INTERCELLULAR RESERVOIR FORMED BY REGENERATION OF THE TUNICA ALBUGINEA IN THE GUINEA PIG AFTER CASTRATION OR HEMICASTRATION.

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ABSTRACT

Guinea pig males were hemicastrated or castrated by a technique which leaves the tunica albuginea *in situ*. The tunica albuginea was implanted with perforated hollow borosilicate ellipsoids. The tunica albuginea healed with 1-2 weeks and encapsulated the implant. In some cases, buds of tunica vasculosa grew through the perforations in the wall of implants and fused. Encapsulation resulted in the formation of an extra-intercellular reservoir containing interstitial fluid and a network of cords of tunica vasculosa. This preparation may serve for further studies on the rate of movement and concentration of either exogenous or endogenous materials in the blood and tissue fluid.

INTRODUCTION

A different castration technique was developed by McGlinn *et al.* (1976), in which the inside of the testis was removed while the tunica albuginea was left *in situ*. Minimal damage to the testicular excurrent ducts, claimed by previous authors, was confirmed by Shepherd and Martan (1976, 1979) who reported extended longevity and fertilizing ability of epididymal spermatozoa in guinea pig males castrated by this technique. A glass implant was inserted into the tunica albuginea of guinea pig males immediately after castration. The results of this experiment and some potential applications of the developed model are reported in this article.

METHODS

Nineteen sexually mature male guinea pigs (SR/Siuz), from a colony in the SIUC Vivarium, were used in this study. The animals were maintained as previously described (Martan, 1966; Martan and Shepherd, 1973). Animals were anesthetized with Metofane (Methoxyflurane), Pittman-Moore, N.J., and the testis(es) removed by the technique reported by McGlinn *et al.* (1976), with the exception that a flame-heated dissecting needle was used to cauterize the connection of the seminiferous tubules with the mediastinum testis. Following

castration, the dense fibrous tunica albuginea lined with the loose, richly vascularized tunica vasculosa remained attached to the intact ducts, nerves, and blood vessels. Glass, hollow ellipsoids (Fig 1a) with perforated walls were then surgically implanted in the empty tunics.

The glass implants measured approximately 1.7 cm x 1.0 cm and had a capacity of approximately 0.4 ml. They were customized in the Research Glassblowing Shop at SIUC from borosilicate. Prior to implanting, the glass was sterilized and rinsed in Tyrode Solution (Difco Laboratories).

Implantation was performed by stretching the cut edge of the tunica albuginea over the ellipsoid using fine forceps and suturing the tunica close with 4-0 silk thread. Implants were placed either bilaterally (6 males) or unilaterally (13 males). Two to 4 sutures spaced 2-3 mm were made to retain the implant and facilitate encapsulation. The musculature of the scrotal pouch and skin were sutured separately, and the closed incision cleaned externally with 95% ethanol. The animals were then returned to individual cages for one to several weeks and euthanatized.

The animals were sacrificed by overdosing with ether and the scrota opened (Fig 2). The general condition of the healed tunica albuginea was observed and the fluid content of the implant was aspirated and measured, using a hypodermic syringe fitted with a gauge 22 needle. Most fat was removed and the epididymis along with the tunica was fixed in Baker's Formol for histological observation. In some instances, the implant was removed prior to fixation and in others after fixation.

Tissues were processed by standard paraffin methods and sectioned at 8 μm. Sections were stained using Mallory's method for collagenic fibers and Gomori's aldehyde fuchsin method for elastic fibers (Lillie, 1965).

RESULTS AND DISCUSSION

Within 1-2 weeks after the operation, the tunica albuginea was completely healed (Fig 1b) and was filled with interstitial fluid containing blood. After 5-6 weeks, each implant contained 0.3 - 0.4 ml of straw-colored interstitial fluid free of blood. By 4-5 weeks post-operation, small buds of connective tissues from the tunica vasculosa had grown through the openings in the wall of the implant. In some cases, these buds elongated and fused in the center of the implant's cavity (Fig 3). The entire ingrowth histologically resembled the tunica vasculosa. It was richly vascularized and contained fibroblasts, and both collagenous and elastic fibers. In a few males, the inner scrotal wall adhered to the tunica albuginea. In one case, the distal tip of the seminal vesicle on the ipsilateral side of the implant adhered to the tunica albuginea. In one male euthanatized 6 weeks post-operation the implant was filled with pus rather than interstitial fluid.

No differences in healing of the tunica albuginea, growth of the tunica vasculosa, and amount of tissue fluid in the "glass testis" were observed between castrated and hemicastrated male guinea pigs. These observations suggest that testicular hormones were not involved. Preliminary experiments with laboratory rats have shown similar results.

The hollow glass implant encapsulated by the tunica albuginea (Figs 1b, 2) provides an extra-intercellular reservoir from which tissue fluid can be collected with a hypodermic needle through the scrotal wall or in the same manner,

hormones and other pharmacological agents introduced. Therefore, this technique may be used for studies involving the rate of movement of materials administered to the animal either orally or by injection, or to determine the differences in concentration of exogenous or endogenous materials between the blood and tissue fluid, by providing easy access to the tissue fluid for analyses. In addition, this technique may be applicable in cosmetic surgeries in cases of accidental injury to the testis or testicular tumors in man or domestic animals.

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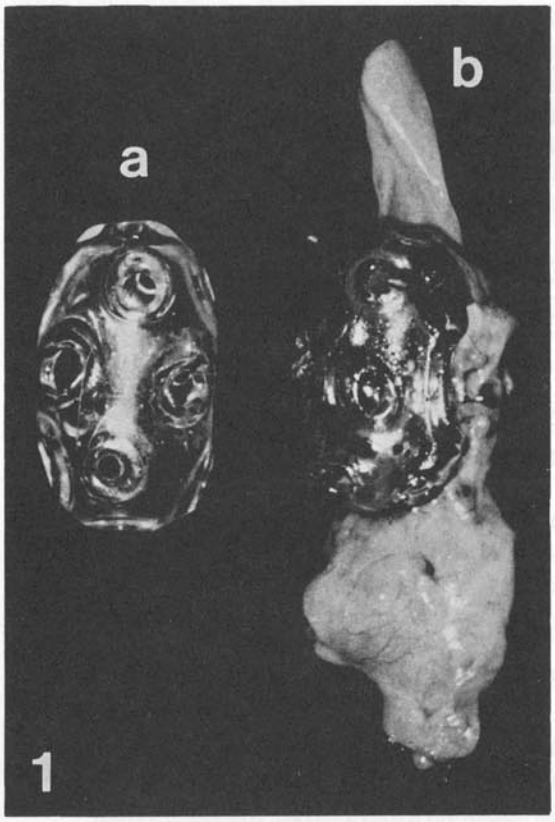
Fig 1a. Borosilicate implant with perforated wall. X 2.5

Fig 1b. Dissected tunica albuginea with attached epididymis and fat, encapsulating a glass implant. X 2.5

Fig 2. Encapsulated inplant *in situ* exposed by dissection of the skin and muscle wall of the scrotal pouch.

Fig 3. Section of the tunica albuginea with the cauda epididymis (right) attached, containing a fused ingrowth of tunica vasculosa (center) which grew inward through perforations in the wall of the implant. X 4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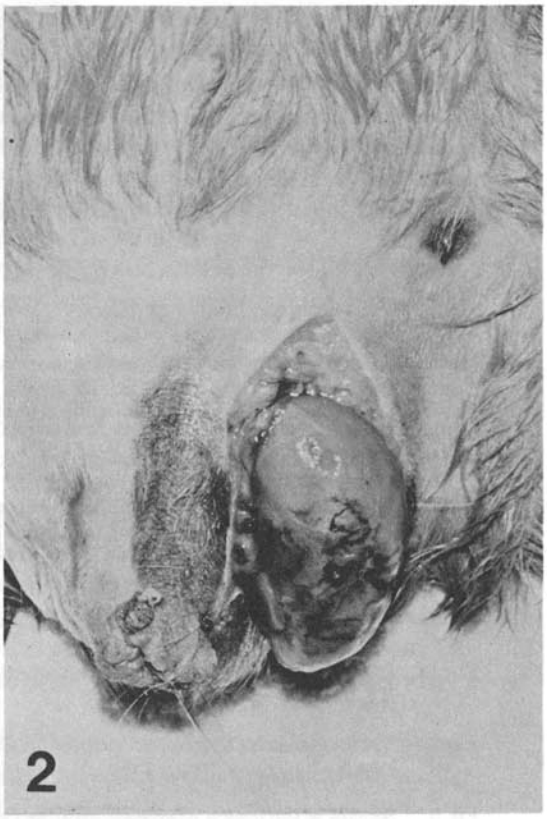
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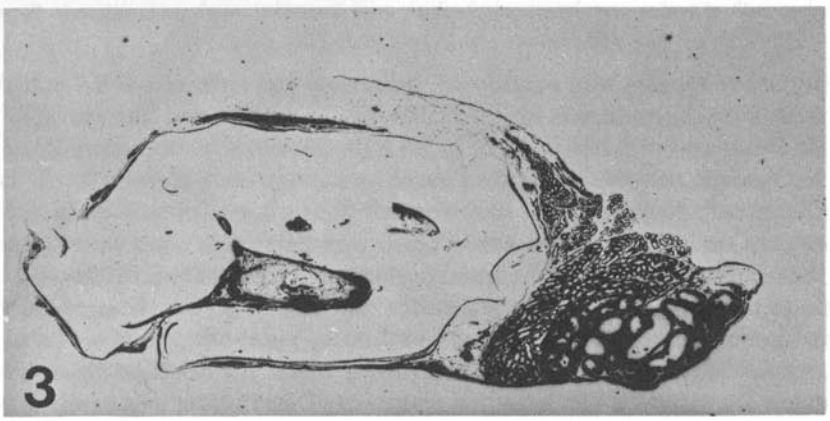
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