AN AUTORADIOGRAPHIC STUDY OF THE POST-NATAL CHANGES IN [³H]LEUCINE INCORPORATION BY SERTOLI CELL NUCLEI IN RAT TESTES

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Summary. Using [³H]leucine and autoradiography, protein synthesis was studied in the post-natal and mature Sertoli cell nuclei of rat testes. It was shown that all nuclei were labelled in all age groups and that the amount of amino acid incorporation was greatest in newborn animals and thereafter declined until about 21 days after birth when adult levels of incorporation were realized. The realization of the adult level of protein synthetic activity coincides with the cessation of mitotic activity in Sertoli cells as well as the quickening of spermatogenic activity in the germinal cells.

INTRODUCTION

Several methods of investigation have been applied to mammalian Sertoli cells. The morphology of the pre- and post-natal development of the Sertoli cell has been well documented at the light microscope level (Clermont & Perey, 1957; Sapsford, 1957, 1962, 1963) as well as with the electron microscope (Flickinger, 1967). Several workers have dealt with the cytochemistry of mature Sertoli cells particularly with regard to lipids (Wislocki, 1949; Lynch & Scott, 1951; Long & Engle, 1952) and various enzymes (Niemi & Kormano, 1965; Jirásek, 1967; Singh & Mathur, 1968).

Using *in-vitro* incubation of testicular slices, Firlit & Davis (1966) examined the degree to which Sertoli cells in normal and artificially cryptorchid rat testes incorporated tritiated lysine. With *in-vivo* autoradiographic techniques, Nagy (1973) found that, both in cryptorchid and scrotal rat testes, not all Sertoli cell nuclei synthesize proteins to the same extent and that there is a spectrum of synthetic activity.

Cell cycle parameters were determined for immature Sertoli cells (supporting cells) in the rat (Nagy, 1972). It was also reported that the mitotic index dropped to nearly zero at 14 days after birth. At the same time, the tritiated thymidine labelling index dropped to almost half the value found in the newborn rat supporting cells and thereafter continued to decline.

The present work is directed towards determining (1) the post-natal age at

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which adult levels of nuclear protein synthesis are realized in rat Sertoli cells, (2) if this age correlates with the decline in mitotic activity in the cells, and (3) if changes in amounts of labelled amino acid incorporation can be correlated with spermatogenic changes taking place in the developing testis.

MATERIALS AND METHODS

The offspring of Sprague-Dawley rats mated in the laboratory were used in all experiments. Animals designated as 'newborn' were killed on the 1st day after birth and other animals were killed at 7, 14, 21, 42, 70 and 77 days after birth. Testes from at least three animals were obtained at each time interval. One hour before autopsy, each animal was given an intraperitoneal injection of [^3H]leucine (sp. act. 5.0 Ci/mmol, New England Nuclear Corp.) in the amount of 5.0 $\mu\text{Ci/g}$ body weight. All injections were made between 10.00 and 11.00 hours. Between the time of injection and autopsy, offspring were returned to their mothers.

Animals were killed with an overdose of ether and the testes were fixed in Bouin's fluid for 12 to 18 hr, washed in Li_2CO_3 in 70% alcohol, dehydrated in a graded series of ethanols and embedded in paraffin wax. With the exception of the newborn, the tunica albuginea was removed from all testes before fixation.

Sections were cut at 5 μm and prepared for autoradiography by coating them with Kodak NTB-2 liquid emulsion. Slides were allowed to expose in black boxes for 7 days before development in Kodak D-19 and fixation in Kodak Fixer. Sections were stained through the emulsion with haematoxylin and chromotrope 2R.

At least 100 nuclei were scored for each animal. Grain counts were expressed as grains per 100 μm^2 nuclear area. As each nucleus was scored for grain counts, a photographic record was made of that nucleus and the area was subsequently determined planimetrically. In these studies, only the nuclei were evaluated because of the difficulty inherent in trying to localize silver grains over the sometimes rather tenuous cytoplasm of the more mature Sertoli cells.

RESULTS

Post-natal changes in the seminiferous cords

Brief descriptions of post-natal testicular changes are included here to present histological pictures prepared at times coincident with fixation of tissue samples for autoradiography.

In the newborn rat testis, the sex cords were composed chiefly of small supporting (presumptive Sertoli) cells and large gonocytes (Pl. 1, Fig. 1). The supporting cell nuclei (Pl. 1, Fig. 2) were irregular in outline, variously sized and shaped, contained prominent nucleoli and rather coarse, granular chromatin. Active mitosis was seen in the supporting cells whose cytoplasm was reticular with ill-defined borders. The gonocytes contained large nuclei with one to four prominent nucleoli. These cells stained more lightly than the supporting cells and displayed clearly defined cytoplasmic borders.

At 7 days after birth, the sex cords were still solid and the appearance of the supporting cells was not appreciably different from that in younger animals (Pl. 1, Fig. 3). Mitotic activity was still frequent in supporting cells in contrast to that of the now sparse gonocytes. Also at this time, a few type A, intermediate and type B spermatogonia were beginning to appear in the epithelium of the sex cords.

The chromatin in the supporting cell nuclei of 14-day-old rats was less granular and more diffuse than in earlier stages. Nuclei were still prominent

TEXT-FIG. 1. Curves illustrating the relative rates and amounts of incorporation with time of tritiated leucine by supporting cell nuclei of newborn (□), 7-day-old (○) and 14-day-old (●) rats.

with a smooth contour (Pl. 1, Fig. 4). Gonocytes were no longer found in the sex cords but spermatogenesis had progressed to the extent that zygotene and occasional pachytene stages of meiosis were found (Pl. 1, Fig. 5).

At 21 days after birth, the supporting cell nuclei (Pl. 1, Fig. 6) contained diffuse, lightly staining chromatin and the nucleoli were assuming the characteristic smooth, round appearance seen in adult animals. Later stages of spermatogenesis were evident and some of the sex cords had begun to canalize.

Subsequently, the supporting cells presented all the features common to mature Sertoli cells and could be rightfully referred to as such. From 6 weeks on, all stages of spermatogenesis were present.

[³H]Leucine incorporation by Sertoli cell nuclei

All supporting cell nuclei regardless of age incorporated tritiated leucine as evidenced by autoradiography.

Grain counts were made over supporting cell nuclei of newborn, 7- and 14-day-old animals at various intervals following the injection of [^3H]leucine (Text-fig. 1). It was the purpose of these counts to determine the time after injection at which maximal incorporation occurred. At 60 min after injection, the level of incorporation began to plateau in the several age groups examined. Computation of the slopes (Table 1) of the ascending portions of the curves in Text-fig. 1 revealed that the slopes are markedly different in all three age groups, decreasing continually until 14 days after birth. The maximum amount

Table 1. Slopes of nuclear incorporation curves from 10 to 60 min following injection of tritiated leucine into rats

<i>Age of animals</i>	<i>Slope</i>
Newborn	0.11
7 days	0.04
14 days	0.01

of incorporation at 14 days after birth was essentially that observed in earlier studies (Nagy, 1973) in mature Sertoli cell nuclei. Therefore, curves for the kinetics of incorporation were not constructed after 14 days. Since maximal incorporation of [^3H]leucine was realized 1 hr after injection, this time interval was used in the remaining experiments.

Incorporation was greatest in the supporting cells of the newborn rat. The level of incorporation then dropped sharply at 1 week after birth (Text-fig. 2) and continued to decline more slowly until the animals reached an age of about 3 weeks. Thereafter, the amount of nuclear incorporation of tritiated leucine did not differ significantly from that found in mature cells.

EXPLANATION OF PLATE I

FIG. 1. Newborn rat testis showing large numbers of centrally situated gonocytes (G) and peripherally located supporting cells (S). Note well developed vascular supply (V). Haematoxylin and chromotrope. $\times 625$.

FIG. 2. Newborn rat supporting cells (S) illustrating polymorphic nuclei, prominent nucleoli (N) and indistinct cellular borders. Haematoxylin and chromotrope. $\times 2600$.

FIG. 3. Tangential section of 7-day testis cord illustrating numerous supporting cells (S) with polymorphic nuclei and prominent nucleoli. Haematoxylin and chromotrope. $\times 2600$.

FIG. 4. Testis cord from 14-day-old rat. The cord is lined by supporting cells (S) and some primary spermatocytes (Sp) in early meiotic prophase. Haematoxylin and chromotrope. $\times 2600$.

FIG. 5. Section of 14-day rat testis cord. Note supporting cells (S) and spermatogonia (Sg). The centre of the cord is occupied by primary spermatocytes (Sp) in the leptotene phase of meiosis. Haematoxylin and chromotrope. $\times 1560$.

FIG. 6. Section of testicular tubule from 21-day-old rat. Note the abundance of supporting cells (S), the primary spermatocyte (Sp) and the lumen within the tubule (top). Haematoxylin and chromotrope. $\times 2600$.

PLATE 1

(Facing p. 356)

TEXT-FIG. 2. Curve illustrating the amount of incorporation of tritiated leucine by rat Sertoli cell nuclei as a function of age. Grain counts were made on animals killed 1 hr after injection of the isotope.

DISCUSSION

It is significant that all Sertoli cells, whether immature or mature, incorporate tritiated leucine into their nuclei, implying that these nuclei throughout all stages of development are actively engaged in the synthesis of proteins (Droz & Warshawsky, 1963). This observation serves to emphasize a difference between this cell type and the gonocytes initially present in the sex cords of the neonatal rat testis. In gonocytes of the 2-day-old rat, Lett (1968) reported that only 55% of the nuclei were labelled following the administration of tritiated phenylalanine. The level increased to 90% in germinal cell nuclei by 8 days after birth. In his study of the synthetic activity of the primordial germinal cells in neonatal rat testes, Lett (1968) used the amount of labelled protein precursor in the supporting cells as an index of the quantity available to each animal. The ratio of the number of silver grains over germinal cell nuclei to the number over Sertoli cell nuclei was used as an indication of the amount of isotope incorporation. It was the assumption that since the supporting cells are not significantly altered morphologically between 2 and 8 days of age, the synthetic activity of these cells was likewise altered very little. The data reported in the present work provide evidence to the contrary. The patterns of incorporation demonstrate that both the relative rates and relative amounts of nuclear incorporation of tritiated leucine are significantly different in supporting cells of newborn, 7- and 14-day-old animals. The trends in both the rate and amount of incorporation are towards a continual diminishing until the animals are between 14 and 21 days of age. At this time, a level of incorporation is reached which is not significantly different from that in mature Sertoli cell nuclei.

The decrease in the amount of protein synthesis in supporting cell nuclei parallels the morphological change in these nuclei which shows less granular chromatin as the animals become older. By 21 days of age, some of the later stages of meiotic prophase are evident in the seminiferous epithelium. The supporting cell nuclei also reach their adult level of synthetic activity at this time, a factor which might be indicative of their functional significance and their rôle in providing metabolic products to the germinal cells. That this actually occurs has been suggested by the studies of Reddy & Svoboda (1967) in which peroxidase was shown to be transferred from Sertoli cells to the germinal cells. Tice & Barnett (1963) suggested that nucleoside phosphatase activity associated with the Sertoli cells might be instrumental in the transfer of materials from the Sertoli cells to spermatozoa. If these phenomena apply to mature Sertoli cells, there is reason to believe that the same holds true for immature cells which are present at the initiation of spermatogenesis.

The observation of the initially greater nuclear protein synthetic activity in younger animals suggests that reserves are being synthesized and stored before the onset of spermatogenesis. Such proteins might be utilized in the initiation of spermatogenesis.

It is interesting that, between 14 and 21 days after birth, nuclear incorporation of tritiated leucine by Sertoli cells closely approximates its adult level of activity, spermatogenic activity quickens and mitotic activity in the Sertoli cells ceases (Nagy, 1972). The functional relationship of these events remains to be elucidated.

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