MITOTIC ACTIVITY IN THE ORAL EPITHELIA OF THE FEMALE RAT

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ABSTRACT

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To study what effect the oestrous cycle had on mitotic activity of the oral epithelia of the female rat, twenty-four Long Evans strain rats having regular oestrous cycles as determined by the vaginal smear technique, were finally sacrificed in four groups at different intervals in the oestrous cycle at the same time of day to avoid a diurnal variation affect. Six hours prior to sacrifice the animals had received colchicine. The mitotic activity during the the six hours prior to sacrifice was estimated for vaginal mucosa, ear epidermis, and several oral epithelia.

The rats were put in sequence in a standard oestrous cycle for comparison of mitotic activity in all the tissues studied.

The mitotic activity in the ear epidermis and the oral epithelia was not significantly affected by the oestrous cycle, unlike the vaginal mucosa which showed marked fluctuations in mitotic activity in conjunction with definite histological changes of the epithelium.

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INTRODUCTION

The process of renewal in epithelial surfaces is one which is basically interesting in several areas of biological investigation. It occupies a central position in the understanding of the mechanisms for maintaining skin and mucous membranes by mitotic activity, cellular differentiation and shedding. And knowledge of the reactions of this process to noxious agents may be expected to contribute to an understanding of various pathological conditions. As an example, the susceptibility of an epithelial surface to atomic radiations is to a large part determined by the rapidity of cell renewal which occurs within it.

In the oral cavity, all the epithelial surfaces are being constantly renewed. The normal lingual mucosa for example maintains a fine complex papillated surface, the epithelium of the gingiva - an integrity of epithelium around and between the teeth. The oral epithelial surfaces are at times the site of pathological alterations and one curious feature is that the oral mucous membranes and the vaginal mucosa may be similarly and concurrently affected. The concurrent affection by familial white folded dysplasia (Cannon 1935) and the susceptibility to concurrent oral and vaginal candida albicans infection are examples. Moreover, several conditions of oral pathology have a suspected ovarian hormone dependence related to their aetiology. Certain gingival pathologies of adolescence, pregnancy and the menopause have been

suggested to be the result, in part at least, of alterations in hormonal physiology at these periods of life.

These and possibly other factors, perhaps merely a certain superficial anatomical and physiological similarity to vaginal epithelium, make the subject of oral mucous membrane susceptibility to hormonal fluctuations an interesting one.

If actual epithelial renewal in the oral cavity was directly affected by hormonal action, one of the components of the renewal mechanism which might be affected, could be mitotic activity.

Experimentally, this can be measured most suitably in the oral epithelia by the use of the drug colchicine which acts on dividing cells and in the appropriate dosage arrests their division at metaphase. Individual cells are counted and the proportion of them that are arrested in metaphase gives an indication of the mitotic activity over the period that the drug has been acting.

If mitotic activity in oral epithelia could then be measured over the regular periodic fluctuation in physiology which accompanies the oestrous cycle of an experimental animal, such as the female rat, an indication might be given of a physiological response of oral epithelium to hormonal stimulation.

RELEVANT CONCEPTS AND METHODOLOGY

A Review of the Experimental Literature

THE PHENOMENON OF CELL RENEWAL

With the exception of the permanent cell populations, e.g. the neurones and cells of cardiac muscle, some degree of replacement of cells lost to the body occurs in most organs throughout life, and moreover, there is continuous, gradual addition to the total cell mass of the body in respect of general body growth. However, in the integument, the linings of epithelial tracts, and in the formed elements of the blood, a more rapid and continued multiplication prevails, unrelated to the general cell multiplication necessary for the growth of the organism. This is offset by a corresponding cell loss by destruction and shedding and it is this process of rapid renewal that characterises the labile cell populations of the body.

It is interesting to note that with the exception of the mesenchymal derivatives the renewing cell populations occupy a biological position between the external environment and the milieu interieur of Claude Bernard (1880). Both Leblond and Walker (1956) and Stevens Hooper (1956) suggest that rapid renewal of epithelial surfaces is probably an inherited characteristic directed towards maintaining the integrity of the integument, rather than a continuous repair process in response to "wear and tear". In situations where external environmental influences are not acting, the capacity

for tissue renewal is unimpaired and persists in a stereotyped manner (Leblond & Walker, 1956). This is evident in the intestinal mucosa, which continues to be renewed even when all irritation due to food digestion is eliminated by fasting, (Leblond & Stevens, 1948).

THE MEASUREMENT OF RENEWAL

Of the several tools for studying renewal in labile cell populations, two most useful and complimentary methods have emerged: The "colchicine method" by which the turnover time of a tissue can be estimated by determining the daily mitotic rate (Leblond & Walker, 1956); and autoradiography by which labeled cells can be observed to undergo the stages of renewal.

Different epithelial surfaces have different mitotic rates and therefore different turnover times. The patterns of differentiation and migration towards the surface vary in different types of epithelia, as do modes of shedding, but for the purpose of investigating renewal phenomena, the important expressions of the process seem to be:

1. The duplication phase of the interphase nucleus prior to the process of mitosis in which sufficient DNA is elaborated to permit reduplication of the chromosome compliment. This is the phase at which radioactive tracers may be incorporated into the nucleus.

2. The mitotic phase when the mitotic index can be calculated and when colchicine may be used to inhibit the process at at metaphase allowing assessment of mitotic activity, daily mitotic rate and turnover time.

3. The stages of differentiation and migration when labelled cells or cell types in various layers may be recorded.

4. The shedding stage which determines the thickness of the epithelial surface at any one time.

Extensive research of cell renewal in organs and tissues with the colchicine technique have been carried out by Leblond and his associates in the epidermis and associated structures by Storey & Leblond (1951), in the gastric mucosa by Stevens & Leblond (1953), and in the lung alveoli by Bertalanffy & Leblond (1953). The mitotic rate and renewal times of the digestive tract and the female genital tract in the rat have been studied by Bertalanffy (1960) and Bertalanffy & Lau (1963).

Cell proliferation and migration as revealed by auto-radiography have been studied by Messier & Leblond (1960). Cameron and Greulich (1963) give a comprehensive account of the interrelationship of the two methods.

THE VARIABILITY OF RENEWAL

From these and other studies emerges the concept that although for any one particular tissue the turnover time is relatively constant, the processes involved are susceptible to individual variations in response to a number of factors. The histology of the vaginal epithelium demonstrates this particularly well (vide infra) in that fluctuations of all the factors concerned in epithelial renewal occur in relation

to the oestrous cycle. Variations in mitotic rate, in the number and thickness of the cell layers, in keratinisation, and in the degree of shedding, all occur at different phases of the cycle.

What then are the factors of the environment or the physiology of the organism which may affect the process of cell renewal in other situations?

SOME FACTORS AFFECTING RENEWAL

Bullough (1949) and Storey & Leblond (1951) have shown that fluctuations of local temperature cause renewal in epidermis to be less rapid at low temperatures and more rapid at high.

Adverse conditions, injury in particular, cause a burst of mitotic activity at the injured site, and even the minor irritation of stripping off scotch-tape from the epidermis, which merely removes the superficial cornified layers, has been shown to have a similar effect (Pinkus, 1951).

The states of shock (Green & Bullough, 1950) and stress (Bullough, 1952 a,b) have a marked depressing effect on epidermal mitotic activity. This has led Bullough (1952 a,b) to postulate that the adreno-cortico-steroid hormones evoked by the stressing conditions act as antimitotic agents.

The mitotic activity in the skin thus may reflect alterations in the physiology of the animal in response to its environment.

OTHER FACTORS AFFECTING RENEWAL

It may likewise respond to other aspects of physiological variance such as age (Bullough, 1949 b), the time of day (Bertalanffy, 1960), and the routine habits of the waking and sleeping periods, (Bullough 1948 a,b). These latter changes in mitotic activity comprise the "diurnal variations". That alimentation and nutrition (Bertalanffy & Lau, 1962) are factors influencing mitotic rate has also been postulated. Bullough & Eisa (1950) relate the diurnal variations in glycogen content of the epidermis to the mitotic activity - an increase in epidermal glycogen content which occurs during sleep being accompanied by an increased mitotic rate.

Lastly, the effect of fluctuations in hormonal physiology on mitotic activity, epidermal thickness and shedding has been extensively investigated.

THE OESTROUS CYCLE AND THE VAGINAL EPITHELIUM

As has been mentioned before, the vaginal mucosa is particularly affected by the fluctuations of hormonal physiology which occur during the oestrous cycle. The observations of Allen (1922) on the oestrous cycle in the mouse and Long & Evans (1922) in the rat, are classical accounts of this interrelationship.

More recently, Bertalanffy & Lau (1963) found pronounced and related cyclical changes in morphology and mitotic activity of the vaginal epithelium. The mitotic rate was found to be at a minimum during prooestrus and at a maximum during oestrus. In contrast, they found that activity in the endometrial surface epithelium was highest in prooestrus and considerably lower in oestrus and pointed out that this indicated that the predominance of one type of hormone at some stage of the oestrus cycle does not necessarily lead to a general simultaneous elevation or decline of mitotic activity in all tissues.

Walker (1960) using autoradiography studied the differentiation of the vaginal epithelium in mice.

The cells in the basal layers, taking up the radioisotope in prooestrus, migrated towards the surface in a wave to be present in, and shed from, the superficial layer by prooestrus of the next cycle.

He also noted that the polymorphonuclear leucocytes which migrate and are shed through the vaginal epithelium at the metoestrous and dioestrous phases, were derived directly from the blood stream and not the vaginal connective tissues.

It is, of course, the variations in the shed cell population of the vaginal lumen which permits the phasing of the oestrous cycle in the live animal.

The phasing of the oestrous cycle in the rat was accomplished with the vaginal smear technique by Long & Evans (1922). They delineated five phases in the oestrouscycle:

Stage one - Prooestrus
Stage two - Oestrus 1
Stage three - Oestrus 2
Stage four - Metoestrus
Stage five - Dioestrus

Carter (1953) for the purposes of her experiment in albino rats recognized four phases:

- Oestrus
- First day of Dioestrus
- Second day of Dioestrus
- Prooestrus

Ebling (1954) recognized four phases in albino rats:

- Oestrus
- Metoestrus
- Dioestrus
- Prooestrus

Bertalanffy & Lau (1963) delineated six phases in the Holtzman-Sprague-Dawley strain of rat:

- Proestrus
- Estrus 1
- Estrus 11
- Metestrus
- Diestrus 1
- Diestrus 11

THE OESTROUS CYCLE AND THE EPIDERMIS

Loeb & Haven (1929 a,b) tentatively delineated a relation between cell proliferation in the epidermis of the female guinea pig and the functional states of the sex organs.

Bullough (1943) studied cyclical changes in mitotic activity and thickness of the skin of the mouse during the oestrous cycle, and found that maximum activity occured during prooestrus and minimum activity on the first day of dioestrus. The thickness appeared to fluctuate with the mitotic activity; being greatest at oestrus, and least on the first day of dioestrus.

The possibility that this was due to the internal secretion of oestrogen was tested by injections of oestrone over a three day period and similar observations of increased mitotic activity and skin thickness were recorded (Bullough, 1947). The increased thickness did not remain and this was attributed to rapid cornification combined with sloughing. Hooker & Pfeiffer (1943) administered oestrodiol benzoate to rats twice weekly over a long period and reported a decrease in epidermal thickness. This was paralleled in male mice by Bullough (1952 b) who showed a depression of epidermal mitotic activity after the subcutaneous implantation of oestradiol. In this last experimental group adrenocortical hyperplasia was noted and Bullough postulated that this was due to stimulation by the high blood levels of oestrogen and resulted in increased adreno-corticoid secretion with depression of mitotic activity similar in nature to that occuring in shock and stress. Although mitotic activity could initially be expected to rise with increased oestrogen action, this would be cancelled out by the antimitotic action of the adrenal

steroids resulting in reduced mitotic activity and thinning of the epidermis.

Carter (1953), however, could find no statistical correlation of mitotic activity in ear, oesophagus or duodenum of female rats during the oestrous cycle and the treatment of spayed rats with oestrone did not appear to affect the mitotic activity.

The work of Ebling (1954) found in relation to the oestrous cycle of the female rat, that the size of the sebaceous glands, and the thickness of the stratum germinativum of the skin of the back were significantly correlated and that they fluctuate together during the oestrous cycle. High values were found during prooestrus, and lower values in both oestrus and the day following it. Mitotic activity, however, showed no correlation with the oestrous cycle or with skin thickness. In administering oestradiol benzoate to immature female rats, a rise in mitotic activity was observed, but there was also a reduction in sebaceous gland size and epidermal thickness.

These results were interpreted as indicating that oestrogens affect both the holocrine secretion of sebaceous glands and epidermal keratinisation and shedding, but not necessarily the mitotic activity. In further studies, Ebling (1955) investigated the interrelationship of oestrogens, adrenal, and pituitary hormones to cell renewal. He suggested that the normal processes of sebaceous gland breakdown,

keratinisation, and shedding were delayed by interference with the pituitary-adrenal axis; whereas, oestradiol accelerated these functions even in the absence of the pituitary and adrenal glands. He concluded, that in the rat at least, mitotic activity in the epidermis did not depend on the action of oestrodiol, although inhibition of mitosis might be a function of the pituitary or adrenal hormones.

So far we have seen that while a relationship between the oestrous cycle, mitotic activity, and epidermal thickness can be demonstrated in mice; only a relationship of the epidermal thickness, and sebaceous gland size can be related to oestrus in the rat.

EPITHELIAL RENEWAL IN THE ORAL CAVITY

The possibility that the oestrous cycle might affect the epithelial surfaces of the oral cavity, has lead most investigators of this area to employ male animals only in studies of mitotic activity and epithelial renewal. Henry, Meyer, Weinmann and Schour (1952) investigated the mitotic activity in buccal mucosa of rabbits. Mühlemann, Zander and Halberg (1954), and Halberg, Zander, Houglum and Mühlemann (1954) found a correlation between 24 hour periodic phenomena, as measured by fluctuation of rectal temperature and eosinophil count from tail blood, with the mitotic activity in the retromolar epithelium in the rat.

Bertalanffy (1960) studying the mitotic rates and renewal times of the epithelia of the digestive tract assessed these factors for lip, buccal mucosa, and tongue.

EPITHELIAL RENEWAL IN THE GINGIVA

However, considerably more attention has been paid in oral biology to the pattern of epithelial renewal in the gingiva. The gingiva is that part of the oral masticatory mucosa, that is attached to the teeth and alveolar processes of the jaws. Where the gingiva encircles the tooth, the gingival epithelium forms a cuff-like fold around the neck of the tooth about the level of the cemento-enamel junction. The potential space between this fold and the tooth is called the gingival sulcus. A comprehensive account of this complex area in the rat is given by Alldritt (1961).

Studies of cell renewal in this area have been particularly directed to determining the pattern and differential rates of renewal of the epithelia concerned at the dento-gingival junction namely, the epithelial cuff, and the down-growing gingival epithelium.

Hirt, Hartl and Mühlemann (1955) recorded the distribution of mitoses, without the use of colchicine in the epithelium of the interdental papilla in male rat material. The epithelium of the gingival sulcus, they divided into two morphological areas, "the downgrowing oral epithelium" and "the epithelial attachment". A high count of mitotic figures was found in both

these areas but only exceptionally was a high count found in the area where they joined.

Studies in mice by Beagrie and Skougaard (1962) and in mice and monkeys by Beagrie (1963), compared the turnover times of the same morphological areas by means of autoradiography. The rate of turnover was found to be approximately twice as great in the epithelial attachment than in the downgrowing and the oral epithelium. By passive movement the cells were shed into the gingival sulcus.

Trott & Gorenstein (1963) estimated the mitotic rates in the oral and gingival epithelium in the male rat with colchicine. The epithelial attachment showed the highest daily mitotic rate of all the epithelia examined.

From these studies a concept of a rapidly renewing epithelial lining to the gingival sulcus emerges, the highest activity being at the lowest point of the sulcus, that is the epithelial cuff. The constant shedding of epithelial cells is into the gingival sulcus. (Löe, 1961). This concept seems incompatible with an anatomical union between the epithelial cuff and the tooth surface, the so called epithelial attachment.

FACTORS AFFECTING RENEWAL IN THE ORAL CAVITY

Relatively little experimental work has been done as yet on the factors causing variations in oral epithelial renewal. The work of Mühlemann et al (1954), and Mühlemann, Ebneter and Rupf (1959), recorded a marked diurnal variation

in mitotic activity in ante-molar epithelium of the lower jaw.

Trott & Gorenstein (1963) found diurnal variations in most of the oral tissues they studied, but especially in the palatal and buccal mucosae. These authors also discussed the relationship of high mitotic activity to high functional demands on particular types of oral mucosa.

Rateitschak and Mühlemann (1957), investigated the effect of cold-stress on the renewal in the oral cavity, but found that rats immersed for 60 secs. in water at -2°C showed no alteration from the mean mitotic rate in the oral epithelium.

Qualitative estimations of the state of oral epithelia in experimental animals subjected to alterations in their ovarian hormonal physiology have been made by several authors.

Ziskin, Blackberg and Stanetz (1936) found degenerative changes and superficial cornification in the gingival epithelia of Rhesus monkeys with altered oestrogen physiology. Stahl, Weinmann, Schour and Budy (1950) in a study of the effect of oestrogen on alveolar bone and teeth of mice, noticed no change in the gingival epithelium but did not study mitotic activity.

Nutlay, Bhaskar, Weinmann and Budy, (1954) in a further study found no change even with large doses of oestrogen in the gingival epithelium of rats, though older mice did show proliferation of the epithelial cuff along the root surfaces of the teeth.

Glickman and Quintarelli (1960) recorded no uniquely

distinguishable microscopic features in the gingiva of ovariectomized animals. Mitotic activity was not studied however.

It may be that there are several other factors of the general environment, of the local oral environment, and of the general physiology of an animal which may influence the mitotic activity of the oral epithelia. The oestrous cycle may be one of them, but as yet no firm experimental evidence of their action has been forthcoming.

OBJECTS OF THE INVESTIGATION

The present study was orientated to test the possibility that mitotic activity in the oral epithelia could be affected by the alterations of physiology which are concurrent with the oestrous cycle in the female rat.

A method was sought to arrange groups of rats sacrificed at intervals over the oestrous cycle to demonstrate fluctuations in mitotic activity and relate them to the oestrous cycle.

The colchicine technique was employed to measure mitotic activity over a standard six hour period of the day in a known phase of the oestrous cycle.

Mitotic activity was measured over six hour periods in the vaginal mucosa to ascertain the effect of the oestrous cycle on mitotic activity in the vaginal mucosa of this experimental group and for comparison with other investigators.

The mitotic activity was measured in the ear epidermis to ascertain the effect of the oestrous cycle on mitotic activity on the skin of this experimental group and to compare with other investigators.

Mitotic activity was to be measured in the lingual mucosae, the buccal and palatal mucosae and the gingiva to give a comprehensive variety of histological configuration for correlation with the oestrous cycle. This was also to give a comparative pattern of mitotic activity within the tissues of the oral cavity for comparison with other investigators.

Fluctuations in mitotic activity of the individual tissues were compared directly to the mitotic activity fluctuation pattern for the vaginal mucosa to ascertain the possibility of a concurrent fluctuation.