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Complete Spermatogenesis in Intratesticular Homotransplants of Fetal and Neonatal Testes in the Rat. (27971)

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It is well known that mammalian testes from fetal and neonatal donors, in contrast to the adult organ, are readily transplantable and that they generally are subnormal with respect to hormone production and germ cell proliferation(1-4). If rejection by the host does not occur, subcutaneous, intramuscular and intraperitoneal transplants may persist for long periods but, being subjected to temperatures higher than those of the scrotum, they are equivalent to cryptorchid testes. Completion of spermiogenesis, with the accumulation of mature spermatozoa, has been reported only in grafts residing in sites providing temperatures approximating those of the scrotum: *viz.*, in postnatal testes sutured to the lining of the scrotum(3,5,6) and in neonatal testes persisting in the anterior chamber of the eye(7). Certain transplantation sites, including the testis, brain, anterior eye chamber, and cheek pouch of the hamster, have been designated "immunologically privileged" environments. Some factor seems to operate in these areas which reduces the capacity of the host for immunological response, permitting prolonged survival of the grafts, and it is possible that the peculiar anatomy of the lymphatic drainage from these areas may be an important consideration(8, 9).

Experiments were designed to test the interior of the testis as a transplantation site for gonads, and to compare the onset and incidence of immunological rejection of transplants from the same donor residing simultaneously in kidneys and testes of the recipient.

Material and methods. Randomly bred rats of the Sprague-Dawley strain were obtained from Hormone Assay Laboratories, Chicago. The 494 grafts recovered for this study are part of the controls being used in another project on experimental induction of actively acquired tolerance. A total of 222 *pairs* of testes from 15- and 16-day fetuses, and 34 *pairs* from neonatal animals were transplanted to the kidneys and testes of normal and unilaterally orchiectomized hosts. As a control procedure, one gonad from each donor was inserted beneath the kidney capsule and the contralateral one was placed under the tunica albuginea of the corresponding testis of the same host. The recipients were healthy adult males ranging in weight from 182 to 376 g.

Fetuses were removed from the pregnant mother under light nembutal anesthesia. Gonads were quickly excised and placed in a Ringer-phosphate solution and carefully freed from all adnexa by means of a fine needle-knife. The host testis was delivered through an abdominal incision, and the donor tissue inserted near the cranial pole by means of a cannula introduced through the opposite pole. Care was taken to prevent extensive hemorrhage within the testis or damage to the rete testis and ductuli efferentes. It was found to be unnecessary to suture the tunica albuginea. Transplants were introduced immediately below the tunica since this facilitated recovery. To prevent epididymitis and other infections, a standard procedure of administering 5000 units of penicillin and 5 mg of streptomycin daily for one week following operation was adopted.

Results. Table I indicates that all trans-

TABLE 1. Testicular Homotransplants Residing in Kidneys and Testes of Adult Hosts.

Site	Age of donor testes	No. inserted	No. recovered	No. and % rejected after 21 days*
Kidney	15-day fetuses	104	104	29 (31.18%)
"	16-day fetuses	118	118	34 (33.72%)
"	Newborn	34	34	8 (23.52%)
Testis	15-day fetuses	104	95	26 (30.95%)
"	16-day fetuses	118	102	28 (31.81%)
"	Newborn	34	34	8 (23.52%)

* Grafts rejected after 21 days:
15-day kidney *vs* testis grafts: t=.03
16-day " " " " : t=.27

plants placed in the kidneys and most of those introduced into the testes were recovered. The 256 grafts remaining in the kidneys for 10-60 days varied in histological appearance. Some showed only traces of inactive seminiferous tubules, often widely separated by invading connective tissue and heavily infiltrated with lymphocytes; others were composed of many compact tubules in which early stages of spermatogenesis were apparent. The best grafts after 60 days had some tubules lined by 3 to 6 layers of cells containing identifiable spermatocytes, but cells nearest the lumina were undergoing varying degrees of pycnosis, sloughing and dissolution. In other tubules of the same grafts, the epithelium was reduced to a single layer consisting of Sertoli cells and possibly a few spermatogonia (Fig. 1). While no attempt was made to measure the Leydig cells, they did not seem to differ appreciably from those of a normal testis. In 2 of the kidney grafts removed on day 50, early spermatid stages were identifiable. Acrosome stages and mature spermatozoa were never encountered in grafts from this site. This is in agreement with other workers(1-4).

Of the 256 fetal and neonatal testes transplanted under the tunica albuginea of adult recipients, 231 were recovered at intervals from 10-60 days. Ninety-one grafts were allowed to remain in the host for 60 days, and 65 (71%) of these survived without histological signs of host rejection. The grafted testes were enclosed in their own tunicae and these clearly delimited them from the surrounding host tissues (Fig. 2). The 65 intratesticular grafts recovered at 60 days were quite uniform in appearance; approximately half of the tubules in each graft contained either late spermatids or mature spermatozoa, and the cells of Leydig did not appear to differ structurally or numerically from those of the host testis (Fig. 2, 3). All the grafts contained some degenerate tubules with reduced epithelia consisting of Sertoli cells and possibly a few spermatogonia.

Seven viable intratesticular grafts were examined after remaining in the host for 5 months. Except for size, these did not differ

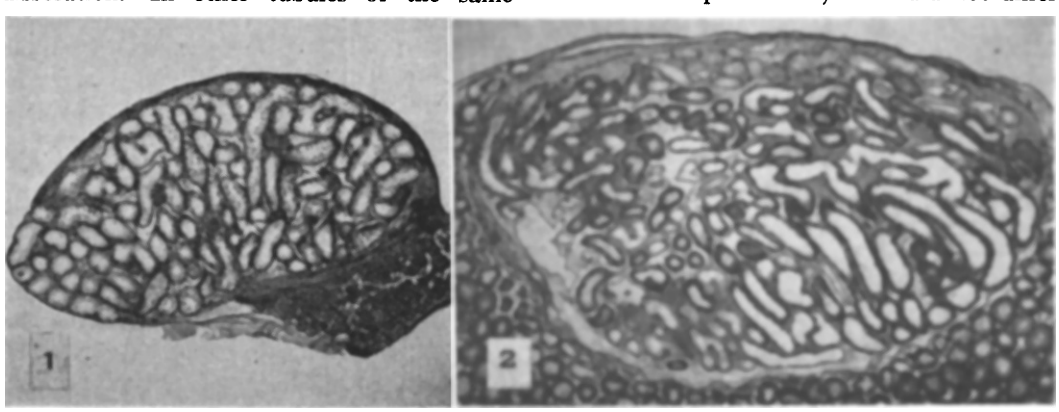

FIG. 1. Testicular graft from a 15-day fetus following persistence for 60 days under the kidney capsule of an adult unilaterally orchiectomized host. 10×.

FIG. 2. Contralateral testis of same donor following persistence within the testis of same host for same length of time. 10×.

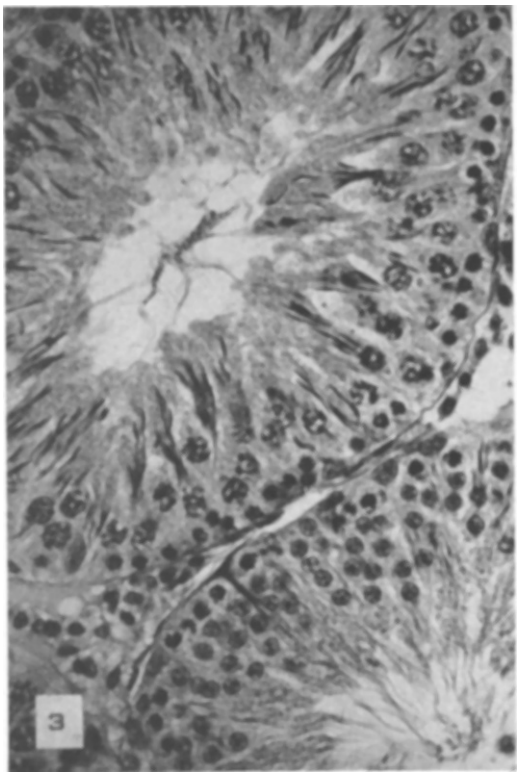

FIG. 3. Small area of intratesticular graft shown in Fig. 2. These seminiferous tubules are normal and contain spermatozoa. 300×.

essentially from those recovered at 60 days; some tubules appeared healthy and were proliferating spermatozoa, whereas others were atrophic. In unilateral orchiectomized hosts, the 5-month grafts occupied approximately one-fourth of the host testis. Grafts of this age, residing in intact hosts, were not so large but were histologically comparable.

Embryonic tissues possess little antigenicity and must undergo a period of aging before they elicit host rejection phenomena(9, 13). Histological indications of rejection were not observed in any of the transplants from fetal donors prior to day 21. Grafts were not recovered frequently enough to determine the exact onset of rejection, but the critical period seems to lie between day 21 and day 40.

Mild or extreme degrees of host rejection were observed in 26 (29%) of the 91 intratesticular transplants recovered on day 60, and no stages of spermiogenesis were found; all tubules were degenerate and many of them appeared to be occupied only by a Sertoli syncytium. The first visible indications that the host was rejecting the grafts were peripheral fibrosis and infiltration of lymphocytes into the tunica of the graft. The seminiferous epithelium was always degenerate by the time large nests of lymphocytes had collected at the periphery of the graft and some degree of connective tissue encapsulation had ensued. Fibrous tissue and lymphocytes eventually infiltrated the intertubular areas and most of the tubules were lost or persisted as calcified areas. In nearly every instance where host rejection was observed in the kidney graft, a comparable picture of fibrosis and lymphocyte aggregation was apparent in the contralateral gonad transplanted to the testis of the same host. These results do not indicate that testis transplants have any better chance of escaping homograft rejection when placed within the testis than when introduced below the kidney capsule (Table I).

Discussion. Opportunities for prompt vascularization and the ease of inserting and recovering the tissues are the main advantages of the kidney capsule as a transplantation site(1,10). While many kinds of tissues incorporate and thrive in this position, one could not expect testicular grafts in the kidneys to function more completely than cryptorchid testes. Experimental cryptorchidism impairs both gametogenic and endocrine functions of the testis(11), and it appears that grafts in the kidney never exceed this order of function.

Several kinds of organs are known to function after transplantation to the interior of the testis(12-14). The present results indicate that because of the thermo-regulatory capacity of the scrotum this site is especially advantageous for full gametogenic expression in transplanted mammalian testes. Though the testis is much less vascular than the kidney, the intratesticular grafts typically incorporated without any central necrosis. Completion of spermiogenesis has never been observed in grafts of the testis unless placed in sites which provide temperatures equivalent to those inside of the tunica vaginalis

(3,5,15). The interstitial cells in the compatible testis homografts reported here do not seem to differ histologically from those of the host, but no way has been found to free the graft of host tissue and establish its endocrinological competence.

Transplants incorporated equally well in intact and unilaterally orchiectomized hosts, and there was no evidence of precocious sperm formation in the latter hosts. Very few compatible grafts removed on day 50 contained spermatid stages, but all such grafts recovered at 60 days and at 5 months contained both sperm heads and mature sperm. It is generally stated that spermatid maturation is completed at approximately 35 days of age in common strains of the laboratory rat, and this suggests that spermiogenesis may have been delayed somewhat in the present transplants.

Intratesticular grafts are completely surrounded by their own tunica albuginea, and the tubules of the graft fail to establish connections with the excurrent duct system of the host. It is probable that the tubules atrophy because of the accumulation of fluid within their lumina, but some may still be able to restore themselves and, eventually, to proliferate another generation of spermatozoa. Most workers agree that ligation of the ductuli efferentes in mammals produces pressure atrophy of the germinal epithelium, whereas ligation of the duct system distal to the epididymis does not. This suggests that the proximal portion of the excurrent duct system of the testis may perform a role in reabsorbing large amounts of fluid from the seminiferous tubules(16,17). While marked accumulations of spermatozoa have never been seen in the grafted testes, it is probable that fluid does accumulate and this, obviously, cannot be drained *via* an excurrent duct system. Peritubular fibrosis, as has been described following ligation of the ductuli efferentes(18), has not been observed in any of the compatible intratesticular grafts containing sperm heads or spermatozoa.

Summary. Fetal and neonatal testes, transplanted to the testes of adult hosts, proliferate mature spermatozoa by 60 days unless prior immunological rejection ensues. Intratesticular grafts of testes are rejected by the host with approximately the same frequency as contralateral testes persisting below the kidney capsule. So far as testis transplants are concerned, the evidence presented does not indicate that the testis is, immunologically, a more privileged site than the kidney.

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