CORNIFICATION OF THE HUMAN VAGINAL EPITHELIUM

By A. W. ASSCHER, C. J. TURNER
*The London Hospital Medical College, London, E.*1
AND C. H. DE BOER
The Women's Hospital, Liverpool

INTRODUCTION

Many authors have described the cyclical changes in the human vaginal epithelium, but the cornification of this epithelium has received less precise attention. It is the purpose of this investigation to study the cornification of the human vaginal epithelium by histochemical and other techniques.

Dierks (1927) considered that cornification occurred in an intra-epithelial zone, the 'Verhornungszone' (zone of cornification), but Stieve (1931*a*, *b*) maintained that the appearance of this zone resulted from mechanical factors. Stemshorn (1928) considered that there was insufficient evidence for the occurrence of cornification in the intra-epithelial zone; he thus proposed the non-committal term 'Verdichtungszone' (zone of densification). Traut, Bloch & Kuder (1936) described the occurrence of cornification in the superficial layers of the epithelium; during the menstrual cycle no consistent changes in these layers were observed. Papanicolaou, Traut & Marchetti (1948) also described cornification of the superficial portion of the epithelium; they recognized the existence of an intra-epithelial zone which was not always well defined and which often gave the impression of an artefact produced by shrinkage of the epithelium. Papanicolaou (1954) stated that the functional significance of this zone is not yet properly understood.

MATERIAL AND METHODS

Twenty-eight biopsy specimens, taken from different subjects at different stages of the menstrual cycle, were used. The subjects included in this investigation showed no evidence of unusual hormonal balance; in eleven cases an endometrial biopsy, taken at the time of the vaginal biopsy, revealed a normal endometrium corresponding in its development to the stage of the cycle determined from the menstrual history. All specimens were taken from the anterior vaginal wall at a level just above the bladder neck; they were immediately fixed in a solution containing 1 g. of trichloroacetic acid dissolved in 100 ml. of 80% ethyl alcohol. The specimens were embedded in paraffin; serial sections, cut at 7μ, were prepared and studied with the following procedures:

(i) Ehrlich's haematoxylin and eosin.

(ii) Heidenhain's haematoxylin.

(iii) Michrome (M.F.4) stain (Edward Gurr, Ltd.). The vaginal smear staining technique described by Gurr (1953) was adapted for use on sections by lengthening the staining period to 5 min.

(iv) Papanicolaou's vaginal smear stain (E.A.36). The modified technique described by Papanicolaou *et al.* (1948) was used; it was adapted for use on sections by lengthening the staining periods in O.G.6 and E.A.36 to 5 min. each.

(v) Inspection of unstained material with polarized light.

(vi) Barrnett & Seligman (1952, 1954) procedure for the demonstration of sulphydryl and disulphide groups. Disulphide groups were localized by comparing preparations, previously reduced for 1 hr. with 0·5 M thioglycollic acid at a pH of 8·5 and a temperature of 37° C., with corresponding sulphydryl group preparations. Controls were incubated for 1 hr. at 50° C. in 0·1 M iodoacetic acid at pH 8.

OBSERVATIONS

(*a*) *The follicular phase epithelium*

Observations were made on ten specimens taken from the 3rd to the 12th day of the menstrual cycle. The deepest six to ten layers of the epithelium show sulphhydryl group positivity of the cellular cytoplasm and intercellular bridges. The superficial portion of the epithelium displays sulphydryl group positivity of the cell walls. An intermediate zone, consisting of flattened acidophilic cells, can frequently be distinguished; its sulphydryl group positivity appears greater than that of the overlying superficial cells. This appearance might be attributable to an optical artefact resulting from the flattening of its cells. The intermediate zone is further distinguished by the presence of sulphydryl groups in the peripheral cytoplasm of its constituent cells. This intermediate zone shows greater contrast to the remainder of the epithelium after thioglycollic acid reduction, indicating the presence of disulphide groups that are localized in the cell walls and peripheral cytoplasm (Pl. 1, figs. 1, 3). This zone can be shown to contain a considerable amount of birefringent material (Pl. 1, fig. 4). The intermediate zone shows great affinity for Heidenhain's haematoxylin, acquiring an intense black colour that frequently extends throughout the cytoplasm (Pl. 1, fig. 2). The intermediate zone assumes an intense yellow coloration with Michrome vaginal smear stain (Pl. 2, fig. 5), whereas it acquires a red coloration with Papanicolaou's stain (Pl. 2, fig. 6). The thickness of the intermediate zone varies in different regions of the same specimen, its average thickness, however, is related to that of the whole epithelium. Thus, since the vaginal epithelium grows in thickness during the follicular phase (Papanicolaou *et al.* 1948), it may be inferred that the intermediate zone also increases in thickness during this phase.

(*b*) *The mid-cycle epithelium*

Observations were made on seven specimens taken between the 12th and 17th days of the menstrual cycle. A disulphide-containing, birefringent zone was found on the surface of the epithelium in places in five of these specimens (Pl. 2, fig. 7), whilst elsewhere this zone was covered by a few layers of cells in which no disulphide groups could be demonstrated. The disulphide-containing, birefringent zone in the remaining two specimens, taken on the 15th and 17th days of the cycle, was found on the surface of the epithelium throughout the specimen. In the superficial zones of these latter specimens disulphide and sulphydryl groups were not merely

confined to the cell walls and peripheral cytoplasm; some of the cells showed disulphide and sulphydryl groups throughout their cytoplasm. Furthermore, in these specimens the superficial zone assumed a more intense crimson coloration with the Barrnett and Seligman procedure, particularly after thioglycollic acid reduction. This superficial zone showed with all other techniques employed properties identical to those of the intermediate zone of the preceding follicular phase.

(*c*) *The luteal phase epithelium*

Eleven specimens taken between the 17th and 28th days of the menstrual cycle were studied. Early luteal-phase specimens showed a disulphide-containing, birefringent zone on the surface of the epithelium; its tinctorial properties with the other techniques employed were similar to those of the intermediate zone of the follicular phase. Some cells in this zone contained disulphide and sulphydryl groups throughout their cytoplasm, in others they were confined to the cell walls and peripheral cytoplasm (Pl. 2, fig. 8). The superficial zone acquired an intense crimson appearance with the Barrnett & Seligman procedure in these specimens, as in the two mid-cycle specimens. A considerable individual variation was found in the thickness of the superficial zone during the early luteal phase.

Late luteal phase specimens did not show the disulphide-containing, superficial zone (Pl. 2, fig. 9). A zone of flattened cells could occasionally be distinguished in these specimens; it was intermediate in position and appeared particularly in areas overlying the subepithelial papillae. This zone showed properties similar to those of the intermediate zone of the early follicular phase, but its staining reactions were patchy and less well defined.

An intense scattered yellowness of the deeper layers of the epithelium, extending to the surface in places, was observed in two of the late luteal-phase specimens when stained with Michrome vaginal smear stain (Pl. 2, fig. 10); with Papanicolaou's stain a similar distribution of red coloration was found. These areas were not stained with Heidenhain's haematoxylin and they showed no birefringence or disulphide groups.

DISCUSSION

The functional significance of the intra-epithelial zone of the human vaginal epithelium has been the subject of much discussion. Stieve (1931*a*, *b*) and Traut *et al.* (1936) attributed no importance to the zone in view of its inconstancy. Stieve pointed out that intra-epithelial zones could be demonstrated at any stage of the menstrual cycle as well as after the menopause and that they could even be found in the buccal and oesophageal mucosae of both male and female subjects. He therefore concluded that its presence was not dependent on endocrine relationships but on mechanical factors.

The present observations show that the follicular-phase human vaginal epithelium possesses a specialized intra-epithelial zone with distinctive chemical and physical properties. Disulphide groups and birefringence are localized in this zone; it shows great affinity for Heidenhain's haematoxylin and can be selectively demonstrated with certain vaginal smear stains. Such properties are unlikely to be those of an artefact; these characteristics of the intra-epithelial zone clearly indicate that it constitutes a site of cornification, thus substantiating the view of Dierks (1927).

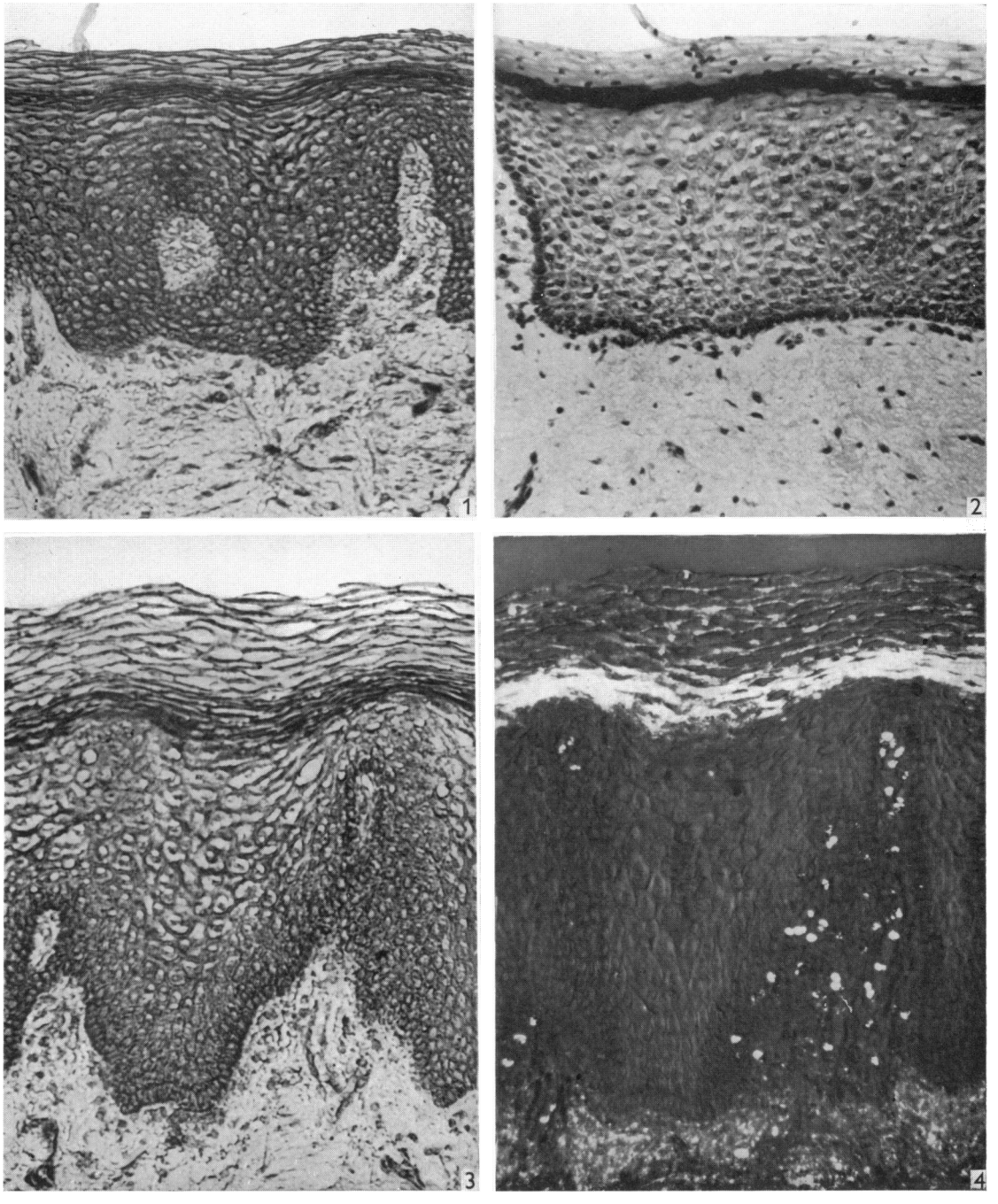
The intra-epithelial zone of the follicular phase closely resembles the intra-epithelial zone of the pro-oestrous mouse vaginal epithelium. Asscher & Turner (1955) showed that during the oestrous cycle of the mouse, disulphide groups first appear in the intra-epithelial zone of the pro-oestrous epithelium. A disulphide-containing, birefringent intra-epithelial zone can be experimentally produced in ovariectomized mice by the administration of oestrogens (Asscher & Turner, unpublished). This comparative histochemical evidence suggests that the existence of an intra-epithelial zone in the human vaginal epithelium might also be dependent on hormonal factors.

The interpretation of cyclical phenomena from random specimens must necessarily be speculative; thus the present observations can do no more than indicate the following tentative sequence of events in the formation and fate of the intra-epithelial zone during the menstrual cycle. This cornification zone appears to increase in thickness during the follicular phase; it attains a more superficial position towards the middle of the cycle and is found in a completely superficial position during the early luteal phase; at this time it possesses the greatest amount of disulphide groups, as judged from the colorations observed with the Barrnett & Seligman technique. This cornification zone appears to be desquamated during the luteal-phase and thus no evidence of it remains at the end of this phase. In late luteal phase specimens evidence of intra-epithelial zone regeneration may frequently be found. The superficial layers of vaginal epithelia may be exposed to a variety of exogenous influences; this may in part account for the variable times at which the cornification zone was found to reach the surface and later to be desquamated. De Allende & Orias (1950) found the highest percentage of cornified cells in vaginal smears taken during the middle of the cycle; the present account of the cornification of the human vaginal epithelium provides a histological basis for this observation, since it is at this stage of the cycle that the cornification zone forms the superficial part of the vaginal epithelium.

The presence of disulphide groups in association with birefringence may be taken to indicate keratin; the use of Michrome and Papanicolaou's vaginal smear stains has produced yellow and red colorations respectively, in all those regions of the epithelium which contained keratin on the basis of the above-mentioned criterion. Red, orange or yellow colorations are considered to indicate the presence of keratin with these stains (Gurr, 1953); the value of these stains in the demonstration of cornified cells in the human vaginal epithelium is thus confirmed. These particular colorations were produced in two specimens, however, in regions in which no keratin could be detected histochemically or by polarized light. It is therefore concluded that some other substance(s) may occasionally imitate the staining reactions of keratin with these vaginal smear stains.

CONCLUSIONS

1. An intra-epithelial zone has been demonstrated in the human vaginal epithelium during the follicular phase. It possesses distinctive chemical and physical properties which indicate that it constitutes a site of cornification. Its development and fate during the menstrual cycle are discussed.



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2. The value of Papanicolaou's (E.A.36) and Michrome (M.F.4) vaginal smear stains in the demonstration of cornified cells in the human vaginal epithelium has been confirmed; anomalous staining reactions were observed in two specimens.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Sulphydryl and disulphide group distribution in the human vaginal epithelium on the 8th day of a 28-day menstrual cycle. Barrnett & Seligman procedure. ×288. Disulphide groups are localized in the cell walls and peripheral cytoplasm of the cells of the intermediate zone.

Fig. 2. Same epithelium as shown in fig. 1 stained with Heidenhain's haematoxylin. ×288. The intermediate zone shows an intense black coloration.

Fig. 3. Sulphydryl and disulphide group distribution in the human vaginal epithelium on the 12th day of a 28-day menstrual cycle. Barrnett & Seligman procedure. ×288. The disulphide-containing, intermediate zone shows greater thickness at this stage of the menstrual cycle.

Fig. 4. Same epithelium as shown in fig. 3 seen under polarized light. ×288. Birefringence is largely confined to the intermediate zone.

PLATE 2

Fig. 5. Same epithelium as shown in fig. 1. × 180. Stained with Michrome vaginal smear stain (M.F.4). The intermediate zone shows an intense yellow coloration.

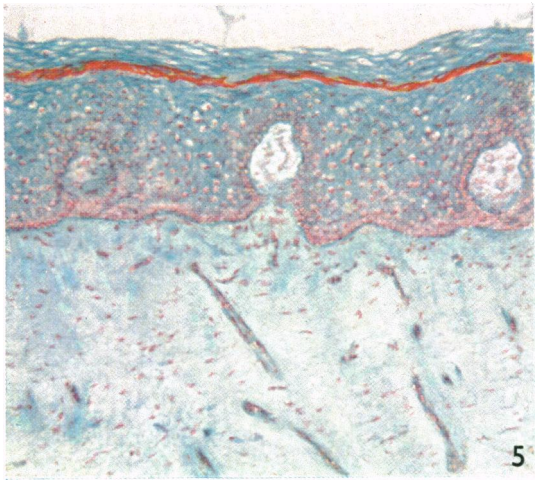
Fig. 6. Same epithelium as shown in fig. 1. × 180. Stained with Papanicolaou's vaginal smear stain (E.A.36). The intermediate zone shows a red coloration.

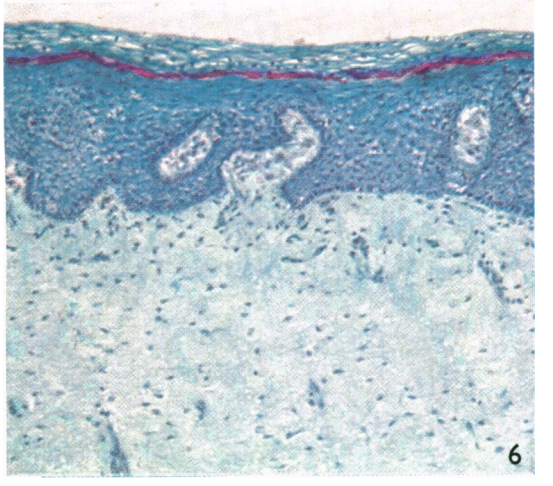
Fig. 7. Human vaginal epithelium taken on the 14th day of a 28-day menstrual cycle. Barrnett & Seligman procedure. × 180. The upper section shows the distribution of sulphydryl groups alone, the lower section shows sulphydryl as well as disulphide groups. A comparison of these sections, which is facilitated by viewing them through a green filter, reveals that the disulphide-containing zone is in a superficial position in most areas of this specimen.

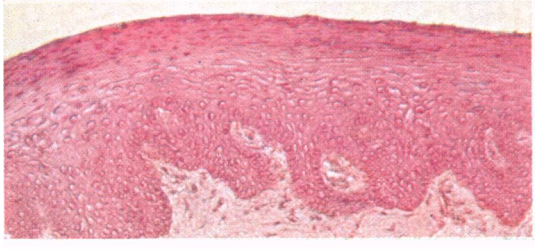
Fig. 8. Distribution of disulphide and sulphydryl groups in a specimen taken on the 22nd day of a 28-day menstrual cycle. Barrnett & Seligman procedure. × 320. The disulphide-containing zone is completely superficial and shows an intense crimson coloration. Some of the cells of the superficial zone possess disulphide groups throughout their cytoplasm; in others these groups are confined to the cell walls and peripheral cytoplasm. The intercellular bridges and some of the nuclei show sulphydryl group positivity.

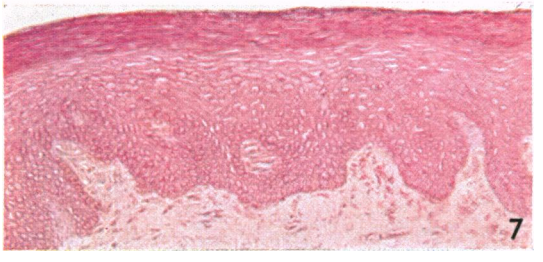
Fig. 9. Human vaginal epithelium taken on the 27th day of a 28-day menstrual cycle. Barrnett & Seligman procedure. × 180. No disulphide groups could be demonstrated in this specimen.

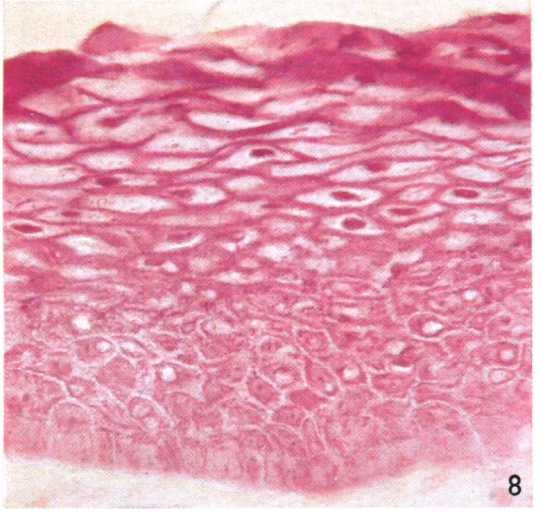
Fig. 10. Same epithelium as shown in fig. 9. × 180. Stained with Michrome vaginal smear stain (M.F.4). Note the intense, patchy yellowness of the deeper layers of the epithelium, extending to the surface in places; in these areas no disulphide groups or birefringence could be demonstrated.

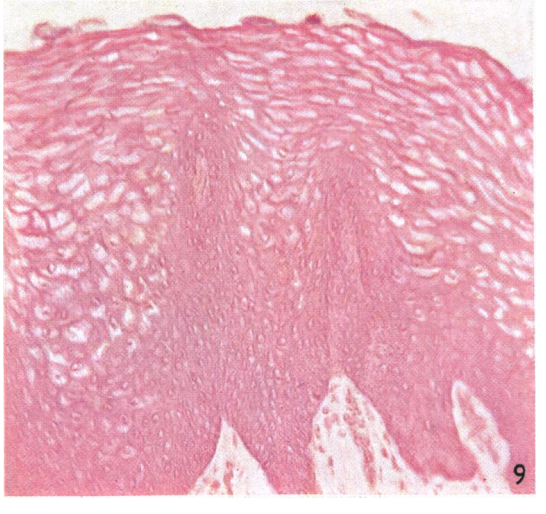


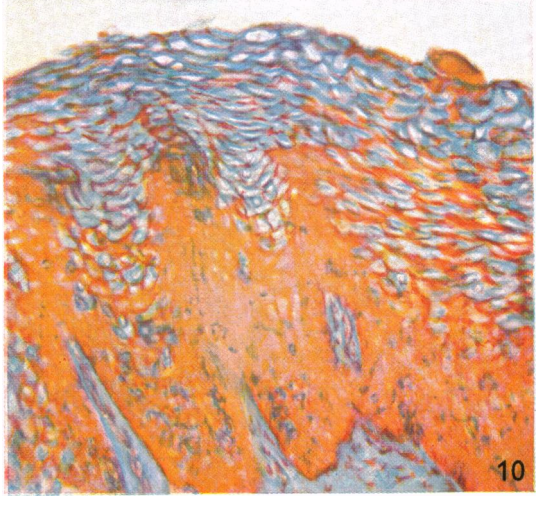












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