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The Reaction of the Cornea *in vivo* and *in vitro* to Thermal Stimulation

A Contribution to the Thesis by Busse-Grawitz

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Summary. In a test series with 15 rabbits a so-called central keratitis by means of a red-hot needle was set in the left and right eye. The cornea of the left eye was immediately extirpated under sterile conditions and was observed in the tissue culture for 6 days. The corresponding right eye of the first rabbit was left within the animal's body, later it was extirpated after $^1/_2$, 1, $1^1/_2$, 2 hours etc. up to 11 days histologically examined. In the explants following results were obtained. After the lesion is set the puncture site is devoid of epithelium. A double wall of cells lies closely around the crater. One-and-one-half hours later these large cells with more or less variably segmented nuclei demonstrate a strong proliferation. The characteristic nucleus form, the weakly positive naphthol-ASD-chloracetate-esterase-reaction and the variable peroxydase-reaction of these cells resemble quite closely leucocytes in phenomenology and ferment-histochemistry. We have therefore chosen the description leucocytoid cells. They are not leucocytes, but cells which originate from basal pluripotent epithelia of the cornea. The so-called central keratitis is not an inflammation in the sense of Marchand, it is rather a regenerative process. The thesis of Busse-Grawitz, that leucocytes in the cornea originate from collagen and elastic fibers or less than cocci-sized transitional forms, cannot be verified.

Key words: Cornea-Lesion — Rabbit-Tissue Culture — Leucocytoid Cells.

Zusammenfassung. In einer Versuchsreihe an 15 Kaninchen wurde an der Hornhaut des linken und rechten Auges mittels einer glühenden Nadel eine sog. zentrale Keratitis induziert. Die Cornea des linken Auges wurde unter sterilen Bedingungen sofort exstirpiert und 6 Tage in der Gewebekultur beobachtet. Das korrespondierende rechte Auge wurde beim 1. Kaninchen sofort, später nach $^1/_2$, 1, $1^1/_2$, 2 Std usw. bis zu 11 Tagen im Tierkörper belassen, dann exstirpiert und histologisch aufgearbeitet. An den Hornhautexplantaten beobachteten wir folgende Veränderungen: Nach gesetzter Läsion ist die Stichstelle ohne Epithel. Direkt um den Krater liegt ein doppelter Zellwall. Nach $1^1/_2$ Std kommt es zu einer starken Vermehrung von großen runden Zellen mit mehr oder weniger segmentierten Kernen. Den gleichen Vorgang findet man an den Explantaträndern. Die charakteristische Kernform, die schwach positive Naphthol-ASD-Chloracetatesterase-Reaktion und einer wechselnde Peroxydase-Reaktion machen diese Zellen phänomenologisch und fermenthistochemisch leukocytenähnlich. Wir nennen sie leukocytoide Zellen. Es sind keine Leukocyten, sondern Zellen, die ihren Ursprung von basalliegenden pluripotenten Epithelien der Cornea nehmen. Die sog. zentrale Keratitis ist keine Entzündung im Sinne Marchands, es handelt sich vielmehr um einen regenerativen Vorgang. Die von Busse-Grawitz vertretene These, daß "Leukocyten" in der Hornhaut aus kollagenen und elastischen Fasern, aus sog. unterkokkengroßen Übergangsformen stammen, kann nicht bestätigt werden.

For over a century the origin of the "inflammation cells" which are already apparent within a few hours surrounding the injury site has been a mystery. The supporters of the inflammation theory of Cohnheim (1867, 1869, 1874, 1914) believe this to be white blood cells which wander into the area either from

episcleral capillaries or tear fluid. In strong contrast to the emigration theory of Cohnheim is the dormant cell school of Grawitz (1893, 1896, 1899, 1913) and his pupils. It is based upon their observations especially on the cornea, an anatomically definded avascular region, that inflammation cells, mainly leucocytes, do not originate intravascularly but rather by metamorphosis and through transitional forms of collagen and elastic fibers in the substantia propria of the cornea.

The recent ergography by Busse-Grawitz (1973) "Die Überwindung der Zellularpathologie" and the challenge by the author to test his results on the cornea were the stimulus for the following research: the goal is to clarify the origin and morphogenesis of these so-called inflammation cells.

Materials and Methods

Fifteen rabbits of the silver-grey race ranging an age from 8 to 24 weeks were anesthatized with nembutal, aquamycetin eye drops applied to both eyes and a lesion placed in the center of the cornea using a sharp, glowing needle and reaching the substantia propria (Table 1 and Fig. 2). The wound was kept free of tears by elevation of the eye using 2 retractors. A square piece (5×5 mm) was removed from the left cornea with the wound in the center. The borders of the explantate were always 4 mm from the episcleral vessels. The tissue was placed in a culture medium (TC 199 + 10% fetal calf serum at 37° C) for further observation.

Table 1. Schematic presentation of the experimental plan

CORNEA (RABBIT)

DATE	AGE OF ANIMALS ca.	LEFT EYE	MEDIUM	FIXATION LEFT EYE ALCOHOL	FIXATION RIGHT EYE ETHER-ALC.	MEDIUM-SEDIMENT METHANOL-FIX.	STAINING	EXPLANT STRIPPED	NOTE
3/4 74	ANIMAL 1 8 WEEKS	EXPLANT IMMEDIATELY	TC 199 + FKS	6 DAYS CULTIVATED	IMMEDIATELY	1½. DAY 3. " 4. " 5. " 6. "	MAY-GRÜNW. FEULGEN PEROXYDASE NAPHTHOL-CHLORACETAT	–	
3/11 74	2 8 WEEKS	"	"	5 DAYS CULTIVATED	½ h	3. DAY 4. " 5. "	"	–	
3/25 74	3 10 WEEKS	"	"	5 DAYS CULTIVATED	1 h	2. DAY 2½. " 3. " 4. "	"	–	
3/29 74	4 10 WEEKS	"	"	7 DAYS CULTIVATED	1½ h	3. DAY 7. "	"	SUBST. PROPRIA WITHOUT LESION	3 WEEKS WITHOUT CELLGROWTH
4/19 74	5 13 WEEKS	"	"	7 DAYS CULTIVATED	2 h	3. DAY 7. "	"	–	
4/29 74	6 15 WEEKS	"	"	5 DAYS CULTIVATED	3 h	2. DAY 5. "	"	SUBST. PROPRIA WITH LESION	3 WEEKS WITHOUT CELLGROWTH
5/2 74	7 15 WEEKS	"	"	6 DAYS CULTIVATED	4 h	3. DAY 6. "	"	–	
5/6 74	8 16 WEEKS	"	"	6 DAYS CULTIVATED	8 h	3. DAY 6. "	"	–	
5/10 74	9 17 WEEKS	"	"	5 DAYS CULTIVATED	16 h	3. DAY 5. "	"	–	
5/13 74	10 18 WEEKS	"	"	6 DAYS CULTIVATED	24 h	3. DAY 6. "	"	–	
5/15 74	11 19 WEEKS	"	"	5 DAYS CULTIVATED	2½ d	3. DAY 5. "	"	–	
6/5 74	12 21 WEEKS	"	"	23 DAYS CULTIVATED	5 d	2. DAY 5. "	"	SUBST. PROPRIA WITH LESION	3 WEEKS WITHOUT CELLGROWTH
6/10 74	13 22 WEEKS	"	"	5 DAYS CULTIVATED	8 d	2. DAY 5. "	"	–	
6/18 74	14 23 WEEKS	"	"	6 DAYS CULTIVATED	9 d	3. DAY 6. "	"	–	
6/20 74	15 23 WEEKS	"	"	6 DAYS CULTIVATED	11 d	3. DAY 5. "	"	–	

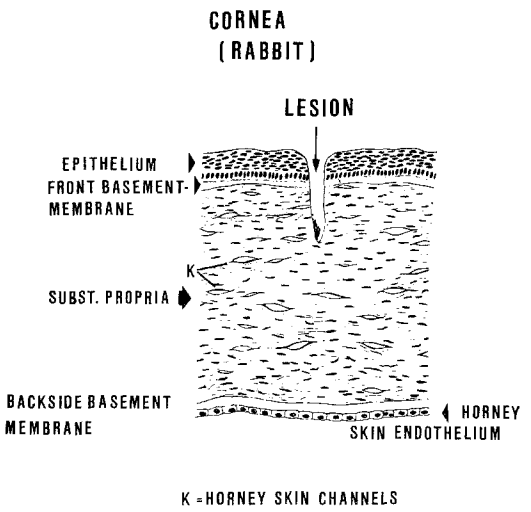

Fig. 1. Cornea after lesion

At predetermined times, an equally large area was removed from the right eye fixed in ether alcohol and prepared for examination. The 6–8 μ thick sections were stained with HE, Giemsa and according to the method recommended by Busse-Grawitz (1973). On every second and fifth day 4 smears each were prepared from one cell sediment. These were fixed in methanol, stained with May-Grünwald-Giemsa and Feulgen and exposed to peroxydase and naphthol-ASD-chloracetate ersterase reaction.

After careful removal of the corneal epithelium and endothelium layers in rabbits number 4, 6 and 12, the connective tissue and collagen-rich middle layer and substantia propria were cultivated for 3 weeks in the same manner.

Results

1. Cell Culture

Immediately following the excision of the 5×5 mm explantate the wound demonstrated a bizarre, ripped crater with charred tissue and homogeneous substance on the floor. A double cell wall is seen surrounding the crater. Thirty minutes after injury to the cornea large, round, chromatin-containing cells with a light cytoplasma ring surrounding the crater edge, in the crater itself as well as further distant on the explantate surface were present. One-and-one-half hours later these cells demonstrate a strong proliferation and after 18 hours the entire explantate surface is covered (Fig. 2). The same process is found on the explantate borders (Fig. 3). The round cells lie either singularly or arranged in drop-like configurations. Only a few of the cells settle to the floor of the culture dish, most prefer to divide while swimming in the medium. A few individual polygonal, squamous and cylindric basal epithelial cells lie scattered throughout. Mitoses are present in large numbers. Together with the large cells with segmented cell nuclei are also numerous smaller cells with large, round nuclei and thin cytoplasma rings. These are found exspecially in the first 2 days.

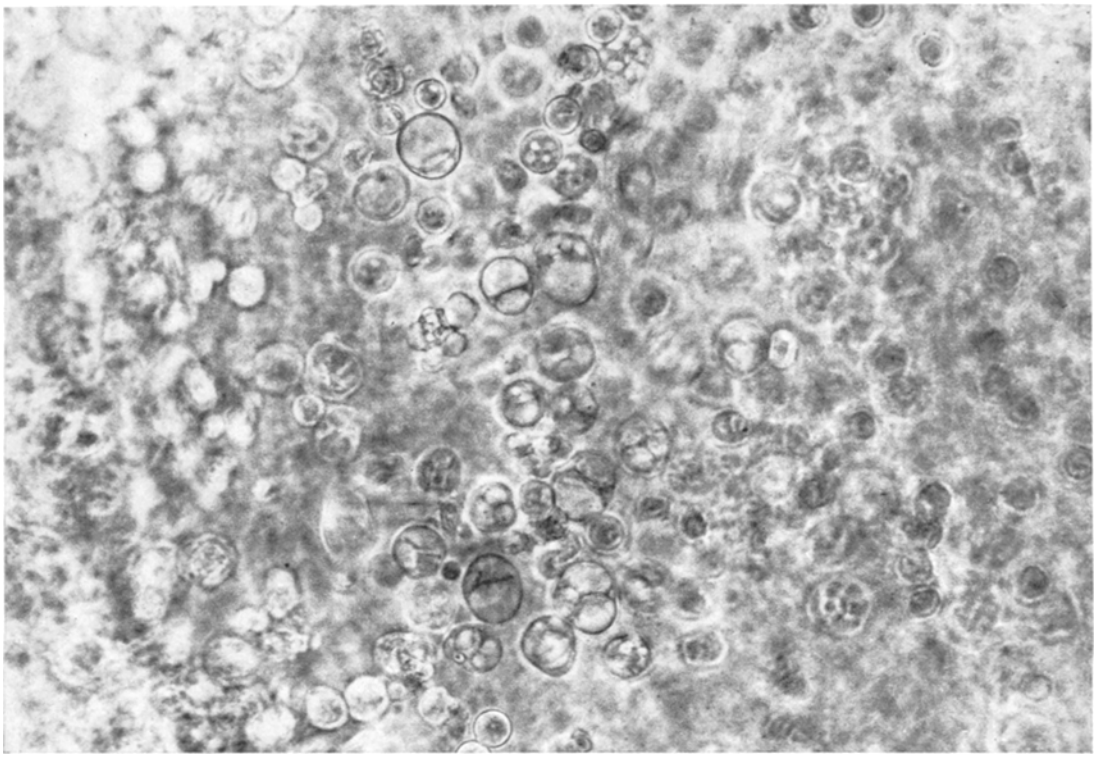

Fig. 2. High proliferation of large round cells on the explant surface 18 hrs after extirpation. (Magn. 320)

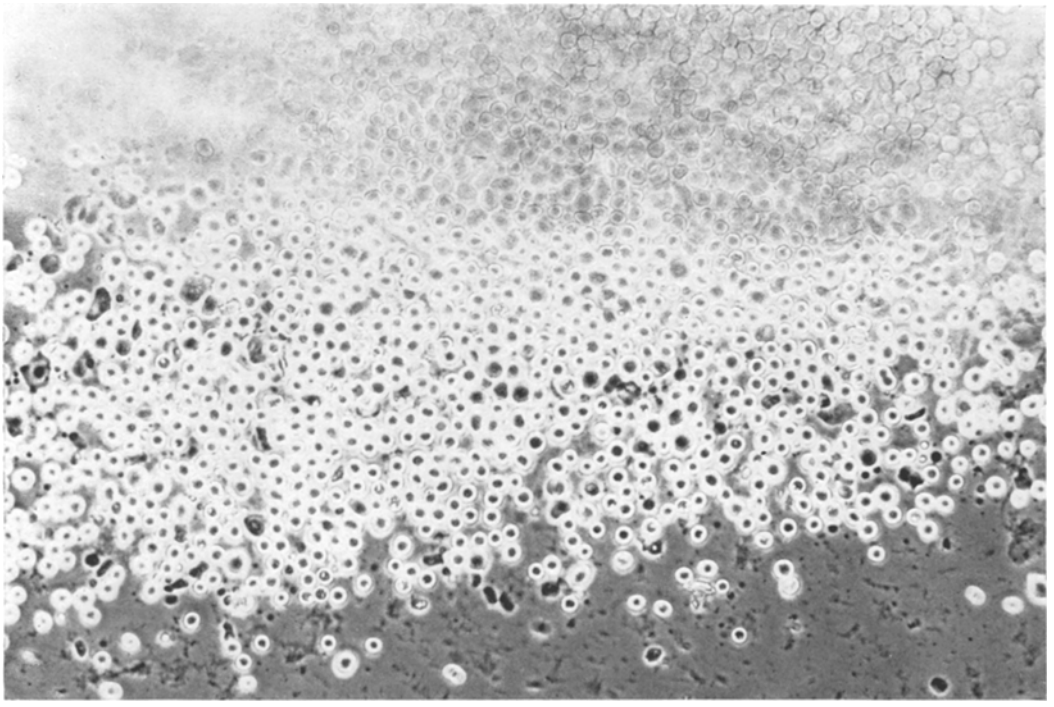

Fig. 3. Explant limit with proliferation of numerous round cells (Magn. 100)

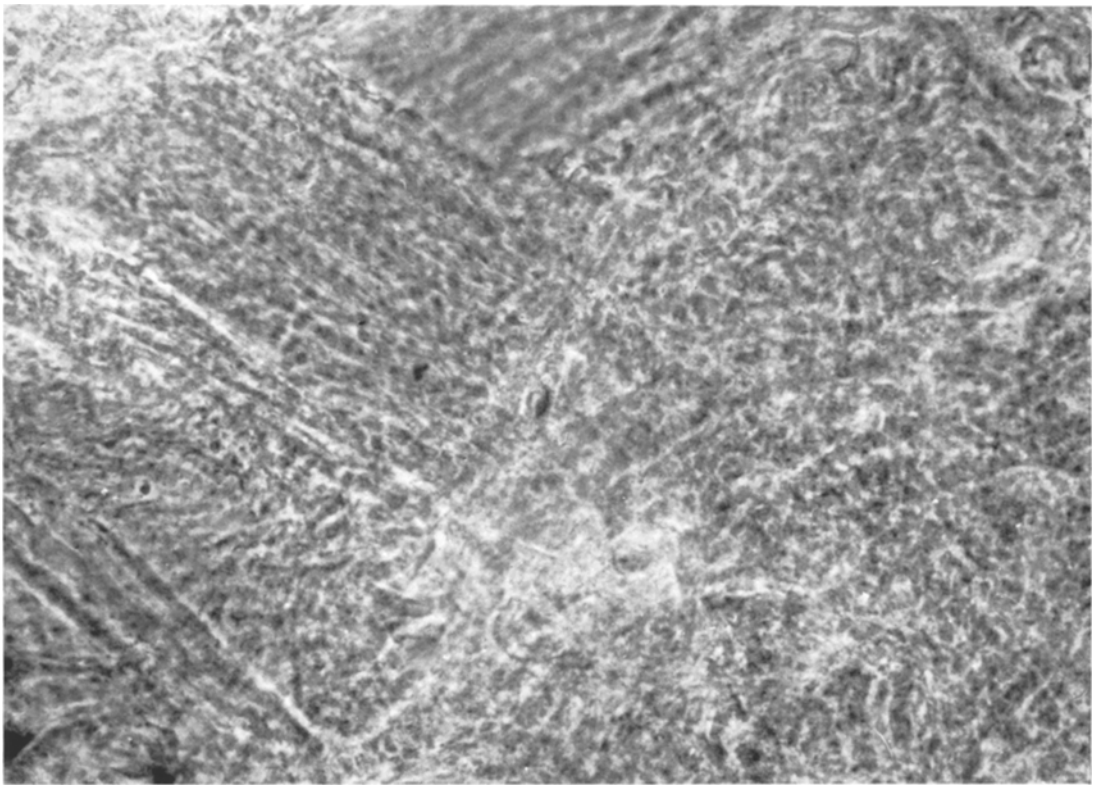

Fig. 4. Collagenous fibres in the fundamental substance after 3 weeks of culture. No cell growth. Traces of the lesion at midst of the lower third of the figure (Magn. 200)

In the first 5 days the large cells numerically proliferate, the nuclei demonstrate stronger segmentation. There after these cells gradually reduce in number.

The substantia propria which has been freed of the epithelial and endothelial cells presents a completely different picture. During the 3 weeks cultivation no new cells have been formed even though the wound reaches down into this layer (Fig. 4).

2. Sediment

Following slow centrifugation of the culture medium and at predetermined times cells were harvested and after staining with May-Grünwald-Giemsa, demonstrate strongly basophilic cytoplasma with blue-violet to reddish colored nuclei. The nucleus appears often loosely structured and contains at least one nucleolus, seldom more. The cell nuclei are either round or variably segmented, rarely rod-shaped (Fig. 5). Both large and small round cells are found in the sediment. The Feulgen stain is positive in both cell forms. The peroxydase reaction according to Osgood and Washborn is only weakly positive. The method according to Avrameas is negative.

The naphthol-ASD-chloracetatesterase reaction according to Leder is weakly positive in both cell forms. Polygonal squamous epithelium cells also demonstrate this phenomenon.

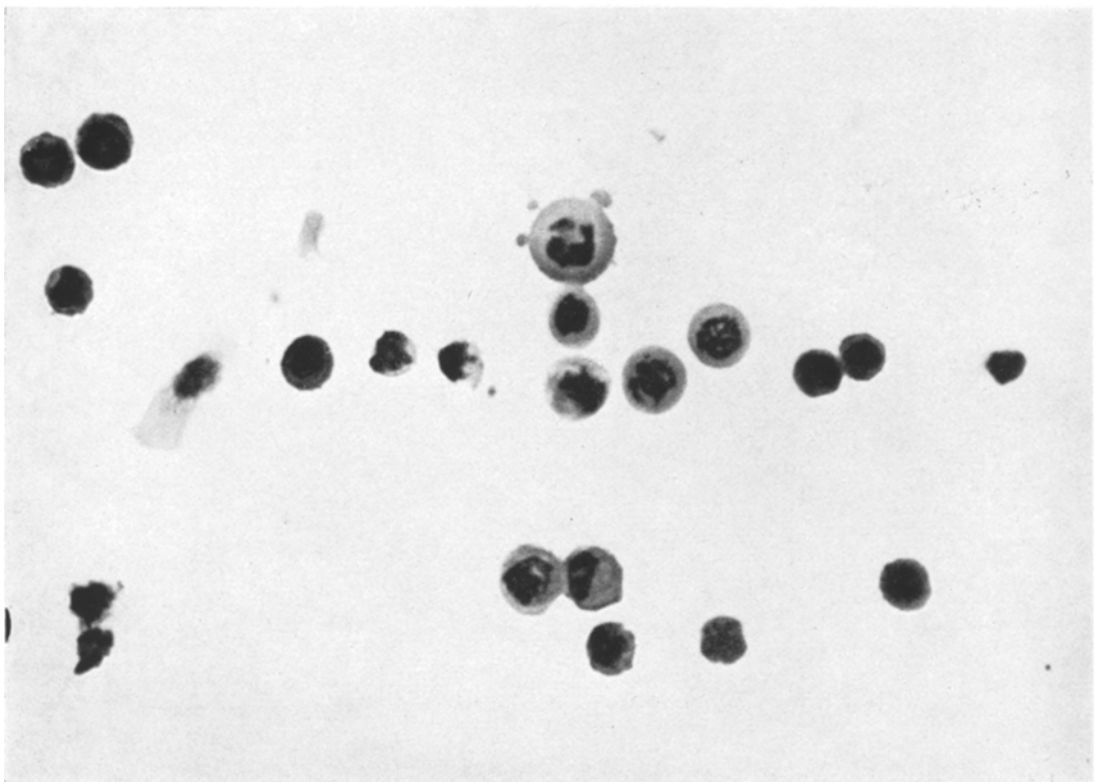

Fig. 5. Harvested cells of the culture medium after centrifugation and staining by May-Grünwald. Nuclei partly round, partly variably segmentated (Magn. 400)

3. Histology

The corneas selected for histological examination were left in the animals as before for periods of time ranging from $^1/_2$ hour to 11 days (Fig. 6). At the edges of the wound the basal epithelial cells are loosened. Staggered sectioning shows that this loosening reaches its maximum in the fifth day. At distances greater than 1.5 mm from the wound edge, the epithelium formation is normal. The epitheliation of the wound is completed in the sixth day.

Discussion

The so-called inflammation cells observed in the tissue cultures and histological sections stem from modified, pluripotent epithelial cells lying in the basal regions. Originally cylindric in shape, they reform themselves quickly into either small or large round cells. From the wound edge they reach the corneal surface and even force themselves to a certain degree between the epithelial cells in the proximity of the wound edges, and some may individually reach the upper layers of the substantia propria (Fig. 7). These cells are primarily capable of division, their

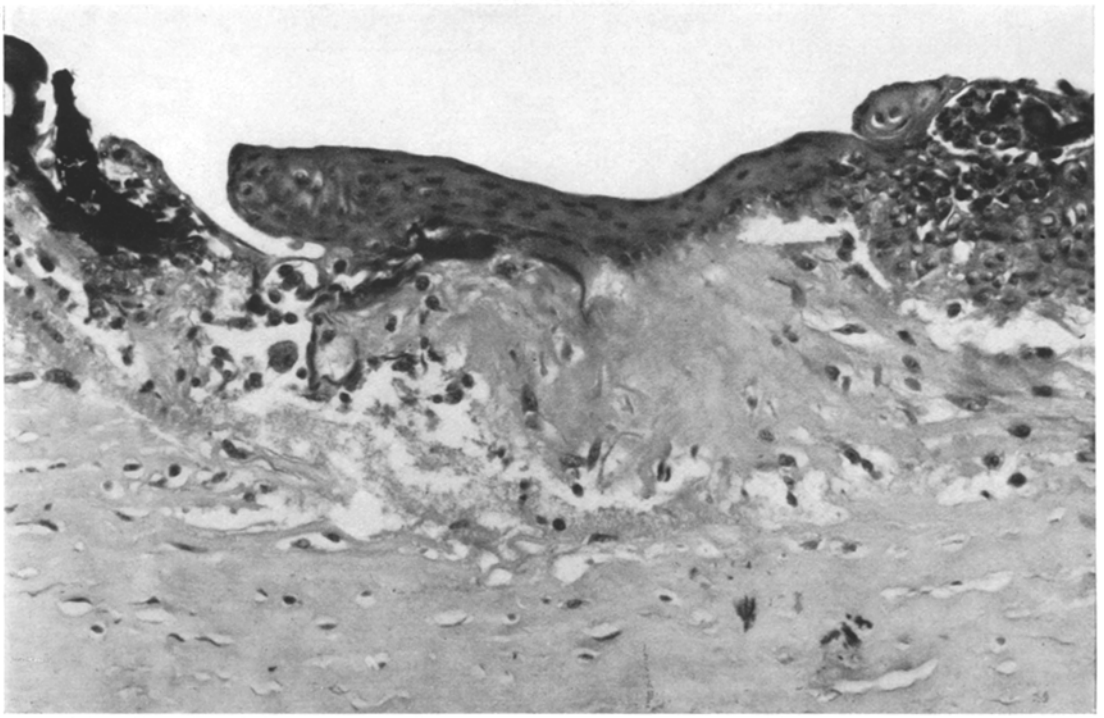

Fig. 6. Epithelial plug growing from right to left over the corneal lesion on the 5th day after applied injury (HE Magn. 200)

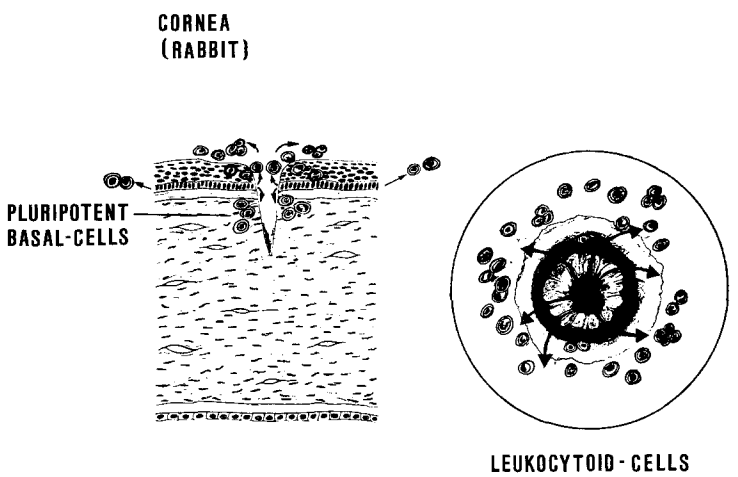

Fig. 7. Schematic presentation of cell migration. At left a cross-section at right a top plan view of the corneal crater

chromatin regroups into irregular formed, more or less segmented nuclei similar to leucocytes. The characteristic nucleus form, the weakly positive naphthol-ASD-chloracetate-esterase reaction and the variable peroxydase reaction of these cells

resemble quite closely leucocytes in phenomenology and ferment-histochemistry. We have therefore chosen the description leucocytoid cells. They do not originate intravascularly, they are not inflammation cells but modified epithelium with a slight capability for phagocytosis.

The so-called central keratitis is not an inflammation in the sense of the definition by Marchand (1921) which is interpreted as the sum answer of reactions in the vascular-connective tissue to an inflammation stimulus. In the cornea there is no definite vascular-connective tissue and therefore there can be no answer from this system to any stimulus. The process observed to take place around the central stimulus locus and which never exceeded a diameter of 3 mm during the maximal observation time of 11 days is better described as a regeneration without inflammation (Göcke, 1896; Neumann, 1918).

The so-called leucocytes seen by Busse-Grawitz and other researchers correspond to our leucocytoid cells.

The smaller than cell-sized or less than cocci-sized basophilic structures or trasitional forms (A, B, C, D, E-forms) of leucocytes which supposedly originate from collagen or elastic fibers and are described by the former were at no time present during our experiments. Knowledge of the chemical composition and tertiary structure of the basic substances collagen and elastic fibers makes it improbable that they contain DNA or RNA in any form. The formation of cell nuclei or a "protoplasmatic" metamorphoses of these structures cannot be realistically considered. The classic statement of Virchow still holds true for the formation of animal or plant cell structures "Omnis cellula e cellula eiusdem generis".

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