IMPLANTATION, TRANSPLANTATION, AND EPITHELIAL–MESENCHYMAL RELATIONSHIPS IN THE RAT UTERUS

By ALAN E. BEER, M.D. and R. E. BILLINGHAM, F.R.S.

(From the Departments of Medical Genetics and Obstetrics and Gynecology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104)

(Received for publication 20 May 1970)

The implantation of a blastocyst in the endometrium represents nature's principal incursion into the field of transplantation biology. The elucidation of the essential endocrinologic, immunogenetic, vascular and other prerequisites in the uterine milieu for the primary "take" or healing-in, of the blastocyst, its cellular interactions with its "bed," the development of the resultant fetal organismic graft and its ultimate prompt "rejection" at term, plays as central a role in reproductive biology as does the elucidation of analogous parameters for the conventional tissue and organ grafts used in replacement surgery.

As a graft, the fertilized egg is clearly not fastidious in terms of its requirements for implanting and developing more or less normally, at least until it has attained a relatively advanced stage. This is evidenced by the behavior of blastocysts transplanted to a variety of ectopic sites including the spleen, the kidney, the brain, the cryptorchid testis, the anterior chamber of the eye, and the mesentery (1–6). By contrast, the endometrium, the natural recipient site for blastocysts, is much more discriminating, as indicated by the relatively short time in the reproductive cycle at which implantation is possible, the apparently restricted distribution of potential "recipient" sites over the endometrial surface, and the fact that the stage of maturation of the blastocyst is a critical factor in determining its acceptance in this site (7–10). Not inappropriately, therefore, has the uterus been depicted as a Procrustean bed (11). One obvious factor that may contribute to its apparent discriminating selectivity as a recipient area is its complete investment at all times, in nonprimate species, by an uninterrupted layer of epithelium. The successful establishment of a "free" graft on an intact, epithelialized surface is an event that no surgeon would expect to be possible.

The experiments described in this communication were carried out with two purposes in mind: (*a*) to study further the uniqueness of the uterus as a graft site by determining the conditions under which free skin grafts and monodisperse suspensions of viable epidermal cells can become established on the endometrium; and (*b*) to investigate the response of the uterus to a skin graft or a pure epidermal graft which has become united to its surface, as well as the reaction of skin epithelial cells to growth upon a uterine mesenchymal substrate.

Skin grafts were employed because of the well-studied, distinctive histologic and migratory properties of their epithelium, and the facility with which skin survives implantation into many different sites in the body. To avoid the intervention of tissue transplantation immunity as a source of complication the work was conducted upon rats of an isogenic strain.

Materials and Methods

The subjects of our experiments were sexually mature female rats weighing 130–150 g, belonging to a domestic subline of the Fischer isogenic strain.

Preparation of Skin Grafts.—After a donor's tail was thoroughly cleaned and shaved, a circumferential incision was made down to the underlying vertebrae near to its base. This enabled the skin to be pulled off as an intact, empty tube of tapering bore. With the aid of fine forceps this was then pulled inside out, so that the raw dermal surface was on the outside and the epidermal surface on the inside. The resultant extroverted skin tube was then cut into short cylinders, 3–5 mm in length, which, after soaking in Hanks' solution containing added antibiotics (penicillin 10,000 U/ml, streptomycin 10,000 μg/ml, and Fungizone [Grand Island Biological Co., Grand Island, N. Y.] 25 μg/ml) for 30 min, were ready for grafting.

Preparation of Lingual Mucosal Grafts.—The donor's tongue was excised and pinned out with the inferior surface uppermost. A longitudinal incision was then made into the muscle which could be carefully dissected away producing a sheet of mucosa with relatively little residual muscle adherent to its thin layer of connective tissue. This mucosa was then cut into strips approximately 5 × 2 mm and treated with antibiotic solution.

Preparation of Vaginal Mucosal Grafts.—This entailed removal of the vaginas of sexually mature females as intact tubes by carefully dissecting the vaginas from their cervical, rectal, and bladder attachments. These were cut into 5 mm long cylinders and soaked in antibiotic solution.

Epidermal Cell Suspensions. Epidermal cell suspensions, free of identifiable dermal components, were prepared from tail skin with the aid of trypsin, according to the procedure described in detail elsewhere (12). After being washed in antibiotic solution and counted in a hemocytometer, these cells were suspended in Hanks' solution to a final concentration of either 5 or 250 × 10^6 malpighian cells per ml.

Grafting Procedures

Skin Grafts.—The recipient animal was anesthetized with ether, and a median longitudinal incision made in the clipped and shaved skin of its abdomen. A 1–2 mm longitudinal incision was then made into the lumen of the right uterine horn near the uterotubal junction. This incision was distended to allow the insertion of a cylindrical graft of tail skin of appropriate diameter, i.e., slightly larger than that of the uterine lumen (Fig. 1). Sutures were not required either to anchor the graft in place or to close the uterine incision. The natural resilience of the graft was such as to maintain its dermal surface in constant and complete contact with the endometrium. The graft was free to move down the uterine lumen until it became implanted, usually in the lower third of the host organ.

Vaginal grafts and lingual grafts were also inserted into the uterine lumen according to the procedure described above.

Epidermal Cell Suspensions.—The recipient's right uterine horn was exposed and a ligature of 6–0 chromic gut suture was placed near the uterine bifurcation, taking care to preserve the organ's blood supply. A standard volume of 0.2 ml of a suspension containing either 1.0

or 50 × 10^6 epidermal cells was injected into the uterine lumen via a No. 25 gauge needle (Fig. 2). The function of the ligature was to prevent loss of the inoculated cells through the cervix or the needle tract.

Other "materials and methods" will be described in the specific sections of the paper to which they relate.

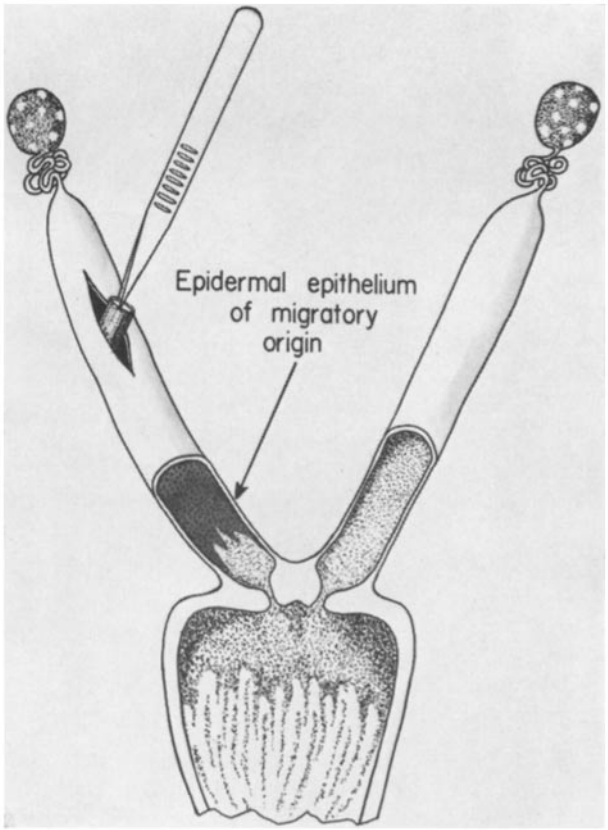

FIG. 1. The insertion of a cylindrical tail skin graft into the lumen of a rat's right uterine horn, and the eventual resurfacing of the endometrium by epidermal epithelium of migratory origin from the established skin graft under estrogenic influence.

EXPERIMENTS AND RESULTS

Fate of Intrauterine Tail Skin Grafts in Animals Treated with Estrogen at the Time of Operation

To provide a base line for the present study, a panel of 50 female rats received a skin graft in their right uterine horn. On conclusion of the operation each

animal was given an intramuscular injection of 50 μg of estradiol benzoate in sesame seed oil to simulate the "surge" of endogenous estrogen believed to be essential for the successful implantation of blastocysts in rats (13–15). Groups of 3–5 animals were subsequently killed at weekly intervals, commencing on the 5th postoperative day. One group of rats was maintained for 100 days.

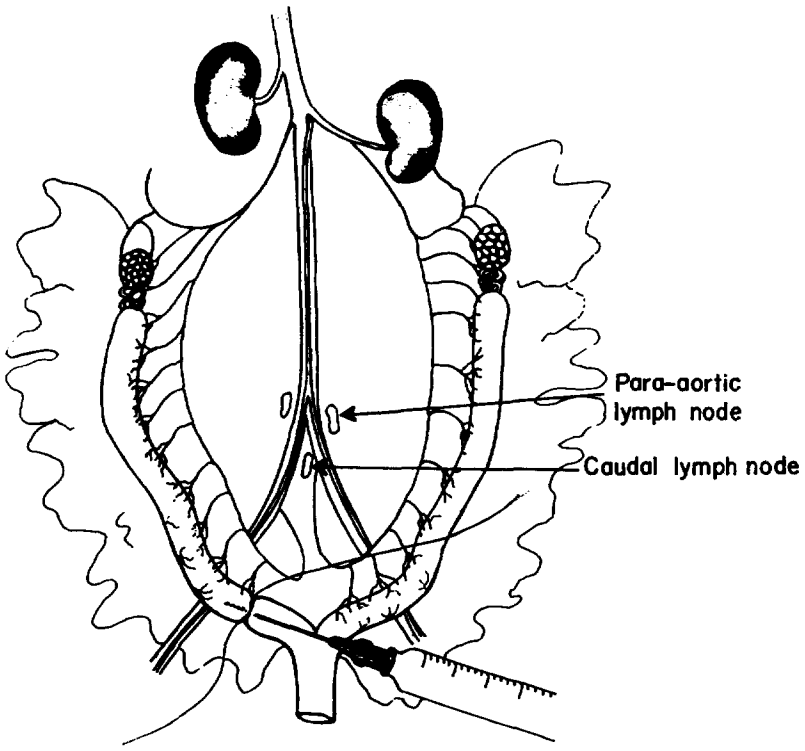

FIG. 2. The procedure employed to inject a suspension of epidermal cells into a rat's right uterine horn. Note the placement of a ligature to prevent loss of the inoculum.

Segments of the uteri bearing the grafts were excised, opened longitudinally, and examined under a dissecting microscope. Subsequently these segments were fixed in Bouin's solution, sectioned, and examined histologically. Specimens were also taken from the equivalent levels of the contralateral, unoperated uterine horns and similarly processed to provide controls.

Of the 50 animals grafted and injected once with estrogen, 48 yielded both macroscopic and microscopic evidence of the primary healing in and survival of their grafts (Table I, group 1). By the 5th postoperative day the grafts had

become attached to the endometrial surface and histological examination revealed that they were already vascularized.

Skin grafts in this ectopic site resembled similar grafts transplanted orthotopically to "full thickness" beds prepared in the integument of the trunk. Without further exogenous estrogen, these intrauterine transplants appeared capable of persisting indefinitely, as evidenced by their consistently healthy appearance after more than 100 days. They soon regenerated sparse hair crops characteristic of the tail and retained all the structural characteristics of this type of skin. The only striking abnormality was hyperactivity of sebaceous glands, as evidenced by their grossly enlarged size and the mitotic activity of their peripheral cells. In no case was there evidence of any tendency on the part of the skin epithelium to migrate outwards from the original graft margins over the surrounding endometrial surface.

TABLE I

Influence of Various Treatments of Host Rats on Their Acceptance of Free Intrauterine Skin Grafts

Experimental group	No. of animals	Additional treatment	No. and percentages of successful implantation	
				%
1	50	50 μg estrogen	48/50	96
2	24	No estrogen	2/24	8
3	6	Bilateral oophorectomy	0/6	0
4	12	Bilateral oophorectomy 50 μg estrogen	12/12	100
5	24	Day 4 or 5 of preimplantation pregnancy	24/24	100

Influence of Maintenance of Chronic Estrus on the Migratory Behavior of Epidermis of Intrauterine Skin Grafts

To investigate the influence of physiologic estrogen dominance on the behavior of skin grafts in utero, a panel of 24 rats received intrauterine skin grafts as before and an injection of 50 μg of estradiol benzoate at operation and weekly thereafter. Subgroups of animals were killed at various intervals for examination of their uterine horns and for the removal of specimens for histological study.

As a consequence of this treatment it was found that from about the 12th postoperative day what appeared to be epidermis began to migrate centrifugally from the graft margins over the adjacent endometrial surface. The advancing edge of putative skin epithelium usually undermined the pseudostratified layer of tall columnar cells comprising the endometrial epithelium, maintaining contact with, and becoming firmly united to, the superficial mesen-

chymal stroma (Fig. 3 *a*). However, in some instances the skin epithelium actually grew *over* the uterine epithelium which subsequently became attenuated and soon disappeared leaving the malpighian layer of skin epidermis juxtaposed to uterine mesenchymal tissue.

Cephalad from the skin graft, migratory activity of the epidermis seemed to undergo complete arrest at the uterotubal junction. Epidermis was never observed in the fallopian tube nor in the ovarian bursa. However, by 21–24 days in most animals, in the caudal direction "tongues" of stratified, well-keratinized skin epithelium were found to have grown down to and passed through the cervical os into the *ungrafted* contralateral uterine horn (Fig. 1). Frequently, given enough time, the native epithelium of this organ too suffered partial replacement by skin epidermis.

No tendency was observed for the skin epithelium to interact with its uterine mesenchymal substrate to generate pilosebaceous units or any other kind of appendage characteristic of skin. However, in addition to its displacement of the superficial endometrial epithelium, skin epidermis grew down into the crypts of the uterine glands, progressively replacing the indigenous columnar epithelium by stratified squamous epithelium (Fig. 3 *b*).

Influence of Host's Hormonal Status on Implantation and Subsequent Behavior of Intrauterine Skin Grafts

To study the influence of the host's hormonal status at the time of transplantation on the competence of the endometrium to maintain a skin graft, 66 sexually mature rats were divided into five groups and subjected to the various experimental treatments and procedures set out in Table I, groups 2–5.

FIG. 3. A. Longitudinal section through a rat uterus grafted with skin 14 days beforehand, showing an advancing "tongue" of stratified epidermal epithelium of graft origin which is firmly attached to the endometrial mesenchymal substrate and is also undermining the "native" endometrial epithelium. × 190.

B. Transverse section through a rat uterus in which epidermis from a skin graft has migrated into and relined a uterine gland. Note the squamous cell debris in the lumen of the gland. × 190.

C. Longitudinal section through a uterine horn injected with a suspension of epidermal cells 12 days previously. Note the two small foci of hyperplastic epidermis which have replaced endometrial epithelium. × 75.

D. Section through uterus grafted with vaginal "skin" 21 days previously to show the advancing edge of migrating vaginal epithelium which is in contact with and displacing columnar endometrial epithelium. × 75.

E. Lingual epidermis of migratory origin from a tongue "skin" graft of 14 days' standing in a rat uterus. Note the typical reentrant pattern presented by the superficial endometrial stroma in contact with the basal layer cells of the epithelium, and the compact laminated structure characteristic of the cuticle of the tongue. × 30.

F. Showing striking hyperplasia of the sebaceous glands in a skin graft growing in the uterus of a pregnant rat. × 75.

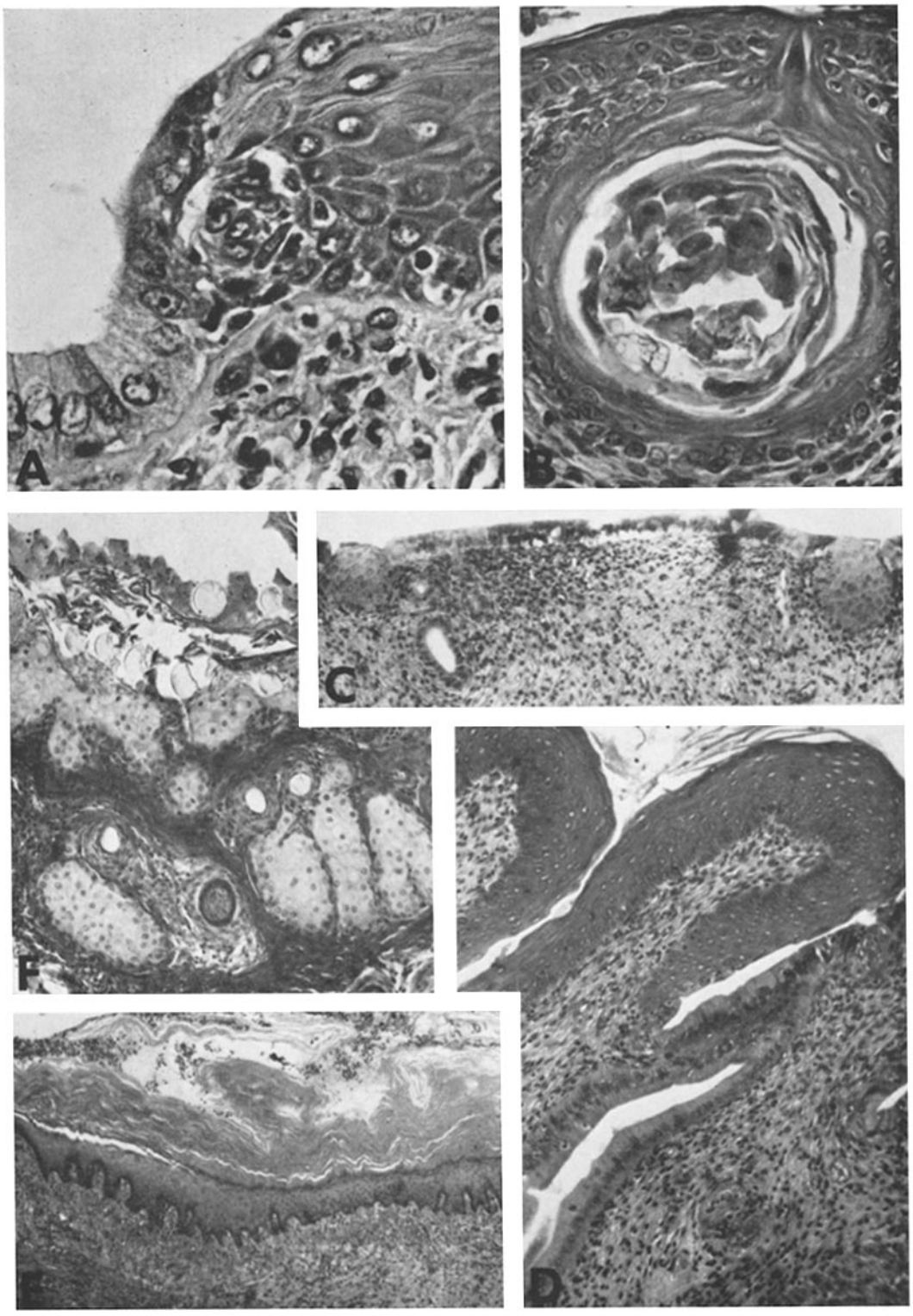

FIG. 3

In the animals in group 2, which received no estrogen treatment, only a very small proportion (8%) of the grafts implanted. The fates of these grafts were studied on a daily basis in a separate panel of 16 virgin females. In the absence of exogenous estrogen, the grafts were extruded into the host's vagina within 48 hr of insertion. Likewise, none of the grafts implanted in animals whose own ovaries were removed at the time of grafting and who received no estrogen. In all the animals which were bilaterally oophorectomized and injected with estrogen at the time of grafting (group 4), the skin grafts were successful. Finally, complete success attended the insertion of skin into the uteri of nonestrogen-treated rats 4 or 5 days *after* what subsequently proved to have been a successful mating (group 5).

These observations indicate that exogenous estrogen is virtually essential for the implantation of free skin grafts in the uteri of nonpregnant rats. The striking consistency with which skin grafts became implanted following insertion into the uterus during the 4th or 5th day of preimplantation pregnancy is consistent with the hypothesis that the immediate preimplantation hormonal milieu includes an estrogen surge that is essential for the primary healing-in of the blastocyst (13–15).

Once skin grafts had become established on the endometrium of normal rats under the influence of a single injection of estrogen, oophorectomy performed 14 days postoperatively had no deleterious effect upon their well being, as evidenced by the examination of six animals subjected to this treatment. In the absence of further estrogen, epidermal migration failed to take place in the uteri of castrated hosts, even from skin grafts that had been in residence for upwards of 100 days. However, epidermis could be caused to migrate from such grafts at will and replace the neighboring endometrial epithelium simply by initiation of chronic estrogen inoculation (50 μg weekly) at *any* time after the grafting operation.

Fate of Epidermal "Cellular" Grafts in the Uterus

The grafts described so far constitute exceedingly crude models of blastocysts in the uterus. In the light of previous findings in rabbits (16), that epidermal cell suspensions "seeded" over the surfaces of extensive full-thickness cutaneous wounds give rise to foci of epidermal outgrowth which eventually coalesce and resurface the entire graft bed, the fate of "cellular" grafts of epidermis in the uterus was investigated.

In the initial series of 20 animals in which no estrogen was given, no evidence was obtained of the successful establishment of epidermal cells. However, when estrogen was given at the time of inoculation of the epidermal cell suspension into the uterine lumen, the presence of numerous small foci of epidermal proliferation was established histologically in 10 out of 10 recipients killed on the 12th postoperative day.

Longitudinal sections of the grafted uterine horns of these animals revealed a linear distribution of small foci or plaques of squamous epithelium, analogous to the distribution of the implanted sites of conceptuses (Fig. 3 *c*). This hinted that epidermal cells might only be capable of "nidation" at certain predetermined sites, possibly those normally destined to receive conceptuses (7, 17). Some support for this premise derives from the observation that the epidermal plaques in the rats that received 50 million epidermal cells were no more abundant nor more extensive 12 days postoperatively than those which developed in rats that received only 1 million cells.

Once established, in the presence of chronically administered estrogen, these epidermal cell "bridgeheads" underwent rapid expansion through mitotic and migratory activity. What is remarkable here is that monodisperse or small clumps of basal layer epidermal cells were able to gain permanent footing on the intact, completely epithelialized endometrial surface.

Analysis of the Modus Operandi of the Estrogen Facilitation of Epidermal Migration in the Uterus

One of the most striking findings described so far was the complete dependence of intrauterine epidermal migratory activity upon maintenance of a state of chronic estrus. The obvious question was whether the hormone acted upon receptors in the uterine substrate, affecting the behavior of the epidermis indirectly, or whether it acted directly upon receptors in the epidermal cells.

In a previous study it had been found that in the rat, unlike the situation in rabbits, guinea pigs, and man, when skin grafts are transplanted to large, full-thickness cutaneous wounds, epithelial migration from the graft margins takes place only fitfully and to a very limited extent (18). There is also usually very little marginal ingrowth of native epithelium across such wound beds. No satisfactory explanation exists for this reluctance of epidermal migration to occur in cutaneous wounds in this species. The present findings suggested that it might be possible to enhance epidermal migration in nonuterine sites in rats by chronic estrogen therapy.

To explore this possibility, small rectangles of tail skin, about 3 × 5 mm, were transplanted to three different sites in panels of rats, some of which were subjected to chronic estrogen treatment, and some of which were untreated controls. These sites were: (*a*) extensive full-thickness cutaneous beds, approximately 3 × 5 cm, prepared in the skin of the lateral thoracic wall according to our standard procedure (18); (*b*) shallow defects prepared by a tangential incision into the renal parenchyma after reflecting the capsule from the anterior pole of the kidney; and (*c*) small, very shallow pockets cut in the surface of the peritoneum. Although the grafts healed-in well and survived in all three sites, estrogen therapy failed to cause the epidermis to wander beyond the limits of its own native mesenchymal (dermal) substrate and to migrate

over the adjacent tissue. These observations sustain the view that the estrogen-dependent migration of epidermis in the uterus results from the action of the hormone on the host organ and not upon the grafted epidermis.

Endometrial Squamous Metaplasia versus Epidermal Migration

Endometrial epithelium undergoes various, well defined cyclical changes of a structural nature during the reproductive phase of life, and displays a more constant structure in the succeeding nonreproductive era. The pseudostratified epithelium of the proliferative estrogenic phase and the secretory activity of the progestational phase of the estrus cycle are two familiar examples of its protean structure. In women the postmenstrual endometrium may undergo epidermoid or squamoid changes during its rapid regeneration (19), and in laboratory animals vitamin A deficiency and exogenous hormone administration may lead to what has been identified as extensive squamous metaplasia by some workers (20–27). In the light of these various reports of the capacity of uterine epithelium to undergo squamous metaplasia, it was necessary to examine critically the possibility that what we had provisionally regarded as epidermis of migratory origin growing on the uterine mesenchymal substrate was, in fact, endometrial epithelium transformed under the influence of chronic estrogen administration.

The simplest experiment involved weekly inoculations of a group of 20 ungrafted female rats with 50 μg of estradiol benzoate for 5 wk, after which their uterine horns were excised for histological study. In every animal this treatment caused the endometrium to become mitotically active, hyperplastic, and pseudostratified. The glands became cystically dilated to varying degrees. However, there was no evidence of squamous metaplasia.

In a second group of six rats given intrauterine skin grafts and subjected to weekly estrogen stimulation for 5 wk, both grafted and ungrafted uterine horns were removed and serial transverse histological sections prepared. These were carefully studied in an attempt to establish whether the putative ectopic squamous epidermis, which appeared in the form of advancing migratory "tongues," did in fact originate from the skin grafts and not from squamous metaplasia of uterine glandular epithelium. In several of these animals it was established that there was complete tissue continuity between the keratinized squamous epithelium present in the ungrafted and untraumatized "control" contralateral uterine horn and the epidermis of the skin graft in the opposite "experimental" horn. Furthermore, in every case in which keratinized, "skin-like" epithelium was demonstrable in the grafted horn at locations remote from the skin graft, epidermal continuity with the skin graft was easily demonstrable.

A further piece of circumstantial evidence against the metaplasia hypothesis is that at all sites in which skin epidermis and uterine epithelium are juxta-

posed, there is an incisive difference between the two cell types and their structural organization into the two distinctive types of epithelia.

The present findings suggest that some reported instances of epidermidization of the uterine epithelium in man and laboratory animals may reflect *migratory displacements* of squamous epithelium from the cervix or vagina into the uterus. Studies on perinatal human females have shown that the squamocolumnar junction of the uterine cervical epithelium advances to the level of the internal os of the uterus during the height of the estrogenic stimulus which reaches the fetus from its mother during gestation (28). A similar inward migration of the cervical squamous epithelium towards the uterus has been shown to take place in mice maintained in estrus (29).

Behavior of Grafts of Vaginal and Lingual "Skin" in the Uterus

To demonstrate the capacity of vaginal epidermis to replace endometrial epithelium, the fate of intrauterine vaginal grafts was studied in rats maintained under estrogen therapy. Under these conditions vaginal epithelium displayed a capacity to migrate vigorously and conserved its distinctive histological specificity (Fig. 3 *d*).

Tongue epidermis is quite distinct from that of skin or vagina in its histological fine structure and in the configurations its basal layer makes by interdigitation with its connective tissue stroma. Heterotypic recombination grafting experiments have shown that when enzymically separated lingual epithelium is recombined with dermis from various types of skin, it conserves its specificity, indicating that this specificity is intrinsically determined by its germinal layer cells rather than an inductive type of response to stimuli from the underlying stroma (30).

Because of these properties, the fate of small grafts of lingual mucosa was studied in the uteri of a group of 12 estrogen-treated rats. The grafts healed-in readily and their epithelium displayed migratory activity that was significantly superior to that of skin epidermis. More important, the specificity of this migratory epithelium was unmistakably that of lingual epidermis (Fig. 3 *e*), a finding that refutes the possibility that it was metaplastic endometrial epithelium in some way transformed through the proximity of a lingual graft.

Reaction of the Endometrium to the Presence of a Skin Graft and Influence of Hormonal Status of the Host on this Reactivity

The presence of a foreign body in a rat's uterus stimulates a pseudodecidual response in the endometrium and a state of pseudopregnancy in the animal. In light of these facts we were surprised to find that skin grafts which implanted successfully in the uterus failed to evoke a similar response. Once the grafts had healed-in, estrus cycles, as evidenced by the appearance of vaginal smears, resumed and the animals displayed normal mating behavior. Histological

studies revealed cyclic changes in both the endometrium beneath the graft dermis as well as in the endometrium resurfaced by epidermis of migratory origin.

During pregnancy, however, the decidual bed beneath both the graft dermis and outlying epidermis of migratory origin was indistinguishable from the decidua in contact with the fetal trophoblast. Furthermore, in animals that became pregnant subsequent to the establishment of a skin graft in one uterine horn, the epidermis migrated in response to the endogenous hormones of pregnancy just as strikingly as in nonpregnant animals treated with estrogen.

The presence of an established skin graft in a uterine horn did not preclude the subsequent natural implantation of conceptuses in *that* horn. Histological studies on uteri bearing both embryos and skin isografts suggested that implantation of the zygotes occurred only in those areas of the endometrium which had not been resurfaced by skin epithelium.

Despite the apparent similarity between the decidual responses to fetal tissue and to skin grafts, respectively, skin grafts proved to be exempt from parturition even when fetuses in the same or in the contralateral uterine horn were delivered.

Finally, it was noted that the sebaceous glands of grafts residing in the pregnant uteri were more active mitotically, more hyperplastic and hypersecretory than in established grafts in nonpregnant animals treated with estrogen alone (Fig. 3*f*).

DISCUSSION

There have been previous desultory attempts to graft skin to the endometrial surface by procedures involving the use of sutures. Interpretation of the results has usually been complicated by a high rate of technical failure or the rejection of homografts which were frequently used instead of immunologically compatible autografts or isografts (31–32). Zipper et al. (31) reported the prolonged survival of skin homografts in the uterus of ovariectomized rats of an outbred population subjected to chronic treatment with *massive* doses of estrogen. They also claimed that the uterine epithelium was replaced by that from the skin, but the authors failed to rule out the possibility that the observed cornification of the uterine lining was due to the administration of estrogen in physiologically outrageous doses.

The present findings show that both free skin grafts and dissociated epidermal cells from genetically compatible donors are capable of implanting and becoming permanently established on the endometrial surface with a high degree of consistency, provided that a transient physiological level of estrogen of exogenous or endogenous origin is furnished. Thus the endocrinologic parameters for the establishment of a skin or an epidermal cell graft as a "placebo embryo" in the uterus closely resemble those needed by nature's

own grafts. It has recently been shown that the ability of Walker carcinoma cells to become established in the rat uterus displays a similar hormonal dependence (34).

Although a skin graft on the endometrium of a pregnant animal attaches to a decidual bed like a conceptus, its decidua constitutes a *permanent* organ of attachment even in animals delivering fetuses from the same or contralateral horn. This suggests that the important parturitional role of the decidua of pregnancy must turn upon unique interrelations between the maternal decidua and the juxtaposed fetal trophoblast.

The observation that, under conditions of chronic estrogen stimulation, the epithelia of skin, tongue, and vagina enjoy a striking selective advantage over native endometrial epithelium which they progressively replace has several interesting implications: (*a*) it reinforces previous conclusions that the various subspecificities displayed by integumentary epidermis on the one hand, and that of lingual epidermis on the other hand, are due to intrinsic differences of cytodifferentiative potentiality; (*b*) the differences between uterine epithelium and the squamous epithelium of the vagina are not due to the influences of different kinds of mesenchymal stroma acting upon a common equipotential lineage of cells, but are due to intrinsic differences between the germinal cells of the two types of epithelia; (*c*) the finding poses the interesting question as to the nature of the estrogen-dependent processes that disrupt the stability of the junctional zone between skin epidermis and contiguous endometrial epithelium at the margin of a skin graft; and finally, (*d*) it raises the possibility that some alleged instances of epidermidization, or metaplasia, of the uterine epithelium resulting from hormonal stimulation or vitamin A deficiency may in fact be due to migration of squamous epithelium from the cervix or vagina.

The origin of the vasculature in free skin grafts has long been the subject of speculation. According to one theory, an early blood supply is obtained by "inosculation" through the establishment of continuity between the severed ends of juxtaposed or almost juxtaposed vessels in the graft and its bed (35). Although previous findings have made this theory seem improbable, the present observation that free skin grafts on an undamaged, completely epithelialized bed in the uterus are rapidly revascularized render it untenable.

SUMMARY

Free tail skin grafts or suspensions of viable epidermal cells have been placed in the atraumatized uterus of isologous rat hosts and allowed to "implant" of their own accord to study the possible uniqueness of this site for other than nature's transplants, i.e. conceptuses, and its response to unnatural grafts.

Despite the presence of an intact endometrial epithelium, free skin grafts heal-in rapidly, provided that a state of estrogen excess is established at the

time of transplantation. In the absence of estrogen most of the grafts failed to implant. Once established, the grafts survive indefinitely without further estrogen. However, if at any stage a state of continual estrus is established, skin epidermis migrates centrifugally from the graft perimeter invasively replacing the native uterine epithelium.

The results of an analysis of the modus operandi of this estrogen-facilitated epidermal migration in utero sustain the view that the hormone acts upon the uterine stroma rather than upon the epidermal cells.

When grafts of lingual mucosa or vaginal "skin" were placed in the uteri of rats maintained in chronic estrus, migration of epidermis took place even more vigorously than from tail skin. These epithelia conserved their distinctive histologic specificities indefinitely when growing as heterotypic recombinants on the alien mesenchymal stroma of the uterus.

Monodisperse suspensions of epidermal cells appear to "implant" and establish small epidermal plaques in the uterus only at sites predestined to accept conceptuses.

That the endocrinologic parameters for the establishment of skin grafts in the uterus are similar to those for blastocysts is suggested by the finding that both kinds of graft can become established in the same uterine horn in the absence of exogenous hormones.

The authors are deeply indebted to Dr. Willys K. Silvers for critically reading this manuscript, to Mrs. Brigitte Koeberlein, Mr. George Sawchuck, and Mr. Robert Hoerr for their invaluable technical assistance, and to Mrs. Pat Cole for her help in preparing the manuscript. This work supported by U. S. Public Health Service Grants 5-T01-GM-00957-08, 01810-05, FR00340-04, AI-07001; also the Lalor Foundation, the American Cancer Society, and the Ford Foundation.

BIBLIOGRAPHY

1. Fawcett, D. W., G. B. Wislocki, and C. M. Waldo. 1947 The development of mouse ova in the anterior chamber of the eye and abdominal cavity. *Amer. J. Anat.* **81**:418.
2. Kirby, D. R. S. 1963. The development of mouse blastocyts transplanted to the spleen. *J. Reprod. Fert.* **5**:1.
3. Kirby, D. R. S. 1963. The development of mouse blastocyts transplanted to the scrotal and cryptorchid testis. *J. Anat.* **97**:119.
4. Kirby, D. R. S. 1960. The development of mouse eggs beneath the renal capsule. *Nature (London)* **187**:707.
5. McLaren, A. and A. K. Tarkowski. 1963. Implantation of mouse eggs to the peritoneal cavity. *J. Reprod. Fert.* **6**:385.
6. Fawcett, D. W. 1950. The development of mouse ova under the capsule of the kidney. *Anat. Rec.* **108**:71.
7. McLaren, A. and D. Mitchie. 1959. The spacing of implantations in the mouse uterus. *Mem. Soc. Endocrinol.* **6**:65.

8. McLaren, A. and C. A. Finn. 1967. A study of the early stages of implantation in mice. *J. Reprod. Fert.* **13**:259.
9. McLaren, A. and D. Mitchie. 1959. Studies on the transfer of fertilized mouse eggs to uterine foster-mothers. II The effect of transferring larger number of eggs. *J. Exp. Biol.* **36**:40.
10. Doyle, L. L., A. H. Gates, and R. W. Noyes. 1963. Asynchronous transfer of mouse ova. *Fert. Steril.* **14**:215.
11. McLaren, A. Maternal factors in nidation. 1965. *In* Symposium on the early conceptus, normal and abnormal. W. W. Park, editor. Williams & Wilkins, Baltimore, Md. 27.
12. Silvers, W. K. and R. E. Billingham. 1966. Further studies on the induction of tolerance of skin homografts in rats. *J. Exp. Zool.* **161**:413.
13. Shelesnyak, M. D. and P. F. Kraicer. 1963. The role of estrogen in nidation. *In* Delayed Implantation. A. C. Enders, editor. University of Chicago Press, Chicago, Ill. p. 265.
14. Nutting, E. F. and R. K. Meyer. Implantation delay, nidation and embryonal survival in rats treated with ovarian hormones. *In* Delayed Implantation. 1963. A. C. Enders, editor. University of Chicago Press, Chicago, Ill. 233.
15. Mayer, G. Delayed nidation in rats. 1963. A method of exploring the mechanism of ova implantation. *In* Delayed Implantation. A. C. Enders, editor. University of Chicago Press, Chicago, Ill. 213.
16. Billingham, R. E. and J. Reynolds. 1952. Transplantation studies on sheets of pure epidermal epithelium and epidermal cell suspensions. *Brit. J. Plast. Surg.* **5**:25.
17. McLaren, A. 1969. Stimulus and response during early pregnancy in the mouse. *Nature (London)* **221**:739.
18. Billingham, R. E. and W. K. Silvers. 1968. Dermoepidermal interactions and epithelial specificity. *In* Epithelial Mesenchymal Interactions. R. Fleischmajer and R. E. Billingham, editors. Williams & Wilkins, Baltimore, Md. 252.
19. Baggish, M. S. and D. J. Woodruff. 1967. The occurrence of squamous epithelium in the endometrium. *Obstet. Gynecol., Surv.* **22**:69.
20. Reiter, R. J. 1965. Uterine keratinizing metaplasia and plasma levels of vitamin A in rats fed vitamin deficient diets. *Tex. Rep. Biol. Med.* **23**:486.
21. McEuen, C. S., H. Selye, and J. B. Collip. 1936. Some effects of prolonged administration of oestrin in rats. *Lancet* **1**:225.
22. Patton, W. T. and G. V. Squires. 1962. Ichthyosis uteri. *Amer. J. Obstet. Gynecol.* **84**:858.
23. Korenchevsky, V., K. Hall and R. Burbank. 1939. The manifold effects of prolonged administration of sex hormones to female rats. *Biochem. J.* **33**:372.
24. Bo, W. J. 1961. The effect of vitamin A deficiency and estrogen on the uterus. *Amer. J. Clin. Nutr.* **9**:13.
25. Bo, W. J. 1956. The relationship between vitamin A deficiency and estrogen in producing uterine metaplasia in the rat. *Anat. Rec.* **124**:619.
26. Bo W. J. 1957. Relation of vitamin A deficiency and estrogen to induction of keratinizing metaplasia in the uterus of the rat. *Amer. J. Clin. Nutr* **5**:666

27. Fluhmann, C. F. 1955. Squamous metaplasia in the rat uterus. *Arch. Pathol.* **59:** 238.
28. Schneppenheim, P., H. Hampert, C. Kaufmann, and K. G. Ober. 1957–58. Die Beziehungen des Schleimepithels zum Plattenepithel an der Cervix Uteri im Lebenslauf der Frau. *Arch. Gynaekol.* **190:**303.
29. Graham, Charles E. 1967. Uterine cervical epithelium of fetal and immature females in relation to estrogenic stimulation. *Amer. J. Obstet. Gynecol.* **97:**1033.
30. Billingham, R. E. and W. K. Silvers. 1967. Studies on the conservation of epidermal specificities of skin and certain mucosas in adult mammals. *J. Exp. Med.* **125:**429.
31. Zipper, J., G. Ferrando, G. Saez, and A. Tchernitchin. 1966. Intrauterine grafting in rats of autologous and homologous adult rat skin. *Amer. J. Obstet. Gynecol.* **94:**1056.
32. Watnick, A. S. and R. A. Russo. 1968. Survival of skin homografts in uteri of pregnant and progesterone treated rats. *Proc. Soc. Exp. Biol. Med.* **128:**1.
33. Kirby, D. R. S. 1968. Transplantation and pregnancy. *In* Human Transplantation. F. T. Rapaport, J. Dausett, editors. Grune and Stratton, N. Y. and London. p. 565.
34. Short, R. V. and K. Yoshinaga. 1967. Hornomal influences on tumor growth in the uterus of the rat. *J. Reprod. Fert.* **14:**287.
35. Haller, A. J. and R. E. Billingham. 1967. Studies of the origin of the vasculature in free skin grafts. *Ann. Surg.* **166.**