THE CANINE CORNEA IN TISSUE CULTURE

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Waldo Frank Keller

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ABSTRACT

THE CANINE CORNEA IN TISSUE CULTURE

by Waldo Frank Keller

The hydrogen ion concentration of canine aqueous humor was determined and an average pH of 7.8 established.

Six mm corneal sections were placed in the Rose perfusion chamber for tissue culture studies.

Two media were used in these experiments. One contained 10% canine serum and 90% Mixture 199, while the other was comprised of 10% canine serum and 90% Puck's medium. The pH was maintained at 7.8 and both media proved capable of stimulating cellular activity. Epithelial-like cells were observed to migrate from the explant after three hours in culture and the greatest cellular activity occurred between the 15th and 20th hours.

Hope is expressed that further experimentation with this vital tissue will make keratoplasty a clinical reality for the veterinarian.

THE CANINE CORNEA IN TISSUE CULTURE

By

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Dedication

To Mary Jane

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INTRODUCTION

In the areas of clinical surgery and medicine there has been an increasing demand on the veterinarian to diagnose and treat various pathological conditions of the canine cornea. Unfortunately, there is a dearth of basic research concerning the canine eye and such understanding must precede constructive experimentation in this area. There are many situations arising which would be amenable to corneal transplantation but thus far the instances of transplantation procedures have been haphazard and without proper documentation.

Before the transplantation studies could be initiated, a basic study of the cells of the cornea seemed appropriate. The most logical approach appeared to be a study of the cellular activity in tissue culture. A considerable number of investigations has been undertaken in this area but most of the reports are concerned with corneas of cats and rabbits. Such work has been directed toward determining viability after the corneal tissues have been handled in various environments prior to transplantation.

The perfusion chamber technique employed in this present study was pursued because of its adaptability to microscopic examination and photomicroscopy without disturbing

the corneal sections. The author also was interested in utilizing a technique that could be expanded into further experimentation in the area of holding and maintaining the donor corneal tissues until needed for surgical transplantation.

Two readily available synthetic media were considered in this study in the hope that they would maintain and stimulate growth of the corneal tissues. This basic information would be of value for future experiments.

REVIEW OF THE LITERATURE

A. TISSUE CULTURE

Lewis and Lewis (1911) initiated investigation of the nutrients necessary for growth of tissues *in vitro*, and since that time experimenters have developed a great variety of media for tissue culture studies. This study was confined to two synthetic growth media, Mixture 199 (Morgan *et al.*, 1950) and the medium used by Puck *et al.* (1958). Fowle and Ormsby (1955) demonstrated Mixture 199 to be insufficient in stimulating adequate growth of monkey corneal cells, but with the addition of horse serum and chick embryo extract, fibroplasia was stimulated greatly and epithelial growth slightly. McPherson *et al.* (1956 a) demonstrated rabbit corneal growth utilizing 40 per cent Earle's balanced saline, 40 per cent horse serum and 20 per cent chicken-embryo extract. This same group of researchers (Draheim *et al.*, 1957) gave evidence of satisfaction in this medium by continuing corneal viability studies using the same mixture. Cockeram *et al.* (1957) examined the viability of rabbit corneal epithelial and stromal cells making use of a medium composed of 30 per cent horse serum, 5 per cent chicken-embryo extract, and 65 per cent Earle's balanced saline. Cockeram *et al.* (1959) again used this

medium in their work with the cornea in tissue culture and it is of interest to note that these experimenters maintained a pH of 7.0 in their cultures. This concern for the pH of the medium appears lacking in the viability studies aforementioned. Perry et al. (1959) experimented with stored human grafts and, in determining viability, exposed the corneal tissues to a mixture of 30 per cent homologous serum, 20 per cent chicken-embryo extract, and 50 per cent Earle's balanced saline. Paul (1959) stated that the components essential for the immediate survival of cells and tissues outside the body have been precisely described, but it is obvious that there will never be a universal chemically defined medium for all cell types. The tissue culture studies of the cornea substantiate this because varied media have produced inconsistent cellular activity. Observations of epithelial activity varied from a few hours to 48 hours in culture, and initial fibroblastic migration was evidenced as early as 48 hours and after as long as 20 days.

Many containers and growth chambers have been utilized in tissue culture from Carrel's (1923) optically thin flasks and Maximow's (1929) hanging drop techniques to perfusion chambers of Buchsbaum and Kuntz (1954) who utilized double coverslips mounted in a stainless steel slide. Christiansen et al. (1953) used a similar perfusion chamber with a plastic or glass slide. Rose (1954) improved upon these devices by creating a chamber which allows for simple media exchange

techniques throughout the entire cultivation period as well as constant optical observation. Further improvements on the Rose chamber have been developed through the Electro-Mechanical Development Company (1957).

B. HISTOLOGY OF THE CORNEA

Jordan (1952) stated that the cornea consists of five layers; the anterior epithelium, the anterior homogeneous membrane, the corneal substance, the posterior homogeneous membrane and the posterior epithelium. Histologists have agreed on the cell types present in these five layers, but they have not been consistent in the names applied to the tissues. The most universally accepted nomenclature for the tissues comprising the cornea was proposed by Maximow and Bloom (1957). These authors designated the layers, from anterior to posterior, as; the epithelium, Bowman's membrane, the substantia propria, Descemet's membrane, and the corneal mesenchymal epithelium. Discordance in naming this innermost layer has led to considerable confusion. Copenhaver and Johnson (1958) referred to this tissue both as mesenchymal epithelium, and corneal endothelium, while Rycroft (1955) and many others writing on keratoplasty refer to this simply as the endothelium. The latter term may be misleading, since endothelium is usually associated with the blood vascular system. Prince et al. (1960) have

provided average thicknesses for the layers of the canine cornea, and these measurements are included in the following descriptions.

1. THE EPITHELIUM. This layer is stratified squamous epithelium with flattened superficial cells which do not show keratinization. Cells of the deepest layers are columnar in shape, with the remaining middle cells appearing polyhedral. Continuous division is observed and Binder (1952) stated that the average life of the cell is about 4 to 8 weeks. Mann (1944) reported the presence of special goblet cells in the rabbit cornea during epithelial regeneration, and Meirowsky et al. (1947) demonstrated the transition of epithelial cells to goblet cells when the rabbit cornea was exposed to various elements. The epithelium is reported to be 0.08 mm thick in the dog.

2. BOWMAN'S MEMBRANE. This structure has been reported as a homogeneous mass by many authors, but Maximow and Bloom (1957) reported the membrane to consist of a feltwork of randomly arranged fibrils, apparently of collagen. The report was based on research utilizing electron microscopy. These authors described the membrane in the human cornea as being 6 to 9 microns thick, and this is much greater than the 1.5 microns observed in the dog.

3. SUBSTANTIA PROPRIA. In the canine cornea, this

layer represents over 85 per cent of the total thickness and is reported as varying from 0.5 to 0.6 mm. Parallel with the surface are many laminae, with corneal corpuscles occupying spaces between the lamellae. These corpuscles are analogous to the fibrocytes of other tissues, and the fibrils contain the typical connective tissue protein, collagen. Wooden (1952) has confirmed the presence of a polysaccharide-protein complex which acts as an adhesive binding the laminae together. Hoffman and Messier (1949) demonstrated the importance of the corneal corpuscles in successful graft techniques.

4. DESCEMET'S MEMBRANE. In the dog, this tissue is 14 microns thick as compared to 6 microns in the human cornea (Rycroft, 1955). There are no microscopic structures observed but, unlike Bowman's membrane, this tissue can be reformed after penetrating damage. McDonald (1957) demonstrated a retraction of this membrane following incisions through the cornea.

5. MESENCHYMAL EPITHELIUM. The innermost surface is recognized as a single layer of epithelial-type cells averaging 4 microns in thickness. These cells are capable of regeneration and many authors feel that they secrete a substance which forms the Descemet's membrane. Investigators have reported a consistent inability to demonstrate mesenchymal epithelial activity in tissue culture. (McPherson et al., 1956 b)

C. EMBRYOLOGY OF THE CORNEA

The cornea is a complex structure which evolves from both ectodermal and mesodermal embryonic cells. Mann (1950) described surface ectoderm as the anlage of the epithelium while the substantia propria and mesenchymal epithelium are derived from the paraxial mesoderm. A condensation of the mesostroma separating the surface ectoderm from the anterior surface of the lens, gives rise to a "directional membrane" which, when invaded by mesodermal cells, is split into Bowman's and Descemet's membranes.

D. NUTRITION OF THE CORNEA

Surgical techniques, though mechanical in appearance, are based upon principals obtained through basic studies in the areas of anatomy and physiology. Confidence in the outcome of his endeavor is a necessary ingredient in the make-up of the successful surgeon, and knowledge concerning the tissues involved will advance this confidence. Techniques of corneal surgery have advanced rapidly, well ahead of the theoretical knowledge of the physiologist, and Rycroft (1955) stated that the point has been reached where real progress in surgical technique will depend on a more intimate knowledge of the corneal physiology. It is therefore important that the nutrition of the cornea be considered, since it is devoid of a true vascular bed and thus must receive

nutrients by other means.

Three routes of supply have been postulated; diffusion from the aqueous humor, from the tears, and from the capillaries of the conjunctiva and sclera. The importance of each route has been argued by many authorities, and while Nonidez and Windle (1953) stated simply that the metabolism is provided for by diffusion through the tissue fluid of the cornea from capillaries located at the junction of the corneal and conjunctival epithelium; other researchers have been striving to follow certain ions into the tissues via all three routes. Potts and Johnson (1950) demonstrated the importance of the limbal route in the transportation of certain ions, by using radioactive isotopes. Potts (1953) presented evidence substantiating the diffusion of inorganic ions through the surface epithelium and mesenchymal epithelium in both directions. In the latter studies the mesenchymal epithelium was shown to be more permeable than the surface epithelium. Bock and Maumenee (1953), in their studies with implanted polyethylene films, have shown a free interchange of stromal fluid with aqueous humor to be absolutely necessary for the survival of stromal and epithelial cells. Diffusion from the limbus did not appear sufficient, by itself, to prevent degeneration of the stromal cells denied aqueous penetration. Rycroft (1955), in comparing routes of nutrition to the cornea, pointed out that the mesenchymal epithelium and stratified squamous epithelium act as selective

membranes, whereas nutrition from the limbus involves passage through unselective membranes of the capillaries. Thus, from the standpoint of permeability, the limbus would be a favorable route of nutrition. It is apparent that the total nutrition of the healthy cornea is dependent upon diffusion of nutrients through all three postulated routes. This would indicate that the corneal cells, when placed in a perfusion chamber and exposed to a synthetic media, will receive adequate nutrition for maintenance and growth.

MATERIALS AND METHODS

A. Aqueous humor pH determinations

Five dogs obtained from a city pound were utilized; the ages varied from 8 months to 3 years. The breeds represented were Beagle, Basset hound, Border Collie (nursing pups), and Boxer.

The animals were anesthetized using intravenous injections of a four per cent thiamylal sodium.[1] An eye speculum was positioned holding the lids apart and a conjunctival flush with sterile .9% solution of sodium chloride[2] followed. A 24 gauge needle was placed two mm from the limbus and forced through the bulbar conjunctive and sclera angling into the anterior chamber in such a manner as to avoid the iris. This technique allowed an average of one ml of aqueous humor to be removed without damage to the eye and without hemorrhage. The aqueous was withdrawn into a two ml syringe and transferred to sterile five ml bottles. Samples were collected and the pH determinations completed on three consecutive days, and this series was repeated one

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[1]Surital Sodium; Parke, Davis and Co., Detroit, Michigan.

[2]Normal Saline Solution; Jensen-Salsbery Laboratories, Inc., Kansas City, Missouri.

week later. Two readings of each sample were taken one hour apart. The Beckman pH meter model G[1] was used in all determinations.

B. Tissue Culture Studies

The dogs used in these experiments represented both sexes, weighed from 18 to 65 pounds, and were from 8 months to 3 years of age. These animals were maintained in the experimental kennel until the corneas were completely clear and the globe apparently normal in all respects.

The animals were anesthetized with intravenous injections of pentobarbital sodium[2] in a quantity sufficient to produce a surgical plane of anesthesia. The hair was clipped from around the eyes and the conjunctival sac flushed three times with sterile .9% solution of sodium chloride. A cotton mass saturated with a 1:1000 dilution of benzalkonium chloride[3] was placed over the clipped area for 15 minutes. Aseptic surgical procedures were employed in performing the subconjunctival enucleations, and the globes were placed in a sterile dish containing 1:1000 Zephiran Chloride. After 10 minutes the eyes were transferred to another sterile dish containing 100 ml of Hank's (1948) balanced salt

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[1]National Technical Laboratories, Pasadena, California.

[2]Halital; Jensen-Salsbery Laboratories, Inc., Kansas City, Missouri.

[3]Zephiran Chloride; Winthrop Laboratories, New York, New York.

solution with 50 mg of dihydrostreptomycin[1] and 2,000 units of penicillin[2] added. This dish remained wrapped in a sterile shroud for transportation to the tissue culture laboratory. Immediately following enucleation, sufficient Halital was administered to effect humane euthanasia of the dogs.

A special surgical pack containing all of the instruments used in trephining the corneal sections and preparing them for the culture chambers was autoclaved. Cap, mask, and sterile gloves were worn by the operator during this entire procedure. A six mm corneal trephine was utilized in cutting the sections, and these were transferred immediately to a Rose Perfusion Chamber.[3] Three sections could be taken from within the limbus of each cornea in such a manner that no conjunctival or scleral tissues were included. In order to minimize trauma and to insure uniformity of the corneal sections, deep lamellar incisions were made for all three sections. The resections were completed after collapse of the anterior chamber. One section in the second trial included both corneal and scleral tissues for comparative study. A number one, 22 mm, circular coverslip

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[1]Dihydrostreptomycin sulfate, Crystalline; Chas. Pfizer and Co., Inc., Brooklyn, New York.

[2]Crystalline penicillin G, Potassium; E. R. Squibb and Sons, New York.

[3]Electro-Mechanical Development Co., Houston, Texas.

was placed in the bottom half of the Rose chamber and fitted with one Silastic gasket.[1] Separate two ml syringes fitted with 24 gauge needles were used to dispense two drops each of chicken embryo extract and chicken plasma on the coverslip. Immediately the first corneal section was trephined and placed in the mixture with the anterior surface epithelium up. A similar circular coverslip was placed over the clot on the gasket and the culture sealed by the addition of a metal gasket and the top half of the chamber (Figure 1). After the last chamber was closed and it was evident that a good clot had formed, medium was added to the chamber by inserting two 24 gauge, one inch needles through the seal gasket 180 degrees apart. The medium was infused into the chamber through one needle fitted to a syringe, while the opposite needle allowed for air pressure release.

Mixture 199 and Puck's synthetic medium were used in each experiment. To these solutions sterile, cell free, canine blood serum was added to make a final concentration of 10 per cent. All media was adjusted to pH 7.8 with sterile 7.5% $NaHCo_3$ and stored at -15° C in five ml amounts. Frequent bacteriological tests of the media at the beginning and at the end of each trial, and of thawed and incubated media revealed no contamination. Any change in pH, indicated by a shift in the color of the phenol red indicator,

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[1]Electro-Mechanical Development Co., Houston, Texas.

was used as a criterion for feeding. The first trial established a feeding schedule which resulted in media changes after the first four to five hours, again after 12 hours in culture, and at 10 to 12 hour intervals thereafter. The media was thawed in the incubator allowing the temperature to reach 37.5 degrees centigrade before use. All feedings were accomplished in the enclosed tissue culture room.

The perfusion chambers were placed in petri dishes and maintained in a 37° C. incubator. The cultures were examined at each feeding period with light field microscopy, and photographic recordings of cellular activity were made. The last two trials were examined by both light field and phase contrast microscopes with photomicrographs taken at various intervals. For phase observations the microscope with the chamber was housed in a special incubator which maintained the temperature at 37° C. This allowed continuous observation and photography over a period of several hours. Table I indicates the handling of individual sections in each experiment. At the end of the culture periods, the coverslips were removed from the chambers and the adhering cells stained with May-Grünwald-Giemsa (Merchant et al., 1960).

RESULTS

A. pH Determinations

Table II presents the results of three consecutive days' examinations of the aqueous humor, with the pH determinations repeated in one week. The puncture technique produced hemorrhage in only one eye; this had completely disappeared before the second series of examinations were initiated. One ml of aqueous humor could be obtained consistently and only in the collie did this amount effect near collapse of the globe. Aqueous clots were experienced and in a few cases did not allow for pH determinations. The aqueous samples were observed in the glass vials and in all cases a partial clot resulted in one hour. After 24 hours the clot appeared to break down; at the end of 48 hours the aqueous once again was in a liquid state. From this study an average pH of the canine aqueous was recorded as 7.8.

B. Tissue Culture Observations

In the first two trials, the corneal sections which were placed in a clot without additional media did show some cellular activity on the surface of the explant, but

in no case did migration of cells into the clot occur. The cellular activity was not evidenced after the first five hours in culture. A section of sclera was allowed to remain intact with the corneal tissue in one culture, but no cellular activity of the sclera was observed over a 44 hour period. The attached cornea gave evidence of activity and followed the general pattern to be discussed. The growth patterns were very consistent and in four to six hours, migration of epithelial-like cells was observed (Figure 2).

The perfusion chamber made possible examination of the epithelial and the mesenchymal epithelial surfaces individually. In the early periods of observation, it was apparent that the cellular activity was confined to the epithelial tissues; this activity was observed on the surface and at the perimeter of the explant. As the cellular migration continued, it was possible, by focusing through the clot, eventually to observe the presence of the epithelial-like cells near the mesenchymal epithelial surface. There was, however, no cellular activity present on this surface at any time.

Because of the difficulty in measuring three dimensional migration, cell rows from the edge of the explant were counted and recorded (Table II). During each period of observation, photomicrographs documented the cellular activity.

Changes in the pH of the media became apparent after four to six hours in the incubator and in nearly all of the cultures a slightly acid medium was observed as evidenced by a change in the color. The chambers containing Mixture 199 invariably demonstrated a more rapid and severe change to an acid reaction than those with Puck's medium.

At the end of 15 hours, the migration resulted in 12 to 16 rows of cells (Figure 3). By this time a few cells were observed in all layers of the clot with the greatest concentration near the epithelial edge of the explant. It was noted that the least active areas were those which exhibited an irregular border, a result of an imperfect incision during the surgical procedure. Color changes in the media again indicated an alteration of the pH; this acid appearance was interpreted as cellular metabolism. Continuous observations revealed the period represented by the 15th to 20th hours in culture to be the most active. It was during this period of time that the cells rapidly reached the edge of the clot.

After 31 hours in culture, the cells had migrated to the edge of the clot and were quite densely packed (Figure 4). Beyond this period very little cellular activity was visible microscopically or by gross observations of the media. At this stage of culture, the pH did not reach the acid scale but 10 or 12 hour feedings were continued. The cellular mass became more dense through the 42nd hour and

there was still only a slight decrease in alkalinity observed. Beyond this point it was impossible to determine cellular activity microscopically and there was no apparent pH change.

Both of the media used in these experiments produced similar stimulation of the explant but it should be noted that Mixture 199 cultures were consistently a few hours ahead, both in the number of cells observed and pH reduction. Following the most active culture period, fewer differences between the two were observed and by the end of 42 hours, Puck's medium was equal to Mixture 199 in cellular stimulation.

The series followed by phase microscopy, demonstrated cellular activity with the first migration of epithelial-like cells noted in three hours (Figure 5). Migration of these cells was a rapid and continuous process and even though observations were accomplished at hourly intervals, dramatic changes in the field under observation were noted. New cells were present and the cells previously observed assumed a new position within the field. Figures 6, 7, and 8 respectively, represent 10, 11, and 12 hours in culture. Again, the most active period observed was between the 15th and 20th hours. Figure 9 represents the cellular migration as it appeared following 20 hours in culture. Because of the packed appearance at this time, further developments in this field could not be shown photographically. By moving the field of observation away from the explant, further

migration could be observed; at the end of 30 hours, the cells were present in all areas of the clot.

The cells attached to the cover slips, which comprise the top and bottom of the perfusion chamber, were fixed in absolute methyl alcohol and the May-Grünwald-Giemsa method for staining whole cultures differentially was utilized. Sheets of epithelial cells and groups of epithelial-like cells are demonstrated in Figures 10 and 11.

DISCUSSION

Examination of the canine aqueous humor was initiated primarily to establish a normal pH range as a basis for adjusting tissue culture media to an optimum pH level. Many interesting observations resulted, namely, increased clotting powers exhibited by the replacement aqueous. The fact that rapid clotting was not as dramatic in the second series of sampling may be attributed to the improved technique of globe puncture. Only when the iris was traumatized did there appear any indication of the anterior chamber invasion. No permanent pathological change was observed in any of the eyes and this should be of interest when considering experiments involving ocular therapeutics.

It was interesting to note that paired eyes did not give identical pH readings (Table I). This observation was also reported by Epstein (1959) who examined 11 dogs between eight and 15 years of age. These dogs were afflicted with various disease processes, some of which were accompanied by corneal lesions. Paper indicators were used in his experiments and the pH ranged from 7.5 to 8.0 with the average approaching 7.5. Most of the ocular preparations on the market have been produced for use in man but are utilized by the veterinarian in treating canine patients. In some

instances these preparations appear to be irritating to the canine eye and at times the reaction reported for the human patient is not observed in the dog. It may well be that further consideration should be given to the relatively high pH observed in the canine aqueous humor when producing a preparation for use in canine ocular therapeutics.

The consistent results observed with the 6 mm corneal sections, indicate the tissues to be easily adapted to cellular activity within the perfusion chamber. No contamination difficulties were experienced when the enucleated globe was immersed immediately in 1:1000 zephiran chloride for a ten minute period. This step was omitted from the technique in handling one globe and culture contamination occurred. The stored media was examined at this time and found to be sterile; thus, it appeared that the contaminants were carried to the chambers on the corneal tissue. An established technique for handling the chambers allowed feeding over long periods of time without contamination or damage to the chambers. The number one cover slips were adequate for visualization and photomicrography, but they were easily broken during the feeding procedure when pressed by the needle or when excessive pressure was applied to the syringe.

Histological sections were obtained from the trephined cornea in an attempt to demonstrate all layers of the cornea. Mesenchymal epithelial cells were demonstrated but never as an intact layer. No mesenchymal epithelial migration

was observed during the study. This parallels the results observed by McPherson et al. (1956).

The very early appearance of cellular activity has not been reported by other investigators but because this identical technique has not been used on other species, it would not be possible to attribute this solely to species variation.

There was no evidence of fibroblastic activity in any of the cultures even when held for periods up to 20 days. One explanation for this may be found in the high pH maintained in the cultures. Paul (1959), in discussing the cell and its environment, places the optimal growth range between pH 7.2 and 7.4. He further stated that it is undesirable to allow the pH to deviate outside the limits of pH 6.8 to 7.6. One of the problems encountered in tissue culture techniques has been that of excessive fibroblastic activity. The experiment presented here demonstrated an overgrowth of the culture by epithelial-like cells at pH 7.8. This early activity by these cells may have contributed to the inhibition of fibroplasia.

Both media demonstrated the ability to stimulate cellular activity. The availability of these media in addition to the extremely small amounts required by the perfusion chamber will make future studies much less difficult and very economical.

It is hoped that the technique of handling large

corneal sections in the Rose perfusion chamber will stimulate further experimentation to the end that keratoplasty in the dog will become a clinical reality.

SUMMARY

The hydrogen ion concentration of canine aqueous humor was determined in order that the media utilized in the perfusion chambers might be adjusted to maintain an environment similar to that enjoyed by the corneal tissues in the normal eye.

The Rose perfusion chamber was shown to be adaptable to the handling of large corneal sections in tissue culture.

Two media were used in this study; one contained 10% canine serum and 90% Mixture 199, while the other was comprised of 10% canine serum and 90% Puck's medium. Both media proved capable of stimulating the migration of epithelial-like cells from the explant.

Uniform activity of the corneal tissue was recorded, with migration observed as early as three hours after the initial feeding of the explants. The greatest cellular activity was observed to occur between the 15th and 20th hours of culture.

Observations of the large corneal sections in tissue culture were encouraging and hope is expressed that further investigations will make keratoplasty a clinical reality for the veterinarian.

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TABLE I

QUANTIFICATION OF CELLULAR MIGRATION

Trial	No. of Explants	Medium Used	Results at Examination Periods (Hours)				
			4-6	15-19	31-34	38-42	52-56
1	2	199	/	4+	7+	7+	7+ T
	2	Puck's	/	3+	5+	6+	6+ T
	1	Puck's no serum	-	-	-	- T	
	1	Clot	-	-	-	- T	
2	2	199	/	4+	7+	7+ T[b]	
	2	Puck's	/	4+	6+	6+ T	
	1	Clot	-	-	-	- T	
	1	199[a]	Cornea /	4+	6+	6+ T	
			Sclera -	-	-	- T	
3	2	199	/	3+	8+	8+ T	
	2	Puck's	/	3+	7+	8+ T	
	1	Clot	-	-	-	-	
4	3	199	/	3+	7+ T		
	2	Puck's	/	3+	6+ T		
5	2	199	/	3+	7+	7+	8+ T
	2	Puck's	/	3+	6+	7+	8+ T
6	3	199	/	3+	8+	8+	8+ T[c]
	3	Puck's	-	2+	7+	7+	8+ T[c]
7	2	199	/	3+	8+	10+	10+[d]
	1	Puck's	/	2+	8+	8+	8+ T

Key: / = 1 row of cells
+ = 4 rows of cells
T = Termination of Culture

a. Corneal and scleral tissue both in culture
b. One culture. Maintained for 20 days - no fibroplasia
c. One culture. Maintained for 12 days - no fibroplasia
d. One culture. Maintained for 10 days - no fibroplasia

TABLE II

pH DETERMINATIONS OF CANINE AQUEOUS HUMOR

Experimental Dog	Day						
	1	2	3	8	9	10	Average
Beagle Female 2 years	R-7.79	8.02 pc-imm	7.87 c in 30 m	7.76	7.74	7.76	7.82
	L-7.89	8.08 pc-imm	7.88 c in 30 m	7.80	7.78	7.79	7.87
Beagle Male 3 years	R-7.68	c	7.65 c in 30 m	7.66	7.85	7.83	7.73
	L-7.93	c	7.64 c in 30 m	7.74	7.77	7.76	7.77
Basset Male 3 years	R-7.82	7.65	7.92	7.91	7.88	7.87	7.84
	L-7.98	c	8.15 sh	7.93	7.92	7.92	7.98
Border Collie Female 1 year (lactating)	R-7.70	7.83	8.45 c in 30 m	7.71	7.72	7.77	7.86
	L-8.08	7.90	8.12	7.89	7.89	7.91	7.97
Boxer Male 8 months	R-7.92	7.70	7.93	7.92	7.87	7.88	7.87
	L-7.96	7.75	8.07	7.89	7.89	7.87	7.90

Key: pc = partial clot
c = clotted
sh = slight hemorrhage
imm = immediate
m = minutes
R = right
L = left

Figure 1

ROSE PERFUSION CHAMBER WITH EXPLANT

X1.8

Bottom view of chamber showing grooves 180° apart, through which needles are inserted for feeding. The explant was centered in the chamber for uniform exposure of the cells to the medium.

Figure 2

FIVE HOUR CULTURE

X447

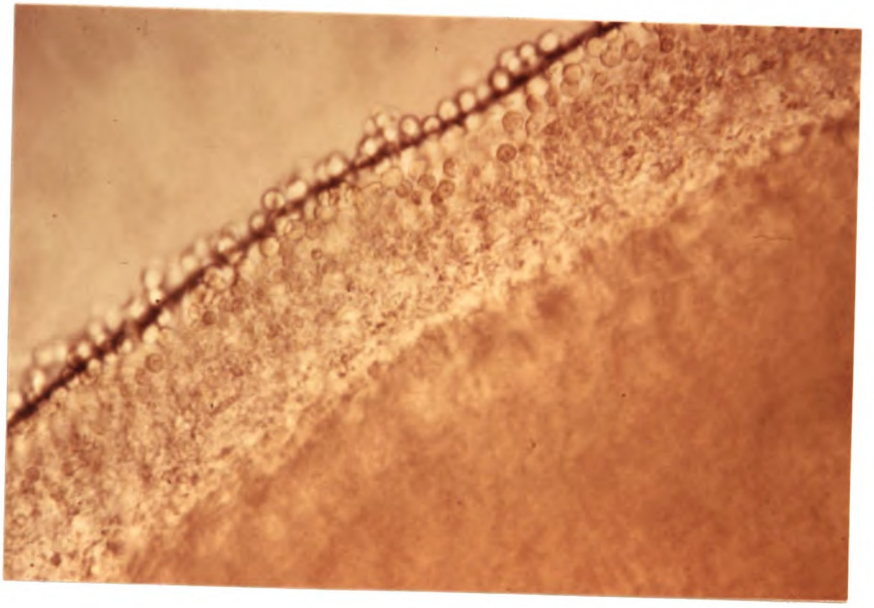
Cellular activity is shown to be present both on the surface and from the cut edge of the explant.

Figure 3

FIFTEEN HOUR CULTURE

X447

Epithelial surface of the explant gives evidence of cellular "packing." As many as 20 rows of cells may now be observed in the clot.



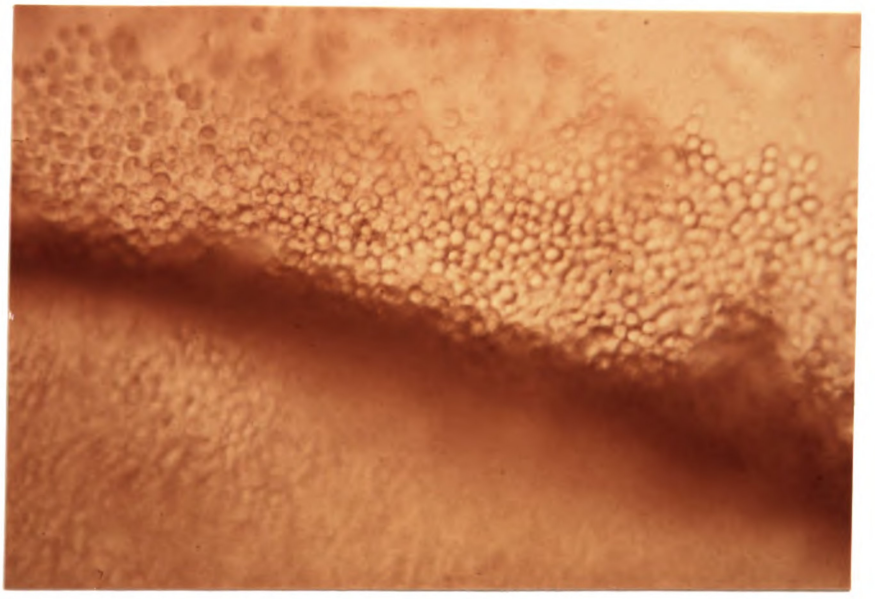


Figure 4

THIRTY-ONE HOUR CULTURE

X447

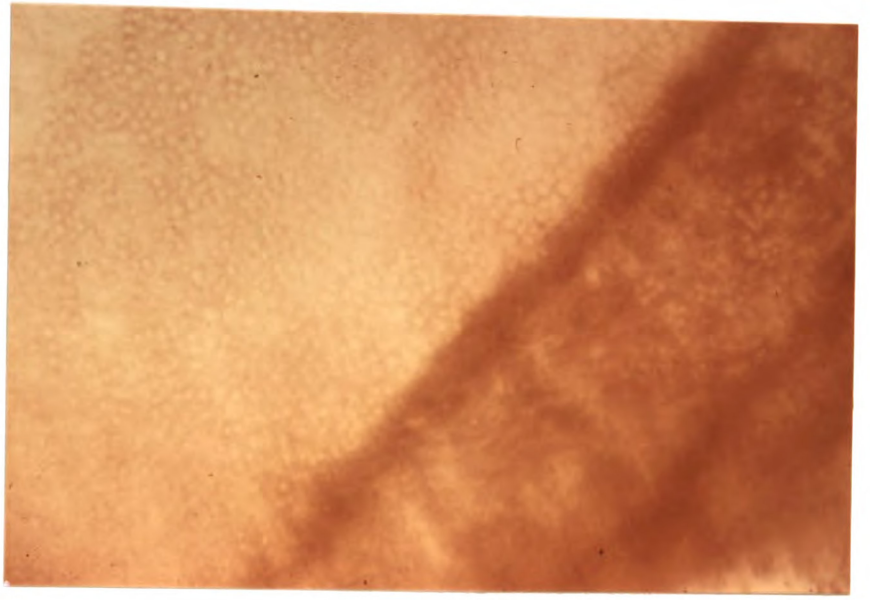
Epithelial-like cells have migrated to the edge of the clot. The packed appearance can only be visualized photographically in one dimension.

Figure 5

THREE HOUR CULTURE

Phase Contrast X495

A typical view of the young cell forms observed in cultures fed with either Mixture 199 or Puck's medium, showing the very early appearance of cellular activity at the explant edge.



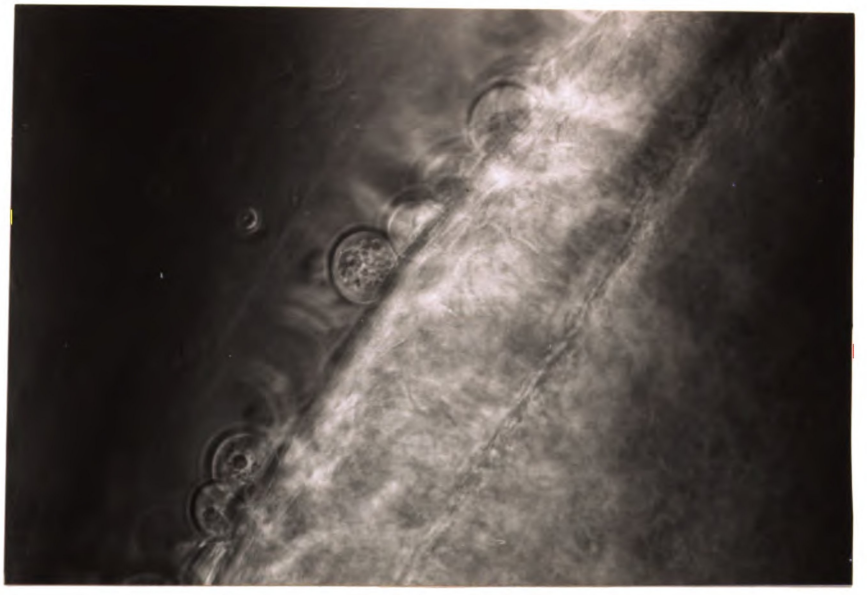


Figure 6

TEN HOUR CULTURE

Phase Contrast X495

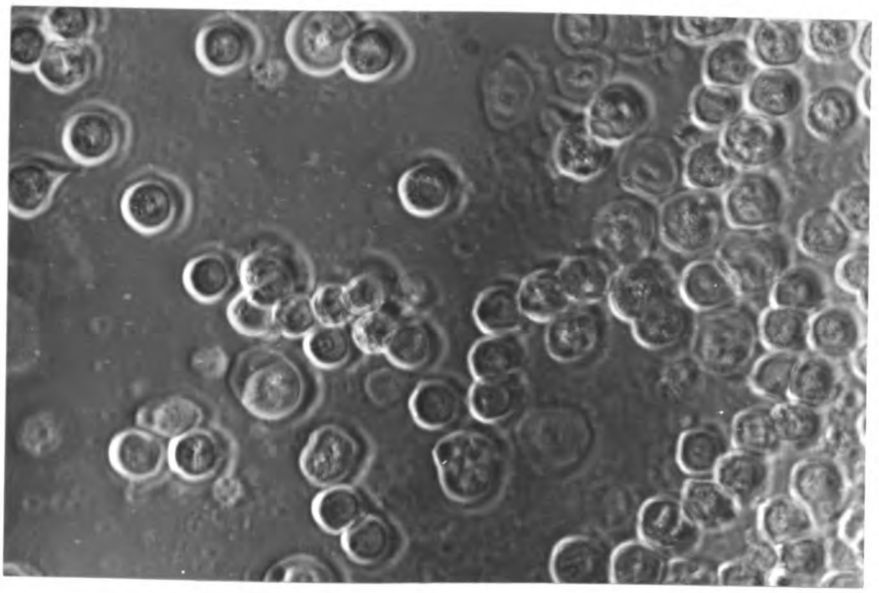
Epithelial-like cells are shown in various stages of activity. True epithelial cells are now observed.

Figure 7

ELEVEN HOUR CULTURE

Phase Contrast X495

Changes in cellular positions within the field are noted and the area near the explant has become more dense.



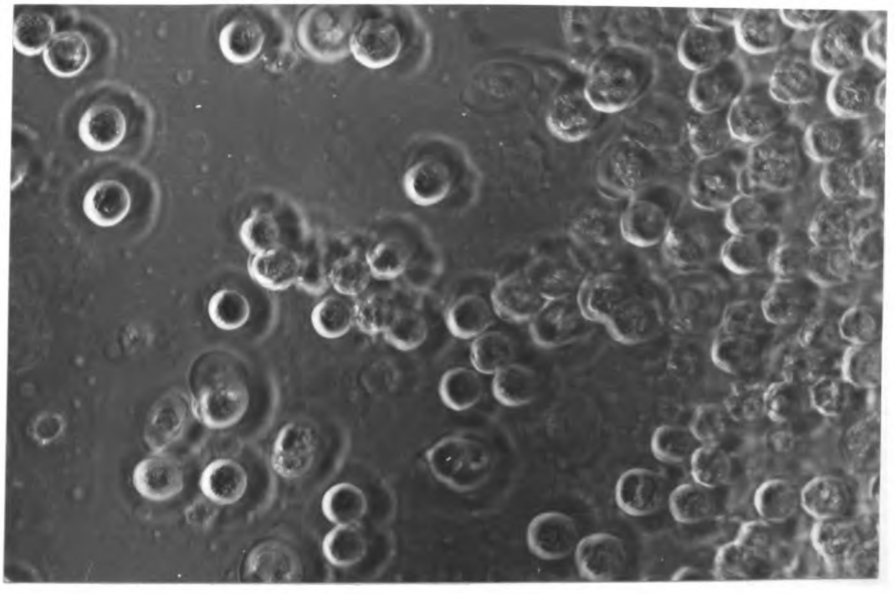


Figure 8

TWELVE HOUR CULTURE

Phase Contrast X495

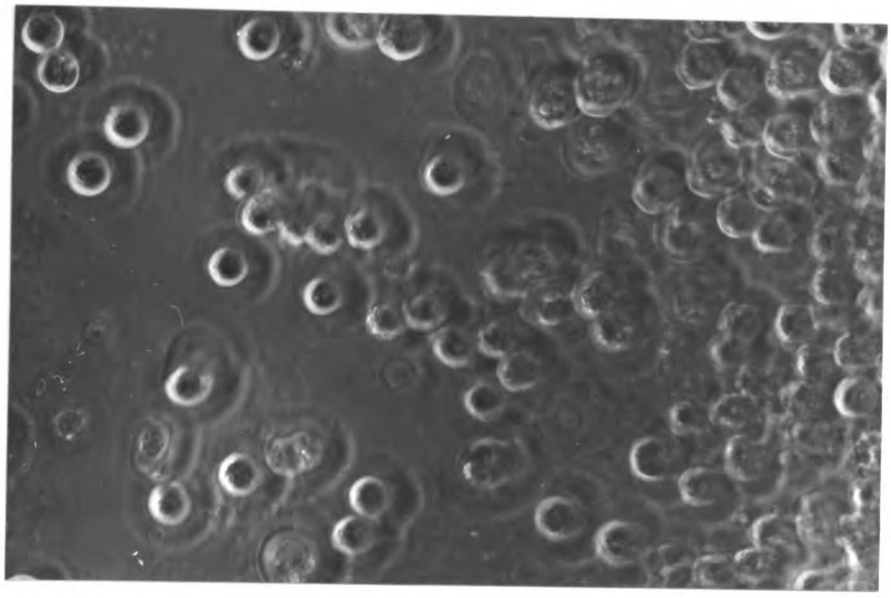
Further cellular movement can be observed and the area near the explant appears to have a more dense background.

Figure 9

TWENTY HOUR CULTURE

Phase Contrast X495

Sheets of cells have created a dense field near the explant, and the nuclear activity is most prominent in the cells furthest from the explant.



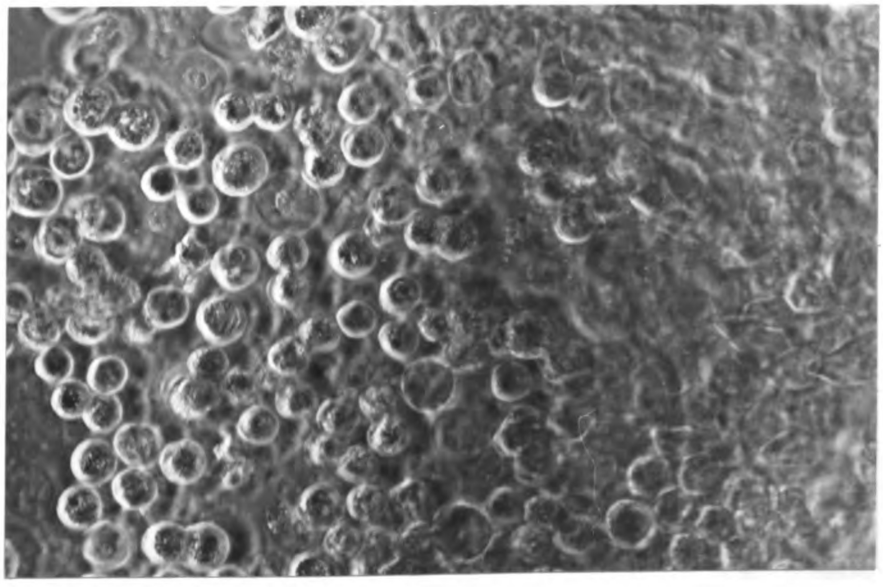


Figure 10

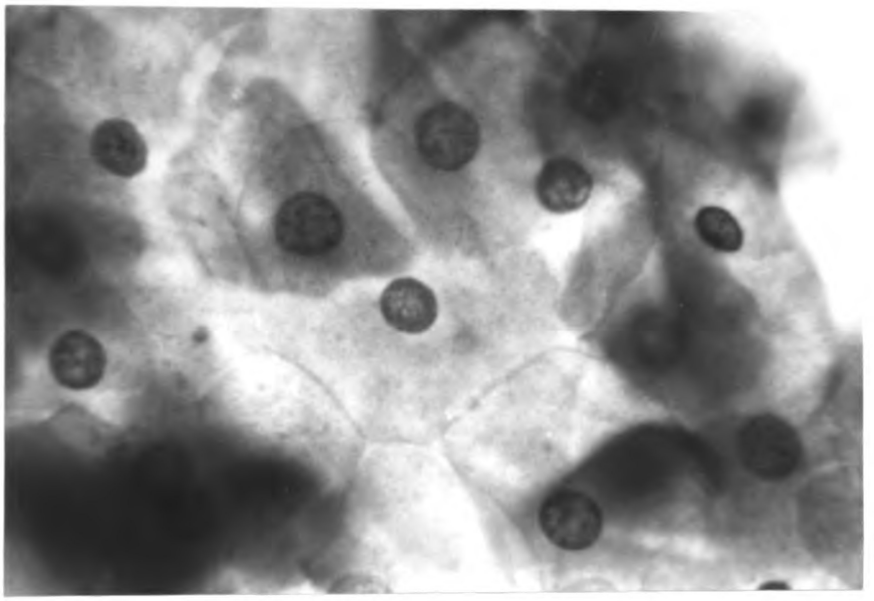
EPITHELIAL SHEET

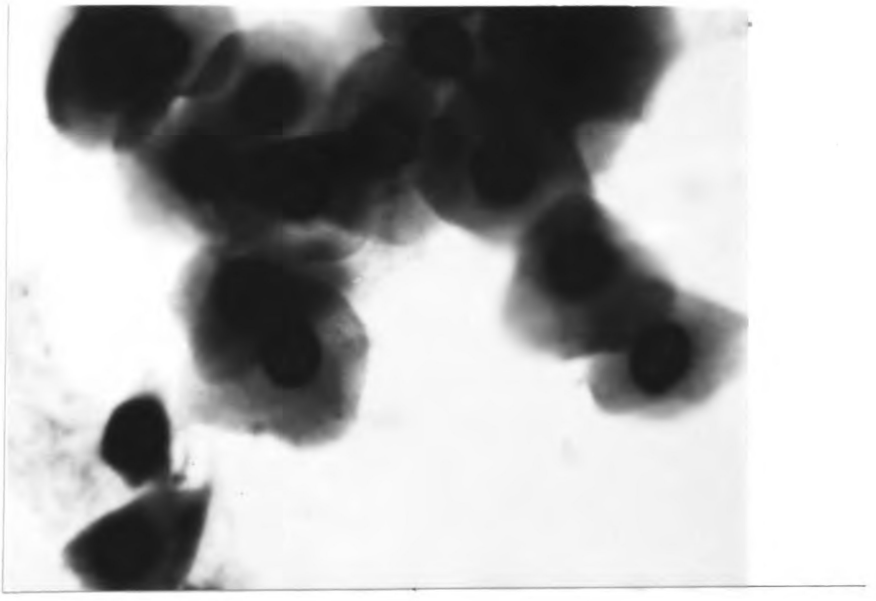
Mature epithelial cells forming a sheet on the chamber coverslip. May-Grünwald-Giemsa; X1117.

Figure 11

EPITHELIAL-LIKE CELLS IN CLUMPS

Clumping of epithelial cells on the chamber coverslip. May-Grünwald-Giemsa; X1117.





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