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## **Amitosis in the Epithelium Lining the Oral Cavity of the Cat, Guinea-Pig and Mouse.**

**(A Preliminary Report.)**

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### **Introduction.**

When occupied with histological investigations of the epithelium in the oral cavity of the guinea-pig I became aware of the presence of a great number of binuclear cells in the labial epithelium. On studying the literature I discovered that a series of investigators have been dealing with the lingual epithelium of this animal, in which binuclear cells, formed by amitotic cell division, are found in great numbers.

Thus SEVERIN observed, besides numerous mitoses, large amounts of binuclear cells, which were present in all the layers from the deepest to the stratum corneum. In some areas almost exclusively binuclear cells were found, while these in other locations were fewer in number; in the deepest layers they were very seldom though. SEVERIN did not see any tri- or quadrinuclear cells. DITLEVSEN was able to demonstrate gemmation (Knospung) of the nuclei in the lingual and oesophageal epithelium of the guinea-pig. On examining the skin and tongue of the rat, mouse, rabbit, horse and cow this peculiarity was not found, nor was it seen in man. DITLEVSEN therefore assumes that gemmation of the nuclei exclusively occurs in the stratified epithelium of the guinea-pig, which is peculiarly cornified and in which cell proliferation to a large extent is effected by amitosis.

The gemmae are found either in a depression on the surface of the nucleus or on to a flatter area of the latter. Whether uni- or binuclear cells, seldom more than one gemma is found. In the case of two gemmae they are as a rule found in juxtaposition. The amount of chromatin contained is but small.

DITLEVSEN also described a peculiar type of cells in the epithelium covering the tongue in the guinea-pig. These cells often have a lumpy inspissation at one pole of the cell and may resemble fibrocytes. The cytoplasm of these cells is so deeply staining that their structure is discerned but with difficulty. Excessive differentiation will turn out two nuclei. Frequently one half of the cell is intensively stained while the other is staining like a usual epithelial cell.

MAXIMOW studied amitosis in embryonic tissue, more precisely in mesenchymatous cells originating from rabbit fetusses from  $11\frac{1}{2}$  to  $13\frac{1}{2}$  days old. MAXIMOW found that the amitoses obviously appeared in a definite period of fetal life. He finds no explanation for the fact that amitosis in embryonic tissue (besides in the rabbit) so far has been observed in the guinea-pig only. In the rat and cat he failed almost entirely to find amitoses.

MAXIMOW describes the gemmation of the nucleus thus: "Der Kern bekommt an irgendeiner Stelle seiner Oberfläche eine kleine knopfförmige Knospe. Es ist schwer zu sagen ob sie zuerst als eine Art Ausstülpung entsteht, oder ob ein kleiner Teil des verlängerten Kerns durch eine zirkuläre Furche abgeschnürt wird. Die Knospe vergrößert sich allmählich, ihre Ansatzstelle an der Hauptmasse des Kerns bleibt immer sehr eng und kann sich schliesslich zu einem ziemlich langen Stiel verlängern. Man bekommt den Eindruck, als ob der Inhalt des Kerns durch die unachgiebige enge, eingeschnürte Stelle allmählich herausgepresst wird, in die Knospe gelangt und sie immer mehr und mehr ausdehnt . . .".

By division of the long stalk two nuclei may be formed either of equal or unequal size. In some cases the new nucleus disintegrates and can then be demonstrated in the cytoplasm in the form of small globular more or less deeply staining bodies.

With respect to the occurrence of amitoses in the covering epitheliums it may be mentioned that PATZELT, basing on investigations of the human epidermis, thinks that amitotic cell division takes place to a considerable extent in the *stratum spinosum*.

According to SCHUMACHER binuclear cells are occasionally present in the human lingual epithelium. In MÖLLENDORFF'S *Handbuch der mikroskopischen Anatomie des Menschen* (1929) WASSERMANN gives an account of the present state of the problem of amitosis after having critically reviewed the previous literature on this subject. On the basis of morphology WASSERMANN distinguishes 6 forms of amitotic nuclear division.

### Own Investigations.

In the light of the literature cited above I will now in a series of micro-photographs show amitoses in the epithelium lining the oral cavity of the cat, guinea-pig and mouse.

The material of the present investigation comprises both embryonic and postnatal tissue samples. The material was treated rather promiscuously as it was gathered partly to study the epithelium of the oral cavity in a general way. For fixation I have in the main used Flemming's and Maximow's solutions besides Bouin's and 8 % formalin. All the samples were imbedded in paraffin. The tissue slices were 4  $\mu$  thick. Most of the samples were stained with Heidenhain's iron-hematoxylin stain and partly bleached. In a few cases I have also used a molybdenium-hematoxylin stain.

Of all the embryos frontal sections through the head were prepared, the head being partly cut in series, and partly only single sections from different parts were taken. The heads of the cats and guinea-pigs were first decalcified with 5 % nitric acid.

As far as I can see embryonic epithelium from the oral cavity of the animals in question has not been studied before with regard to amitosis. In the postnatal stages epithelium from the upper and lower lip and from the hard and soft palate were examined separately in sagittal sections from these regions. Besides I had a complete series of frontal sections through the head of a newborn mouse.

Figs. 1 and 2 show respectively the hard and soft palate of a fullgrown mouse in one and the same sagittal section. In the epithelium of the hard palate an abundance of binuclear cells is to be seen, while such cells are very few in number in the epithelium covering the soft palate. Now, the epithelium of the hard palate is far more cornified than that of the soft, and thus

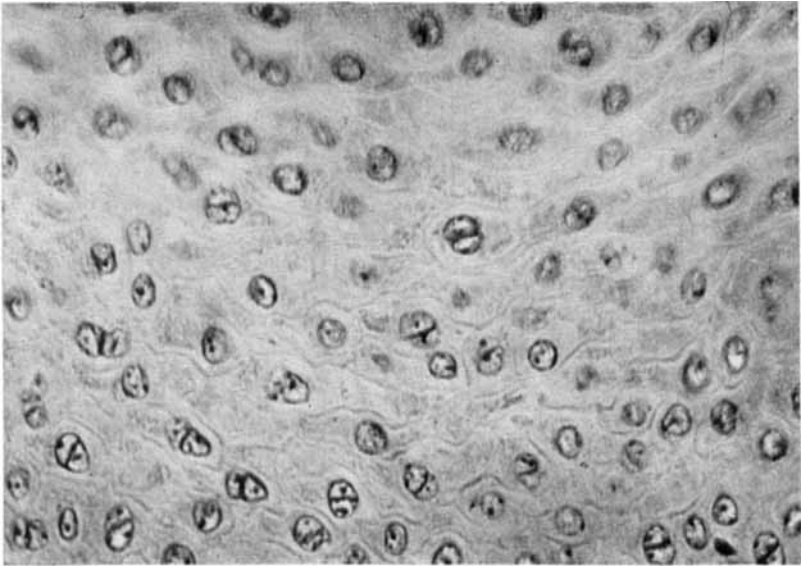


Fig. 1. Adult mouse. Epithelium of hard palate. *Stratum spinosum*. Sagittal section. Fixed with 8 % formalin and stained with HEIDENHAIN's iron-hematoxylin stain.  $\times 450$ .

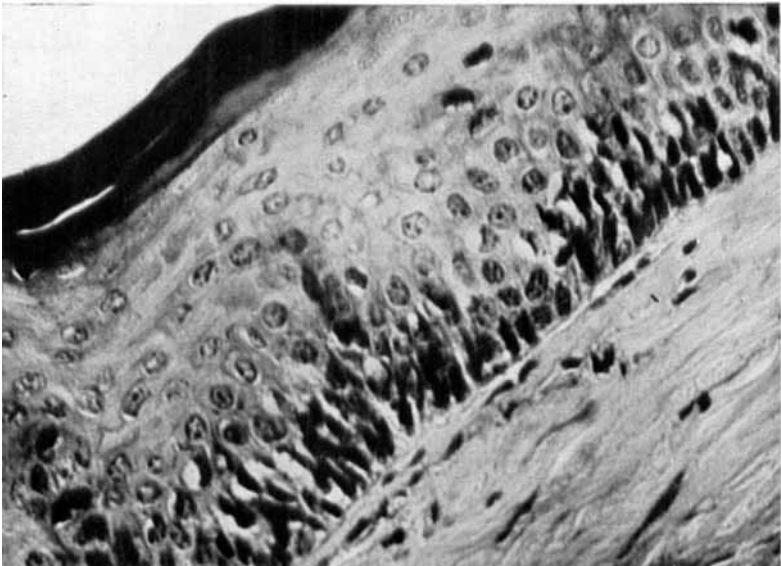


Fig. 2. Same section as in fig. 1. Epithelium of soft palate.  $\times 450$ .

HALS: Amitosis in the Epithelium.

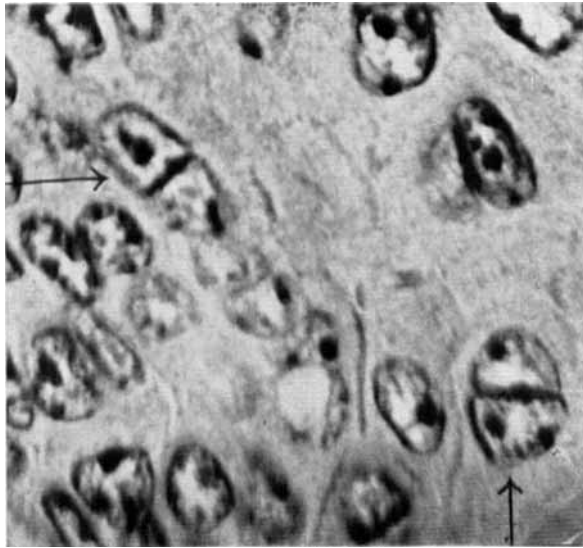


Fig. 3. Adult guinea-pig. Epithelium of lower lip. *Stratum spinosum*. Fixed with FLEMING'S fluid, and stained with HEIDENHAIN'S iron-hematoxylin stain.  $\times 1350$ .

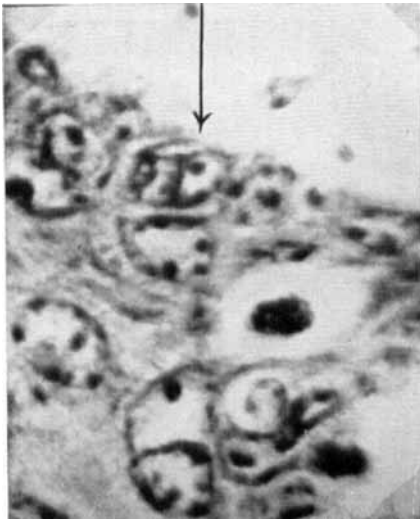


Fig. 4. Adult guinea-pig. Epithelium from the fold of mucous membrane between upper lip and hard palate. *Stratum cylindricum*. Fixed with 8% formalin, and stained with HEIDENHAIN'S iron-hematoxylin stain.  $\times 900$ .

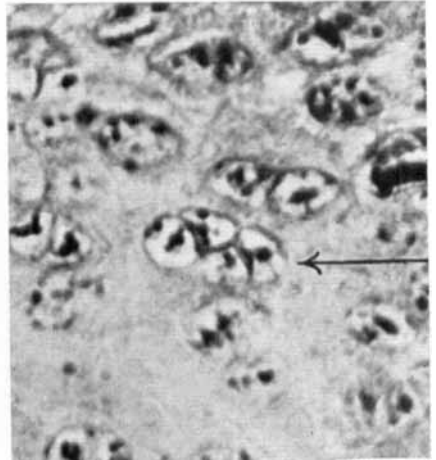


Fig. 6. Same material and technique as in fig. 4.  $\times 900$ .

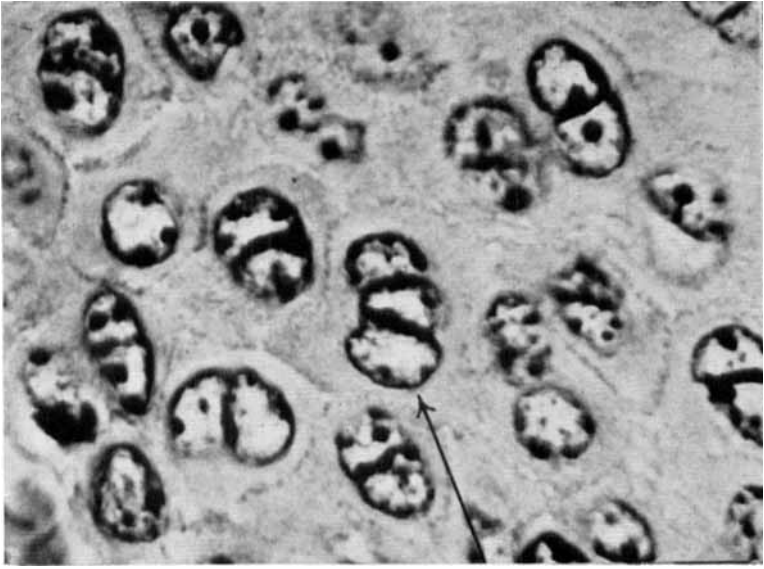


Fig. 5. Same material and technique as in fig. 1. *Stratum spinosum*.  $\times 1350$ .

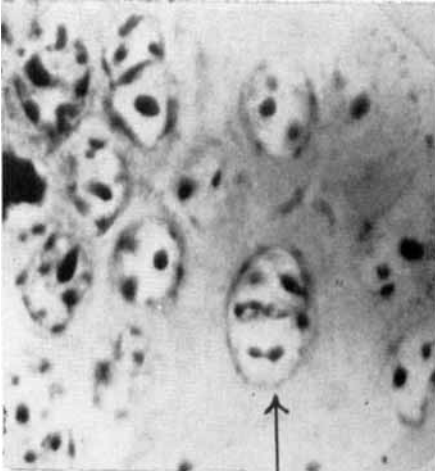


Fig. 7. Same material and technique as in fig. 4.  $\times 1350$ .



Fig. 8. Cat. Fetus (8 cm.). Epithelium from the upper surface of the tongue. Fixed with MAXIMOW's fluid and stained with HEIDENHAIN's iron-hematoxylin stain.  $\times 1350$ .

HALS: Amitosis in the Epithelium.

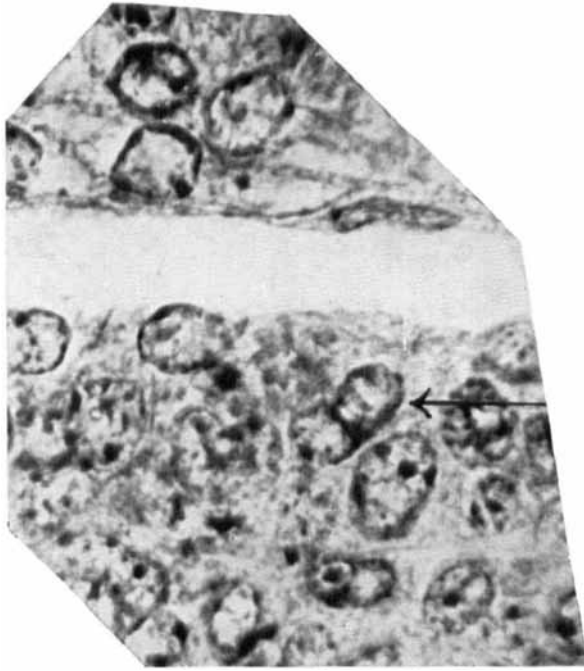


Fig. 9. Mouse. Fetus (12 mm.). Epithelium from the lower surface of the tongue. Fixed with MAXIMOW's fluid, and stained with HEIDENHAIN's iron-hematoxylin stain and with bleaching.  $\times 1350$ .

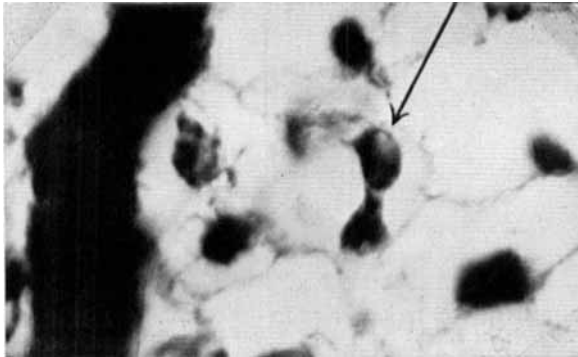


Fig. 10. Guinea-pig. Fetus (8 cm.). Epithelium from upper lip. *Stratum spinosum*. Fixed with MAXIMOW's fluid, and stained with molybdenum-hematoxylin stain.  $\times 1350$ .

it seems in this case as if the number of binuclear cells was directly proportional to the degree of cornification.

Fig. 3 is a high power picture of a cell, the nucleus of which is undergoing amitotic division. The nucleus of this cell has — compared with the resting nucleus — suffered a change of form; it has become oval. Across the center of the nucleus runs a distinct line.

This type of amitosis is the one characteristic of my material. A few exceptions will be discussed below. When trying to classify this type of amitosis I want to quote WASSERMANN:

“6. Eine besondere Form der Kernteilung, die man den bisher genannten Typen gegenüberstellen müsste, beschreibt PATTERSON (1908, S. 118). Neben der Durchschnürung findet er eine Form, bei der anscheinend quer durch den Kern eine Scheidewand (‘a nuclear plate’) gebildet wird. Die Teilung soll in einer Spaltung der Kernplatte bestehen, wofür Zustandsbilder wie das der Abb. 379 zu sprechen schienen. Über ähnliche Beobachtungen, die er an den Kernen der glatten Muskelzellen des menschlichen Uterus gemacht habe, berichtet BAST (1921). Da die Kernplattenbildung aber nicht genauer studiert worden ist, wird man damit rechnen müssen, dass auch hier eine Durchschnürung ohne vorausgehende Verlängerung des Kerns vorgelegen hat und durch die Einfaltung der Kernwand lediglich der Anschein einer Scheidewandbildung erweckt worden ist. Es ist aber auch nicht ausgeschlossen, dass hier doch eine besondere Art von Kernteilung vorliegt von der Art, wie sie von SCHÜRHOFF (1915) bei Pflanzenzellen genauer beschrieben worden ist (s. unten S. 578). Diese Möglichkeit sichert den noch unzureichenden Beobachtungen eine gewisse Beachtung.”

On studying the papers of PATTERSON and BAST referred to by WASSERMANN, it is not difficult to understand that the amitosis shown in fig. 3 is identical with WASSERMANN's type 6.

I believe though, as does WASSERMANN, that we here need not have to deal with a form of amitosis fundamentally different from the five other types, as the “nuclear plate” very well may be the nuclear membrane seen in cross-section as a result of a division perpendicular to the surface of the nucleus. Phases of this division of the nucleus — necessarily to be regarded as a division of the nuclear plate (of PATTERSON) — I have not been able to observe either. About this point BAST only says the following:

"In the early stages of division a plate-like structure marks the plane of cleavage."

Morphologically these amitoses are however so characteristic that they ought to be regarded as a separate type.

Fig. 4 shows an amitosis in the stratum cylindricum. We notice that the plane of cleavage forms a right angle with the border between the epithelium and the underlying connective tissue. It was found that amitosis is extremely seldom in the stratum cylindricum in all of the three animal species examined (with one exception which I will recur to below).

Fig. 5 shows a nucleus divided into three by amitosis.

In fig. 6 is seen a cell containing two nuclei undergoing amitotic division. SEVERIN, as mentioned, was unable to discover cells with three or four nuclei in the lingual epithelium of the guinea-pig.

With respect to the behavior of the nuclei in amitotic nuclear division the views held in literature differ from one another. The majority of the authors however thinks it probable that a division of the nucleolus precedes the cleavage of the nucleus as such.

Fig. 7 shows a nucleus in an early stage of nuclear division, the nuclear plate being not very distinct. In either of the nucleus one sees a nucleolus in the state of division assuming the shape of a dumb-bell.

In the discussion of the following micro-photographs I will make reference to what the investigations showed with regard to the quantitative occurrence of amitoses in the three animal species studied. The material examined is rather insufficient but in spite of that the result will be able to give us some idea.

#### **Cat.**

Samples from 4 animals of different age and a fetus of 8 cm. were examined. Amitoses and binuclear cells were found only quite exceptionally.

In fig. 8 is reproduced an amitosis of the type shown in fig. 3 from the fetus examined.

#### **Mouse.**

The material studied in this animal comprises 6 stages of fetal development (6, 8, 10, 12, 13 and 16 mm. respectively), as well as two newborn animals and one full-grown.

In the fetal material the amitoses were rare though more easily discovered than in the cat fetusses examined. Vide the amitosis in fig. 9. In the two earliest stages I was however unable to demonstrate amitoses. In the newborn animals the amitoses were somewhat more frequent, while they were numerous in the adult animal (fig. 1). The number of amitoses to be found in the different stages of development is in accordance with the result of CLARA's investigations on amitoses in liver cells (table after MÜNTZER).

An estimation of the precise number of the amitoses was not attempted, neither by simply counting the amitoses in one section nor by computation using PFUHL's formula. The difference of the number of amitoses in the three groups (fetusses, newborn and fullgrown animals) however was rather obvious.

As is known, the so-called "nucleus centre distance" (Kernmittelpunktabstand) is one of the fixed values in the formula of PFUHL. The larger this distance, the less probable it is that the both nuclei be seen in one and the same section and the more easily the cell will be taken for a mononuclear cell. It is characteristic of my whole material that the two nuclei after the amitosis lie close together. Often they are situated in two different optical planes so that the nuclear membranes intersect when the slide is examined under the microscope. The sections being thin, the distance between the nuclei consequently is also minimal in the direction perpendicular to the surface of the slice.

The nuclei — usually of equal size — either touching or partly covering one another, the Kernmittelpunktabstand (being a magnitude of plane geometry) will consequently become either equal to or less than the diametres of the nuclei.

#### Guinea-Pig.

The guinea-pig material is derived from 2 fetusses (6 and 8 cm respectively) and from 2 full-grown animals. In all the samples examined numerous amitoses were found, in largest number these were however present in the adult animals. Of some interest was the demonstration of amitoses in the two embryos. The direct form of division proved here to be the usual form of tissue proliferation. Only in a few cases I found mitoses on examining a large number of sections from one series. In the adult animals, on the other hand, mitoses in the stratum cylindricum were not

so seldom. This occurrence of mitosis in the two developmental stages is in fact the opposite of what one would have expected. The two fetal stages examined in my material were also characterized by variations in the types of the amitoses observed. Besides amitoses of WASSERMANN's type 6 I saw also representatives of WASSERMANN's type 1 (sand-glass or dumb-bell form). An amitosis of this kind is represented in fig. 10.

Fig. 11 shows a series of amitoses lying side by side. These amitoses are characterized by one half of the nucleus being more deeply stained than the other. Similar findings were made by PATTERSON in the blastoderm of the pigeon egg. This author claims that there be not nucleoli in the deeply staining half of the nucleus, and thinks therefore that the chromatin — otherwise bound to the nucleoli — in these cases be scattered over this half of the nucleus. My sections however show that there are nucleoli in the deeply staining half of the nucleus too; PATTERSON's explanation thus is not sufficient. Possibly this type of amitosis may be seen as the expression of changes really taking place in the structure of the nucleus during the direct cleavage, a view which, according to WASSERMANN, seems rather plausible. This author thinks it namely to be improbable that the nuclear reticulum should remain entirely unaffected by the process of amitosis while the nucleoli (being related structures) are subject to changes.

Also in amitoses of WASSERMANN's type 1 I found the above mentioned peculiarity that the two halves of the nucleus were staining differently. The resting nuclei showed either an entirely normal picture or they were so deeply stained that the different formal elements could only be discerned with difficulty or not at all.

Fig. 12 shows a nucleus with a long outgrowth, the terminal section of which shows a noose-like enlargement. This I took as a gemmation of the type described by MAXIMOW (WASSERMANN's Type 4). The noose stains less deeply than the nucleus as an expression of the fact that at this stage none of the chromatin has passed into the outgrowth. Peculiar filiform structures, which I frequently was able to observe in these sections, and which seemed to protrude from the nucleus, on the basis of this picture receive their natural explanation. This form of gemmation is quite different from the one described by DITLEVSEN for the guinea-pig tongue.

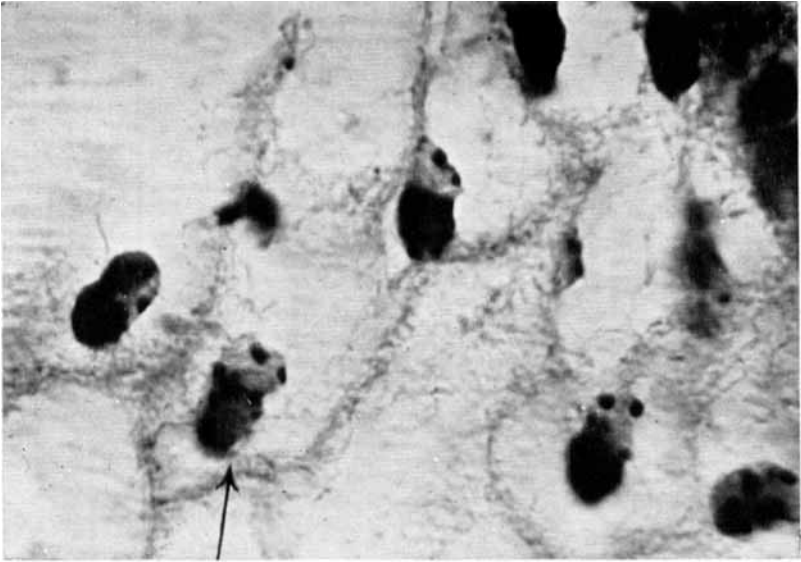


Fig. 11. Material and technique as in fig. 10.  $\times 1350$ .

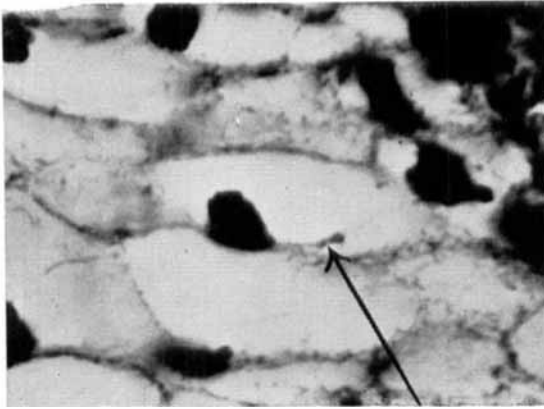


Fig. 12. Material and technique as in fig. 10.  $\times 1350$ .

HALS: Amitosis in the Epithelium.

I am greatly indebted to dr. B. BERGERSEN, professor at Norges Tannlægehøiskole, for facilities of research, and to miss A. BEHRENTZ, cand. real., on the staff of his department for useful advice.

### Summary.

Fetal and postfetal epithelium from the oral cavity was examined in the cat, guinea-pig and mouse.

1. The number of amitoses respectively binuclear cells seems to be proportional to the degree of cornification of the epithelium of the palate in the full-grown mouse.

2. Apart from the epithelium in guinea-pig embryos (6 and 8 cm. respectively) only one type of amitosis was seen, characterized by a more or less oval nucleus with a distinct division line running across its centre WASSERMANN's type 6).

3. The Kernmittelpunktastand (PFUHL) in the binuclear cells formed by this type of direct division of the nucleus as a rule is equal to or less than the diameters of the two (equal) nuclei of the cell.

4. The guinea-pig fetusses showed a variation with respect to the type of amitosis. Most frequent was WASSERMANN's type 6. Besides this both WASSERMANN's types 1 and 4 could be demonstrated. In these preparations amitoses are seen where the two halves of the nucleus are staining differently.

5. One amitotic cell with three nuclei and one cell with two nuclei in amitosis could be demonstrated in a full-grown mouse and guinea-pig respectively.

6. The number of amitoses and binuclear cells in the different developmental stages is different in the three animal species studied:

In the cat amitoses are very rare both in the embryonic and post-natal epithelium,

In the mouse they are seldom in the fetal stages and in newborn animals, numerous however in adult animals, and

In the guinea-pig they are frequent in the embryonic stages and numerous in the adult animals.

### Zusammenfassung.

Untersucht wurde fötales Mundhöhlenepithel bei Katze, Meerschweinchen und Maus.

1. Die Zahl der Amitosen bzw. zweikernigen Zellen scheint im Gaumenepithel der voll entwickelten Maus der Verhornungsgrad direkt proportional zu sein.

2. Abgesehen von dem Epithel der Meerschweinchenembryonen (6 bzw. 8 cm) kam überall nur ein Amitosentypus vor, der gekennzeichnet war durch einen mehr oder weniger ovalen Kern, über dessen Mitte eine deutliche Teilungslinie lief (Typus 6 von WASSERMANN).

3. Der Kernmittelpunktabstand (PFUHL) in den durch diese Form der direkten Kernteilung gebildeten Zellen ist in der Regel kleiner als der Durchmesser der gleichgrossen Zellkerne oder gleich demselben.

4. Die Meerschweinchenembryonen zeigten bezüglich der Amitosentypen ein abweichendes Verhalten. Am häufigsten kam der WASSERMANNsche Typus 6 vor. Daneben liess sich aber auch der Typus 1 und 4 von WASSERMANN nachweisen. In diesen Präparaten sieht man auch Amitosen, wo die beiden Kernhälften ungleich stark gefärbt sind.

5. Ein amitotisch in drei geteilter Kern und eine Zelle mit zwei in Amitose befindlichen Kernen wurden bei einer erwachsenen Maus bzw. einem erwachsenen Meerschweinchen nachgewiesen.

6. Die Zahl der Amitosen und der zweikernigen Zellen in den verschiedenen Entwicklungsstufen war bei den drei untersuchten Tierarten verschieden:

Katze: Amitosen äusserst selten sowohl im embryonalen als auch im postnatalen Mundhöhlenepithel.

Maus: Amitosen selten in den Embryonalstadien und bei neugeborenen Tieren, zahlreich bei den voll entwickelten Tieren.

Meerschweinchen: Amitosen kommen häufig vor in den Embryonalstadien und sind zahlreich bei den voll entwickelten Tieren.

### Résumé.

L'auteur a examiné l'épithèle foetal et postfoetal chez le chat, le cochon d'Inde et le souris.

1. Le nombre d'amitoses respectivement de cellules à deux noyaux semble être directement proportionnel au degré de cornification de l'épithèle du palais chez le souris adulte.

2. Sans compter l'épithèle chez les embryos du cochon d'Inde (6 et 8 cm.), l'auteur ne trouva qu'un seul type d'amitose charac-

terisé par un noyau plus ou moins oval avec une ligne de division distincte traversant la partie centrale (Type 6 de WASSERMANN).

3. Le Kernmittelpunktabstand (PFUHL) (distance entre les centres des deux noyaux) dans les cellules à deux noyaux, formées par division directe de noyau à laquelle on a fait mention au point 2, est habituellement égal au diamètre des noyaux (égaux) ou moins que celui-ci.

4. Les embryos du cochon d'Inde montrent une variation quant aux types d'amitoses. Le plus fréquent est le type 6 de WASSERMANN. A côté de celui-ci l'auteur put démontrer les types 1 et 4. Il put aussi observer des amitoses où les deux moitiés étaient colorées différemment.

5. Les nombres d'amitoses et de cellules binucleaires dans les différentes phases du développement était différent chez les trois espèces d'animaux étudiées:

Chat: Les amitoses sont très rares dans l'épithèle oral fétal et postnatal.

Souris: Les amitoses sont rares pendant la période embryonale et chez les animaux nouveau-nés, nombreuses cependant chez les animaux adultes.

Cochon d'Inde: Des amitoses sont trouvées fréquemment pendant la période embryonale, et chez les animaux adultes elles sont nombreuses.

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