The vaginal changes in rodents have been known for over sixty years.

To Stockard & Papanicolaou (1917) is due the credit of having established a connection between these variations and the ovarian changes.

In the course of histo-physiological investigations we have studied the vagina of the mouse, the rat and the cat for 25 years. During the last four years we have also investigated the vaginal cytology of women by different methods and particularly by means of differential staining.

We should like to compare these clinical and experimental data and try and demonstrate their fundamental biological basis.

Two facts must be taken into account:

1° Only Hartmann (1944) and Jaworski (1950) have used differential staining for their experiments on the female rat. This is due to the fact that the variations in this animal are so conclusive that specific staining did not prove necessary.

2° For a very long time, vaginal smears from women have not been considered reliable from a hormonal point of view, even
after the discoveries of Stockard & Papanicolaou (1917) and in spite of the work of pioneers such as Pouchet (1847), who 100 years ago already noted important changes in the cellular contents of the human vagina at different stages of the cycle.

The forerunner was Lehmann (1921); Papanicolaou in 1924 again studied the subject from a different angle. Four years later, in 1928, Ramírez, a Mexican, published a most important work on cyclic variations and in 1933 Papanicolaou published his monograph on the vagina and vaginal smears, which is now universally known. In our country, Schockaert & Férin have been the first, since 1939, to make an important contribution on this question.

Scepticism, however, still persists in a decreasing number of countries, regarding the value of this method from an endocrinological point of view.

Thus if vaginal smears have been used readily for experimental purposes in the rodents, it has been quite different in women.

This results from the apparent uniformity of the desquamated products of the human vagina and the absence of specific dyes in Europe before 1944. Indeed, though it is true that monochromic methods of staining has already given useful indications, they are in fact incomplete and much more difficult to read than is differential staining.

Papanicolaou's method of staining was time consuming and complicated and in spite of much patience it was sometimes difficult to interpret the different colours because they did not stand out sufficiently clearly. Shorr (1940) has contrived a new stain which, being much simpler and more rapid, has allowed of a considerable extension of the method and naturally brought important progress.

Consequently we may say that, for the time being, the greater use of this method in the diagnosis and control of patients is due equally to the staining method and to the biological principle in itself.

Of course the simplicity and harmlessness of taking smears in every case have also been a predominant factor.
MODIFICATIONS OF THE VAGINAL EPITHELIUM

First of all it must be stated that the vaginal mucosa is extremely variable; and this applies to all animal species. This variability appears in two ways viz. the intensity and the speed of the changes.

Each reaction is in fact limited at the outset by possibilities peculiar to itself and a castrated woman will not be able to reach the full oestrous condition in the vagina as quickly as a female rat; and it is quite conceivable that if the epithelium of the rat may contain say 15 to 20 cellular layers, that of the woman may contain for instance as many as 40 to 50.

But apart from these variations in the quantity and quality of the changes in the mucosa, which in short represent its basic inertia, three essential potentialities of the vaginal modifications must be considered, i. e. 1° proliferation; 2° differentiation; 3° desquamation.

Proliferation is always based on the same principle: the increase by mitosis or perhaps amitosis at the expense of the deep basal layers. The first two or three layers alone have this mitotic power; as soon as the cells are more differentiated, however, they lose the power of reproduction.

This implies that the first hormonal stimulus will necessarily take place in the same cellular elements, involving stimulation of mitosis and this happens whether oestrogens, progestogens or androgens are given. Hence all steroids have a similar and superimposable action: they start mitosis in the cells of the basal layers. If mitoses are still found in the mucosa of menopausal women, and even of castrated women*) this is because in these conditions a reduced hormonal action may still persist which is able to induce this fundamental process or process n° I, on which all the others depend.

Differentiation: let us admit that this process is highly variable from one animal to another; but it is always based

*) It must be noted that these mitoses may have a degenerative character.
on the same possibilities which are after all always limited.

It consists of glycogenisation, keratinisation and mucification.

_Glycogenisation_ is distinctly predominant in women; it starts in the juxta-basal layers and spreads to the superficial layers.

This process is essential and fundamental for the cytological changes we found, because its importance varies as does probably its morphological aspect, according to the hormonal conditions; this glycogenisation certainly occurs in the flat cells found at the oestrus and in both navicular and external basal cells.

Moreover Mack's reaction, with its iodine vapors, uses this fundamental property of the human vaginal mucosa in cytology.\(^*)\)

In female-rats, on the contrary, glycogenisation is not strongly marked but it is different from _keratinisation_. It is found in the zone of Diercks in the shape of some layers of cells, already flattened, the cytoplasms of which become loaded with large granules, vividly stained with hematoxylin. This process does not go beyond this stage in women. In female-rats and guinea-pigs, on the contrary, it goes further and transforms the cell into a squame that is comparable with the cutaneous-squame, with a uniform cytoplasm and devoid of nucleus.

Between those cells, both containing granules of kerato-hyalin and the non-nucleated squames, other cells appear, the nuclei of which are shrunk, condensed, pycnotic, as well as

\(^*)\) Recently, however, _Vokaer (1952)_ has demonstrated that, when fixing vaginal smears with Gendre at \(-73^\circ\) C., the degree of intensity of the glycogenic contents in the vaginal cells depends only on its differentiation.

A superficial cell contained in a vaginal smear with feeble oestrogenic activity is equivalent with that technique to that noted after the desquamation of a mucosa in intense folliculinic stimulation.

This does not seem to prove that Mack's reaction has no practical value.
other elements in which only a clear spot persists, indicating the place previously occupied by the nucleus.

Full-oestrus, i.e. the peri-ovulatory (pre- and post ovulatory) follicular maturation, is characterized by tightly packed squames. Total keratinisation is only found in women when irritation transforms the physiological evolution of the superficial layers.

This may happen in case of prolapse, dyskeratosis or pathological leucoplasia due to various causes: trophic, avitaminosis (vit. A deficiency) of chronic inflammations. *)

We have seen these squames rather frequently in negroes of the extra-customary center of Elizabethville (Belgian Congo) but this may be due to the sexual habits of the negroes.

*Mucification* is very important in rats. It assumes two distinct aspects: the pro-oestrous phase and the pseudo-pregnant or pregnant.

In the first case only the superficial cellular layer becomes cylindro-cubic, while the mucus forms a deposit in the cell. This layer desquamates at the end of pro-oestrus when the epithelial growth has produced multiple layers, the most superficial of which become flat and assume an appearance of the pre-keratinic or keratinic type.

The mucification of pseudo-pregnancy on the other hand attacks the whole of the mucosa: only one or two basal layers remain unchanged. The higher layers prepare the formation of a mucous globe that grows larger, drives back the nucleus, flattens it and finally occupies the whole of a highly hypertrophied cell.

Several superposed layers are then transformed into a mucous coating.

*) Some keratinisation noted in the vagina of female-rats could also result from an equivalent dystrophy (for instance vit. A deficiency) or even from an intoxication. There is hardly any possible distinction between these reactions and those of elements with oestrogenic activity. This results from the fact that the power of differentiation of the vaginal epithelium is necessarily limited at the outset.
If the pro-oestrous type of mucification may be induced by oestrogen, pseudo-pregnancy mucification is only induced by progesterone or steroids with progesterone-like activity. Thus this modification is specific to a gravidic hormonal action, that is to the functional corpus luteum. It has the same value as Loeb's experimental deciduoma, but it represents a spontaneous reaction of the tract. Ultimately it denotes the presence of a luteotrophic factor or prolactin which has transformed a non-functional cyclic c. 1. into an active c. 1.

The significance of this particular histological aspect derives from this fact.

There is no equivalent process in women. Pregnancy is not accompanied by mucification; the mucosa is markedly hypertrophied, the superficial layers have desquamated and the intermediary layers form the coating in contact with the outside.

Let us now consider desquamation.

It includes noble elements and so-called trivial elements such as leucocytes, histiocytes and blood-corpuscles.

In female-rats this process is very active during pro-oestrus and met-oestrus and very polymorphic. It is active and uniform during oestrus and practically non-existant during dioestrus and after castration.

But if keratinisation or pro-oestrous mucification do not interfere with desquamation, pseudo-pregnant mucification literally stops it and the corresponding smears are very poor if not entirely deprived of cellular elements.

In women, in whom only glycogenisation constitutes the differentiation of the mucosa, desquamation is always abundant, except in cases of marked atrophy of Türner's amenorrhea or in cases of old castration or senility.

This explains why vaginal biopsies are often very difficult to read and on the whole little differentiated (with the exception of extreme cases) while cytological examinations are rich, polymorphic, diverse, transitional or clearly differentiated.
The whole clinical interest of cytology is derived from the importance of desquamation of the human vaginal mucosa.

Considering the cellular contents particularly leucocytes, we notice in both species that, during the periods of growth, infiltration is generally poor; while on the other hand, during the phases which are regressive, on the way back-to-normal, destructive and lacking in differentiated layers, it is plentiful. But, in this respect, many supplementary data are needed.

Does this mean that in women, this desquamation presents a total and perfect differential character? It is undeniable that the histological aspect of the mucosa is widely different from that of the desquamated products studied by differential methods. Let us admit, however, that we should like more particularly to find during the progesterone period of the cycle or at the beginning of gestation more distinctive and definite cytological characters, which would make vaginal cytology an ideal method for the normal and pathological hormonal study. Those characters probably exist. There is good ground for carrying on investigations.

We can also summarize those three fundamental properties of the vaginal mucosa at three distinct stages of sexual life: castration, oestrus and pregnancy or pseudo-pregnancy:

Castration.

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<tr>
<td>Rat</td>
<td>0</td>
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<td>Woman</td>
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Oestrus or Ovulation.

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<tr>
<td>Woman</td>
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Pseudo-pregnancy or Pregnancy.

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Thus we can see that the evolution of the vaginal mucosa in women is characterized by its particular property of abun-
dant desquamation during the different periods of sexual life. This property, however, is least developed during castration.

We would moreover like to point out that this problem is more complicated for two reasons: 1° the same differentiation, for instance mucification in rats and glycogenisation in women, may correspond to hormonal states, which are essentially distinct. But under those conditions, these histological and cytological aspects are also different; 2° because two antagonistic differentiations may coexist at the same time, either at the same place or in adjacent places in one histological preparation.

We shall study that particular cytological problem presently.

It is an established fact that the vaginal cytology of woman enables us to appreciate the existing hormonal state and that it is therefore possible to establish a cyto-hormonal diagnosis. This includes not only the determination of the day of the normal cycle but also the diagnosis of pathological hormonal conditions which either fall short of or exceed what is required.

The histology of the vaginal mucosa of female-rats as well as the differential staining of its contents also allow of the demonstration of abnormal cycles as compared with the complete normal cycle of an adult.

Jaworski (1950) has shown that, during puberty or after the injections of small doses of oestrogen into castrated animals, commencing incomplete cycles could be noted. This can be demonstrated by the acidophilic index and that of the nuclear degeneration (homologous with the karyopycnotic index of woman).

The anucleated squames may be absent; the leucocytes persist during the different takings. It is difficult not to draw a parallel between the physiological or experimental observations found in female-rats, and the vaginal smears of amenorrhea or hypooestrogenic oligomenorrhoea, met with in women. On the contrary, during permanent oestrus associated with an exaggerated or abnormally prolonged folliculiny, the vagina is thick, Diercks's zona is clear and well-marked and
contains several layers; the keratinized layers are abundant and the vagina is filled with typical desquamated tightly packed cells. The oestrogenic hyperactivity therefore can sometime be recognized histologically and cytologically.

Pseudo-pregnancy, on the contrary, cannot be distinguished from pregnancy, or normal lactation and the vaginal smears are not more significant during these periods than during dioestrus or the period of rest.

Yet the negativity of cytology in some definite experimental conditions allows us to suppose that this state exists, but nothing more.

It must be noted, however, that modern cytology employing the proper methods has not yet been sufficiently used in experiments that aim at producing extra-physiological reactions.

Let us even say, that generally speaking, histology shows too that errors of estimation must necessarily result from the cytological study of the vaginal contents under certain conditions.

For instance, in female-rats, we can induce particular types of reactions that we have called intricate or mixed reactions (Bourg, 1952).

In the first case, under the muciparous layers, there is a beginning of oestrus, more or less advanced, from which consequently no product of desquamation derive. This oestrus may reach the differentiation of the keratinized cells underlying the mucus layers.

In the second case, side by side with pseudo-pregnancy like recesses and crypts or in front of them, other recesses and crypts, in typical oestrus can be noted. There we observe a peculiarity which is similar to the one that may be expressed in the human endometrium in which side by side with glands in a typical secretory phase, there are others in a state of cystic dilatation.

Under these circumstances, the vaginal content shows an oestrus condition since the pseudo-pregnant zones do not desquamate. And this conclusion may not correspond with reality.

It is also possible that in women, all vaginal zones do not
react equally or may even show opposed hormonal conditions. Under such conditions smears made at different levels could similarly give rise to diagnoses quite different from one another.

These are errors connected with the method, the possibilities and basal conditions of which must be carefully kept in mind.

In women cytological reactions will be different according to the site in the vagina with regard to the vulva where the smears has been taken.

Factors which alter the cytological aspects observed are: cytological alterations of old cells, extrinsic influence, the easier contamination by cutaneous-vulvar squames, oxygenation and the easier desiccation of the contents. This is the reason why the smears must be taken in the lower part of the vagina with a speculum and preferably from a lateral cul-de-sac so as to avoid the mucous secretions of the cervix, which are sometimes so abundant.

Dystrophic states such as a polycystic degeneration, of the epithelium can be produced in the vagina of female-rats. The cells are then changed into small microscopic cysts which often possess very definite outlines; the leucocyte infiltration is abundant and invades the cysts and the mucoid reactions are non-existent.

These states are always artificial; they are experimentally created hormonal disequilibria and perhaps the expression of a miscarried progesterone phase.

Similar dystrophic are liable to exist in women, for instance after an irradiation or under some inflammatory or pathological conditions.

Most of the facts concerning the cyto-chemical mechanism of the morphological modifications noted in female-rats are still to be elucidated and would institute a most interesting field of study. As for women, elaborate investigations in connection with this have already been made and they will certainly lay down new bases for a method that is applicable to clinical work.
SUMMARY

The author has studied by the history of the evolution of experimental and clinical cytology, the reasons why this method of diagnosis has developed so slowly in human pathology.

He draws a parallel between the different types of reactions of the vaginal epithelium: proliferation, the varied differentiation and desquamation in rats and women.

He demonstrates that, in the vagina of the latter, desquamation is the most important process.

This observation indicates from a general biological point of view why cytology must necessarily be of great clinical importance.

He establishes the concept of basal inertia of the epithelium which always limits its possibilities. Experimentation is able to determine in the vagina of rats particular cytological aspects. Yet histology also enables us to detect in the vagina of this animal various modifications which the cytological study of the vaginal contents will not always be able to detect with precision even with differential stainings.

For these biological reasons too, inevitable errors will always be associated with this method, which, however, may and will progress by the use of new techniques.

RESUME

L'auteur étudie par l'historique de l'évolution de la cytologie expérimentale et clinique les raisons pour lesquelles cette méthode de diagnostic a tant tardé à se développer, en pathologie humaine.

Il fait un parallèle entre les diverses modalités de réactions de l'épithélium vaginal: la prolifération, les différenciations variées et la desquamation, chez la rate et chez la femme et démontre que chez cette dernière, c'est la desquamation qui est la plus importante.

Cette constatation fait pressentir pourquoi d'un point de vue
biologique général déjà, la cytologie chez la femme doit nécessairement avoir une importance très grande.

Il établit la notion d’inertie de base de l’épithélium qui limite toujours ses possibilités. L’expérimentation peut déterminer chez la rate des aspects cytologiques particuliers. Cependant, l’histologie permet également de repérer chez cet animal des modifications variées qui ne seront pas toujours susceptibles d’être repérées avec exactitude par l’étude cytologique du contenu vaginal, même avec les procédés de colorations différentielles.

Il y aura donc toujours, pour ces raisons biologiques également, des erreurs inévitéables attachées à la méthode, qui peut et doit cependant faire encore de grands progrès grâce à des techniques nouvelles.

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