ON MERISTEMATIC CELLS IN STRATIFIED EPITHELIA OF MAMMALS

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in the American University of Beirut

by

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Introduction.

Since the work of August Weismann it is known that protozoa and reproductive cells of metazoa are potentially immortal. More recently it has been proven that somatic cells of metazoa have the same property; their potential immortality has been demonstrated by the cultivation of tissue-cell colonies *in vitro* through an unlimited number of passages. The potential immortality of tissue cells *in vitro* is apparently a result of their arrangement as a colony and of the favorable media. Due to these factors the cells *in vitro* keep their capacity of dividing for an unlimited period.

In plants those somatic cells which retain the ability of dividing are called "meristematic cells". This term has been introduced into the biology of metazoa by K. Bělař (1) and Edm. Mayer (7). Accordingly in the present paper the term meristematic cells will denote somatic cells which retain the ability of dividing throughout the life of the individual. The fact that the somatic cells in tissue-cell colonies *in vitro* are meristematic, leads to another problem: How to explain that in the natural arrangement and situs, i.e., in the intact organ some somatic cells retain this meristematic ability while

others are subject to ageing and death (Edm. Mayer, 16).

Before the study of the problem of meristematic cells in metazoan organisms can be undertaken, it is necessary to know, (1) what methods are available to demonstrate the meristematic nature of a cell in the natural arrangement and situs, and (2) where in the tissues the meristematic cells are located. Is it possible to demonstrate that a cell has kept the ability of dividing throughout the life of the individual? At the present time the only way we have of testing for this meristematic nature is to see these cells in the process of mitosis, either in the adult or in the senile organism. For it is quite clear that if a cell is able to divide in the adult or senile stage of the individual, it must have had that ability in all previous stages. It is not possible to lose a potentiality and then regain it, for, by definition, loss of potentiality is irreversible.

In the present study we shall attempt to locate the presence of meristematic cells in some mammalian stratified epithelia. At the same time we shall try to analyse the possible factors responsible for the mitotic activity. The nictitating membrane of adult rabbits will be the basis for this investigation, because it is particularly suitable for experimentation.

The stratified epithelium of the nictitating membrane has about 5 nuclear layers. Therefore, it seemed useful to also study the distribution of mitosis in a tissue in which the number of nuclear layers is extremely high. A condyloma

acuminatum of man containing in many places more than 30 nuclear layers was used for this purpose.

Finally an attempt was made to study the distribution of mitoses in an epidermoid carcinoma. This type of growth is abnormal in many respects, but still has a stratified epithelial arrangement.

Literature.

In the literature, the work on "meristematic cells" is to be found mainly under the heading of regeneration and particularly the source of the regeneration. Most of the work has been done on invertebrate animals, amphibians and reptiles (cf. the review of Goetsch, 8).

As warm-blooded animals do not regenerate organs, experiments by which the presence and location of meristematic cells can be tested are confined to the regeneration of tissues. This means an increase in difficulties, because the methods for studying the development of tissues are not as advanced as those for studying the development of organs (Fischer and Mayer, 7).

In 1905 an attempt was made by Schaper and Cohen (21) to find the places where cell-division occurs in epithelial tissue of embryos, of regenerative growth, and of tumors of warm-blooded animals. They studied the following embryonic tissues: small intestines, enamel organ, eye, labyrinth, and central nervous system of a number of vertebrates such as man, guinea pig, rabbit, mouse, etc. In some cases they extended their investigations to fishes, amphibians, reptiles, and birds for comparative studies. They also made comparative studies as to the places of cell-proliferation in the epithelium of the small intestines of adult mammals. The investigators concluded that there are "centers of proliferation" in the tissues of vertebrate animals and that these "centers of

proliferation" are approximately the same for all animals examined: i.e., the deeper parts of the crypts of Lieberkühn are the centers of cell-proliferation for the epithelium of the small intestines of cats, rabbits, rats and ground-squirrels. No experimental attempts were made to analyse the factors which may account for the "centers of proliferation".

For regeneration, or what Schaper and Cohen called typical growth as a result of pathological injuries, liver of sheep containing flukes was studied. The authors found that there was a proliferation of bile ducts as a response to the atrophy of liver-cord cells. No other investigations were made; but the literature existing on regeneration is discussed in detail.

The third and last part of the work of Schaper and Cohen deals with malignant tumors, or as they call it, atypical pathological growth. No investigations of their own were made but they discussed the histogenesis of malignant tumors with special reference to Cohënheim's theory, in the light of their conception of "centers of cell-proliferation".

As to the stratified epithelium, Schaper and Cohen realized its particular problems. They did not study it, but they give a schematic drawing which is a "diagramatic representation of the well known conditions of stratified epithelium. The lowest layer which is the focus of proliferation contains the mitoses".* This traditional view that only the basal layer or deep layers of the stratum spinosum contain the mitoses is still found in most textbooks to-day, ~~i.e.~~ e.g., in

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* Verbal translation

the textbook by Maximow and Bloom (15, on page 325, 327, 328), Hartridge and Haynes (10, on page 180), Petersen (20, on page 674) and Darier, Sabouraud, Gougerot, Sézary, Clémont Simon (5, Vol. I, on page 45). More references are found in a paper of Thuringer (22) appearing in 1924, in which is listed an additional number of books and publications maintaining this traditional view.

Thuringer showed definitely that the traditional view was wrong and that mitoses occurred throughout the stratum spinosum as well as in the basal layer of the human epidermis. He divided the stratum spinosum of the human epidermis into inner, middle and outer thirds, and stated the number of mitoses found in each. Unfortunately, however, it is not mentioned how many cell layers there were in each third. In a later investigation Thuringer (23) demonstrated that the younger the individual the greater was the ratio of dividing to non-dividing cells in the epidermis; he also stated that in the prepuce, the mitoses were localized in definite areas. "These growth waves reached a maximum diameter of about 100 μ containing in the principal area as high as fourteen mitotic figures in a single high-powered field". Aside from the variation in age factor, Thuringer* made no attempt to analyse the factors responsible for cell-divison.

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* Thuringer calls the intervals between removal of tissues and fixation also a "factor" which influences mitotic frequency. This point has, however, no bearing upon the problem of the factors responsible for cell-division.

A greater variety of factors which may influence mitotic activity were studied by L. Loeb and collaborators (12,13). These workers published, in a series of articles, their results on the proliferative activity of the epidermis of various warm-blooded animals under different conditions. Their work was based mainly on the epidermis of the ear of the guinea pig under various conditions of age, weight, seasonal temperature, sex, and diet. What is of particular interest, is that these workers tried to define the location of mitoses more accurately by dividing the epidermis into lower and upper layers and stating how many mitotic figures there were in each. L. Loeb and collaborators did not state definitely how many cell layers were to be found in their division of lower and upper layers but the number of cell layers must no doubt be much less than in the human epidermis. Most of the factors studied by this last group of workers are factors that act on the whole animal. Though some regional differences were considered, the aim of their work was not to analyse the local mechanisms of mitotic activity but to see the effect of the mitotic frequency on the structure of the epidermis.

As mentioned before most of the biological papers, which are in some way related to the problem of meristematic cells, deal with regeneration. Other papers, nearer to the realm of medicine, concern wound-healing and tumor-formation: it is difficult to isolate the problem of meristematic cells from this type of publication. Finally there is a large group of papers dealing with "growth". Growth means either

increase in weight or increase in volume. The increase in volume depends on the following factors: (1) cell-division, (2) increase in cell volume, and (3) cell migration. A considerable amount of work has been done on the effects produced on complex phenomena like wound-healing by various chemical, physical, and biological agents. On the other hand, the effect of different agents on single factors of growth has been carefully studied, particularly the effect on mitotic division. It would be beyond the scope of this paper to discuss this enormous literature, as the present study is confined to stratified epithelium of mammals.

Material and Methods.

A stratified epithelium consists of layers of cells or layers of nuclei superimposed one on top of another. We have used the layers of nuclei throughout. The first nuclear layer is considered the one proximal to the corium, the other nuclear layers are successively numbered from the corium to the free surface. By recording the number of the nuclear layer in which a mitosis occurs, we not only characterize the mitoses for comparable studies by localizing them but at the same time their distance from the corium is stated approximately. Amitosis is not to be considered in this paper.

A. The nictitating membrane of the adult rabbit. The stratified epithelium of the nictitating membrane of the adult rabbit is used to study the distribution of mitoses under the normal conditions and under some abnormal conditions. The membrane not touched in any way, except for its removal from the animal at the time of study, is the one under normal conditions, i.e., control membrane. To produce abnormal conditions in the membrane, either an incision was made, or a "hyperemia"* caused by "foreign body" irritation was caused. The latter two are called the experimental membranes.

1. Nictitating membrane under normal conditions.

a. The nictitating membrane of the adult rabbit

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* "Hyperemia" is the term used in the language of pathology to denote increased blood supply. The increased blood supply to the tissue may result either from an increase in the number of patent capillaries or from a dilatation of the capillaries or from both.

is macroscopically a more or less triangular structure situated in the medial side of each eye (See Fig. 1.). It is approximately 1 cm. in its largest cephalic-caudal direction and 1 cm. in its medial-lateral direction. Conditions of blood circulation can be easily estimated because there are no hairs, ducts, keratinization, or other disturbing factors present on the nictitating membrane. It is well protected from injuries, due to the eyelids and its position.

b. Microscopically, the histology consists of: (Fig. 2.)

(1) Anterior and posterior surface of stratified epithelium continuing into each other over the free border. The anterior surface has an average of five nuclear layers, and the posterior surface an average of three nuclear layers. Goblet cells may be present, in greater numbers on the posterior surface than on the anterior surface. Melanin pigment is present mostly on the free margin. Morphologically, the cells in the higher layers including the top, except for some flattening, are the same as those in the lower cell layers.

(2) Corium: Consisting of loose connective tissue, blood vessels, and some mucous glands.

(3) Central plate of cartilage, which supports the nictitating membrane by extending through-

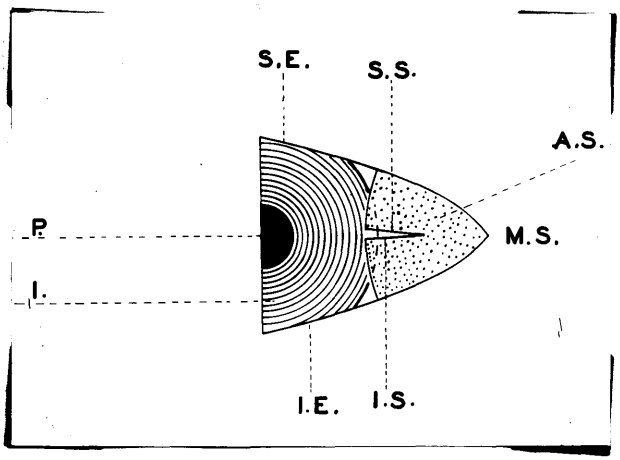

Fig. 1 . Macroscopical representation of the right nictitating membrane of the rabbit , illustrating its position and the place of incision (about x 2) .
A.S. anterior surface ; I. iris ; I.E. inferior eyelid ; I.S. inferior edge produced by incision ; M.S. medial side of the eye ; P. pupil ; S.E. superior eyelid ; S.S. superior edge produced by incision.

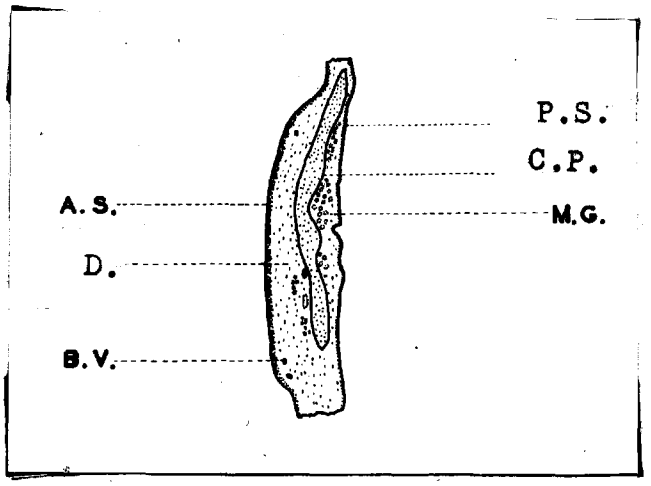

Fig. 2 . Diagrammatic histological section of the nictitating membrane in sagittal plane. All control and experimental membranes were sectioned in sagittal plane (about x 5) .
A.S. anterior surface ; B.V. blood vessel ; C.P. cartilage plate ; D. corium ; M.G. mucous glands ; P.S. posterior surface.

out its length.

c. Type of animal used: Adult rabbits of both sexes, ranging in weight from 1600 to 2600 grams.

d. Food: Bran and barley mixtures, about 125 grams per rabbit, were given once a day; greens were given two or three times a week. Fresh water was given daily.

e. Quarters: Bins were cleaned once a week; cages twice a week. No heating facilities were used during the rainy season or winter.

2. Nictitating membrane under abnormal conditions:

a. Experimental procedure for experiments on incised membranes. The procedure for anesthesia used throughout, was as follows: an assistant held the animal down on a table by its legs and 1 c.c. of nembutal per kilogram of body weight was injected intraperitoneally. About twenty minutes later, ether was administered to complete the anesthesia. The animals seldom squealed, which may be used as an indication that the animals, though they struggled, were not handled too roughly.

One nictitating membrane of each of three rabbits was incised at about the center reaching from the free border to 3 to 5 mm. toward the medial end (see Fig. /.); no aseptic technique was attempted, the incision was merely washed with Ringer's solution several times immediately after the incision. At

some definite interval of time, 31 to 72 hours after the incision, the incised and non-incised membranes of each rabbit were removed under nembutal and ether anesthesia and treated as follows:

(1) Either placed onto pieces of cork and held there with glass pins or placed on pieces of filter paper.

(2) Fixed in Bouin's or Zenker's solution.

(3) Paraffin embedded and sectioned (not in serial order) 7 to 10μ in thickness.

(4) Stained with hematoxylin and eosin solutions.

(5) Mitoses: the phase, location, and number in the anterior surface of a number of sections of each nictitating membrane was recorded.

For further studies on mitoses:

(1) Mitotic index of a few sections of a few nictitating membranes was recorded, the whole anterior surface of each section of a membrane was used for these counts.

(2) Distribution of mitoses in relation to the incised surface of 4 sections was recorded.

b. Experiments on hyperemia without wounds: Hyperemia was produced in nictitating membranes for the study of the factors responsible for mitoses. Only a few rabbits were used in these experiments, and of these, the hyperemia produced by chemical irritants such as croton oil, proved to be unsatisfactory

because of necrosis of the epithelium. In one rabbit, hyperemia of one nictitating membrane was caused by placing a piece of iron wire into the conjunctiva of the superior eyelid, at some distance from the nictitating membrane so as not to injure the membrane when the lids were moved. The piece of iron wire did not exceed 5 mm. in length and was less than a millimeter in diameter. The ends were not very sharp. After piercing the conjunctiva the wire was held in place by bringing the ends together by means of pincers.

About 17 hours later the hyperemic nictitating membrane and the membrane of the other side were removed under nembutal and ether anesthesia, and were fixed, sectioned, and stained as described above. The mitotic index of several sections of each membrane was recorded.

B. To study the distribution of mitoses in a stratified epithelium with an enormous number of nuclear layers, human venereal wart (condyloma acuminatum) was used.

Biopsy Number: 9417.

Sex: Male.

Age: 54 years old.

Treatment of specimen:

1. Formalin fixation.
2. Paraffin embedding: serially sectioned about 10μ in thickness.

3. Stained with hematoxylin and eosin solutions.

4. Mitoses: number, phase, and location in the different nuclear layers were recorded. If a mitotic figure was found above the 6th nuclear layer, the mitosis or the area in which it was found was searched for in adjacent sections. In those cases in which a marked difference was noted, as the disappearance of area, or the encroachment of the corium on that region, the original nuclear layer in which the mitosis was found was reduced to a lower level.

C. To study the distribution of mitoses in a stratified epithelium of a type of growth which is different in some respects from the human condyloma acuminatum and nictitating membrane of the rabbit, human epidermoid carcinoma was used.

Biopsy Number: 8970.

Sex: Female.

Age: 60 years old.

Treatment of specimen:

1. Formalin fixation.

2. Paraffin embedding: serially sectioned about 10μ in thickness.

3. Stained with hematoxylin and eosin solutions.

4. Mitoses: number, phase, and location in the different nuclear layers were recorded. If a mitosis was found above the 4th nuclear layer, the mitotic figure or the region in which it was found was

searched for in adjacent sections. In those cases in which a marked difference was noted, as the disappearance of the region, or the encroachment of the corium on that region, the original nuclear layer in which the mitosis was found was reduced to a lower level. No distinction was made in considering the mitoses, whether they were found in (1) definite carcinoma islands, (2) epidermis which morphologically appeared normal, or (3) strands of epithelium connecting (1) and (2).

Observations and Discussion

A. Nictitating Membrane of the Rabbit.

1. Observations and discussion on the incised nictitating membranes:

a. Macroscopical findings: The experimental membranes, i.e., incised membranes, rarely showed any signs of suppuration in the interval between incision and removal. The condition of blood circulation of each experimental and each control membrane was recorded immediately before the administration of ether for their removal. In the controls, very few vessels were visible whereas the experimental membranes were definitely hyperemic or red. On the administration of ether the control nictitating membrane sometimes became slightly hyperemic.

b. Microscopical findings:

(1) Many blood vessels of the experimental nictitating membranes were dilated and congested. In the controls, on the other hand, the number of patent and dilated vessels was less and it is not excluded that this might be due to the ether.

(2) There was a cellular and fluid exudate in the corium of the experimental but not in the control membranes.

(3) There was an infiltration with leucocytes of the stratified epithelium of the experimental membranes in many places which was not present in the controls.

(4) There were crevices or spaces between the epithelial cells in the experimental membranes which were practically absent in the controls. Most probably these crevices were due to fluid exudate. At the time of the investigations, namely 31 hour s, 48 hours, and 72 hours, there was practically no necrosis of the stratified epithelium of either the control or experimental membranes. At these respective periods, the separated surfaces, produced by the incision in all the experimental nictitating membranes, were completely covered over with epithelium of approximately the normal number, or more, of nuclear layers. Therefore, since morphologically the separated surfaces produced by the incision appeared fully healed, we designated the above experimental membranes as "post-regenerative", so that one may not confuse it with the process of wound healing proper.

c. The distribution of the mitoses in the different nuclear layers is recorded in Table 1.

Table I. Absolute number and percentage of mitoses occurring in the different nuclear layers of normal (C) and post-regenerative (E) stratified epithelium of the nictitating membrane of the rabbit. The nuclear layers recorded in the table are the "corrected layers" (i.e. reduced to a comparable basis, cf. page (8).

Rabbit No.	Sex, Weight (gms.)	Interval between incision and removal.	Category E-experim. C-control	Total No. mitoses	1st N.L.*		2nd N.L.		3rd N.L.		4th N.L.		5th N.L.	
					absolute No.	Per cent	absolute No.	Per cent	absolute No.	Per cent	absolute No.	Per cent	absolute No.	Per cent
No.6	♀ 2600	2 days	C (g17)	7	5	71	2	29	—	—	—	—	—	—
			E (g18)	282	144	51	90	32	32	11	12	4	4	1
No.7	♂ 1700	3 days	C (g21)	5	5	100	—	—	—	—	—	—	—	—
			E (g22)	98	62	63	28	29	8	8	—	—	—	—
No.9	♀ 1 week pregnant 1700	31 hrs.	C (g23)	7	4	57	3	43	—	—	—	—	—	—
			E (g24)	163	104	64	50	30	8	5	1	1	—	—
No.10	♂ 1650	—	C (g26)	70	39	56	26	37	5	7	—	—	—	—
No.11	♂ 1600	—	C (g27)	112	58	52	48	43	6	5	—	—	—	—
Total for control			5 rabbits.	201	111	55	79	39	11	5	—	—	—	—
Total for experiment			3 rabbits	543	310	57	168	31	48	9	13	2	4	1

* N.L. = nuclear layer.

(1) Table 1. Explanatory notes: There are two factors that might make comparison between experiments and controls difficult. These are, (a) elastic retraction of the epithelium due to removal of the membrane from the situs, and (b) tangential or oblique sectioning. Both might lead to an artificial increase in the number of nuclear layers or to a false recording of the nuclear layer in which a mitosis occurred (see Fig. 3.). To overcome this difficulty we have "corrected", to a comparable basis, the absolute nuclear layers in which the mitoses

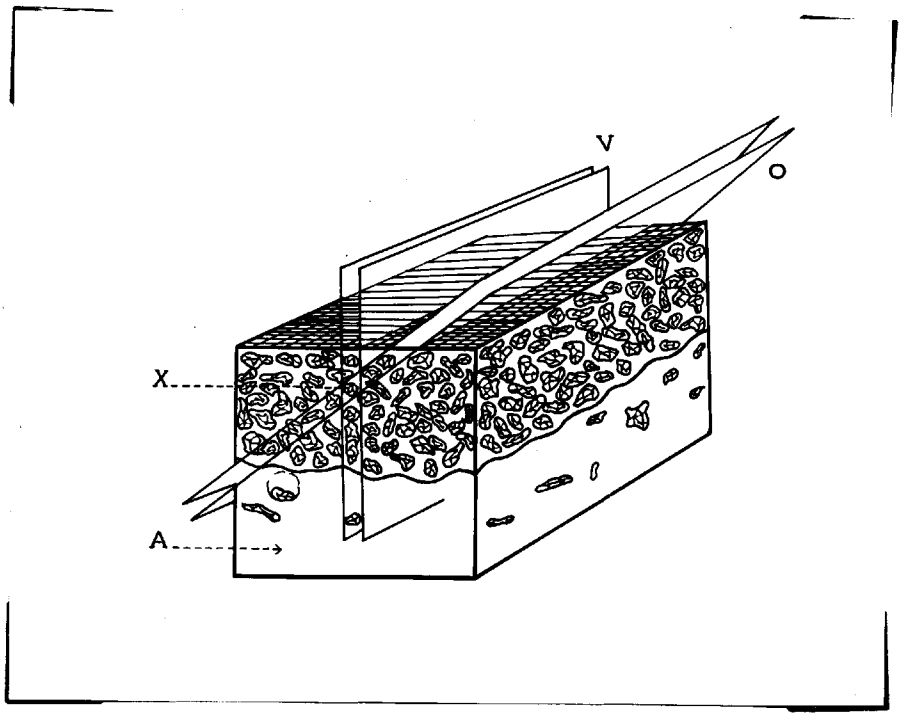

Fig. 3 . Diagram illustrating the procedure by which nuclear layers (**N.L.**) are corrected for oblique sectioning.
A. plane used for illustrating the different effects of section V and section O ; O. section oblique to surface of the membrane ; V. section vertical to surface of the membrane. X. a nucleus in 4th N.L. of section V , but 6th N.L. of section O . 5 - 6 is the total number of N.L. in section V. 5 N.L. are used as standard. Total number of N.L. in section O is 8 .

$$\frac{\text{standard number of N.L.}}{\text{absolute number of N.L.}} \times \text{N.L. in which mitosis is found}$$

= "corrected" nuclear layer in which mitosis is found.

Therefore in this case $\frac{5 \times 6}{8} = 3\frac{3}{4}$ or 4th N.L. is the corrected N.L. in which nucleus X in the oblique section O would be recorded in Table 1 .

occurred, as follows: there were many places in the controls and experimental membranes which were obviously vertically cut, the average number of nuclear layers of these places was five; absolute range is 2 to 12 nuclear layers. All the nuclear layers, which contained mitoses and which were found in places where the total number of nuclear layers was greater than five, were reduced proportionally to this standard-5 (see Fig. 4). No correction was made if the mitosis was found in the first nuclear layer; obviously it could not be reduced lower.

The sum of the number of mitoses of the control membranes of rabbits Nos. 6, 7, and 9 is 19, while the sum of the number of mitoses of the experimental membranes of the same rabbits is 543. Therefore, we have used the membranes under normal conditions, of two other rabbits (Nos. 10 and 11) to raise the total number of mitoses for the controls. Though 201 mitoses for the controls are still not equal to the number in the experiments still the discrepancy is by no means as great as before. The control nictitating membranes of rabbits No. 10 and No. 11 are comparable to the other three controls because their mitotic indices are of the same order of magnitude (cf. Table 2 and discussion). The number of sections of each nictitating membrane of rabbits No. 10 and No. 11 counted, was greater than those of controls of

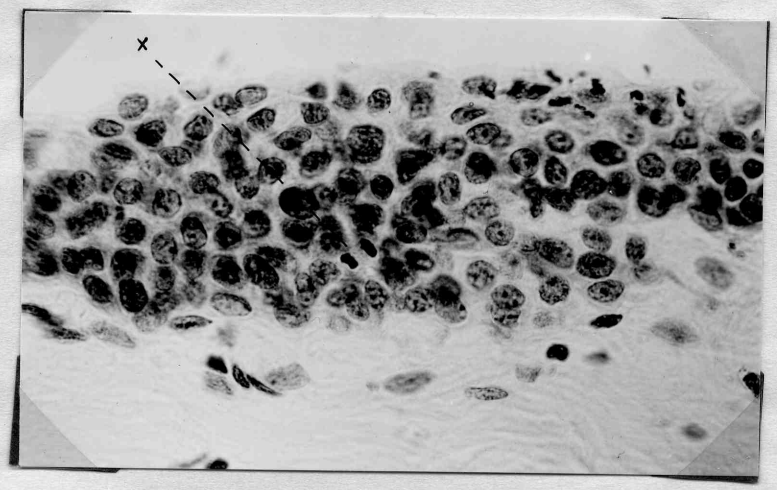

Fig. 4 . Photomicrograph of control nictitating membrane of Rabbit No.9 illustrating the necessity of correcting nuclear layers (N.L.) in case of oblique sectioning (cf. Fig.3) . x 500 .
X mitotic figure in telophase in 2nd N.L.
Total absolute number of N.L. in this region is 7 ; at either end of the photomicrograph the total absolute number of layers is about 5.
The correction is as follows :

$$\frac{\text{standard number of N.L.}}{\text{absolute number of N.L.}} \times \text{N.L. in which mitosis is found}$$

$$= \frac{5 \times 2}{7} = 1\,3/7 = \text{1st "corrected" N.L. as recorded in Table 1 .}$$

of Rabbits Nos. 6, 7, and 9. This accounts for the greater number of mitoses in each of the nictitating membranes of Rabbits Nos. 10 and 11.

In the column headed category, the figures in parentheses indicate the label of the paraffin block in which the nictitating membrane was embedded.

(2) Table 1. Results and discussion:

(a) The percentage of mitoses in the different "corrected" nuclear layers in both the control and experimental nictitating membranes, decreases as one proceeds from the corium towards the free surface of the membrane.

(b) In the control membranes, the mitoses are found in the lower three out of five "corrected" nuclear layers, while in the experimental membranes mitoses are found in all five of the "corrected" nuclear layers. Out of the four mitoses recorded in the 5th "corrected" nuclear layer, one was situated in the very top layer (see Fig. 5.) and the other three were situated in other layers (a mitosis in the 9th absolute nuclear layer would be reduced to the "corrected" 5th nuclear layer if the total number of nuclear layers at that particular region was 10). Assuming that the number of mitoses for the controls is sufficient, and that we are justified in reducing the absolute nuclear layers

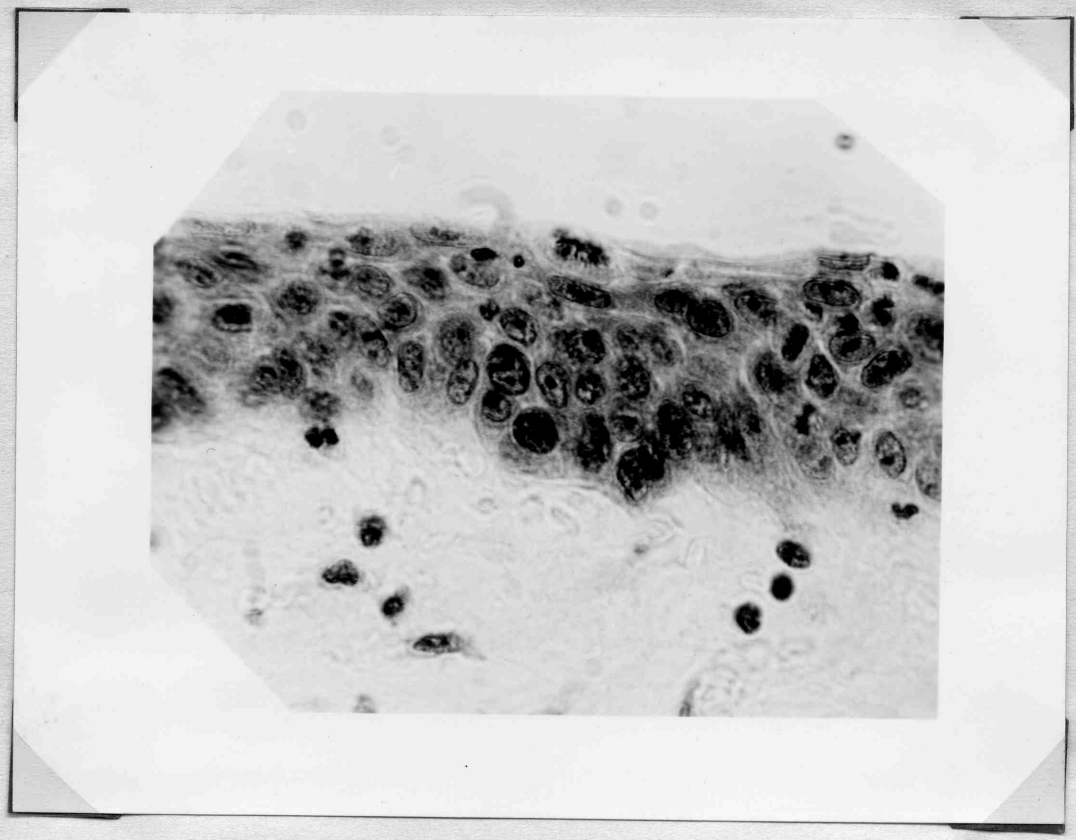

Fig. 5 . Photomicrograph of experimental nictitating membrane of Rabbit No. 6 illustrating a mitosis in metaphase in the very top nuclear layer (5th nuclear layer). x 700 .

to some common basis, what conclusions can be drawn? Is it justified to state that all of the cells, for example, in the basal layer or in the first nuclear layer of a stratified epithelium are able to divide because some cells are found in that layer in the process of division? It is impossible to decide this question by fixed and stained histological sections. To decide it, it is necessary to observe the epithelial cell layer in the living animal. It is best to do this in a single cell layer, as for example, the surface of the cornea or, at most, in a two or three cell layered epithelium, but this is technically rather difficult. The traditional view seems to be that it is justifiable to consider the whole epithelial layer as being able to divide if some cells in that layer are seen in division. In keeping with the general opinion, we will say, that since all the nuclear layers showed some mitoses, all the cells of that stratified epithelium are able to divide. It will be recalled that in the introduction, it was stated that meristematic cells are those somatic cells which retain the ability of dividing throughout the life of the organism. Since these experimental membranes are from the adult animal it is perhaps warrantable to say that all the cells in

this stratified epithelium of the nictitating membrane of the rabbit are meristematic.

In the controls only the inner three-fifths of the epithelium is meristematic, while in the experimental membranes all of the epithelium is meristematic. Therefore, by producing abnormal conditions, it is demonstrated that more cells have the potentiality of dividing than one would conclude from observations under normal conditions. This is true of other tissues as well. Liver-cord cells are seldom seen in division under normal conditions, but if a partial hepatotomy is done, or injury of liver-cord cells is produced, there is a vigorous regeneration of the remaining liver-cord cells (2, 6, and 11). The tubular epithelial cells of a kidney will divide if the other kidney is removed or destroyed (MacCallum, 14, on page 75).

The next problem that comes up is what are the conditions or factors which are responsible for the release of the mitotic division of the meristematic cells of the upper two-fifths of the stratified epithelium of the nictitating membrane of the rabbit. A comparison of the experimental to the control membranes brings out a number of possible factors which are present in the experimental membranes, but which are

either not present or are present to a very limited degree in the control nictitating membranes. We shall divide these factors into visible morphological factors and non-visible factors. The visible factors are (1) increased blood supply, called "hyperemia" (see Figs. 6. and 7.), (2) formation of crevices between the epithelial cells (see Figs. 8. and 9.), and (3) the appearance of leucocytes in the stratified epithelium. The possible non-visible factors are (1) re-arrangement of the different components of the tissue due to its elastic retraction after the incision, (2) liberation of "special substances" resulting from tissue-destruction in the place of the vicinity of the incision, and (3) nervous influences. The factors mentioned in both groups are caused by the "complex stimulus" applied, namely the incision.

d. In order to analyse the factors above it is necessary to characterize the mitotic frequency in a better way than by stating the total number of mitoses and their percentage distribution per layer. The standard which is best suited for this type of investigation is the "mitotic index" of Champy. This index is expressed by: $\frac{\text{number of mitoses} \times 1000}{\text{total number of nuclei}}$. In Table 2 the mitotic indices are given for, (1) four sections from the experimental nictitating membrane of Rabbit No. 6, removed 2 days after incision; (2) four sections from the

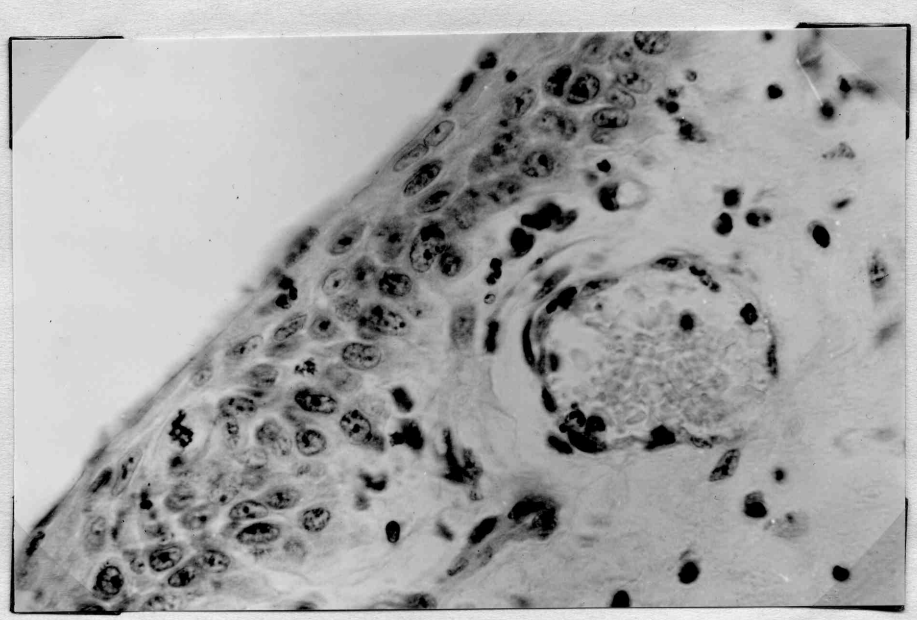

Fig. 6 . Photomicrograph of experimental nictitating membrane of Rabbit No. 6 illustrating 1) dilated blood vessel in corium , 2) two mitotic figures in metaphase in the 4th nuclear layer , 3) leucocytes in the corium . x 500 .

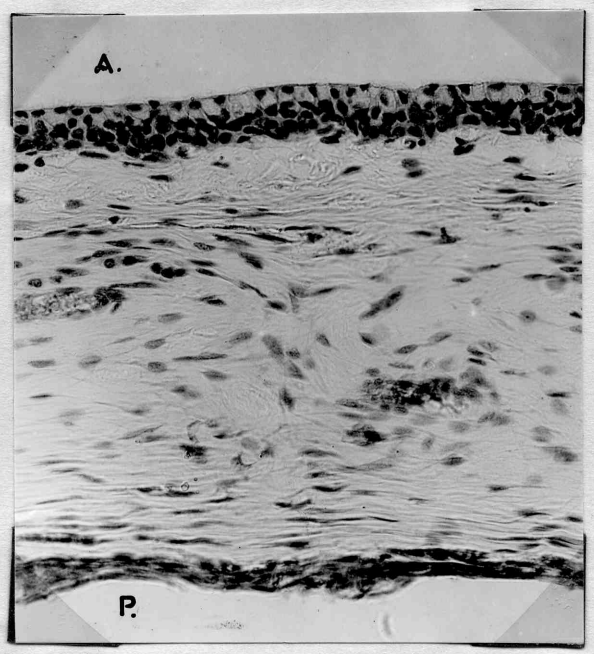

Fig. 7 . Photomicrograph of control nictitating membrane of Rabbit No. 11 illustrating 1) corium - no dilated blood vessels ; no leucocytes.
2) Goblet cells : A. anterior surface, P. posterior surface of membrane .
x 230 .

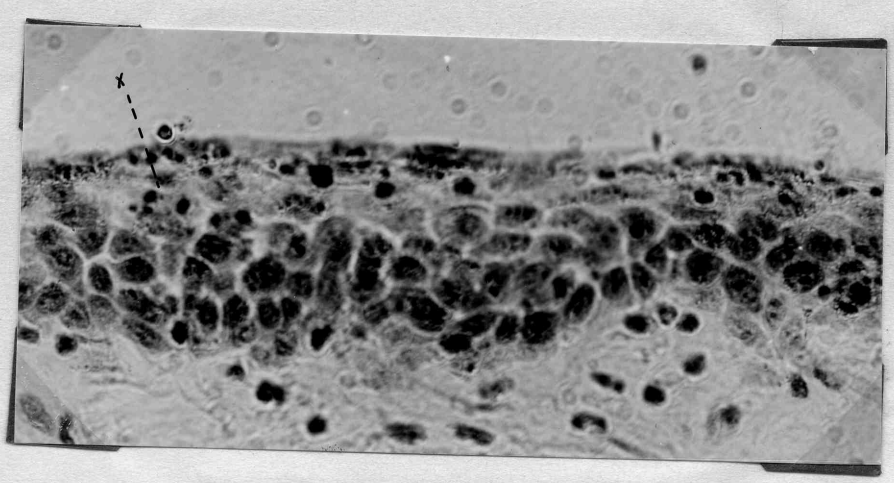

Fig. 8 . Photomicrograph of experimental nictitating membrane of Rabbit No. 6 illustrating the presence of crevices between epithelial cells .
X mitosis in telophase in 5th nuclear layer. Leucocytes in more superficial layers of the epithelium . x 500 .

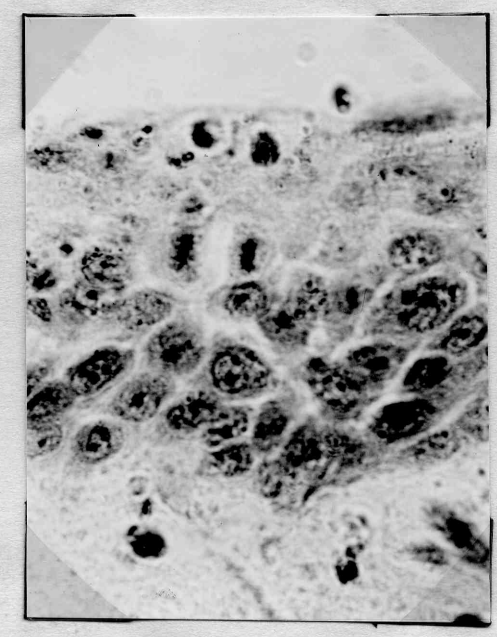

Fig. 9 . Photomicrograph of experimental nictitating membrane of Rabbit No. 6 illustrating the presence of crevices between epithelial cells. Mitosis in telophase in 4th nuclear layer. x 700 .

control membrane of the same rabbit; and (3) one section each from the control nictitating membranes of Rabbits Nos. 10 and 11 respectively.

Table 2. Mitotic indices of normal (control) and post-regenerative (experimental) stratified epithelium without considering the nuclear layers. Nictitating membranes of rabbits No: 6 (control and experimental) and Nos: 10 and 11 (controls only – cf. table 1 and page 24) were used.

$$\text{Mitotic index} = \frac{\text{number of mitoses} \times 1000}{\text{total number of nuclei}}$$

control				experimental			
rabbit (R) section (S)	No: of nuclei	No: of mitoses	mitotic index	mitotic index	No: of mitoses	No: of nuclei	rabbit (R) section (S)
R. No: 6 S. No: 16	5135	3	0.6	8.5	32	3768	R. No: 6 S. No: 18a
R. No: 6 S. No: 80	5538	3	0.5	11.1	53	4780	R. No: 6 S. No: 18b
R. No: 6 S. No: 82	5422	4	0.7	8.5	53	6199	R. No: 6 S. No: 20a
R. No: 6 S. No: 83	5344	3	0.6	7.1	52	7300	R. No: 6 S. No: 20b
R. No: 10 S. No: 46	8946	7	0.8	—	—	—	———
R. No: 11 S. No: 70a	6725	11	1.6	—	—	—	———
total	37110	31	——	——	190	22047	total
average	——	——	0.8	8.6	——	——	average

(1) Table 2. Explanatory notes:

(a) The experimental nictitating membrane of Rabbit No.6 is used because here mitoses were found in all of the 5 "corrected" nuclear layers of the stratified epithelium. The control nictitating membranes of Rabbits Nos. 6, 10, and 11 are used in order to compare them with each other and with the experimental membrane of Rabbit No. 6.

(b) The mitotic index recorded refers to the entire anterior surface of each section. This proved to be necessary because it was found that there are "waves" of mitoses along this surface (see Figs. 10 and 11). Therefore, by choosing microscopic fields at random, extremes may be struck which would make results non-comparable, especially if the fields counted were few.

(2) Table 2. Results and discussion:

(a) The order of magnitude of all the control sections is the same.

(b) The order of magnitude of all the experimental sections is ten times that of the controls. At the time of the examination, 2 days after the incision of this experimental nictitating membrane, the wounded surface was found to be completely covered with epithelium. It is surprising to find a mitotic index ten times higher than that of the controls in a period which is "post-regenerative" rather than regenerative. Consequently, the same experimental nictitating membrane offers two different problems: (1) How to account for the occurence of mitoses in all 5 of the "corrected" nuclear layers when in the controls they only occur in the inner 3 layers? and (2) How to account for the mitotic index being ten times that of the controls?

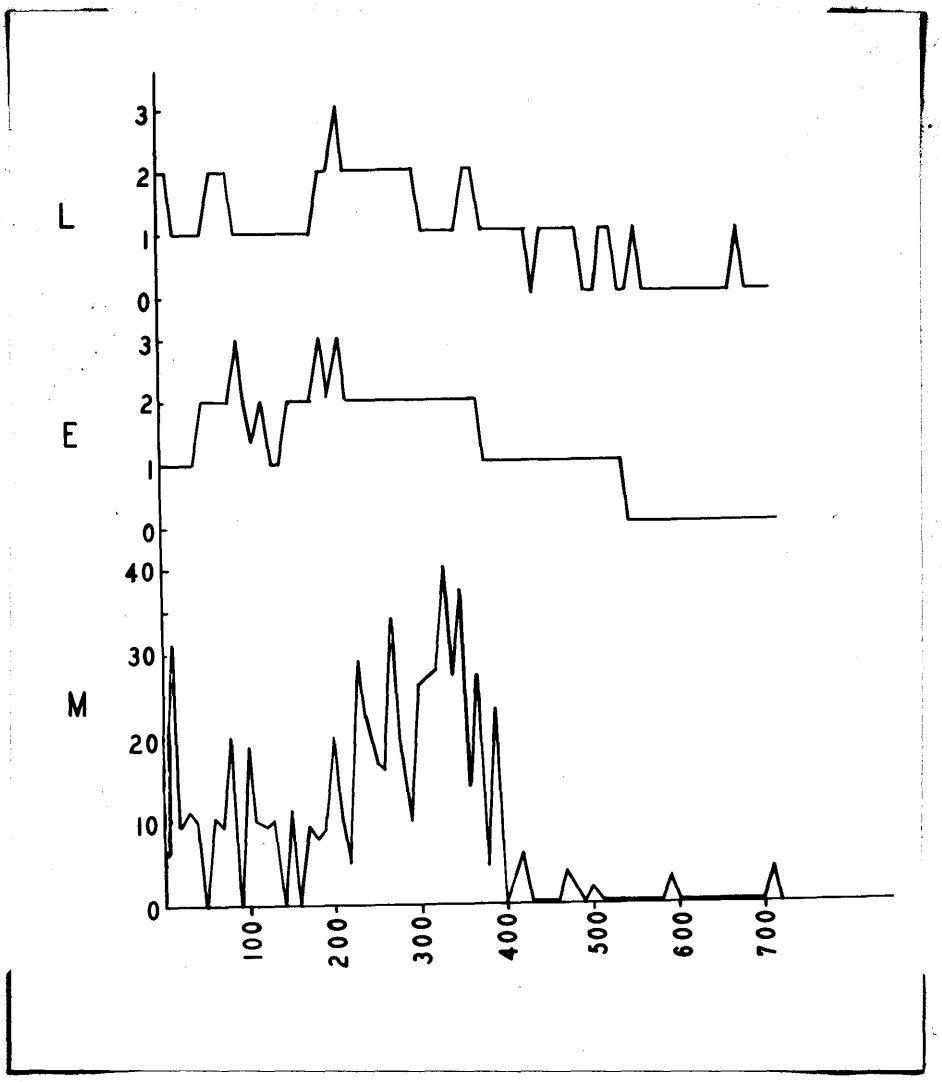

Fig. 10 . Abscissa = distance in ocular micrometer units from the artificial superior edge produced by incision towards superior eyelid (see Fig. 12,part A).
1 ocular unit = 12 µ .
Ordinates :
M = mitotic index
E = "degree" of looseness of epithelium
L = "degree" of leucocytic infiltration.

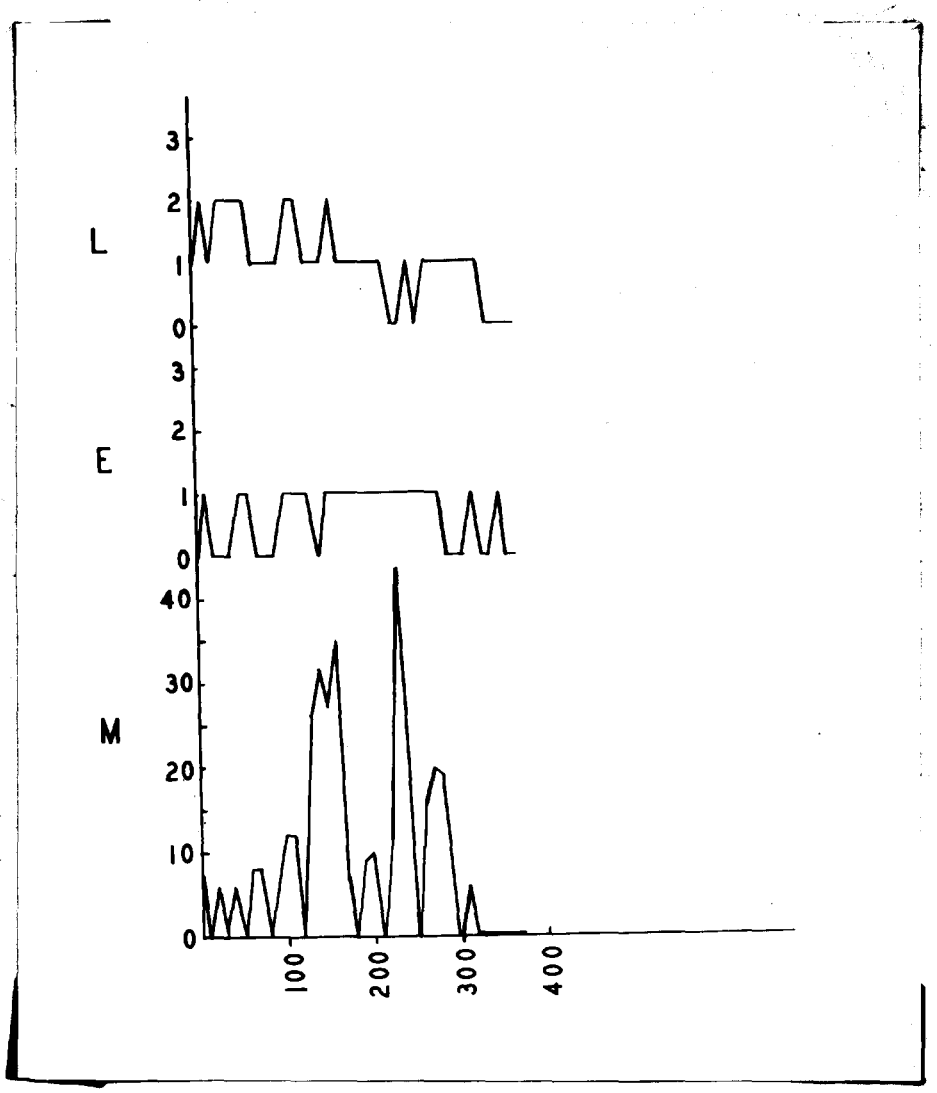

Fig. 11 . Abscissa = distance in ocular micrometer units from the artificial inferior edge produced by the incision toward inferior eyelid (see Fig. 12,part B).
1 ocular micrometer unit = 12 µ .
Ordinates :
M = mitotic index
E = "degree" of looseness of epithelium
L = "degree" of leucocytic infiltration.

It is very probable that both problems are related to the same factor or same set of factors. Using the mitotic index, the difference in the order of magnitude between the controls and experimentals was very large. This method was used to study and analyse the factors which might be responsible for the increase, not only in the mitotic index, but also in the increase of the number of nuclear layers in which the mitoses are found, providing it is justified to assume that they are related.

e. The first two factors studied were the crevices between the epithelial cells and the leucocytic infiltration of the stratified epithelium.

(1) Figures 10 and 11 Explanatory notes:

In addition to the mitotic index, the degree of "looseness" of the epithelium and the degree of leucocytic infiltration of the epithelium for each successive microscopical field of the anterior surface of 4 sections were recorded. Each microscopical field was a strip of epithelium 120μ in length. The sections were from the experimental nictitating membrane of Rabbit No. 6.

(a) The degree of "looseness" of the epithelium was recorded as follows:

o degree..... no crevices between cells.

1st degree..... few and narrow crevices between cells.

2nd degree..... crevices wider and more numerous

than those of the 1st degree.

3rd degree..... spaces between the epithelial cells so great that a network arrangement of the epithelial cells substituted for the usual mosaic arrangement.

(b) The degree of leucocytic infiltration of epithelium was recorded as follows:

o degree..... no leucocytes were present.

1st degree..... few leucocytes, i.e., up to 5, were present.

2nd degree..... moderate number of leucocytes, i.e., 5 to 15 were present.

3rd degree..... a great number of leucocytes, i.e., above 15 were present.

In many places the degree of leucocytic infiltration and of "looseness" of the epithelium are parallel but they are not necessarily identical (see Figs. 10. and 11.), because in some places crevices may be seen with no leucocytes infiltrating and vice versa, leucocytes without crevices. That the presence of crevices is not all due to technique can be demonstrated by the fact that the control membranes, treated in the same manner, showed little or no crevices. Of course, leucocytic infiltration cannot be a matter of technique. Figures 10. and 11. give the averages of the observations of 4 sections plotted against

the distance, in ocular micrometer units, from the incised surface of the membrane towards superior and inferior eyelid respectively. Each ocular micrometer unit is equivalent to 12μ. Fig. 10. represents part A in Fig. 12. and Fig. 11. represents part B in the Fig. 12. There are no definite limits to either superior or inferior surface produced by the incision; the limit of the original wounded surface could not be ascertained, therefore, the limits were arbitrarily chosen as those places where the surfaces no longer were markedly curved (places marked with x in Fig. 12.).

(2) Figures 10. and 11. Results and discussion:

(a) The distribution of mitoses, along the anterior surface of the sections of the experimental nictitating membranes studied, is not a uniform one. There are definite "waves" or crops of mitoses. Furthermore, the largest waves of mitoses are not at the surfaces produced by the incision, as one might expect, but at some distance inferiorly or superiorly from the incised surface, i.e., 3 to 4 mm. for the incised surface in Fig. 10. and 2 to 3 mm. in Fig. 11.

(b) There seems to be no direct relation between the degree of "looseness" of the epithelium and the mitotic index. A low degree

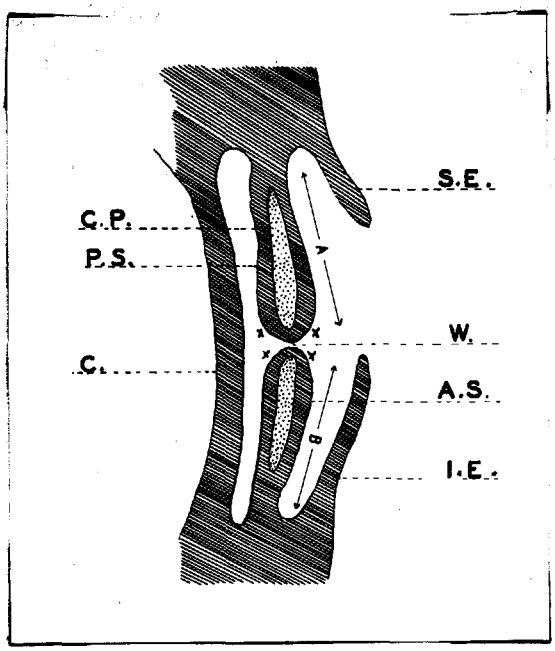

Fig. 12. Diagrammatic sagittal section through incised nictitating membrane, 2 days after incision, illustrating surface used for Figs. 10 and 11 . About x 5 .
A anterior surface used for Fig. 10.
B anterior surface used for Fig. 11.
A.S. anterior surface ; C. cornea ; C.P. cartilage plate ; C.S. conjunctival sac ; I.E. inferior eyelid ; P.S. posterior surface ; S.E. superior eyelid ; XX chosen limits of superior and inferior surfaces of W. place of incision.

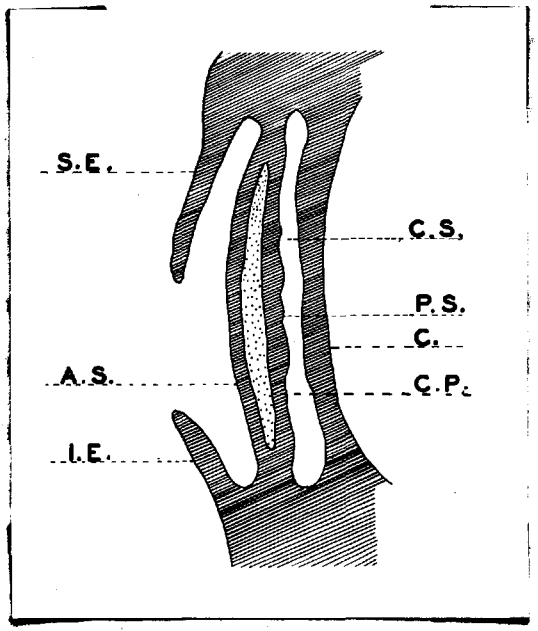

Fig. 13 . Diagrammatic sagittal section of normal nictitating membrane illustrating relation to eyelids. About x 5 .
A.S. anterior surface ; C. cornea ; C.P. cartilage plate ; C.S. conjunctical sac ; I.E. inferior eyelid ; P.S. posterior surface ; S.E. superior eyelid.

may coincide with a high mitotic index and vice versa.

(c) There seems to be no direct relation between the degree of leucocytic infiltration and the mitotic index.

The last two observations do not exclude the possibility that there may be an indirect effect or an optimal degree which may be important. Crevices or spaces increase the rate at which substances may diffuse in and out of the cells as well as in between the cells; and also both from the corium outward as in the reverse direction. The exchange of substances, whether they be the normal nutritional ones, oxygen, wastes, or "special substances" is materially favored. Therefore, if not the only factor, crevices or spaces in between the cells may be a contributing factor to the increase of mitoses in the experimental conditions. Leucocytic infiltration may have the effect of helping to produce crevices; they may further alter the environment of the cells by (1) possibly producing "special substances" favorable to cell-division, and (2) interfering with the nutrition of the epithelial cells if too many of the leucocytes are present in the vicinity. It is not possible to measure directly the duration of either the leucocytic infiltration or the presences of crevices; obviously they are neither permanent nor static factors. Therefore, some caution is needed in drawing conclusions from these experiments; other factors should also be considered.

2. Observations and discussion on the hyperemic nictitating membranes: As mentioned above, one of the visible factors of the "complex stimulus", i.e., the incision and its consequences, is the hyperemia. Therefore, it seemed useful to produce hyperemia alone without producing any incisions or wounds of the nictitating membrane. In this way, it was hoped that the rôle of the different factors could be narrowed down.* The use of chemical irritants was not feasible, for though they produced a very marked hyperemia they also caused some necrosis of the epithelium of the membrane. Therefore, "foreign" bodies were used in order to produce the hyperemia. A piece of iron wire 5 mm. long and less than 1 mm. thick, was placed into the conjunctiva of the eyelid, at some distance from the nictitating membrane to avoid injuring it.

a. Macroscopical findings: After some time a hyperemia resulted not only of the conjunctiva but of the nictitating membrane of that side as well. The number of visible blood vessels of the nictitating membrane on the side of the wire, though not as great as that found in membranes treated with chemical irritants, was markedly greater than that of the control nictitating membrane of the opposite eye. A few preliminary experiments showed that the hyperemia of the nictitating membrane appeared about 1 hour after the wire was in place and that it continued as long as the wire remained in its place. For mitotic counts the experimental and control

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* From another point of view, Dr. O. Krayer had also suggested that we might examine the effect of circulatory factors on mitotic activity.

nictitating membranes of only one rabbit (No. 52) were used. The membranes were removed 17 hours after the wire had been placed in the conjunctiva of one eyelid.

b. Microscopical findings:

(1) The control nictitating membrane did not differ from the controls of all the previous experiments.

(2) The experimental hyperemic membrane showed the following characteristics:

(a) No necrosis of the epithelium.

(b) Some exudation of leucocytes and fluid.

(c) Leucocytes in the stratified epithelium.

(d) Crevices between the epithelium.

The degree of the last two observations was not recorded but the impression was that their intensity was by no means as great as that found in the incised experimental membrane of Rabbit No. 6, which was studied previously. So that except for the incision and perhaps intensity of (c) and (d) above, this hyperemic membrane appeared very similar to the experimental nictitating membrane of the incision type.

This similarity suggests that the following factors are excluded from being responsible for the increased mitotic activity: elastic retraction of tissue, tissue-destruction with liberation of "special substances", probably also nervous influences. The factors which are now remaining

are the hyperemia, the presence of crevices, and the slight amount of leucocytic infiltration. It is very probable that the last two are a result of the hyperemia.

c. The mitotic index was determined in three of the experimental sections and two of the control sections (Table 3). The procedure of counting was the same as above.

Table 3 Mitotic index of the stratified epithelium of the normal (control) and hyperemic (experimental) nictitating membrane of a rabbit (No:52)

control				experimental			
slide number	No: of nuclei	No: of mitoses	mitotic index	mitotic index	No: of mitoses	No: of nuclei	slide number
85	5801	8	1.4	3.4	11	3273	76
88	6395	1	0.2	6.5	25	3859	79
—	—	—	—	2.9	13	4421	86
total	12196	9			49	11553	
average			0.8	4.2			

Table 3. Results and Discussion:

(a) The order of magnitude of these controls is the same as that of the other control sections (see Table 2).

(b) The average mitotic index of the experimental hyperemic nictitating membrane is about five times that of the controls; it is, however, only one-half that of the incised experimental membrane. Definite conclusions can, of course, not be drawn from one experiment. The results of

Table 3 seem, however, to encourage further experimentation along this line.

3. A suggested programme for future experiments on the nictitating membrane of the rabbit: The number of experiments done for these investigations are few, therefore, more experiments, where statistical evaluation and calculation of errors are desired, are necessary.

a. Distribution of mitoses and mitotic index: It may be of value to give not only the location of the mitoses in the different layers but to give as well, a topographical distribution of the "centers of proliferation" or "waves" of mitoses on the entire surface of the membrane, under different conditions. Figures 10. and 11. were a first attempt in this direction. New experiments of the following type may improve the value of the controls and at the same time help to analyse the factors favorable to mitoses.

(1) Variations of ages, weights, and sizes. For the purpose of locating meristematic cells, older animals should be used.

(2) Sex.

(3) Different physiological conditions as phases of the oestrus cycle, pregnancy.

(4) Periodicity: either daily or of longer periods. Studies of this type have been made by Alice Carleton (3) on the epidermis of new born mice. She states that, "under normal conditions of light, there seems to be a 24-hourly rhythm in the mitotic division of animal cells, with a maximum from 8 o'clock in the evening to midnight, and a minimum

about noon".

(5) Effect of different diets, temperature and moisture of atmosphere.

In case of future use of the incision method, it might be of value to time the process by investigating the onset, duration and subsidence of the increased mitotic index in the vicinity of the wound. It is likely that the first step in covering the wound with epithelium consists of a migration of epithelial cells from the margin of the wound towards its center (cf. Oppel, 19). This migration may lead to another re-arrangement of the epithelial cells along the surface in addition to the previous re-arrangement resulting from retraction. This may be the mechanism by which the "stimulus" is transmitted to some distance away from the incision causing an increased mitotic index far from the wound.

b. <u>"Looseness" of the epithelium and leucocytic infiltration of the epithelium</u>: The degree of "looseness" of the epithelium and leucocytic infiltration was an arbitrary estimation. It may be necessary to use or devise a more accurate method for recording the degree. For example, for measuring the degree of "looseness" of the epithelium Okkel's (18) photometric method may be used where the ratio of dark (crevices or spaces) to light areas (cells, etc.) is obtained. On the other hand, there may be a relation between the crevices in between the epithelial cells and the number of nuclei per unit area. The number of nuclei per unit of area depends on:

(1) Size of nuclei.

(2) Nucleo-cytoplasmic ratio.

(3) Degree of "looseness" (= presence of crevices)

(4) Infiltration with leucocytes.

(5) The presence of goblet cells.

(6) Variation of thickness of different sections.

Having excluded the factors (5) and (6) by selecting suitable sections, the following figures were calculated from one experimental section and one control section of Rabbit No. 6.

Experimental Membrane

Degree of "looseness" of epithelium	Number of nuclei per sq. mm.
o degree	23,200
1st degree	15,500
2nd degree	9,100
3rd degree	6,400

Control Membrane

o degree	27,000

There seems to be an inverse proportion between the number of nuclei per unit of area and the degree of "looseness" of the epithelium. Whether the relation is really so simple or not can be ascertained by measuring the degree of "looseness" as suggested above and by increasing the counts. It is interesting to note that Wigglesworth (24) in his studies on

wound-healing in insects, states that, "The occurrence of mitoses seems to be determined by sparseness among the activated cells". He means by sparseness, that the distance between epithelial cells has increased above that of the normal.

As for the leucocytic infiltration, the ratio of leucocytes to epithelial cells may be recorded.

c. The factor of hyperemia: It has already been remarked that the results of the one experiment on hyperemia of the nictitating membrane, though not sufficient to draw any conclusions, were encouraging enough to continue experimentation along this line. It may be useful to produce hyperemia by means other than foreign bodies, e.g., section of the sympathetic nerve supply or injection of histamine or histamine-like substances.* Furthermore, it may be advisable to record the hyperemia in a more quantitative manner, e.g., making sketches at various intervals or by a colorimetric method.

The question arises as to whether studies made on one type of stratified epithelium can be applied to other types of stratified epithelia. Again, the first point to be settled should be the distribution of mitoses in the different layers of different types of stratified epithelia. Whether or not there are general rules of distribution will appear from the examination and discussion of two extreme variations of stratified epithelium, namely a condyloma acuminatum and an epidermoid carcinoma.

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* We are indebted to Dr. J. O. Pinkston for these suggestions.

Observations and Discussion

(continued)

B. Condyloma acuminatum. Biopsy specimen (No. 9417) from a 54 year old male person: Microscopically, the specimen consisted of a very much corrugated epidermis, with broad and deep papillae and a portion of the underlying corium. The corium was very vascular; many patent and congested blood vessels were visible. There were a great number of leucocytes scattered throughout the corium; in a few places the epithelium was infiltrated to some extent with leucocytes.

Maximow and Bloom (15 on page 323) state that the normal "epidermis varies from 0.07 to 0.12 mm. in thickness on most parts of the body", i.e., not including the palms and soles, but the number of cell or nuclear layers is not given. The epidermis of this specimen was in many places 0.4 mm. or more in thickness, with the number of nuclear layers of the whole epidermis ranging from 5 to over 30 layers. The number of nuclear layers was in most places much nearer to 30 than to 5. This enormous increase in epidermis was entirely due to the prickle cell layer, stratum spinosum. The strata granulosum, lucidum, and corneum were exceedingly inconspicuous consisting of practically in all places of only a few cell layers. There was no necrosis of the epithelium in the sections studied except for the normal keratized layer. Mitoses were so numerous that only a few sections were necessary to obtain the number equal to that found in the

experimental nictitating membranes of the rabbit. The number of mitoses per section was approximately the same. The distribution of the mitoses in the different nuclear layers and inner, middle, and outer third of the epithelium is recorded in Table 4.

Table 4. Distribution of mitoses in the stratified epithelium of a condyloma acuminatum (Biopsy No: 9417). Total epithelium ranging between 10–30 or more nuclear layers.

A. Absolute number and percentage of mitoses occurring in the different nuclear layers.

Nuclear layer	Absolute number	%
1	83	16
2	118	22
3	93	17
4	71	13
5	43	8
6	31	6
7	26	5
8	17	3
9	11	2
10	10	2
11	5	1
12	6	1
13	2	< 0.5
14	4	0.5
15	6	1
16	2	< 0.5
17	1	< 0.5
total	529	99

B. Absolute number and percentage of mitoses occurring in the inner, middle and outer third of the epithelium; the third proximal to the corium is considered the lower.

	inner third	middle third	outer third
total	483	40	——
%	92	8	——

1. Table 4, A and B. Explanatory notes: The nuclear layers recorded in the Table are the absolute ones.

If a mitotic figure was found in a nuclear layer above the 6th, the adjacent sections were examined to see whether the mitosis or region in which it was found, changed in respect to the distance from the corium. If the distance did change markedly, then that particular mitosis was reduced to a lower level, depending on the individual case. Few such cases occurred so that on the whole, the figures recorded in the table are the original ones. Though the nuclear layers did range from 5 to 30 or over, on the whole, the number of layers was surprisingly uniform. The whole epithelium in each microscopical field was divided into thirds, and the mitoses found in that field were recorded according to the third in which they were found. Thuringer, instead of dividing the whole epithelium into thirds, divided only the stratum spinosum into thirds. Therefore, when he states that all the thirds of the stratum spinosum showed some mitoses, it is not at variance with our observations, because the absolute nuclear layer in which mitoses were found in our case was no doubt higher than in his. He does not give the number of nuclear layers in his specimen. A rough estimate from photographs of normal epidermis in Thuringer's paper, as well as in other places, leads us to conclude that the total number of nuclear layers in the stratum spinosum is about ten or less, at least not much more. Therefore, the stratum spinosum in our specimen was 3 to 4 times as large as the normal.

2. Table 4, A and B. Results and discussion:

Mitoses were found in the inner 17 nuclear layers of the

epidermis. Proceeding from the corium towards the free surface, the percentage of mitoses in the nuclear layers decreases. The location of the mitoses in the outer layers is not as certain as that of the lower layers, but we can assume that the first twelve nuclear layers are fairly accurate.

The assumption was made (see page 21 for discussion) that all the cells in these 12 layers are able to divide. Consequently, it is likely that all the cells in the lower twelve nuclear layers of the epidermis are meristematic; this is already equal to more than all the cells in the prickle cell layer of a normal epidermis. Can we therefore, say that the entire stratum spinosum of the human epidermis is meristematic? For the present, the answer is yes. If this is so, then how is one to account for the loss of the meristematic cells of the stratum granulosum and outwards, and what factors are responsible for the mitosis of the meristematic cells. It will be recalled that this second point is also common to the nictitating membrane of the rabbit.

3. Petersen (20, on page 674) emphasizes the fact that, due to the papillary architecture, the corium surface of the epidermis is much larger than the air-surface. The point in which Petersen is interested is the great "resevoir" of basal cells. However, the papillary architecture is also important for the problems of this paper. The diagram Fig. 14 serves for illustrating these things. With the aid

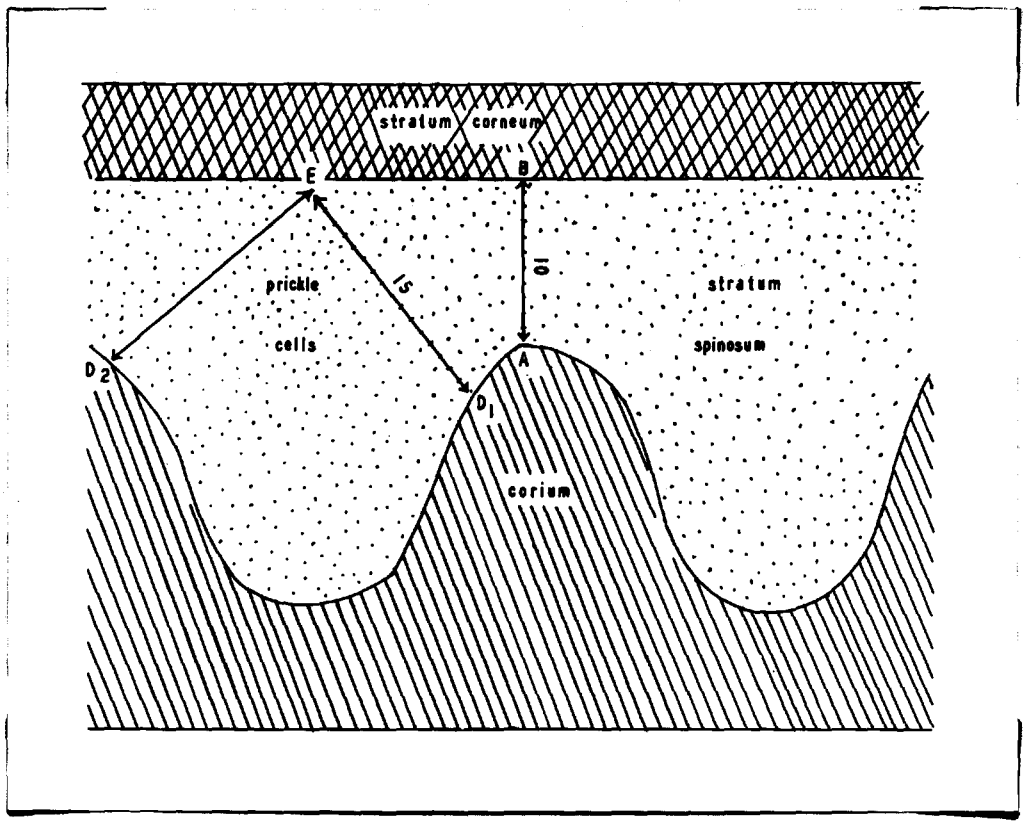

Fig. 14 . Diagrammatic representation of the normal skin illustrating 1) corrugated corium-surface of the epidermis 2) relative distances of different points from the corium.
AB smallest distance (10 N.L.) , D_1E and D_2E medium distance (15 N.L.) .

of Fig. 14. we shall attempt a theoretical explanation which fits to some extent with the results found in the nictitating membrane, and which at the same time is open to further investigations. The following explanation can, for the moment, apply only to stratified epithelium of the type studied.

Fig. 14. represents a diagram of the normal epidermis. B and E are the boundaries between the stratum spinosum and the stratum granulosum. AB represents ten prickle cell layers, and D_1E or D_2E represent fifteen prickle cell layers. It is assumed that all the cells of the stratum spinosum of the normal epidermis are meristematic (see above), and it appears that the cells of the stratum granulosum have lost the meristematic ~~nature~~ ability. If we proceed further out towards the surface we come to the corneum layer which consists of dead cells. There are two different gradients which have been described by Cowdry (4 , on page 453), as follows: "We may imagine fluid and nutritive substances leaving the capillaries and percolating between the densely massed cells towards the surface*. The further they go the slower will be the movement and the more they will be depleted of life-giving and sustaining materials. A compensating diffusion, at first sluggish but increasing in speed and volume, of by-products of cellular metabolism proceeds in the opposite direction." We can conceivably suppose that the concentration of the "substances" (life giving and sustaining) is dependent

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* Of the epidermis.

on the blood supply to the corium.

The concentration of "substances" at B is ΔAB; at E it is $\Delta D_1E + \Delta D_2E$. Since E and B look morphologically alike, and their meristematic ability is the same, then we assume that the concentration of ΔAB is similar to $\Delta D_1E + \Delta D_2E$. At the same time the concentration above B and E should be less than ΔAB; finally, when we reach the stratum corneum the concentration is least, or practically zero. Assuming that the concentration ΔAB, at B or E is the minimum concentration that will enable a cell to retain its morphological appearance as a prickle cell, then above B and E the cells will change morphologically to stratum granulosum cells. This change must be irreversible for this cell type can only extend outwards where the cells are definitely necrotic. On the other hand, starting from the outer third of the stratum spinosum and proceeding towards the corium, the concentrations of "substances" becomes higher and is highest in the very lowest of the epidermal cells next to the corium. We note that the number of mitoses increases as we approach the corium. It is possible therefore, that there are the following concentrations of "substances":

a. Minimal concentration ΔAB, to maintain or sustain a prickle cell as such.

b. Below this minimal concentration ΔAB, the prickle cell changes irreversibly into a stratum granulosum cell.

c. Above this minimal concentration ΔAB, the

conditions become favorable for mitotic division.

We say favorable to mitotic division, because probably factors other than the immediate environment, such as the previous history of the cell, its rate of metabolism, and size, enter into consideration; because the environment is favorable does not mean that the cell must divide at that moment. If the concentrations of the substances in the different nuclear layers of the epidermis are increased, e.g., by hyperemia, we would expect to find a greater increase of nuclear layers of prickle cells and at the same time, an increase of mitoses not only in absolute number, but also in the higher levels of the epidermis. Though we did not calculate the mitotic index for the sections of the condyloma studied, it was definitely much greater than in the normal, judging from results of Thuringer. At the same time the number of prickle cell layers was enormously increased and the nuclear layers in which mitoses were found extended further out than in the normal.

We may sum up by saying that a somatic cell of the stratified epithelium studied, retains its meristematic ability because the environmental conditions are favorable; and conversely looses that ability because they are unfavorable. In other words, meristematic potentiality is a function of the environment.

Observations and Discussion

(continued)

C. Epidermoid carcinoma. Biopsy specimen (No. 8970) from a 60 year old female person: Microscopically, the eleven sections studied consisted of: (1) non-ulcerated surface epidermis, part of which appeared morphologically normal, and part of which showed long and interlacing papillae connecting at times with the carcinoma islands; (2) definite carcinoma islands, some of them horny pearls (some carcinoma islands were infiltrated with leucocytes to some extent), the centers of some of them contained hyalinized and necrotic material; (3) strands of epithelial cells connecting (1) and (2); and (4) corium which was very vascular, containing many leucocytes, mainly lymphocytes.

The distribution of mitoses in the different nuclear layers and the inner, middle, and outer third of the epithelium is given in Table 5.

Table 5. Distribution of mitoses in the stratified epithelium of an epidermoid carcinoma (Biopsy No: 8970). Total epithelium ranging between 3 – 25 nuclear layers.

A. Absolute number and percentage of mitoses occurring in the different nuclear layers (N.L.).

Slide No:	1st. N.L.	2nd. N.L.	3rd. N.L.	4th. N.L.	5th. N.L.	6th. N.L.	7th N.L.	8th N.L.	9th N.L.	10th N.L.	11th N.L.	12th. N.L.	total
1	26	6	2	1	—	1	—	—	—	—	—	1	37
2	42	16	4	—	—	—	—	—	—	1	—	—	63
3	38	11	1	2	1	—	1	—	—	—	—	—	54
4	33	4	—	—	2	—	—	1	—	—	—	—	40
5	33	6	3	2	—	—	—	1	—	—	—	—	45
6	30	8	5	2	—	—	1	—	1	—	—	—	47
7	45	10	1	1	1	—	—	—	—	—	—	—	58
8	25	6	3	3	—	—	—	—	—	1	—	—	38
9	37	7	—	2	—	1	—	—	—	—	—	—	47
10	28	5	3	—	1	—	1	—	—	—	—	—	38
11	44	10	5	3	—	—	—	—	—	—	—	—	62
total	381	89	27	16	5	2	3	2	1	2	0	1	529
%	72	17	5	3	1	<0.5	0.5	<0.5	<0.5	<0.5	0	<0.5	

B. Absolute number and percentage of mitoses occurring in the inner, middle and outer third of the epithelium; the third proximal to the corium is considered the lower.

	inner third	middle third	outer third
total	470	60	—
%	89	11	—

1. Table 5, A and B. Explanatory notes:

No distinction was made between:

a. Definite carcinoma islands,

b. normal morphologically appearing surface epidermis, and

c. strands of epithelial cells connecting a. and b.

The number of nuclear layers of all three regions ranged from 3 to over 25. The number of the nuclear layers given in the table are the absolute ones. If, above the 4th

nuclear layer, the mitoses or the area in which they were found was searched for in adjacent sections to see if their distance from the corium had changed. If the distance had changed markedly, that particular mitosis was reduced to a lower level. The number of such cases was few.

It is very difficult to divide the epithelium of the carcinoma islands and the long papillae into inner, middle, and outer thirds, comparable to the surface epidermis. We divided the diameter of the islands into thirds and recorded the mitoses found in each third.

2. Table 5, A and B. Results and discussion: Mitoses were found in the first twelve nuclear layers, taken as a whole, of the stratified epithelium. From the corium, outwards to the surface, the percentage of the mitoses in the different nuclear layers decreases. Though it is not seen in Table 5A or 5B, the majority of the mitoses are found in the islands of the carcinoma. Some of the mitoses are found in or near the center of an islands. In 1902, Von Hansemann (9 , on page 30 - 33) called attention to the fact that mitoses occur in the different "layers" of hornified epidermoid carcinoma. However, he neither defined the "layers" nor gave the number of mitoses in the "layers". In this specimen, the inner, and middle thirds only of the epithelium contained mitoses. The distribution of the mitoses in the different nuclear layers and inner and middle thirds of the epithelium of this specimen compare favorably with that found in the Condyloma acuminatum. The percentages of mitoses in

the different nuclear layers differ somewhat, but the number of layers in which mitoses are found are approximately the same. Though there is obviously a great difference in their mode of growth, there is no apparent difference as to the distribution of the mitoses. As in the Condyloma acuminatum, the first twelve nuclear layers of this epidermoid carcinoma are meristematic.

CONCLUSIONS.

In the normal nictitating membrane of the rabbit, as in the "post-regenerative" phase after incision, the mitotic frequency decreases with the distance from the corium. The same rule seems to apply to the Condyloma acuminatum of man which is a moderate deviation from the normal stratified epithelium. Finally, it seems to apply as well to an extreme variation of stratified squamous epithelium, namely an epidermoid carcinoma. This similarity of behavior, in all cases, is obviously a result of the combination of the vascular corium with the non-vascular stratified epithelium.

Although our observations on hyperemia without a wound are based on one experiment only, they permit the formulation of a working hypothesis, namely that the conditions of blood-circulation in the corium plays a predominant rôle in governing the distribution and mitotic activity of meristematic cells in stratified epithelia.

SUMMARY.

1. The distribution of mitoses was studied in three types of stratified epithelia, namely:

   a. Nictitating membrane of the rabbit.

   b. Condyloma acuminatum of man.

   c. Epidermoid carcinoma of man.

2. The reliability of the records of this paper is greatest for the nictitating membrane of the rabbit and least for the epidermoid carcinoma.

3. In the normal nictitating membrane of the rabbit, mitoses are found in the basal three-fifths of the epithelium.

4. In the nictitating membranes which are in a "post-regenerative" phase after an experimental incision, mitoses are found in all nuclear layers of the epithelium, including the very top layer.

5. The possible error in numbering layers which may be caused by folds or oblique sectioning is eliminated by a correction method.

6. In order to analyse the factors which are favorable for mitotic division a comparison is made of the normal membrane, incised membrane, and a membrane made hyperemic by a "foreign body".

7. The hyperemia caused by a foreign body is considered as comparable to the hyperemia which is one of the consequences of the incision.

8. The mitotic index of the incised membranes is ten times that of the controls, while the mitotic index of

the hyperemic (foreign body) membrane is five times that of the controls.

9. The factors which seem responsible for mitotic activity are:

a. Hyperemia.

b. "Looseness" (presence of crevices) of the epithelium.

c. Leucocytic infiltration.

10. In the stratified epithelium of a human Condyloma acuminatum mitoses are found in the inner 17 nuclear layers or basal two-thirds of the epithelium.

11. In the stratified epithelium of a human epidermoid carcinoma, mitoses are found in the inner 12 nuclear layers or basal two-thirds of the epithelium.

12. A working hypothesis is formulated that the distribution of meristematic cells and mitotic activity of stratified epithelia of mammals are a function of the environment, particularly the "milieu interne" created by the blood-circulation of the corium.

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