THE DEVELOPMENT OF THE HUMAN VAGINA

By D. BULMER

Anatomy Department, University of Aberdeen

The literature on the development of the human vagina is already so abundant that this further contribution is made only with some trepidation. However, the findings which form the substance of this paper seem of sufficient significance to be recorded, since they clearly demonstrate a method of vaginal development which has not so far been generally accepted in this country. The material studied, while not always forming as complete a series as might be wished, has in most cases been in excellent condition for detailed histological examination, and it is possible that the previous confusions and disagreements on this subject were largely due to deficiencies in the material available to earlier workers.

Bloomfield & Frazer (1927) pointed out that the descriptions of vaginal development in the literature could be divided into three main groups, depending on the structures which their authors believed to take part in the formation of the vagina, and it will be convenient to employ this classification here.

In the first group are those accounts which have attributed the origin of the entire vagina to the lower end of the Müllerian utero-vaginal canal. This opinion first gained general acceptance from the demonstration by Thiersch and others, work which is well summarized by Banks (1864), of the fusion of the Müllerian ducts in the genital cord, and in this century has been supported by Felix (1912), Bloomfield & Frazer (1927), Hunter (1930) and von Lippmann (1939). It may be regarded as the 'classical' account of vaginal development, and was for many years the standard textbook description.

The second group consists of those descriptions which supposed that the vaginal epithelium was formed in part (Tourneux & Legay, 1884; Mijsberg, 1924) or in whole (Hart, 1896, 1901 and 1911; Kempermann, 1931) from the lower ends of the Wolffian ducts.

The third group includes the accounts of all those workers who believed that the epithelium of the urogenital sinus made a contribution to the vagina. Before the time of Thiersch it was accepted that the entire vagina was derived from the sinus (Müller, 1830; Rathke, 1832; Valentin, 1835), but this opinion was discarded when the fusion of the Müllerian ducts became known. Retterer (1891), however, suggested that while most of the vagina was formed from the utero-vaginal canal a short lower segment arose by the splitting of the upper part of the sinus into dorsal and ventral channels, and this view was later supported by Bolk (1907). Koff (1933), whose work is recognized by most current British and American textbooks, believed that the lower one-fifth of the vagina was derived from the sinus by the growth of paired epithelial 'sino-vaginal bulbs' from the dorsal sinus wall, these bulbs fusing together to form a lower vaginal segment. On the other hand, Spuler (1930), Vilas (1932), Kempermann (1935) and Meyer (1934–38)

believed that a proliferation of cells from the dorsal wall of the sinus gave rise to the epithelial lining of the entire vagina, displacing the epithelium of the utero-vaginal canal as far as the cervix. This opinion has recently been supported by Politzer (1955), and Zuckerman (1940) showed that there was a considerable body of endocrinological evidence in its favour.

MATERIAL

The material consists of thirteen embryos and foetuses, ranging from 28 to 375 mm. crown–rump length, and an infant of 1 month. The majority of the specimens were already sectioned and stained with haematoxylin and eosin, alternately with haematoxylin and eosin and a trichrome stain, or by the de Castro method of silver impregnation. The remainder were sectioned at 6–10 μ, either transversely or coronally, and the sections stained mainly with haematoxylin and eosin or a trichrome stain. With some of the larger specimens slides were also stained with Best's ammoniacal carmine and by the periodic acid-Schiff technique (Gomori, 1952).

28 *mm. embryo*

In the lower part of the genital cord the Müllerian ducts are in apposition with each other, their medial walls forming a septum between the two lumina. They are lined by a closely packed columnar epithelium and lie between the two Wolffian ducts, rather larger structures with a cubical epithelium. The caudal ends of the Müllerian ducts do not quite reach the dorsal wall of the sinus, remaining separated from the Müllerian tubercle by a small mass of mesoderm, but on either side they are in close contact with the lower ends of the Wolffian ducts (Pl. 1, fig. 1).

Most of the pars pelvina of the sinus is lined by an epithelium consisting of three or four layers of small, darkly staining cells with closely arranged nuclei, but in the region of the Müllerian tubercle the epithelium of the dorsal sinus wall is differentiated into a thin basal layer of deeply staining cells overlain by one to three layers of larger and very pale staining cells with relatively smaller nuclei. At the Wolffian openings, on either side of the Müllerian tubercle, these pale cells seem to be compressed together, and they extend backwards for a short distance into the lower ends of the Wolffian ducts themselves.

42 *mm. foetus*

The lower portions of the Müllerian ducts are completely fused together as the utero-vaginal canal and the caudal tip of the Müllerian epithelium forms a solid mass in contact with the dorsal wall of the sinus, though a small central mass of mesoderm interrupts this contact in the midline. On either side of the Müllerian tubercle the Wolffian ducts enter the sinus, and are now of about the same calibre as the utero-vaginal canal. The sinus epithelium presents the same features as in the 28 mm. specimen (Pl. 1, fig. 2).

50 *mm. foetus*

This specimen shows no significant developmental change, except that the sinus epithelium in the region of the Müllerian tubercle is now considerably thicker. This epithelium, divided into basal deeply staining and superficial pale-staining zones, will in future be referred to as the differentiated type of sinus epithelium.

65 *mm. foetus*

The Müllerian utero-vaginal canal is a large structure, oval in cross-section and with its long axis lying transversely (Pl. 1, fig. 3). Throughout most of its extent it is lined by a closely packed columnar epithelium, but about 0·3 mm. above the caudal end the lining cells lose their columnar arrangement and form a thick stratified polygonal epithelium, which at the lowermost tip completely occludes the lumen. The Wolffian ducts are very small structures, lined by a cubical epithelium, and lie on either side of the utero-vaginal canal. There is a short segment where the Wolffian ducts have completely disappeared, just above their lower ends, but they reappear below this to join the dorsal wall of the sinus.

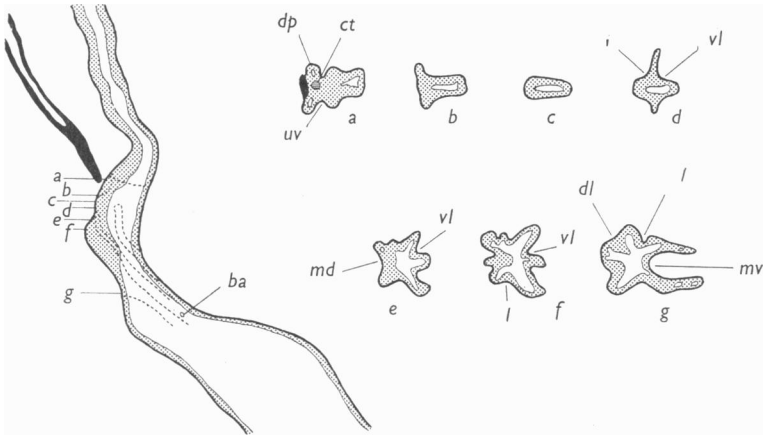
The urogenital sinus shows a very significant advance in the region previously occupied by the Müllerian tubercle (Pl. 1, figs. 4, 5, 6). On either side the Wolffian ducts join 'dorso-lateral projections' of the sinus, solid epithelial structures which prevent the communication of the ducts with the sinus lumen and are in contact on their dorso-medial aspects with the solid mass of Müllerian epithelium at the caudal end of the utero-vaginal canal. More medially, the Müllerian epithelium is displaced dorsally by a proliferation of darkly staining cells from the dorsal sinus wall, between the bases of the two dorso-lateral projections (Pl. 1, fig. 4). The dorso-lateral projections are each formed by a central mass of pale-staining cells, with small nuclei, surrounded by a thin basal layer of more darkly staining cells, and the cubical cell lining of the lower ends of the Wolffian ducts is applied to their dorsal aspects. Caudal and ventral to the proliferation of darkly staining cells from the dorsal sinus wall, the pale-staining cells extend forwards and medially to form a crest projecting into the dorsal aspect of the sinus lumen (Pl. 1, figs. 5, 6)—the structure identified by Kempermann (1931) as the *Wolffsche Kamm*. Apart from this differentiation in the region previously occupied by the Müllerian tubercle, the remainder of the pars pelvina is lined by a darkly staining stratified epithelium of six or seven layers of small cells.

In this specimen, therefore, the sinus cells in the region which was previously lined by the differentiated type of epithelium form a proliferative zone, apparently composed of three elements. On either side are the dorso-lateral projections, associated with the Wolffian openings, while between them is a proliferation of darkly staining cells from the dorsal sinus wall.

68 *mm. foetus*

This specimen is at only a slightly later developmental stage than the 65 mm. foetus, but several interesting differences may be noted. The Wolffian ducts are present throughout the whole of the genital cord as small, but easily recognizable structures. The occlusion of the lumen of the caudal portion of the utero-vaginal canal reaches further cranially than in the 65 mm. specimen, to a distance of about 0·3 mm. above the junction of Müllerian and sinus epithelia. A small mesodermal mass is enclosed in the root of the proliferation of darkly staining cells from the dorsal wall of the sinus, splitting it into bilateral elements which meet each other behind the mesodermal mass and are there in contact with the caudal end of the Müllerian epithelium (Text-fig. 1*a*).

The most interesting feature is the appearance of a characteristic system of longitudinal folds in the sinus wall (Text-fig. 1), foreshadowed at the 65 mm. stage but now very much more distinct. It may be noted that there is a pronounced fold—termed the 'urethro-vaginal fold'—demarcating from the rest of the sinus its cranial and dorsal portion which forms the zone of contact with the Müllerian tissue. The dorsal margins of the urethro-vaginal folds run from the dorsal wall of the sinus, below the dorso-lateral projections, to meet each other behind the lower end of the urethra, and limit the area, dorsal and cranial to them, which is occupied by the



Text-fig. 1. 68 mm. foetus. The figure on the left is a graphic reconstruction (× 20) of a median sagittal section through the lower end of the utero-vaginal canal and the urogenital sinus. The figures on the right, *a–g*, are transverse sections at the levels indicated in the sagittal section. The dorsal aspect of the sections is towards the left. The Müllerian epithelium is shown by the solid black shading, and the sinus epithelium by the stippling. A small area of connective tissue, *ct* in fig. 1*a*, interrupts the sinus proliferation in the midline. The folds of the sinus wall are indicated in the transverse sections, and their relative positions in the sagittal section are shown by the dotted lines. *dl*, dorso-lateral fold; *md*, median dorsal fold; *l*, lateral fold; *vl*, ventro-lateral fold; *uv*, urethro-lateral fold; *v*, median ventral fold. The dorso-lateral sinus bay lies between the lateral and dorso-lateral folds (figs. 1*f*, 1*g*) or between the lateral and median dorsal folds (fig. 1*e*), and is joined at *ba* by the duct of Bartholin's gland. The ventro-lateral bay is between the lateral and ventro-lateral folds. It will be noticed that the relief of the sinus wall compares very closely with that described by Mijsberg (1924) and Politzer (1952) at similar developmental stages.

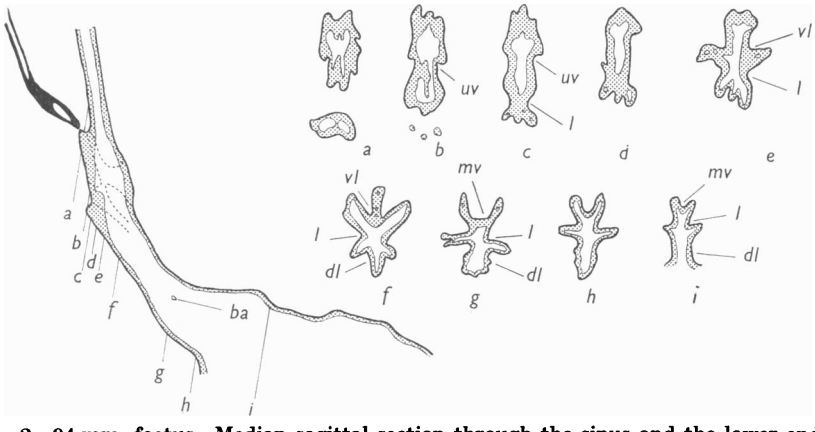
differentiated type of sinus epithelium. The pattern of the folds in the wall of the human sinus has been described previously by Mijsberg (1924) and Politzer (1952). They are indicated in the diagrams in Text-figs. 1–4, and a more detailed account has been given elsewhere (Bulmer, 1955).

14 *week foetus*

The dorso-lateral projections can be identified, similar in structure to those of the 65 mm. stage, and their dorso-medial aspects are in contact with the Müllerian epithelium. Between their bases is the mass of darkly staining cells proliferating from the dorsal wall of the sinus, ventral to the Müllerian epithelium and now more extensive than in the 65 mm. foetus.

94 mm. foetus

There is a considerable increase in size of the sinus compared with the 68 mm. stage (Text-fig. 2), but the arrangement of the longitudinal folds remains essentially unchanged. The urethro-vaginal fold is a marked feature, accentuated by the enlargement of the sinus which lies dorsal and cranial to it (Text-fig. 2*b*). This enlargement is associated with a considerable thickening of the epithelium, which now consists of a basal zone of five or six layers of small, darkly staining cells, with relatively large nuclei, clearly demarcated from a superficial zone of three or four layers of larger, clear staining polygonal cells with relatively much smaller nuclei. There is a striking distinction between the two zones in the low-power view (Pl. 2, fig. 7), and this epithelium is markedly different from the much thinner, undifferentiated type of epithelium, of four or five layers of smaller, rather darkly staining cells, which lines the rest of the pars pelvina.



Text-fig. 2. 94 mm. foetus. Median sagittal section through the sinus and the lower end of the vagina, with the corresponding coronal sections ($\times 13\frac{1}{3}$). Shading and lettering as for Text-fig. 1. The dotted lines in the sagittal section indicate the relative positions of the folds in the upper part of the sinus wall. The coronal sections, *a–i*, are shown with their cranial ends (i.e. the ventral wall of the sinus) towards the top.

The enlargement of the sinus behind the urethro-vaginal folds also forms the root of a short 'sinus upgrowth', projecting dorsally and cranially from the sinus to meet the caudal end of the Müllerian epithelium. The lower end of the sinus upgrowth contains paired lumina, continuous with the sinus lumen, and the epithelium is of the same differentiated type as that of the sinus enlargement. Followed further cranially the lumina disappear, and the sinus upgrowth forms a solid crescentic mass of epithelium in which the basal and superficial zones are still distinct (Pl. 2, fig. 8), though the basal cells are not so prominent as they are more caudally. In addition, a small mass of darkly staining cells, the caudal end of the Müllerian tissue, is embedded in this cranial end of the sinus upgrowth.

As the vaginal mass is followed still further cranially the left side of the sinus upgrowth is joined by a short persistent segment of the lower end of the left Wolffian duct, and the Müllerian epithelium comes to occupy a gradually increasing area in

the centre. Eventually, about 240 μ above the root of the sinus upgrowth, the sinus cells are completely replaced by the solid Müllerian epithelium, which is canalized a short distance above this as the lower end of the utero-vaginal canal. This part of the canal is lined by a three- or four-layered stratified polygonal epithelium, which extends cranially to meet the columnar cell lining of the upper part. The level of junction between these two types of Müllerian epithelium coincides with a fusiform swelling of the genital cord, corresponding with that identified by Koff (1933) as the site of the future cervix.

It can be appreciated that several changes have occurred to reach this stage of development. The three components which formed the proliferation of the sinus from the 65 mm. stage onwards—the two dorso-lateral projections and the darkly staining cells between them—have apparently fused together to form a single mass which extends dorsally and cranially as the sinus upgrowth. The lower ends of the Wolffian ducts have disappeared except for a short persistent segment on the left side, which, as might be expected, joins the side of the sinus upgrowth. In addition, the 'vaginal' portion of the utero-vaginal canal, so far as this can be defined, is now entirely lined by a stratified polygonal epithelium, presumably derived from the original columnar Müllerian epithelium. One of the most interesting features, however, is the differentiation of the sinus epithelium behind and above the urethro-vaginal folds. A similar differentiation, though less marked, has been noted since the 28 mm. stage, always confined to this particular region of the sinus, and it is from this differentiated type of epithelium that the sinus upgrowth appears to arise.

112 *mm. foetus*

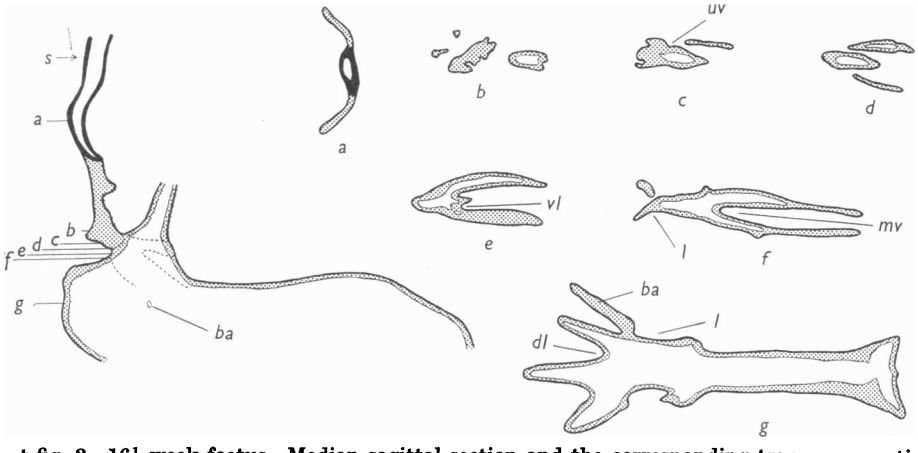
The configuration of the urogenital sinus shows little change from the 94 mm. stage, though the pars phallica is now becoming more dorso-ventrally elongated. The sinus upgrowth extends for about 570 μ above the dorsal wall of the sinus, as a transversely elongated epithelial plate, and its cranial end reaches up for a short distance as tapering 'wings' on either side of the lower end of the utero-vaginal canal. Caudally it is continuous with the dorsal wall of the sinus, but its root is split into two by a small mesodermal septum (Pl. 2, fig. 9). Dorsal to this septum the two roots join each other to form the sinus upgrowth, while on its ventral aspect they are continuous with the enlarged dorsal and cranial portion of the sinus which lies above and behind the urethro-vaginal folds. This arrangement obviously cannot have arisen from conditions such as have been described in the 94 mm. foetus. Apparently, as in the 68 mm. specimen, a small mesodermal septum must have divided the root of the sinus upgrowth at the time of its initial formation.

The epithelium of the sinus upgrowth stains rather more deeply than in the 94 mm. foetus, but the basal and superficial zones can be readily recognized and it can be distinguished from the Müllerian epithelium with which it is in contact (Pl. 2, fig. 10). At the caudal end of the upgrowth, and in the enlargement of the dorsal portion of the sinus from which it arises, the epithelium is of the same differentiated type, but here the pale-staining cells are much larger and more prominent than they are further cranially, their cytoplasm eosinophilic and their nuclei very small (Pl. 2, fig. 9). The remainder of the pars pelvina is still lined by the same undifferentiated type of epithelium as in the earlier stages.

The lower part of the utero-vaginal canal is lined by a stratified polygonal epithelium, and the first sign of the differentiation of the surrounding mesoderm which marks the position of the future external os (Bulmer, 1955) indicates that this epithelium lines the entire 'vaginal' portion of the canal, the uterine segment retaining its original columnar epithelium.

16½ *week foetus*

The crown–rump length of this specimen is unfortunately not known, but it shows a slightly later stage of development than the 112 mm. foetus. There is now a marked dorso-ventral elongation of the pars phallica and a relative and absolute shortening of the pars pelvina compared with the 94 mm. foetus. Though this change of shape has slightly modified the relative positions of the folds in the sinus wall their pattern remains essentially the same (Text-fig. 3).



Text-fig. 3. 16½ week foetus. Median sagittal section and the corresponding transverse sections through the sinus and the lower end of the utero-vaginal canal (× 13½). The arrow at *s* indicates the level of the cranial tips of the sinus upgrowth, on either side of the lower end of the utero-vaginal canal. Other lettering and shading as for Text-fig. 1.

The sinus upgrowth extends cranially for a distance of about 1·6 mm.—approximately half the extent of the future vagina as indicated by the site of the external os. In most of its extent the upgrowth forms a solid transversely elongated epithelial plate, and cranially it extends for a short distance as bilateral wings on either side of the lower end of the utero-vaginal canal. Its epithelium is again differentiated into basal and superficial zones, and the thickness of section (12 μ) seems to accentuate the distinction between Müllerian and sinus epithelia (Pl. 2, fig. 11). The 'vaginal' portion of the utero-vaginal canal is lined by a four- or five-layered stratified Müllerian epithelium, and the superficial cells are now becoming more flattened.

At its caudal end the sinus upgrowth joins the enlargement of the sinus which lies cranial and dorsal to the urethro-vaginal folds, unsplit by any mesodermal septum, and this lower end of the upgrowth now shows three swellings of its epithelial plate, one centrally and one on either side. In these swellings, and in the dorsal

enlargement of the sinus, the epithelium is rather different from that in the cranial part of the sinus upgrowth. The internal cells are larger and the nuclei smaller, so that the distinction between basal and superficial zones is much more apparent (Pl. 2, fig. 12). In addition, particularly in the swellings of the sinus upgrowth, the cytoplasm of the internal cells is eosinophilic, and the cell walls deeply stained. The remainder of the pars pelvina, below and ventral to the urethro-vaginal folds, is still lined by an undifferentiated type of epithelium, consisting of four or five layers of small, darkly staining cells.

140 *mm. foetus*

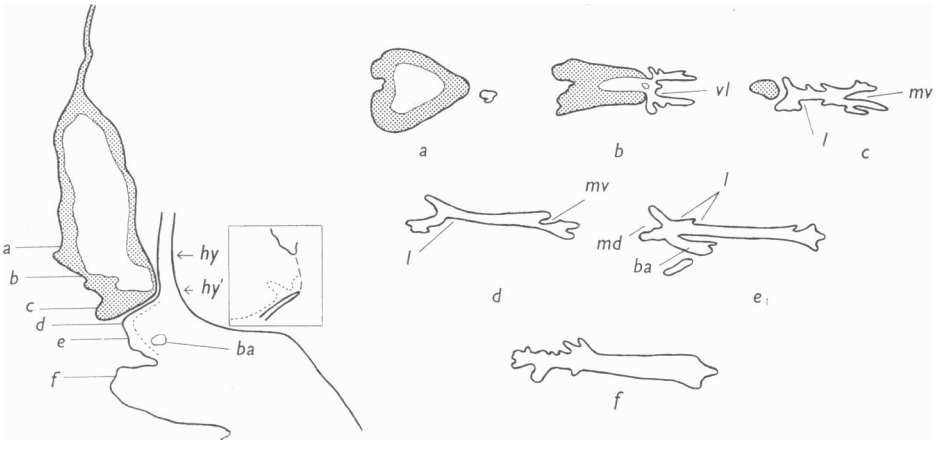
In this specimen many further developments are apparent. The sinus upgrowth extends to the lower end of the cervical canal as a solid, transversely elongated epithelial plate in which the basal and internal zones of cells can be distinguished. Here it meets a stratified squamous Müllerian epithelium (Pl. 2, fig. 13), which consists of very much smaller cells and lines the lower portion of the cervical canal immediately above the sinus upgrowth, intervening between this and the columnar epithelium of the rest of the uterus above. The vaginal fornices have not yet appeared.

As the sinus upgrowth is followed caudally, it develops, about the middle of its cranio-caudal extent, enlargements similar to those seen at the lower end of the upgrowth in the 16½ week foetus—a large swelling centrally, and smaller swellings at each lateral extremity. Traced further caudally these swellings become larger, until eventually they merge together to form a single mass occupying about the lower one-third of the vagina, rather heart-shaped in cross-section and with a large central lumen (Text-fig. 4). In the swellings the vaginal epithelium consists of a narrow basal zone of darkly staining cells, with relatively large nuclei, and a mass of large internal pale-staining cells with very small nuclei, eosinophilic cytoplasm and very deeply stained cell walls (Pl. 3, fig. 14). In the canalized portion of the vagina the epithelium is very similar, with a thick zone of internal cells of which the superficial layers are flattened. The lumen is filled by a mass of desquamated material, and the PAS technique demonstrates that all but the basal cells of the epithelial lining are loaded with glycogen.

The lower end of the vagina communicates with the sinus by paired hymeneal orifices (Text-fig. 4*b* and Pl. 3, fig. 15), separated from each other by a small median mesodermal septum, and the great enlargement of the lower end of the vagina has resulted in the extension of its area of contact with the sinus, particularly on the lateral and caudal aspects of the orifices (Text-fig. 4*c*). In this way the hymen is formed, consisting of a plate of dense connective tissue, lined above by vaginal epithelium and below by the undifferentiated type of sinus epithelium which still occupies the upper part of the sinus (Pl. 3, fig. 17).

Associated with the increasing area of contact between the vagina and the sinus which gives rise to the hymen, the configuration of the sinus itself shows a considerable change from the stage represented by the 16½ week foetus. The pars pelvina, as judged by the site of entry of the ducts of Bartholin's glands (Text-fig. 4), is now very short, and the original system of longitudinal folds, though it can still be followed, has become modified by the change in shape of this part of the sinus.

A striking feature, which seems to be associated with the invagination of the upper part of the dorsal wall of the sinus by the lower end of the vagina, is the accentuation of the upper end of the lateral fold, now lying parallel with the posterior portion of the hymen and separated from it by an upward extension of the dorso-lateral sinus bay—the bay which, at a lower level (Text-fig. 4), receives the ducts of Bartholin's glands. The dorso-lateral bay extends cranially and ventrally on the lower aspect of the hymen as far as the hymeneal orifices, where it terminates as the lateral fold becomes continuous with the hymeneal fold. In addition, it gives off a cranial diverticulum which ascends for a short distance on the lateral aspect of



Text-fig. 4. 140 mm. foetus. Median sagittal section through the vagina and the sinus, with the corresponding transverse sections ($\times 6\frac{2}{3}$). The stippling indicates the vaginal epithelium, and the vestibule is shown only in outline. Because of the dilatation of the upper end of the sinus it is not practicable to indicate the folds of its wall in the median sagittal section. However, the dorsal margin of the lower part of the lateral fold is shown by the dotted line, and the small inset figure shows the outline (dotted line) of the upper end of the dorso-lateral sinus bay and its cranial diverticulum in relation to the margins of the vaginal and sinus epithelia (continuous lines) and to the hymeneal orifices (interrupted line). *hy–hy'* indicates the level of the hymeneal orifices in relation to the median sagittal section. Other lettering as for Text-fig. 1. It will be seen that the change in the position of the lateral fold in this specimen, and to an even greater extent in the next (Text-fig. 5), differs from that described by Mijsberg (1924). The dorso-lateral bay is rotated relatively upwards and backwards with the downgrowth of the vagina into the sinus. According to Mijsberg, this bay is rotated upwards and forwards, an opinion for which the present material offers no evidence.

the lower end of the vagina (Pl. 3, figs. 15, 16), and presumably represents the cranial extremity of the dorso-lateral bay of the $16\frac{1}{2}$ week stage, maintaining its position despite the relative downgrowth of the lower end of the vagina.

The hymeneal folds, bounding the outer margins of the paired hymeneal orifices, represent the dorsal margins of the urethro-vaginal folds of the earlier stages. In the same way they form the boundary between the differentiated and undifferentiated types of sinus epithelium, and it is by the enlargement of the area of differentiated epithelium—now the lower end of the vagina—and its downgrowth relative to the urogenital sinus that the hymen is formed. The mesodermal septum between the two hymeneal orifices seems to differ in origin from the remainder of

the hymen, and probably results from the further development of a septum such as occurs in the 112 mm. foetus, splitting the root of the sinus upgrowth. By the enlargement and downgrowth of the lower end of the vagina the septum is projected forwards to divide the space between the dorsal margins of the urethro-vaginal folds—in other words, to split the hymeneal orifice. The mesodermal septum of the 140 mm. stage is therefore lined on both its ventral and dorsal aspects by vaginal epithelium.

180 *mm. foetus*

The enlargement and canalisation of the vagina now extends throughout its entire length, and the fornices are well established. An important change is the disappearance of the zone of stratified squamous Müllerian epithelium from the lower portion of the cervical canal, and the sinus epithelium of the vagina meets the columnar epithelium of the uterus just inside the external os. Here there is a very clear line of distinction between the two epithelia (Pl. 3, fig. 18). Both give a strongly positive PAS reaction, granular in the vaginal epithelium and diffuse in the columnar cells of the cervix, but in the latter this reaction is not affected by previous salivary digestion.

The vagina is lined by a very thick stratified squamous epithelium (Pl. 3, fig. 19), in which it is possible to distinguish four cellular zones. The basal zone is formed by three or four layers of small cubical cells, with relatively large nuclei and basophil cytoplasm. The next zone consists of seven or eight layers of much larger cells, with relatively much smaller nuclei and pale, acidophil cytoplasm. The deeper layers of this zone consist of polygonal cells, with deeply stained cell walls, but the three or four superficial layers are formed by very flattened cells, with much thicker cell walls. The third zone consists of about ten layers of large, clear-staining polygonal cells, less flattened and with thinner cell walls than the most superficial cells of the second zone. The nuclei are very small, and are absent from many of the cells. The innermost zone is formed by four or five layers of cells, similar to those of the third zone but more flattened. Many of the cells are without nuclei, and the nuclei which are present are very small and pyknotic.

Glycogen is extremely abundant in the vaginal epithelium in all but the basal zone of cells, as shown by staining with Best's ammoniacal carmine and by the PAS technique. It is generally located as large granules, sometimes almost filling the cytoplasm of the cell, and is usually, but not always, restricted to the part of the cell on the proximal side of the nucleus. In addition, the cell walls of the three superficial zones show a positive PAS reaction after previous salivary digestion, similar to that demonstrated by Wislocki, Fawcett & Dempsey (1951) in the vaginal epithelium of the adult.

A further development from the 140 mm. stage is the lining of the under surface of the hymen and the adjacent portion of the vestibule by an epithelium similar to the vaginal epithelium, though rather thinner. Associated with the further relative downgrowth of the lower end of the vagina, and the resulting more horizontal position of the hymen (Text-fig. 5), the pars pelvina has almost disappeared. In fact, the shape of the sinus is so distorted from its original form that the distinction between pars pelvina and pars phallica now has little meaning.

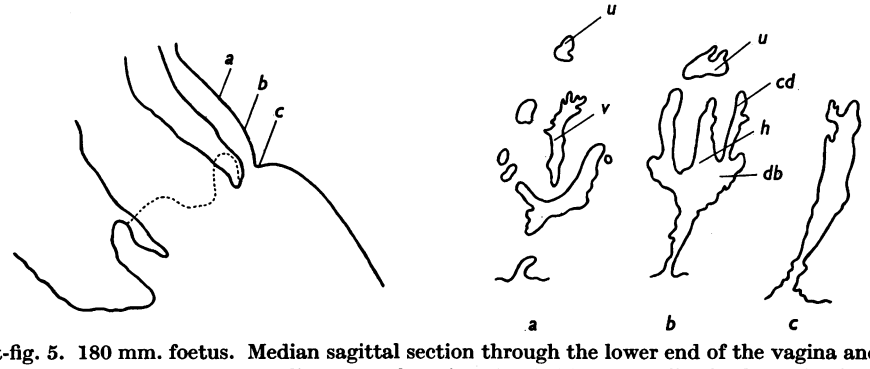
By this stage the sinus extends further dorsally in relation to the under surface of the hymen, and the dorso-lateral sinus bays are very prominent, forming a dilatation of the vestibule immediately beneath the hymen (Text-figs. 5*a*, *b*). They extend further ventrally than in the 140 mm. foetus, reaching forwards around the sides of the anterior portion of the hymen. As before, they send short diverticula cranially, on either side of the lower end of the vagina. The hymenal orifice is unsplit.

200 mm. foetus

This specimen shows little further change, but the sagittal plane of section gives a very clear picture of the penetration of the lower end of the cervical canal by the vaginal epithelium, to a distance of about 3 mm. above the external os.

375 mm. foetus

The vaginal epithelium is now considerably thinner, but the lumen is filled with cell debris, suggesting that the decrease in thickness may be largely due to desquamation of the superficial cell layers. Only three cellular zones can be distinguished (Pl. 3, fig. 20). In the basal zone there are usually three layers of small cubical cells, with large nuclei and basophil cytoplasm. The intermediate zone is



Text-fig. 5. 180 mm. foetus. Median sagittal section through the lower end of the vagina and the vestibule, with the corresponding coronal sections (× 4). The dotted line in the sagittal section shows the relative extent of the dorso-lateral bays. The coronal sections are shown with their cranial ends towards the top. *u*, urethra; *v*, vagina; *h*, hymeneal orifice; *db*, dorso-lateral sinus bay; *cd*, cranial diverticulum of dorso-lateral sinus bay.

formed by four or five layers of much larger polygonal cells, with relatively smaller nuclei and deeply staining cell walls. Superficially there are two or three layers of large flattened cells with small pyknotic nuclei. The superficial and intermediate zones are not clearly distinct from each other, and both are rich in glycogen granules. It is possible that these two zones represent the second zone of the epithelium of the 180 mm. foetus, the third and fourth zones having been desquamated. Again the cell walls give a positive PAS reaction after salivary digestion.

The sinus shows the further progress of the developmental changes noticed at the 180 mm. stage. The dorso-lateral bays extend still further forwards, now reaching around the urethral orifice, and the dorsal extension of the sinus in relation to the under surface of the hymen is still more marked. The whole of the upper part of the

sinus is lined by an epithelium similar to that of the vagina, and now of about the same thickness.

1 *month infant*

Little change is noticed in this specimen, except in the character of the vaginal epithelium (Pl. 3, fig. 21). This is considerably thinner than in the later foetal stages and consists mainly of polygonal cells with relatively large nuclei, deeply staining cytoplasm and indistinct cell walls. Glycogen is no longer present, and the cell walls no longer give a positive PAS reaction.

DISCUSSION

There can be little doubt that the epithelium of the human vagina is entirely derived from an upgrowth of sinus cells, as Vilas (1932), Kempermann (1935) and Meyer (1934–38) claimed, but the manner of formation of this upgrowth does not seem to be completely in accord with their findings. Vilas believed that the epithelium in the dorsal wall of the sinus proliferated in two different ways. An inner proliferation of pale-staining cells grew ventrally and medially in the internal layers of the dorsal sinus wall, meeting its fellow to form a crest which projected ventrally into the sinus lumen. An outer proliferation of darkly staining cells grew dorsally, displaced the Müllerian cells from the Müllerian tubercle and extended cranially as an epithelial plate, subsequently canalized to form the vagina. The views of Meyer and Kempermann were essentially similar.

It is interesting to compare this with Koff's account (1933) of the origin of the 'sino-vaginal bulbs', evaginations of the dorso-lateral aspect of the sinus on each side. Koff found that their epithelium was different from that of the rest of the sinus, consisting of well-marked basal and superficial zones, the basal cells darkly staining and the superficial cells pale-staining. The bulbs became larger and eventually fused with each other to form a solid cellular mass, which displaced the Müllerian tissue dorsally and cranially and eventually became canalized as the lower one-fifth of the vagina. The sino-vaginal bulbs seem to be the structures which have here been termed the dorso-lateral projections. In the same way they were joined by the lower ends of the Wolffian ducts, when these persisted, and carried the attachments of the ducts with them as they extended cranially. Koff did not mention any proliferation of darkly staining cells from the intermediate portion of the dorsal wall of the sinus, such as occurs in the 65 mm., 68 mm. and 14-week foetuses of this present collection, and would presumably regard this proliferation as an early stage in the fusion of the sino-vaginal bulbs.

Up to a point, the findings of this present investigation combine both these views on the initial origin of the sinus contribution to the vagina, the sinus upgrowth taking origin from all three of the elements associated with the activity of the dorsal wall of the sinus, first noticed in the 65 mm. foetus. The junction of the persistent lower end of the left Wolffian duct with the sinus upgrowth in the 94 mm. specimen indicates that, at any rate in this foetus, the dorso-lateral projection must have participated in the formation of the upgrowth. On the other hand, in the 65 mm. foetus there seems no doubt as to the entity of the intermediate proliferation of darkly staining cells, and their contribution to the sinus

upgrowth cannot be ignored. Only by the fusion of the three initial elements, by the 94 mm. stage, is the single sinus upgrowth produced. It is interesting that the formation of the sinus upgrowth in the human foetus bears such a close similarity to the initial origin of the lower vaginal segment in the sheep (Bulmer, 1956). There may be no great significance in the distinction between the dorso-lateral projections and the intermediate proliferation of darkly staining cells, all three representing a continuous cellular proliferation from this area of the sinus wall. There seems little doubt, however, of the identification of the dorso-lateral projections with Koff's sino-vaginal bulbs, and of the intermediate proliferation with the outer sinus proliferation of Vilas.

In many instances, it appears, the root of the sinus upgrowth is split at its origin from the dorsal wall of the sinus by the inclusion of a small mesodermal septum. Vilas pointed out that in his early foetuses, from the 38 mm. stage onwards, a small mass of mesoderm interrupted the contact between the Müllerian epithelium and the dorsal wall of the sinus in the midline, and this has been noticed here in the 28, 42 and 50 mm. foetuses. Vilas described the initial bilateral origin of the darkly staining sinus proliferation, the two origins then fusing to form a single sinus upgrowth. In the 65 mm. foetus, described here, there is no sign of such a bilateral origin, but in the 68 mm. foetus the proliferation is split by a small mesodermal septum. The inclusion of a mesodermal septum in the root of the sinus upgrowth is most probably a result of the persistence of such a septum at the Müllerian tubercle, and this would suggest that the darkly staining proliferation only arises where the sinus is actually in contact with Müllerian cells. The persistence of such a septum, as in the 112 mm. foetus, and its development into a hymeneal septum, as in the 140 mm. foetus, must be fairly common, and occurred in many of the specimens examined by Meyer (1934–38). The persistence of a more extensive septum in the utero-vaginal canal would account, in a similar manner, for a congenital duplication of the vagina.

The further development of the sinus upgrowth, after the 94 mm. stage, is of some interest. Vilas found that as the Müllerian epithelium receded before the advancing plate of sinus epithelium the lower end of the utero-vaginal canal, including its whole 'vaginal' portion, became completely occluded by the proliferation of its lining cells. A solid vaginal plate was thereby formed, composed of Müllerian tissue above and sinus tissue below, but the sinus epithelium gradually extended further and further cranially until eventually the Müllerian epithelium was completely displaced from the vaginal plate. In the series of foetuses examined here the occlusion of the lower end of the utero-vaginal canal is not a prominent feature. It is first seen at the 65 mm. stage and persists until the Müllerian epithelium is completely excluded from the vagina, but it never involves more than a short segment. It is to be noted, however, that the stratification of the epithelium of the utero-vaginal canal does extend, apparently by the 94 mm. stage, throughout the entire 'vaginal' portion of the canal.

Koff also described a 'primitive vaginal plate', formed in its lower portion by the solid epithelial mass of the sino-vaginal bulbs, and in its upper portion from the occlusion of the 'vaginal' portion of the utero-vaginal canal by the proliferation of its lining cells. In this solid vaginal plate Koff claimed that he was able to distinguish histologically between the sinus and Müllerian components, and only after

the 142 mm. stage was this distinction no longer apparent. He assumed that the relative proportions of the two epithelia remained unchanged after this stage, the sinus epithelium being restricted to the lower one-fifth of the vagina.

Unfortunately, Koff showed no microphotograph of the epithelial distinction between the components of his primitive vaginal plate, but the criterion which he used for the identification of the sinus epithelium was its differentiation into external darkly staining and internal pale-staining zones. It has been noticed in the series of foetuses described here that after the 94 mm. stage the internal cells in the cranial part of the sinus upgrowth are much less pale-staining than they are further caudally. For instance, in the 16½ week foetus the large, markedly pale-staining cells are restricted to the enlarged portion of the dorsal wall of the sinus and to the swellings at the caudal end of the sinus upgrowth. Indeed, Koff seems to have identified the two laterally placed swellings with the sino-vaginal bulbs themselves—a conclusion which is difficult to follow and for which there is no evidence in the material described here. The epithelium at the cranial end of the sinus upgrowth is of the same essential character as that further caudally. There is no evidence of any sharp histological distinction in the vaginal mass except the one which has been identified as the junction of the cranial end of the sinus upgrowth with the caudal end of the Müllerian tissue. Koff's description offers no explanation for the very marked distinction between the two, occurring about half-way up the 'vagina' in the 16½ week foetus.

The most likely explanation for the histological differences between the cranial and caudal ends of the sinus upgrowth is that the differentiation of its cells progresses from below upwards. Thus, the cells of the caudal end of the upgrowth in the 112 mm. and 16½ week foetuses, when the internal cells are larger, their nuclei smaller and the cell walls deeply stained with eosin, are similar to those occupying the whole of the lower half of the vagina in the 140 mm. foetus. The transversely elongated plate of the vagina appears to differentiate, at each particular stage, by first enlarging to form swellings. The epithelium correspondingly proliferates and the internal cells become large, with small nuclei and deeply stained cell walls. The swellings become still larger, and confluent with each other, the central cells then desquamating to form the vaginal lumen. The 140 mm. foetus shows later and later stages of this process as the sinus upgrowth is followed further and further caudally. The vaginal epithelium continues to proliferate, presumably under the influence of hormonal stimulation, and it is interesting to compare its structure in the two older foetuses with the adult vaginal epithelium as described by Papanicolou, Traut & Marchetti (1948), and more particularly, in view of the probable hormonal influences at work, with the vaginal epithelium of the pregnant woman, described by Smith & Brunner (1934).

As indicated above, many earlier workers have denied the participation of sinus epithelium in the formation of the vagina. Berry Hart (1896, 1901 and 1911) and Mijsberg (1924) described a contribution to the vagina from the 'Wolffian bulbs', proliferations of the epithelium of the lower ends of the Wolffian ducts. There seems no doubt that the Wolffian bulbs were the dorso-lateral projections, and there is no evidence that these latter arise from Wolffian epithelium. Their cells are unlike the cubical Wolffian cells which lie dorsal to them, and much more closely

resemble those of the dorsal sinus wall. Berry Hart believed that the epithelium of the Wolffian bulbs extended throughout the entire vagina, while Mijsberg, in a sense less correctly, restricted the Wolffian contribution to a lower vaginal segment. His reasons for this were similar to those which influenced Koff to believe that the sino-vaginal bulbs formed only the lower end of the vagina.

Other authors have held to the view that the human vagina is entirely derived from the Müllerian utero-vaginal canal. Bloomfield & Frazer (1927) illustrated a section rather similar to the ones shown in Pl. 1, figs. 4, 5 and 6, for the 65 mm. foetus, but their interpretation was very different, as they believed that the pale-staining cells in the dorsal sinus wall were the Müllerian cells breaking through into the sinus at the Müllerian tubercle. It is clear that the findings in the 65 mm. foetus make such a view untenable, and this misinterpretation presumably led Bloomfield & Frazer to think that the whole extent of the differentiated type of sinus epithelium was of Müllerian origin.

While the sinus origin of the vaginal epithelium appears to be established, one or two points of detail merit further consideration. From the earliest stage examined, represented by the 28 mm. foetus, the epithelium which lines the part of the sinus from which the sinus upgrowth later arises differs from that of the rest of the pars pelvina in consisting of two cellular zones—darkly staining cells deeply and pale-staining cells superficially. So far, it has been tacitly assumed that both these cell types are of sinus origin, but other workers have placed different interpretations upon them. Vilas (1932) believed that the darkly staining cells were of sinus origin, but was prepared to accept that the pale-staining cells were Wolffian, growing ventrally and medially in the dorsal wall of the sinus to form the ventrally projecting *Wolffsche Kamm*, identified by Kempermann (1931) and evident in the 65 mm. foetus of this present investigation. Kempermann (1935) also believed that the pale-staining cells were Wolffian in origin, but that they later completely disappeared. The pale-staining cells in the internal layers of the sinus upgrowth at a stage such as that represented by the 94 mm. foetus of this collection were not the Wolffian cells, but a new generation of cells derived from the sinus. Meyer (1934–38), however, believed that the pale-staining Wolffian cells did persist into later foetal life, identifying them with the large pale-staining cells such as occur at the root of the sinus upgrowth in the 112 mm. and 16½ week foetuses.

This question does not seem possible of solution by ordinary histological methods. Nevertheless, the pale-staining cells in the dorsal wall of the sinus from the 28 mm. stage onwards bear no obvious similarity to the cubical cells which line the Wolffian ducts, and are distinct from the Wolffian cells which lie dorsal to them. It appears just as reasonable to suppose that the pale-staining cells are derivatives of the darkly staining basal cells, and that there is no incorporation of Wolffian cells in the dorsal wall of the sinus at this stage. No such ingrowth occurs in other mammals, and it seems unnecessary to postulate it in the human foetus. Nevertheless, it must be admitted that the differentiation of the sinus epithelium from which the sinus upgrowth arises is a remarkable feature, and it too has not been described in other mammals.

Also of some significance is the problem of the behaviour of the lower ends of the Wolffian ducts during the development of the vagina. Descriptions of the lower ends

of the Wolffian ducts in female foetuses fall generally into two main categories. In the first are those which maintain that the Wolffian ducts continue to open into the sinus at their original site, immediately lateral to the position of the Müllerian tubercle. Thus, Meyer (1909) described the persistence of the lower ends of the Wolffian ducts in a large number of older foetuses and new-born children, running into the substance of the hymen and opening into the vestibule close beside the hymeneal orifice. On the other hand, many other workers (Tourneux & Legay, 1884; van Ackeren, 1889; Mijsberg, 1924; Koff, 1933) have described the cranial migration of the lower ends of the Wolffian ducts to join the lower end of the vagina itself, though the ducts degenerated very soon after and did not persist into late foetal life.

At first sight, the present investigation supports this latter view. In the 94 mm. foetus the lower end of the left Wolffian duct joins the sinus upgrowth, indicating that the dorso-lateral projection has carried the Wolffian remnant with it as it grew cranially, while in none of the older foetuses was there any trace of the lower ends of the Wolffian ducts. Nevertheless, this evidence is insufficient to suggest that the lower ends of the Wolffian ducts never maintain their openings into the sinus beside the original site of the Müllerian tubercle, particularly in view of the large number of such cases described by Meyer (1909). At that time Meyer regarded these Wolffian remnants as indicative of the entirely Müllerian origin of the vagina, but in his later publications (1934–38) pointed out that they were equally in accord with the account of vaginal development given by Vilas. The Wolffian ducts were not involved in the proliferation which Vilas believed to form the epithelial plate of the vagina, and were therefore left behind near the hymen. Nevertheless, the structures which Meyer now identified as persistent Wolffian remnants do not appear to occupy the same site as those which he had described earlier, but join the lower end of the vagina on the upper aspect of the hymen. In other words, they have been carried cranially for a short distance during the formation of the sinus upgrowth.

It may be that in some instances the lower ends of the Wolffian ducts are not carried cranially with the sinus upgrowth to such a great extent as they have been in the 94 mm. foetus, or indeed that they are not carried cranially at all. This may be because the dorso-lateral projections do not play such a large part in the formation of the sinus upgrowth in these cases, or because the manner of their growth is such as not to involve the lower ends of the Wolffian ducts. In other words, it is possible, as von Lippmann suggested (1939), though with rather a different purpose, that there is some variability in the behaviour of the lower ends of the Wolffian ducts during the early stages of vaginal development. This is supported by the fact that an ectopic ureter, presumably opening by a persistent Wolffian remnant, may join either the vagina or the vestibule (Kermauner, 1909). It is also possible that many of the structures which have been identified as persistent Wolffian remnants were, in fact, not so. In the 140 mm. foetus the dorso-lateral sinus bays end above by sending short diverticula upwards on either side of the lower end of the vagina, lined by the undifferentiated type of sinus epithelium. In the 180 mm. foetus these diverticula are still lined by the undifferentiated epithelium, though the dorso-lateral bays themselves are occupied by an epithelium very similar to that of the vagina. The diverticula are presumably associated with the relative downgrowth of the lower end of the vagina, as has already been pointed out. They are, however,

very similar to the 'Wolffian remnant' described by Bloomfield & Frazer (1927), in a 170 mm. foetus of their collection, but their lining epithelium leaves no doubt of their origin (Pl. 3, fig. 16).

An interesting viewpoint on the development of the vagina was suggested by Zuckerman (1940), who believed that any epithelium of the adult genital tract which responded to oestrogenic stimulation by a stratified squamous proliferation—a 'squamous response'—was a derivative of the sinus epithelium of the foetus. The conclusion of this present investigation, that the entire vaginal epithelium is derived from the sinus upgrowth, might appear to support Zuckerman's hypothesis. There is, however, a considerable body of embryological evidence which indicates that in many other mammalian forms the upper vaginal segment, though it is lined by a stratified squamous epithelium and gives a squamous response to oestrogenic stimulation, is a Müllerian derivative. Thus, it seems likely that when the Müllerian epithelium does persist in the vagina it tends to be of stratified squamous form and its response to oestrogenic stimulation squamous in type. Of some interest in this respect is the lining of the 'vaginal' portion of the utero-vaginal canal in the human foetus by a stratified squamous epithelium of Müllerian origin, and the disappearance of this epithelium after the 140 mm. stage, when it is displaced into the cervical canal. In the absence of any firm knowledge of the hormonal conditions in female human foetuses, we do not know how this stratified Müllerian epithelium responds to oestrogenic stimulation. Probably, however, there is some controlling factor, producing its effect in foetal life in the human subject and throughout life in many other mammals, which stimulates Müllerian cells in the vagina, but not in the uterus, to form a stratified squamous epithelium.

One of the most interesting problems is that of the extent to which the development of the human vagina is influenced by hormonal factors. It is a reasonable supposition that the enormous activity of the vaginal epithelium in the older foetuses is a result of stimulation by maternal oestrogens, as Fraenkel & Papanicolou (1938) suggested. The gross differences between the vaginal epithelium in these foetuses and that of the 1-month old infant imply that this oestrogenic stimulation must extend over a considerable period of foetal life—at any rate from the 112 mm. stage onwards. The question of the hormonal control of genital development has introduced a new field of experimental embryology, in which a very large amount of work has already been carried out (for bibliography see Jost, 1948), and it raises many interesting problems concerning the development of the human vagina. It may be that the enormous enlargement of the lower end of the vaginal mass at the 140 mm. stage, associated with the formation of the hymen, is a result of hormonal stimulation to which only the differentiated type of sinus epithelium is sensitive. At an earlier stage, the extension of the sinus upgrowth itself may be due to a similar selective response of the differentiated epithelium. Some of the marked differences between vaginal development in man and in other mammals may result from differences in the degree of hormonal stimulation at various critical periods of foetal life, rather than from inherent differences in the structures which go to form the vagina. On the other hand, the histological differentiation of the epithelium of the sinus upgrowth in the human foetus may be associated with an increased sensitivity to hormonal stimulation, compared with other mammalian forms. While

such suggestions can be only tentative, it is, nevertheless, very likely that the endocrinology of vaginal development holds the key to many of these outstanding problems.

SUMMARY

1. The development of the human vagina has been studied from a series of female foetuses ranging from 28 to 375 mm. crown–rump length, and from a 1-month old infant.

2. An upgrowth arises from an area of 'differentiated' epithelium in the dorsal wall of the sinus, in a manner which might be said to combine the descriptions of Vilas (1932) and Koff (1933).

3. The sinus upgrowth extends throughout the entire region of the vagina by the 140 mm. stage, and forms the whole of its epithelial lining. The changes in the vaginal epithelium of older foetuses are also described.

4. The findings are discussed in relation to the question of an early ingrowth of Wolffian epithelium into the dorsal wall of the sinus, the problem of the behaviour of the lower ends of the Wolffian ducts in female foetuses, and recent opinions on the significance of the sex hormones in genital development.

My thanks are due to Prof. J. D. Boyd, for the loan of most of the material which I was able to study. I am also indebted to Messrs J. F. Crane and A. Cain for the microphotographs, and to Mr W. Cruickshank for his assistance with the diagrams.

REFERENCES

ACKEREN, F. VAN (1889). Beiträge zur Entwicklungsgeschichte der weiblichen Sexualorgane des Menschen. *Z. wiss. Zool.* **48**, 1–46.

BANKS, W. M. (1864). *On the Wolffian Bodies of the Foetus.* Edinburgh.

BLOOMFIELD, A. & FRAZER, J. E. (1927). The development of the lower end of the vagina. *J. Anat., Lond.*, **62**, 9–32.

BOLK, L. (1907). Beiträge zur Affenanatomie VI. Zur Entwicklung und vergleichenden Anatomie des Tractus urethro-vaginalis der Primaten. *Z. Morph. Anthr.* **10**, 250–316.

BULMER, D. (1955). *The Development of the Vagina.* Thesis for the degree of M.D., University of Cambridge.

BULMER, D. (1956). The early stages of vaginal development in the sheep. *J. Anat., Lond.*, **90**, 123–134.

FELIX W. (1912). The development of the urogenital organs. In Keibel and Mall, *Manual of Human Embryology*, Vol. II, p. 752. Philadelphia and London.

FRAENKEL, L. & PAPANICOLOU, G. N. (1938). Growth, desquamation and involution of the vaginal epithelium of human foetuses and children, with a consideration of the related hormonal factors. *Amer. J. Anat.* **62**, 427–451.

GOMORI, G. (1952). *Microscopic Histochemistry.* University of Chicago Press.

HART, D. B. (1896). Preliminary note on the development of the clitoris, vagina and hymen. *J. Anat., Lond.*, **31**, 18–28.

HART, D. B. (1901). Morphology of the human urogenital tract. *J. Anat., Lond.*, **35**, 330–375.

HART, D. B. (1911). Adenoma vaginae diffusum (adenomatosis vaginae), with a critical discussion of present views of vaginal and hymeneal development. *Edinb. med. J.* **6**, 577–590.

HUNTER, R. H. (1930). Observations on the development of the human female genital tract. *Contr. Embryol. Carneg. Inst.* **22**, 91–108.

JOST, A. (1948). Le contrôle hormonal de la différenciation du sexe. *Biol. Rev.* **23**, 201–236.

KEMPERMANN, C. T. (1931). Beitrag zur Frage der Genese der menschlichen vagina. *Gegenbaurs Jb.* **66**, 485–531.

KEMPERMANN, C. T. (1935). Beiträge zur Entwicklung des Genitaltraktus der Säuger. III. Das Schicksal der kaudalen Enden der Wolffsche Gänge beim Weibe und ihre Bedeutung für die Genese der Vagina. *Gegenbaurs Jb.* **75**, 151–179.

KERMAUNER, F. (1909). Die Missbildungen der weiblichen Geschlechts-organe. In Schwalbe, *Die Morphologie der Missbildungen des Menschen und der Tiere*, Jena. T. III, p. 253.

KOFF, A. (1933). Development of the vagina in the human foetus. *Contr. Embryol. Carneg. Instn*, **24**, 59–91.

LIPPMANN, R. VON (1939). Beitrag zur Entwicklungsgeschichte der menschlichen Vagina und des Hymen. *Z. Anat. EntwGesch.* **110**, 264–300.

MEYER, R. (1909). Zur Kentniss des Gartnerschen (oder Wolffschen) Ganges besonders in der Vagina und dem Hymen des Menschen. *Arch. mikr. Anat.* **73**, 751–792.

MEYER, R. (1934–1938). Zur Frage der Entwicklung der menschlichen Vagina. I. *Arch. Gynaek.* **158**, 639–738; II, *Arch. Gynaek.* **163**, 205–308; III, *Arch. Gynaek.* **164**, 207–357; IV, *Arch. Gynaek.* **165**, 504–590; V, *Arch. Gynaek.* **167**, 306–338.

MIJSBERG, W. A. (1924). Über die Entwicklung der Vagina, des Hymen und des Sinus urogenitalis beim Menschen. *Z. Anat. EntwGesch.* **74**, 684–760.

MÜLLER, J. (1830). *Bildungsgeschichte der Genitalien.* Dusseldorf: Arnz.

PAPANICOLOU, G. N., TRAUT, H. F. & MARCHETTI, A. A. (1948). *The Epithelia of Woman's Reproductive Organs.* Commonwealth Fund, New York.

POLITZER, G. (1952). Das Schicksal des Sinus urogenitalis beim Weibe. *Z. mikr-anat. Forsch.* **59**, 6–28.

POLITZER, G. (1955). Zur normalen und abnormen Entwicklung der menschlichen Scheide. *Anat. Anz.* **102**, 271–278.

RATHKE, H. (1832). *Abhandlungen zur Bildungs- und Entwicklungsgeschichte des Menschen und der Thiere.* Leipzig.

RETTERER, E. (1891). Sur l'origine du vagin de la femme. *C.R. Soc. Biol., Paris*, Ser. 9, **3**, 291–293.

SMITH, B. G. & BRUNNER, E. K. (1934). The structure of the human vaginal mucosa in relation to the menstrual cycle and to pregnancy. *Amer. J. Anat.* **54**, 27–85.

SPULER, A. (1930). *Entwicklungsgeschichte des weiblichen Genitalapparates.* In Veit-Stoeckel, *Handbuch der Gynäkologie*, 3rd ed., Band I, p. 470 (quoted by Vilas). Wiesbaden.

TOURNEUX, F. & LEGAY, C. (1884). Mémoire sur le développement de l'uterus et du vagin. *J. Anat. Paris*, **20**, 330–386.

VALENTIN, G. G. (1835). *Handbuch der Entwicklungsgeschichte des Menschen.* Berlin.

VILAS, E. (1932). Über die Entwicklung der menschlichen Scheide. *Z. Anat. EntwGesch.* **98**, 263–292.

WISLOCKI, G. B., FAWCETT, D. W. & DEMPSEY, E. W. (1951). Staining of stratified squamous epithelium of mucous membranes of Man and Monkey by the periodic acid-Schiff method. *Anat. Rec.* **110**, 359–376.

ZUCKERMAN, S. (1940). The histogenesis of tissues sensitive to oestrogens. *Biol. Rev.* **15**, 231–271.

EXPLANATION OF PLATES

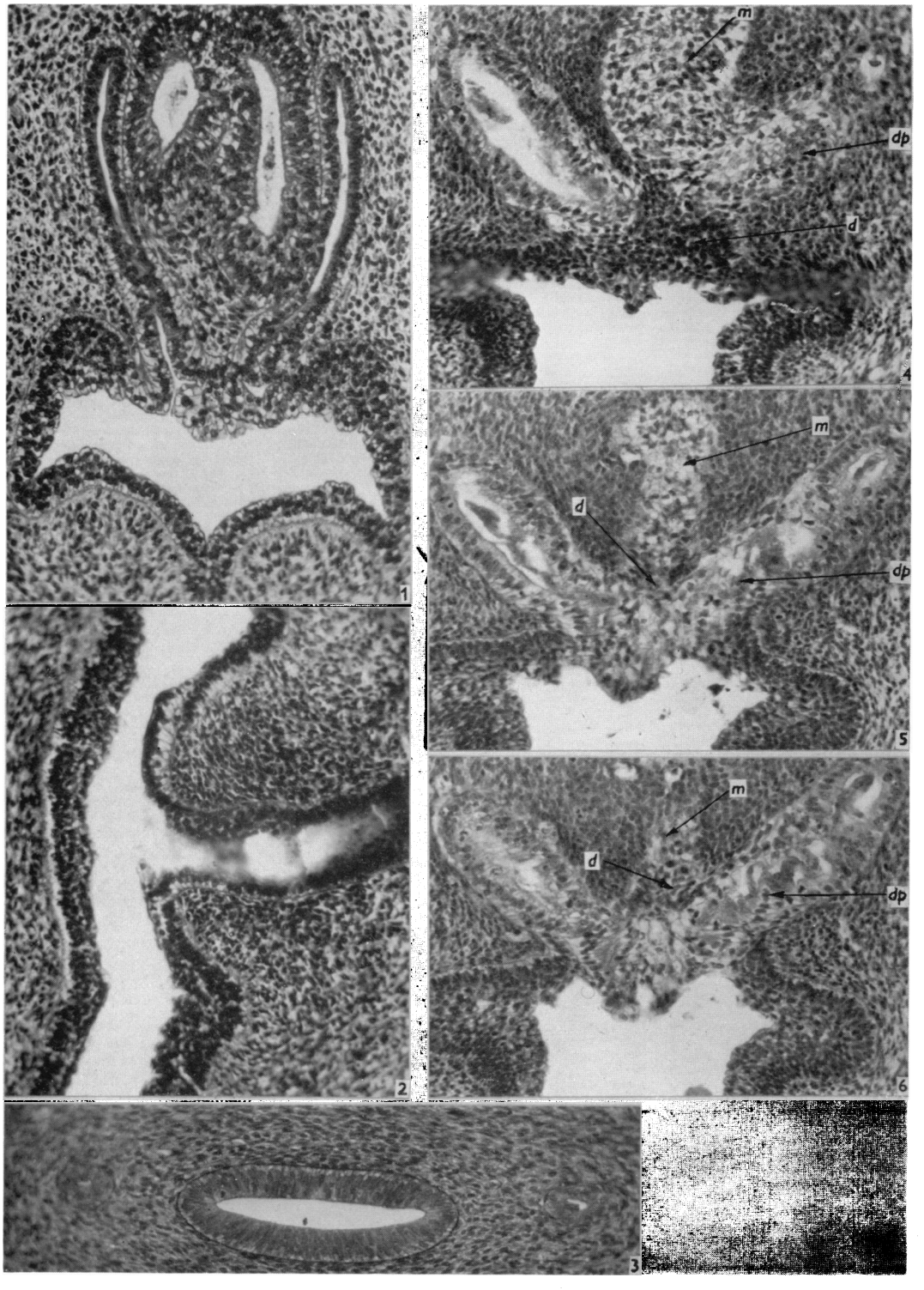
PLATE 1

Fig. 1. 28 mm. embryo. Transverse section through the openings of the Wolffian ducts into the urogenital sinus. The sinus epithelium consists of basal deeply staining and superficial pale-staining cells, and the pale-staining cells extend for a short distance into the lower ends of the Wolffian ducts. The Müllerian ducts are in close relation with the Wolffian ducts, but a small mass of mesoderm separates them from the sinus. (×215.)

Fig. 2. 48 mm. foetus. Sagittal section through the opening of the right Wolffian duct. The differentiation of the sinus epithelium in this region can be seen, and the extension of the pale-staining cells into the lower end of the Wolffian duct. (×215.)

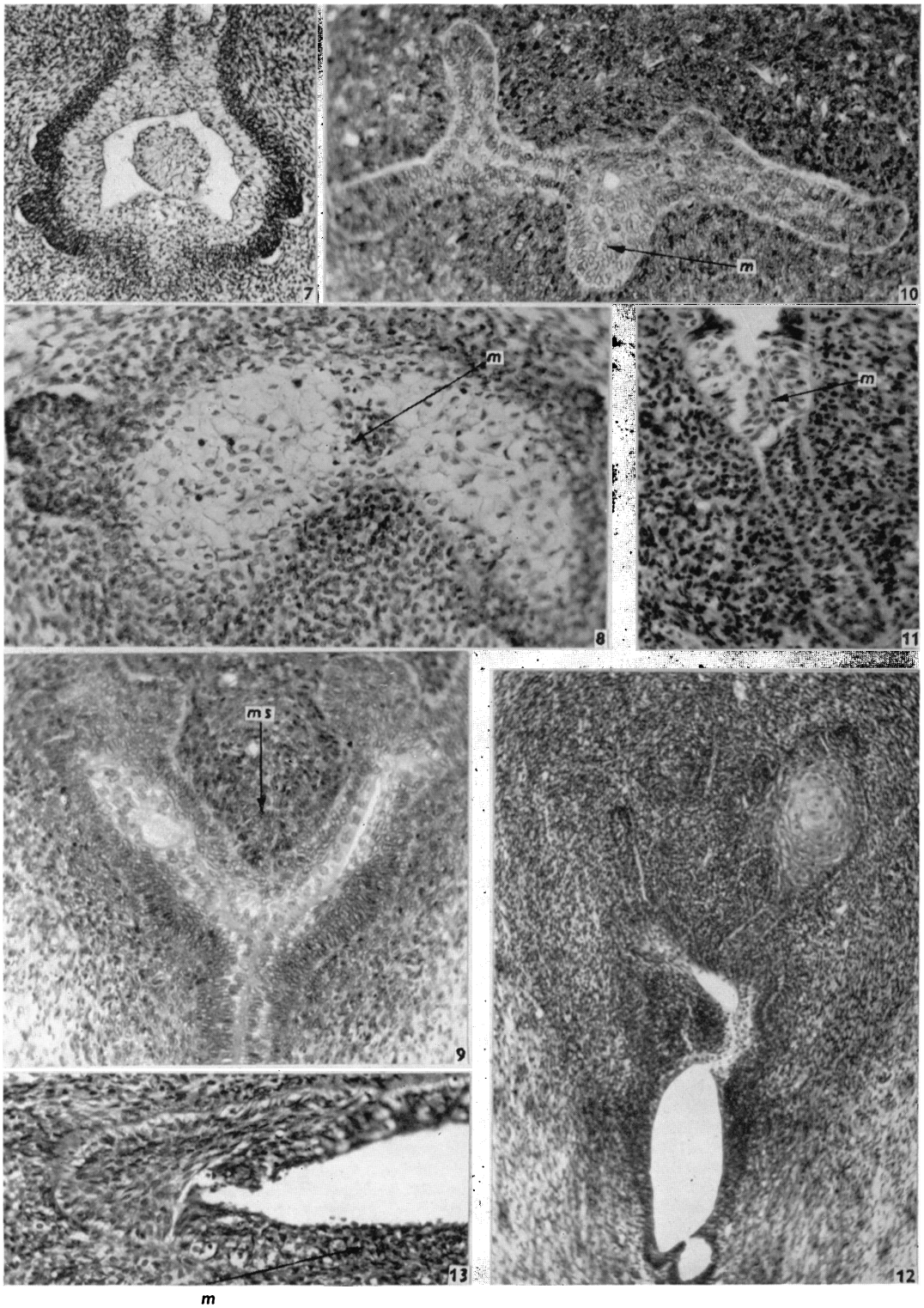
Fig. 3. 65 mm. foetus. Transverse section through the genital cord. The Wolffian ducts are very small structures, on either side of the utero-vaginal canal. (×215.)

Fig. 4. 65 mm. foetus. Transverse section through the junction of Müllerian and sinus epithelia. A mass of darkly staining cells (*d*), arising from the dorsal wall of the sinus, separates the caudal end of the Müllerian epithelium (*m*) from the sinus lumen. On the right side the section passes below the bulk of the dorso-lateral projection, and the Wolffian duct is large. On the left the Wolffian duct is a small structure, applied to the dorsal aspect of the dorso-lateral projection (*dp*). (×215.)

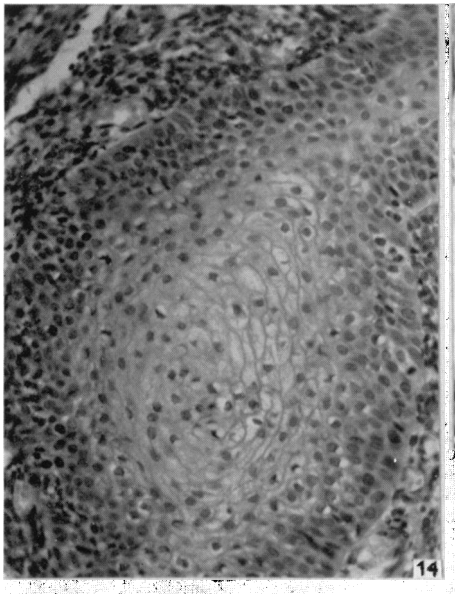


BULMER—THE DEVELOPMENT OF THE HUMAN VAGINA

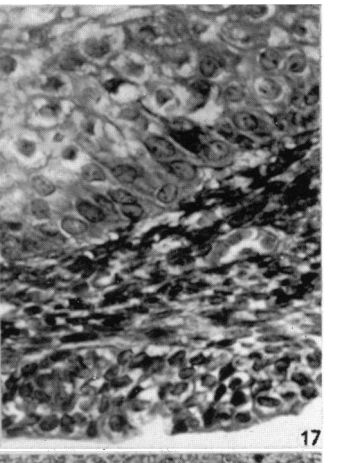
(*Facing p.* 508)

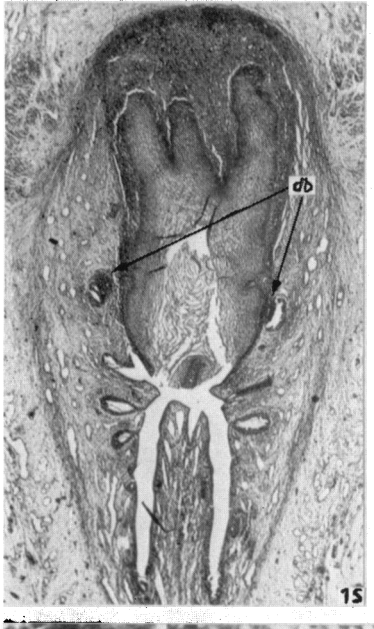


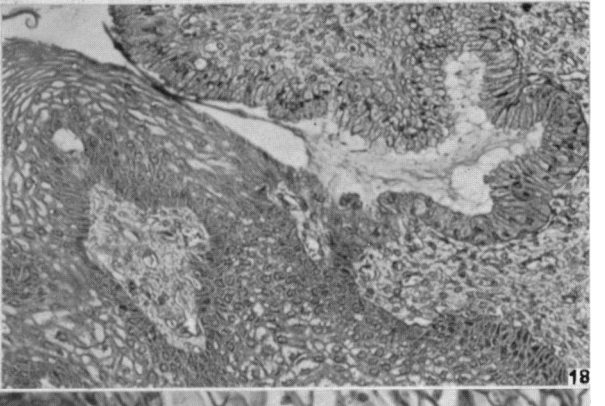
BULMER—THE DEVELOPMENT OF THE HUMAN VAGINA

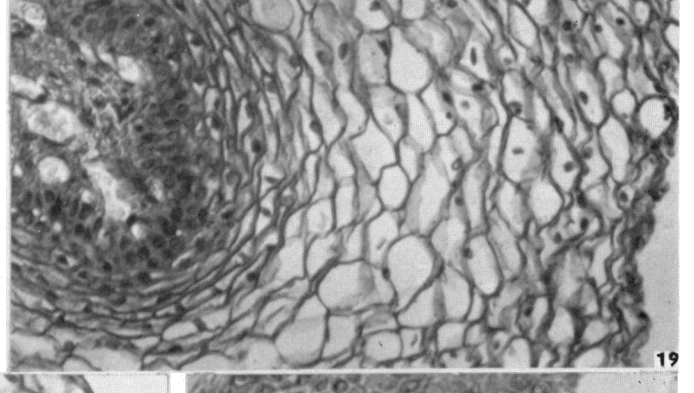

14

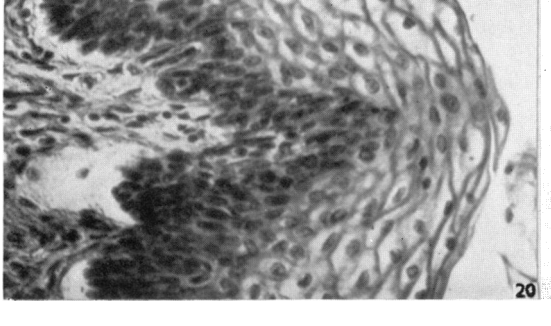

16


17


db
15


18


19


20

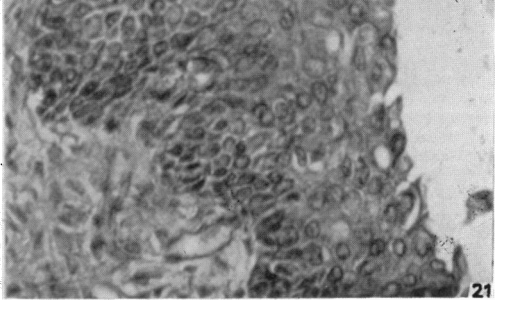

21

Fig. 5. 65 mm. foetus. Transverse section 16 μ caudal to Fig. 4. The Müllerian epithelium now forms a smaller mass dorsally, and a few of the darkly staining sinus cells separate it from the pale-staining sinus epithelium ventrally. ($\times 215$.)

Fig. 6. 65 mm. foetus. Transverse section 8 μ caudal to Fig. 5. ($\times 215$.)

PLATE 2

Fig. 7. 94 mm. foetus. Coronal section through the cranial and dorsal enlargement of the sinus which forms the root of the sinus upgrowth. The epithelial differentiation is very well marked. ($\times 84$.)

Fig. 8. 94 mm. foetus. Coronal section through the sinus upgrowth, 90 μ dorsal to Fig. 7. The small darkly staining cells in the centre of the section (*m*) represent the caudal tip of the Müllerian epithelium. A thin layer of darkly staining cells forms the outer layer of the sinus upgrowth, and there is a small mass of these cells on the right (to the left of the photograph). 90 μ further dorsally, a similar small mass, on the other side, is joined by the persistent lower end of the left Wolffian duct. ($\times 215$.)

Fig. 9. 112 mm. foetus. Transverse section through the dorsal part of the sinus, showing the two roots of the sinus upgrowth separated from each other by the mesodermal septum (*ms*). The pale-staining internal cells of the sinus are very prominent. ($\times 215$.)

Fig. 10. 112 mm. foetus. Transverse section through the vaginal mass, 288 μ cranial to Fig. 9. The sinus upgrowth forms two lateral wings, while the central portion (*m*) is formed by the paler-staining polygonal cells of the caudal end of the Müllerian tissue. ($\times 215$.)

Fig. 11. 16½ week foetus. Transverse section through the junction of the Müllerian epithelium of the utero-vaginal canal (*m*) with the cranial wing of the sinus upgrowth on the left side. ($\times 215$.)

Fig. 12. 16½ week foetus. Transverse section through the sinus showing the junction of its dorsal wall with the caudal end of the sinus upgrowth. The central enlargement of the upgrowth is immediately dorsal to the sinus lumen, and a further enlargement projects dorsally from it. The large clear-staining internal cells are prominent in the central enlargement. In the dorsal enlargement the cytoplasm and cell boundaries of the internal cells are markedly eosinophilic. ($\times 84$.)

Fig. 13. 140 mm. foetus. Transverse section through the left side of the cervical canal, showing the junction between the stratified Müllerian epithelium (*m*) and the cranial tip of the sinus upgrowth. ($\times 160$.)

PLATE 3

Fig. 14. 140 mm. foetus. Transverse section through the left vaginal enlargement, just below the middle of the cranio-caudal extent of the vagina. The internal cells are large, their nuclei relatively small and the cell-boundaries markedly eosinophilic. ($\times 215$.)

Fig. 15. 140 mm. foetus. Transverse section through the urogenital sinus and the lower end of the vagina, at the level of the paired hymeneal orifices. On each side of the ventro-lateral aspect of the vagina is the small cranial extremity of the dorso-lateral sinus bay (*db*). ($\times 30$.)

Fig. 16. 140 mm. foetus. Transverse section through the wall of the dorso-lateral sinus bay, showing its lining of undifferentiated sinus epithelium. ($\times 450$.)

Fig. 17. 140 mm. foetus. Transverse section through the lower portion of the hymen, showing the vaginal epithelium above, separated by the hymeneal connective tissue from the undifferentiated sinus epithelium below. ($\times 450$.)

Fig. 18. 180 mm. foetus. Transverse section through the junction of the vaginal epithelium with the columnar epithelium of the cervix. ($\times 120$.)

Fig. 19. 180 mm. foetus. Section through the vaginal epithelium. ($\times 280$.)

Fig. 20. 375 mm. foetus. Section of the vaginal epithelium overlying a mesodermal papilla. ($\times 280$.)

Fig. 21. Vaginal epithelium in the 1-month old infant. The epithelium is not of uniform thickness, and this photograph is taken from an area where it is rather thicker than usual, in order to give a better impression of its histological structure. ($\times 320$.)