On the histological figures and the glycogen in the mucous epithelium of the hymen during the period of pregnancy

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Introduction

Dierks (1927) and Adler (1928) reported that the histological figures of the mucous epithelium of the human vagina is subjected to cyclic menstrual changes, on the contrary Lindemann (1928) and Pankow (1952) denied the opinion mentioned above. It is strange that the absolutely opposed results are reported on the observation of the vagina, of which the structure is seemingly so simple that the histological observation is thought to be very easy. These differences seem to be due to the difficulty of the collection of the materials excised from the adult women and also to the fact that these studies described above are practised by employing the smear method.

Yamasaki (1950), using the mucous epithelium of the human vagina as the material, observed histologically on the distribution of glycogen and on the cyclic changes of the amount of it and concluded that the mucous epithelium of the vagina was closely related to the ovarial function and that the manifestation of glycogen in the mucous epithelium of the vagina underwent cyclic changes. The hymen shows the most similar histological structure to the vagina. In this study the authors made a survey of the histological transition and of the quantitative change of glycogen, and compared the results with the reports mentioned above, using the hymens excised from the women who were ranged in the months of pregnancy from the first to the tenth month and were in childbed.
Material and Method

The materials used comprised thirty eight hymens excised from the women who were ranged in pregnancy from the first to the last month and also were in childbed, ranging in age from 20 to 35 years. The materials were fixed in alcohol immediately upon excision, embedded in paraffin and sectioned 5μ thick. The method used for revealing the site of glycogen was PAS technique. Some specimens were fixed in formalin and stained with the allochrome method for connective tissues (Lillie 1951).

Observation

The mucous epithelium of the vagina is distinguished various layers on the standpoint of the histological structure. Dierks (1930) noticed that the vaginal epithelium consisted of three layers, namely, functional layer, horny layer and basal layer. Tani (1952) said that the vaginal epithelium consisted of the layer of flattened cells, the keratohyaline layer, the layer of vesicular cells, the prickle cell layer and the layer of basal cells. In this classification the basal layer, which is designated by Dierks, is divided furthermore into three layers, the layer of vesicular cells, the prickle cell layer and the layer of basal cells. But there have not been any reports on the structural classification of the hymen. The authors designated both the functional layer and the horny layer which were named Dierks as functional horny layer. The reason why the authors call these two layers together as functional horny layer is that these two layers are very difficult to differentiate from each other on the preparations which are stained by PAS method. The authors divide the layer beneath the former layer into three layers, that is to say, the layer of vesicular cells, the prickle cell layer and the layer of basal cells. The observation on the glycogen contents of each layer in each month of pregnancy reveals that the prickle cell layer is richest in glycogen and the layer of vesicular cells is the next, throughout the period of pregnancy. The functional horny layer is less abundant than the layer of vesicular cells. The layer of basal cells contains the least amount of all the layers, and very poor distribution of glycogen is observed throughout the period of pregnancy.

1. the basal cell layer

This layer, adjacent to the lamina propria consists of cylindrical cells lining up in three or four files and is differentiated from prickle cell
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layer with great ease because of the difference of the stainability by PAS method. In other words, the prickle cell layer is stained strongly by PAS method but the basal cell layer gives only feeble stainability. The large and oval nuclei are encountered. The narrow fibrous layer, which is stained red and bends towards the lamina propria mucosae, forms the boundary between the basal cell layer and the lamina propria mucosae.

2. **the prickle cell layer**

This layer consists of round, oval and polygonal cells, of which the nuclei are large according to the size of the cytoplasm. The cytoplasm of the prickle cell stains dark red by PAS method. Many cells which contain fine granules stained dark red are often encountered.

3. **the layer of vesicle cells**

Above the prickle cell layer, there are cells with large size, weak stainability, vesicular appearance and with large nuclei. It is difficult to differentiate this layer from the prickle cell layer definitely. The intercellular borderline is stained red in a line.

4. **the functional horny layer**

In the functional horny layer which is situated nearest to the free surface of the epithelium, there are flattened cells which are distributed almost parallel to the free surface. These cells are stained so strongly by PAS method that they are easily differentiated from the cells which belong to the layer of vesicular cells. It is difficult to differentiate the functional layer from the horny layer on the preparations stained by PAS method. The horny layer, adjacent to the layer of vesicular cells, is thin and is situated beneath the functional layer. The horny layer alters its own thickness. Sometimes the functional horny layer consists only of the horny layer, of both layers or of neither of the two layers. The wide intercellular spaces are clearly recognized in the preparations which are stained by the allochrome method. There are definite, longitudinal interstices between the basal cells, into which the wandering cells which possess the refrangibility invade. In the prickle cell layer and in the layer of vesicular cells, there are slightly wider interstices than in the basal cell layer. Many wandering cells are also encountered. The interspaces in the functional horny layer are not so obviously observed as in the other layers. The wandering cells are observed but very few in number.
There were no glands in all the preparation of the hymen. When the preparations which are stained by PAS method after diastase digestion are compared with those which are stained without digestion, the decrease of the stainability in all the layers of the epithelium is recognized. The feeble stainability of the borderlines between the cells in the basal cell layer, in the layer of vesicular cells and in the prickle cell layer still remain. As the exception, the stainability of the PAS positive substance remains a little stronger in the functional horny layer than in the other three layers. From the observations described above, it was concluded that the most of the PAS positive substances in the epithelium might be glycogen.

The case in the first month of pregnancy. (Fig. 1)

The functional horny layer lies at the most upper part of the mucous epithelium and has so strong stainability that it appears as if it were a dark red belting. This layer is thickest in all the layers throughout the period of pregnancy. The thickness of this layer occupies one-third of the total thickness of the mucous epithelium. The free surface of the mucous epithelium of this case appears in a clear straight line and is seen to be more strongly built than that of the third month, though the free surface of the epithelium in the case of the third month is uneven because some parts of the epithelium have fallen off and the other parts are going to fall off. The detail on the epithelium in the case of the third month will be mentioned later.

The layer of the vesicular cells, just beneath the functional horny layer, is not strictly differentiated from the prickle cell layer and gives the same stainability by PAS method as the functional horny layer, so the cells of this layer contain a moderate amount of glycogen.

In the cytoplasm of the cells which are found at the lower parts of the prickle cell layer or near the basal cell layer, small granules which are stained dark-red are recognized. The basal cell layer gives feeble stainability and only the intercellular borderlines are stained weakly, so that this layer is easily differentiated from the prickle cell layer.

The case in the third month of pregnancy (Fig. 2). The functional horny layer decreases in its thickness, when compared with that of the first month. Some uneven parts of the free surface are going to fall off. The stainability by PAS method becomes weaker than in the case of the first month, namely the decrease of the amount of glycogen is recognized.

The layer of vesicular cells and the prickle cell layer give weaker
stainability than those in the first month and the sites where the stainability is recognized decrease. The basal cell layer shows the same feeble stainability by PAS method as in the case of the first month.

The case in the fifth month of pregnancy (Fig. 3).

The functional horny layer is less thick in thickness and weaker in the stainability by PAS method than in the case of the third month, so the decrease of of the amount of glycogen is recognized. The free surface of the epithelium is uneven just as in the case of the third month. In the layer of vesicular cells, underlying the functional horny layer, the cells occupied with vacuoles are encountered and only the intercellular borderline is stained well by the PAS method to show the structure like network.

The prickle cell layer gives weaker stainability by PAS method than in the case of three months but contains a richer amount of glycogen than the layer of vesicular cells.

The basal cell layer shows the same feeble stainability by the PAS method as in the case of the third month.

The case in the seventh month of pregnancy (Fig. 4).

There are not any remarkable differences, when compared with the case in the fifth month. The stainability by PAS method in all the layers of the epithelium become furthermore weaker. The thickness of the functional horny layer is very thin.

The case in the eighth month of pregnancy (Fig. 5).

The stainability by PAS method in the epithelium becomes still weaker, when compared with the case in the seventh month. The breadth of the functional horny layer becomes very narrow.

The amount of glycogen in the prickle cell layer decreases. The cells which have vacuoles, such as seen in the cells of the layer of vesicular cells, increase in the prickle cell layer. So it became too difficult to differentiate the prickle cell layer from the layer of the vesicular cells.

The case in the tenth month of pregnancy (Fig. 6).

The stainability by the PAS method in all the layers of the epithelium becomes remarkably weaker than in the case of the eighth month. Most parts of the functional horny layer have fallen off with the exception of some surviving parts to show nakedly the layer of vesicular cells. The cells, with vacuoles in their cytoplasm, in the prickle cell layer are more numerous in number than in the case of the eighth month.

Glycogen still remains in the cytoplasm of the cells in three or
four files, which lies near the basal cell layer, of the prickle cell layer. Not any remarkable changes are found at all in the basal cell layer, when compared with the case of the early stage of the pregnancy.

The case in the fiftieth day of puerperium. (Fig. 7).

The figures of the functional horny layer and the stainability by the PAS method of all the layers of the epithelium are similar to those in the case of the third month of pregnancy. Namely, the functional horny layer becomes thicker and the stainability by PAS method in the layer of vesicular cells and in the prickle cell layer become stronger than those in the case of the tenth month of pregnancy. So the difference between the layer of vesicular cells and the prickle cell layer became difficult to find out.

Discussion

Dierks (1927) discussed the changes, which occurred both in the functional layer and the horny layer in accordance with the menstrual cycle, dividing the mucous epithelium of the vagina in three layers, namely basal cell layer, horny layer and the functional layer. He insists that the horny and functional layers are not recognized on the first day of the menstrual period, but on the seventh day a thin functional layer is found for the first time. He said that it became possible to differentiate the functional layer from the horny layer on the seventh day. He said also, on the twenty second day of the menstrual period the thickness of the functional layer became as thick as that of the basal cell layer. He noticed that the functional and horny layer fell off and only the basal cell layer remained, just after the beginning of the menstrual period. According to Dierks, the changes which are observed in the hymen seem to be almost similar to those occurred in the uterine cycle. On the other hand, Lindemann (1928), Pankow (1928), Smith (1929) and others denied the opinion offered by Dierks, as the authors have already referred to in the introduction.

In the present study on the hymen, the changes of the structure and those of the stainability by PAS method are encountered, in the epithelium as described above. In other words, the functional horny layer is remarkably thick in the early stage of pregnancy, becomes thinner with the proceeding of the period of pregnancy; then some parts of the epithelium fall off. This layer increases its thickness in a lying-in period and resembles to that in the case of the third month, in the fiftieth day of puerperium. As the hymen shows changes
occurred in the vaginal epithelium in every month of pregnancy can be surmised. The changes recognized in the epithelium are supposed to be intimately related to the changes occurring in the vaginal epithelium in accordance with the menstrual cycle. Yamazaki (1950) observed the distribution of glycogen and its cycle change on the human vaginal epithelium. According to Yamazaki, glycogen is most abundantly manifested in the prickle cell layer, next in the horny layer and least in the basal cell layer throughout the period of pregnancy.

The prickle cell layer in the classification by Yamazaki corresponds to both the prickle cell layer and the layer of vesicular cells in our classification, the horny flattened layer in the former is equivalent to the functional horny layer in the latter. So the figures of the distribution of glycogen in the vaginal epithelium corresponds to that in the epithelium of the hymen. Yamazaki also recognized the manifestation of the superabundance of the amount of glycogen in the vaginal epithelium in the menstrual period. That is to say, even in the basal cell layer the manifestation of the moderate amount of glycogen is noticed in menstruation, though the glycogen contents in the basal cell layer are very poor in the other period except menstruation.

Yamazaki concluded that glycogen manifested itself in the epithelium in the cyclic change, from the fact that glycogen was most abundantly distributed in the vaginal epithelium of the pregnant women, and was less abundantly in the epithelium of the women at menopause and of the girls in their period of puberty.

Ikeda (1951) recognized the intimate relationship between the distribution of glycogen in the vaginal mucous epithelium and the female estrogentic substance, from the results that the amount of glycogen in the vaginal epithelium of old women increased and showed the appearance like that of the women in pregnancy by the injection of the substance mentioned above. The authors noticed in this study that glycogen in the mucous epithelium of the hymen was very rich in the early stage of pregnancy, decreased with the proceeding to the course of pregnancy, was least in amount in the tenth month of pregnancy and again increased in a lying-in period.

Comparing these results with the pattern of the distribution of glycogen in the vaginal epithelium in the menstrual cycle, on which Yamazaki reported and with the changes in the vaginal epithelium occurred in accordance with the menstrual cycle, which Dierks noticed, it is supposed that the mucous epithelium of the hymen had an intimate relationship with the ovarial function.
Conclusion

The hymens taken from pregnant women and from women who were in lying-in period were fixed both in alcohol and in formalin, sectioned 5μ thick and were stained with PAS method and with the allochrome method for connective tissues (Lillie 1951).

The functional horny layer was thickest in the first month of pregnancy, became thinner with the proceeding of the period of pregnancy.

Some parts of the thick layer fell off in the tenth month of pregnancy. In the puerperal stadium this layer became thicker again. The mucous epithelium was richest in an amount of glycogen in the first month of pregnancy, decreased with the development of the months, but increased in the fiftieth day of puerperium, just like in the third month of pregnancy. From the results mentioned above, it was concluded that the changes which occurred both in the functional horny layer and in the manifestation of glycogen in the epithelium had a close relationship with the cycle of the ovarian function.

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Literature


Smith, G., 1929. Histological changes in the epithelium of the human vagina, correlated
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Explanation of Figures

Fig. 1. The hymen of a woman who is in the first month of pregnancy.

The functional horny layer, the layer of vesicular cells and the the prickle cell layer are stained dark red. The free surface of the functional horny layer appears as a definite straight line.

The staining method is PAS technique from Fig. 1. to Fig. 7.

Fig. 2. The hymen of a women who is in the third month of pregnancy.

The functional horny layer becomes weaker in the stainability by PAS method and the free surfaces which are uneven because some parts of the epithelum begin to fall off.

The stainability by PAS method in all the layers of the mucous epithelium became weaker.

Fig. 3. The hymen of a women who is in the fifth month of pregnancy.

The functional horny layer became thinner than that of the case in the third month of pregnancy.

Some cells with vacuoles in them are found in the layer of vesicular cells, showing the appearance of the network.

Fig. 4. The hymen of a woman who is in the seventh month of pregnancy.

Fig. 5. The hymen of a woman who is in the eighth month of pregnancy.

The functional horny layer is thicker than that showed in fig. 4. The stainability by PAS method decreases in all the layers of the mucous epithelium.

Fig. 6. The hymen of a woman who is in the tenth month of pregnancy.

Some parts in the functional horny layer fall off, so the layer of vesicular cells appears nakedly.

The cells in the prickle cell layer gives remarkably weak stainability, because the vacuoles appear in the cytoplasm of these cells.

Fig. 7. The hymen of a woman who is in the fiftieth day of puerperium.

The functional horny layer becomes thicker.

In the layer of vesicular cells and in the prickle cell layer, glycogen is abundantly encountered.

All specimens are stained with PAS method.
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