An experimental approach to the problem of the derivation of the vaginal epithelium

by JOHN-GUNNAR FORSBERG

From the Department of Anatomy, University of Lund

WITH ONE PLATE

In earlier papers the present author (Forsberg, 1963, 1965a, b; Forsberg & Olivecrona, 1964) has presented studies of the derivation and differentiation of the vaginal epithelium by morphological, histochemical and autoradiographic methods and by counts of mitotic rates. These investigations favour the view that the vaginal epithelium in mouse and rat has a dual origin: the anterior part being derived from the Müllerian epithelium and the posterior part from the sinus epithelium. This means that in these species the pseudostratified columnar Müllerian epithelium in the vaginal region undergoes a transformation into a stratified squamous epithelium. The possible mechanism behind this transformation has been discussed (Forsberg & Olivecrona, 1965). Results from estradiol treatment of neonatal mice during the differentiation of the vaginal epithelium support the above-mentioned investigations. (For a detailed review on the problem of the derivation of the vaginal epithelium, see Forsberg, 1963.)

Juillard & Delost (1963a, b) broadly agree with the present author on the morphological development of the vaginal epithelium in mouse. However, after a study of estradiol-treated mouse young these authors (1964) describe the sinus epithelium as growing anteriorly and replacing the Müllerian epithelium in the anterior part of the vagina. Raynaud (1942, 1962) is of the opinion that the whole mouse vaginal epithelium may be a derivative of the sinus epithelium.

The problem of the origin of the epithelium in the anterior part of the vagina has a great interest since the generally accepted view is that the sinus epithelium, but not the Müllerian epithelium, is capable of giving a stratified squamous response to estrogens. The Müllerian epithelium should, on this view, give a glandular response (see Zuckerman, 1940; Raynaud, 1962).

In this paper, the author presents the results of a study of the developmental capacities of the Müllerian epithelium in the anterior part of the mouse vaginal anlage, both in the presence and in the absence of sinus epithelium.

Author's address: Department of Anatomy, University of Lund, Biskopsgatan 7, Lund, Sweden.
MATERIAL AND METHODS

The material for this investigation consisted of newborn female young from albino mice kept at this institute. Within twelve hours of birth, in most cases immediately after partus, either the major part of the vaginal anlage or only its anterior part, was transplanted from one female young into the muscles of the thigh of a female littermate. By using newborn young immunological reactions are avoided.

The bladder and the urethra, down to its opening into the urogenital sinus, were removed from the donor animal. All the operations were carried out under a dissecting microscope. In newborn young there is a histological border be-

Text-fig. 1. Diagram of the vaginal anlage in a newborn young. c cervix; mv Müllerian vagina proper; sc cord of sinus epithelium, the anlage of the sinus vagina; u urethra; us urogenital sinus; ws wedge of sinus epithelium protruding into the lumen of the Müllerian vagina. The part of the anlage between A and B was grafted under the designation ‘Müllerian vagina’, the part between A and C was grafted as ‘the whole vaginal anlage’. 

between the anterior part of the vaginal anlage containing Müllerian epithelium and the posterior part containing sinus epithelium. The border can also be seen under the dissecting microscope after removal of the bladder and urethra: the sinus vagina is thin and ‘compact’, the Müllerian vagina is wider and more ‘transparent’. The vaginal anlage was divided above the border between the sinus epithelium and the Müllerian epithelium (see Text-fig. 1). The next step was to cut through at the junction of the uterine horns. Thus the cervical region is also included in the preparations of the anterior part of the vaginal anlagen
used for transplantation; in what follows this part is called 'the Millerian vagina' in spite of the inclusion of the cervix. In order to exclude any contamination of sinus epithelium in the preparation of the grafted Millerian vagina, the posterior remaining part of the vaginal anlage was fixed in Bouin's fluid, paraffin embedded, and serially sectioned for further study.

In other cases, the vaginal anlage was freed from the uterine horns at the same level as described above. Posteriorly, the vaginal anlage was cut through posterior to the junction between the urethra and the urogenital sinus (see Text-fig. 1). The part of the vaginal anlage between the two cuts (also including the cervix) is called in the following 'the whole vaginal anlage'.

The host females were anaesthetized by being put on ice. When they no longer reacted to stimulus, they were put under the dissecting microscope. An incision was made parallel to the left femur and anterior to it. Thereafter, a muscular incision was made (care being taken to avoid bleeding), also parallel to the femur and just anterior to it. The graft was put into the incision which was closed by a suture; 7-0 silk on a 3/8 circle taper. The skin incision was closed by two similar sutures. In some cases, the females had to be re-chilled during the operation which took about 5-7 min. After the females had been warmed in the hand for a few minutes, they showed spontaneous movements and were put back with their mother. The survival of the operated young was 100 per cent and they showed no signs of retarded growth.

One group of hosts received subcutaneous injections of 5 µg. estradiol-17β dissolved in 0.02 ml. olive oil for 5 or 6 days, beginning on the day of operation. In the last case, the animals were killed 6-8 hr. after the last injection. The injections were given on the side of the body opposite to that on which the transplant lay.

After 6 or 14 days, the hosts were killed and the thigh musculature containing the graft was fixed in Bouin's fluid, paraffin embedded, and serially sectioned in 5 µ sections. The host's own vaginal anlage was also removed and treated in the same way. All sections were stained in haematoxylin and eosin.

NORMAL DEVELOPMENT

A detailed account of the morphological development of the mouse vagina has been given earlier (Forsberg, 1963). At birth, the Millerian epithelium in the anterior part of the vaginal anlage is pseudostratified columnar. This vaginal part is called the Millerian vagina. The Millerian epithelium is attached to a short solid cord of sinus epithelium, which in turn is attached to the urogenital sinus (see Text-fig. 1). The latter structure undergoes a frontal division and gives rise to a ventral urethral part and a dorsal vaginal part. The part of the vaginal anlage containing sinus epithelium is called the sinus vagina.

Beginning about 3 days after birth, a change occurs in the pseudostratified columnar epithelium in the Millerian vagina; it divides into two zones: one
superficial, one basal. This change begins in the border region to the sinus vagina and progresses in an anterior direction. The basal zone consists of cells of the same type as those seen in the basal layer in the sinus vagina, whereas the superficial zone for some time retains its character of a columnar epithelium. Later, the superficial zone, too, changes character and the cells become of the same type as those in the corresponding level of the epithelium in the sinus vagina. As a result of these epithelial changes, the border between the sinus vagina and the Müllerian vagina disappears. The controversial problem is whether the cells in the basal zone in the Müllerian vagina are anteriorly growing sinus cells, which later also invade the superficial zone, or whether the epithelial changes are due to a transformation within the Müllerian epithelium itself.

RESULTS

Morphological appearance of the untreated host vaginal anlagen at 6 days after birth

The urogenital sinus is completely divided into a urethral part and a vaginal part. The most posterior part of the vaginal anlage is solid. In the part of the sinus vagina with a lumen, the epithelium is low with two to three layers of palely stained cells. There is a gradual transition into the epithelium of the Müllerian vagina. The cells of this epithelium are arranged in the two above-mentioned zones: one superficial zone with high, darkly staining cells; one basal zone with low, more palely stained cells, similar to those in the basal layer in the sinus vagina. These differences are most pronounced in the anterior part of the vaginal anlage: more posteriorly, the superficial zone too has low, palely stained cells, and thus the two zones merge. Anteriorly, the zones can be followed into the posterior part of the unpaired portion of the cervix.

Differentiation of the epithelium in grafts of the whole vaginal anlage, studied 6 days after birth (nineteen grafts)

Most of these grafts show a very good growth. Compared with the host vaginal anlagen there is some lag in the development. Thus the urogenital sinus has not completely divided into a urethral part and a vaginal part. Further, there is a distinct border between the Müllerian vaginal epithelium with its dark stained superficial zone and the solid sinus vagina with palely stained cells. The epithelium in the Müllerian vagina has differentiated into the two zones, but these have not merged as they have in the host preparations. The cells in the basal zone have the same appearance as those in the basal layer in the sinus vagina.

In two of the studied grafts, the two epithelial zones are seen in the posterior part of the unpaired region of the cervix; in ten, the zones are found in the whole Müllerian vaginal part but have not extended into the cervix. In five, the zones are seen only in a part of the Müllerian vagina; finally, in a further two, no development of the zones can be seen. In the latter two, the epithelium in the Müllerian
vagina shows a poor survival: instead of the high pseudostratified epithelium normally seen, there is only a low columnar epithelium, mostly single-layered. Those preparations showing two zones in only a part of the Müllerian vagina seem to have grown poorly and in the lumen lots of debris is found.

**Differentiation of the epithelium in grafts of the Müllerian vaginal part, 6 days after birth (twenty-three grafts)**

One graft was necrotic and is excluded from the following description.

The criterion for grafts to be included in this group is that the pseudostratified Müllerian epithelium in the posterior, non-grafted part of the vaginal anlage showed no morphological signs of contamination with sinus epithelium in the cut region. At the newborn stage, a short wedge of sinus epithelium containing degeneration granules extends from the solid sinus vagina into the lumen of the Müllerian vagina (see Text-fig. 1). In seven cases, the anterior tip of this wedge has been seen floating free in the Müllerian vaginal lumen in the cut region of the non-grafted part of the vaginal anlage, but it has never been seen in the sections of the grafts. (In some excluded cases where sinus epithelium has been included in a part of the wall, there has always been a distinct border between this and the Müllerian epithelium.) At 6 days no difference can be seen in the differentiation of the epithelium in the grafts, whether or not the tip of the wedge has been seen in the cut region of the non-grafted vaginal part.

As a rule the grafts show a very good growth. Whereas the Müllerian epithelium in the sections from the posterior non-grafted part of the vaginal anlage in the newborn stage is a pseudostratified columnar one, the epithelium in the grafted part of the Müllerian vagina is characterized at 6 days by a basal and a superficial zone of the type described above (see Plate, Fig. 1). The zones can be seen not only in the whole Müllerian vagina (10 cases), but also extends into the posterior region of the unpaired part of the cervix (6 cases). In another five grafts, the basal zone is lacking in a part of the circumference of the epithelial wall but is seen in the rest. In one graft, there was no development of the zones. This seems to be related to the growth of the pseudostratified Müllerian epithelium or of the superficial zone. If these show a retarded growth in a part of the wall (lower cell density than normal, lower epithelial height) the basal zone is either absent or contains only a single layer of low cells.

**Differentiation of the epithelium in the host vaginal anlagen and in grafts of the Müllerian vagina at 14 days after birth (ten grafts)**

Four grafts were more or less necrotic and are excluded from the following description. Six grafts show good growth. On study of the posterior non-grafted part of the vaginal anlage from the newborn stage, the tip of the wedge of sinus epithelium was seen floating free in the lumen of the Müllerian vagina in three cases.
The epithelium in the grafts has the same appearance as that in the host vaginal anlagen: the epithelial wall consists of three to four layers of small cells (Plate, Fig. 2). The same epithelial type is also seen in the cervical canals, bordering columnar epithelium anteriorly. Epithelial zones in the sense mentioned above can no longer be seen.

**Differentiation of the vaginal epithelium in hosts and grafts of the Müllerian vagina after estradiol treatment, 6 days old young (twenty-four grafts)**

The host vaginal anlagen show a very pronounced reaction to estradiol. The basal zone in the Müllerian vaginal part is hyperplastic, and the major part of it is differentiated into a stratified squamous epithelium. It is covered by the superficial zone which posteriorly consists of low cells anteriorly increasing in height to high columnar cells.

The posterior non-grafted part of the vaginal anlagen used in this group do not show any morphological contamination of the Müllerian wall epithelium with sinus epithelium in the cut region. In four cases, the tip of the wedge of sinus epithelium described above has been seen floating free in the lumen of the Müllerian vagina.

In the grafts, the epithelial reaction to estradiol is seen in the basal zone in the form of hyperplasia and differentiation into a stratified squamous epithelium (Plate, Fig. 3). This differentiation, however, is seen only in a greater or lesser part of the epithelial wall; in contrast to the conditions in the majority of the grafts of the Müllerian vagina in untreated hosts, the basal zone is not seen in the whole Müllerian vagina. Where it is not developed the wall may well consist of a high pseudostratified columnar epithelium. In ten cases the grafts are more
or less dilated forming a cyst-like structure but the epithelial differentiation described above can still be seen. In two cases, there was no development of the basal zone and in a further case, the basal zone was developed, but it was not hyperplastic. No differences related to the dose of the hormone are seen.

DISCUSSION

A comparison made 6 days after birth between the host vaginal anlagen and the grafts of the whole vaginal anlage shows that the grafts differentiate normally even if the development is somewhat retarded as shown by an incomplete division of the urogenital sinus and a remaining distinct border between the sinus vagina and the Müllerian vagina. In the latter, the epithelial differentiation proceeds normally. The simple pseudostratified columnar epithelium seen in the newborn has been replaced by an epithelium consisting of two zones: one superficial, one basal. The latter consists of low cells of the same appearance as those in the basal layer in the sinus vagina. The superficial zone contains high columnar cells. In regions where the pseudostratified epithelium or the superficial zone shows a poor growth, the basal zone is either lacking or poorly developed. This condition may well be explained by interference with nutrition. The muscular milieu itself has not interfered with the development, as can be seen by comparing the host vaginal anlagen with the grafts. Nor did Planel et al. (1964) find any influence from skeletal muscles on the rat vaginal epithelium in vitro. On the other hand, kidney tissue, for example, gave rise to a hyperplasia and also stimulated keratinization.

The results from this investigation show that the epithelium in the Müllerian vagina, deprived of contact with sinus epithelium, has the capacity to undergo a transformation from a pseudostratified columnar epithelium into an epithelium consisting of a superficial zone and a basal zone. The latter, which later forms the basal layer in the cranial 3/5 of the mouse vagina, must thus be derived from the Müllerian and not from the sinus epithelium as supposed by Juillard & Delost (1964). At 14 days after birth, the vaginal epithelium, now consisting of three to four layers of small cells, has a similar appearance in both the hosts and the grafts of the Müllerian vagina. Thus the superficial zone has by then also undergone a transformation and this in the absence of sinus epithelium.

It must be pointed out that the basal zone appears at about three days in normal young. The transplantations were carried out in most cases immediately after and in no case later than twelve hours after birth. It may be argued that already at the time when the operations were made there might be an invisible infiltration of the Müllerian epithelium with sinus epithelium. This, however, is improbable as at this stage there is a distinct difference between the two and the border between them is distinct. A histological examination of the Müllerian epithelium does not reveal any signs of a cell population which might come from the sinus epithelium. As the basal zone in the grafts of the Müllerian vagina is
capable of giving a squamous response to estrogen, there are no reasons for the belief in a wave of sinus epithelium growing anteriorly and replacing the Müllerian epithelium from the vagina later than 6 days after birth. Up to and including 13 days after birth, when there is one and the same epithelium in the whole vagina, mitotic rate studies have not revealed any signs of such a wave (Forsberg, 1965a).

Apart from a cellular contribution, it is not yet possible to exclude the possibility of the sinus epithelium having any influence on the differentiation of the Müllerian epithelium. There are circumstances (Bulmer, 1964; Forsberg, 1965b) which might suggest that the inductive stimulus for the transformation of the Müllerian epithelium originates in the sinus epithelium and then spreads through the Müllerian vagina. It is possible that this induction began to act before the transplantations were made. Further investigations may throw light on this problem.

The results of the experiments in which the hosts were given estradiol in daily subcutaneous injections show that the basal Müllerian epithelial zone in the grafts can give a squamous response to estrogen. This zone undergoes a hyperplasia and differentiates into a stratified squamous epithelium. The covering superficial zone does not give this response; instead, the cells become loaded with mucin, and when the basal zone cornifies, the superficial zone is shed (Forsberg & Olivecrona, 1965). The view of Zuckerman (1940) and Raynaud (1962) that the sinus epithelium may give a squamous response to estrogen and the Müllerian epithelium a glandular response must thus be reassessed. The Müllerian epithelium in the vaginal region is capable of giving a squamous response.

The basal zone has a considerably greater extension in grafts of the Müllerian vagina in untreated hosts than in estradiol treated hosts. Judging from the histological appearance of the epithelium, this difference cannot be explained by nutritive factors. Instead, it might be possible that there is a lag phase in the inductive mechanisms in the grafts after operation. During this time estradiol might induce a precocious differentiation of the columnar epithelium (cf. Witschi, 1959; Forsberg & Olivecrona, 1965), rendering it resistant to the stimulus to form the basal zone.

**SUMMARY**

In order to investigate the developmental capacities of the Müllerian epithelium in the vaginal anlage in mouse, the author transplanted the Müllerian epithelium alone, and in combination with the sinus epithelium, to the thigh muscles of female littermates. The transplantations were made within twelve hours of birth and the grafts and the host vaginal anlagen were fixed at 6 or 14 days. Care was taken to exclude any contamination by sinus epithelium in the grafts of Müllerian epithelium alone. In one experimental series, the hosts of the grafts of the Müllerian epithelium were given daily subcutaneous injections of 5 μg. estradiol-17β.
The results of this investigation show that the intramuscular milieu does not influence the epithelium in the grafts. Further, the Müllerian epithelium combined with sinus epithelium undergoes the same differentiation as the Müllerian epithelium alone. Thus, in both cases, there are formed in the Müllerian epithelium a superficial zone and a basal zone, and it is the latter which gives rise to the basal layer in the anterior part of the vagina. It can therefore be concluded that the mouse vaginal epithelium has a dual origin: the posterior part from the sinus epithelium, the anterior part from the Müllerian epithelium. The estradiol treatment showed that the Müllerian epithelium is able to give a stratified squamous response.

RÉSUMÉ

Abord expérimental du problème de l’origine de l’épithélium vaginal chez la Souris

Pour étudier les potentialités du développement de l’épithélium Müllerien dans l’ébauche vaginale de la Souris, l’auteur a transplanté l’épithélium Müllerien, seul ou en combinaison avec l’épithélium du sinus, dans les muscles de la cuisse de femelles d’une même portée. Les transplantations ont été faites dans les douze heures suivant la naissance; les greffons et les ébauches vaginales des porte-greffes ont été fixés à 6 ou 14 jours. On a pris soin d’exclure toute contamination par l’épithélium du sinus dans le cas des greffes d’épithélium Müllerien seul. Dans une série expérimentale, on a administré aux porteurs de greffons d’épithélium Müllerien des injections quotidiennes sous-cutanées de 5 μg. d’oestradiol-17β.

Les résultats montrent que le milieu intramusculaire n’influence pas la différenciation épithéliale des greffons. De plus, l’épithélium Müllerien associé à l’épithélium du sinus subit la même différenciation que l’épithélium Müllerien seul. Ainsi, dans les deux cas, il se forme dans l’épithélium Müllerien une zone superficielle et une zone basale et c’est cell-ci qui donne naissance à la couche basale de la partie antérieure du vagin. On peut ainsi conclure que le vagin de la Souris a une origine double: la partie postérieure provient de l’épithélium du sinus, la partie antérieure de l’épithélium Müllerien. Le traitement par l’oestradiol a montré que l’épithélium Müllerien peut donner par réaction des structures squameuses stratifiées.

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REFERENCES


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