Functional Survival of Ovarian Homografts within Millipore Filter Chambers in the Castrate Rat.* (23015)

SOMERS H. STURGIS AND HECTOR CASTELLANOS. (Introduced by R. O. Greep)
Peter Bent Brigham Hospital, Boston, Mass.

Homografts in rats are known to survive and function unless the animals have been immunized by previous transplants. After such immunization, grafts become rapidly replaced by fibroblasts and soon nothing but a scar is left. Algire and associates(1) however, found that when they excluded the host's cells from contact with their transplants by embedding the latter between two special filters, they obtained satisfactory survival. For this purpose they used millipore filters† with pore sizes of 0.45 and 0.8 μ. Mammary carcinoma transplants with a survival of only 5.8 days in immunized hosts were shown to be viable at least 60 days after transplantation in such a system.

This suggested to us the possibility that homografts of endocrine glands in primates might perhaps be protected from destruction if similar filters were used to prevent leucocytic infiltration and vascularization. To maintain function as well, the filters would have to allow the inward passage of tropic hormones from the pituitary and the outward elimination of secretions from the grafted transplants. This is a preliminary report with ovarian homografts in the non-immunized rat.

Material and methods. Millipore filters are made of intermeshed cellulose ester filaments having extremely uniform pore size that are manufactured in various dimensions. Chambers for the grafts were constructed similar to those used by Algire, by cutting thin cross-sections from a ½ inch lucite tube. A section of the filter membrane was cut and glued with Duco cement to one side of a cross-section, and then after sterilization in ethylene oxide gas, 2 such sections were glued together after inclusion of a desired piece of fresh tissue under aseptic conditions. Preliminary tests showed that pituitary gonadotropins contained in urine, and plasma‡ of a young human castrate could be drawn by suction through the HA millipore filter (pore

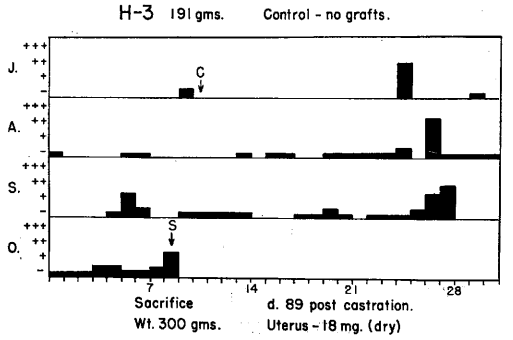

FIG. 1. Cornification of vaginal smear of castrate control. In 89 days after castration, only occasional and sporadic positive smears (++ or +++) were found, probably due to traumatization. Uterine weight at autopsy 18 mg. C = castration; G = graft; S = sacrifice; J, A, S, O = July, Aug., Sept., Oct.; baseline in days of month.

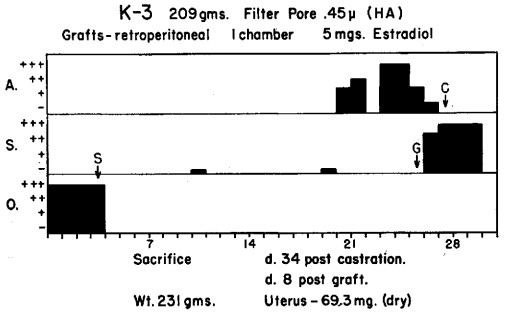

FIG. 2. Vaginal cornification is maintained at full estrus when estradiol crystals are embedded in an HA millipore filter chamber one month after castration. At sacrifice 8 days later the uterus weighed 69.3 mg.

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† Millipore filter membranes were obtained from the Millipore Filter Corp., Watertown, Mass.

‡ Estrogen-free human plasma was contributed through the courtesy of Dr. H. Antoniades of Protein Foundation, Jamaica Plain, Mass. It was assayed after filtration by its effect on uterine weight of immature mice according to standard procedures.

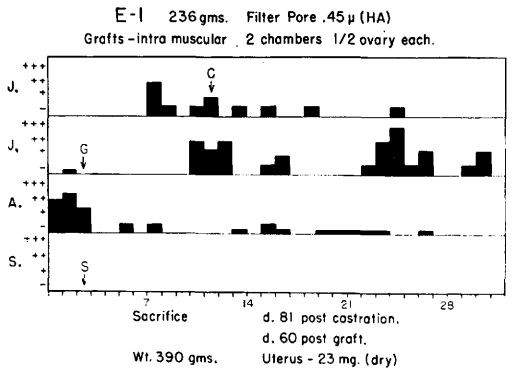

FIG. 3. Cornification is produced only temporarily by ½ an ovary embedded in an HA filter chamber after castration. G = date of grafting.

size 0.45 μ) so we chose this membrane for the experiments below. Adult female albino rats weighing approximately 200 g were observed through one or 2 estrous cycles by daily vaginal smears. Castration was performed and one month later, sections of ovary from another adult rat were introduced within millipore filter chambers in various positions — intramuscular, intraperitoneal and retroperitoneal — and in varying sizes. Vaginal smears were again followed up to about 60 days when the animals were sacrificed, the uteri dissected and their dry weight recorded. In each group of experiments control animals were prepared with no tissue transplants for comparison. Histologic sections stained with H and E were then prepared from the transplants recovered at sacrifice.

Results. Control animals invariably showed the expected atrophic vaginal smear of anestrus. After 60 days, the dry uterine weight ranged from 16-20 mg (Fig. 1). When 5 mg estradiol crystals were enclosed in an HA filter chamber in a castrate, the vaginal smear showed a full estrous effect from the day after operation through the next 8 days, and the uterine weight was maintained at over 60 mg (Fig 2). Two chambers each containing ½ an ovary produced a vaginal smear response suggesting some functional survival for a few weeks, but this dwindled away, and at sacrifice the uterus was again atrophic (Fig. 3).

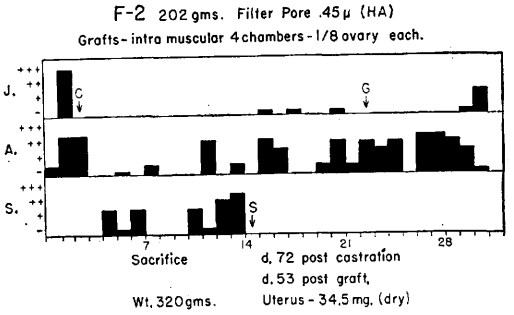

FIG. 4. Homografts of approximately ⅛ of ovary showed some evidence of steroid production in vaginal smears for 53 days when transplanted intramuscularly, and uterine weight at sacrifice was 34 mg.

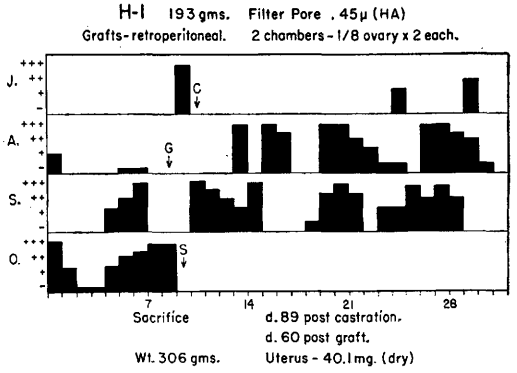

FIG. 5. Transplantation of ⅛ ovary in filter chamber retroperitoneally one month after castration produces cycles of cornification approximating at interval of 4½ days typical for estrous cycle in this animal. Uterus weighed 40.1 mg at sacrifice.

TABLE I. Uterine Dry Weights in Castrate Rats with Filter Chamber Ovarian Grafts.

Preparation	Site	Rat wt	Days P castrate	Days P graft	Filter, pore μ	Uterus wt, mg
Intact		189*				63.5*
H3—control		300	89			18.0
Estradiol	RP	231	34	8	.45	69.3
H2—4 × ⅛ ov.	"	274	89	60	.8	16.0
E1—2 × ½ ov.	IM	390	81	60	.45	23.0
F2—4 × ⅛ ov.	"	320	72	53	.45	34.5
H1—4 × ⅛ ov.	RP	306	89	60	.45	40.1

* Avg.

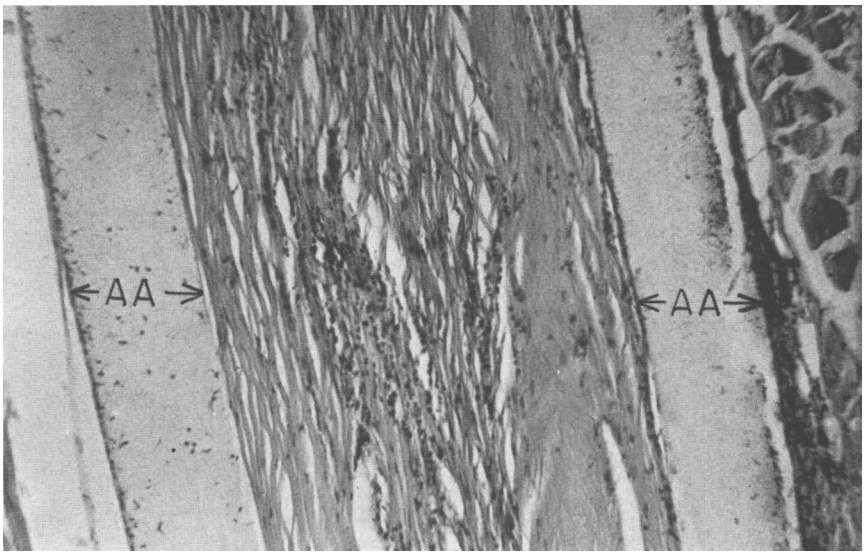

FIG. 6. Section through an AA filter chamber (pore size 0.8 μ) to show membranes on each side infiltrated with cellular debris.

When 1/8 of an ovary was used, the vaginal smear and uterine weight were more satisfactory (Fig. 4). In both the latter experiments the chambers were embedded in the muscles of the back. The best results were obtained with chambers containing 1/8 fragments of ovary placed retroperitoneal. After 60 days a continuing estrous cycle approximating the 4½ days typical for this strain was seen, and the uterine weight showed definite estrogenic support (Fig. 5).

Table I summarizes the dry uterine weights of intact and castrate control rats and the experimental animals recorded in Fig. 1-5.

Histologic sections demonstrate that the AA filter (pore size 0.8 μ) becomes infiltrated with cellular debris (Fig. 6) in contrast to the HA filter (pore size 0.45 μ) which remains clear of cell fragments (Fig. 7). Although ovarian function appeared to be maintained

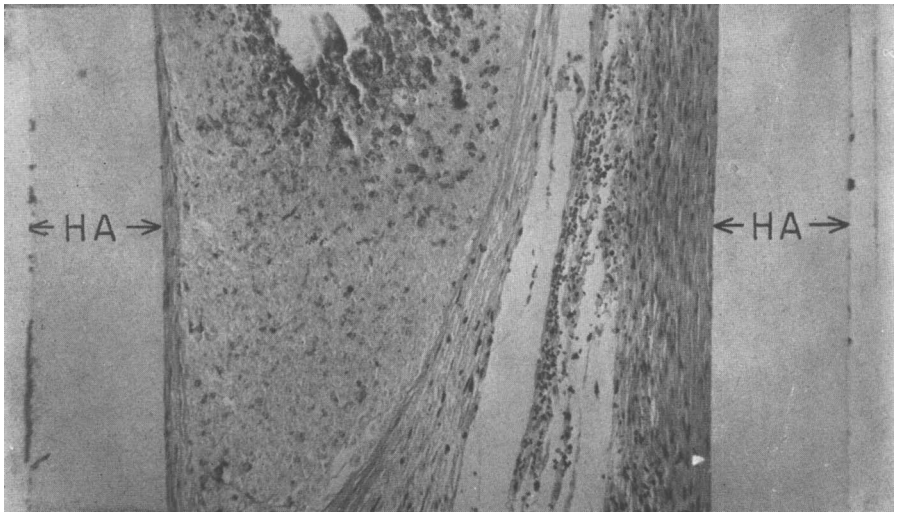

FIG. 7. Section through an HA filter chamber (pore size 0.45 μ) to demonstrate filters on each side entirely clear of cell fragments. Ovarian graft within is non-vascularized; there is no leucocytic infiltration.

in chambers of the latter (Fig. 5) yet the sections in Fig. 7 show a complete degeneration of follicles and ova 60 days after grafting.

Conclusions. Homografts of the rat ovary do not depend on vascularization to maintain the production of estrogenic substances up to 60 days. Semipermeable millipore filter membranes were used to form a chamber in which to embed ovarian transplants in previously castrated recipients. These chambers excluded leucocytes and prohibit vascularization, yet permitted passage of gonadotropic hormones. Vaginal smears and uterine weights demonstrated the production of estrogens from these grafts two months later. The functional survival of similar preparations in the monkey is now under investigation.

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1. Algire, Glenn H., Weaver, J. M., and Prehn, R. T., *J. Nat. Cancer Inst.*, 1954, v15, 493.

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Hypophysectomy and the Tail Darkening Reaction in *Xenopus.** (23016)

JOSEPH T. BAGNARA.† (Introduced by Arthur R. Kemmerer)
Department of Zoology, State University of Iowa.

It has long been known that if the hypophyseal placode is removed from amphibian embryos, the resulting larvae will be very pale in appearance and vary in color in different species from white or silver, to golden yellow(1,2). Pallor generally results from persistent contraction of epidermal melanophores and expansion of both dermal and epidermal guanophores(2). It is therefore surprising to observe that in hypophysioprivic *Xenopus* larvae, melanophores of the tail are capable of considerable expansion or contraction under appropriate conditions. The experiments reported here were designed to study the influence of the hypophysis on the behavior of some of the chromatophores of *Xenopus* larvae, and to gain some insight into the peculiar activity of melanophores in the tail.

Methods. All tadpoles were raised from naturally spawned eggs of *Xenopus laevis* Daudin. At an early tailbud stage, the hypophyseal placode was removed from 378 embryos. Judging from the number of pale larvae resulting, removal of the hypophysis was accomplished in about 85% of the operated animals. Only about 25% of these larvae survived after 2 weeks, because of severe damage to their mouth parts.

Melanophore counts were made in the ventral fin of both normal and hypophysioprivic larvae of the same egg batch. An area about one-third the distance from the anterior margin of the pigmented region of the tail and midway between fin and somites was selected for the counts. These were made using an ocular micrometer on 6 larvae from each group. Under magnification of 45X, melanophores enclosed in a square, one-sixteenth of a square cm in size, were counted for 25 squares and the average number per square was then determined for each tadpole.

Results. In hypophysioprivic larvae a very great reduction in number of melanophores was noted in all regions except the tail, and those that were present were maximally contracted. This was especially true of melanophores present on thymus, nerves, and blood vessels (Fig. 1, 2). In the tail, melanophore number was not greatly influenced by hypophysectomy (Fig. 3, 4, 5). A reduction of about 30% occurred in the number of tail melanophores in hypophysioprivic tadpoles. This is a remarkably small percentage compared with the great reduction observed in other parts of the tadpole.

In normal *Xenopus* larvae, guanophores are

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† Present address: Dept. of Zoology, Univ. of Arizona.