

## THE DEVELOPMENT OF THE LOWER END OF THE VAGINA

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ALTHOUGH a considerable amount of work has been done on the development of the lower end of the vagina and its associated structures, such as the hymen, it is nevertheless true that uniformity of opinion on the subject has not been reached. Partly, no doubt, this is due to much of the research having been carried out on non-human material, the human processes being assumed to be parallel if not identical, a supposition which appears to contain many fallacies. Possibly, also, some of the suggestions put forward may have been advanced on insufficient grounds, such as scantiness of material, or want of careful examination by serial sections of the parts above and below the region concerned. Whatever may be the cause, it is evident that not only descriptive discrepancies in detail, but utterly opposed differences in fundamental origins are to be met with by any who are seeking guidance in this developmental field. Clinicians and pathologists have been suggesting for many years that, from their respective standpoints, there is reason to suppose that the lower end of the vagina and hymen may have a developmental value which differs from that of the vagina higher up, and it is no doubt owing to this clinical impression that so much work has been done on the matter. The results of this work may be shortly summed up for present purposes by saying that—disregarding differences in detail—three main views emerge—the vagina may be held to be purely Müllerian in its origin, or it may be mainly Müllerian, but with an additional (Wolffian) factor present and taking part in the formation of its lower part, or, lastly, it may be largely if not altogether a derivative of the urogenital sinus. We will consider these views more fully in the final part of this paper, it being enough at the moment to recall the apparently irreconcilable differences between them, and thus to have a conception of the main questions at issue.

As we were engaged on certain investigations concerned with the development of the female genital system, and were hindered to some extent by the evident want of reliable data on the formation of the lower part of the genital tract in women, we attempted to determine the matter conclusively for ourselves. The present paper gives our results in a condensed but, we hope, useful form. The research was carried out exclusively on human material, and by the method of examination of serial sections and by reconstructions.

There is little or no question about the parts played by the Müllerian and Wolffian ducts during the first three months of intra-uterine life. The Wolffian duct is present at a very early stage, and soon reaches and opens into the cloacal cavity. The Müllerian duct, appearing first at its proximal end at or before the 10 mm. stage, extends gradually distally in close relation with the Wolffian duct: it lies at first rather lateral and ventral to this last-named tube, but in the pelvis comes to lie to its inner side, in contact with its fellow. The two Müllerian ducts come into relation with each other in the pelvis about the stage of 28–30 mm., and then, following the curved course of the Wolffian ducts between which they lie, they extend toward the posterior wall of the urogenital sinus, which they reach at about 36–38 mm. The four ducts, grouped in this way, are surrounded by a mesodermal condensation, the so-called “genital stalk.” The Müllerian ducts, within the

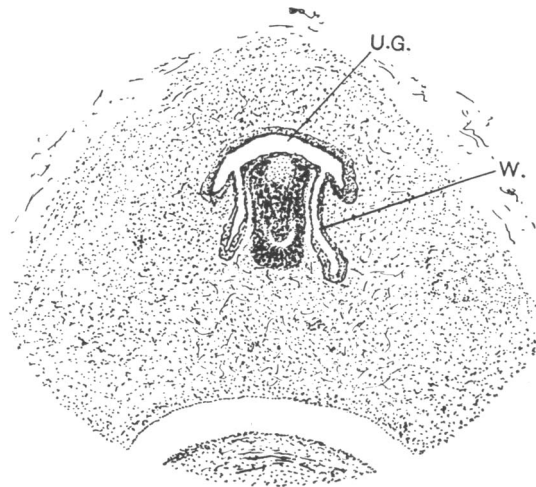


Fig. 1. Section through the terminal parts of the Wolffian and Müllerian ducts in an embryo of 38 mm. *U.G.*, urogenital sinus; *W.*, Wolffian duct. The double nature of the Müllerian terminations between the two Wolffian ducts is apparent. The surrounding condensation is the genital stalk.

“stalk,” have well-marked lumina, and are in contact at first with one another. Their inner walls, however, quickly fuse and disappear, so that a simple tube appears in this part, the change seemingly taking place from above downwards, but not affecting so completely the terminal parts close to their attachment to the wall of the sinus.

Fig. 1 is from a section from an embryo of 38 mm. and shows the four ducts and the urogenital sinus. The two Wolffian ducts have clear but small openings into the sinus, while the Müllerian ducts appear at first to be represented by a mass of darkly-staining cells: closer examination of this mass, however, reveals the separate tracks of the two ducts, the thickened walls of which—fused where they are in contact—make the cellular mass.

An examination of the whole length of the utero-vaginal portion of the Müllerian structures in this embryo shows very clearly how the double tube forms a single median channel below the level of the future uterine cornua, but it can be seen that the fusion, or, more accurately, the disappearance of the basic double nature of the structure, does not take place at the lower end (which is shown in fig. 1) in contact with the wall of the sinus.

We have no desire at present to suggest any reason for the partial persistence of the paired condition at the lower end of this Müllerian structure, as just described, but we may point out here that the somewhat paired appearance (which we are about to describe) of the earlier bulbous growths at the lower end of the vagina may be due to their origin from this still double rudiment.

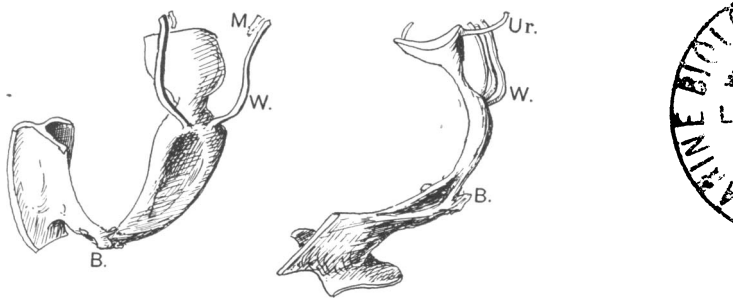


Fig. 2. Sketches from "cast" reconstructions, to show the general form of the urogenital sinus in 28 mm. and 38 mm. embryos. The younger specimen, seen from the left and behind, is shown at more than twice the magnification of the other, viewed from the left front. Upper and lower parts of the sinus (p. pelvina and p. phallica of Felix) are easily distinguished. B., the rudiment of Bartholin's gland, is at the upper end of the lower part. This part has its roof depressed in a longitudinal "keel" (38 mm.) which later extends into the upper part: in this there is an impression on the floor (28 mm.) of uncertain meaning. Ur., ureter; W., Wolffian duct; M., lower end of Müllerian duct, not yet reaching sinus.

Fig. 1 shows the dorsal projection into the cavity of the urogenital sinus, at the level of the ducts, which is termed the Müllerian tubercle. The sinus is really crescentic on section at this part, perhaps owing to the presence of the more solid condensation in relation with its posterior wall.

The sinus urogenitalis presents most interesting changes during development, and the history of this region deserves fuller consideration than it seems to have received. We are only concerned indirectly, however, with this part in the present paper and cannot enter into any long description of it. Its continuation above the level of attachment of the ducts leads, of course, to the bladder, and may be termed without prejudice the urethra. Below this level the sinus appears to consist of two parts: a "lower," opening on the surface and relatively wide, with a longitudinal and broad keel-like ridge along the roof, and an "upper" part, at first fairly long and with a narrower lumen. Bartholin's glands begin as solid outgrowths from the upper end of the lower part—about the stage of 30 mm., or perhaps before this. This spot,

marked in subsequent stages by the position of the junction of the gland duct with the sinus, is, from our present point of view, the important feature of the sinus. It can be taken as practically fixed in position and unaffected by subsequent longitudinal foldings or groove-formations in the wall of the sinus. Fig. 2, from a couple of "cast" reconstructions of the sinus in embryos of 28 mm. and 38 mm., will give an idea of the appearance of the region and the condition of the Bartholin rudiments at these stages.

Slow growth *in situ* now goes on. It is not necessary to describe these successive slow changes, and it will suffice to say that, by the time that the stage of 55 mm. is reached, certain alterations have occurred in the lower end of the utero-vaginal canal: the upper part of the canal has its lumen

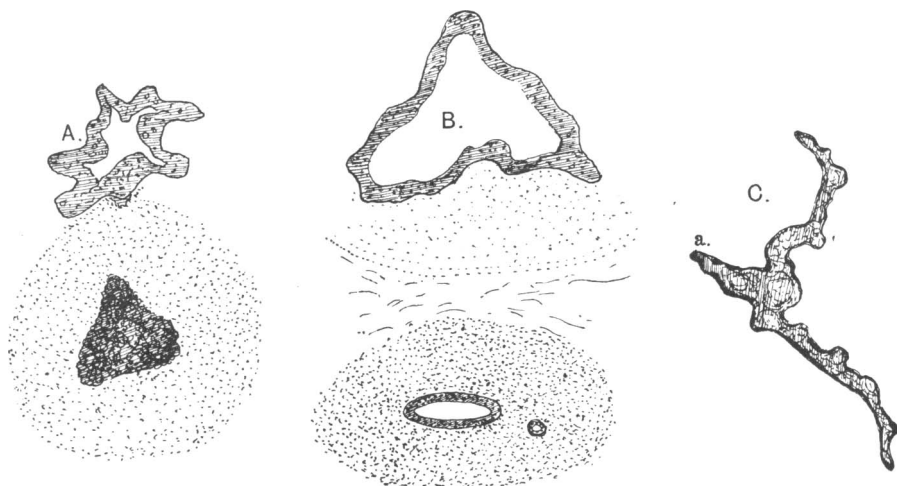


Fig. 3. A. and B., sections at different levels through the utero-vaginal canal of an embryo of 55 mm. In the higher section (B.) a Wolffian duct is seen. The lower section (B.) is a very little distance above the site of contact with the sinus. C., section through the canal in a specimen of 79 mm., above the region of main enlargement, to show the triradiate shape and the beginnings of distinct areas of cell proliferation.

lined by high columnar epithelium, and is surrounded by a definite mesodermal condensation distinct from that surrounding the bladder. As it is traced down towards the region where, in the 38 mm. specimen (fig. 1), the original two lumina were still distinguishable, we find that the single lumen becomes smaller, finally disappearing in a central solid plug of cells which lies between two somewhat rod-shaped lateral cell masses. These last evidently represent the double rudiments of the earlier state, now become altogether solid from proliferation of their lining cells. The central plug is apparently the direct derivative of the fused mesial walls of the two tubes, undergoing proliferation, and forms a ridge projecting forward and giving a form to the whole which is somewhat triangular or T-shaped on section.

Fig. 3 (A. and B.) shows sections through this region. The anterior process reaches the wall of the sinus further on, and extends a little way downwards

along it. Here it is to be noticed that the cellular mass has also broken into the sinus, as shown in fig. 4. This is an important fact, distinguishing this stage from that last figured, in which the vaginal and sinus epithelia came into contact and blended, but there was no true communication between the cavities: in the 55 mm. specimen there is an actual disruption of the sinus epithelium covering the site of contact, and therefore a true—though for the time being only a potential—communication is formed between the cavity of the sinus and the utero-vaginal canal, here represented by a solid epithelial mass.

The Wolffian duct can be traced for a few sections in this 55 mm. specimen as a patent tube lined with a sharply-cut epithelial layer, lying lateral to the

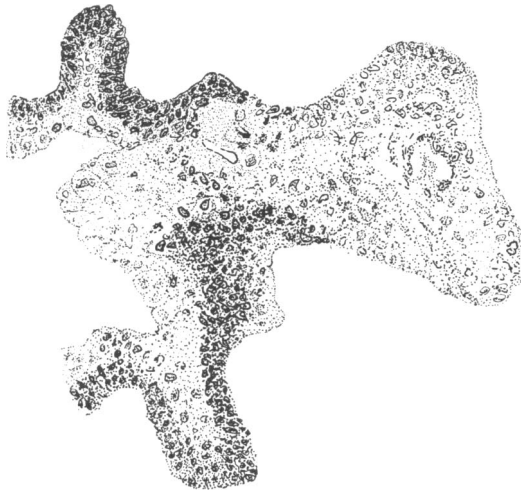


Fig. 4. Section showing vaginal epithelial mass (on right) breaking the epithelial lining of sinus (left), 55 mm.

utero-vaginal canal, perfectly distinct from it but situated within the condensation surrounding it. No trace of the Wolffian duct could be found on either side in the neighbourhood of the communication between sinus and vagina.

As exemplified in this specimen, we have found in later stages the presence and persistence of the Wolffian duct to be very variable. Very occasionally its lower end may persist in its entirety, at any rate on one side. More often it can be picked up in serial sections for a short distance and on one side only, to be lost further on, while occasionally at a lower level, it, or the duct of the other side, may be traced again for a little distance.

The sinus, still relatively long and narrow, has a well-marked groove on each side wall, into which the duct of Bartholin's gland opens. The gland itself is represented by a small mass of rudimentary alveoli lying in the mesoderm lateral to the sinus. The position of the opening of the duct, and

its relation to the site of contact of the vaginal structures is very much the same as before, about half-way up the sinus: this situation holds for some time, and the state of the parts at 55 mm. is practically the same as that seen in fig. 6.

A specimen of the 65 mm. stage may be mentioned here, not because any marked change has taken place in the parts, but because one Wolffian duct remnant can be traced in it in the neighbourhood of the greater part of the length of the utero-vaginal canal. It is first picked up (fig. 5) as a small distinct cord (? tube with very small lumen) lying laterally: traced down, it can be seen to enter the sinus just below and lateral to the junction between the latter and the utero-vaginal canal. The remnant is some little distance

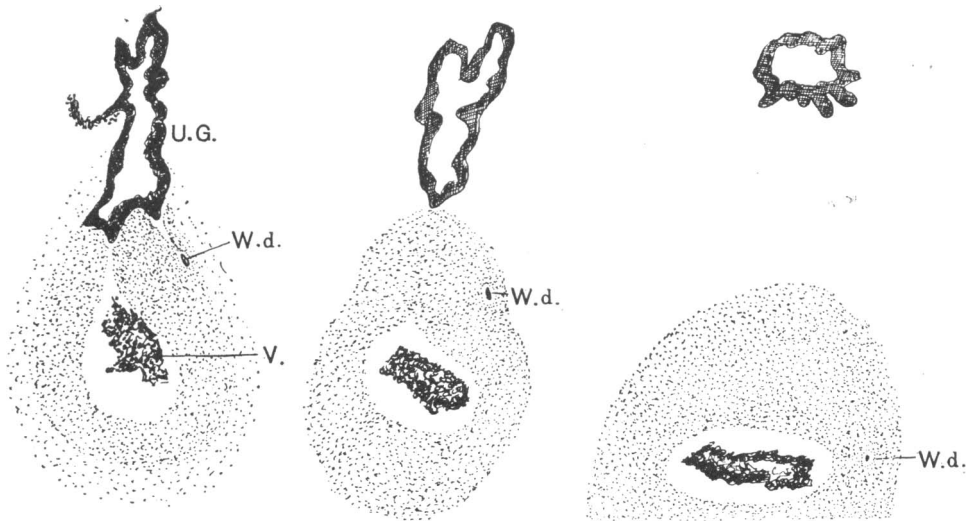


Fig. 5. Sections to show remnants of Wolffian duct on one side, 65 mm. V., vaginal epithelium, shrunken; U.G., urogenital sinus; W.d., Wolffian duct.

from the Müllerian structures, although embedded in the surrounding condensation. It is evidently in an atrophic state, presumably about to disappear.

The central fact, at the stage reached so far, is the evidence of a certain amount of activity in the Müllerian derivatives at, and for a little distance above, their area of contact with the sinus. A stage a little later, one of 79 mm., shows the beginning of a further and definite evolution in this activity. In this specimen the epithelial proliferation at the lower part of the utero-vaginal canal has assumed certain definite characters, progressing mainly at three "centres" which, however, are naturally continuous. Of these two are laterally and the third centrally placed, so that three bulbous swellings, of irregular outlines, are produced: the lateral "bulbs" are somewhat larger than the central one, which projects more like a keel on the front surface, and makes the contact with the wall of the sinus. The cells forming these

bulbs assume a somewhat concentric arrangement, the innermost cells becoming large, rounded, and vacuolated, with a tendency to break down, the whole bulb giving the impression on section of a "cell-nest." Although there are three main "centres," it must be understood that there are many small and separate ones as well, and the area of this growth extends for a considerable distance up the vaginal canal.

The triradiate or T-shaped form of the vaginal mass, as seen on section and mentioned in earlier stages, is preserved in this, the definite bulbar formations being essentially only further elaborations of the former central and lateral thickenings (see fig. 3, C.).

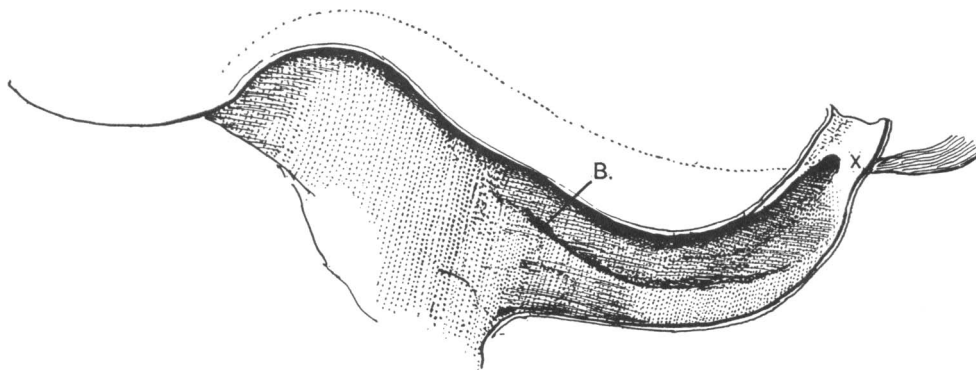


Fig. 6. Sagittal section of sinus of 65-70 mm. specimen. Reconstruction. B., opening of duct of Bartholin's gland; X., site of vaginal contact. The dotted line marks the level of the longitudinal sinus running beside the "keel" of the roof, which is cut along its length.

In this particular specimen there is an interesting condition to be noted. The Wolffian duct on the left side, which can be traced as a patent tube for some sections above the level mainly concerned in the description just given, can be seen to come into direct contact, lower down, with the outer aspect of the left lateral vaginal bulb, but without in any way participating in the formation of this structure. Fig. 7 gives the serial sections in which this occurs, and it can be seen that the remnant fades away without any sign of epithelial activity or bulb formation at its lower end. This is the only example we have in our specimens of a Wolffian duct coming close to, or in contact with, the Müllerian cells, and there seems to be no reason to doubt that the condition in this case is an accidental combination of a localised irregular growth of vaginal cells with a persistent Wolffian remnant.

It will be remembered that it was shown in earlier specimens that the utero-vaginal canal comes into contact by its epithelium with the upper and posterior wall of the urogenital sinus. It is by means of the vaginal bulbs which have now been mentioned, that this area of contact is maintained and increased, the vaginal cells pushing their way, by this proliferation, downwards along the posterior wall of the sinus,

Formation of the vaginal bulbs progresses steadily, and, by the stage of 110 mm., has reached considerably greater proportions and more complicated appearance than in former specimens, without yet attaining that exaggeration

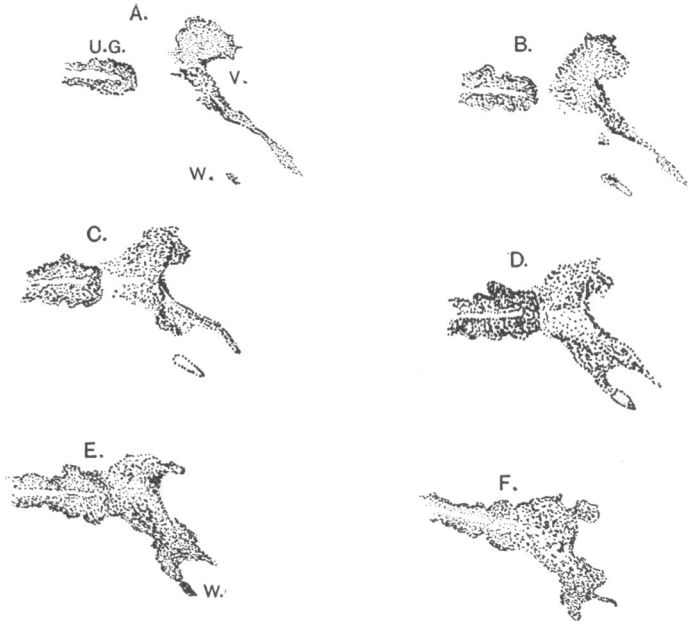


Fig. 7. Successive sections, 79 mm., to show a Wolffian rudiment (*W.*) in contact with the Müllerian vagina, *V.* *U.G.*, urogenital sinus. The rudiment enlarges in *C.*, but its wall remains a single-celled layer. It comes into contact with the vaginal mass in *D.* and begins to fade in *E.* Sections  $15\mu$ .

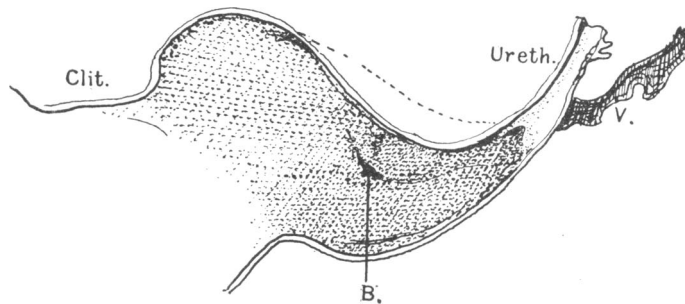


Fig. 8. 110 mm. Reconstruction of sinus. Sagittal section. *V.*, vagina; *B.*, orifice of duct of Bartholin's gland.

of growth which marks the later stages. As in the earlier specimens, this epithelial proliferation is associated with the triradiate shape of the mass, on section, already alluded to, and the anterior central portion reaches and



establishes contact with the upper and posterior part of the sinus wall. There is no evidence whatever of any intervention of Wolffian duct or bulb.

After this there is a progressive actual and relative increase in size of the lower end of the utero-vaginal canal. The general nature of this growth has been indicated in the description given above, but may with advantage be repeated here with some additional details. Just above its lower end, the lumen of the canal is occluded as the result of the activity of its lining cells (fig. 9). As it is followed down, this activity is seen to become concentrated mainly in three "centres," a single median and two lateral, and the three "vaginal bulbs" formed in this way project into the surrounding mesoderm

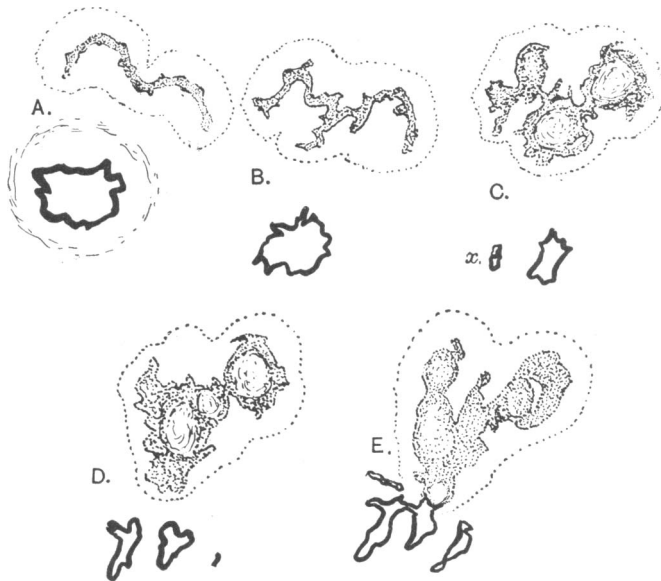


Fig. 9. Several sections showing the enlargement, from above down, of the lower end of the vaginal structures, 135 mm. The three main bulbs are seen in C. *x* is the upper end of a recess of the sinus.

as rounded masses possessing vacuolating centres. The anterior or median bulb is in contact with the posterior sinus wall, invaginating it slightly and thus producing a low projection of vaginal epithelium covered by the lining cells of the sinus. At the apex of this projection the cells are breaking down, allowing communication between the sinus and the cavity which is appearing within the bulbs as a result of the disintegration of their central cells. This marks the site of the hymeneal orifice—limited for the moment, of course, in extent—while the area of contact of vaginal and sinus cells forms the upper central part of the hymen, just below and behind the urethral orifice.

That this appearance, becoming more evident and important as development goes on, is the result of a true invagination and infolding of the posterior

wall of the sinus, and not merely produced by a growth of this wall to keep pace with the bulging epithelial vagina, is shown by the altered relation of the hymeneal site to the various grooves on the lateral wall of the sinus.

The configuration of the lateral wall has already been briefly noticed, and one groove in particular—that into the depths of which Bartholin's duct opens—has been pointed out. As development proceeds, the relief of this wall becomes more complicated, with the formation of additional grooves and folds. Certain grooves, however, can be found at all stages, and among these is that just mentioned as receiving the duct of Bartholin's gland. The gland and its duct remain in the same relation to each other, the gland deeply fixed in surrounding structures, and the duct running upwards and forwards from it. The duct aperture can therefore be taken as maintaining a constant position in the sinus, and affording a definite level from which relative measurements may be made. It will be found that, as growth goes on, the duct opening and the hymeneal level get nearer: as the duct is fixed, it follows that the hymeneal level is being brought down, and this implies invagination of the upper part of the sinus.

The additional folds in the wall of the sinus, which appear as development proceeds and seem to be somewhat variable, are probably associated with this inturning of the upper part of the sinus. It is interesting to observe that they appear higher up than the orifice of the duct, and that there is a tendency to the formation of a groove in a transverse plane as growth goes on, just below the level of the hymen, where in fact such a groove might be expected to form with increasing vaginal pressure from behind.

As development proceeds, we find the vaginal bulbs—and, to a lesser extent, the whole vagina—growing at a rate quite out of proportion to the growth of neighbouring structures. This can be shown by comparing the two stages, 135 mm. and 170 mm., in fig. 10. The reconstructions were made at the same magnification, and the vaginal formations in the older specimen are enormous compared with those in the earlier one: yet the urethrae are little different in size and the differences in the sinus are only in proportion to the general increase, although this point is not very evident in the figure, only a small part of each sinus having been modelled.

Fig. 11 gives the sagittal section of the parts in the 170 mm. specimen. The magnification is, of course, much less than in the figures already given of one or two earlier stages, but, bearing this in mind, it is evident at once that the orifice of Bartholin's duct (*B.*) is much nearer the hymeneal level than in those stages (figs. 6, 8). In other words, the invagination of the wall of the sinus has progressed considerably in connection with the great growth of the vaginal bulbs. The line of inflection, along which the wall is invaginated to form the covering of the hymen, is indicated by the interrupted line *Y*. Thus the distance from *B.* to *Y.*, plus that from *Y.* to the crest of the hymeneal prominence, is the distance between the Bartholin site and the posterior wall

of the sinus of the earlier stages, modified now (see also fig. 12) by progressive in-folding.

At *a.*, below the bulbs, are certain diverticula or prolongations from the sinus: these can be seen also in fig. 11, and seem to be associated with the invagination.

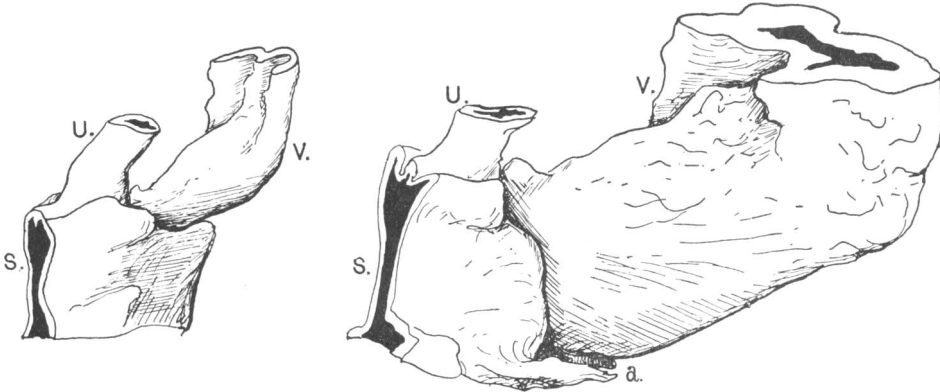


Fig. 10. The lower end of the vagina (*V.*) at 135 mm. compared with one of 170 mm., showing the relatively enormous growth. Drawn from models made at the same magnification. *S.*, part of the sinus, invaginated above and behind by the vaginal growth; *U.*, urethra. The "outgrowths" from the sinus below the bulbs are indicated at *a.*

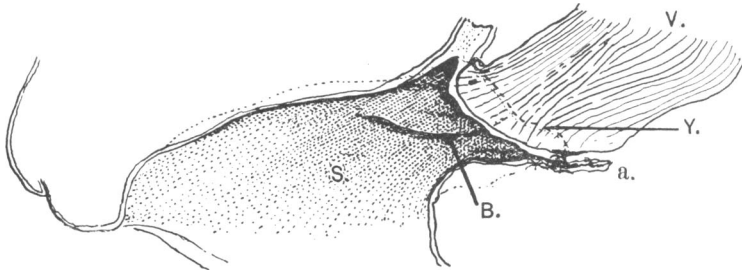


Fig. 11. Sagittal section, 170 mm. Reconstruction. *B.*, Bartholin's duct; *Y.*, line of inflection of wall of sinus, *S.* Outgrowths (?) are shown at *a.*

These were noticed by Mijsberg, who looked on them as active outgrowths. This was, to some extent, also our view so far as their extremities, at any rate, were concerned, but we have the impression that in their greater part they are probably only side-effects of the invagination: the question, however, is not easy to solve, and it is fortunate that it does not appear to be of primary importance.

This 170 mm. specimen presents conditions so suggestive and important for the present study, that further consideration of it is pardonable. It has been seen that the very large bulbous end of its vagina has markedly invaginated the posterior wall of the sinus, and this leads to a somewhat cone-shaped projection into this cavity (fig. 12). This receives a covering of the

lining cells of the sinus on its surface. The central cells of the vagina are breaking down, and the cavity so formed communicates with that of the sinus at the apex of the projection, so marking the site of the hymeneal opening. The basic structure of the hymen is seen in fig. 12, where the central portion of the upper part of that structure is already made. It is seen to consist of cells derived from the vaginal epithelium, covered superficially by sinus epithelium, and possesses an intervening layer of vascular mesoderm derived from the condensations round the walls of the cavities.



Fig. 12. Section, 170 mm., showing invagination of wall of sinus by vaginal bulbs, with formation of hymen. In the process the lower end of a persistent Wolffian duct, *W.d.*, has been turned in and opens on the hymen. The composition of the hymen is also seen, as well as the cellular conditions within the vagina.

One of the most interesting features of this specimen is the presence of a Wolffian duct on one side, which reaches the sinus and extends for a considerable distance upwards beside the vagina. The lumen of this rudiment is small but clear, and is surrounded throughout by a single layer of cubical cells, there being no indication whatever of any cellular activity in the direction of division or hypertrophy: the layer is only a persisting simple lining layer. When followed down, the duct, embedded as in all cases in the lateral part of the thick condensation surrounding the vagina, is found to open on the surface of the hymen (fig. 12, *W.d.*) a little distance from its "free border" or opening. It is evident that that part of the wall of the sinus on which the duct originally opened has been invaginated, turned in by the growth of the vaginal bulbs behind it, and has thus come to form part of the superficial

aspect of the hymen. Lastly, it has to be noted that the Wolffian tube nowhere in its course shows any sign whatever of contact or fusion, past or present, with the vaginal epithelium, nor (as has been pointed out) is there any smallest indication of past or present bulb-formation about it: it is a separate structure, not concerned with the activities in progress in the vagina, and its presence in this case is a happy accident which in its conditions bears out to the full the inferences drawn from examination of stages in which no such rudiment was to be found.

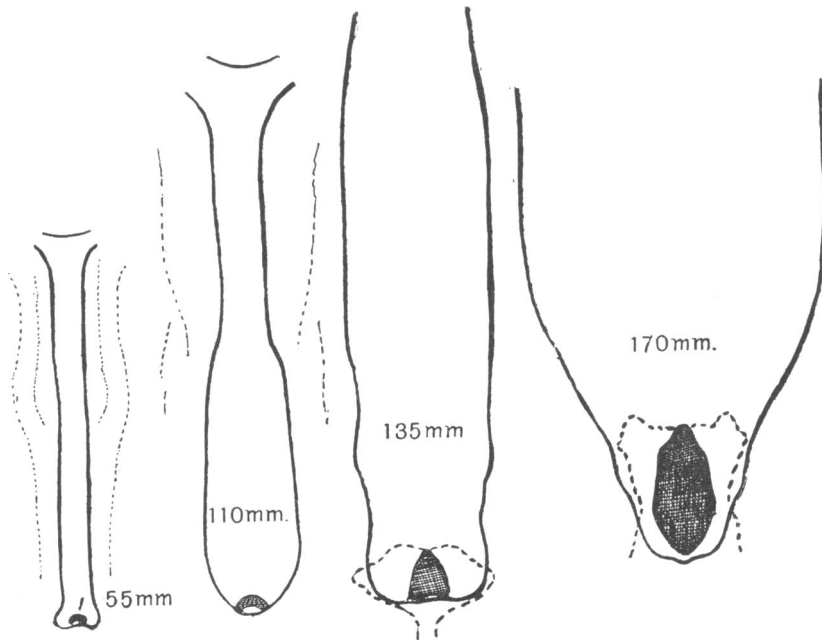


Fig. 13. Schematic reconstructions. The utero-vaginal length is shown in the first two diagrams the vagina only in the third, and its lower portion only in the last. The area of contact with the sinus is shaded, and the outline of the neighbouring part of the sinus given in interrupted lines. Magnification the same in all.

The relatively enormous bulbous growth in this foetus is only the modified representative at the lower end of the vagina of a disproportionate increase in size found in the canal above this. The striking size of the bulbs in fig. 10 would be lost to a large extent if they were viewed from behind, for there is marked side-to-side compression. The vagina higher up, on the other hand, is widened laterally to a considerable degree and compressed from before backwards. Fig. 13 represents these relations by schematic reconstructions of four stages: it can be seen that, whereas the increasing vaginal breadth in the first two stages does not markedly exceed a growth proportionate to the general increase, that of the 170 mm. stage is quite disproportionate. At the same time the lower end, at first wider than the rest of the tube, becomes

relatively narrower. This is associated with the beginning of bulb-formation, these structures being compressed laterally: subsequently they extend downwards by their continued growth and in so doing increase the area of contact with the wall of the sinus in a downward direction.

The bulbs show, centrally, large rounded vacuolated cells staining poorly, which are disintegrating, while the more externally placed cells are small and stain well, and are apparently growing rapidly and pushing their way into or within the surrounding mesoderm. A question arises at once as to the source of these cells, but there does not seem to be any reasonable doubt about the matter. Apart from the fact that this epithelial plug exists at a time when the Wolffian ducts are present and open separately in the sinus, we have seen that in all the subsequent stages mentioned in this paper there has been no evidence or suggestion of any sort of bulb-formation in the Wolffian ducts, when these could be traced, and, *a fortiori*, no hint of any participation of these ducts in the formation of vaginal bulbs. The same can be said about the suggestion that the cellular bulbs might be derived from the urogenital sinus: the vaginal structures are solid masses of cells before the blending of the respective epithelia occurs, and in none of our sections of later stages is there any appearance of ingrowth from the sinus, but rather of vaginal projection into this cavity. The utero-vaginal canal and the Wolffian duct remnants, when present, are surrounded by a condensation of mesoderm which is directly derived or continued from the condensation of the genital stalk. With the appearance of the bulbs, it has to be decided whether their form is the result of a high degree of activity of the vaginal cells, or is due to an invasive action of the surrounding cellular condensation. When we see the relative increase in size of the two contrasted regions, and observe the way in which the condensation layer is moulded on the contained vaginal growth, there seems no reason to doubt that the former is the correct explanation. If the irregular bulbar outline were due to invasion by the surrounding cells, one would not expect such altered shape and accommodation to the bulbar outline, but rather the maintenance of the original regular form.

The method by which the lower end of the vagina is formed and its area of contact with the wall of the sinus is enlarged, as we have described it in this paper, progresses up to birth at least, and the increasing invagination of the upper and back part of the sinus wall causes an increase in size of the hymen and brings it nearer to the surface. Fig. 14 shows the condition in a specimen of 240 mm.; this is enlarged only half the amount of the 170 mm. foetus in fig. 11, but comparison of the two figures will show at once how much progress has been made in the growth of the bulbs and in the invagination. This last process has gone on to such an extent that the orifice of the duct of Bartholin's gland is now hidden by the projecting hymen, and is not very far from the base or line of inflection of the hymen: it is interesting to observe, in this foetus, that the upper end of the long groove, into the depths of which the duct opens, reaches and is turned down on the side of the hymen.

The second section shown in fig. 14 is that of a foetus at full term, in which the orifice of the duct is close to the base of the hymeneal inflection.

It shows the final foetal stage of the invaginating process which was initiated some four or five months previously.

There are many matters and questions associated more or less closely with the development of the vagina which have not been mentioned, or at most very shortly—such as the development of the urogenital sinus, the changes occurring in the growths from this, the formation of the urethra, the extension of condensations, etc. We have purposely refrained from introducing these subjects, leaving them for further consideration at a future time, and have

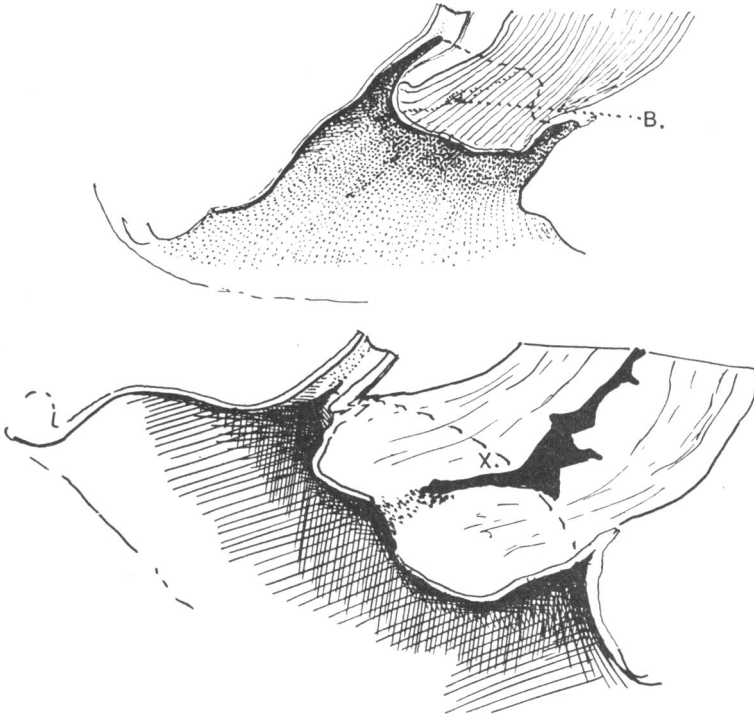


Fig. 14. Sagittal sections of (above) 240 mm. foetus, and (below) foetus at term, at similar magnification. *B.* and *X.* indicate the situations of the openings of the ducts, deep to the hymeneal projection.

endeavoured to confine ourselves to the main general question—the origin of the lower end of the vagina, and the mode by which it attains the position of its final opening—without going into details which are not essential.

The views which we hold, as the result of our observations on the material, may now be briefly summed up. They are as follows:

1. The lower end of the vagina, like the rest of that structure, is purely a Müllerian derivative.
2. It establishes contact with the urogenital sinus at an early stage, at the apex of the “Müllerian tubercle.” This site is that of the upper part of the future hymen.

3. Its lumen is for a time filled up by a plug of epithelial cells, derived from the lining cells of the canal. Later, by their active growth, these cells lead to the formation of the so-called *vaginal bulbs*. These bulbs increase the area of vaginal contact with the sinus (and become the hymeneal area) by their extension in a downward direction along its posterior wall. At the same time they invaginate this wall from behind, so that the sinus undergoes a relative diminution in length and a change in direction of its long axis, a corresponding increase occurring in its opposite diameter. The hymeneal site is thus made to approach nearer the surface.

4. The hymen is formed passively by the invagination of the posterior wall of the sinus, as just described. Thus it consists of three layers—a layer of vaginal cells from the invaginating “bulbs,” a covering layer of epithelium from the sinus, and an intervening stratum of vascular mesoderm which appears to be derived from the vascular condensations surrounding the walls of both these cavities.

5. The Wolffian ducts take no part in the formation of any portion of the vagina and hymen. They disappear in their lower parts (as a rule) at a fairly early stage, and, when persistent, can be followed as patent small tubes which open near the free border of the hymen. This position on the hymen is due to their terminal openings being invaginated with that part of the sinus wall in which they are situated, and any secondary change leading to an opening beside the hymen would evidently be due to disappearance of the thin covering of sinus cells which lies over their invaginated ends.

In comparing these results with those of other observers we are faced with the difficulty of classifying the diverse opinions for purposes of review. It is only possible to do this in a very general way, owing to the overlap in many details, and perhaps the large division into three main classes, which we mentioned earlier in this paper, affords as good a basis as any, on which we can make the comparison. These three headings were:

(a) Descriptions which give the derivation of vagina and hymen as purely Müllerian.

(b) Those which make it mainly Müllerian, but bring in an additional element in the development of its lower part. This new factor may be derived from the Wolffian ducts or from the urogenital sinus, entailing an associated origin for the hymen.

(c) Those which derive the vagina mainly or altogether from the urogenital sinus, with similar hymeneal origin.

The difficulty of placing any individual opinion in its proper class can be exemplified in the present case: the views we advocate would certainly seem to belong to class (a) in the list just given, but, strictly speaking, the sinus-element in the hymen would give some reason for placing them also in (b).

(a) R. Nagel<sup>(1, 2)</sup> is one of the main supporters of the entirely Müllerian origin of the vagina. He described it as arising wholly from the united Müllerian ducts, the lower ends of which, becoming solid, grow rapidly in



length through hypertrophy and multiplication of their lining cells, and come to invaginate the posterior wall of the urogenital sinus. The apex of the projection so formed breaks down to allow communication between vagina and sinus, this marking the site of the future hymeneal orifice—in the position of the Müllerian tubercle of earlier specimens. In this way the lower solid portions of the Müllerian ducts form the vagina, a lumen being absent throughout. This view, it is evident, is, in essentials, the same as that which we put forward, as is also his description of the formation of the hymen, which he considers to arise passively by the bulging of the Müllerian vagina into the sinus, invaginating it. He considers that *active* growth of the hymen may take place later, as shown by the fact that the hymeneal orifice may be relatively smaller in the new born child, than at a period in foetal life. This is, however, difficult of proof, and does not affect the main issue as to the mode of development of the hymen. While Nagel describes a bulging of the lower end of the vagina, the vaginal bulb-formation, which is so striking in our specimens, is not mentioned, nor the process of spread of the vaginal area of contact with the sinus, brought about by this means.

Wood Jones<sup>(3)</sup> describes this Müllerian downgrowth as double, and in the form of bulbs. According to him, the fused Müllerian ducts reach and open into the upper end of the sinus at a comparatively early stage. Later this opening is lost, to be regained at an advanced stage of foetal life, this second opening being brought about by a paired bulbar downgrowth from the fused Müllerian ducts. These bulbs tunnel their way along the mesoderm behind the posterior sinus wall, to open at a much lower level than the primary opening, the hymen being that part of the mesoderm lying between this paired bulbar downgrowth and the surface, and at first unaffected by it.

While this view is distinctly comparable with ours as here expressed, we should like to say that we have never in any of our specimens seen any trace of a paired downgrowth in the mesoderm posterior to the sinus and not in contact with its wall. Wood Jones does not make it clear, when he states that the primary opening between vagina and sinus is lost, whether an epithelial contact is maintained, but we take his meaning to be that all communication between the two channels and their epithelia is lost, an opinion which we have found by our specimens to be erroneous. Contact having once been established between the vagina and sinus, at the 30–38 mm. stage, is never again lost, but continues to increase in area by the formation of the vaginal bulbs and their downgrowth along, and in contact with, the posterior sinus wall.

According to other authors (with whom we do not disagree) the original utero-vaginal canal takes a much larger share in the formation of the vagina, which possesses at first a lumen, lost through transition of the simple lining epithelium, into a multilayered squamous epithelium, the lumen of the canal being lost through growth of its anterior and posterior walls.

Felix<sup>(4)</sup> describes this epithelial transition also, mentioning the appearance

here of large rounded cells, which fill up the utero-vaginal canal and render it solid. The origin of these cells has caused much speculation, Felix considering that they arise from the cells lining the Müllerian ducts, while Berry Hart (5, 6) derives them from the so-called *Wolffian Bulbs*, and Van du Hoeven, as quoted by Mijsberg (7), derives them from the lining membrane of the urogenital sinus. This places the views of these last two observers in our next group (b).

Before passing on to consider this group in detail, a few further remarks must be made on the "vaginal" view of the origin of the *hymen*.

Taussig (8, 9) in his original paper bases his views on five embryos examined by serial section, and considers the hymen has a *vaginal* origin independent of the place at which the vagina breaks into the urogenital sinus. Within this latter site arises a fold of vaginal tissue, the hymen. At the point where the vaginal bulbs break through, i.e. at the Müllerian tubercle, another more or less well-marked fold of tissue is left, which becomes obliterated as development proceeds. Taussig thinks that the bi-lamellate *utero-vaginal hymen*, which Schaeffer (10) describes as being found in so large a proportion of his specimens, is due either to an abnormal persistence of these two folds, or to the fact that many of the specimens were examined at a stage when both folds would be normally present.

Taussig in a later paper (9) states that the hymen is not a passively formed structure, but that it is an actively proliferating outgrowth, and considers that such variations in the normal hymen as *hymen cribriformis* can only be explained on such an hypothesis. We consider that such a variation as a cribriform hymen, could very readily arise by a process of passive bulging of the posterior sinus wall by the actively bulging vaginal bulbs, rupture and communication of the two contiguous lumina taking place not at one point but at several, on the apex of the epithelial covered promontory so formed.

Taussig's second paper is largely based on vaginal and hymeneal abnormalities and, as these will be discussed later, nothing further need be said now about them.

Blair Bell, when asked to discuss Taussig's (9) view, stated that he considered the hymen to be the remains of the urogenital plate, or anterior portion of the original cloacal membrane, but considered the question not proven as to whether it were a new formation or a disappearing membrane.

Gellhorn (11) is in agreement with our view and with that of Nagel, that the development of the hymen is inseparable from that of the vagina, and is formed by a bulging of the *vaginal bulbs* into the sinus. He, however, derives practically the whole hymen, with the exception of a thin epithelial covering from the sinus lining, from the vagina, considering that mesoderm from the vagina only grows into this. We are of the opinion that a true *ad hoc* invagination of the posterior sinus wall occurs, and that the *mesoderm* of the hymen is derived both from that of the vagina and of the urogenital sinus.

We have never seen any evidence of a *vaginal* hymen, arising as an active fold of mucous membrane, and consider that the microscopical appearances

are quite against such a view. The lumen of the lower end of the vagina (i.e. in the region of the vaginal bulbs) is filled with vacuolated desquamating epithelium, all activity of growth taking place at the outer limit of the epithelial mass, towards the surrounding mesoderm and the urogenital sinus.

Among the more recent observers whose views must be placed in this class, are Küstner<sup>(12)</sup> and Lubosch<sup>(13)</sup>. The former considers that the hymen arises at the site of the early Müllerian tubercle, taking as proof of this the fact that, where the Wolffian duct is persistent, it opens on the hymen, i.e. its original site in embryonic life. His paper is largely based on a study of abnormalities, and he considers that such an error of development as the presence of the hymen in the absence of vagina is rather against his views expressed above. He states, however, in this connection, that the hymenical relations vary with the degree of development of the Müllerian ducts.

Lubosch (writing in Halban and Seitz's text-book of Gynecology) describes the formation of a bulging lower end of the vagina, which invaginates the posterior wall of the urogenital sinus, the hymen thus coming to be formed passively, as we have described in our paper. He describes, however, the limiting of the spread of the bulging epithelial vagina, by a ring-like narrowing, which is continuous with the columnae rugarum and which he calls the "plica retrohymenalis." We have remarked no such specialised structure in our specimens.

We now come to the second class (*b*) of hypotheses about the development of vagina and hymen, namely that the former is mainly a Müllerian structure, but an additional factor is present in its formation and in that of the hymen.

(*b*) Berry Hart<sup>(5, 6)</sup> is one of the best-known exponents of this view. He considers that the upper two-thirds of the vagina arises wholly from the fused Müllerian ducts, while he ascribes to the "Wolffian bulbs"—i.e. the lower ends of the Wolffian ducts, which he describes as becoming solid and developing bulbar enlargements on their extremities—the formation of the lower third of the vagina, the lining membrane of the entire vagina and the hymen, which therefore, in his opinion, forms below the site of the former Müllerian tubercle, i.e. at the original site of entry of the Wolffian ducts into the urogenital sinus.

Before proceeding to show how entirely we disagree with this view, those of some other observers, holding somewhat similar opinions, must be examined.

Tourneux and Legay<sup>(14)</sup> also mention the presence of "Wolffian bulbs" and consider they form the lower third of the vagina.

Mijsberg<sup>(7)</sup>, whose recent and most interesting paper was published while this research was in progress, is of the opinion that the upper two-thirds of the vagina is Müllerian while the lower third is formed by the fusion of the Wolffian bulbs with each other, with the Müllerian vagina above, and the sinus below, while the hymen forms as the result of the reaction of the sinus to the growth in length of the vagina—here, in a limited sense, in agreement

with our view—and is therefore in actual structure, according to Mijsberg, partly of Wolffian and partly of sinus origin.

Against these views the very definite evidence of our specimens and reconstructions must be placed. At no stage and in none of our specimens have we seen any evidence whatever of Wolffian bulb-formation. In many of our specimens it was possible to trace the Wolffian duct to a lesser or greater extent. In the former case, with one exception, the Wolffian duct was always lost sight of as a patent duct, possessing a lumen, lined by a single layer of cubical epithelium, and without any evidence of proliferation of these lining cells or of bulb-formation. In the exceptional case, the Wolffian duct was seen on one side to join, or rather come into contact with, the outer side of the Müllerian bulb-formation. No cells, however, were given from the Wolffian duct, to this bulb-formation, which had already reached a developed state above the level of contact, and the duct was completely lost sight of in the next—serial—section. In cases where the duct could be followed to the sinus its lumen was maintained throughout, and it opened into the urogenital sinus below the opening of the fused Müllerian ducts. This is particularly well seen in one—170 mm.—specimen, where a patent Wolffian duct on either side is seen to open into the sinus on the surface of the hymen. R. Meyer<sup>(15)</sup> has also traced the duct opening in this position.

In addition, against the view put forward by Berry Hart—that the hymen forms below the site of the original Müllerian tubercle—we consider that we have conclusive evidence in our specimens and the reconstructions made therefrom, that the fused Müllerian ducts, having once established contact with the sinus epithelium at the apex of the Müllerian duct, never again lose that contact (and at this site the hymen is formed) but continue to increase it, and the area of the hymen, by the formation of the Müllerian or vaginal bulbs.

Other workers who in addition to R. Nagel, already quoted, agree with us in attributing a “unilamellate” vulvo-vaginal origin for the hymen, are Budin<sup>(16)</sup> and Webster<sup>(17)</sup>. Budin, basing his work on dissections of adult specimens, considers that the hymen does not exist as an independent structure, but that it is merely the projecting lower and anterior end of the vagina, while Webster describes the hymen as the septum which is left between the urogenital sinus, and the lower part of the vagina, as the latter becomes channelled, the opening of the hymen being formed by the involution of the epithelium on the urogenital surface of the hymen.

Spuler<sup>(18)</sup> considers the vagina mainly Müllerian, but thinks that the lower fused ends of the Müllerian ducts, here called the “Conus Vaginalis,” derive cells from the urogenital sinus, and that the lower third of the vagina arises by a frontal division of the urogenital sinus, which brings this worker into line with the next group, (c), in which the sinus is held to play a large and important part in the development of the vagina.

(c) Retterer<sup>(19)</sup> describes lateral folds arising in the sinus wall, about the

fourth month of foetal life. By union of these folds from above downwards, the lower portion of the vagina is formed, the upper part, i.e. that in relation to the base of the bladder, being formed from the Müllerian ducts.

Pozzi (20) also agrees that the lower third of the vagina is derived from the urogenital sinus.

The latter bases his belief largely on a study of vaginal and vulvar abnormalities, especially on a case in which a hymen was present but no vagina. The vagina was represented by a small pocket only, which he considers to be the lower part of the vagina, derived from the urogenital sinus, which also gives rise to the hymen. Other abnormalities of development put forward to support this view are:

(1) The presence of a single hymen with a double vagina.

(2) The presence of a hymen or fold which surrounds urethral as well as vaginal orifice.

On the general question we would like to point out that theories of development based on mal-developments are only satisfactory when the assumptions are borne out by normal embryological investigation. At least, they should not be in opposition to what is observed in normal development. It is not usual to find the hymen in the absence of the vagina, but there is nothing in its presence under these conditions to invalidate or disprove in any way the facts observed in the study of a normal sinus. The urogenital sinus has many folds, and these can be followed from their inception to what is practically the attainment of the definitive condition, but we have certainly not seen any stage, nor have we been able to come across any description, in which one or more of these folds is clearly shown to form a hymen. On the other hand, the hymen can be seen to be formed at the contact-region of vagina and sinus. It would seem much more likely that, in these cases, the lower end of the vagina and hymen were made in the way indicated above and that subsequently and for some reason in any case unknown, vaginal atrophy occurred. So far as we know, no dissection has been undertaken on such an abnormality to determine the presence or absence of remnants of a vagina.

With regard to the second abnormality, double vagina and single hymen, this would seem to admit of a very simple explanation. The double character belongs to the vagina, and, as has been shown, is still evident to some degree at the lower end, even when this is growing. Nevertheless, the growth, as a whole, invaginates the wall of the sinus and produces the hymen, and it does not require much imagination to see that it is quite possible for the double character of the vagina to be much more marked and persistent, while the effect of the growth of the structure, as a whole, is exercised in the production of a practically unpaired projection into the sinus. Still more marked division, or want of fusion, of the vagina, and distinction of its bulbs, would lead to a definitely double invagination, from which would be derived the commoner variety of double hymen with double vagina.

The presence of a hymen surrounding the urethral orifice as well as that of the vagina does not appear to invalidate in any way the account given of the formation of the parts. In fact, during the course of this investigation, we have noted with interest the presence of a definite invagination of the posterior and upper part of the sinus wall implicating the urethra as well as the vagina. In this way a common hymen might be formed, although, as a rule, the structure ultimately becomes vaginal. We have made some observations on this matter, but have not paid particular attention to the details, as they seemed to be rather outside the more fundamental scope of the questions we were trying to solve. An investigation of the whole development of the female urethra, particularly of its lower part, is needed: we did not go into the question, but what we observed has satisfied us that the presence of a hymen which includes the urethra may be an indication of an inhibition, but at any rate does not clash with the vaginal productional value of the ordinary hymen.

With regard to Retterer's view, we know that septum formation and division of the sinus are said to occur in certain animals (Mijsberg<sup>(7a)</sup>). We have not studied these, and are of course in agreement with most other observers, that such a method of development never takes place in the human embryo. The site of the opening of the Wolffian ducts into the sinus below the Müllerian vaginal orifice, disproves this theory. If the lower third of the vagina arose from the sinus, the Wolffian ducts should open into the vagina—unless indeed, as suggested by one author, they deviate from their close relation to the vagina at this point, extend down in the mesoderm external to the sinus, and come to open below the vaginal orifice formed as above. It seems hardly necessary to say that this effort of the imagination lacks any support whatever from developmental observation.

Before bringing this account to an end, we would like to call attention once more to the Wolffian ducts. On general principles one would expect to find the female tract associated with the Müllerian ducts, but, on some views as to their possible evolution, it does not seem justifiable to deny theoretically the possibility of some Wolffian intervention at the lower end. With such outlook we naturally paid particular attention to the nature and condition of any remnants of the Wolffian ducts with which we came in contact. In this matter we were struck by one common character possessed by all these remains: there was never any sign whatever, in any place, of any activity of the lining cells of the ducts. Their lining was formed by a single layer of cubical cells, surrounding a small lumen, except in those sections where the lumen was disappearing, the cells here coming into contact and getting smaller, and the whole structure plainly on the point of vanishing. In fact the only signs of activity seen were retrograde and directed towards atrophy and disappearance. At no time could we see the faintest sign that either duct might be about to form a bulb, was forming it at the time, or had formed it, and, whenever that duct could be traced to the sinus, it ran to its original site

without any enlargement, and unconnected with the vaginal structures. We have spoken of the one case (fig. 7) which seems an exception to this last statement, but the fact that it was the only case, and the appearance presented by the remnant on examination, make it in our opinion clearly a case of accidental contiguity. Here and there, along the sides of the vaginal bulbs, are processes directed outwards and backwards, which owe their direction doubtless to the fixation of their ends in the surrounding condensation while their bases are carried forward by the growth of the bulbs. Such processes, when cut longitudinally, may be taken—in our opinion—for Wolffian remnants, and we cannot help thinking that some such mistaken reading of a section may account for some of the descriptions of “Wolffian bulbs<sup>1</sup>.” The fact that they may be fairly numerous, and that they may co-exist with the real duct, disposes of them at once. The successive stages that we have examined have given us pictures in which the outstanding impressions we have gained have been those of great activity in the Müllerian structures, and complete absence of activity of any sort, except atrophy, in the Wolffian ducts.

#### SUMMARY

1. The subject was investigated on human material only, by means of serial sections.

2. The Müllerian ducts, fused, reach the wall of the urogenital sinus between 30 and 38 mm. (probably a little variable).

3. Slowly, after this, the lower end of the vagina begins to grow, thus enlarging the originally small area of contact with the wall of the sinus.

4. The growth is due to multiplication of vaginal cells, and is already evident before the end of the third month. It progresses slowly, assuming the form of “vaginal bulbs,” paired structures fused in front with a common median swelling. These are well formed by the fifth month, and now begin to grow much more rapidly.

5. The bulbous growth of the lower end of the vagina is particularly effective in two directions: (*a*) it pushes in the posterior wall of the sinus, invaginating it into the cavity, and (*b*) it extends down along this posterior wall, thus increasing markedly the contact area between vagina and sinus.

6. The progressive invagination of the wall of the sinus makes this cavity relatively shorter: this seems to go on up to birth at least.

7. The hymen is produced by the meeting of vaginal and sinus structures, and therefore corresponds with the area of contact, and increases with it. It bulges into the sinus as this area is invaginated; its lower surface is made by the invaginated wall of the sinus, its upper surface by evaginated vaginal cells, and a vascular mesoderm partly separates these.

<sup>1</sup> I remember seeing the drawing of a section many years ago, where such a projection was labelled “Wolffian.” I remember the section clearly, although I cannot recall the paper, and at the time I wondered—without doubting—on what grounds it was recognised as Wolffian. I concluded that the writer had inside information on the matter, but I am inclined now to think he was simply mistaken. (J. E. F.)

8. The central part of the area of contact, where there is no intervening vascular layer, breaks down, and the cavity of the sinus thus has opening into it the cavity of the vagina, made by breaking down of the central cells of the bulbs. The opening thus made is the hymeneal orifice.

9. The duct of Bartholin's gland, at first far down in the sinus, gets relatively higher and higher. This is not due to any change in position of the duct, but to the increasing invagination shortening the sinus from above, and bringing the hymen to an increasingly lower level. Finally, the upper part of the long groove into which the duct opens is turned down on to the side of the hymen, and the duct comes to open near its margin.

10. The Wolffian ducts take no part in the formation of the vagina and hymen. They usually disappear. If they persist their lower ends are invaginated, and open on the hymen: secondary degeneration of their walls may lead to a persistent opening being situated beside the hymen.

11. Published views on the formation of the parts have been shortly summarised and considered, with the possible bearing of certain malformations.

#### REFERENCES

- (1) NAGEL, R. (1891). i. *Arkiv. für mikros. Anat.* vol. XXXVII, p. 620.
- (2) — (1895). ii. *Centralblatt für Gynäk.* vol. XIX, p. 46.
- (3) WOOD JONES (1904). *British Med. J.* vol. II, p. 1630, Dec. 17.
- (4) FELIX. In Keibel and Mall. vol. II, pp. 921 *et seq.*
- (5) BERRY HART, D. (1901). *Journal of Anat. and Phys.* vol. XXXV, p. 330, May 22.
- (6) — (1896). *Ibid.* vol. XXXI, p. 18.
- (7) MJSBERG (1924). *Zeitschrift für Anat. und Entwicklungsgeschichte*, vol. LXXIV, p. 684.
- (7 a). — (1925). *Ibid.* vol. LXXVII, p. 630.
- (8) TAUSSIG (1908). *American J. of Anatomy*, vol. VIII, p. 89.
- (9) — (1921). *American J. of Obstet. and Gynaecology*, vol. III, No. 5, November.
- (10) SCHAEFFER (1890). *Archiv für Gynäk.* vol. XXXVII, p. 199.
- (11) GELLHORN (1904). *American J. Obstet.* vol. I, No. 2, p. 145.
- (12) KÜSTNER (1919). *Zeitschrift für Geburtshilfe und Gynäk.* vol. LXXXI, p. 353.
- (13) LUBOSCH (1923-24). In Halban und Seitz. *Biologie und Pathologie des Weibes*, vol. I, p. 268.
- (14) TOURNEUX and LEGAY (1884). *Journal de l'Anatomie et de la Physiologie*, Tome xx, p. 376.
- (15) MEYER, R. (1909). *Arkiv für Mikros. Anat.* vol. LXXXIII, p. 751.
- (16) BUDIN, P. (1879). *Progrès Médicale*, p. 677.
- (17) WEBSTER, J. C. (1898). *Trans. of the American Gynecol. Society*, vol. XXIII.
- (18) SPULER (1910). *Handbuch der Gynäk. J. Veit.* vol. V, p. 602.
- (19) RETTERER (1891). *Comptes Rendus et mémoires Société de Biologie*, Série 9, 3, p. 291.
- (20) POZZI (1884). *Annales de Gynecol.* vol. XXI, p. 268.