THE INFLUENCE OF A CRYPTORCHID MILIEU ON THE INITIATION OF SPERMATOGENESIS IN THE RAT

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Summary. The influence of a cryptorchid milieu on the initiation of spermatogenesis was studied, utilizing the technique of transplantation of newborn rat testes into cryptorchid testes of adult hosts. The histological changes in the host and the transplanted testes were evaluated at various periods after transplantation. The transplant showed progressive growth and differentiation of the germ cells up to mid-pachytene stage within 30 days of transplantation into the cryptorchid testes. Thereafter, further progression of spermatogenesis did not occur and the germ cells gradually degenerated. Within 60 days, the seminiferous tubules in the transplant were lined by a layer of Sertoli cells and were comparable in appearance to the host tissue. In another group of animals where the cryptorchid testis was returned to the scrotum at the time of transplantation, both initiation and completion of spermatogenesis were observed. The results indicate that a cryptorchid milieu does not interfere with the initiation of spermatogenesis, but prevents its progression and maintenance.

INTRODUCTION

In spontaneously occurring cryptorchidism in mammals, the germinal epithelium is essentially absent and the testes fail to produce spermatozoa. When a normal adult testis is surgically translocated into the abdomen, the germinal epithelium promptly degenerates. The details of structural changes in the seminiferous epithelium and their temporal sequence have been extensively documented (Moore, 1924; Nelson, 1951; Steinberger & Nelson, 1955; Clegg, 1963; Niemi & Korman, 1965). Little attention, however (Davis & Firlit, 1966) has been paid to the effect of intra-abdominal milieu on the process of initiation of spermatogenesis.

There is a considerable body of evidence suggesting that the initiation of spermatogenesis in an immature testis differs from maintenance in its physiological requirements (Steinberger & Steinberger, 1969). It has been shown by Turner & Asakawa (1963) and Steinberger & Steinberger (1967) that a testis from newborn rats transplanted into a normal rat testis continues its development, the seminiferous epithelium differentiating and producing spermatozoa.

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We utilized the transplantation technique to investigate the effect of a cryptorchid milieu on the initiation of spermatogenesis in the testes of newborn rats.

MATERIALS AND METHODS

Long Evans hooded rats, bred and maintained in our laboratory, were used. Adult rats, weighing about 250 to 300 g, served as recipients for the transplants.

Two sets of experiments were set up. In one, the testes of newborn rats were transplanted into the testes of recipients rendered cryptorchid 15 days earlier. The 15-day interval was selected because by this time most of the germinal epithelium cells are absent from the cryptorchid testis (Steinberger & Nelson, 1955), and the gonadotrophins are already elevated (Steinberger & Duckett, 1967). In the second group of experiments, after a 30-day sojourn in the abdomen, the testes of host animals were replaced in the scrotum and at that time testes from newborn rats were transplanted.

Surgical procedure

The animals were rendered cryptorchid through an abdominal incision, the testes being anchored to the dorsolateral wall with a suture placed in the epididymal fat pad. The transplantation procedure was performed by two individuals. One prepared the host animal by exposing the intra-abdominal testis, while the other removed the testis from a newborn rat and placed it into the open end of a 17-gauge trocar. The trocar was inserted into the host (cryptorchid) testis through the tunica albuginea. The tip of the trocar was then guided under the tunica albuginea as far as possible from the site of insertion and the donor testis was deposited within the host testis. The utmost care was taken to avoid any further injury to the recipient or the donor testis during the transplantation procedure. After transplantation, the host testis was either allowed to remain anchored to the abdominal wall, or it was released from the anchorage and returned to the scrotum. Animals were killed at various time intervals after transplantation. The testes were fixed in Bouin's solution and processed, utilizing conventional histological techniques. Sections were cut at 4 μ m and stained with PAS-haematoxylin.

RESULTS

The growth of the transplant in the host testis showed considerable variation among animals within the same experimental group. In some instances, excessive leucocytic infiltration of the parenchyma was observed; in others, connective tissue growth obliterated the transplant. Spermatogenic activity within the transplant also varied so that only 30% of the specimens showing minimal leucocytic infiltration or fibrotic changes were utilized for detailed histological studies. However, in cases where the host testis was returned to the scrotum at the time of transplantation, more than 70% of the transplants showed uncomplicated growth and differentiation.

Differentiation of newborn testis within a testis of a cryptorchid host

Ten days after transplantation. The interstitial tissue appeared normal, the seminiferous tubules were comparable in size to those found in 10-day-old rats,

and were lined by supporting cells, type A spermatogonia, intermediate spermatogonia and a few primitive type A spermatogonia. Numerous mitotic figures were observed. In a few tubules, newly formed type B spermatogonia were present. The spermatogenic activity in the transplant was similar to that found in a testis of a 6- to 8-day-old rat. The testis of the host showed the typical picture of cryptorchidism. The seminiferous tubules were lined by a layer of Sertoli cells, and only a few scattered type A spermatogonia could be observed. A 10-day growing implant in an atrophic host testis is shown in Pl. 1, Fig. 1.

Twenty days after transplantation. The tubules showed a further increase in diameter. In most tubules, type A and intermediate spermatogonia were present; in some, type B spermatogonia and resting spermatocytes were observed. In a few tubules, spermatogenesis had proceeded up to the early pachytene stage of the primary spermatocytes (Pl. 1, Fig. 2). The progression of spermatogenesis was similar to that found in a 13- to 14-day-old rat; however, the total number of cells was considerably smaller.

Thirty days after transplantation. In this group of animals, the transplants showed maximum growth and differentiation of the seminiferous tubules. Typical adult type Leydig cells were present in the interstitial areas and there was a further increase in the diameter of the seminiferous tubules. According to their appearance, the seminiferous tubules could be divided into three groups: (1) tubules lined by Sertoli cells and spermatogonia, mostly type A and intermediate, with a few type B spermatogonia (Pl. 1, Fig. 3), (2) tubules showing a layer of spermatogonia, as well as young primary spermatocytes in resting, leptotene and occasionally early pachytene stages (Pl. 1, Fig. 4), (3) tubules showing several layers of germinal cells and a large number of free cells in the lumen. Many tubules contained two generations of primary spermatocytes. The differentiation of pachytene spermatocytes proceeded up to the mid-pachytene stage (Pl. 1, Fig. 5). The host testes showed completely atrophic seminiferous tubules lined with Sertoli cells and very few abnormal type A spermatogonia.

Forty days after transplantation. The spermatogenic process in the transplant failed to proceed beyond pachytene spermatocytes. Actually, fewer tubules contained spermatocytes as compared with 30-day transplants; many tubules showed degenerating pachytene spermatocytes (Pl. 2, Fig. 6). The Leydig cells appeared to be numerous and well differentiated. The host tissue showed the typical cryptorchid picture.

Sixty days after transplantation. The seminiferous tubules within the transplant were lined mostly by Sertoli cells. Scattered degenerating germinal cells were seen only in a few tubules. The Leydig cells appeared morphologically normal (Pl. 2, Fig. 7).

Differentiation of newborn testis transplanted into cryptorchid adult testes which were replaced within the scrotum at the time of transplantation

In this series of experiments, the host testes were allowed to remain in the abdomen for 30 days before the transplantation procedure. At the time of transplantation, they were replaced in the scrotum. Samples obtained during the first 30 days after transplantation revealed a picture similar to that observed

in transplants which remained cryptorchid (see above). However, at a later time interval a marked difference was observed.

Forty-five days after transplantation. The transplant was large, occupying more than 75% of the area of the host testis. The transplanted testis was separated from the host parenchyma by a well defined tunica albuginea. The diameter of the seminiferous tubules was comparable to that of a normal adult testis. More than 50% of the tubules in the transplanted testis showed active spermatogenesis (Pl. 2, Fig. 8). Spermiogenesis reached up to step 14; spermatids at steps 15 to 19 were absent from tubules at stages I to VIII (Pl. 2, Fig. 9). A small number of tubules showing atrophic changes were lined by a layer of Sertoli cells and a few scattered type A or intermediate spermatogonia. The Leydig cells appeared normal.

The parenchyma of the host testis showed patchy areas of regeneration, only a few tubules showing reinitiation of spermatogenesis and progression up to spermatids.

DISCUSSION

The degenerative changes occurring in germinal cells of artificially cryptorchid testes have been described in detail (Moore, 1924; Nelson, 1951; Davis & Firlit, 1966). Abnormalities in the seminiferous epithelium appearing within 2 days of translocation of adult testes into the abdomen have been noted (Davis & Firlit, 1966). Abdominal retention for 10 to 15 days resulted in the disappearance of all the spermatids and most of the spermatocytes (Steinberger & Nelson, 1955; Clegg, 1963). When the immature testes of 20-day-old rats were rendered cryptorchid, the pachytene spermatocytes failed to differentiate and degenerated within 30 days of intra-abdominal retention (Davis & Firlit, 1966).

These observations indicate that both the spermatids, as well as the primary spermatocytes, are readily damaged by the cryptorchid state. While the adult and young rat testes show almost complete degeneration of germ cells 30 days

EXPLANATION OF PLATES 1 AND 2

All sections are stained with PAS-Weigert haematoxylin; the arrow denotes demarcation between the host and the transplant rat testis tissues.

PLATE 1

FIG. 1. Section of transplant in cryptorchid host-testis 10 days after transplantation. The transplant (T) shows growth and differentiation with numerous spermatogonia. The host (H) testis shows tubules lined only with Sertoli cells. $\times 250$.

FIG. 2. Section of transplant in cryptorchid host-testis 20 days after transplantation. The tubules show marked increase in diameter and further differentiation of the germ cells up to the early pachytene stage (P). The host tissue (H) consists of degenerated atrophic seminiferous tubules lined by Sertoli cells. $\times 250$.

FIG. 3. Section of transplant in cryptorchid host-testis 30 days after transplantation. The tubules of the transplant are lined with a layer of Sertoli cells and few spermatogonia (A). $\times 250$.

FIG. 4. Section of transplant in cryptorchid testis 30 days after transplantation. The tubules are lined by a basal layer of Sertoli cells, spermatogonia and a layer of primary spermatocytes at the leptotene (L) and zygotene stages. $\times 250$.

FIG. 5. Appearance of the transplant 30 days after transplantation. The tubule shows two generations of primary spermatocytes at the leptotene (L) and pachytene (P) stages. $\times 250$.

PLATE 1

(Facing p. 176)

FIG. 6. Section of transplant in cryptorchid testis 40 days after transplantation. The tubules show atrophy of the seminiferous epithelium. Most of the germinal cells are pycnotic. $\times 250$.

FIG. 7. Section of transplant in cryptorchid host-testis 60 days after transplantation. The transplant tissue (T) shows complete absence of germinal cells. The host-tissues (H) show typical atrophic tubules. $\times 180$.

FIG. 8. Section of transplant in cryptorchid host testis returned to scrotum at the time of transplantation. The tissue was examined 45 days after transplantation; the transplant (T) showing normal spermatogenic activity in more than 50% of the tubule. $\times 150$.

FIG. 9. Higher magnification of a 45-day transplant showing two atrophic tubules of the host-testis (H), and two tubules of the transplant (T), one showing stage VI of spermatogenesis but absence of step-18 spermatids. $\times 250$.

after surgery (Davis & Firlit, 1966), the testes of newborn rats transplanted into cryptorchid testis showed a maximal degree of differentiation during this period of time and produced late stages of pachytene spermatocytes. This apparently paradoxical observation can be explained only by assuming that there is a difference between the physiological requirements for the process of initiation of spermatogenesis in the testis of newborn animals and for its maintenance in immature or adult rats.

A suggestion that this may be the case has been made in the past (Steinberger & Steinberger, 1969), and a considerable amount of supportive evidence exists in the literature. The first wave of spermatogenesis requires less time for its completion than do the subsequent waves (Huckins, 1965). The hormonal requirements for the initiation of spermatogenesis are different from those required for its maintenance (for review of this, see Steinberger, 1971). The present finding that the higher ambient temperature and altered hormonal milieu of the cryptorchid host testis did not interfere with the initiation of spermatogenesis in the transplants of newborn testes further supports the hypothesis that the first wave of spermatogenesis differs from that of the subsequent waves.

In studies where the cryptorchid host testes were returned to the scrotum at the time of transplantation, the cell differentiation in the transplant during the first 30 days was comparable to that obtained in transplants into the cryptorchid testis. Thereafter, the cell differentiation in the transplant residing in a scrotal environment proceeded normally. The milieu of the cryptorchid testis replaced into the scrotum supported the initiation of spermatogenesis, as well as completion of the process in the transplant, in spite of the fact that the host testis remained mostly atrophic. This finding suggests that the lack of uniform regeneration in the host testis replaced into the scrotum is, most likely, due to destruction of the stem cells under the cryptorchid condition rather than to changes in the hormonal milieu.

The delay in the differentiation of germ cells in the transplant was uniform in a cryptorchid or scrotal environment. Turner & Asakawa (1963) also reported such a delay in the onset of spermatogenesis when newborn rat testis was transplanted in a normal adult host testis. This delay seems to represent the time taken for the transplant to establish a proper vascular supply. It is probable that during this interim period, many stem cells degenerate and differentiation is restricted to a limited part of the transplant.

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