MITOTIC ACTIVITY IN THE ORAL EPITHELIA OF THE FEMALE RAT

A Thesis Presented To

The Faculty of Graduate Studies
The University of Manitoba

In Partial Fulfillment of the
Requirements for the Degree

MASTER OF SCIENCE

William G. Young, B.D.S.

Teaching and Research Fellow
Department of Oral Pathology
August, 1965

ABSTRACT

M.Sc. THESIS

MITOTIC ACTIVITY IN THE ORAL EPITHELIA OF THE FEMALE RAT

by

William G. Young

Department of Oral Pathology
Faculty of Dentistry, University of Manitoba

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.

ACKNOWLEDGEMENTS

This thesis was prepared in the Department of Oral Pathology, Faculty of Dentistry, University of Manitoba. I wish to acknowledge the assistance given by Mrs. O. Pace in preparing the histological material and by Mrs. B. Tiller who typed the manuscript.

The work was supported by grants to Mr. A. T. Ball and Mr. M. Helper from the Canadian Dental Association and the School of Graduate Studies. The Canadian Department of National Health and Welfare also assisted by a grant to Miss R. Chappell and Miss L. Borrow. These four students provided technical assistance, in addition to helpful and humorous comment. Also, I would like specially to thank Mr. A. Domokos for his preparation of the figures used in this thesis.

Lastly, the constructive criticism, helpful guidance, and constant encouragement of Dr. J. R. Trott, as my supervisor, have combined to make this project a rewarding and educating experience.

CONTENTS

RESULTS AND OBSERVATIONS

FIGURES

VAGINAL SMEARS

HISTOLOGY OF THE OTHER EPITHELIA

HISTOLOGY OF THE GINGIVAL EPITHELIA

SEQUENTIAL VAGINAL SMEARS

VAGINAL HISTOLOGY AT SACRIFICE

VAGINAL HISTOLOGY

TREND OF THE MEAN MITOTIC ACTIVITY IN THE TISSUES

VAGINAL MITOTIC ACTIVITY - COMPARISON WITH OTHER TISSUES

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.

ANIMAL MATERIAL

The experimental animals were adult female rats of the Long-Evans strain. At the start of the experiment most of the rats weighed between 200 and 250 gms, though three rats of 187, 262 and 270 gms were also included. This range of body weight has been previously considered optimal for studies of mitotic activity related to the oestrous cycle by Bertalanffy & Lau (1963). An average gain of 7 gms of body weight per week was taken as an indication of health, but not of rapid growth, of this experimental group. In all, forty-six potentially suitable rats were kept under similar conditions of environment, in an isolated room with artificial lighting only, for 12 hours daily, 6 a.m. - 6 p.m., controlled by an electrically operated time switch. The temperature in the room was continuously recorded by means of a Taylors weekly recording thermometer. It remained fairly constant at around 73°F ± 2°F.

The rats had continuous access to Victor fox chow and water ad libidum and, in addition, received a dietary supplement of a carrot once a week, lest vitamin A deficiency affect the epithelial surfaces under study. The rats, which were used to communal living, were kept in an uncrowded manner, two to a cage, as the stress of overcrowding has been shown to affect mitotic activity (Bullough, 1952 a). Long & Evans (1922) however, have noted that there was no difference between the ovulation periods of females kept in solitary confinement for

about a month and females allowed to live together in small numbers.

VAGINAL SMEARS

A period of five days prior to commencing vaginal smears was allowed to adjust the rats to the conditions of their environment. Smears were then taken twice daily, generally at 9 a.m. and 3 p.m. The technique employed a fine moistened cotton wool swab on a small toothpick carefully inserted 1 cm into the vagina and gently rotated out. The desquamate was then smeared on slides appropriately marked with the rat's record number, the date, and the time of sampling. These were then stained with methylene blue for four minutes (A.A.F.I.P., 1960), rinsed in tape water, and examined under the microscope at a field magnification of X100.

INTERPRETATION OF SMEARS

The following phases of the oestrous cycle were identified by the fluctuations of the vaginal cell population.

Prooestrus: Here the small round intermediate squamous cells, singly or in clumps, comprised the entire smear. Their vesicular nuclei occupied one-third of their cell volume. In late prooestrus some superficial squamous cells were superadded as the vaginal mucosa matured into early oestrus. (Fig. 1).

Oestrus: This smear was almost exclusively non-nucleated superficial squames (Fig. 2), which accumulated in the vagina towards late oestrus as curd-like masses which were

readily visible to the naked eye on the smeared slide.

Metoestrus: This was characterized by two features; the appearance of polymorphonuclear leucocytes, and the reappearance of intermediate cells. These were interspersed in approximately equal proportions with persisting clumps of degenerating squames, (Fig. 3).

Dioestrus: The most marked expression of this phase was a preponderance of polymorphonuclear leucocytes, with numbers of intermediate squamous cells and nucleated squames increasing proportionally as the phase approached prooestrus (Fig. 4). The smears of dioestrus, in our group of rats, had a tendency to vary in their quantity. The complete exclusion of cornified elements from the smear, as observed by Long & Evans (1922), was not a consistent finding. This may have been due to the frequency of smearing (Mandl, 1951).

SELECTION FOR SACRIFICE

By this method of delineation, individual oestrous histories were recorded on stenciled charts for each animal, for at least seven days and in some cases for as long as four weeks. This continued until an accurate prediction could be made of the vaginal phase on the day of sacrifice or more precisely between the administration of colchicine and the time of sacrifice.

FIGURE 1

A vaginal smear of rat in the prooestrous phase. Predominantly round intermediate epithelial cells and occasional superficial squamous cells are visible. Polymorphonuclear leucocytes are absent. Orig. Mag. 80X. Stain methylene blue.

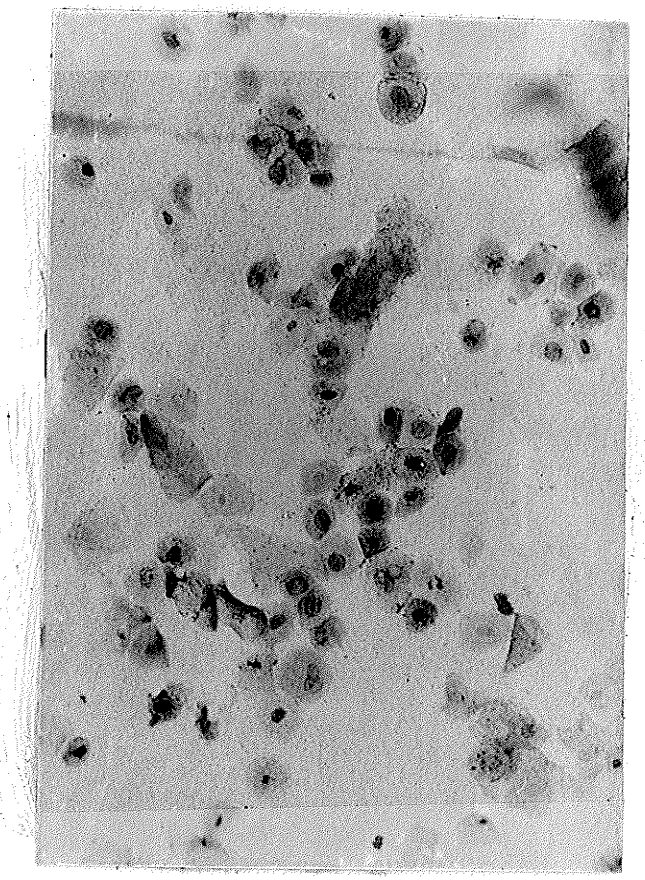


FIGURE 3

A vertical smear of tar in the osseous phase.

Non-uncleaved subcortical edematous cells only are present. Some open cytoplasm. Orig. Mag. 80X. Stain methylene blue.

FIGURE 3

A vaginal smear of rat in the metoestrous phase. Degenerating superficial squamous cells and occasional round intermediate cells are interspersed with polymorphonuclear leucocytes. Orig. Mag. 80X. Stain methylene blue.

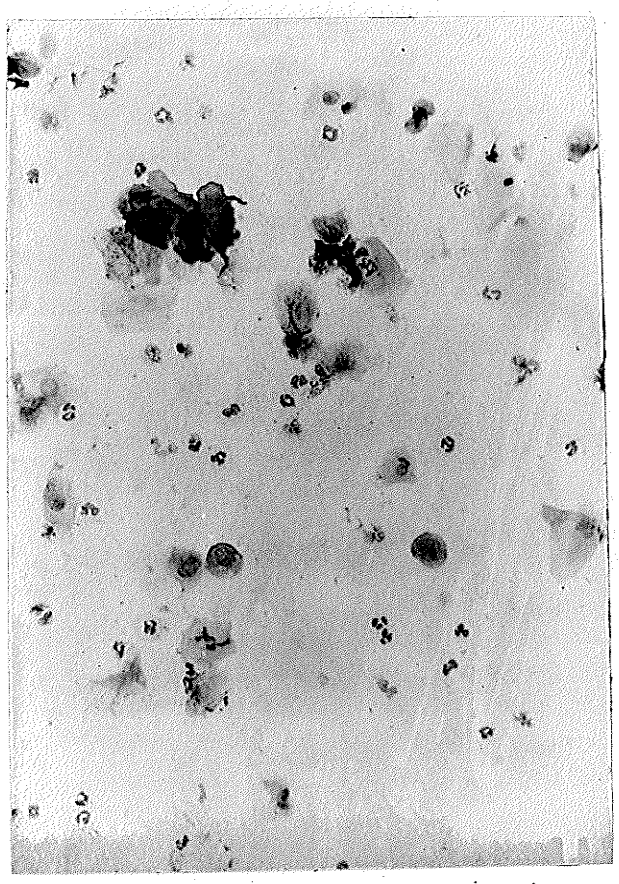
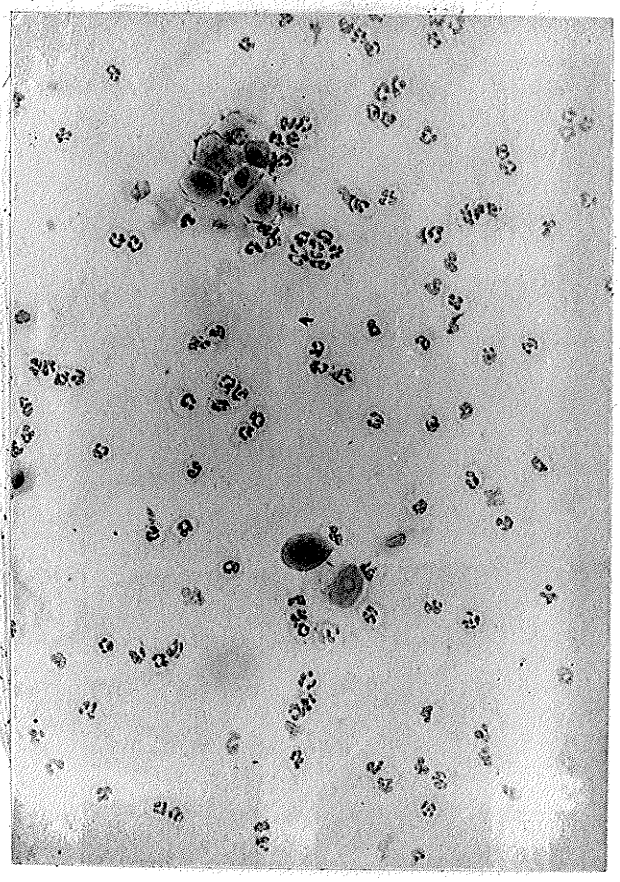


FIGURE 4

A vaginal smear of rat in the dioestrous phase. Polymorphonuclear leucocytes, some with the annular nucleus found in the rat, are present in abundance with small clumps of intermediate epithelial cells. Orig. Mag. 80X. Stain methylene blue.



From the total group of forty-six, twenty-five were selected as having regular cycles of four to five days and clear delineation of individual phases.

The twenty-five selected rats then fell into four broad groups for sacrifice as in Table I.

COLCHICINE

The preparation used for this study was U.S.P. Colchicine (Inland Alkaloid Co., Tipton, Indiana). Dosage 0.1 mg per 100 gm body weight (See Appendix). Dilution 0.1 mg per 1.0 cc distilled water.

PROCEDURE ON THE DAY OF SACRIFICE

A preliminary vaginal smear was taken and if the rat was in the forecasted phase of the oestrous cycle, the rat was then weighed and administered the appropriate dose of colchicine. Then the rat was replaced in its regular environment and not disturbed until the final vaginal smear prior to sacrifice.

The animals were subjected to the action of colchicine during the same period of the day 10.00 - 16.00 hours as far as possible consistent with handling procedures. This avoids the differences in mitotic activity which occur due to diurnal variation (Bullough, 1950; Ebling, 1954; Trott, 1963). In all cases the afternoon sleep period, circa 14.00 hours was covered by the action of the drug when maximum mitotic activity has been observed to occur in the mouse (Bullough 1950)

TABLE I

RATS AS SACRIFICED

No. of Rats	Forecast In	Rat Numbers	Wt. at Sacrifice
6	Prooestrus	A25	242 Gm
		A30	234
		B14	206
		A13	278
		A11	256
		A24	255
6	Oestrus	A18	265
		B10	240
		A1	248
		A4	251
		A9	234
		B6	239
6	First Day After Oestrus	(A19)	215
		A23	250
		B15	213
		A28	277
		(B3)	220
		A26	270
7	Second Day After Oestrus	A12	257
		B5	273
		B12	226
		B8	233
		B13	236
		A8	234
		A10	250

This table outlines the four broad groups in which the selected rats were forecast for sacrifice using the vaginal smear technique. No attempt was made at this stage to delineate more precisely the position of individual rats in the oestrous cycle. Two rats, (numbers bracketed) later proved to have 5-day cycles.

and similarly in the rat (Bertalanffy, 1960).

Precisely six hours after the administration of colchicine, the rat was killed by an overdose of ether anaesthesia.

HISTOLOGICAL PROCEDURES

The following tissue samples were dissected out immediately and fixed in acetic acid-alcohol-formalin (Lillie, 1954).

1. The entire maxilla.
2. The mucous membrane of the inner surface of the cheek (buccal mucous membrane).
3. A coronal segment of tongue (at rest) anterior to the lower first molar teeth, but not the tip.
4. The auricle of right ear.
5. A transverse section of the anterior portion of the vagina.

The mucous membranes were laid flat on moistened blotting paper prior to fixation. The maxillae were left overnight in the fixative and then transferred to formic acid-sodium citrate decalcifying solution for not less than eighteen hours. (AAFIP 1960).

A coronal segment of maxilla containing the right and left first molars with their periodontium intact, and the intervening vault of the hard palate, was chosen for examination and trimmed accordingly.

The specimens were imbedded in paraffin wax in a regular fashion together in one block. Sections were cut at 7μ to avoid counting the same mitotic figure twice, only every third section was mounted. At least twenty such sections were prepared from each block.

Haematoxylin and Eosin was selected as the stain of choice, for in addition to giving clear indication of colchicine metaphases, it permitted ready recognition of polymorphonuclear leucocytes and lymphocytes within the epithelia.

COUNTING TECHNIQUE

Counts were done with binocular microscopes at a field magnification of X400. A fine bristle was incorporated in each ocular and used to delineate fields and assist counting. The total cell counts of interphase nuclei were done for selected fields and concurrently the number of colchicine arrested mitoses were recorded on hand tally counters.

No information of the phases of the oestrous cycle was given to the observers while they were counting.

Prophase nuclei and resting basal cell nuclei were included in the total cell counts. The count of colchicine metaphases was taken as the number of mitoses. For each tissue, the ratio of the number of colchicine metaphases to the total cells counted from randomly selected areas from each of twenty different slides was determined and expressed as a percentage. Polymorphonuclear leucocytes and lymphocytes in the epithelia were excluded. This percentage was taken as representing, for the particular tissue, the mitotic activity within the six hour period prior to sacrifice.

The number of cells counted per slide in any one tissue was determined by the histology of the area under examination. Thus, in the ear epidermis (Fig. 5), 125 - 175 cells per slide were counted in random fields. Sebaceous glands, ducts and hair follicles were avoided. In the vaginal epithelium 125-175 cells per slide were counted in random fields. Care was exercised in metoestrus and dioestrus to discriminate migrating polymorphonuclear leucocytes. In the buccal mucous membrane (Fig. 6) 100 - 150 cells per slide were counted. In the tongue, (Fig. 7 & 8), the superior and inferior surfaces were recorded separately, and 100 - 200 cells per slide were counted. Counts were made between papillae in the upper surface, and in the lower surface, in random fields, always lateral to the mid-line area.

In the palate (Fig. 9) approximately 300 cells per slide were counted in the mid-line region of the vault. In the latter tissues where keratinisation and parakeratosis occurred, distinct cell nuclei only were recorded.

In the gingiva, the buccal and not the palatal gingiva was used for the estimate.

The gingival epithelium was divided into three arbitrary zones for counting, (Fig. 10).

1. The epithelial attachment
2. The downgrowing gingival epithelium
3. The outer gingival epithelium

FIGURE 5

In this section of ear epidermis of rat, three well-defined colchicine metaphases are present.

Orig. Mag. 80X. Stain H & E.

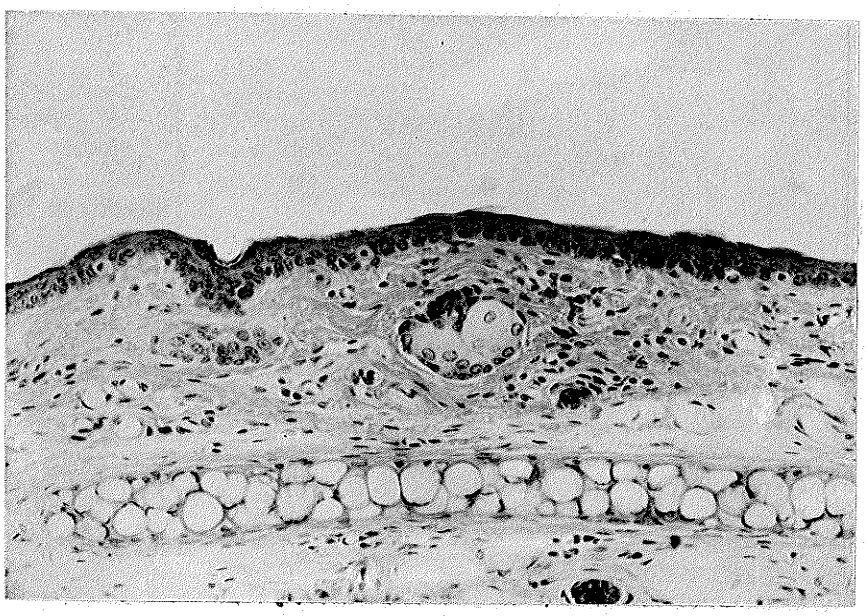


FIGURE 6

In this section of buccal mucous membrane of rat, numerous colchicine metaphases are seen in the basal layers. The total cell count would include cells in the stratum granulosum where the nuclei are discernible. Orig. Mag. 80X. Stain H & E.

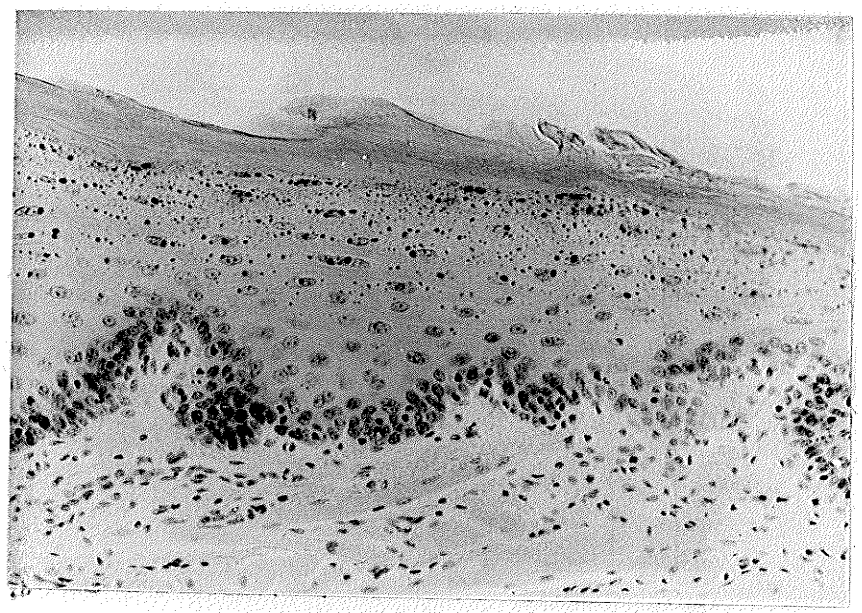


FIGURE 7

In this section of superior lingual mucosa of rat the papilliform nature of this area restricted counting to the regions between papillae. Colchicine metaphases are readily visible. Orig. Mag. 80X. Stain H & E.

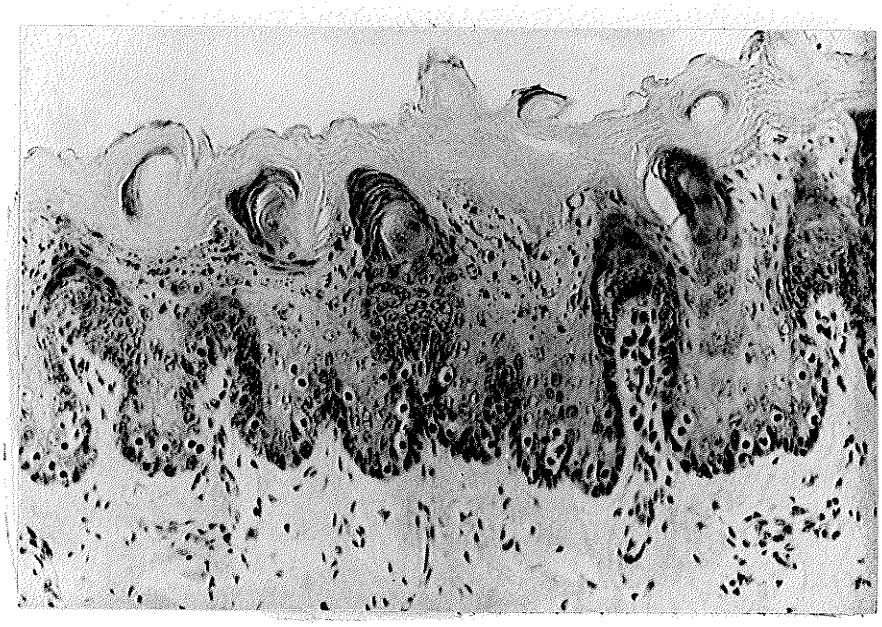


FIGURE 8

In this section of inferior lingual mucosa of rat the total cell count in such an area would be taken to the stratum granulosum. Orig. Mag. 80X. Stain H & E.

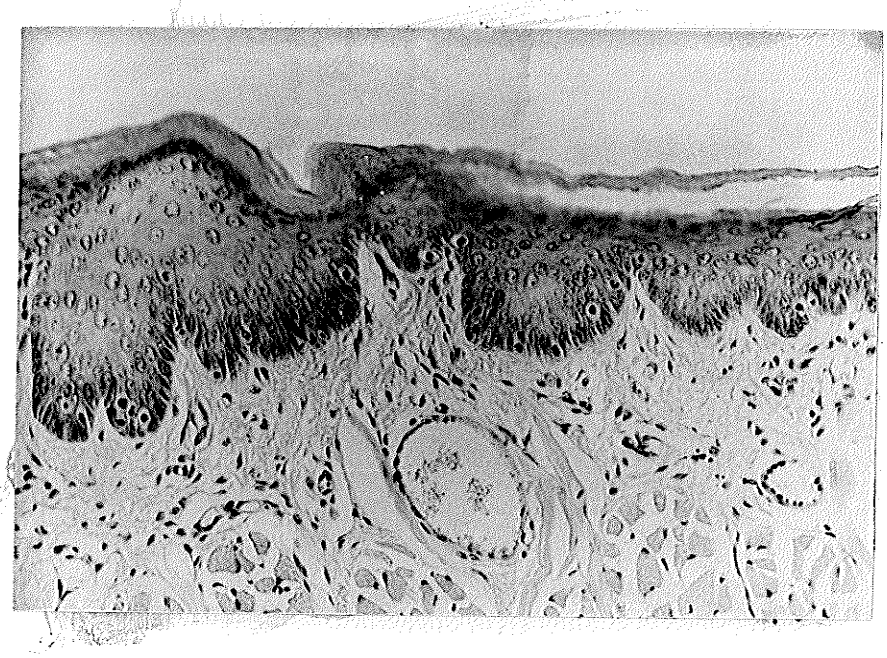


FIGURE 9

In this section of palatal mucosa of rat the rugae generally were excluded from the areas counted. Colchicine metaphases are seen in the basal layers. Orig. Mag. 80X. Stain H & E.

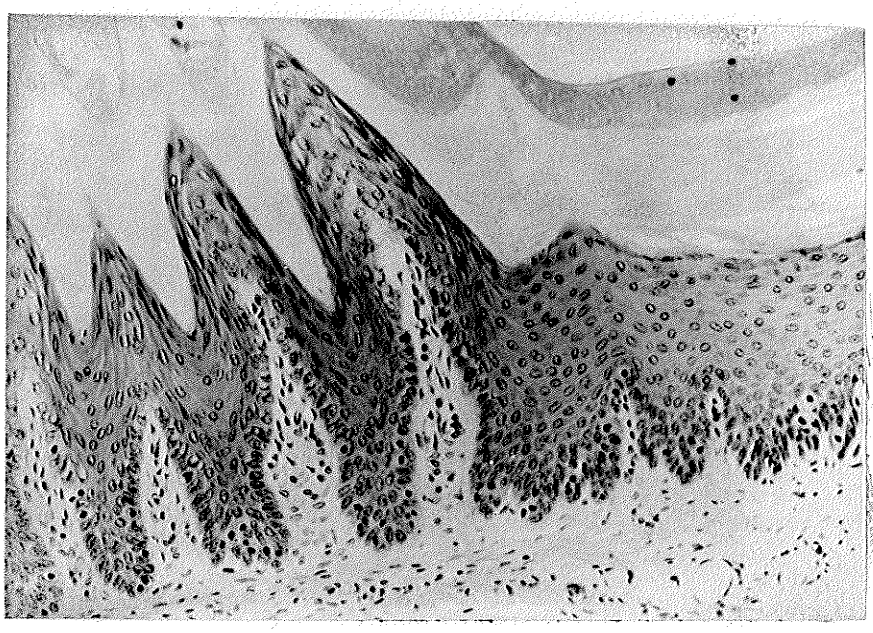


FIGURE 10

In this section of gingival epithelium of rat the epithelium was divided for purposes of counting by the lines VW and XY into the epithelial attachment E, the downgrowing gingival epithelium D, and the outer gingival epithelium O. The latter extends as far as the mucogingival junction M. Orig. Mag. 40X. Stain H & E.

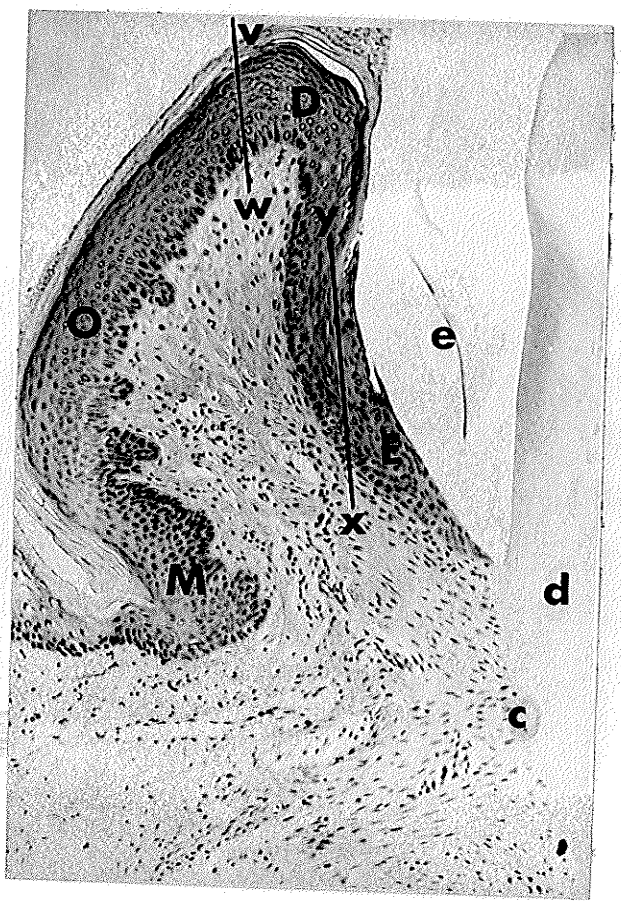
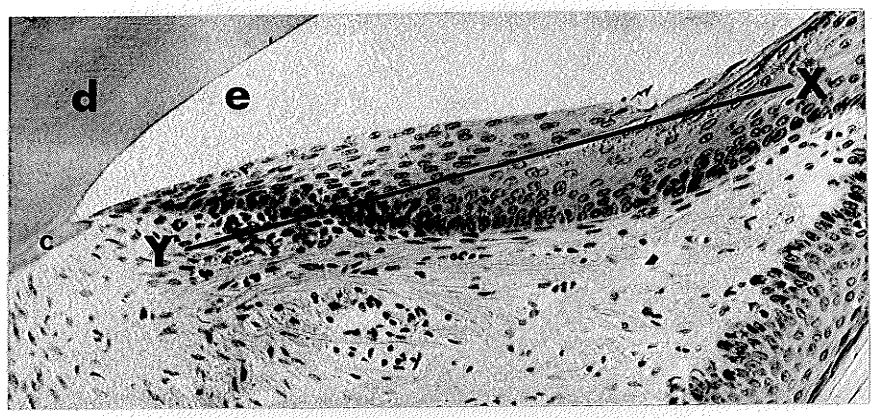

v
D
w
y
O
e
E
x
M
d
c

FIGURE 11

In this section of the gingival sulcus of rat the epithelial attachment was divided from the downgrowing gingival epithelium by the line XY drawn along the line of keratinisation of the downgrowing epithelium. The cementum c, on the dentine d, slightly overlaps the enamel space e. The epithelial attachment does not encroach on the cementum. Only this configuration was counted. Orig. Mag. 80X. Stain H & E.


d
e
X
c
Y

This zoning was basically similar to that employed by Hirt, Hartland Mühlemann (1955), and Beagrie and Skougaard (1962). The division between the epithelial attachment and the downgrowing gingival epithelium was delineated with the ocular line-marker along the line of keratinisation of the down-growing gingival epithelium (Fig. 11). The division between the down-growing and outer gingival epithelium was taken at the gingival crest, and the outer limit of the outer gingival epithelium at the muco-gingival junction.

The total cell population in the epithelial attachment area and in the down-growing gingival epithelium was counted in each slide. No slide was counted where the epithelial attachment was in contact with the tooth apical to the cemento-enamel junction. Thus, only the epithelium of the gingival sulcus, in apposition to enamel was recorded.

Approximately 300 cells per slide were counted in the outer gingival epithelium commencing at the gingival crest and counting towards the muco-gingival junction.

METHOD OF CYCLE PHASE DETERMINATION

Previous estimates of the mean length of the oestrous cycle in the rat have been made by Long and Evans (1922) as 4.6 days, by Astwood (1939) as 4.5 days, and by Mandl (1951) as 4.4 $\pm$ 0.04 days. The mode, however, of both the first and last sets of results was four days (Mandl, 1951). Ebling, (1954) found the average length of the cycle to be 4.15 $\pm$ 0.064 days.

Bertalanffy and Lau (1963) estimated the mean duration of the oestrous cycle of the Holtzman-Sprague-Dawley strain of rat to be 104.83 hours ± 4.68 hours or 4.37 ± 0.20 days. The mode being 102 hours or 4.25 days.

In a further series of rats they found the duration of the four phases of the oestrous cycle to be:

	MEAN	STANDARD DEVIATION
Prooestrus	14.91 hours	2.24 hours
Oestrus	30.67 "	2.16 "
Metoestrus	13.33 "	2.44 "
Dioestrus	48.50 "	2.54 "

The total duration of the cycle: 107.41 hours ± 4.70 hours or 4.48 days.

The 2.58 hour difference between the two estimates of the total length of the cycles was considered insignificant.

Thus we can say that the prooestrus phase lasts for at least twelve hours, oestrus for at least thirty, metoestrus for at least twelve hours, and dioestrus for at least forty-eight hours. And the relative durations of the phases can be looked on as two six-hour units of prooestrus, five six-hour units of oestrus, two six-hour units of metoestrus, and eight six-hour units of dioestrus. This gives a cycle length of 4.25 days which is between the mode and the mean of the studies of Long and Ewans (1922) and Mandl (1951) and corresponds to the mode in the first series of Bertalanffy and Lau (1963).

And it provides a suitable "standard oestrous cycle" with which two daily smears taken six hours apart with an intervening period of 18 hours, as done in this study, can be conveniently correlated.

It may be objected that the figure of 14.91 hours given above for the average duration of prooestrus is almost three hours longer than two six-hour periods. However, it was found in this series of rats that an allowance for this three hour period in the standard cycle did not materially assist the correlation of individual oestrous histories with the standard cycle. On the basis of the individual oestrous history and with special reference to the smears on the day before and the day of sacrifice, the smear phases for each rat were plotted into this standard cycle, ending their forward progression in the cycle over that six-hour period of the phase which corresponded to the six hours of colchicine administration before sacrifice (Table II).

To take a specific example in the case of rat number B12, the oestrous history indicated that on the day before sacrifice, 19/5/65, this rat had entered the second six-hour period of the metoestrous phase at the time of the first smear 9 A.M. (Fig. 12). This was the only position that correlated the previous oestrous history with the standard cycle. At 3 P.M. the same day the smear still showed the picture of metoestrus (Fig. 13). The following day, 20/5/65, at 9 A.M. the smear was that of dioestrus (Fig. 14). Colchicine was

administered and allowed to act for six hours. The smear at 3 P.M. was also that of dioestrus (Fig. 15). Confirmation of phase was obtained from the vaginal mucosa (Fig. 16) which showed the picture of dioestrus. This forward progression of phase is illustrated graphically in Table II.

It is submitted that:

1. This gives a valid sequential arrangement of the series of rats in the standard cycle.

2. The rats are placed in the cycle in an equivalent relationship one to another as regards their phase, but that

3. only an approximate interrelationship in time exists between their position in an phase, as the standard cycle length is only an average measurement, and individual cycle lengths vary.

All but two rats in the series could be plotted in this manner. The two exceptions (A19; B3) had prolonged cycles of around 5 days, and their positions were plotted on an equivalent 5 day cycle. This was derived from the 4 day cycle by an increase in phase length proportional to the length of each phase in the four day cycle. This does not accord with the suggestion of Mandl (1951) that the differences in length of the whole cycle in different animals is due to the variation in dioestrus, and that the duration of the oestrous phase is more or less constant. However, neither of the two five-day cycle rats correlated with the cycle in which the length of dioestrus only was increased, whereas the former five-day cycle permitted correlation. The validity of scaling down this five-day cycle to permit the incorporation of the

results of the two five-day cycle rats in the four-day standard cycle is thus questionable and therefore the results for the two five-day rats were included for comparison only, and were not included in the computation of mean values.

A further indication of phase position was afforded by the histology of the vaginal mucosa at sacrifice, which was found to accord with the sequential arrangement arrived at by the individual oestrous histories.

Table II illustrates the method employed to place individual rats in sequence in the standard oestrous cycle. The first two columns record the smear phases at 9 AM and 3 PM on the day prior to sacrifice and the second two columns the smear phases on the day of sacrifice. The line joining these points indicates the forward progression of the rat in the cycle shown in the fifth column. The sixth column is the rats numbered, in the sequence by six hour stages of the oestrous phases in which they fell during the six hour period prior to sacrifice. The phases of the oestrous cycle and the day groups in which they were divided for the calculation of mean values is indicated in the fifth and seventh columns. The eigth column records vaginal histological phase. The five-day cycle rats are ringed.

TABLE II: RAT SEQUENCE IN STANDARD OESTROUS CYCLE

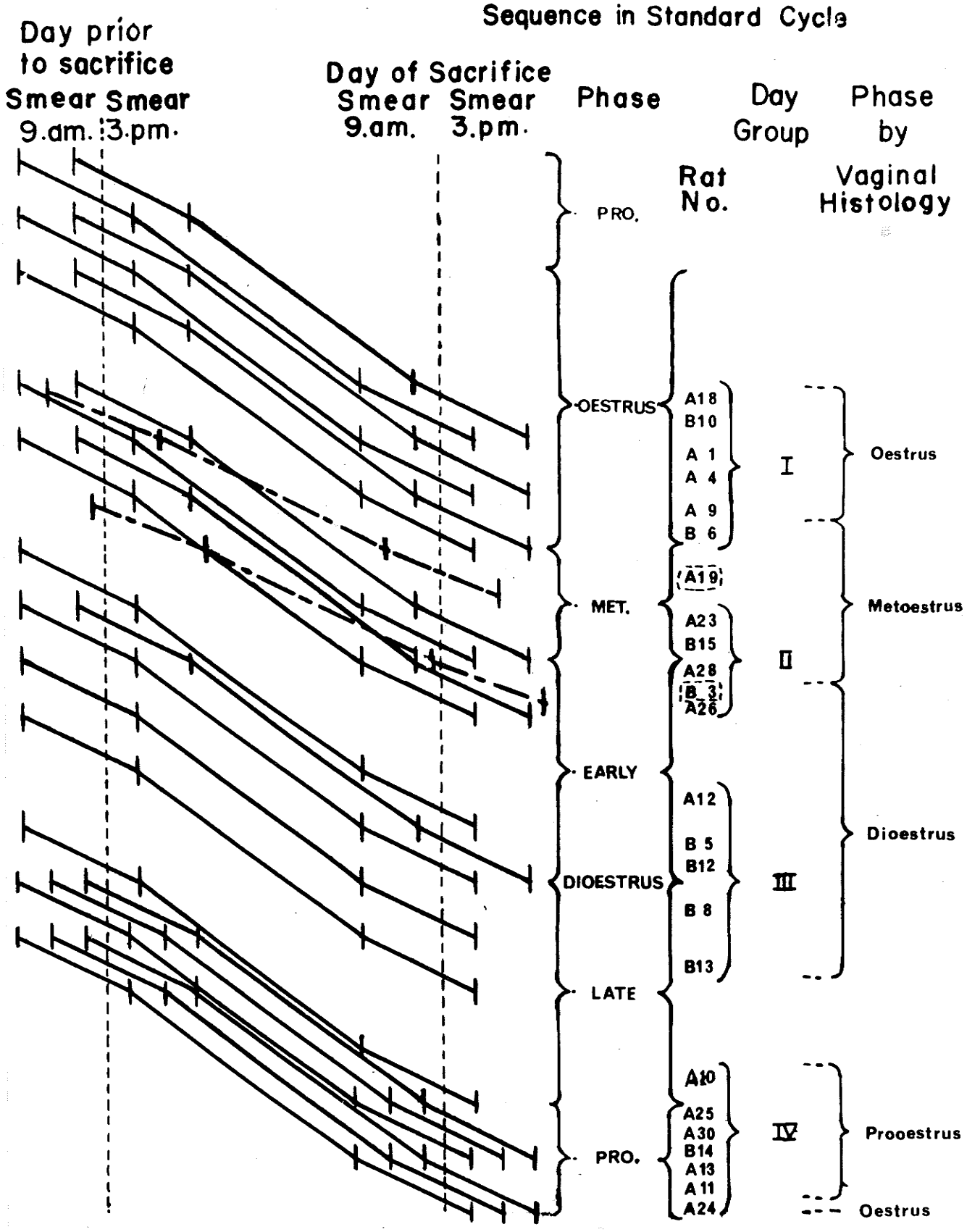


FIGURE 12

A vaginal smear of rat B12, at 9 AM the day before sacrifice, in metoestrous. The phase is indicated by the proportion of non-nucleated superficial squamous cells to polymorphonuclear leucocytes. Several intermediate cells are present. Orig. Mag. 60X. Stain methylene blue.

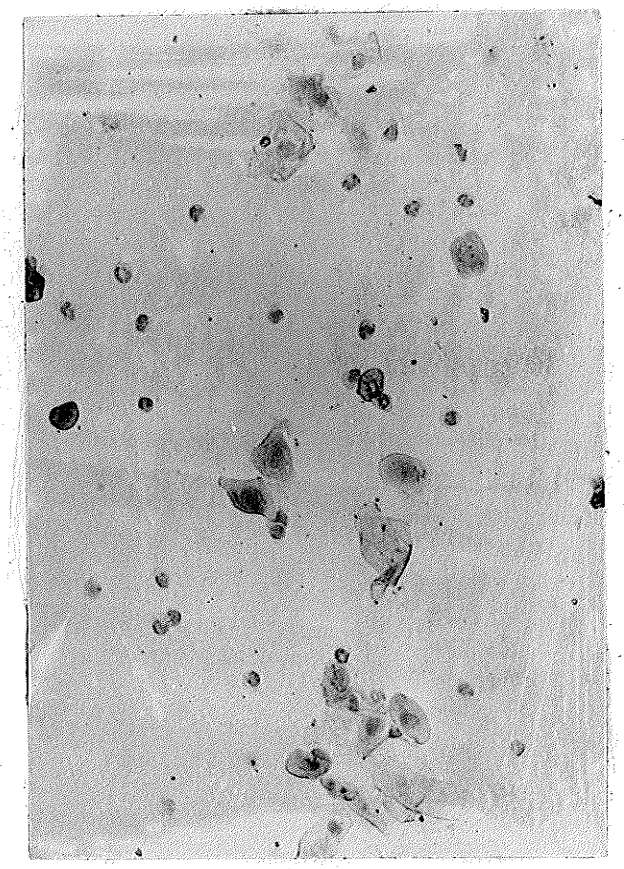


FIGURE 13

A vaginal smear of rat B12, at 3 PM the day before sacrifice, in metoestrus. The phase has continued and superficial squames are still present while the spread of polymorphonuclear leucocytes remains fairly sparse. Orig. Mag. 60X. Stain methylene blue.

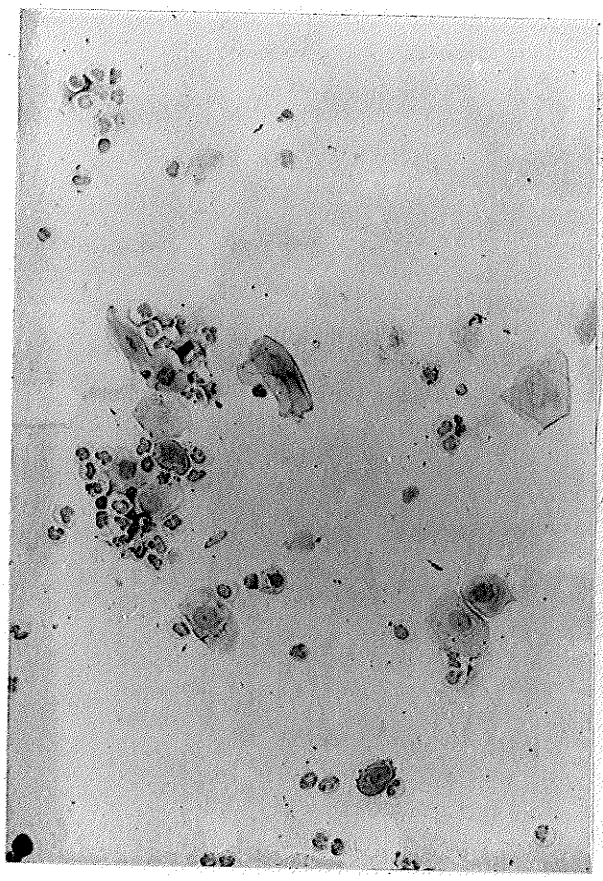


FIGURE 14

A vaginal smear of rat B12, at 9 AM the day of sacrifice, in dioestrus. The phase is indicated by the density of polymorphonuclear leucocytes and occasional intermediate cells. Non nucleated squames are absent. Orig. Mag. 60X. Stain methylene blue.

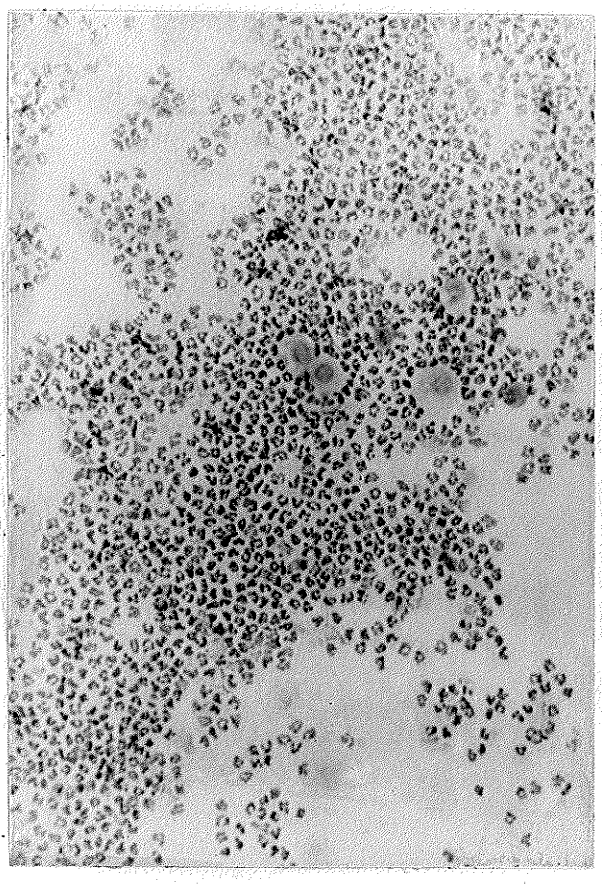


FIGURE 15

A vaginal smear of rat B12, at 3 PM the day of sacrifice in dioestrus. The phase has continued and the proportion of intermediate cells has increased while the polymorphonuclear leucocytes are still abundant. Orig. Mag. 60X. Stain methylene blue.

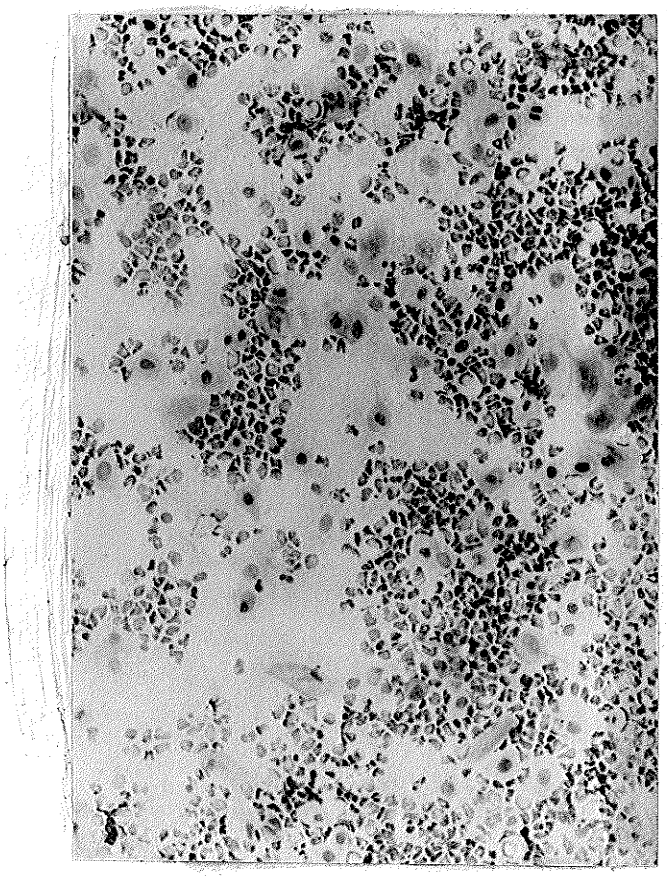
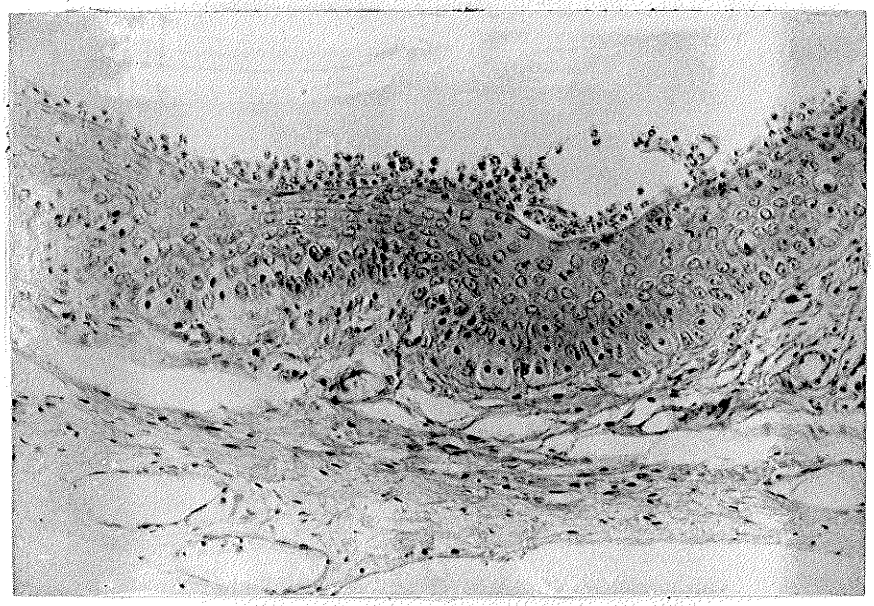


FIGURE 16

A section of vaginal mucosa of rat B12 showing dioestrous histology. The epithelium is 8 - 10 cells thick; the uppermost stratum being intermediate squamous cells. Colchicine metaphases are abundant in the basal and parabasal layers, and polymorphonuclear leucocytes, in a mucinous discharge, coat the surface. Orig. Mag. 60X. Stain H & E.



SEQUENCE OF THE EXPERIMENTAL GROUP IN THE OESTROUS CYCLE

The sequential arrangement of the animals in the standard oestrous cycle is laid out in Table II. It will be seen that the six rats forecast as being in the oestrous phase all fell into the last eight hours of the oestrous phase of the standard cycle - rats Nos. A18, B10, A1, A4, A9 and B6. The first 12 hours of the oestrous phase thus was not covered by a group of rats.

Of the six rats forecast for the first day after oestrus, two rats, Nos. A23 and B15, fell into the last six hours of metoestrus, and two into the first six hours of dioestrus (Nos. A26 and 28). The remaining two, A19 and B3, were found to have five-day cycles and were deleted from the calculations of mean values. They were, however, included in the scatter graphs for comparison only. Of the seven rats forecast as being in the second day after oestrus, all seven fell within the dioestrous phase. (Rat. Nos. A12, B5, B12, B8, B13, A8 and A10). Of the six rats forecast as being in the prooestrous phase, three, Nos. A25, A30 and B14, fell into the first six hour period and three in the second, Nos. A11, A13 and A24.

PHASING AND VAGINAL HISTOLOGY

During the prooestrous phase the vaginal epithelium was at its maximum thickness and was a fairly uniform layer of eight to twelve cells deep. The superficial layers were loosely

attached nucleated squamous cells with voluminous transparent cytoplasm (Fig. 17). As these were shed, flattened squames with pyknotic nuclei were exposed which differentiate to the keratinised and non-nucleated squames which characterise the desquamate of oestrus. Mitotic activity is noticeably scant.

In oestrus the progressive keratinisation and desquamation of several superficial layers resulted in thinning of the vaginal epithelium to five to eight cell layers. Feathery layers of translucent non-nucleated squames lay on the surface, and mitotic activity was increased from prooestrus (Fig. 18).

The shedding finally resulted in the thin denuded epithelium of the metoestrus phase reduced to four to seven cell layers, its surface composed of the intermediate cell layers (Fig. 19). Migration of polymorphonuclear leucocytes was noticeable through the epithelium and in the underlying connective tissue.

In dioestrus there was a gradual reconstitution of the epithelial thickness. Intermediate cells still occupied the superficial layers, and polymorphonuclear leucocytes were abundant in migration and on the surface (Figs. 16 and 20) for the greatest part of the dioestrous phase.

The renewing epithelium of the dioestrous phase had a certain irregularity in thickness which tended to disappear as the mucosa approached the configuration of prooestrus. In both metoestrus and dioestrus mitotic activity in the basal

and parabasal layers was obviously greater than in prooestrus.

On comparing the vaginal histology with the phase arrived at by the final smear, one rat in the last six hours of each phase showed the vaginal histological phase of the subsequent smear phase, Rats. Nos. B6, A26, A10 and A24 (Table II). The remainder of the rats had a vaginal histological configuration according with their final smear phase.

PHASING AND THE HISTOLOGY OF THE OTHER EPITHELIA

There were no cyclical changes observed in any of the other epithelia examined. The thickness of the various epithelia and the degree of their keratinisation did not appear to differ from phase to phase although no systematic measurement of these factors was carried out.

RESULTS OF COLCHICINE ADMINISTRATION

In every case but one, the drug successfully blocked mitotic activity at metaphase. Few telophase or anaphase nuclei were noticed. Nuclear fragmentation of the colchicine metaphases was minimal in the vagina, ear and all of the oral tissues with the exception of the lingual mucosae. In the tongue, the incidence of nuclear fragmentation was high. The appearance of clumps of pyknotic chromatin as opposed to the discrete oval appearance of typical colchicine metaphases probably reflect the relatively high mitotic activity of this organ. Presumably a shorter time interval between the

FIGURE 17

The vaginal mucosa of rat in the prooestrous stage. The epithelium is thick and the surface comprises loosely attached nucleated squamous cells. Two colchicine metaphases are seen in the Malpighian layer. Orig. Mag. 80X. Stain H & E.

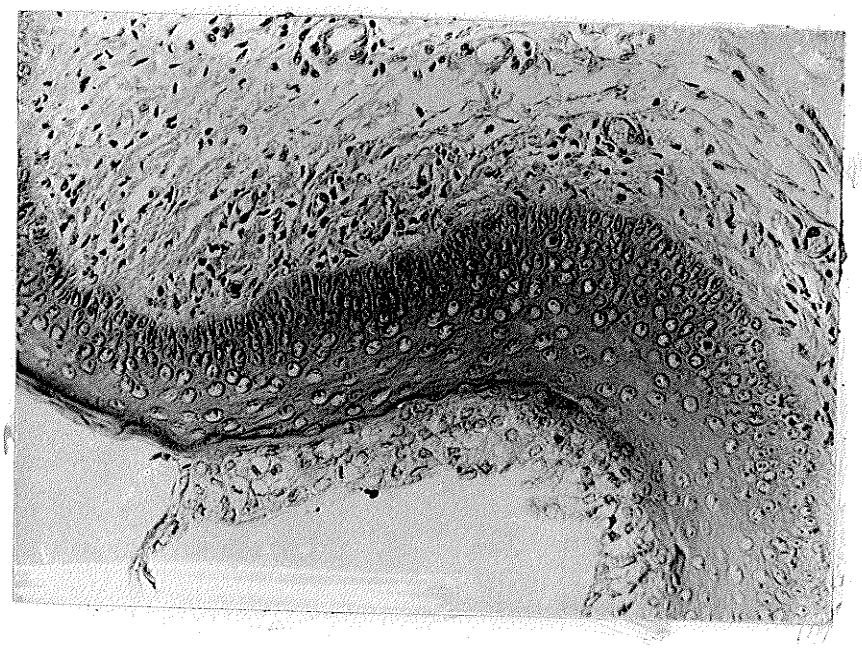


FIGURE 18

The vaginal mucosa of rat in the oestrous stage. Marked desquamation of non-nucleated squamous cells and some thinning of the epithelium are obvious features. Colchicine metaphases are numerous in the basal and parabasal cell layers. Orig. Mag. 80X. Stain H & E.

FIGURE 19

The vaginal mucosa of rat in the metoestrous stage. The epithelium is thin and the surface is formed of intermediate squamous cells. Colchicine metaphases are numerous in the basal layer.
Orig. Mag. 80X. Stain H & E.

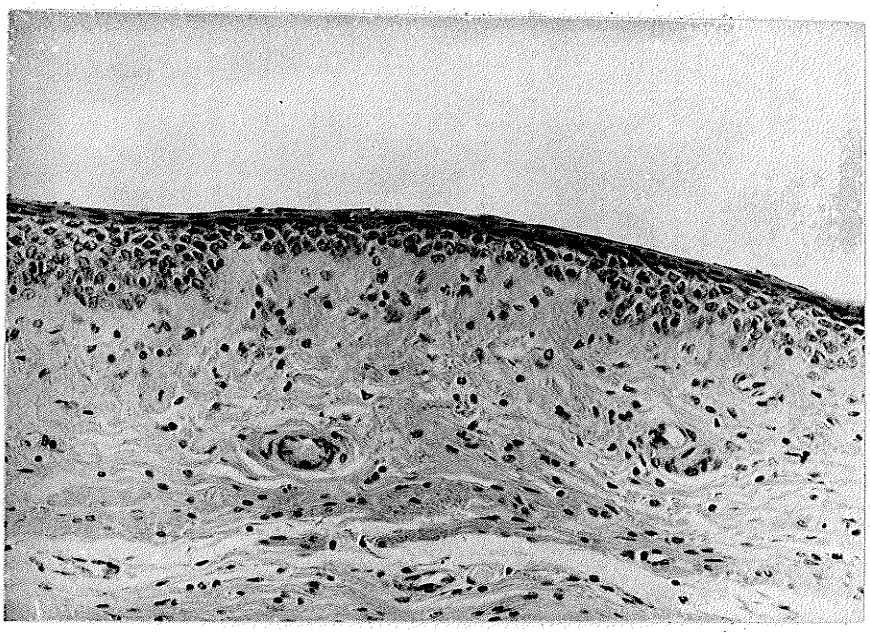
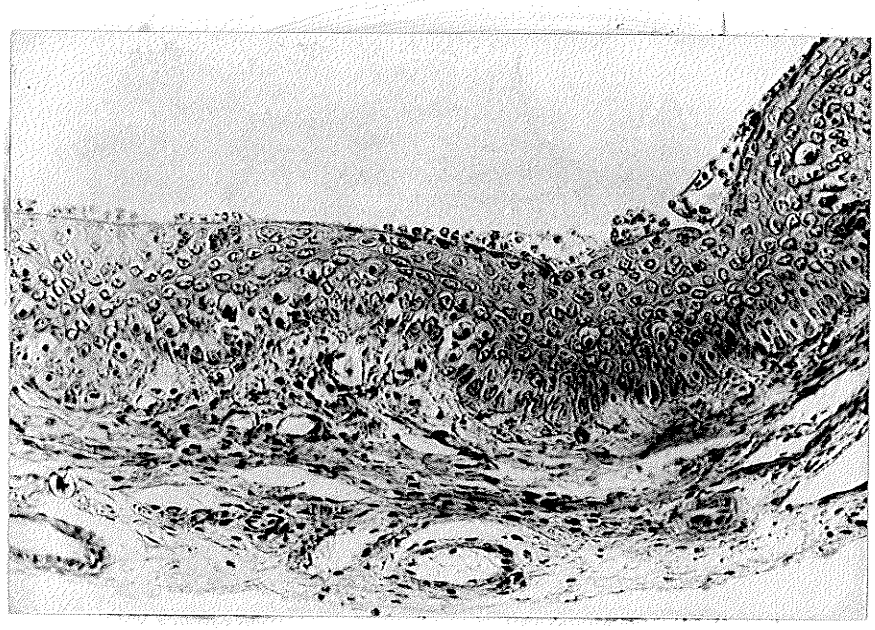


FIGURE 20

The vaginal mucosa of rat in the dioestrous stage. A layer of polymorphonuclear leucocytes is present on the surface of the epithelium which is increasing in thickness. Colchicine metaphases are abundant, but must be distinguished from migrating leucocytes within the epithelium. Orig. Mag. 80X. Stain H & E.



administration of the drug and time of sacrifice would have eliminated this feature. (See Appendix). In one animal, rat A8, arrest of mitoses in metaphase did not occur. This was accounted for by the recorded fact that difficulty in handling this particular animal had been experienced at the time of injection which resulted in an inexact dose of colchicine being administered. This animal was deleted from the experimental group.

Colchicine metaphases appeared in the other oral epithelia uniformly in the basal and parabasal cell layers in all phases of the oestrous cycle.

In the epithelium of the gingival sulcus, metaphases were uniformly distributed in the basal layer of the upper two-thirds of the downgrowing gingival epithelium. The lower third of the downgrowing gingival epithelium was frequently the site of numerous metaphases occasionally in apposition to invaginations of connective tissue. Metaphases also occurred in the basal layers of the epithelial attachment but never in the flattened stratified squamous epithelium in apposition to the enamel.

EXPLANATION OF TABULATION

The mitotic activity for each tissue in each rat was calculated as the percentage of cells undergoing mitotis during the six hour of colchicine administration (10.00 - 16.00 hours) (Tables III a and III b).

TABLE III

In this table the mitotic counts, total cell counts, and resulting percentages of mitotic activity are given for each tissue of each rat studied. The percentage mitotic activity was calculated as the percentage of cells undergoing mitosis during the six hours of colchicine administration (10.00 - 16.00 hours). The two five-day cycle rats have been bracketed to distinguish them from the remaining four-day cycle rats. The rat numbers are presented in succession from oestrus (A18) to late proestrus (A24). The gingival sulcus results are a composite result of the downgrowing gingival epithelium and the epithelial attachment.

TABLE III a

DATA OF MITOTIC ACTIVITY *
FOR VARIOUS RAT TISSUES

Rat No.	VAGINAL MUCOSA			EAR EPIDERMIS			SUPERIOR LINGUAL MUCOSA			INFERIOR LINGUAL MUCOSA			BUCCAL MUCOSA		
	Mit.	Tot.C.	%	Mit.	Tot.C.	%	Mit.	Tot.C.	%	Mit.	Tot.C.	%	Mit.	Tot.C.	%
A18	354	2,850	12.43	108	2,136	5.06	379	2,600	14.58	245	2,785	8.80	158	2,475	6.38
B10	59	3,140	1.88	94	2,985	3.15	228	3,005	7.60	315	2,955	10.66	224	2,895	7.74
A1	251	2,210	11.36	125	2,390	5.23	255	2,425	10.52	242	2,430	9.96	233	2,265	10.29
A4	272	3,085	8.81	62	2,995	2.07	301	3,040	9.90	309	3,085	10.02	188	2,990	6.29
A9	628	3,145	19.90	49	3,140	1.56	338	2,895	11.70	398	3,035	13.22	176	2,970	5.94
B6	313	3,055	10.25	217	3,080	7.05	171	3,100	5.52	154	2,960	5.20	207	3,025	6.84
(A19)	416	2,785	14.90	50	3,005	1.66	204	2,795	7.29	183	2,825	6.48	282	2,850	9.89
A23	393	2,980	13.18	63	3,145	2.00	218	3,040	7.17	236	2,945	8.01	195	2,915	6.69
B15	344	3,100	11.1	67	3,350	2.00	214	2,870	7.46	301	2,900	10.38	198	3,010	6.58
A28	344	3,230	10.65	55	3,445	1.59	308	2,945	10.46	240	2,930	8.19	225	2,885	7.80
(B3)	230	3,000	7.67	82	3,040	2.70	260	2,965	8.77	298	3,010	9.89	179	2,950	6.07
A26	213	2,745	7.76	62	2,665	2.33	254	2,785	9.12	222	2,877	7.72	233	2.705	8.61
A12	159	2,810	5.66	55	3,000	1.83	226	2,840	7.96	211	2,905	7.26	172	2,920	5.89
B5	348	3,070	11.33	75	3,095	2.42	127	3,150	4.03	102	3,135	3.25	170	3,075	5.53
B12	399	3,015	13.23	58	3,225	1.80	198	2,995	6.61	212	2,895	7.32	161	3,165	5.09
B8	533	2,895	18.41	85	2,845	2.99	214	2,840	7.54	220	3,005	7.32	146	2,905	5.03
B13	192	2,985	6.43	38	3,325	1.14	223	2,945	7.57	297	2,950	10.07	219	2,935	7.46
A10	166	2,880	5.76	127	3,035	4.18	278	2,930	9.48	218	2,830	7.70	194	2,945	6.59
A25	34	3,055	1.11	43	2,685	1.60	198	2,540	7.79	168	2,555	6.58	165	2,565	6.93
A30	126	2,900	4.34	109	2,835	3.84	234	2,580	9.07	220	2,705	8.13	105	2,485	6.04
B14	156	3,070	5.07	63	3,285	1.90	198	3,085	6.41	141	2,935	4.79	177	2,915	6.05
A13	116	2,965	3.91	92	2,950	3.12	373	2,965	12.57	330	2,885	11.42	210	2,825	7.44
A11	144	2,880	5.00	48	3,160	1.52	351	2,870	12.23	290	3,045	9.52	246	2,985	8.24
A24	186	3,025	6.15	48	3,070	1.56	194	2,830	6.86	247	2,970	8.32	249	3,005	8.29

* Expressed as the percentage of mitoses per total cells counted.

TABLE III b

Rat No.	PALATAL MUCOSA Mit.	Tot.C.	%	OUTER GINGIVAL EPITHELIUM Mit.	Tot.C.	%	DOWNGROWING GING. EPITH. Mit.	Tot.C.	%	EPITHELIAL ATTACHMENT Mit.	Tot.C.	%	GINGIVAL SULCUS Mit.	Tot.C.	%
A18	238	4,849	4.90	322	5,860	5.50	146	3,398	4.29	131	1,970	6.65	277	5,368	5.47
B10	223	4,539	4.91	354	4,629	7.64	107	2,953	3.63	41	1,402	2.93	148	4,355	3.28
A1	321	6,518	4.92	151	4,305	3.51	121	2,415	5.01	156	2,371	6.58	277	3,586	5.80
A4	325	4,364	7.45	168	4,450	3.77	130	2,959	4.39	53	1,415	3.74	183	4,374	4.07
A9	237	4,320	5.49	200	4,409	4.54	197	2,632	7.49	89	1,579	5.63	286	4,211	6.56
B6	101	4,512	2.24	101	4,257	2.37	162	2,659	6.09	47	1,528	3.07	209	4,187	4.58
(A19)	294	6,411	4.58	168	6,175	2.72	155	2,033	7.63	89	2,030	4.38	244	4,063	6.01
A23	177	4,437	3.99	151	4,540	3.32	212	3,124	6.78	62	1,720	3.61	274	4,844	5.20
B15	178	4,015	4.45	85	4,016	2.12	171	2,770	6.18	69	1,599	4.31	240	4,369	5.25
A28	230	6,011	3.83	113	6,511	1.74	143	2,282	6.26	110	1,797	6.13	253	4,079	6.20
(B3)	267	4,270	6.25	160	4,326	3.70	194	2,534	7.66	60	1,447	4.15	254	3,981	5.91
A26	245	6,387	3.83	166	7,035	2.36	101	2,364	4.27	125	1,493	8.37	226	3,857	6.32
A12	208	3,702	5.62	227	5,060	4.49	186	2,322	8.02	56	1,676	3.34	242	3,998	5.68
B5	123	4,511	2.73	92	4,486	2.05	85	2,730	3.11	37	1,538	2.40	122	4,268	2.76
B12	147	4,356	3.37	100	4,411	2.27	180	2,968	6.06	84	1,596	5.26	264	4,564	5.66
B8	98	4,298	2.28	103	4,410	2.33	157	2,775	5.66	22	1,456	1.51	179	4,231	3.59
B13	194	4,482	4.33	212	4,429	4.79	221	2,941	7.52	81	1,625	4.98	302	4,566	6.25
A10	226	5,435	4.16	186	5,888	3.16	124	1,828	6.78	73	1,594	4.58	197	3,422	5.68
A25	203	5,133	3.90	117	4,586	2.55	88	2,780	3.16	80	2,325	3.45	168	5,105	3.31
A30	327	7,254	4.51	243	6,913	3.51	149	2,274	6.55	167	2,502	6.67	316	4,776	6.61
B14	145	4,365	3.32	123	4,301	2.86	151	2,349	6.43	62	1,366	4.54	213	3,715	5.49
A13	267	4,381	6.10	193	4,360	4.43	161	2,395	6.73	48	1,485	3.23	209	3,880	4.98
A11	356	4,645	7.66	371	4,511	8.23	358	3,030	11.80	115	1,548	7.43	473	4,578	9.62
A24	217	4,541	4.78	187	4,215	4.44	244	2,712	9.01	108	1,447	7.47	352	4,159	8.24

Mit. = No. of Mitotic Figures

Tot. C. = Total No. of Cells Counted

TABLE IV

COMPARISON OF TOTAL MEAN PERCENTAGES AND STANDARD DEVIATIONS OF MITOTIC ACTIVITY IN THE SEPARATE TISSUES

TISSUES	MEAN	STANDARD DEVIATION
Superior Lingual Mucosa	9.20	2.56
Inferior Lingual Mucosa	8.75	1.96
Vaginal Mucosa	8.38	4.93
Buccal Mucosa	7.20	1.32
Downgrowing Gingival Epithelium	6.15	1.89
Epithelium of the Gingival Sulcus	5.48	1.62
Epithelial Attachment	4.81	1.92
Palatal Mucosa	4.49	1.41
Outer Gingival Epithelium	3.72	1.76
Ear Epidermis	2.85	1.49

The total mean and standard deviations of the percentage mitotic activity for all the rats in the standard cycle was calculated for each tissue separately. Standard deviations were calculated according to the formula (Bradford Hill 1956) for small numbers in groups.

The total mean and standard deviations of the percentage mitotic activity for all the rats in the standard cycle taken together was calculated for each tissue separately (Table IV).

Standard deviations were calculated throughout according to the formula for small numbers in groups, Bradford Hill (1956):

$$SD = \sqrt{\frac{\sum (x - m)^2}{n-1}}$$

Where SD is the standard deviation

x is the individual percentage

m is the mean of the sample

and n is the number in the sample

For each tissue, means and standard deviations of percentage mitotic activity at different intervals of the oestrous cycle were calculated by dividing the oestrous cycle in two fashions. Firstly, the animals were grouped by phase of the oestrous cycle (Table II):

Oestrus	A18, B10, A1, A4, A9, B6
Metoestrus	A23, B15
Early Dioestrus	A28, A26, A12, B5, B12
Late Dioestrus	B8, B13, A10
Prooestrus	A25, A30, B14, A13, A11, A24

and, secondly, by day groups beginning at the day of oestrus (Table II).

I - The day of oestrus - A18, B10, A1, A4, A9, B6

II - The first day after oestrus - A23, B15, A28, A26

III - The second day after oestrus- A12, B5, B12, B8, B13

IV - The third day after oestrus - A10, A25, A30, B14 A13, A11, A24

This grouping is shown in Table II, and it will be noted that the "oestrus" group and day group I were effectively the same group of rats. The mean and standard deviation for each of these groups were then calculated for each tissue - Tables V to XIII. A similar table was prepared for the epithelium of the gingival sulcus as a whole by averaging the combined percentages for the downgrowing gingival epithelium and the epithelial attachment (Table XIV).

MITOTIC ACTIVITY - GENERAL OBSERVATIONS

Although the highest values for percentage mitotic activity occurred in the vaginal mucosa, Rats Nos. A9, 19.9%; B8, 18.4%; and A19, 14.90% (Table IIIa) its total mean mitotic activity 8.38% (Table IV) was less than those of both lingual mucosae 9.20% and 8.75%. The total standard deviation for the vagina was the largest of all the tissues, 4.93 reflecting the high values found in oestrus, metoestrus and dioestrus and the low values in prooestrus.

The superior lingual mucosa had the highest total mean mitotic activity of the tissues studied 9.20% and was

just higher than the inferior lingual mucosa, 8.75%. Both the lingual mucosae had high total standard deviations, 2.56 and 1.96 respectively (Table IV).

Of the other oral tissues the buccal mucosa showed the highest total mean mitotic activity 7.20% and lowest standard deviation, 1.32. The total mean values for the two divisions of the epithelium of the gingival sulcus fell in as a group below this, 6.15% and 4.81%. The total mean percentage of the palatal mucosa was of the same order 4.49% and had a low standard deviation, 1.41. The outer gingival epithelium had the lowest total mean 3.72% and standard deviation of the gingivae, 1.76 (Table IV).

The ear epidermis had the lowest total mean mitotic activity of the tissues 2.85% and a moderate standard deviation, 1.49 (Table IV).

On considering if any individual rat showed consistently high or consistently low results for all its tissues, no instance of either constancy was found (Tables IIIa &b).

The results of individual percentage mitotic activity recorded for the two five-day cycle rats A19 and B3, included for comparison, were found to accord with adjacent rats in the same phase of the cycle (Tables IIIa &b).

TABLE V

MITOTIC ACTIVITY DURING OESTROUS CYCLE IN VAGINAL MUCOSA

PHASE	Oestrus	Met.	Dioestrus Early		Late		Pro.	
Day Group	I		II		III		IV	
	19.90	13.18	13.18	13.23	18.41	18.41	6.15	6.15
	12.43	11.1	11.10	11.33	13.23	6.43	5.76	5.07
	11.63		10.65	10.65	11.33	5.76	5.07	5.00
	10.25		7.76	7.76	6.43		5.00	4.34
	8.81			5.66	5.66		4.34	3.91
	1.88						3.91	1.11
							1.11	
MEAN	7.83	12.14	10.67	9.73	7.01	10.20	4.48	4.26
Standard Deviation	5.83	1.47	2.23	3.00	2.88	7.45	1.66	1.68

Data of percentage mitotic activity of individual rats in the vaginal mucosa, and the means and standard deviations by phase and day group.

TABLE VI

MITOTIC ACTIVITY DURING OESTROUS CYCLE IN EAR EPIDERMIS

PHASE	Oestrus	Met.	Dioestrus Early		Late		Pro.
Day Group	I		II		III		IV	
	7.05	2.00	2.33	2.33	2.99	4.18	4.18	3.84
	5.23	2.00	2.00	2.42	2.42	2.99	3.84	3.12
	5.06		2.00	1.83	1.83	1.14	3.12	1.90
	3.15		1.59	1.80	1.80		1.90	1.60
	2.07			1.59	1.14		1.60	1.52
	1.56						1.52	1.56
							1.56	
MEAN	4.02	2.00	1.98	1.99	2.04	2.77	2.53	2.26
Standard Deviation	2.11	0.00	0.30	0.36	0.70	1.53	1.16	0.98

Data of percentage mitotic activity of individual rats in the ear epidermis, and the means and standard deviations by phase and day group..

TABLE VII

MITOTIC ACTIVITY DURING OESTROUS CYCLE IN SUPERIOR LINGUAL MUCOSA

PHASE	Oestrus	Met.	Dioestrus Early		Late		Pro.	
Day Group	I		II		III		IV	
	14.58	7.46	10.46	10.46	7.96	9.48	12.57	12.57
	11.7	7.17	9.12	9.12	7.57	7.57	12.23	12.23
	10.52		7.46	7.96	7.54	7.54	9.48	9.07
	9.90		7.17	6.61			9.07	6.86
	7.60			4.03	6.61		6.86	7.79
	5.52				4.03		7.79	6.41
							6.41	
MEAN	9.97	7.32	8.55	7.64	6.74	8.20	9.20	9.16
Standard Deviation	3.16	0.06	1.53	2.46	1.60	1.11	2.44	2.67

Data of percentage mitotic activity of individual rats in the superior lingual mucosa, and the means and standard deviations by phase and day group.

TABLE VIII

MITOTIC ACTIVITY DURING OESTROUS CYCLE IN INFERIOR LINGUAL MUCOSA

PHASE	Oestrus	Met.	Dioestrus Early		Dioestrus Late		Pro.	
Day Group	I		II		III		IV	
	13.22	10.38	10.38	8.19	10.07	10.07	11.42	11.42
	10.66	8.01	8.19	7.72	7.32	7.70	9.52	9.52
	10.02		8.01	7.26	7.32	7.32	8.32	8.32
	9.96		7.72	7.32	7.26		8.13	8.13
	8.80			3.25	3.25		7.70	6.58
	5.20						6.58	4.79
							4.79	
MEAN	9.64	9.20	8.56	6.75	7.04	8.36	8.07	8.13
Standard Deviation	2.61	1.68	1.22	1.98	2.43	1.49	2.12	2.26

Data of percentage mitotic activity of individual rats in the inferior lingual mucosa, and the means and standard deviations by phase and day group.

TABLE IX

MITOTIC ACTIVITY DURING OESTROUS CYCLE IN BUCCAL MUCOSA

PHASE	Oestrus	Met.	Dioestrus Early		Late		Pro.	
Day Group	I		II		III		IV	
	10.29	6.69	8.61	8.61	7.46	7.46	8.29	8.29
	7.74	6.58	7.80	7.80	5.89	6.59	8.24	8.24
	6.84		6.69	5.89	5.53	5.03	7.44	7.44
	6.38		6.58	5.53	5.09		6.59	6.43
	6.29			5.09	5.03		6.43	6.04
	5.94						6.04	6.05
							6.05	
MEAN	7.25	6.64	7.42	6.58	5.80	6.36	7.01	7.08
Standard Deviation	1.62	0.08	0.96	1.53	0.99	1.23	0.97	1.05

Data of percentage mitotic activity of individual rats in the buccal mucosa, and the means and standard deviations by phase and day group.

TABLE X

MITOTIC ACTIVITY DURING OESTROUS CYCLE IN PALATAL MUCOSA

PHASE	Oestrus	Met.		Dioestrus Early		Late		Pro.
Day Group	I		II		III		IV	
	7.45	4.45	4.45	5.62	5.62	4.33	7.66	7.66
	5.49	3.99	3.99	3.83	4.33	4.16	6.10	6.10
	4.92		3.83	3.83	3.37	2.28	4.78	4.78
	4.91		3.83	3.37	2.73		4.51	4.51
	4.90			2.73	2.28		4.16	3.39
	2.24						3.39	3.32
							3.32	
MEAN	4.99	4.22	4.03	3.88	3.67	3.59	4.85	4.96
Standard Deviation	1.62	0.23	0.29	1.08	1.33	2.28	1.55	1.70

Data of percentage mitotic activity of individual rats in the palatal mucosa, and the means and standard deviations by phase and day group.

TABLE XI

MITOTIC ACTIVITY DURING OESTROUS CYCLE IN OUTER GINGIVAL EPITHELIUM

PHASE	Oestrus	Met.	Dioestrus Early		Late		Pro.	
Day Group	I		II		III		IV	
	7.64	3.32	3.32	4.49	4.79	4.79	8.23	8.23
	5.50	2.12	2.36	2.36	4.49	3.16	4.44	4.44
	4.54		2.12	2.27	2.33	2.33	4.43	4.43
	3.77		1.74	2.05	2.27		3.16	3.51
	3.51			1.74	2.05		3.51	2.86
	2.37						2.86	2.55
							2.55	
MEAN	4.56	2.72	2.39	2.58	3.19	3.43	4.17	4.37
Standard Deviation	1.84	0.85	0.82	1.23	1.30	1.14	1.98	2.06

Data of percentage mitotic activity of individual rats in the outer gingival epithelium, and the means and standard deviations by phase and day group.

TABLE XII

MITOTIC ACTIVITY DURING OESTROUS CYCLE IN DOWNGROWING GINGIVAL EPITHELIUM

PHASE	Oestrus	Met.	Dioestrus Early		Dioestrus Late			Pro.
Day Group	I		II		III		IV	
	7.49	6.78	6.78	8.02	8.02	7.52	11.80	11.80
	6.09	6.18	6.18	6.26	7.52	6.78	9.01	9.01
	5.01		6.26	6.06	6.06	5.66	6.78	6.73
	4.39		4.27	4.27	5.66		6.73	6.55
	4.29			3.11	3.11		6.55	6.43
	3.63						6.43	3.16
							3.16	
MEAN	5.15	6.48	5.87	5.54	6.07	6.65	7.21	7.28
Standard Deviation	1.43	0.42	1.10	1.90	1.91	0.94	2.64	2.88

Data of percentage mitotic activity of individual rats in the downgrowing gingival epithelium, and the means and standard deviations by phase and day group.

TABLE XIII

MITOTIC ACTIVITY DURING OESTROUS CYCLE IN EPITHELIAL ATTACHMENT

PHASE	Oestrus	Met.	Dioestrus Early		Dioestrus Late		Pro.	
Day Group	I		II		III		IV	
	6.65	4.31	8.37	8.37	5.26	4.98	7.43	7.43
	6.58	3.61	6.13	6.13	4.98	4.58	7.47	7.47
	5.63		4.13	5.26	3.34	1.51	6.67	6.67
	3.74		3.61	3.34	2.40		4.58	4.54
	3.07			2.40	1.51		4.54	3.45
	2.93						3.45	3.23
							3.23	
MEAN	4.77	3.96	5.61	5.10	3.50	3.69	5.34	5.47
Standard Deviation	1.73	0.49	2.11	2.32	1.64	1.90	1.82	1.96

Data of percentage mitotic activity of individual rats in the epithelial attachment, and the means and standard deviations by phase and day group.

TABLE XIV

MITOTIC ACTIVITY DURING OESTROUS CYCLE IN THE EPITHELIUM OF THE GINGIVAL SULCUS

PHASE	Oestrus	Met.		Dioestrus Early		Late		Pro.
Day Group	I		II		III		IV	
	6.56	5.25	6.32	6.25	6.25	6.25	9.62	9.62
	5.80	5.20	6.20	6.20	5.68	5.68	8.24	8.24
	5.47		5.25	5.68	5.66	3.59	6.61	6.61
	4.58		5.20	5.66	3.59		5.68	5.49
	4.07			2.76	2.76		5.49	4.98
	3.28						4.98	3.31
							3.31	
MEAN	4.96	5.23	5.59	5.31	4.79	5.17	6.28	6.38
Standard Deviation	1.21	0.04	0.63	1.45	1.52	1.40	2.10	2.26

Data of the percentage of mitotic activity of individual rats in the epithelium of the gingival sulcus, and the means and standard deviations in the phase and day group. The percentages expressed here are the averages of combined values for the downgrowing gingival epithelium and the epithelial attachment.

MITOTIC ACTIVITY IN THE VAGINAL MUCOSA

The stratified squamous epithelium of the vaginal mucosa showed considerable variation of individual values of percentage mitotic activity (Table V) over the period of colchicine administration. Within the oestrous phase the largest range of 1.8 to 19.9 percent was recorded.

In early and late dioestrus the ranges 13.23% - 5.66%, 18.41% - 5.76% and standard deviations 3.0 and 7.45 were large in comparison with the other tissues studied but in metoestrus and prooestrus the standard deviations were relatively small 1.47 and 1.68.

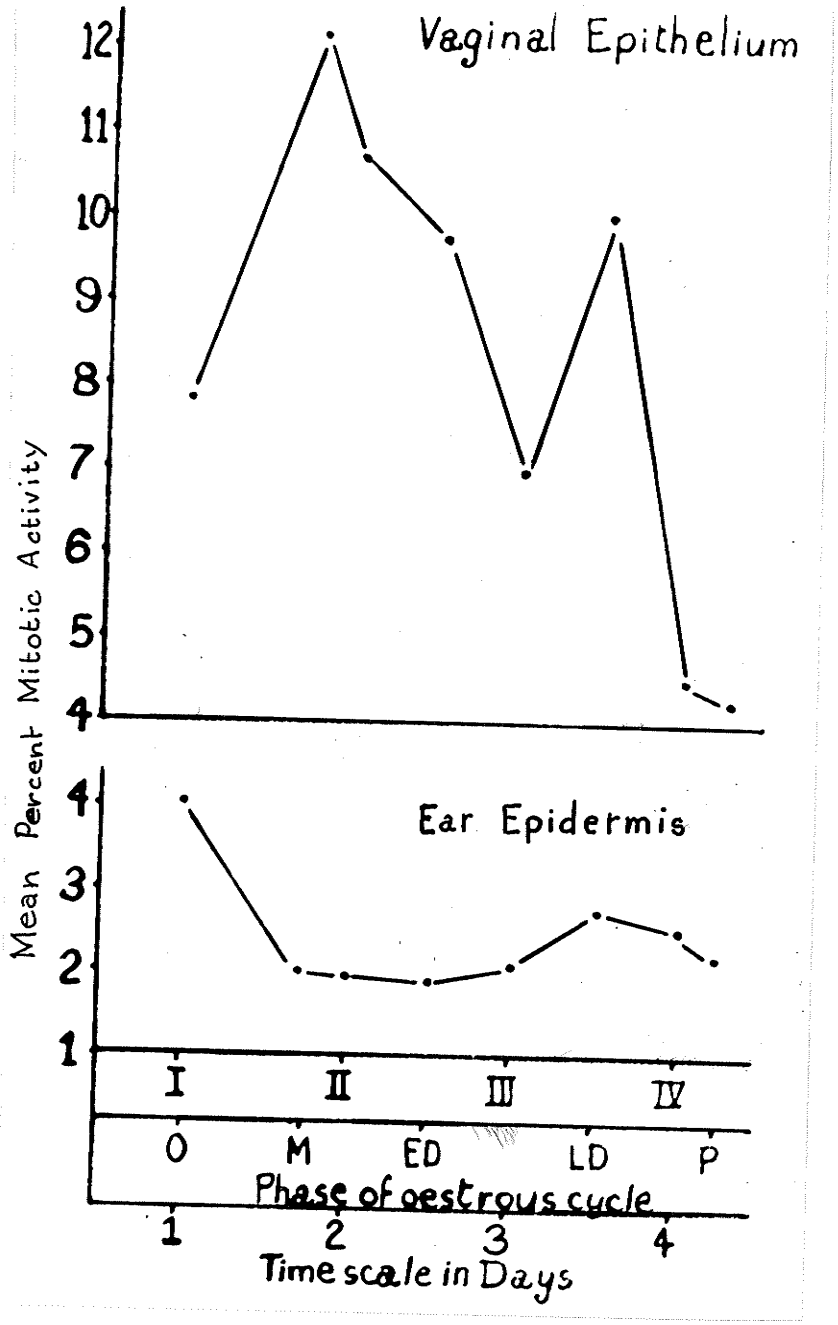
The trend of the mean values for phase and day group over the oestrous cycle (Fig. 21) commenced at an intermediate value in oestrus on the first day, 7.83%, rose to a peak in metoestrus 12.14%, fell gradually on the second day to 10.67%, in early dioestrus to 9.73% and on the third day to 7.01%, rising again in late dioestrus to 10.20%, before falling to low values on the fourth day 4.48% and in proestrus 4.26%. Thus, it may be said that even con sidering the high standard deviations of the phases and day groups the mitotic activity in the vaginal mucosa showed a marked fluctuation over the oestrous cycle.

MITOTIC ACTIVITY IN THE EAR EPIDERMIS

In contrast to the vagina, the range of the individual percentages for this thin epidermis kept within small limits

FIGURE 21

This graph gives the trend of the means of mitotic activity in the phases of the oestrous cycle and the day groups of the vaginal mucosa and the ear epidermis as calculated in Tables V and VI.


Vaginal Epithelium
12
11
10
9
8
7
6
5
4
Mean Percent Mitotic Activity
Ear Epidermis
4
3
2
1
I
II
III
IV
O
M
ED
LD
P
Phase of oestrous cycle
1
2
3
4
Time scale in Days

except in the oestrous phase. In the oestrous phase three higher values were recorded 7.05%, 5.23%, 5.06% and the standard deviation increased to 2.11 (Table VI). The trend of the means (Fig. 21) showed a definite peak in oestrus but owing to the values of the standard deviation little significance can be attached to this variation.

MITOTIC ACTIVITY IN THE SUPERIOR LINGUAL MUCOSA

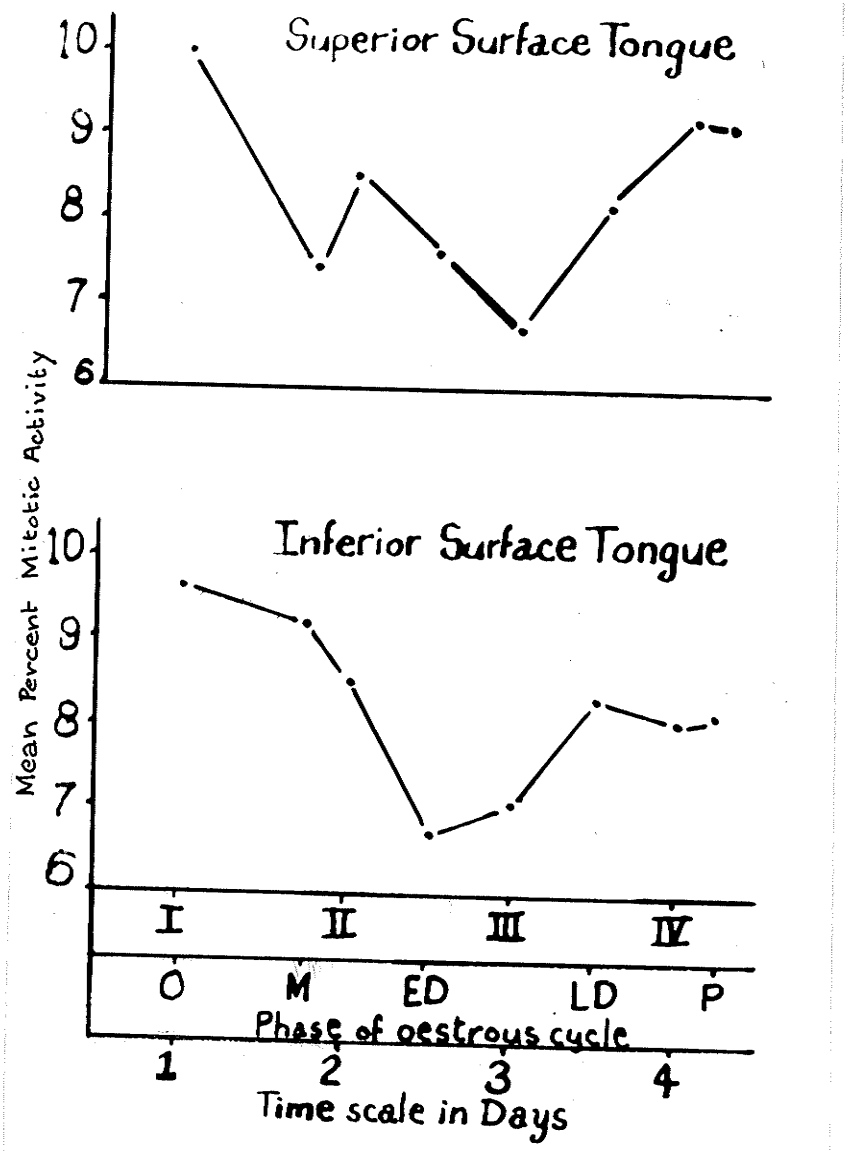
The range of individual percentages of mitotic activity over the whole cycle and within all the groups of the cycle was considerable in this tissue (Table VII). The standard deviation values were high in consequence 2.56 (Table IV). The trend of the means (Fig. 22), showed peaks at oestrus 9.97% and proestrus 9.16% with lows at metoestrus 7.32% and in the third day group 6.74%. Owing to the high values of the standard deviations no relationship of the mitotic activity of this epithelium to the oestrous cycle could be deduced.

MITOTIC ACTIVITY IN THE INFERIOR LINGUAL MUCOSA

As in the superior lingual mucosa, the range of individual percentages of mitotic activity varied noticeably (Table VIII). They were generally lower than the values for the superior lingual mucosa and the standard deviations were not so marked, 1.96 (Table IV). The trend of the means, Fig. 22, showed a slight dip in early dioestrous 6.75% but this was not considered sufficient to denote a definite fluctuation in relation to the oestrous cycle, because of the

FIGURE 22

This graph gives the trend of the means of mitotic activity in the phases of the oestrous cycle and the day groups of the superior and inferior lingual mucosa as calculated in Tables VII and VIII.


Superior Surface Tongue
10
9
8
7
6
Inferior Surface Tongue
10
9
8
7
6
Mean Percent Mitotic Activity
I
II
III
IV
O
M
ED
LD
P
Phase of oestrous cycle
1
2
3
4
Time scale in Days

high standard deviations of the phases.

MITOTIC ACTIVITY IN THE BUCCAL MUCOUS MEMBRANE

The range of variation in individual percentages for this tissue was small (Table IX). The standard deviation was low 1.32 (Table IV) and the trend of the means, Fig. 23 fluctuated only slightly. The buccal mucosal mitotic activity thus appeared to be relatively stable during the oestrous cycle.

MITOTIC ACTIVITY IN THE PALATAL MUCOSA

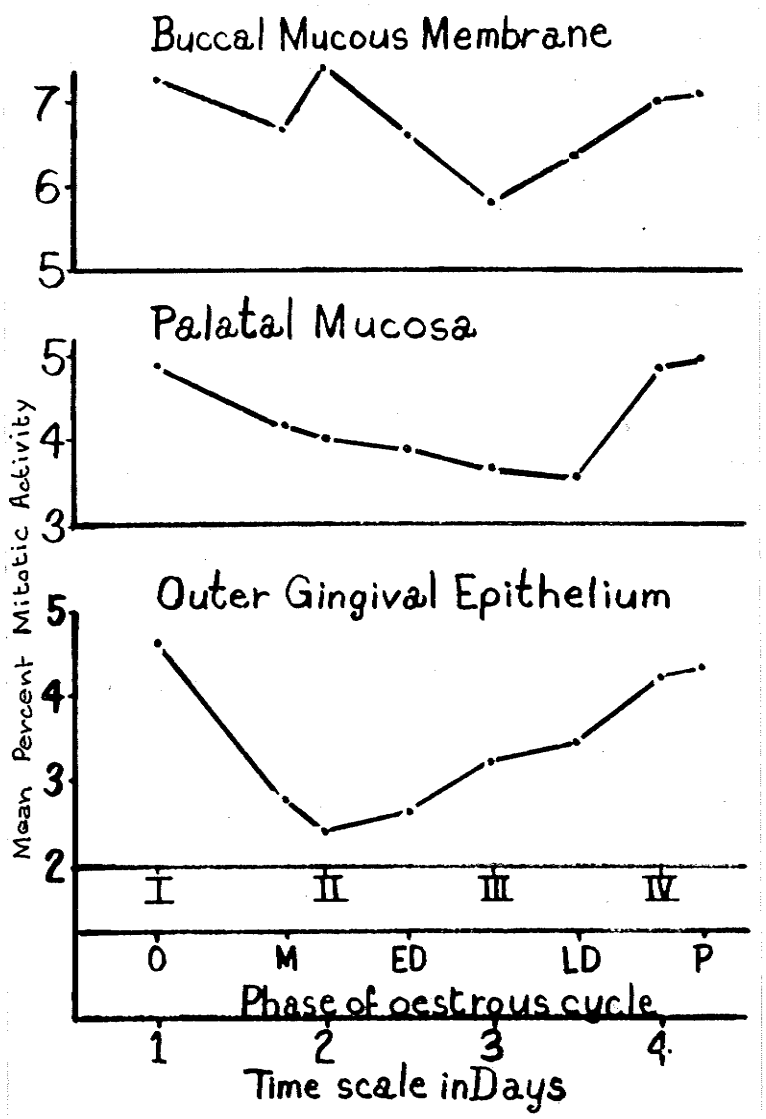
In this tissue the ranges of individual percentage mitotic activity grouped by phase and day group were larger in oestrus 7.45% - 2.24% and proestrus 7.66% - 3.32% than in the other divisions of the cycle (Table X). The standard deviations per phase were moderate or low. The trend of the means (Fig. 23) showed a shallow dip over dioestrus 3.59% but to all intents and purposes the mitotic activity of the palatal mucosa was stable over the oestrous cycle.

MITOTIC ACTIVITY IN THE OUTER GINGIVAL EPITHELIUM

The range of individual percentages was greater in oestrus 7.64% - 2.37%, and proestrus 8.23% - 2.55%, and higher values were recorded in these phases. In metoestrus and group II the mean values were low 2.72% and 2.39% as were the standard deviations 0.85 and 0.82 (Table XI).

FIGURE 23

This graph gives the trend of the means of mitotic activity in the phases of the oestrous cycle and the day groups of the palatal, buccal mucosa and outer gingival epithelium as calculated in Tables IX, X and XI.


Buccal Mucous Membrane
7
6
5
Palatal Mucosa
5
4
3
Outer Gingival Epithelium
5
4
3
2
Mean Percent Mitotic Activity
I
II
III
IV
O
M
ED
LD
P
Phase of oestrous cycle
1
2
3
4
Time scale in Days

A definite dip in the trend of means (Fig. 23) at metoestrus and group II would seem to indicate that the outer gingival epithelium does vary slightly with the oestrous cycle but this cannot be stated catagorically due to the relatively high values for the standard deviation in oestrus 1.84 and prooestrus 2.06 (Table XI).

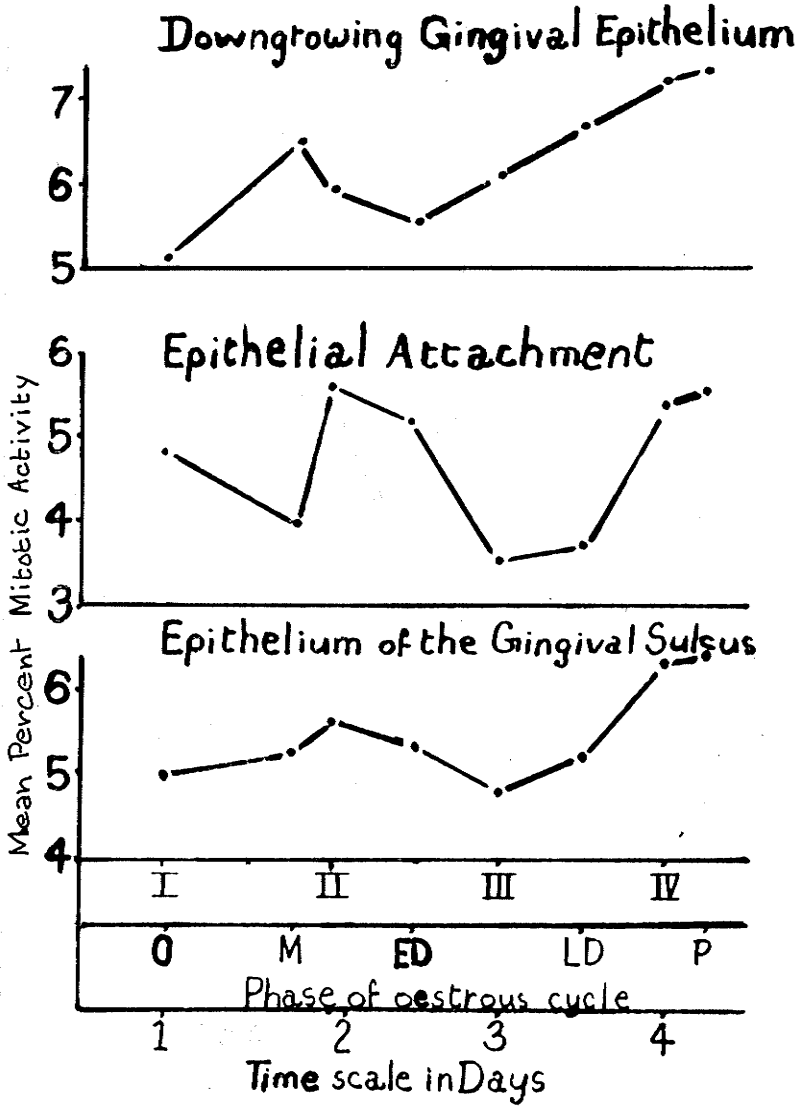
MITOTIC ACTIVITY IN THE EPITHELIUM OF THE GINGIVAL SULCUS

Mitotic activity in the downgrowing gingival epithelium: The range of the individual percentages of mitotic activity showed no more than a fair variation in this area and the standard deviations were moderate except in prooestrus 2.88 (Table XII). The percentages throughout were generally higher than the values for the corresponding epithelial attachment areas. The trend of the means (Fig. 24) showed a tendency to rise gradually from oestrus 5.15% toward prooestrus 7.28% but no significant fluctuation related to the oestrous cycle could be deduced.

Mitotic activity in the epithelial attachment: As in the case of the downgrowing gingival epithelium within the phases and day groups the range of percentages of mitotic activity was fair and standard deviations were small (Table XIII). The trend of the means showed a fluctuation which roughly reciprocated that of the downgrowing gingival epithelium in such a way that high values in one correlated with low values

FIGURE 24

This graph gives the trend of the means of mitotic activity in the phases of the oestrous cycle and the day groups of the components of the epithelium of the gingival sulcus as calculated in Tables XII, XIII and XIV.


Downgrowing Gingival Epithelium
7
6
5
Epithelial Attachment
6
5
4
3
Epithelium of the Gingival Sulcus
6
5
4
Mean Percent Mitotic Activity
I
II
III
IV
O
M
ED
LD
P
Phase of oestrous cycle
1
2
3
4
Time scale in Days

in the other with the exception of the prooestrous phase (Fig. 24). Accordingly, when the two sets of values were compounded in the values for the epithelium of the gingival sulcus, the trend of the means (Fig. 24) approached a straight line with a slight elevation at prooestrus.

The standard deviations of this combination were lower than the two tissues taken singly in all the phases and day groups except prooestrus (Table XIV).

It may be concluded that the component parts of the epithelium of the gingival sulcus respond little as a unit to the oestrous cycle.

COMPARISON OF FLUCTUATIONS IN MITOTIC ACTIVITY

In order to compare directly the slight fluctuations in mitotic activity of the oral epithelia and of the ear epidermis with the marked fluctuation observed in the vaginal epithelium over the oestrous cycle, scatter-graphs for each tissue were prepared comparing them with vaginal epithelium (Fig. 25 - 31). These show the individual values of percentage mitotic activity and the trend of their means. An indication of fluctuation over the oestrous cycle was given by a smooth curve fitted by eye relative to the trend of the means.

These served to emphasize that:

1. The amplitude of the fluctuations of mitotic activity in the oral epithelia and ear epidermis were small in comparison with that of the vaginal mucosa.

2. In all the tissues studied, with the exception of the vagina, the direction of fluctuation tended to lower values in metoestrus and dioestrus than in oestrus and prooestrus although no conclusive significance can be attached to this when one considers the values of standard deviations already discussed. This was particularly well seen in the case of the outer gingival epithelium.

3. No striking correlations between the mitotic activity of the vagina and the epithelia of the oral cavity or the ear were discernible.

Thus, from a consideration of the data by the methods so far described, it would appear that at most the mitotic activity in the oral cavity is only slightly and not significantly affected by the oestrous cycle. Accordingly, it was not considered worthwhile to pursue a more complex statistical analysis of the data.

FIGURE 25

Individual mean percentages of mitotic activity are plotted for the two tissue for the individual rats in sequence in the standard oestrous cycle as delineated in Table II. The straight lines representing the trend of the means in the oestrous phases and day groups (Fig. 21) for each tissue are superimposed. The smooth curve, fitted by eye to the trend of the means, indicates the fluctuation in mitotic activity in the individual tissues over the oestrous cycle. The amplitude of the fluctuation in mitotic activity of the ear epidermis compared with that of the vagina is small.

The four reversed symbols indicate values for the two rats with 5 day cycles.

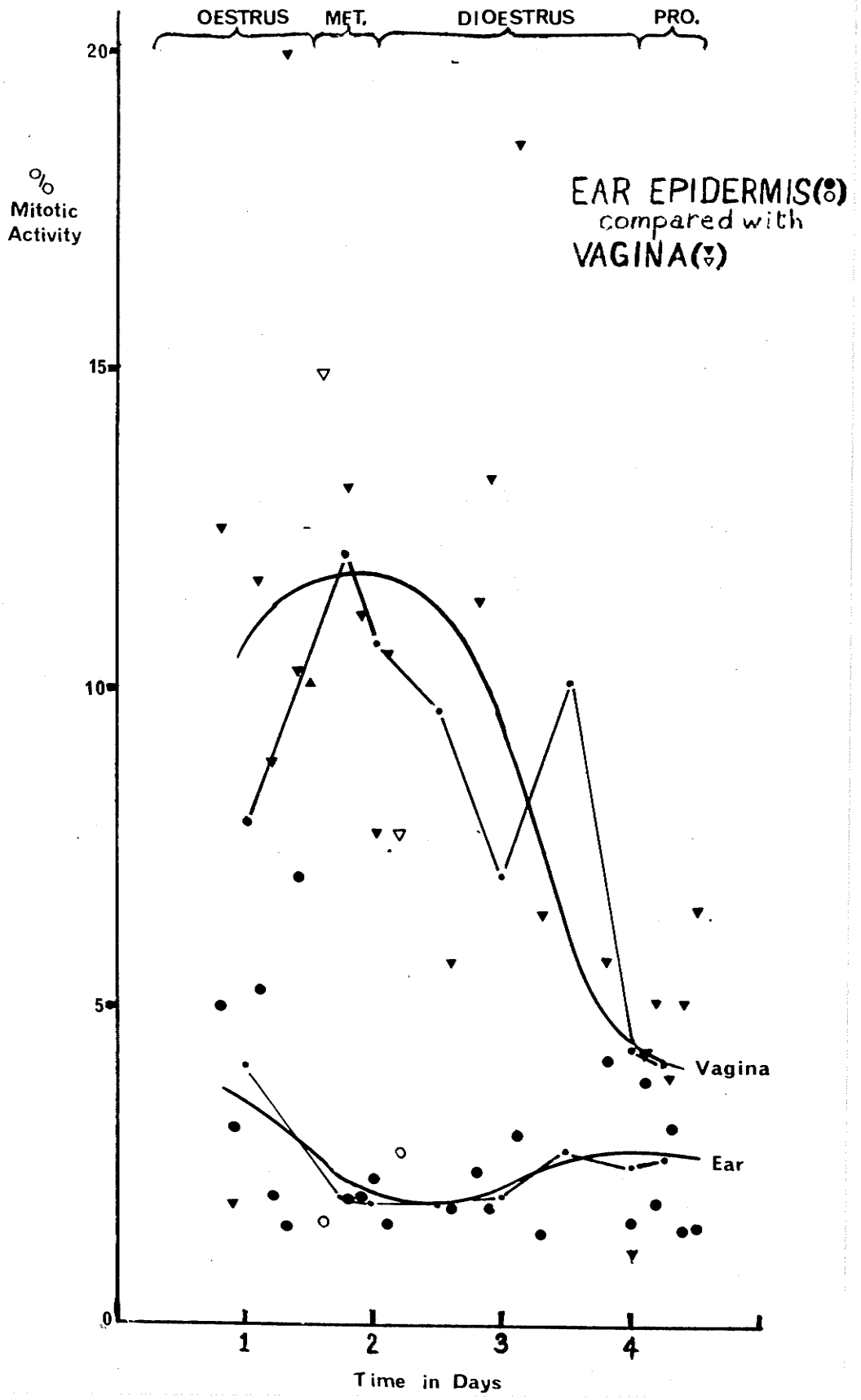

OESTRUS
MET.
DIOESTRUS
PRO.
20
15
10
5
0
% Mitotic Activity
EAR EPIDERMIS(●)
compared with
VAGINA(▼▽)
Vagina
Ear
1
2
3
4
Time in Days

FIGURE 26

Individual mean percentages of mitotic activity are plotted for the two tissues for the individual rats in sequence in the standard oestrous cycle as delineated in Table II. The straight lines representing the trends of the means in the oestrous phases and day groups (Figs. 21 and 22) for each tissue are superimposed. The smooth curve, fitted by eye to the trend of the means, indicates the fluctuation in mitotic activity in the individual tissues over the oestrous cycle. The amplitude of the fluctuation in mitotic activity in superior lingual mucosa is not as marked as in the vagina. An apparent downward fluctuation is present in dioestrus.

The four reversed symbols indicate values for the two rats with 5 day cycles.

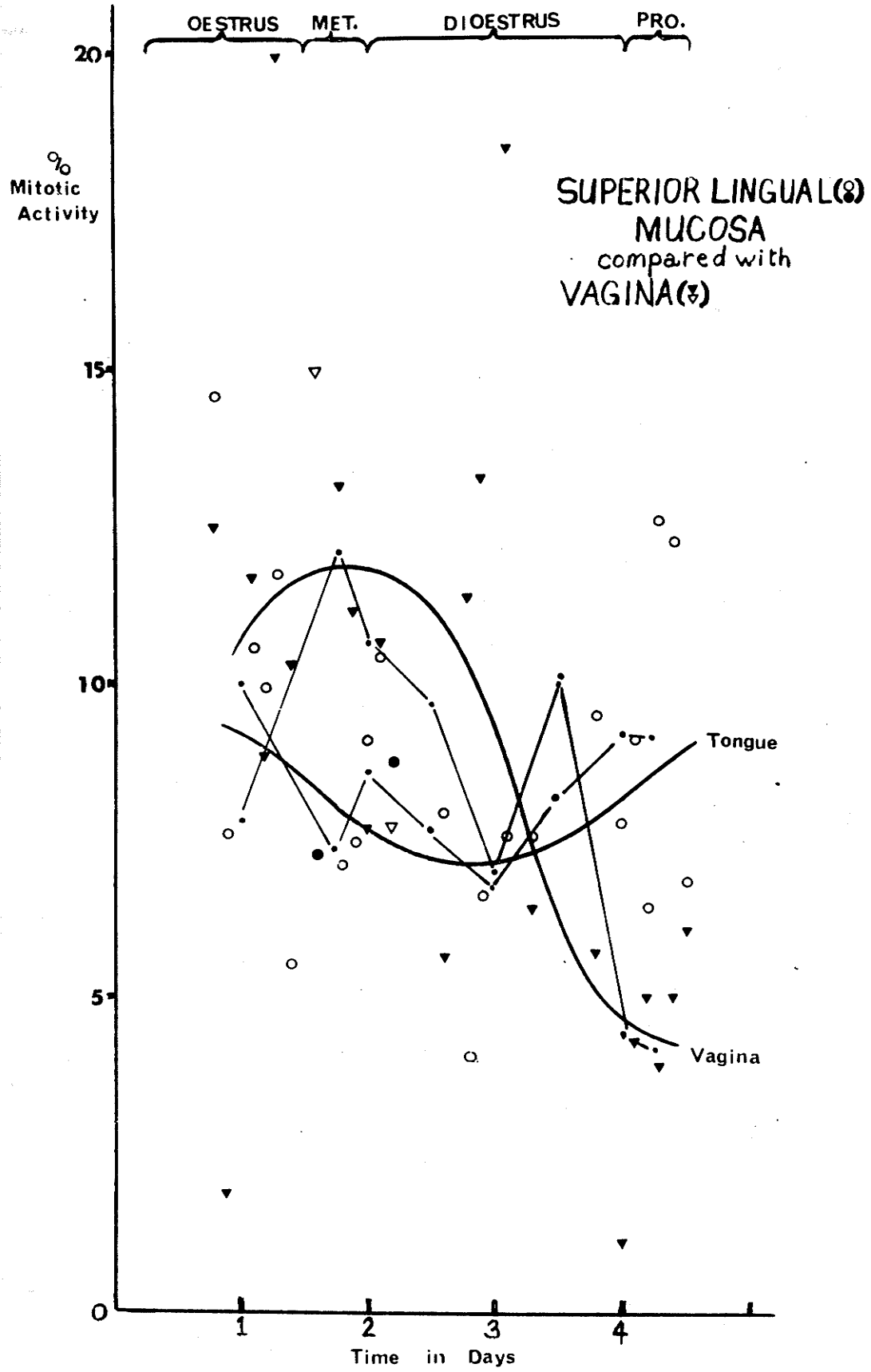

OESTRUS
MET.
DIOESTRUS
PRO.
20
15
10
5
0
% Mitotic Activity
SUPERIOR LINGUAL(♂) MUCOSA compared with VAGINA(♀)
Tongue
Vagina
1
2
3
4
Time in Days

FIGURE 27

Individual mean percentages of mitotic activity are plotted for the two tissues for the individual rats in sequence in the standard oestrous cycle as delineated in Table II. The straight lines representing the trends of the means in the oestrous phases and day groups (Figs. 21 and 22) for each tissue are superimposed. The smooth curve, fitted by eye to the trend of the means, indicates the fluctuation in mitotic activity in the individual tissues over the oestrous cycle. The amplitude of the fluctuation in mitotic activity in inferior lingual mucosa is not as marked as in vagina. An apparent downward fluctuation is present in early dioestrus.

The four reversed symbols indicate values for the two rats with 5 day cycles.

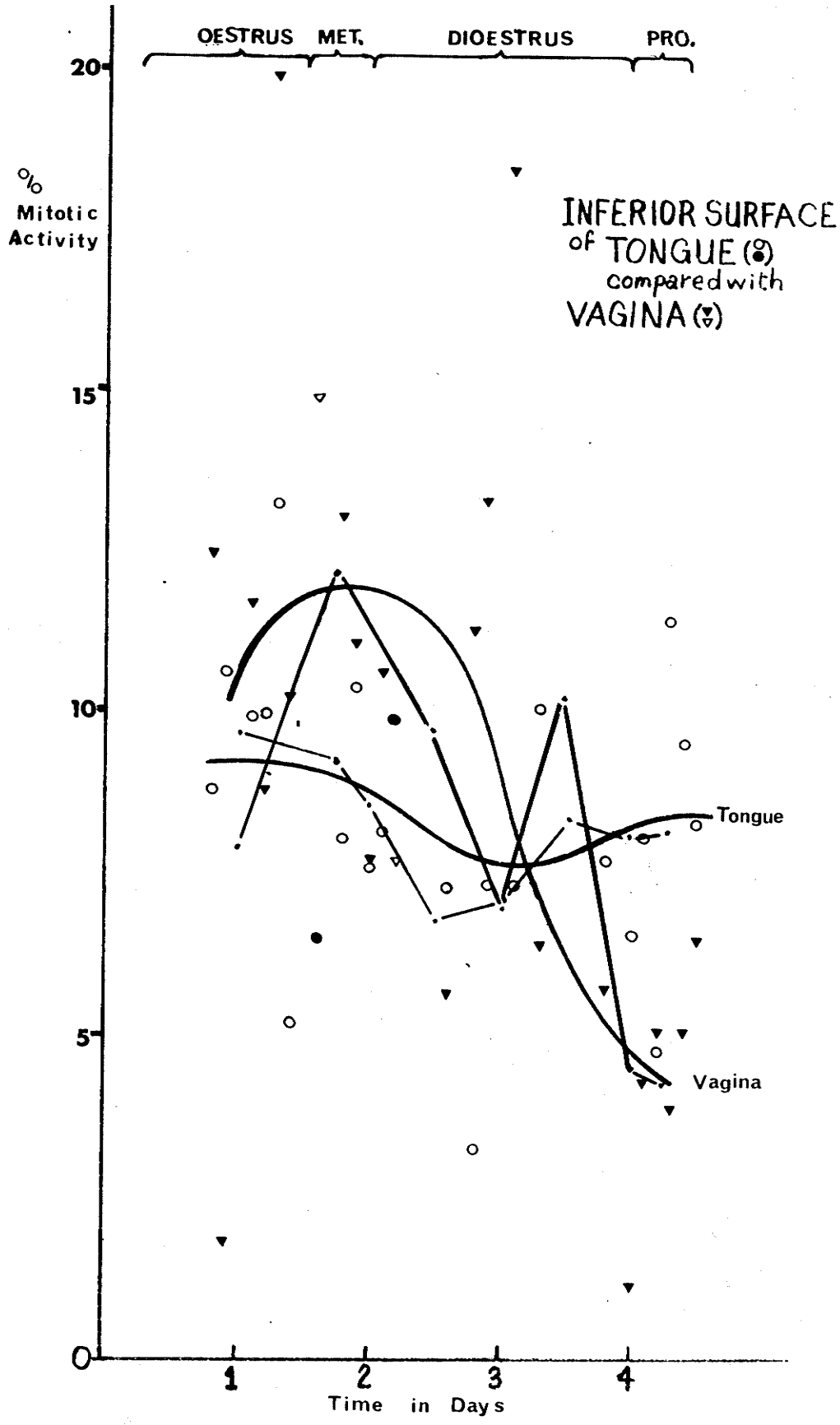

OESTRUS
MET.
DIOESTRUS
PRO.
20
15
10
5
0
% Mitotic Activity
INFERIOR SURFACE of TONGUE (●) compared with VAGINA (▼)
Tongue
Vagina
1
2
3
4
Time in Days

FIGURE 28

Individual mean percentages of mitotic activity are plotted for the two tissues for the individual rats in sequence in the standard oestrous cycle as delineated in Table II. The straight lines representing the trends of the means in the oestrous phases and day groups (Figs. 21 and 23) for each tissue are superimposed. The smooth curve, fitted by eye to the trend of the means, indicates the fluctuation in mitotic activity in the individual tissues over the oestrous cycle. The mitotic activity in the buccal mucosa appears relatively stable, compared with that of the vagina over the oestrous cycle.

The four reversed symbols indicate values for the two rats with 5 day cycles.

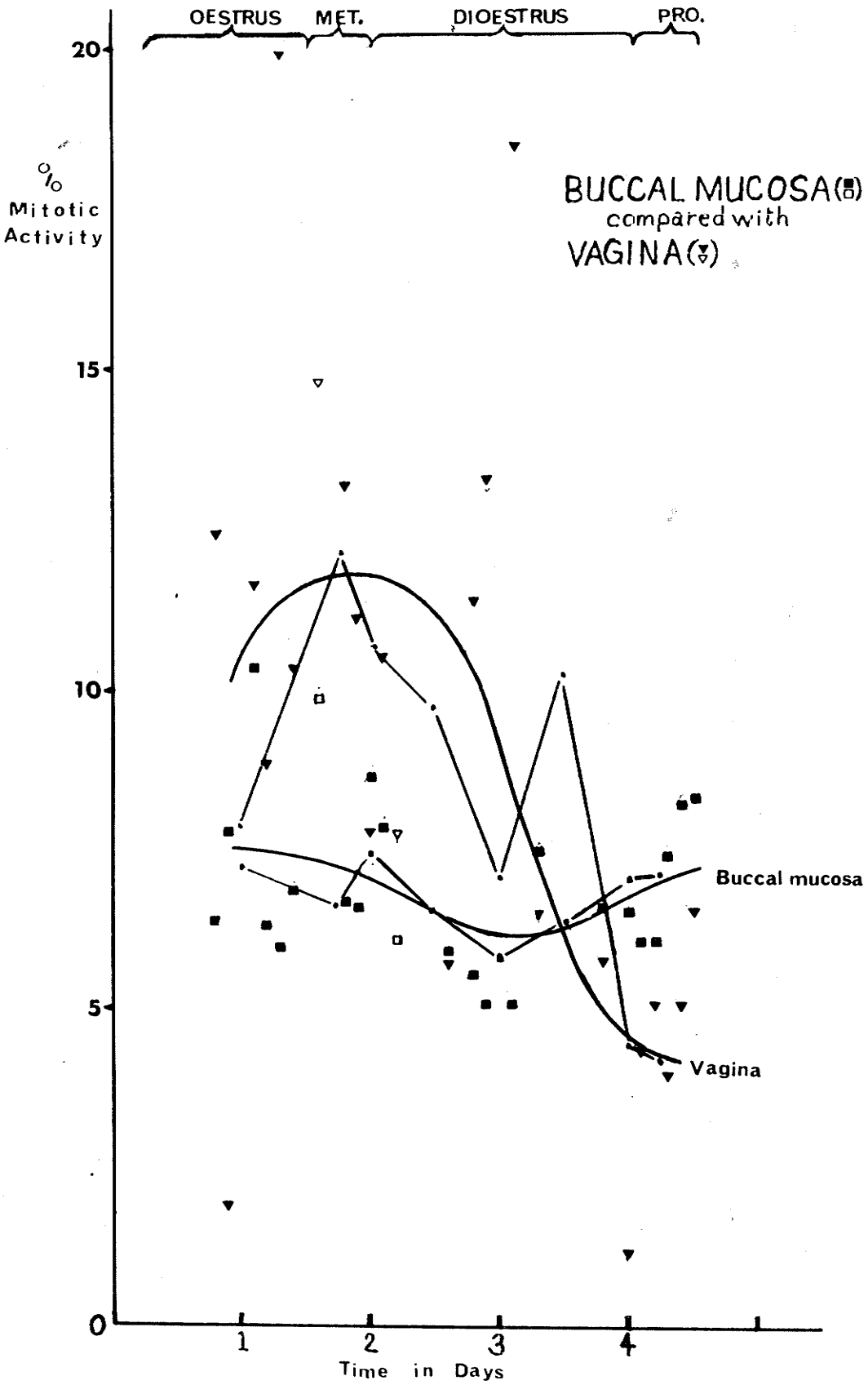

OESTRUS
MET.
DIOESTRUS
PRO.
20
15
10
5
0
%
Mitotic
Activity
BUCCAL MUCOSA(■)
compared with
VAGINA(▼)
Buccal mucosa
Vagina
1
2
3
4
Time in Days

FIGURE 29

Individual mean percentages of mitotic activity are plotted for the two tissues for the individual rats in sequence in the standard oestrous cycle as delineated in Table II. The straight lines representing the trends of the means in the oestrous phases and day groups (Figs. 21 and 23) for each tissue are superimposed. The smooth curve, fitted by eye to the trend of the means, indicates the fluctuation in mitotic activity in the individual tissues over the oestrous cycle. In comparison with the vagina, the palatal mucosa is relatively stable.

The four reversed symbols indicate values for the two rats with 5 day cycles.

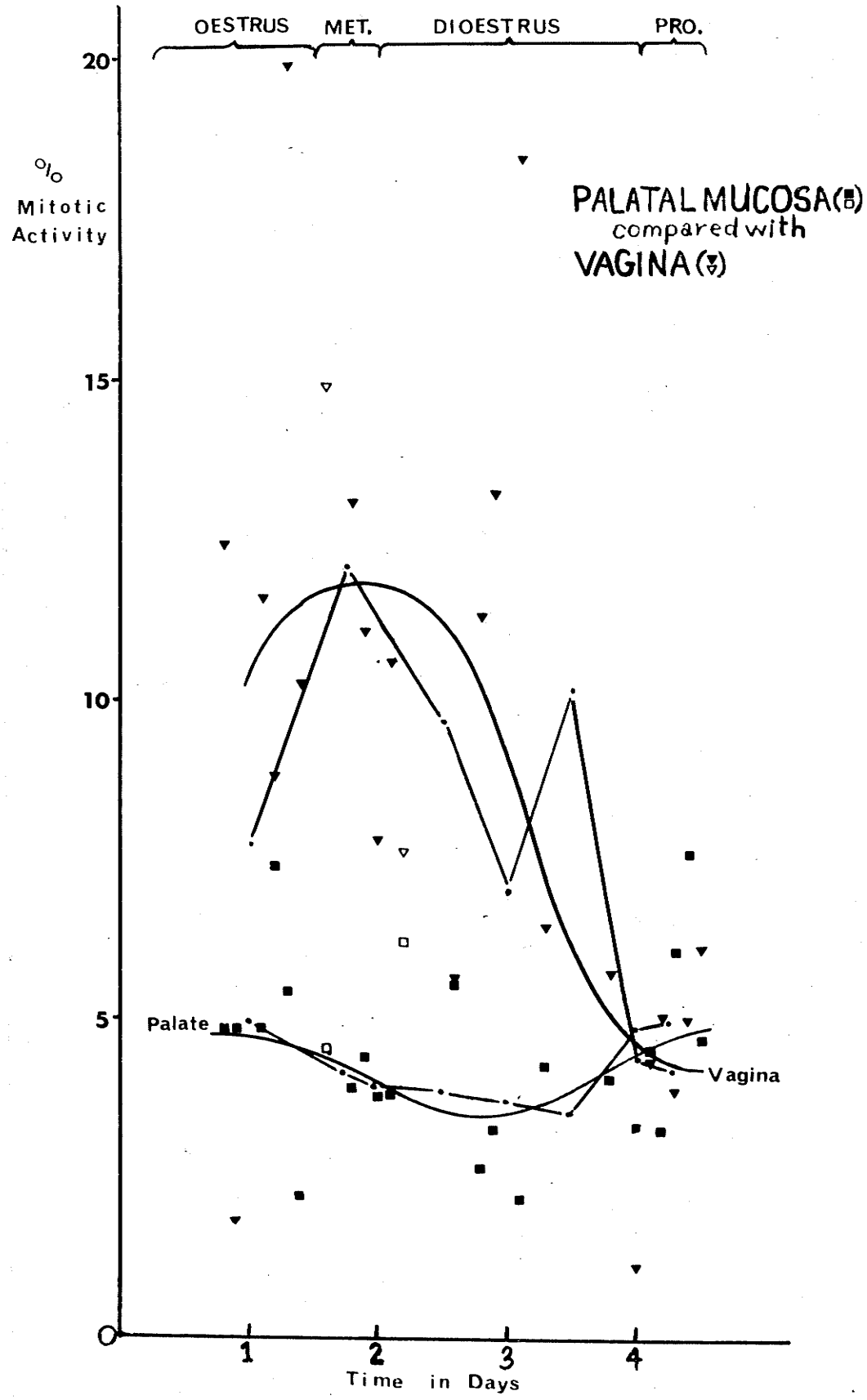

OESTRUS
MET.
DIOESTRUS
PRO.
20
15
10
5
0
% Mitotic Activity
PALATAL MUCOSA(■)
compared with
VAGINA (▼)
Palate
Vagina
1
2
3
4
Time in Days

FIGURE 30

Individual mean percentages of mitotic activity are plotted for the two tissues for the individual rats in sequence in the standard oestrous cycle, as delineated in Table II. The straight lines representing the trends of the means in the oestrous phases and day groups (Figs. 21 and 23) for each tissue are superimposed. The smooth curve, fitted by eye to the trend of the means, indicates the fluctuation in mitotic activity in the individual tissues over the oestrous cycle.

A definite dip in the trend of mitotic activity at metoestrus, would seem to indicate that the outer gingival epithelium does vary with the oestrous cycle, but this is not of the same order of amplitude as the vagina.

The four reversed symbols indicate values for the two rats with 5 day cycles.

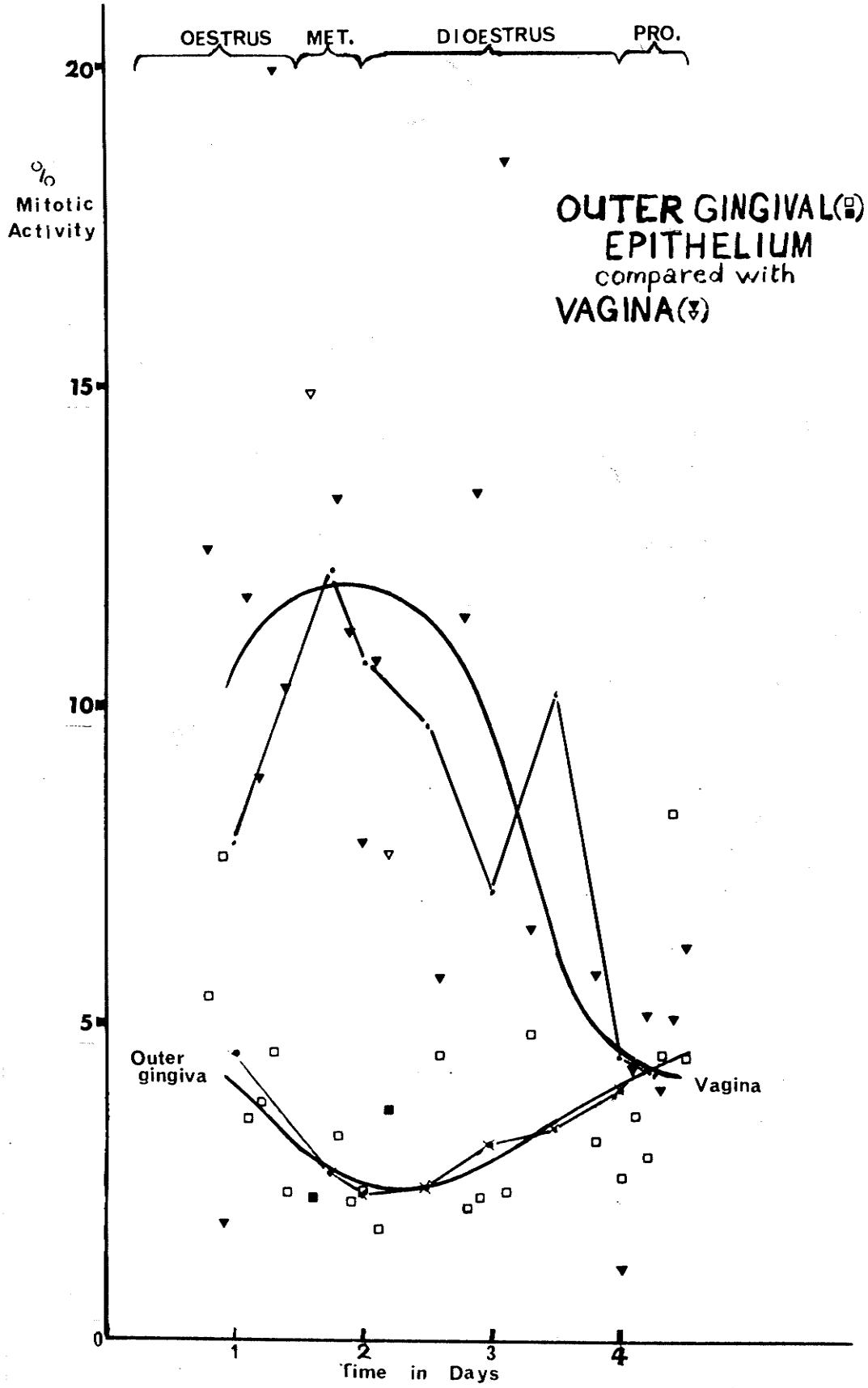
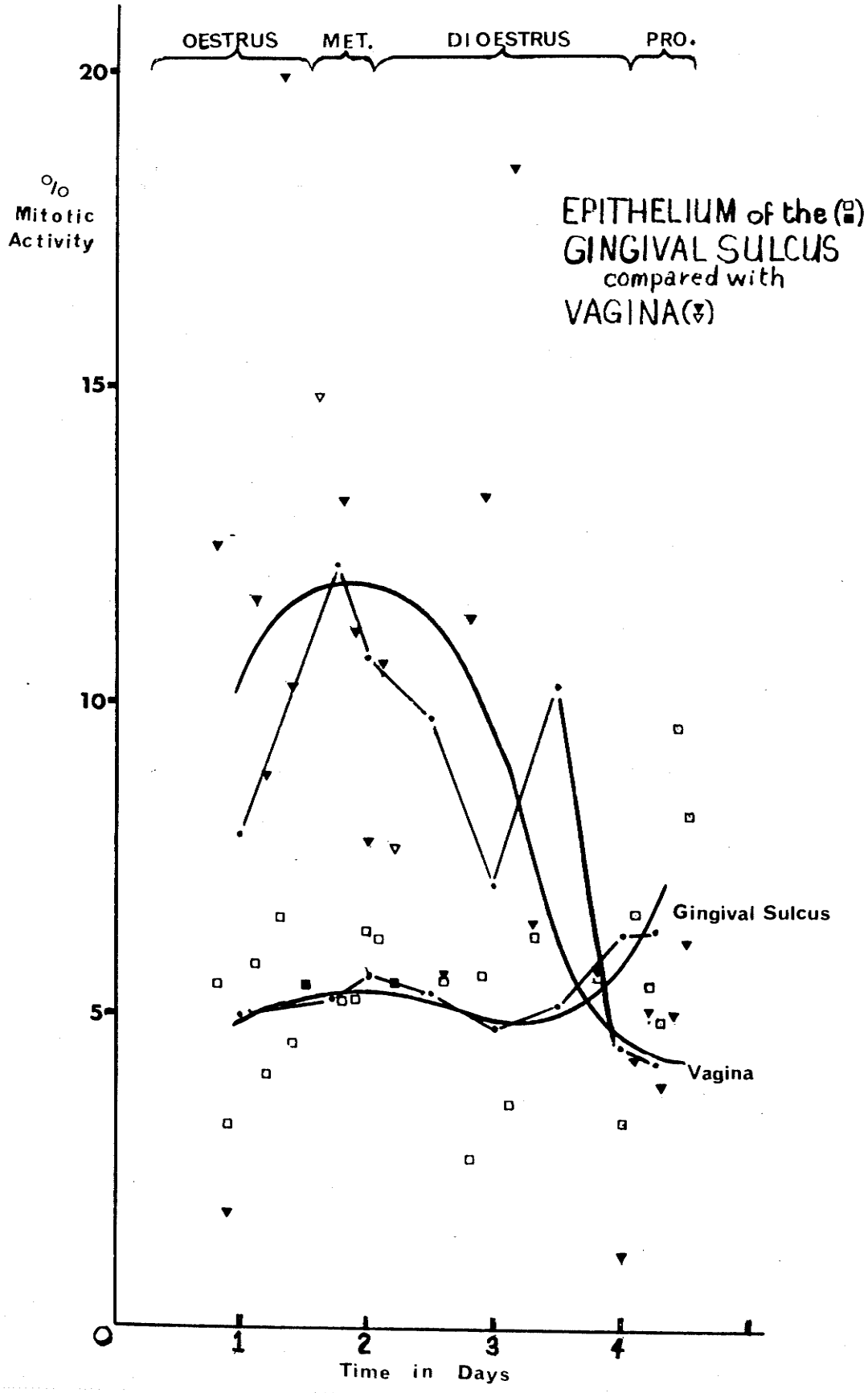

OESTRUS
MET.
DIOESTRUS
PRO.
20
15
10
5
0
% Mitotic Activity
OUTER GINGIVAL(▼) EPITHELIUM compared with VAGINA(▽)
Outer gingiva
Vagina
1
2
3
4
Time in Days

FIGURE 31

Individual mean percentages of mitotic activity are plotted for the two tissues for the individual rats in sequence in the standard oestrous cycle as delineated in Table II. The straight lines representing the trends of the means in the oestrous phases and day groups (Figs. 21 and 24) for each tissue are superimposed. The smooth curve, fitted by eye to the trend of the means, indicates the fluctuation in mitotic activity in the individual tissues over the oestrous cycle. The fluctuation of mitotic activity of the epithelium of the gingival sulcus showed only a slight rise in proestrus and was otherwise stable.

The four reversed symbols indicate values for the two rats with 5 day cycles.


OESTRUS
MET.
DIOESTRUS
PRO.
20
15
10
5
0
% Mitotic Activity
EPITHELIUM of the (□)
GINGIVAL SULCUS
compared with
VAGINA(▼)
Gingival Sulcus
Vagina
1
2
3
4
Time in Days

DISCUSSION

Because of the method adopted in this experiment for forecasting the stage of an individual rat in the oestrous cycle, prior to sacrifice, it was inevitable that no group of rats fell within the first two six hour periods of oestrus in the standard cycle. Forecasts of oestrus were made on the basis of prooestrous smears on the day prior to sacrifice, so that on the day of sacrifice the stage was unavoidably that of the later part of oestrus. In order to forecast the stage of early oestrus reliance would have had to have been placed on the much less well-defined smear picture of late dioestrus. This latter smear, noticeably sparse in its formed elements, comprises occasional small oval intermediate cells and a few polymorphonuclear leucocytes with variable amounts of amorphous debris. Astwood (1939) has termed this phase "preestrus".

The importance of this missing stage may be considerable as it corresponds to the follicular phase of the ovary, prior to the time of ovulation, as determined by Long and Evans (Astwood, 1939).

As a result, the oestrous phase could not be divided into early and late oestrus for the purposes of calculation of means, etc., as was done by Bertalanffy and Lau (1963). It is unfortunate, therefore, that the procedure followed in this experiment left a small but important gap in the cycle. The procedure was also responsible for only three rats being placed in the short metoestrous phase.

The histological findings in the vaginal mucosa as recorded in this investigation are practically identical to those of Long and Evans (1922) and Bertalanffy and Lau (1963).

One rat in each of the four phases of the cycle demonstrated that the vaginal histological configuration in the last six hour period could change to that of the subsequent phase. This was accepted as evidence of the gradual change occuring in the vaginal mucosa slightly in advance of the typical smear phase. It also indicates that every rat in a six hour group is not necessarily exactly at the same stage in the oestrous cycle.

II

When the marked fluctuation, which was observed in the mitotic activity of the vaginal mucosa, was compared to the changes in histology of the vaginal mucosa over the oestrous cycle, higher values occurred when the mucosa was at its thinnest in metoestrus, and while it was building up through dioestrus to the thickest configuration at prooestrus. During the prooestrous phase mitotic activity was low. No results were obtained for the early oestrous phase but in the latter part of this phase mitotic activity appeared to be intermediate with a wide range.

These findings are only in partial agreement with those of Bertalanffy and Lau (1963). They found that the mitotic rate was lowest during prooestrus; highest particularly during early oestrus (estrus I), and that the mitotic rate declined subsequently, remaining fairly constant, during metoestrus and dioestrus.

Bullough (1946) found that in the vaginal mucosa of the mouse, mitotic activity was least in the first day of dioestrus, followed by a rapid rise to reach a maximum in the third day of dioestrus. Relatively slight activity was found in the prooestrous period. High mitotic activity was again found by Bullough in midoestrus just prior to ovulation but not, however, as high as that seen on the third day of dioestrus.

It would thus appear that failure in this study to find a high peak of mitotic activity in the vagina over oestrus could be explained by failure to cover the first twelve hour period of oestrus. The high mitotic activity found in this study in metoestrus, in day group 2, in early dioestrus, interestingly occurs at the phase of vaginal histology when the epithelium is at its thinnest. This finding does not, however, correspond to the findings of Bertalanffy & Lau (1963) or Bullough (1946).

The peak of mean mitotic activity at late dioestrus, probably, is not very significant, as the standard deviation in this phase is high, but it is interesting to note that it might correspond to the maximum peak on the third day of dioestrus observed by Bullough (1946) in the mouse.

The occurrence of low mitotic activity in the prooestrous phase, when the vaginal mucosa is at its thickest, seems to be a universal finding. In this regard it is interesting to note that Rat A24 which had the highest percentage mitotic activity in the prooestrous phase was the one rat in the prooestrous phase to show differentiation of histology of the vaginal mucosa to the oestrous configuration.

In relation to the fluctuations of mitotic activity in the vagina perhaps the differences observed in this study as compared with those previously quoted merely reflects: The methods used to phase the rats in the standard cycle; the biological variability of Long-Evans strain as compared with the Holtzman-Sprague-Dawley strain; or the interspecies difference between rats and mice.

III

The preparation of colchicine used in this study was administered at the dosage of 0.1 mg per 100 gm body weight and was allowed to act for 6 hours. This was found to be emminently suitable in arresting mitoses at metaphase in all

of the tissues studied. It was noted that the degree of disintegration of colchicine metaphases was often marked in the epithelia of the tongue, and it was suggested that a shorter time interval between the administration of the drug and the time of sacrifice would have been preferable in studying this organ alone.

It was felt that using a high dilution of colchicine would be less susceptible to inaccuracies of dosage. Therefore, the drug was administered at a dilution of 0.1 mg per ml, a 250 gm rat receiving 2.5 ml of solution subcutaneously.

IV

No gross histological changes were noted in thickness, keratinisation or size of the sebaceous glands over the oestrous cycle in the epidermis of the ear. The mitotic activity in the ear epidermis was not found to vary significantly according to the stages of the oestrous cycle. This is in agreement with the findings of Carter (1953) on ear and indirectly with the findings of Ebling (1954) on skin. It was found, however, that a slight peak in the trend of means occurred at oestrus. Although the standard deviation in this phase was high, it is tempting to speculate whether a higher and significant mean value might not have occurred in early oestrus in the same phase as Bullough (1950) who found a peak of mitotic activity in the ear epdiermis of the mouse.

Carter, in her experiments on the ear epidermis of the rat, did not divide the oestrous phase into early and late oestrus. Bullough (1950) found also that the maximum mitotic activity in the ear of the mouse occurred on the third day of dioestrus, but this was not supported by the findings of this present study.

In agreement with the findings of Bertalanffy (1960), in the male albino rat, the superior aspect of the tongue showed a higher mitotic activity than the lower surface during the same period of the day (Table XV).

TABLE XV

MITOTIC ACTIVITY IN LINGUAL MUCOSAE 10 AM - 4 PM

TISSUE	BERTALANFFY 1960	YOUNG 1965	
	Mean %	Mean %	S.D.
Superior Lingual Mucosa	7.3	9.2	2.56
Inferior Lingual Mucosa	5.9	8.75	1.96

This has been related by Bertalanffy to the degree of mechanical stress to which the papillary surface is subjected during mastication. The high values for the standard deviation in the superior lingual mucosa over the total cycle (2.56) and within the divisions of the cycle may be a reflection of the difficulties inherent in counting this complex specialised mucosa. The value of the standard deviation of the simpler lower surface is noticeably smaller (1.96).

Whereas the results of the lingual mucosae in this study were slightly higher than those of Bertalanffy (1960) the results for buccal mucosa were of the same order but slightly lower (Table XVI).

Compared with the equivalent mean percentage mitotic activity over the same six hour period 10.00 - 16.00 hours of Trott and Gorenstein (1963), the results of the present study were considerably higher.

TABLE XVI

MITOTIC ACTIVITY IN BUCCAL MUCOSA 10 AM - 4 PM

	Bertalanffy 1960				
	1st Exp.	2nd Exp.	TROTT 1963	YOUNG 1965	
TISSUE	Mean %	Mean %	Mean %	Mean %	S.D.
Buccal Mucosa	8.3	9.7	1.69	7.2	1.32

Comparable values for mitotic activity in the buccal mucosa determined over the same time interval by Bertalanffy, 1960; Trott and Gorenstein, 1963 and this study.

Trott and Gorenstein (1963) found lower mitotic activity in the palatal mucosa than in the buccal mucosa and the findings of the present study are in agreement with this comparison. However, once again the values were not of the same order during the same 6 hour period (Table XVII).

TABLE XVII

MITOTIC ACTIVITY IN PALATAL MUCOSA 10 AM - 4 PM

TISSUE	TROTT 1963 Mean %	YOUNG 1965 Mean %	S.D.
Palatal Mucosa	1.36	4.49	1.41

This present study found the outer gingival epithelium to have the lowest mitotic activity of the oral epithelia studied, and this agrees with the findings of Trott and Gorenstein (1963) insofar as they found that the areas measured as "attached gingivae" and "crestal gingivae" had the lowest mitotic rate of their divisions of the gingiva. These two areas are adjacent to and contain the area described here as the outer gingival epithelium. Again, however, the values of mitotic activity were of a slightly different order, (Table XVIII).

TABLE XVIII

MITOTIC ACTIVITY IN THE GINGIVA 10 AM - 4 PM

TISSUE	TROTT 1963 Mean %	YOUNG 1965 Mean %	S.D.
Attached Gingivae	1.06	-	-
Crestal Gingivae	2.14	-	-
Outer Gingival Epithelium	-	3.72	1.76

Before entering into the discussion of the findings related to the epithelium of the gingival sulcus it is important to underline that the two lingual and the buccal mucosae appear to have high mitotic activity compared with the palatal and the outer gingival epithelium. This is substantiated by comparison with the other studies already quoted. The differences in the order of measurements of mitotic activity between the present study and the studies being compared may well be accounted for by: Differences in observers, differences in methods of counting, differences in strain of rats, differences in sex of rats, or differences in age of rats.

For example, in this regard, it is important to note that Bertalanffy (1960) used male albino rats average weight 300 gms and over; Trott and Gorenstein (1963) used male Holtzman-Sprague-Dawley rats average weight 350 - 500 gms.

Considering now the component parts of the gingivae, the greatest difference in mitotic activity in the same time period of six hours was found between the outer gingival epithelium and the downgrowing gingival epithelium although they are contiguous at the gingival crest.

The higher mitotic activity of the downgrowing gingival epithelium was, however, mainly due to a preponderence of mitoses in the lower third of this area. The area of the epithelial attachment showed a slightly lower mitotic activity

than the downgrowing gingival epithelium.

When the individual percentages of mitotic activity in the epithelial attachment were compared to the corresponding individual percentages of mitotic activity in the downgrowing epithelium (Table IIIb), it was noted that the percentages in the epithelial attachment were generally lower than in the downgrowing epithelium. Where they were higher, however, the percentages for the downgrowing epithelium tended to decrease. This reciprocation was also reflected in the trend of the means of the two epithelia (Fig. 24). When the percentages of the two epithelia were compounded as the epithelium of the gingival sulcus, a more uniform set of percentages emerged. It would thus appear that the arbitrary division of the epithelium of the gingival sulcus resulted in two complimentary sets of percentages.

A state of affairs which would suggest that little was gained by attempting to differentiate the two areas in regard to their mitotic activity, and the most meaningful figures are those for the epithelium of the gingival sulcus as they represent the percentage ratio of a larger number of mitoses to total cells counted in what is virtually the same anatomical area. However, for the sake of comparison with other studies the distinction remains.

In the mouse Beagrie and Skougaard (1962), found that the epithelial attachment area had a renewal time of 24 hours - 5 days and that their downgrowing oral epithelium and oral epithelium, (equivalent to the present downgrowing gingival and outer gingival epithelia) had a renewal time of 10 - 12 days.

Beagrie (1963) in comparing the gingivae of mice and marmosets suggests that the whole of the epithelial cuff is replaced every six days, and the oral epithelium of the gingivae takes longer and is replaced in 10 - 12 days.

From the results of this present study it is not possible to assess accurately the renewal time. The percentages of mitotic activity which have been here determined represent the mitotic activity only during one six hour period of the day.

During the remaining three six-hour periods the diurnal variation in mitotic activity would in all probability produce lower values as the period chosen encompasses the time when maximum mitotic activity occurs according to Bullough (1950).

The results of this present study do, however, indicate that in the female rat the epithelium of the gingival sulcus either as a unit or divided into the two components has a higher renewal time than the outer gingival epithelium. This is in agreement with the findings of Beagrie and Skoogard (1962), Trott and Gorenstein (1963) and Beagrie (1963).

The finding, in the study, that the epithelial attachment area had, if anything, a slightly lower mitotic activity than the downgrowing gingival epithelium may be a reflection of the manner of delineation of the two areas. It does indicate, however, that the lowest epithelium in the sulcus, delineated in this study as the epithelial attachment, is not the site of highest mitotic activity in the epithelium of the gingival sulcus; particularly as in this study where the epithelium is in contact with enamel and not cementum.

The site of highest mitotic activity was found to occur in the basal layers of the lower third of the area here delineated as the downgrowing gingival epithelium. It is possible that the inclusion of more of this area in the epithelial attachment as done by Trott and Gorenstein (1963) would have elevated the percentages for the epithelial attachment area at the expense of the downgrowing gingival epithelium.

Thus, once again the apparent differences in contrasting the downgrowing epithelium with the epithelial attachment are reliant on where the arbitrary division is made. The concept of the so called epithelial attachment as having a separate mitotic rate to that of the rest of the epithelium of the gingival sulcus was thus not found particularly helpful in this study in the female rat.

V

In the discussion so far a comparison has been made between the present study and previous studies merely on the relative mitotic activity of the various oral epithelia. No attempt has been made to calculate renewal times partly because of the diurnal variation but principally because this experiment was not designed to do so.

The percentages of mitotic activity merely represent activity over the six hour period in the day 10.00 - 16.00 hours. No measurements were made over a twenty-four hour period and, therefore, comparisons of mitotic activity only can be made.

In comparing the relative mitotic activity of the various oral epithelia together, the interesting impression emerges that the mobile papillated superior lingual mucosa which during mastication and the daily cleaning habits of the rat is exposed to frequent minor abrasions, exhibits the highest mitotic activity. Also, mobile and relatively lax, the lingual and buccal mucosae have the next highest mitotic activity. The palatal mucosa and the outer gingival epithelium which in contrast are bound down mucoperiostea exhibit lower mitotic activity.

The epithelium of the gingival sulcus is a unique cuff in apposition to tooth substance. The demands placed on it by function in mastication, for example, are hard to assess

but in terms of what Loe (1961), has called the physiology of the gingival sulcus the rapid mitotic activity of its cells and their coronal migration and shedding in the gingival sulcus doubtless play an important part.

Thus, although the mitotic activity of various regions appears to be related to the genetically determined specialised morphology which is related to function in the oral epithelia; to what extent the actual stresses of function influence mitotic activity of the oral tissues has not been determined.

VI

So that turning lastly to the main object of the experiment which was to determine whether the oestrous cycle had any influence on the mitotic activity of the oral tissues, it may be interesting to bear in mind the morphology of these tissues.

Firstly, no gross cyclical changes in the histology of any of the oral epithelia were noticed. Systematic mensuration was not, however, employed to compare the thickness of epithelia, the degree of keratinisation or the changes in e.g. the salivary glands.

No relationship of the mitotic activity in the tongue epithelia to the oestrous cycle could be deduced owing to the high range of variation in individual percentages of mitotic activity in the cycle.

The buccal mucosa whose histological morphology, superficially at least, most resembles the vaginal mucosa proved to have a relatively stable mitotic activity throughout the

oestrous cycle.

Both the palatal and the outer gingival epithelia did appear to be slightly affected by the oestrous cycle by a downward trend in their mean values of mitotic activity in the metoestrous - dioestrous period. Though little significance can be attached to this owing to the size of the standard deviations during the periods of apparent rise at the oestrous and proestrous phases, the similarity of this trend to the findings of Bullough (1943), in skin is interesting.

In the other portions of the gingiva, however, the pattern of the trend of the means in the outer gingival epithelium over the oestrous cycle was not repeated. The component parts of the epithelium of the gingival sulcus responded little as a unit to the oestrous cycle.

It was concluded, that the oral epithelia are not significantly affected by the oestrous cycle in the female rat. Is it a coincidence that several of the trends of the means showed a tendency to dip in the metoestrous or dioestrous phases? Possibly not, but if this does indicate some interrelationship of the oral epithelia to the oestrous cycle the method here adopted was not sufficiently precise to define it.

However, even considering the limitations of this experiment a fair indication is given that the oral epithelia do not show a significant fluctuation in relation to the oestrous cycle.

SUMMARY AND CONCLUSION

It was proposed to study what effect the oestrous cycle might have on the mitotic activity of the oral epithelia of the female rat. To this end the literature was approached to derive information regarding the oestrous cycle and the renewal of epithelial surfaces. A review of the relevant concepts and methodology from the experimental literature was incorporated in the introduction to this thesis.

Forty-six Long-Evans strain rats were kept under stable conditions of environment, and were followed over several oestrous cycles by the vaginal smear technique. Twenty-five rats having regular oestrous cycles were selected and sacrificed in four groups at different intervals in the oestrous cycle. Six hours prior to sacrifice the animals received colchicine. The animals were sacrificed at the same time of day to avoid the effect of the diurnal variations on mitotic activity.

On the basis of the individual oestrous history of each rat, as ascertained by vaginal smear, the selected animals were arranged in sequence on a standard oestrous cycle. The histological configuration of the vaginal mucosa at sacrifice varied with the phase of the oestrous cycle and correlated well with the sequence on the standard oestrous cycle. The histological configuration of the other epithelial surfaces studied showed no apparent change over the oestrous cycle.

The sequence of the groups of rats in the standard oestrous cycle thus derived was used to ascertain the effect of the oestrous cycle on the mitotic activity of the vaginal mucosa, the ear epidermis, and the oral epithelia.

The mitotic activity during the six hours prior to sacrifice was estimated in the vaginal mucosa, the ear epidermis, and several oral epithelia for all the groups of rats sacrificed. The oral epithelia studied were the superior and inferior lingual mucosae, the palatal and buccal mucosa, and the gingiva.

It was found, within the limits of this experiment, that the oestrous cycle had a marked effect on the mitotic activity of the vaginal mucosa in conjunction with definite histological changes of the epithelium.

The mitotic activity in the ear epidermis was not significantly affected by the oestrous cycle.

No apparent histological changes were noted in any of the oral epithelia studied over the oestrous cycle. The mitotic activity in the various divisions of the oral epithelia was not significantly affected by the oestrous cycle.

A comparison of the fluctuations of mitotic activity in the vaginal mucosa and the other epithelia led to the conclusion that the mitotic activity in the oral epithelia and ear epidermis is not affected by the oestrous cycle as is the vaginal epithelium.

These results and observations were compared with those of previous authors on mitotic activity and cell renewal of the epithelial areas under study.

APPENDIX

Colchicine

In reviewing the literature in regard to what dosage of colchicine to employ and for how long to allow it to act, we found the following relevant features:

Although H. F. Bullough (1943 & 1947) and W. S. Bullough (1946) utilised a dosage of 0.1 mg of colchicine and allowed it to act for 9½ hours before sacrifice, W. S. Bullough (1949) in investigating the action of the drug on epidermal mitosis concluded that at least in the case of ear epidermis, for a study of normal mitotic activity (in the mouse) colchicine must not be allowed to act for more than 5 hours before sacrifice. Where the drug was allowed to act up to 9½ hours to 20 hours, the observed mitotic activity merely represented that during the five hour period, as further mitoses were not arrested in metaphase.

Henry, Meyer, Weinmann, and Schour (1952) working on oral epithelium in rabbits made use of a dosage 1 mg/kilogram of body weight allowing it to act in two groups for 3 hour and 6 hour periods. They concluded that the accumulation of mitoses in the first and second three hour periods was practically identical.

Bertalanffy and Leblond (1953) investigating the renewal of alveolar cells in the lung of the rat, produced experimental evidence for the use of a 0.10 mg/100 gm body weight dosage acting over a six hour period. If allowed to act for 9 hours many colchicine metaphases had become pyknotic, completely disintegrated or even disappeared. Ideally the animal should

be sacrificed at a time when the number of accumulating metaphases is still increasing without an appreciable loss of mitotic figures, i.e. certainly up to six hours.

If the dosage was altered to 0.05 mg/100 mg body weight arrest of cell division did not occur whereas 0.20 mg/100 mg wt proved toxic with extensive cell damage and pronounced pyknosis.

The dosage of 0.10/100 mg body weight arrested many metaphases but demonstrated neither anaphases or telophases.

There was no significant difference whether the drug was given as a single dose or as two separate half doses.

Ebling (1954) studying changes in the sebaceous glands and epidermis during the oestrous cycle of the rat used 0.1 mg/100g body weight given intraperitoneally acting over 5 hours before sacrifice in accordance with the findings of Bullough (1949).

Leblond and Walker (1956) in their comprehensive article on the renewal of cell populations discussed the colchicine method and drew the conclusion that although "the number of colchicine metaphases would tend to be lower than the total number of mitoses initiated after injection of the drug" (in the usual dosage) "counts of colchicine blocked mitoses gave a minimum figure for the extent of this activity over the period of action of the drug (4 - 6 hours)."

Hooper (1961) reports further work on the intestinal epithelium of rats to determine the suitability of colchicine in the assessment of mitotic rates. In her studies, the period of action of the drug ranged over 3 hours. She said that results employing more than four hours would not be reliable due to the high proportion of disintegrating metaphases. This had been previously observed in the intestinal epithelium at 6 hours by Leblond & Stevens (1948). She further noted, however, that tissue sensitivity to colchicine is not uniform and quotes the work of Storey and Leblond (1951) who observed no significant deterioration of the arrested metaphases in the Malpighian layer of the plantar epidermis of rats 6 hours after colchicine injection.

She suggested that tissues with an extremely active mitotic rate would respond best to intravenously injected colchicine and a short treatment period whereas subcutaneous injection and a longer experiment might benefit a tissue with a slow mitotic rate. Bertalanffy (1960) in studying lip, buccal mucosa and tongue as part of the digestive tract, and Bertalanffy & Lau (1963) studying female genital tract in the rat used 0.10 mg/100g body weight administered subcutaneously to act for 6 hours before sacrifice. They found good definition of colchicine metaphases and no anaphases or telophases. This was in a tissue with a mitotic activity of around 30% per day or less. In intestinal epithelium where

the mitotic activity was considerably higher (79% in jejunum) sufficiently large number of dividing cells were present to make the use of the colchicine technique unnecessary.

Accordingly, the subcutaneous administration of 0.10 mg/100 gm body weight and a period of 6 hours before sacrifice seemed justifiable for use in studying oral and vaginal epithelium and has been used in this manner by Trott and Gorenstein (1963) in studying the oral and gingival epithelium of the male rat.

BIBLIOGRAPHY

Alldritt, W.S. (1961): "Epithelia in the Dento-gingival junction", Dent. Practit. & Dent. Record, 11, 213-223.

Allen, E. (1922): "The Oestrous Cycle in the Mouse", Am. J. Anat. 30, 297-371.

American Armed Forces Institute of Pathology (1960): "Manual of Histologic and Special Staining Technics", 2nd Ed. Blakiston, New York, pp. 4, 38.

Astwood, E.B. (1939): "Changes in the Weight and Water Content of the Uterus of the Normal Adult Rat", Am. J. Physiol. 126, 162-170.

Beagrie, G.S. (1963): "Autoradiographic Study of the Gingival Epithelium of Mice and Monkeys with Thymidine H^3" Dent. Practit. 24, 18-26.

Beagrie, G.S. & Skougaard, M.R. (1962): "Observation on the Life Cycle of the Gingival Epithelial Cells of Mice as Revealed by Autoradiography", Acta Odont. Scand. 20, 15-31.

Bernard, C.L. (1880): "Leconcs de Pathologie experimentale", (Au College de France) Paris: J.B. Bailliere et Fils. "The protection of the internal environment of organisms".

Bertalanffy, F.D. (1960): "Mitotic Rates and Renewal Times of the Digestive Tract Epithelia in the Rat". Acta Anat. 40, 130-148.

Bertalanffy, F.D. and Lau, C. (1962): "Cell Renewal", Int. Rev. Cytol. 13, 357-366.

Bertalanffy, F.D. & Lau, C. (1963): "Mitotic Rates, Renewal Times and Cytodynamics of the Female Genital Tract Epithelia in the Rat". Acta Anat. 54, 39-81.

Bertalanffy, F.D. & Leblond, C.P. (1953): "The Continuous Renewal of the Two Types of Alveolar Cells in the Lung of the Rat", Anat. Rec. 115, 515-541.

Bullough, H.F. (1943): "Cyclical Changes in Skin of Mouse During the Oestrus Cycle", J. Endocrin. 3, 280-287.

Bullough, H.F. (1947): "Epidermal Thickness Following Oestrone Injections in the Mouse", Nature, London 159, 101-102.

Bullough, W.S. (1946): "Mitotic Activity in the Adult Female Mouse, Mus Musculus L. A Study of Its Relation to the Oestrus Cycle in Normal and Abnormal Conditions", Royal Soc. of Lond. 231, 453.

Bullough, W.S. (1948a): "Mitotic Activity in the Adult Male Mouse, Mus Musculus L, the Diurnal Cycles and Their Relation to Waking and Sleeping", Proc. Royal Soc. 135, 212-232.

Bullough, W.S. (1948b): "The Effect of Experimentally Induced Rest and Exercise on the Epidermal Mitotic Activity of the Adult Male Mouse, Mus Musculus L." Proc. Royal Soc. 135, 233-242.

Bullough, W.S. (1949a): "The Effects of High and Low Temperatures on Epidermal Mitotic Activity of the Adult Male Mouse, Mus Musculus L", J. Exp. Biol. 26, 76-82.

Bullough, W.S. (1949b): "Age and Mitotic Activity in the Male Mouse, Mus Musculus L", J. Exp. Biol. 26, 261-286.

Bullough, W.S. (1950): "Epidermal Mitotic Activity in the Adult Female Mouse", J. Endocrin. 6, 340-349.

Bullough, W.S. (1952a): "Stress & Epidermal Mitotic Activity I. The Effects of the Adrenal Hormones", J. of Endocrin. 8, 265-274,

Bullough, W.S. (1952b): "Stress & Epidermal Mitotic Activity II. The Effects of Sex Hormones", J. Endocrin. 8, 365-376.

Bullough, W.S. & Eisa, E.A. (1950): "The Diurnal Variations in the Tissue Glycogen Content and Their Relation to Mitotic Activity in the Adult Male Mouse". J. Exp. Biol. 27, 257-263.

Cameron, I.L. & Greulich, R.C. (1963): "Evidence for an Essentially Constant Duration of D.N.A. Synthesis in Renewing Epithelia of the Adult Mouse", J. Cell Biol. 18, 31-40.

Cannon, A.B. (1935): "White Sponge Nevus of the Mucosa", Arch. Dermat. & Syph. 31, 365-369.

Carter, Sheila B. (1953): "The Influence of Oestrone on the Division of Cells", J. Endocrin. 9, 19-29.

Ebling, F.J. (1954): "Changes in the Sebaceous Glands and Epidermis During the Oestrus Cycle of the Albino Rat", J. Endocrin. 10, 147-154.

Ebling, F.J. (1955): "Endocrine Factors Affecting Cell Replacement and Cell Loss in the Epidermis and Sebaceous Glands of the Female Albino Rat", J. Endocrin. 12, 38-49.

Glickmann, I. & Quintarelli, J. (1960): "Further Observations Regarding the Effects of Ovariectomy Upon the Tissues of the Periodontium", J. Periodont. 31, 31-37.

Green, H. N. & Bullough, W.S. (1950): "Mitotic Activity in the Shock State", Brit. J. Exp. Path. 31, 175-182.

Halberg, F., Zander, H.A., Houglum, N.W. and Muhlemann, H.R. (1954): "Daily Variations in Tissue Mitoses, Blood Eosinphils and Rectal Temperatures of Rats", Amer. J. Physiol. 177, 361-366.

Henry, J. L., Meyer, J., Weinmann, J.P. and Schour, I. (1952): "Pattern of Mitotic Activity in Oral Epithelium of Rabbits", AMA Arch Path. 54, 281-297.

Hill, A. Bradford (1956): Principles of Medical Statistics 6th Ed. Oxford Univ. Press, 257.

Hirt, C.M., Hartl, S. and Muhlemann, H.R. (1955): "The Distribution of Mitoses in the Epithelium of the Interdental Papillae of the Rat Molar", J. Periodont. 26, 229-232.

Hooker, J. and Pfeiffer, C. (1943): "The Effect of Sex Hormones Upon Body Growth, Skin, Hair and Sebaceous Glands in the Rat", Endocrin. 32, 69-76.

Hooper, C.E. Stevens (1956): "Cell Turnover in Epithelial Populations", J. Histochem & Cytochem. 4, 531.

Hooper, C.E. Stevens (1961): "Use of Colchicine for the Measurement of Mitotic Rate in the Intestinal Epithelium", Amer. J. Anat. 108, 231-244.

Leblond, C.P. & Stevens, C.E. (1948): "The Constant Renewal of the Intestinal Epithelium in the Albino Rat", Anat. Rec. 100, 357-377.

Leblond, C.P. & Walker, B.E. (1956): "Renewal of Cell Populations", Physiol. Rev. 36, 255-276.

Lillie, R.D. (1954): "Histopathologic Technic and Practical Histochemistry", 2nd Ed. p. 35, Blakiston, New York.

Löe, H. (1961): "Physiological Aspects of the Gingival Pocket", "An Experimental Study", Acta. Odont Scand. 19, 387-395.

Loeb, L. & Haven F.L. (1929a) "Quantitative Studies of Growth of Epidermis", Anat. Rec. 42, 217-241.

Loeb, L. & Haven, F.L. (1929b): "The Relation Between Functional States of the Sex Organs in the Female Guinea Pig and the Cell Proliferation in the Epidermis", Anat. Rec. 43, 1-26.

Long, J.A. and Evans, H. McL. (1922): "The Oestrous Cycle in the Rat and Its Associated Phenomena", Univ. of Calif. Press, Berkeley, California.

Mandl, A.M. (1951): "The Phases of the Oestrous Cycle in the Adult White Rat", J. of Exper. Biol. 28, 576-584.

Messier, B. & Leblond, C.P. (1960): "Cell Proliferation and Migration as Revealed by Radioautography After Injection of Thymidine H^3 Into Male Rats & Mice", Amer. J. Anat. 106, 247-285.

Mühlemann, R.H., Ebneter, M. and Rupf, W. (1959): "Diurnal Variation in Mitotic Activity of Oral and Corneal Epithelium in Colchicinized Albino Rats", Helvet. Odont. Acta. 3, 30-34.

Mühlemann, H.R., Zander, H.A. & Halberg, F. (1954): "Mitotic Activity in the Periodontal Tissues of the Rat. Molar", J.D. Res. 33, 459-467.

Nutlay, A.G., Bhaskar, S.N., Weinmann, J.P. & Budy, A.M. (1954): "The Effect of Estrogen on the Gingiva and Alveolar Bone of Molars in Rats & Mice", J.D. Res. 33, 115-127.

Pinkus, H. (1951): "Examination of the Epidermis by the Strip Method of Removing Horny Layers. I Observations on Thickness of the Horny Layer and on Mitotic Activity After Stripping", J. Invest. Dermat. 16, 383-386.

Rateitschak, K.H. & Mühlemann, H.R. (1957): "The Effect of Cold Stress at Two Different Times of Day on the Oral Epithelium 24-Hour-Mitosis-Periodicity in Albino Rats", Helvet. Odont. Acta. 1, 19-21.

Stahl, S.S., Weinmann, J.P., Schour, I. and Budy, A.M. (1950): "The Effect of Estrogen on the Alveolar Bone and Teeth of Mice and Rats", Anat. Rec. 107, 21-41.

Stevens, C.E. & Leblond, C.P. (1953): "Renewal of the Mucous Cells in the Gastric Mucosa of the Rat", Anat. Rec. 115, 231-245.

Storey, W.F. and Leblond, C.P. (1951): "Measurement of the Rate of Proliferation of Epidermis and Associated Structures", Ann. N. York Acad. Sc. 53, 3, 537-545.

Trott, J.R. & Gorenstein, S.L. (1963): "Mitotic Rates in the Oral and Gingival Epithelium of the Rat", Arch. Oral Biol. 8, 425-434.

Walker, B.E. (1960): "Renewal of Cell Populations in the Female Mouse", Amer. J. Anat. 107, 95-105.

Ziskin, D.E., Blackberg, S.N. and Slanetz, C.A. (1936); "Effects of Subcutaneous Injections of Estrogenic and Gonadotrophic Hormones on Gums and Oral Mucous Membranes of Normal and Castrated Rhesus Monkeys", J. Dent. Res. 15, 407-428.