h at 4°C. The tissues were rinsed for about 9 h in three changes of cold phosphate buffer in order to remove soluble RNA precursor molecules. Postfixation was carried out in 2% osmium tetroxide. The specimens were dehydrated with alcohol and embedded in Maraglas according to the usual procedure. Sections displaying light gold interference colors were obtained on a Porter-Blum ultramicrotome, using glass knives.

The flat substrate method for electron microscope autoradiography was used according to Salpeter and Bachmann (1972), as modified by Wisse and Tates (1968). The slides with the sections were stained and then dipped in Ilford L4 emulsion in adequate dilution to yield a monolayer of silver halide crystals. After exposure for 25 days to 5 mo, the slides were developed with the gold latensification[1] Elon ascorbic acid developing technique (EAA) or with Microdol-X and then fixed for 1 min in 20% sodium thiosulfate or Kodak Fixer. The slides were carefully rinsed and the sections detached from the slide. Copper grids (150 mesh) were placed over the sections. The grids were examined with a JEM 100B electron microscope operated at 80 kv.

For *autoradiography of thick sections*, sections of about 0.5 μm thickness were deposited on a glass slide and dipped in Kodak NTB3 emulsion. After exposure for 10–20 days at 4°C, the preparations were developed in Kodak D-19 for 30 sec and fixed in Kodak Fixer for 2 min. Once rinsed and air-dried, the sections were stained through the emulsion with 1% toluidine blue in 1% sodium borate, washed in distilled water and mounted under cover slips.

For *quantitative analysis* of the results, the distribution of the developed grains was analyzed according to the principles, determinations, and procedures described by Salpeter and Bachmann (1972). The grain distribution over *relative* surface areas (10 μm^2) of the cytoplasm, nucleoplasm (nucleolus not included), nucleolus, and region of interchromatin granules were scored in 30 autoradiograms of different Sertoli cells printed at magnification ×7500. The area was taken by superimposing over the autoradiograms a lattice with regularly spaced points. The grain density distribution over each compartment was counted and divided by the number of lattice points found in the same compartment.

The distribution of radioactivity in the cytoplasm of 15 vacuolated Sertoli cells was evaluated according to the *radial grain distribution method* (Salpeter and Bachmann, 1972). The purpose of this quantitative evaluation was to determine whether the cytoplasmic grains are due to radiation which spread from the cytoplasmic matrix or from the dilated endoplasmic reticulum cisternae. The determination of the midpoint of each silver grain was done regarding the *half distance* value. Half distance, as defined by Salpeter and Bachmann (1972), is the distance from a line source in a autoradiographic preparation within which half of the developed grains fall. Experimental values for half distance have been obtained (Salpeter and Bachmann, 1972) and for the method used in this study the corresponding value should be 160 nm. The midpoint of each silver grain was taken by placing over the autoradiograms (×7500) a transparent celluloid sheet with rings of set radius. A comparison with rings of equal size around random points was done. The center of the ring enclosing the grain was punctured with a needle. Different sheets were used for either the cytoplasmic matrix or the dilated endoplasmic reticulum cisternae. The number of grains thus recorded over each compartment of a given cell was expressed as a percentage of total number of grains over all the compartments. Mean results shown in Figs. 10 and 11 and in Table 1 were adjusted to standard deviation estimates.

The background grain density was established in nonradioactive control sections and in experimental autoradiograms.

RESULTS

Several reports dealing with electron microscopic studies (reviewed recently by Fawcett, 1970), have stressed the cytoplasmic features of Sertoli cells as well as

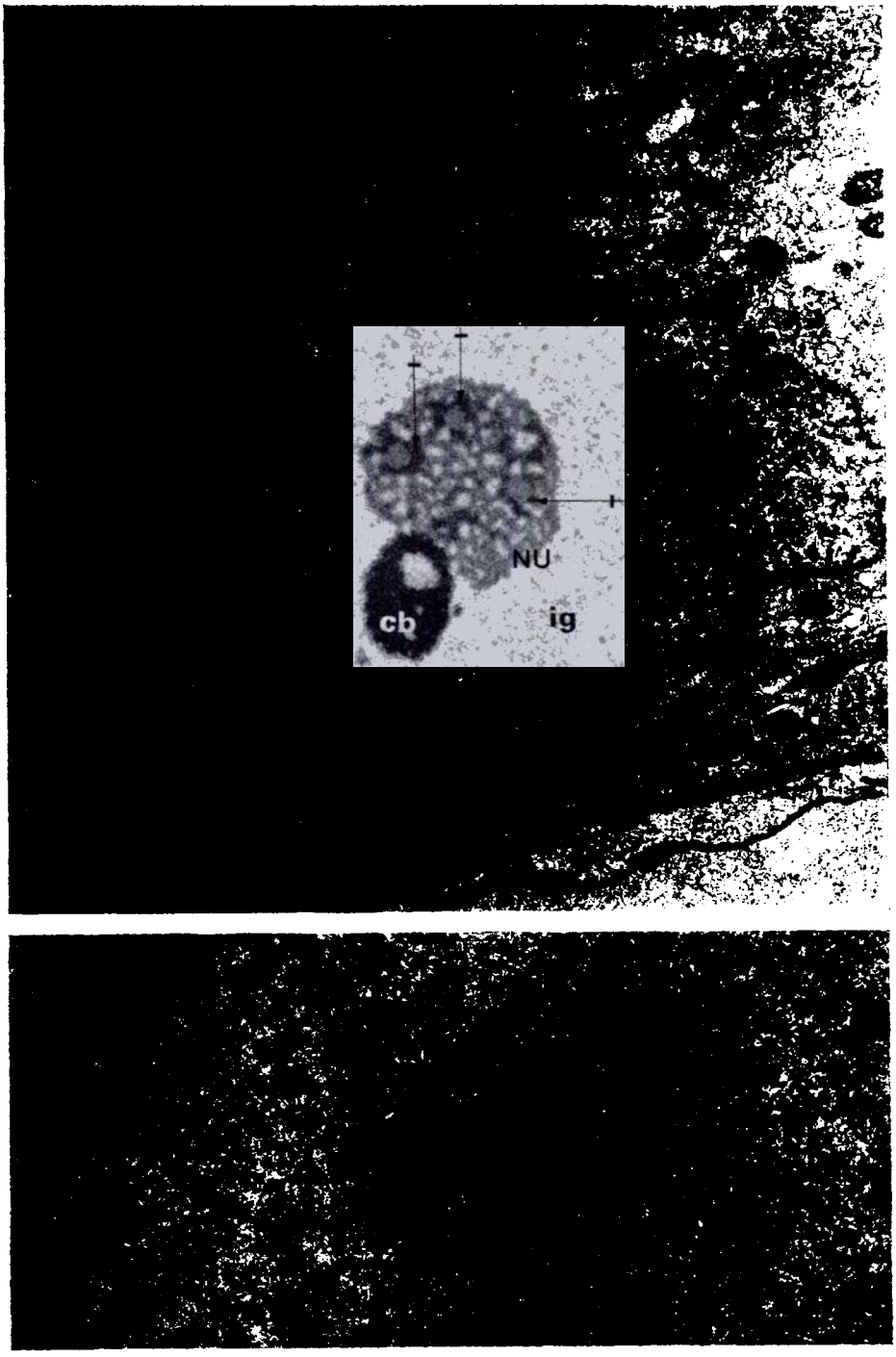


FIG. 1. Sertoli cell closely related to the basal lamina (bl) of the seminiferous epithelium. The cytoplasm shows dilated cisternae of the endoplasmic reticulum, dense bodies and ribosomal aggregates. Chromatin is uniform extended. Several clusters of interchromatin granules (ig), a nucleous (NU) with three ring-shaped fibrillar components (↔) enclosing amorphous central areas and a network of granular components are associated with a condensed chromatin body (cb). ×12,000.

FIG. 2. High magnification of a cluster of interchromatin granules (ig) near the nuclear envelope (ne). ×20,000.

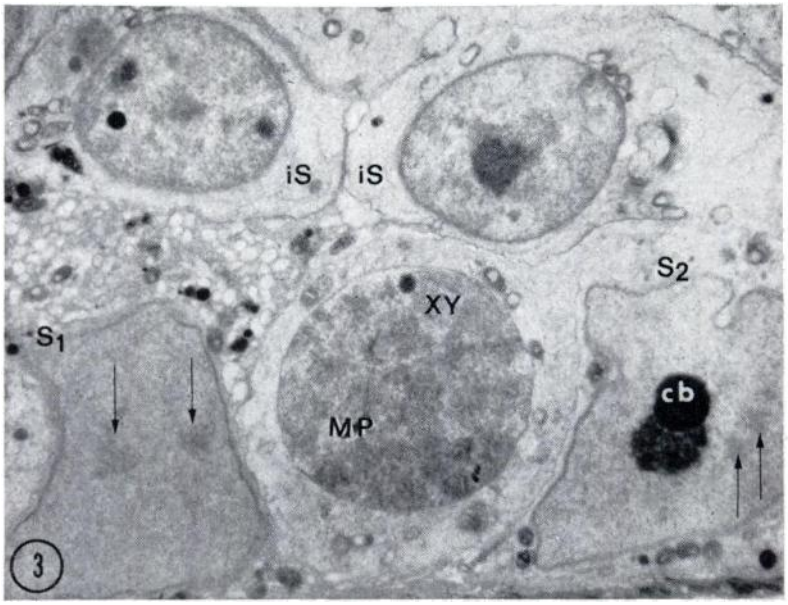


FIG. 3. Two types of Sertoli cells: an extensive vacuolated type (S_1) with greater nuclear density, as contrasted with a less nonvacuolated Sertoli cell type (S_2). Arrows point to accumulations of interchromatin granules in both cellular types. cb, condensed chromatin body attached to the nucleolus; XY, sex chromosomes in a middle pachytene spermatocyte (MP); iS, immature spermatid. ×5200.

specialized plasma membrane junctions at interfaces between Sertoli–Sertoli and Sertoli–germinal cells. In a previous study (Tres *et al.*, 1972) some nuclear features of Sertoli cells were described.

Sertoli cells are recognized in the seminiferous epithelium of the mouse by their apposition along the basal lamina and their characteristic irregularly shaped nuclei, which show several invaginations of the nuclear envelope (Figs. 1, 5). Sertoli cells can be recognized differing from each other in cytoplasmic patterns (Fig. 3). One type shows even dilatations of endoplasmic reticulum cisternae, whereas the other lacks such dilatations. Both types can be found in the same seminiferous tubule, suggesting an asynchronous differential function of these cells. Observations supporting this hypothesis are set forth later.

The most conspicuous feature of the nuclei is the presence of a nucleolus located distant from the nuclear envelope and generally associated with two condensed chromatin bodies (Fig. 6). As pointed out in an earlier report (Tres *et al.*, 1972), the nucleolar fibrillar component, usually ring-shaped, surrounds an amorphous area (fibrillar center, Recher *et al.*, 1970), whereas the granular component is arranged in a network (Fig. 1). In the nucleoplasm, chromatin is evenly dispersed. Against this background, several clusters of interchromatin granules scattered widely throughout the nucleus are clearly visible at light (Fig. 9) and electron microscopic levels (Figs. 1, 3). A small number of perichromatin granules (Watson, 1962; Monneron and Bernhard, 1969) surrounded by clear halos have been found in the nucleoplasm (Fig. 7). Fibrillar structures compatible with perichromatin fibers are recognized with the uranyl acetate–EDTA–lead citrate technique (Bernhard, 1969; Monneron and Bernhard, 1969). However, insufficient contrast between extended chromatin and perichromatin fibers defeats efforts to distinguish the two clearly. Inter-

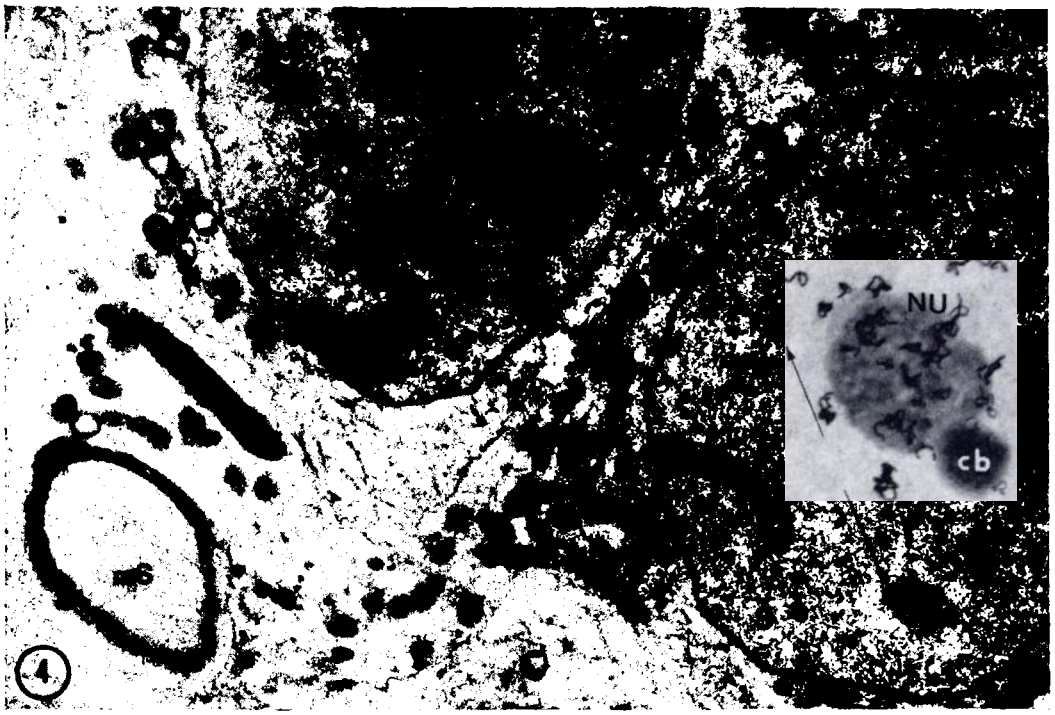


FIG. 4. Stage X. Labeling time: 3 h. Developer: Microdol-X. Nonvacuolated Sertoli cell type (S) showing nucleolar (NU) and extranucleolar labeling. Cytoplasm is free of silver grains. Arrows indicate interchromatin granular areas. A late pachytene (LP) spermatocyte shows a few silver grains at perichromosomal localization. Sex chromosomes (XY) and mature spermatids (mS) are unlabeled. cb, condensed chromatin body. ×8500.

FIG. 5. Stage I. Labeling time: 3 h. Developer: Microdol-X. Vacuolated Sertoli cell type showing silver grains in nucleolus (NU) and nucleoplasm. One sees clearly grains distributed close to the nuclear envelope and in the cytoplasmic sites surrounding dilated cisternae of the endoplasmic reticulum. Interchromatin granules (arrow) are unlabeled. SC, synaptonemal complex in an early pachytene spermatocyte. cb, condensed chromatin body. ×7600. Insert: Nucleolus of the same Sertoli cell in an adjacent section. ×15,300.

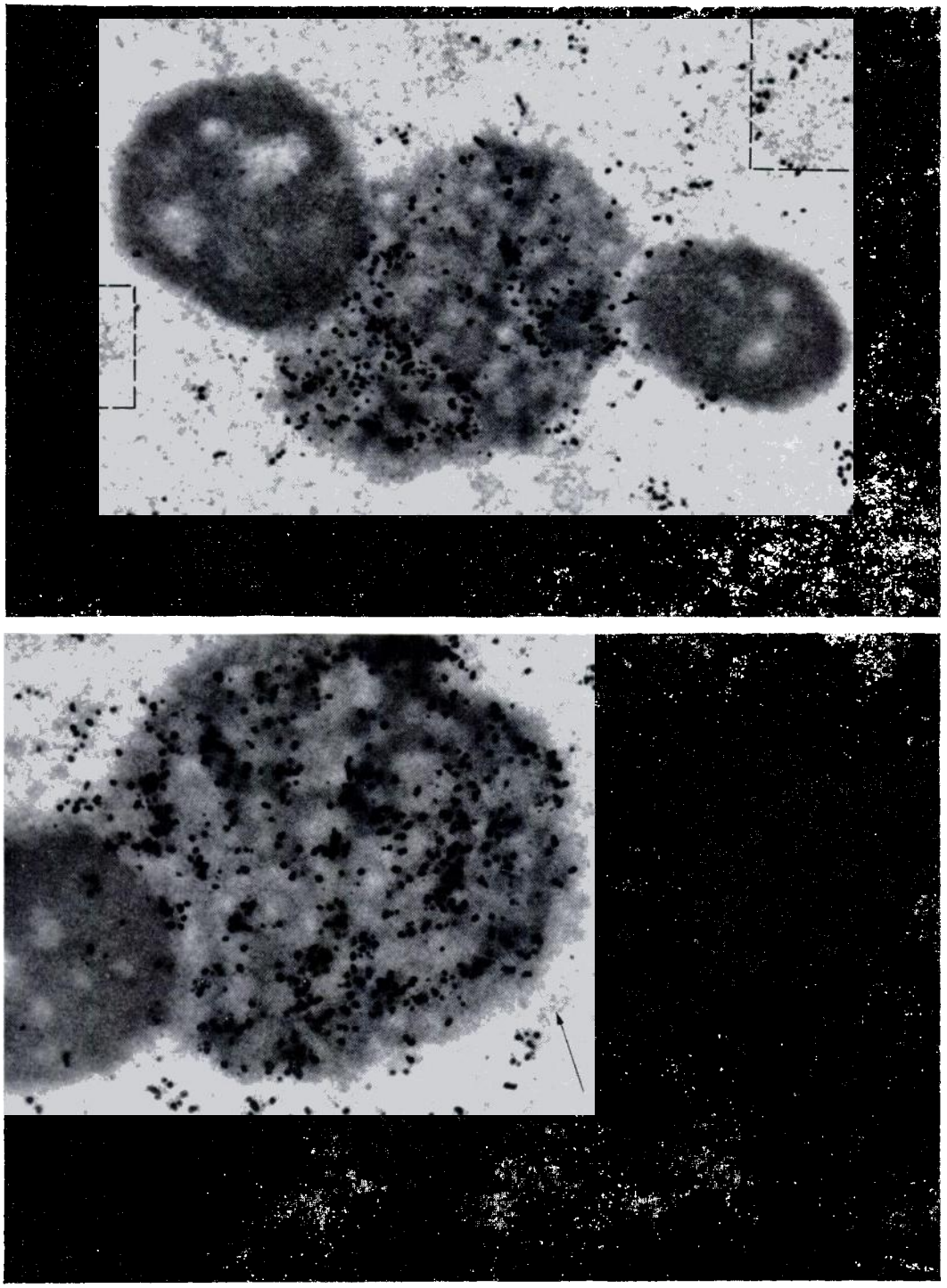


FIG. 6. Sertoli cell. Labeling time: 3 h. Developing procedure: EAA. Nucleolus with two associated condensed chromatin bodies. Areas of interchromatin granules are framed. Nucleoplasm shows scattered silver grains. ×28,900.

FIG. 7. Sertoli cell. Labeling time and developing procedure as in Fig. 6. Arrows point to an apparent connection of a partially labeled cluster of interchromatin granules with the nucleolus. A perichromatin granule is indicated (‡). ×31,200.

chromatin granules, perichromatin granules, and perichromatin fibrils were described as ribonucleoprotein carrying structures (Monneron and Bernhard, 1969).

Electron Microscope Autoradiography

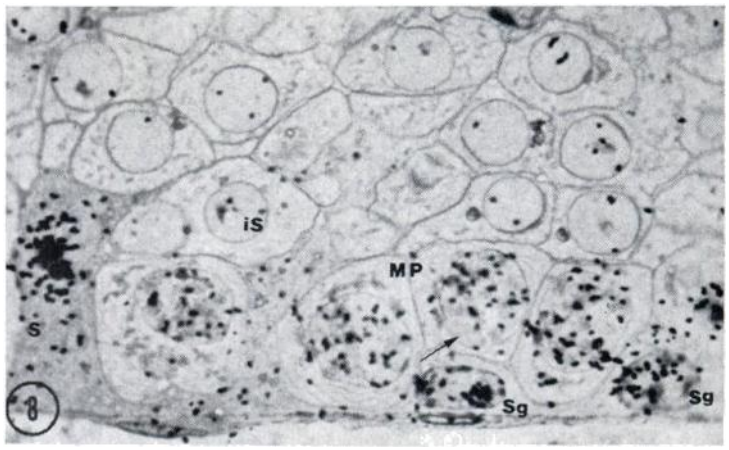
As previously described (Kierszenbaum and Tres, 1974), in sections of seminiferous tubules fixed 5 min after labeling with [^{3}H]uridine, nearly all radioactivity is localized in the nucleoplasm of Sertoli cells and in some spermatogonial types. After 15 min, silver grains can be seen particularly in the nucleoplasm and nucleoli of Sertoli cells and spermatogonia. Three hours after labeling (Fig. 4), the nucleolus of a Sertoli cell shows a high grain density. A chromatin body attached to the nucleolus remains unlabeled, whereas some silver grains are seen scattered on the nucleoplasmic area. A neighboring late pachytene spermatocyte shows an unlabeled nucleolus attached to the XY pair. A few silver grains can be seen at the perichromosomal locus. Mature spermatids are totally unlabeled, as is to be expected from the lack of transcriptional activity at this spermiogenic step (Monesi, 1965; Galdieri and Monesi, 1973). A distinct labeling pattern is seen in a Sertoli cell of another seminiferous tubule (Fig. 5). The nucleolus is heavily labeled (insert, Fig. 5) and several silver grains are clearly observed in the proximity of the nuclear envelope. Cisternae of endoplasmic reticulum appear considerably distended and the labeled compound is restricted to the immediate region around the dilated structures. This evidence for labeled RNA in the vacuolated Sertoli cell cytoplasm contrasts with the scarcity of silver grains in the cytoplasm of germinal cells (Fig. 5). It should also be noted that the cytoplasmic vacuolation pattern in the Sertoli cell is uniform and should be distinguished from the dilatation and vesiculation of endoplasmic reticulum cisternae described as a nonspecific response to injurious stimuli in mouse and guinea pig Sertoli cells (Kierszenbaum, 1970; Kierszenbaum and Mancini, 1973).

Figure 7 presents the silver grain localization in the fibrillar and granular areas of the nucleolus in a Sertoli cell after 3 h labeling. Beside this, dense chromatin bodies show also silver grains over their main portions (Fig. 7) and at their periphery (Figs. 6, 7), thus suggesting sites for nucleolar RNA synthesis.

Many silver grains are seen over the nucleoplasm. Some of them are scattered within clusters of interchromatin granules (Fig. 7). The interchromatin granules show a paranucleolar distribution (Figs. 1, 6, 7) and are apparently associated with the nucleolus through a path of granular elements similar to those forming the cluster (Fig. 7).

Autoradiography of Thick Sections

The labeling pattern in a thick section of a mouse seminiferous epithelium taken 3 h after administration of [^{3}H]uridine, can be observed in Fig. 8. The nucleus and nucleolus of a Sertoli cell are well labeled. Scattered silver grains are identified in middle pachytene spermatocytes, whereas they are scarce over immature spermatid nuclei. The cytoplasm of a Sertoli cell is vacuolated and a few silver grains are recognized throughout the structure. Moreover, the cytoplasm is dense when compared with that of neighboring spermatocytes. Eight days after labeling (Fig. 9), the Sertoli cell nucleus, including the well-recognized interchromatin granules, is almost free of silver grains, thus contrasting with middle pachytene spermatocytes, which show silver grains in their nuclei. This finding suggests a higher rate of turnover of the newly synthesized RNA species Sertoli cells as compared to that in meiotic prophase spermatocytes. Furthermore, the absence of silver grains in the region of interchromatin granules at very late labeling times (i.e., 8–12 days) suggests that labeled RNA molecules are not accumulating at those sites.



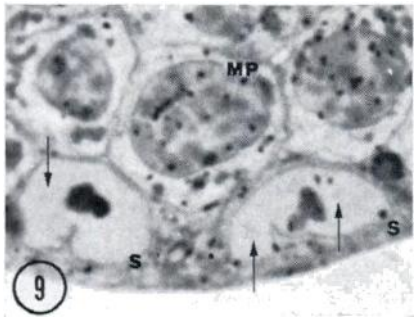


FIG. 8. Stage V. Labeling time: 3 h. Developer: Kodak D-19. A labeled Sertoli cell (S) of the vacuolated type showing considerable density of the cytoplasmic structure, contrasting with the neighboring middle pachytene (MP) spermatocytes which are almost lacking in silver grains in their lighter cytoplasmic areas. iS, immature spermatid. Sg, spermatogonia. XY chromosomes are indicated with an arrow. ×3050.

FIG. 9. Stage V. Labeling time: 8 days. Developer: Kodak D-19. Sertoli cell nuclei are free of label and contrast with the still labeled middle pachytene spermatocytes. Unlabeled interchromatin granular areas are indicated with arrows. ×4000.

Quantitative Analysis

The results of grain density determinations shown in Fig. 10 correspond to nonvacuolated and vacuolated Sertoli cells observed in the same seminiferous tubule, thus avoiding possible error due to a variable rate of incorporation of [^{3}H]uridine into different seminiferous tubules. Four conclusions can be reached from the quantitative analysis of relative surface areas (Figs. 10, 11). First, cytoplasmic labeling rises to a maximum after 3 h in both types of Sertoli cells—comparison of the peaks reflects higher values in the grain density for the vacuolated type. Secondly, the nucleoplasmic label is almost the same in both types. Main counts from specimens fixed 7 days after isotope injection show clear differences of nucleoplasmic labeling between middle pachytene spermatocytes and Sertoli cells. Thirdly, the nucleolus is the most prominent labeled structure inside the nucleus. No appreciable differences among Sertoli cell types have been detected. Fourthly, about 40 areas of interchromatin granules were scored in 30 autoradiograms of different Sertoli cells. The data indicates measurable grain densities after 3 h labeling. Background grain density plotted in this graph (Fig. 11) was taken as control.

The radial grain distribution in vacuolated Sertoli cells (Table 1) shows that almost all the silver grains over the cytoplasm are located in the cytoplasmic matrix.

DISCUSSION

These autoradiographic observations of [^{3}H]uridine uptake in Sertoli cells of the mouse provide indications for various localizations of the RNA synthesis. It is of particular interest that nucleolar and nucleoplasmic transcriptional activities are rapidly displayed after labeling pulses. Moreover, the differential cytoplasmic labeling pattern in some Sertoli cells at later times becomes significant in the presence of structural changes, and may reflect variations in metabolic activities associated with support of spermatogenesis.

RNA synthesis during meiotic prophase in mouse testis has been described in a previous paper (Kierszenbaum and Tres, 1974). It was shown that RNA synthesis varies with different meiotic prophase stages. Moreover, a nucleolar and perichromosomal labeling of the autosomes was noted, contrasting with an inconspicuous labeling of the sex chromosomes. These findings indicate that the ribosomal RNA fractions recently observed by Galdieri and Monesi (1973) in mouse testis, attributed by them mainly to spermatogonia and Sertoli cells must also be attributed in part to spermatocytes.

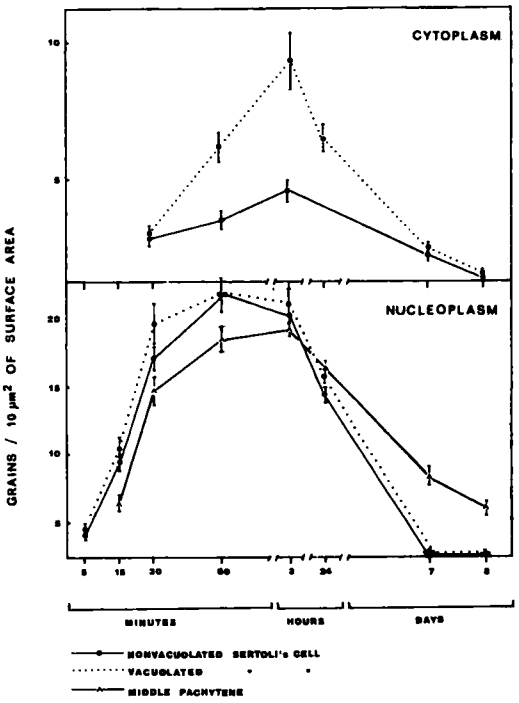


FIG. 10. Comparison of relative grain densities over cytoplasmic and nucleoplasmic regions in nonvacuolated and vacuolated Sertoli cells after different [$_3$H]uridine labeling times. The bars represent the standard deviation.

In the present study, it seems apparent that differing labeling patterns in Sertoli cells within the same seminiferous tubule may reflect differential metabolic activities. That Sertoli cells may show different cellular activity stages has been suspected for some time. Johnsen (1969) described in

TABLE 1

RADIAL GRAIN DISTRIBUTION IN ELECTRON MICROSCOPE AUTORADIOGRAMS OF 15 VACUOLATED SERTOLI CELLS AT VARIOUS INTRATESTICULAR INJECTION TIMES

Time after [^{3}H]uridine injection	Total number of grains	
	Cytoplasmic matrix[a]	Endoplasmic reticulum cisternae[a]
30 min	85% ± 2.4	15% ± 0.7
60 min	90% ± 4.1	10% ± 0.9
3 h	88% ± 2.4	12% ± 0.4
24 h	84% ± 3.1	16% ± 1.0

[a] Values are means, ± is the SEM.

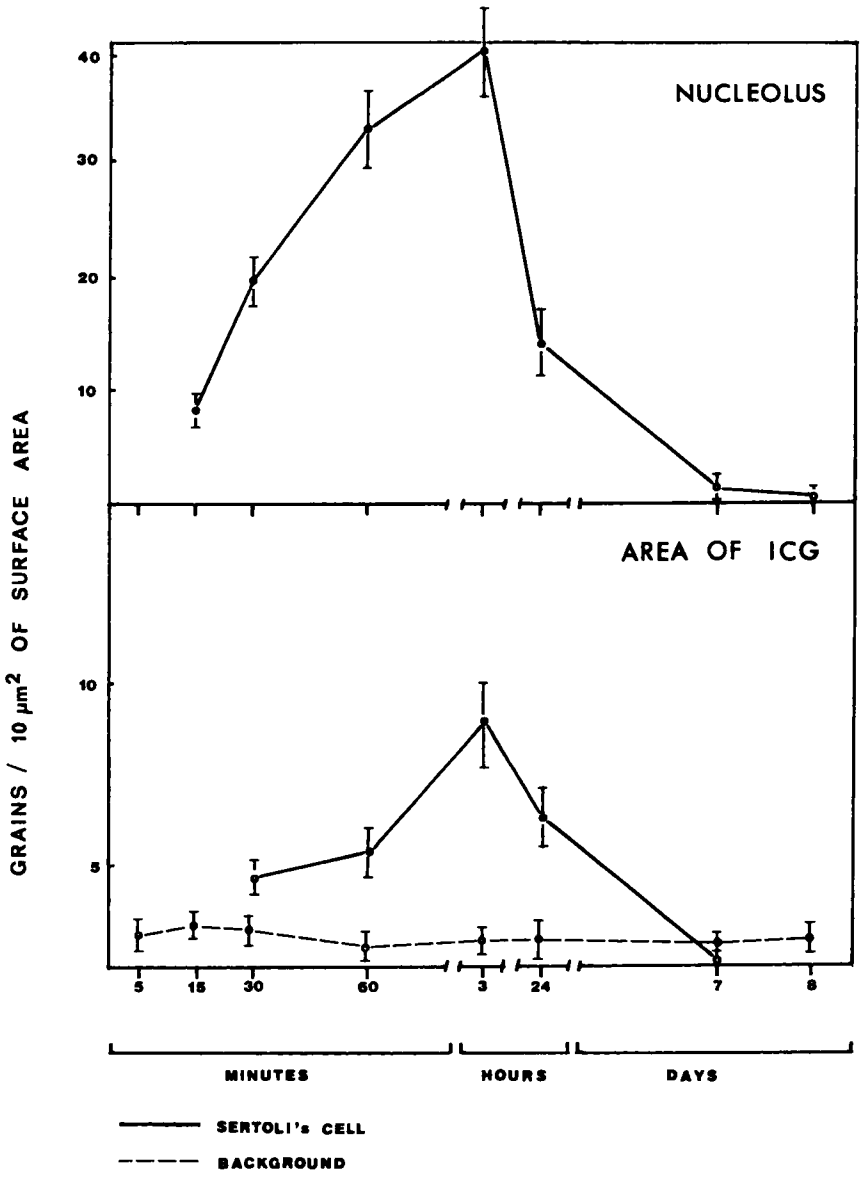


FIG. 11. Relative grain densities in randomly chosen Sertoli cells over the nucleolar and interchromatin granular regions. The bars represent standard deviation.

the human testis differences in the nuclear staining of Sertoli cells such as might signify differences in nucleic acid content.

The Sertoli cytoplasmic vacuolation together with the labeling raise the possibility that a translational product is being transported perhaps toward the germinal cells. However, it cannot be denied that vacuolation may represent some other activity, such as storage of some hormone or its metabolites.

It is conceivable that nuclear RNA synthesis in Sertoli cells might precede protein synthesis which, in turn, would mediate gonadotropin induction, i.e., FSH and/or LH (Mancini, 1970). Furthermore, the translational product might be a direct consequence of gonadotropin induction and

destined to provide a hypothetical "promoting factor" for gametogenic events. In support of this last suggestion is the observation of testicular protein synthesis following enhanced incorporation of uridine into nuclear RNA after FSH administration in the rat (Means, 1971). Thus, reversible Sertoli cell activation represented by increases and decreases of RNA synthesis could account for the different labeling patterns observed in these cells.

Early RNA labeling in the nucleoplasm of Sertoli cells is followed at later times by the appearance of silver grains close to the nuclear envelope and then further in the cytoplasm. Some ribosomal RNA synthesized in the nucleolus is also expected to migrate to the cytoplasm and it is assumed that some nuclear silver grains are associated with this class of RNA.

Remarkable facts arise from the comparison of results in the labeling patterns of spermatocytes and Sertoli cells. First, in spermatocytes, the perichromosomal labeling, attributed to large nuclear heterogeneous RNA molecules (Muramatsu *et al.*, 1968), persists at longer times, when almost all radioactivity has been lost in Sertoli cells. This finding was assigned to degraded sequences of heterogeneous RNA molecules remaining inside the nuclei of spermatocytes (Kierszenbaum and Tres, 1974). Moreover, it also suggests a more rapid turnover of RNA species in Sertoli cells. Secondly it has been indicated that the nucleolar fibrillar component in spermatocytes is labeled earlier than is the granular one (Kierszenbaum and Tres, 1974). Similarly, labeling of the fibrillar nucleolar component occurred first in Sertoli cells in agreement with other reports in different cells (Granboulan and Granboulan, 1965; Recher *et al.*, 1970). Thirdly, in some autosomes of spermatocytes, condensed chromatin regions, called basal knobs (Woollam and Ford, 1964), are closely related to nucleolar structures. In mouse Sertoli cells, the nucleolus is associated with condensed chromatin bodies which take little label and which probably contain the cistrons coding for ribosomal RNA. In this sense, it is quite possible that these bodies are functionally similar to the basal knobs and that they may represent fused paracentromeric regions of chromosomes (Pardue and Gall, 1970).

Clusters of interchromatin granules are very prominent in Sertoli cells and are easily recognized because of the disperse distribution of chromatin fibers. The incorporation of labeled uridine at these clusters shows a distribution similar to the distribution observed under *in vitro* conditions (Fakan and Bernhard, 1971, 1973). When labeling is followed by a long period of time (3 h), some silver grains are visible within the cluster and in more peripheral locations. At later times (8–12 days), there is no labeling at all inside the nucleus. It is now appropriate to raise the question whether the 3-h labeling signifies a delayed RNA synthesis or an accumulation of RNA molecule(s) synthesized in another nuclear region. In a previous study, Tres *et al.* (1972) described electron-opaque precipitates in Sertoli cells compatible with the presence of inorganic cations in sites coincident with clusters of interchromatin granules. Specific nucleolar and perichromosomal localizations of similar precipitates were related to presumed RNA polymerase(s) activity dependent in part on divalent cations, either Mg^{2+} or Mn^{2+}.

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REFERENCES

BERNHARD, W. (1969). A new staining procedure for electron microscopical cytology. *J. Ultrastruct. Res.* **27**, 250–265.

CLEGG, E. J., AND MACMILLAN, E. W. (1965). The uptake of vital dyes and particulate matter by the Sertoli cells of the rat testis. *J. Anat.* **99**, 219–229.

FAKAN, S., AND BERNHARD, W. (1971). Localization of rapidly and slowly labeled nuclear RNA as visualized by high resolution autoradiography. *Exp. Cell Res.* **67**, 129–141.

FAKAN, S., AND BERNHARD, W. (1973). Nuclear labelling after prolonged ^{3}H-uridine incorporation as visualized by high resolution autoradiography. *Exp. Cell Res.* **79**, 431–444.

FAWCETT, D. W. (1970). Interrelations of cell types within the seminiferous epithelium and their implications for control of spermatogenesis. *In* "The Regulation of Mammalian Reproduction." (S. J. Segal, R. Corzier, P. A. Corfman, and P. G. Condliffe, eds.), pp. 116–135. Charles C Thomas, Springfield, Illinois.

GALDIERI, M., AND MONESI, V. (1973). Ribosomal RNA synthesis in spermatogonia and Sertoli cells of the mouse testis. *Exp. Cell Res.* **80**, 120–126.

GRANBOULAN, N., AND GRANBOULAN, P. (1965). Cytochimie ultrastructurale du nucléole. II. Etude des sites de synthèse du RNA dans le nucléole et le noyau. *Exp. Cell Res.* **38**, 604–619.

JAMES, T. H. (1948). The site of reaction in direct photographic development. II. Kinetics of development initiated by gold nuclei. *J. Colloid Sci.* **3**, 447–451.

JOHNSEN, S. G. (1969). Two types of Sertoli cells in man. *Acta Endocrinol.* **61**, 111–116.

KIERSZENBAUM, A. L. (1970). Effect of Trenimon on the ultrastructure of Sertoli cells in the mouse. *Virchows Arch. Ast. b Zellpath.* **5**, 1–12.

KIERSZENBAUM, A. L., AND MANCINI, R. E. (1973). Structural changes manifested by Sertoli cells during experimental allergic orchitis in guinea pigs. *J. Reprod. Fertil.* **33**, 119–122.

KIERSZENBAUM, A. L., AND TRES, L. L. (1974). Nucleolar and perichromosomal RNA synthesis during meiotic prophase in the mouse testis. *J. Cell Biol.* **60**, 39–53.

MANCINI, R. E. (1970). Effect of gonadotropin preparations and of urinary FSH and LH on human spermatogenesis. *In* "The Regulation of Mammalian Reproduction." (S. J. Segal, R. Crozier, P. A. Corfman, and P. G. Condliffe, eds.), pp. 151–162. Charles C Thomas, Springfield, Illinois.

MEANS, A. R. (1971). Concerning the mechanism of FSH action: Rapid stimulation of testicular synthesis of nuclear RNA. *Endocrinology* **89**, 981–989.

MONESI, V. (1965). Synthetic activities during spermatogenesis in the mouse. *Exp. Cell Res.* **39**, 197–224.

MONNERON, A., AND BERNHARD, W. (1969). Fine structural organization of the interphase nucleus in some mammalian cells. *J. Ultrastruct. Res.* **27**, 266–268.

MURAMATSU, M., UTAKOJI, T., AND SUGANO, H. (1968). Rapidly labeled nuclear RNA in chinese hamster testis. *Exp. Cell Res.* **53**, 278–283.

PARDUE, M. L., AND GALL, J. G. (1970). Chromosomal localization of mouse satellite DNA. *Science*, **168**, 1356–1358.

RECHER, L., WHITESCARVER, J., AND BRIGGS, L. (1970). A cytochemical and radioautographic study of human tissue culture cell nucleoli. *J. Cell Biol.* **45**, 479–492.

SALPETER, M. M., AND BACHMANN, L. (1972). Autoradiography. *In* "Principles and Techniques of Electron Microscopy. Biological Applications." (M. A. Hayat, ed.), Vol. 2, pp. 221–278. Van Nostrand Reinhold Company, New York.

TRES, L. L., KIERSZENBAUM, A. L., AND TANDLER, C. J. (1972). Inorganic cations in the cell nucleus. Selective accumulation during meiotic prophase in mouse testis. *J. Cell Biol.* **53**, 483–493.

UTAKOJI, T. (1966). Chronology of nucleic acid synthesis in meiosis of the male chinese hamster. *Exp. Cell Res.* **42**, 585–596.

VILAR, O., PEREZ DEL CERRO, M. I., AND MANCINI, R. E. (1962). The Sertoli cell as a "bridge cell" between the basement membrane and the germinal cells. Histochemical and electron microscopic observations. *Exp. Cell Res.* **27**, 158–161.

VITALE-CALPE, R., AND BURGOS, M. H. (1970). The mechanism of spermiation in the hamster. I. Ultrastructure of spontaneous spermiation. *J. Ultrastruct. Res.* **31**, 381–393.

WATSON, M. L. (1962). Observations on a granule associated with chromatin in the nuclei of cells of rat and mouse. *J. Cell Biol.* **13**, 162–167.

WISSE, E., AND TATES, A. D. (1968). A gold latensification-Elon ascorbic acid developer for Ilford L4 emulsion. Proc. 4th. Europ. Reg. Conf. E.M., Vol. 2, pp. 465–466. Tipografia Polyglotta Vaticana, Rome.

WOOLLAM, D. H. M., AND FORD, E. H. R. (1964). The fine structure of the mammalian chromosome in meiotic prophase with special reference to the synaptinemal complex. *J. Anat.* **98**, 163–173.

RECOMMENDED REVIEWS

BUSCH, H., AND SMETANA, K. (1970). "The Nucleolus." Academic Press, New York and London.

ROGERS, A. W. (1973). "Techniques of Autoradiography." Elsevier Scientific Publishing Co., Amsterdam–London–New York.