RADIO-AUTOGRAPHIC INCORPORATION OF L-LYSINE-$^3$H INTO PROTEIN OF CELLS OF THE GERMINAL EPITHELIUM IN CRYPTORCHIDISM

CASIMIR F. FIRLIT AND JOSEPH R. DAVIS

Department of Pharmacology and Therapeutics, Stritch School of Medicine, and Graduate School, Loyola University, Chicago, Illinois, U.S.A.

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Summary. Unilateral cryptorchidism has been induced in adult rats by transferring the testis from the scrotal sac into the abdominal cavity. The cell types found in the abdominal testis 30 days after the surgical procedure were ‘crust’ spermatogonia, pachytene primary spermatocytes and Sertoli cells, accounting for 31%, 3% and 66%, respectively, of the total cells remaining in the germinal epithelium.

The incorporation of L-lysine-$^3$H into protein of these cells has been studied in vitro. It was found that cryptorchidism resulted in a forty-fold increase in the incorporation of tritiated lysine into protein of the Sertoli cells as compared to the protein labelling of the Sertoli cells found in the corresponding scrotal testis of the same animal. In addition, a four-fold increase in protein labelling of both ‘crust’ spermatogonia and pachytene primary spermatocytes was also observed in the abdominal testis. These data indicate that the greatest enhancement due to an increased abdominal temperature of protein labelling in the remaining cells of the germinal epithelium of the cryptorchid rat testis occurs in the Sertoli cells.

INTRODUCTION

Previous data from this laboratory have indicated that the incorporation of L-lysine-U-$^{14}$C into protein of slices of cryptorchid testes of the rat is markedly greater than the incorporation of L-lysine-U-$^{14}$C into protein of slices of scrotal testes obtained from the same animal (Davis, Morris & Hollinger, 1964; Davis, Morris & Hollinger, 1965). It is well known that the predominant histological feature of the cryptorchid testis is the absence of all of the spermatids (Moore, 1924; Nelson, 1951; Clegg, 1963b; Niemi & Korman, 1965). The question arose as to whether the increased protein labelling of the cryptorchid testis of the rat was, on the one hand, a reflection of this altered histological architecture with a resulting unmasking of the spermatogonia, primary spermatocytes and Sertoli cells or, on the other hand, represented a true change in the protein-synthesizing capacity of the remaining testicular cell types of the germinal epithelium. The present experiments were designed to ascertain which of the cells of the seminiferous tubules of the rat testis remained 30 days after the
experimental-induction of cryptorchidism. The incorporation of tritiated lysine into protein of the remaining cells of the germinal epithelium of the abdominal testis was determined and a comparison made of the incorporation of tritiated lysine into protein of the corresponding cells found in the scrotal testis of the same animal.

MATERIALS AND METHODS

Experimental induction of cryptorchidism

The animals used in the present studies were 60-day-old male Sprague-Dawley rats weighing 185 to 220 g and fed unlimited Rockland rat diet. Unilateral cryptorchidism was produced by transferring the right testis from the scrotal sac into the abdominal cavity. A midline abdominal incision was performed under ether anaesthesia and gentle pressure applied to the bottom of the scrotal sac. The right testis was then sutured to the dorsolateral abdominal wall with a fine 6-0 surgical silk suture passed just under the tunica albuginea beyond the point where the testicular artery entered the parenchyma of the testis. The left testis of each animal was allowed to remain in the scrotal sac to serve as a control.

Incubation of testis slices with L-lysine-3H

The animals were killed by decapitation 30 days after the surgical procedure and both scrotal and abdominal testes rapidly dissected and decapsulated. Slices of testes which averaged 0.5 mm in thickness were obtained with the aid of a Stadie-Riggs microtome at 4°C. Incubation of the tissue slices was performed at 37.5°C in a Warburg apparatus as previously described (Davis, Firlit & Hollinger, 1963). The main chamber of the Warburg flask contained 150 to 200 mg of tissue in 3.0 ml of Krebs’-Ringer bicarbonate buffer at pH 7.4 (Davis & Morris, 1963a). The sidearm contained 100 µc of tritiated lysine in a volume of 0.2 ml. The L-lysine-3H was obtained from the Nuclear-Chicago Corp. and had a specific activity of 91 mc/mmole. The final concentration of L-lysine-3H in the incubation flask was 3.4 × 10⁻⁴ M. The gas phase was 95% O₂ and 5% CO₂. At the end of a 1-hr incubation period, the flask was removed from the manometer and the flask contents poured through a 90-mesh stainless steel sieve to collect the testicular slices. The tissue held back by the sieve was washed three times with Krebs’-Ringer buffer and gently placed in cold Carnoy’s solution by means of a forceps.

Preparation of radio-autograms

Following fixation in Carnoy’s solution overnight, the tissue was dehydrated, cleared, embedded in paraffin and sectioned at 5 µ. The sections were freed from paraffin, dipped in molten Kodak nuclear track emulsion type NTB3 and stored in the dark for 2 days. The exposed slides were developed, stained in haematoxylin solution (Harris) and examined. Alternate sections which were not dipped with nuclear emulsion were stained with either haematoxylin alone, haematoxylin and eosin or the periodic acid–Schiff reagent (Leblond & Clermont, 1952).
Grain counting

Grain counting was performed under oil immersion using a Whipple micrometer eyepiece grid. Grains were counted within four squares in the Whipple micrometer and recorded as grains/200 µ². Background counts were made on areas of emulsion not directly over tissues incubated with tritiated lysine and was found to average 4 grains/200 µ². The data have been expressed as the mean number of grains/200 µ² above background ± the standard error over a given area of cells. A total of four individual animals were used in the present experiments.

RESULTS

Plate 1, Fig. 1 demonstrates the three types of seminiferous tubules which are found in the cryptorchid rat testis 30 days after abdominal transplantation and they have been designated in the present report as types A, B and C cryptorchid tubules. Type A tubules contain spermatogonia of the 'crust' variety as described by Leblond & Clermont (1952), primary spermatocytes in the pachytene stage of meiosis and Sertoli cells. Type B tubules contain only spermatogonia of the 'crust' variety and Sertoli cells, with the cytoplasm of the Sertoli cell extending as an apparent syncytium from the basement membrane to the lumen of the seminiferous tubule. Type C tubules contain essentially only Sertoli cells with pleomorphic-appearing nuclei and fibrotic, hyalinized cytoplasm.

The percentage distribution of the three types of seminiferous tubules found in the cryptorchid rat testis 30 days after transference to the abdomen is indicated in Table 1. Type A tubules constitute almost 31% of the total seminiferous tubules of the cryptorchid rat testis. Type B tubules account for 30% of the total seminiferous tubules of the cryptorchid rat testis while the remaining 39% of the tubules consist of Type C tubules. It would therefore appear that the cryptorchid rat testis 30 days after transference of an adult testis from the scrotal sac to the abdominal cavity is composed of approximately equal numbers of each of the three types of seminiferous tubules described in the present experiments.

<table>
<thead>
<tr>
<th>Tubules</th>
<th>Distribution (%)</th>
</tr>
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<tbody>
<tr>
<td>Type A</td>
<td>30.9 ± 2.1</td>
</tr>
<tr>
<td>Type B</td>
<td>30.1 ± 0.7</td>
</tr>
<tr>
<td>Type C</td>
<td>39.0 ± 1.6</td>
</tr>
</tbody>
</table>

A total of 130, 195, 206 and 164 seminiferous tubules were observed in each of the four animals used.

All figures are percentages of total seminiferous tubules ± standard error.
Table 2 presents the percentage distribution of the cell population of these three types of cryptorchid seminiferous tubules. The epithelium of Type A tubules consists of 55% spermatogonia of the 'crust' variety, 8·6% pachytene primary spermatocytes and 36·4% normal-appearing Sertoli cells. The epithelium of Type B tubules consists of 43% 'crust' spermatogonia, a total absence of primary spermatocytes while the remaining 57% of the epithelium of Type B tubules is made up of normal-appearing Sertoli cells. The epithelium of Type C tubules consists essentially of atrophic-appearing Sertoli cells with only 3·6% spermatogonia and no primary spermatocytes or normal Sertoli cells present. These data indicate that the germinal epithelium of the cryptorchid rat testis 30 days after abdominal transplantation is composed of only four cell types, namely, 'crust' spermatogonia, pachytene primary spermatocytes, normal-appearing Sertoli cells and atrophic-appearing Sertoli cells.

Plate 1, Fig. 2 demonstrates representative radio-autograms following incubation of slices of cryptorchid rat testes with tritiated lysine. A very dense labelling pattern was observed over both the spermatogonia and the Sertoli cell cytoplasm in Type A tubules. The labelling of the Sertoli cell cytoplasm in Type B tubules was found to be quite similar to the labelling found over the Sertoli cell cytoplasm present in Type A tubules. However, the atrophic-

**Table 2**

**PERCENTAGE DISTRIBUTION OF CELL POPULATION IN SEMINIFEROUS TUBULES OF THE CRYPTORCHID RAT TESTIS 30 DAYS AFTER ABDOMINAL TRANSPLANTATION**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Type A tubule</th>
<th>Type B tubule</th>
<th>Type C tubule</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Crust' spermatogonia</td>
<td>55·0±0·8</td>
<td>43·0±2·7</td>
<td>3·6±0·3</td>
</tr>
<tr>
<td>Pachytene 1° spermatocytes</td>
<td>8·6±1·1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Normal Sertoli cells</td>
<td>36·4±1·4</td>
<td>57·0±3·0</td>
<td>0</td>
</tr>
<tr>
<td>Atrophic Sertoli cells</td>
<td>0</td>
<td>0</td>
<td>96·4±0·3</td>
</tr>
</tbody>
</table>

The number of cells were determined in approximately twenty-five seminiferous tubules of each type for each of the four animals used.

All figures are percentages of total tubule cells ± standard error.

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**EXPLANATION OF PLATE 1**

**FIG. 1.** Representative photomicrographs of the three types of seminiferous tubules found in the cryptorchid rat testis 30 days after abdominal transplantation of an adult testis. The tissues were sectioned at 5 μ and stained with haematoxylin and eosin (magnification x 700). A. Type A tubule containing 'crust' spermatogonia, pachytene primary spermatocytes and normal-appearing Sertoli cells. B. Type B tubule containing 'crust' spermatogonia and normal-appearing Sertoli cells. C. Type C tubule containing essentially only atrophic-appearing Sertoli cells with pleomorphic nuclei and fibrotic, hyalinized cytoplasm.

**FIG. 2.** Representative radio-autograms following incubation with tritiated lysine of the three types of seminiferous tubules found in the cryptorchid rat testis 30 days after abdominal transplantation of an adult testis (magnification x 700). A. Type A tubule demonstrating a dense labelling pattern over 'crust' spermatogonia and the Sertoli cell cytoplasm. B. Type B tubule demonstrating a labelling pattern over the Sertoli cell cytoplasm which is similar to that seen in Type A tubules. C. Type C tubule demonstrating a marked decrease in labelling over the cytoplasm of atrophic Sertoli cells.
PLATE 1

(Facing p. 128)
appearing Sertoli cells found in Type C tubules were characterized by a marked decrease in the uptake of tritiated lysine into cellular protein.

Table 3 presents the radio-autographic incorporation of tritiated lysine into each of the remaining cells of the cryptorchid rat testis 30 days after transference transplantation of the testis to the abdomen as compared to those cells found in the normal, scrotal testis. The data are presented as grains/200 µ² above background over each of the designated cell types. The induction of cryptorchidism was found to produce an approximate four-fold increase in protein labelling of both 'crust' spermatogonia and pachytene primary spermatocytes. However, the induction of cryptorchidism was also found to result in a forty-fold increase in protein labelling from tritiated lysine in the normal-appearing Sertoli cells of both Type A and Type B cryptorchid tubules. Protein labelling from tritiated lysine in the atrophic-appearing Sertoli cells found in Type C cryptorchid tubules was, however, observed to be almost one-third that which was seen over the normal-appearing Sertoli cells of Types A and B cryptorchid seminiferous tubules.

**DISCUSSION**

Unilateral cryptorchidism has been induced in adult rats by transplanting a mature testis from the scrotal sac into the abdominal cavity. Following exposure to an abdominal environment for 30 days, three distinct types of seminiferous tubules were found to occur in approximately equal numbers in the cryptorchid testis based on the cellular composition of their germinal epithelium. Only four cell types were found to remain in the germinal epithelium of the abdominal testis 30 days after the experimental induction of cryptorchidism. These cell types were 'crust' spermatogonia, pachytene primary spermatocytes, normal-appearing Sertoli cells and atrophic-appearing Sertoli cells, accounting for 31%, 3%, 28% and 38%, respectively, of the total cells remaining in the germinal epithelium of the seminiferous tubules of the abdominal testis.
A comparison of the in-vitro capacity to incorporate a radioactive amino acid into protein of the remaining cells of the germinal epithelium of the cryptorchid rat testis with the corresponding cells found in the scrotal testis has been carried out. Lysine was chosen as a particularly useful amino acid for this purpose inasmuch as lysine does not readily undergo transamination and has been found to produce negligible amounts of tricarboxylic acid cycle intermediates during the course of its catabolism (Davis & Morris, 1963b). The data of the present experiments indicates that the experimental induction of cryptorchidism results in a forty-fold increase in the in-vitro incorporation of tritiated lysine into protein of the normal-appearing Sertoli cells of the abdominal testis and a twelve-fold increase in the in-vitro incorporation of tritiated lysine into protein of the atrophic-appearing Sertoli cells of the abdominal testis as compared to protein labelling of the Sertoli cells found in the corresponding scrotal testis of the same animal. In addition, cryptorchidism was found to produce a four-fold increase in protein labelling of both 'crust' spermatogonia and pachytene primary spermatocytes as compared to the corresponding cells found in the scrotal testis.

Little is known concerning the function and metabolic activity of the Sertoli cell, especially in cryptorchidism, other than their involvement in the phagocytosis of both dead germ cells (Clegg & Macmillan, 1961) and the residual bodies discarded by maturing spermatids (Kingsley Smith & Lacy, 1959; Firlit & Davis, 1965). Teperman, Teperman & Dick (1949) have attempted to relate an increase in oxygen uptake by the cryptorchid testis of the rat in vitro to a possible persistence of the Sertoli cells in the germinal epithelium following destruction of some of the other testicular cell types. Clegg (1963a) has reported that the induction of artificial cryptorchidism in the rat results in a significant increase in the number of Sertoli cells with the cytoplasm of the Sertoli cell becoming more prominent as the maturing cells of the seminiferous epithelium disappear. Inasmuch as no mitoses were seen in the Sertoli cells, the possibility of amitotic division occurring in the Sertoli cells was offered as a partial explanation for the apparent increase in Sertoli cells in cryptorchidism. The fact that cryptorchidism also results in an increased incorporation of radioactive lysine into the Sertoli cell cytoplasm as observed in the present studies further suggests that amitosis may occur.

The data of the present experiments indicate that the previously-reported increase in labelling of perchloric acid-precipitable protein from $^{14}$C-labelled lysine in the cryptorchid rat testis in vitro has been confirmed on a cellular level employing radio-autographic techniques and tritiated lysine. In addition, a comparison of the capacities of the remaining cells of the germinal epithelium of the cryptorchid rat testis to incorporate tritiated lysine into protein indicates that the greatest enhancement of protein labelling due to an increased abdominal temperature occurs in the Sertoli cells.

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Radio-autographic protein labelling in cryptorchidism

REFERENCES


