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# The Effects of Prostaglandin E2 on the Healing Processes of Rat Perforated Tympanic Membranes

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# THE EFFECTS OF PROSTAGLANDIN E2 ON THE HEALING PROCESSES OF RAT PERFORATED TYMPANIC MEMBRANES

by

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# THE EFFECTS OF PROSTAGLANDIN $E_2$ ON THE HEALING PROCESSES OF RAT PERFORATED TYMPANIC MEMBRANES

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Prostaglandin $E_2$ ($PGE_2$) was topically administered to perforated rat tympanic membranes in an attempt to facilitate regrowth of the membranes. Every 24 hours for the first four postperforation days, two groups of tympanic membranes were processed and examined histologically for differences between $PGE_2$ treated and control animals.

At 24 hours postperforation granulocyte infiltration at the perforation site was greater in the test animals. By 48 hours blood clots were larger in the $PGE_2$ treated animals. Finally, the epithelium was thinner in test animals than in control animals at 96 hours.

Slight tendencies for the test animals to develop mature collagen, smaller perforation sizes, and increased vascularity were noted at 48 hours. The $PGE_2$ treated animals demonstrated slightly thinner regrowing membranes by 72 hours and larger blood clots by 96 hours. No definitive conclusions could be drawn from the results, however $PGE_2$ may slightly accelerate healing of the perforated tympanic membrane.

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Diane Sue Saylor

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# CHAPTER I

## INTRODUCTION

Chronic perforations of the tympanic membrane are long standing problems in the field of otolaryngology. Frequently these perforations fail to heal spontaneously or completely in older persons. The remaining defect in the tympanic membrane causes hearing loss in affected individuals and is an access route for microorganisms and the migration of cornified epithelium into the middle ear (cholesteatoma) (Schuknecht, 1974).

During the past fifteen years numerous techniques and procedures have been evaluated in an attempt to overcome the problems of chronic tympanic membrane perforations. The success rates with these methods have been limited. Topical application of prostaglandin $E_2$ to the perforation site may offer an alternative approach to treatment if the prostaglandin is found to facilitate regrowth of the tympanic membrane.

Prostaglandins are derived from arachidonic acid (20:4) which is derived from membrane phospholipids. $PGE_2$ is an autacoid with numerous and diverse effects and has been tested for use in the treatment of gastric ulcers, bronchial asthma, and as an early abortifact (Behrman & Anderson, 1974). Its pharmacologic properties make it a good candidate for the treatment of perforated tympanic membranes.

## CHAPTER II

## REVIEW OF SELECTED LITERATURE

The tympanic membrane is composed of three layers of tissue: the meatal epithelium, the connective tissue layer (also known as the collagen layer), and the middle ear epithelium (Schuknecht, 1974). McMinn and Taylor (1966), experimenting with guinia pigs, found that within a few hours postperforation blood clots and granulocytes, mainly polymorphonuclear leukocytes, gather at the site of the perforation. Blood vessels dilate in response to the injury, the meatal epithelium hypertrophies, and the connective tissue layer adjacent to the defect is punctuated with loose collagen. Fibroblasts also increase in number at the wound site. Within a few days the hypertrophied meatal epithelial layer returns to preperforation thickness, and the collagen matures to a denser than normal state. McIntire and Benitez (1970) perforated the tympanic membranes of cats and demonstrated the following regrowth pattern. Generally, the meatal epithelium hypertrophies at the edge of the gap, and it is usually this epithelial layer that is the first to close the defect. The other layers follow the course of the meatal epithelium to regenerate the intact tympanic membrane. During the regrowth process the connective tissue layer usually retains its normal thickness except for a "curling effect" at the edge of the defect, and the middle ear epithelium remains largely unchanged except at the margin of the perforation where it intermingles with the hypertrophied meatal epithelium. All of the three layers of the tympanic

membrane may intermingle with each other. McIntire and Benitez (1979) found considerable variation in the final thickness of the healed perforation but could not demonstrate any difference in the threshold of hearing with the different thicknesses of tympanic membranes.

The failure of the perforated tympanic membrane to heal spontaneously or adequately can be a problem in the elderly. Several techniques have been developed and tested to encourage new growth in the perforated area (Shambaugh & Glasscock, 1980). Surgical excision of the tissue directly peripheral to the perforation, acid burning of the adjacent tissue, and myringoplasty are three methods developed to enhance healing of the perforated tympanic membrane. None of these methods are without problems in the effective management of the defect. Surgical excision and acid burning require careful and frequent application over a long period of time. Myringoplasty is not always successful and can be complicated by infection (Shambaugh & Glasscock, 1980). Therefore, an alternative and noninvasive treatment for the injured tympanic membrane was sought.

Prostaglandin $PGE_2$ was chosen as the test theraputic agent because it is endogenous to the middle ear (Jung, Juhn, & Gerrard, 1981) and because it possesses many properties which may facilitate more rapid and complete healing of the perforated membrane. $PGE_2$ is a known vasodilator (Nakano, 1973) and may increase blood flow to the tympanic membrane. It has also been demonstrated that the addition of $PGE_2$ to polymorphonuclear leukocytes prevents the release of the lysosomal hydrolases (Weissmann et al., 1971) and thereby limiting the destruction of healthy tissue during infiltration by these gran-

ulocytes. In addition, $PGE_2$ increases capillary permeability (Mody, 1972). Finally, platelet aggregation is enhanced by $PGE_2$ (Allen & Valeri, 1974) and thereby facilitates a resumption of normal blood flow in vessels damaged by the perforation and provides the framework for fibroblasts to repair the perforation (Guyton, 1976).

The exact mechanisms of action of prostaglandins are still a matter of conjecture. It is believed that the metabolic functions of prostaglandins are somehow related to cyclic AMP and calcium levels. It is suggested that prostaglandins produce their metabolic effects by either stimulating or inhibiting the accumulation of cyclic AMP. Calcium levels also appear to have an effect on the mechanisms of prostaglandins but how the two are involved remains unclear (Goodman & Gilman, 1975).

Based upon the above characteristics, topical administration of $PGE_2$ was tested in a pilot study to determine its effects on perforated rat tympanic membranes (Appendix A). The results of the study demonstrated an increase in the size of blood clots in test animals compared to controls. Active fibroblasts appeared to be more abundant in the $PGE_2$ treated animals. The remaining gaps in the test animals were smaller in area than the controls. Also, the new collagen appeared to be more organized in the $PGE_2$ treated tympanic membranes. After considering the findings of the pilot study, a full size experiment was designed to test $PGE_2$ as a possible agent to facilitate the healing of perforated tympanic membranes.

# CHAPTER III

## MATERIALS AND METHODS

### Animals

Forty-eight nine week old Sprague-Dawley male rats were divided by random selection into eight groups of six animals per group. Four of the groups consisted of test animals, and the remaining four groups were used as control animals. All animals were housed individually in metal cages with wire mesh bottoms. Water and Purina Rat Chow were given ad libitum. The housing room was thermostatically controlled at a temperature of 72° Fahrenheit. The light periodicity was automated at twelve hours of light followed by twelve hours of darkness.

### Perforations

Due to limited housing space and to the length of time necessary to perforate the tympanic membranes, the experiment was run in two equal segments with a time separation of exactly one week. In each segment the animals had their tympanic membranes perforated on day zero and were subsequently treated over the following four days.

Thus on day zero, each rat underwent bilateral tympanic membrane perforations. While under light chloroform anesthesia each rat was placed on its side under an American Optical dissecting scope with overhead illumination. Curved Molony forceps were inserted into the outer portion of the external auditory canal and manipulated sufficiently to reveal the tympanic membrane. Due to the contortions of the external

auditory canal, visualization of the tympanic membrane was difficult and therefore the rat had to be aligned with the light so that the membrane reflected the maximum light to the lens of the dissecting scope. The pilot study (Appendix A) demonstrated that this orientation could be achieved with each animal and that consistent perforations could be obtained.

The forceps were lined up along the manubrium of the malleus so that the end of one tong of the forceps lay at the end of the quadrant line which separates the anterior superior quadrant from the posterior inferior quadrant. (The quadrant system used for the present study is the same system that is used clinically for humans.) A 1.5 inch 18 G needle was hand fed between the tongs of the forceps, and a perforation was made in the pars tensa area of the posterior superior quadrant. The diameter of the needle was used as a measurement guide for placing the perforation two "diameter units" posterior to the umbo (Figure 1).

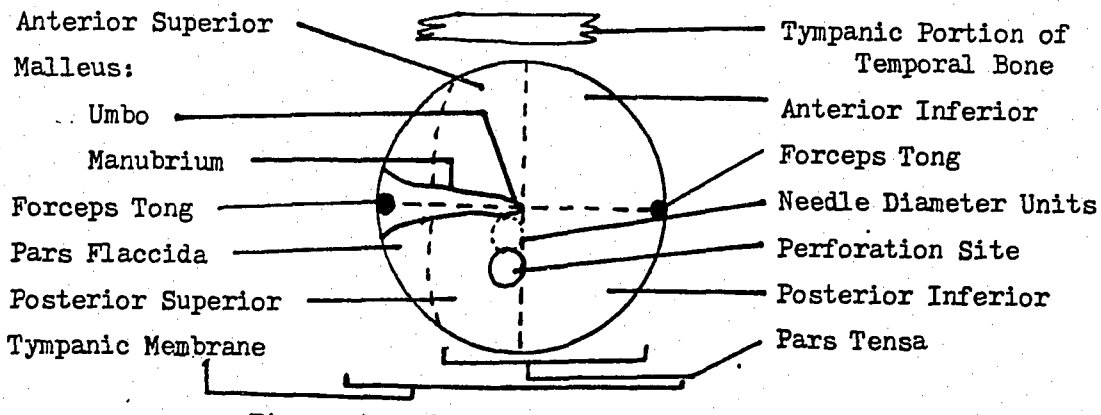



Figure 1. Placement of the perforation

The angle of approach to the tympanic membrane was kept constant by maintaining the needle in a plane which was parallel to the tongs of

the forceps. The tongs were positioned at an eighty degree angle to the fibrous annulus of the pars tensa. With the point of the needle directed toward the posterior most aspect of the perforation site, the needle was inserted through the tympanic membrane and pushed across the middle ear fossa until it contacted the medial wall of the middle ear. The needle was then rotated 360° clockwise. Perforations performed in this manner produced defects which measured 1 mm in diameter. Pilot studies (Appendix A) showed that perforations of consistent size with minimal distortion due to ragged edges could be produced (Figure 2).

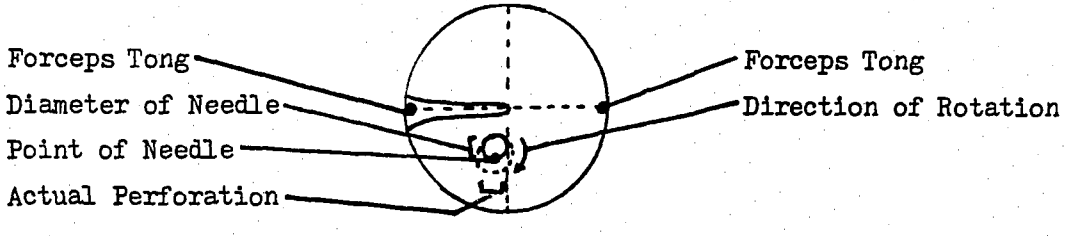



Figure 2. Diameter of the perforation

Drug Treatment

Immediately following the perforation procedure and while the rats were still anesthetized, a 0.2 cc solution of 0.5 mg/kg body weight $PGE_2$, an effective topical dosage to elicit physiological response (American Medical Association, 1980), mixed in isotonic saline (pH 6.95) was made up. The solution was administered topically through a long nosed 1 cc pipette. The delivery end of the pipette was inserted two-thirds the length of the external acoustic meatus. The pipette was then evacuated of its contents with great care to prevent further damage to the membrane. The rats were placed on their sides and kept

immobilized during the $PGE_2$ administration and were maintained in that position for two minutes to assure adequate time for the solution to cover the perforation area. Subsequent applications of the prostaglandin were administered without the use of anesthetic but in an otherwise similar manner. Pilot studies (Appendix A) demonstrated that application of $PGE_2$ in this manner was feasible and did not cause damage to the perforation site. Since unabsorbed prostaglandin may become inactive after 24 hours of its make up, the prostaglandin was reapplied bilaterally every twelve hours until sacrifice (Table 1).

Table 1

Application and sacrifice schedule for test animals

| (HOURS) | 0 | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 |
|---|---|---|---|---|---|---|---|---|---|
| GROUP 1 | P | P | S | | | | | | |
| GROUP 2 | P | P | P | P | S | | | | |
| GROUP 3 | P | P | P | P | P | P | S | | |
| GROUP 4 | P | P | P | P | P | P | P | P | S |

P = PGE2 administered S = Sacrifice

Pilot studies showed that tympanic membrane closure was complete by 72 hours postperforation in control animals. A maximum of 96 hours postperforation was used in the present study because histological assessment disclosed that closure occurred before the repair process was complete (Appendix A).

The control animals were given bilateral applications of 0.2 cc of isotonic saline (the vehicle for the $PGE_2$) in the manner prescribed

for the test animals. The schedule for the control groups was identical to that of the $PGE_2$ treated animals.

## Histological Assessment

At the end of the treatment period, animals were anesthetized with chloroform and sacrificed by cervical dislocation. Following sacrifice, the tympanic rings (fibrocartilagenous rings to which the tympanic membrane attaches) were surgically removed and rapidly fixed in 10% formalin.

Two reference measurements of each observed tympanic membrane defect were made at this time. The superior most point of the tissue was placed at the border of a straight edge. The straight edge was oriented parallel to a line which bissected the inferior half from the superior half of the tympanic membrane (Figure 3). The distance from the superior most point of the deficit to the straight edge was measured with a ruler and recorded. The distance from the inferior most point of the deficit to the straight edge was also measured and recorded. Since the tissues were embedded in paraffin so that the superior most part of the tympanic membrane would be cut first, the reference measurements were used to estimate the depth of the beginning and the ending of the deficit in the block of tissue. The reference measurements were useful for more effecient localization of the deficit in the tissues to be cut.

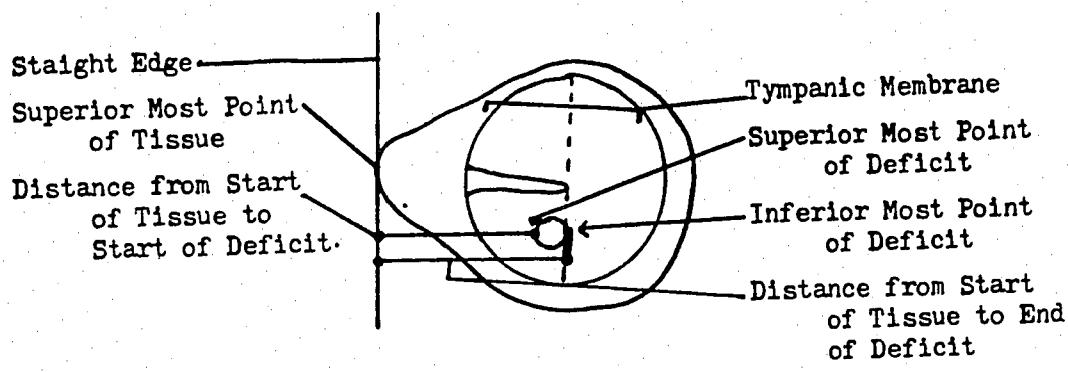



Figure 3. Reference measurements

After fixation the tissues were decalcified in a mixture of formic and hydrochloric acids, dehydrated in ethyl alcohol, cleared in xylene, and embedded in paraffin. The resulting blocks of tissue were sectioned serially at six micrometers. One hundred micrometers before reaching the depth of the block where the perforation began (as noted by the reference measurements), the fifth and sixth sections of each ten section ribbon were saved for staining. Tissue sections were collected in such a manner until one hundred micrometers beyond the ending of the deficit in the tissue. The fifth sections were stained with hematoxylin and eosin (for cellular detail), and the sixth sections were stained with Mallory's connective tissue stain (to demonstrate collagen).

The stained histological sections were evaluated using a random number, blind paradigm to determine any differences in the rate or completeness of the healing processes between the prostaglandin treated animals and the control animals. Evaluations were based on several criteria: the degree of closure observed by histological measurements of the remaining gaps in the tissue, maturity of collagen at the perforation site as assessed by the degree of organization and density,

the quantity of fibroblasts as an indication of active collagen growth, the degree of epithelial hypertrophy, vascularity and the extent of blood clot formation at the perforation site, the thickness of the regrowing membrane relative to the the adjacent unperforated membrane, the quantity of exudate in the middle ear, and the infiltration of granulocytes into the middle ear.

## Statistics

Data from the preceding criteria were recorded in numerical form using the key found in Table 2. The raw data (Appendix B) were analyzed statistically using a two-tailed Student T test.

Table 2

Key for numerical assessment of data

| | |
|---|---|
| Exudate quantity: | 1 (no exudate in middle ear cavity) to 4 (middle ear filled with exudate) |
| Epithelium thickness of regrowing perforation: | 1 (epithelium thickness same as epithelium thickness at one mm anterior to the umbo) to 4 (epithelium thickness at least four times the thickness as the epithelium thickness at one mm anterior to the umbo) |
| Relative thickness of regrowing perforation: | 1 (entire regrowing tympanic membrane the same thickness as tympanic membrane one mm anterior to umbo) to 4 (entire regrowing tympanic membrane at least four times as thick as tympanic membrane one mm anterior to umbo) |
| Diameter of remaining gap in micrometers: | diameter in micrometers of opening in tympanic membrane |

Vascularity of perforation: 1 (quantity and size of blood vessels less than quantity and size of blood vessels one mm anterior to umbo) to 4 (quantity and size of blood vessels at least four times quantity and size of blood vessels at one mm anterior to umbo)

Collagen regrowth at perforation: 1 (loose and disorganized fibers) to 4 (mature, compact, and maximum organization)

Granulocyte infiltration at perforation: 0 (no granulocytes present) to 4 (area filled with granulocytes)

Fibroblast infiltration at perforation: 0 (no fibroblasts present) to 4 (area filled with fibroblasts)

Blood clot quantity: 0 (no blood clot present) to 4 (perforation sealed with blood clot)

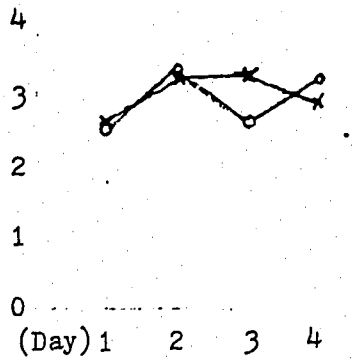
# CHAPTER IV

## RESULTS

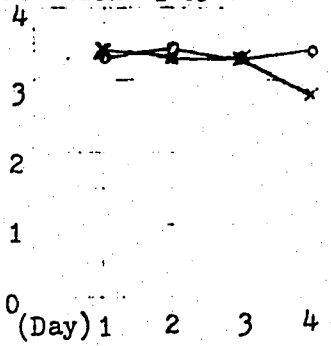
Generally both the control and the test perforations healed in a manner that was consistent with the findings of McMinn and Taylor (1966) and McIntire and Benitez (1970). Gaps appeared to close in a concentric fashion over time, and by day four most of the gaps had been filled with tissue.

The pattern of healing usually began with a marked infiltration of granulocytes, an increase in vascularity, and enlargement of blood vessels. A blood clot was usually present at the perforation site, and the meatal epithelium hypertrophied and formed into several layers. This epithelium was usually the first to span the deficit. The collagen layer and middle ear epithelium layer had usually regrown by the fourth day. The regrowth of collagen was characterized by loose and disorganized fibers with many fibroblasts. This newly formed collagen later matured into a denser collagen, although by four days, it still was not as dense as the collagen in the nonperforated area of the tympanic membrane. By the end of day four, the meatal epithelium at the perforation site had not yet returned to the thickness of the epithelium in the nonperforated area, and consequently the tympanic membrane remained thickened at the perforation site past the end of the study period. The resolution of the blood clot began by day four, but fibroblasts and granulocytes were still present in high numbers at that time (Figure 4).

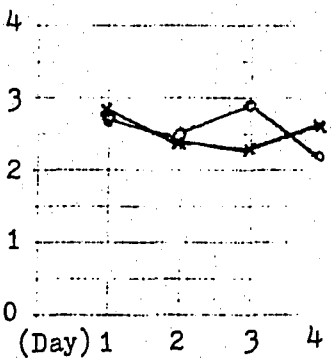
Exudate quantity 1 to 4



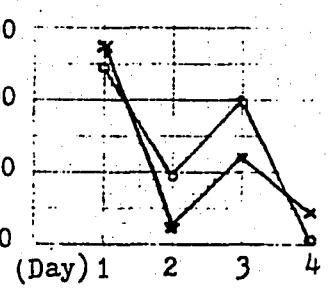

Epithelium thickness of regrowing perforation 1 to 4



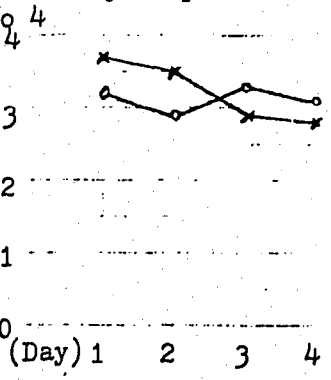

Relative thickness of regrowing perforation 1 to 4



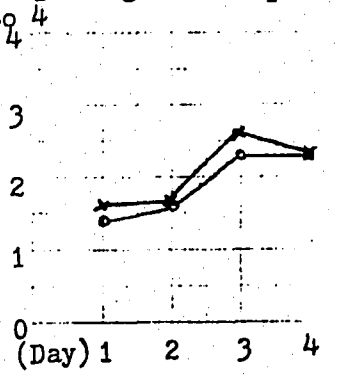

Diameter of remaining gap in micrometers




Vascularity of perforation 1 to 4




Collagen regrowth at perforation 1 to 4




x = mean value for $PGE_2$ treated animals for specified day
o = mean value for control animals for specified day

Figure 4. Graphical representations of mean values

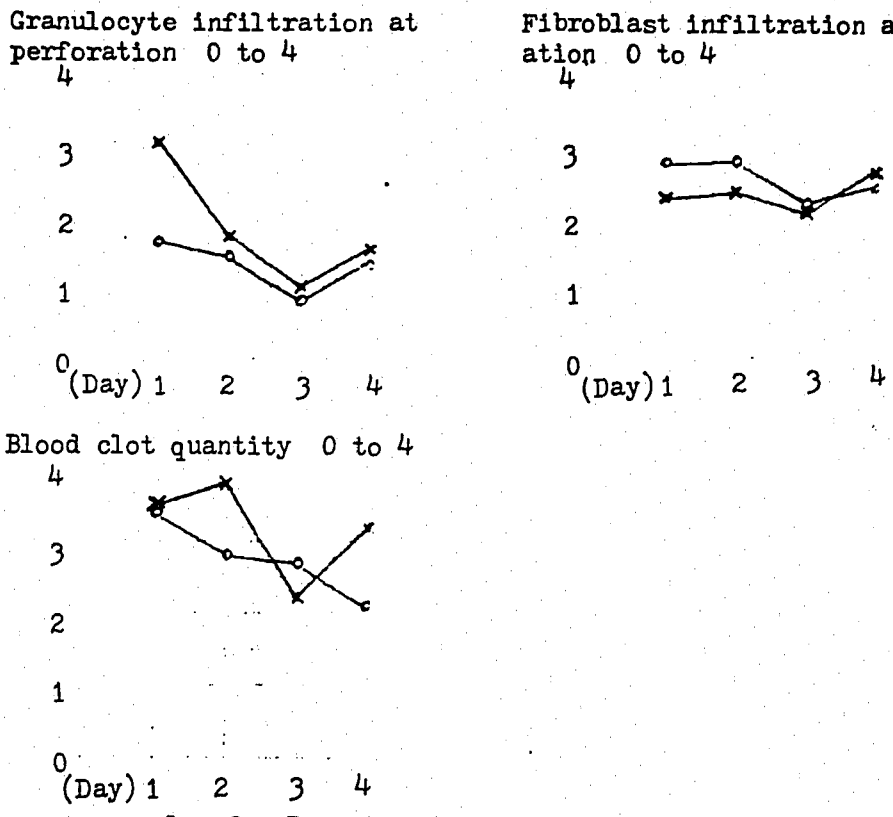



Figure 4. continued

In comparing the $PGE_2$ treated animals with the saline treated animals, several statistically significant results were noted (Winkler & Hays, 1975). First, at 24 hours granulocyte infiltration at the perforation was greater for the $PGE_2$ treated animals. Larger blood clots were noted 48 hours postperforation in the test animals. Finally, the epithelium was thinner 96 hours after the perforation procedure in the $PGE_2$ treated animals (Appendix C).

Certain results indicated tendencies for $PGE_2$ to be correlated with enhanced healing of the perforated tympanic membranes, although the differences were not significant at the $p = .05$ level. These results

included the following findings. At 48 hours postperforation, the test groups demontrated more mature collagen regrowth at the site of the perforations. In addition, the area of the remaining gaps in the perforated tympanic membranes was smaller on the second day compared to the area remaining in the controls. The vascularity, determined by the number and size of blood vessels, did increase slightly at 24 and 48 hours in the test animals over the vascularity of the control animals. At 72 hours the thickness of the regrowing perforations of the tympanic membranes showed tendencies to be thinner in the test animals. Finally, mean blood clot size at 96 hours was slightly larger in the test group. No differences were noted in the mean infiltration values for fibroblasts between the test and the control animals in any of the four days of the study.

## CHAPTER V

## DISCUSSION AND RECOMMENDATIONS

In an analysis of the results of the experiment, most observations of differences between the test and control animals occurred 48 hours following the perforations. At the end of the second day, the blood clots were significantly larger in the test animals than in the controls (a result that was repeated at 96 hours). The increase could be the result of $PGE_2$'s properties to increase capillary permeability and cause vasodialation. The increased size of the blood clots could indicate an increase in blood flow to the treated area which could be beneficial to the reparative processes by carrying more hematologic factors to the affected area. A slight increase in the number and size of blood vessels in the tissues surrounding the perforation could also be demonstrated in the test animals at 48 hours (as well as at 24 hours) possibly indicating an increase in blood flow to the perforation.

The mean gap diameter was smaller at 48 hours in the test animals, but the difference was not statistically significant. The decrease in the gap size could be due to the slightly more mature collagen that was deposited at 48 hours in the test animals as compared to the controls. By 72 hours the difference between the size of the gaps in the test and control animals was no longer demonstrable, and most of the gaps in the tympanic membranes had closed. Theœobservations at 48 hours suggest that the $PGE_2$ may facilitate the closure of the defect slightly sooner than treatment with only saline.

It was also noted that at 72 hours postperforation the thickness of the regrowing perforated tympanic membranes was slightly thinner in the test animals. At 96 hours the test animals demonstrated significantly thinner meatal epithelium at the regrowing perforation site. Based on the findings of McMinn and Taylor (1966) that resolution of the healing of the perforated tympanic membrane was characterized by thinning of the membrane back to the preperforation thickness, it may be suggested that $PGE_2$ facilitates the resolution of the healing process of the injured area over that of the saline treatment.

It was also noted that granulocyte infiltration into the tissues surrounding the perforation was significantly greater in test animals than in controls. This could be the result of increased vasodilation and capillary permeability carrying more of the granulocytes to the affected area.

There was also a slight increase in the amount of exudate (of mainly granulocytes) in the middle ear cavity at 72 hours in the PGE2 treated animals. This too could be explained by an increase in vasodilation and capillary permeability.

Within the parameters of the present study it is not possible to state that the major characteristics of tympanic membrane repair are significantly correlated with topical administration of $PGE_2$. However with the tendencies that the test animals demonstrated for slightly faster regrowth, it remains possible that $PGE_2$ may accelerate healing under optimal conditions.

It is suggested that a modified research design which utilizes different dosage levels of $PGE_2$ be considered. Since the use of $PGE_2$

to treat perforated tympanic membranes had not been previously evaluated, the selected dosage was based upon recommendations for topical delivery of $PGE_2$ for the treatment of other pathologies. It is therefore possible that an effective dosage could be greater or smaller than what was used in the present study. It is also possible that given a longer study time (beyond four days) or the use of a different vehicle, more differences between the groups could have been demonstrated.

Another consideration which would make the study reflect more closely the human condition would be to use older rats (e.g. three years or older) instead of the nine week old rats utilized in this study. The older rats would better typify the reaction of the chronic tympanic membrane perforation in the older person. However, care would have to be taken to eliminate animals with preexisting otitis media (a common ailment in older rats) since infections of the middle ear may alter findings by masking significant differences with previous pathologies.

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# APPENDIX A

## Pilot Study

### Introduction

Chronic perforations of the tympanic membrane present significant problems to aging people. Frequently, these perforations result in hearing loss in affected individuals. The following pilot study was designed to test the therapeutic effects of prostaglandin $E_2$ on the healing processes of mechanically induced perforations of the tympanic membrane in rats.

### Materials and Methods

Nine rats were etherized and given pentobarbital I.P.. While under anesthesia, the rats were placed on their sides so that the tympanic membranes could be seen through a dissecting scope. The membranes were promptly perforated bilaterally in the pars tensa area by rotating the end of an 18 G needle 360°. The punctures measured approximetely 1.0 mm in diameter. Six rats were then given 30 $\mu$g/kg body weight $PGE_2$ dissolved in 100% ethanol. The $PGE_2$ was administered by putting 1.0 ml of the solution into a syringe (without the needle attached) and applying it into the deep part of the ear canals of both ears of each rat. The rats were held on their sides during application of the solution and for two minutes thereafter to assure adequate exposure of the tympanic membranes to the test agent. The other rats were used as control rats and were given 1.0 ml of 100% ethanol in the same man-

ner that the prostaglandin was administered. Application of the $PGE_2$ and of the 100% ethanol were repeated every twelve hours until sacrifice of the animals. Two test animals and one control animal were sacrificed every 24 hours postperforation. Therefore, all animals were sacrificed by the end of 72 hours postperforation.

After sacrifice (through exposure to ether), the tympanic membranes were surgically removed with the surrounding rings attached. The membranes were then fixed in 10% formalin, dehydrated in alcohol, cleared in xylene, and embedded in paraffin. Sections of tissue were cut serially at 6 micrometers. Two of every fifteen sections were mounted on slides. One of the sections was stained with hematoxylin and eosin, and the other section was stained with Mallory's connective tissue stain. The sections were examined for differences in the healing processes between the test and the control animals.

Results

At 24 hours test rats demonstrated larger blood clots than the controls. The clots also tended to be more localized in the region of the perforation in the $PGE_2$ treated animals. More active fibroblasts were observed in the test animals. At the edges of the perforations, more collagen had been laid down in the $PGE_2$ animals thereby making the remaining gaps smaller. A similar decrease in the size of the perforations of the control tympanic membranes was not demonstrable. Granulation tissue was present in both test and control ears, but differences were not discernible.

At 48 hours, preexisting otitis media caused much distortion in

the interpretation of results. However, greater organization of collagen was noted in the test animals. The $PGE_2$ treated perforations were closed or nearly closed with new collagen tissue. The control perforations demonstrated new collagen also, but the new tissue was usually limited to the periphery of the gap and had not yet spanned the perforation. Blood clots in both test and control animals appeared to be dissolving by the second day postperforation.

By 72 hours, the collagen appeared to be more mature in the test groups. Perforations appeared to be closed in both the test and control animals. Finally, resolution of granulation tissue appeared to be accelerated in the test animals over that in the control animals.

Discussion and Recommendations

Based on the results of this pilot study, a deeper investigation into the merits of $PGE_2$ therapy for the treatment of perforated tympanic membranes is warranted. However, several problems were noted with the mechanics of this study. For future studies, portions of this experiment need to be redesigned.

The primary problem was with the rats used. Six month old rats were used in this project. However, these rats presented chronic otitis media. The presence of effusion in the middle ear interfered with interpretation of the histological sections. Older rats are therefore deemed unsuitable and should be replaced with younger rats in following studies. Younger rats (nine week old) are significantly less likely to have developed otitis media.

The 1.0 ml of fluid applied to each ear appeared to be in excess

of the capacity of the ear canal. A volume of 0.2 cc of fluid would be better for the application of the test and control agents. This volume is approximately equal to the volume of space beyond the end of the syringe and to the tympanic membrane.

Another problem is the fact that 100% ethanol will cause some damage to the margin of the perforation, and therefore it should be replaced by a nonoffensive vehicle such as isotonic saline (in which the prostaglandin will dissolve). The $PGE_2$ is labile in solution (converting to $PGA_2$), but the $PGE_2$ is usually stable if used within 24 hours. To assure that an effective dosage of $PGE_2$ reaches the tympanic membrane, the prostaglandin should be prepared preceding each application and used immediately.

The pentabarbital used in this study was found to be unsuitable. The use of this anesthetic resulted in animals that were not relaxed sufficiently to allow for the necessary immobilization for perforation of the tympanic membranes. Chloroform or ether cones were found to be more effective in immobilizing the animals. Although chloroform may cause some liver damage, it is of value as an anesthetic in studies of this nature because the exposure time is short.

The dosage of $PGE_2$ used in this study was recommended by the Upjohn Company. However, reviews of the literature suggest more effective dosages for topical delivery of $PGE_2$. Thus, the dosage should probably be changed in subsequent studies.

# APPENDIX B

## Raw Data

| Animal | Controls (Day) | | | | $PGE_2$ Tested (Day) | | | |
|---|---|---|---|---|---|---|---|---|
| Exudate Quantity | 1C | 2C | 3C | 4C | 1T | 2T | 3T | 4T |
| (1) | 2 | 2 | 1 | 3 | 3 | 3 | 1 | 2 |
| (2) | 2 | 4 | 3 | 4 | 4 | 4 | 4 | 3 |
| (3) | 3 | 4 | 2 | 3 | 4 | 4 | 1 | * |
| (4) | 3 | 3 | 4 | 3 | 3 | 3 | 3 | 4 |
| (5) | * | 2 | 2 | 3 | 2 | 3 | 3 | 2 |
| (6) | 2 | 3 | 3 | 3 | 2 | 2 | 4 | 4 |
| (7) | 2 | 4 | 2 | 2 | 2 | 3 | 4 | 3 |
| (8) | 3 | 4 | 3 | 4 | 3 | 4 | 3 | 3 |
| (9) | 3 | 3 | 3 | 3 | 1 | 2 | 4 | 3 |
| (10) | 3 | 3 | 2 | 4 | 3 | 4 | 4 | 2 |
| (11) | 2 | 4 | 2 | 4 | 1 | 3 | 3 | 3 |
| (12) | 2 | * | 4 | 2 | 3 | 3 | 4 | 3 |
| Mean | 2.5 | 3.3 | 2.6 | 3.2 | 2.6 | 3.2 | 3.2 | 2.9 |
| Epithelium Thickness of Regrowing Perforation | | | | | | | | |
| (1) | * | 4 | 4 | 4 | 4 | 4 | * | 3 |
| (2) | 4 | 3 | 4 | 3 | 4 | 3 | 2 | 2 |
| (3) | * | 4 | * | 4 | * | 2 | 3 | |
| (4) | * | 3 | 4 | 3 | 3 | 4 | 3 | 3 |
| (5) | 2 | 3 | 4 | 4 | 3 | 4 | 3 | 3 |
| (6) | 3 | 4 | 3 | 3 | 3 | 3 | 4 | 3 |
| (7) | * | 4 | 3 | * | * | 3 | 4 | 1 |
| (8) | 3 | 3 | 3 | * | 4 | 3 | 3 | 3 |
| (9) | 3 | 3 | 3 | 4 | 3 | 4 | * | 3 |
| (10) | 4 | 3 | * | 3 | 3 | 3 | * | 4 |
| (11) | 3 | 3 | 2 | * | 4 | 3 | * | 3 |
| (12) | 4 | * | * | 3 | 3 | 3 | 4 | 3 |
| Mean | 3.3 | 3.4 | 3.3 | 3.4 | 3.4 | 3.3 | 3.3 | 2.8 |

*Information unavailable due to histological artifact

| | Animal | Controls (Day) | | | | $PGE_2$ Tested (Day) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Relative Thickness | | 1C | 2C | 3C | 4C | 1T | 2T | 3T | 4T |
| of Regrowing | (1) | 2 | 3 | 3 | 4 | 4 | 3 | 2 | 3 |
| Perforation | (2) | 4 | 2 | 2 | 2 | 3 | 2 | 2 | 2 |
| | (3) | 4 | 4 | 3 | 1 | * | 1 | 2 | * |
| | (4) | * | 2 | 2 | 2 | 4 | 2 | 3 | 2 |
| | (5) | 2 | 1 | 3 | 4 | 4 | 2 | 1 | 2 |
| | (6) | 1 | 3 | 4 | 1 | 2 | 3 | 3 | 3 |
| | (7) | * | 2 | 2 | * | 2 | 2 | 2 | 4 |
| | (8) | 3 | 4 | 4 | * | 4 | 4 | 2 | 2 |
| | (9) | 3 | 3 | 4 | 2 | 3 | 3 | * | 1 |
| | (10) | 4 | 2 | * | 2 | 3 | 2 | 2 | 4 |
| | (11) | 2 | 2 | 2 | * | 3 | 2 | * | 4 |
| | (12) | 2 | * | * | 2 | 2 | 3 | 4 | 2 |
| | Mean | 2.7 | 2.5 | 2.9 | 2.2 | 2.8 | 2.4 | 2.3 | 2.6 |
| Diameter of Remaining Gap in Micrometers | | | | | | | | | |
| | (1) | * | 60 | 300 | 60 | 60 | 0 | * | 120 |
| | (2) | 240 | 0 | 0 | 0 | 240 | 0 | 0 | 0 |
| | (3) | * | 180 | * | 0 | * | 0 | 0 | * |
| | (4) | * | 180 | 0 | 0 | 660 | 0 | 0 | 0 |
| | (5) | 960 | 0 | 480 | 0 | 240 | 0 | 0 | 0 |
| | (6) | 0 | 0 | 0 | 0 | 60 | 0 | 0 | 420 |
| | (7) | * | 420 | 0 | * | * | 0 | 240 | 0 |
| | (8) | 60 | 0 | 0 | * | 240 | 0 | 0 | 0 |
| | (9) | 180 | 120 | 480 | 0 | 420 | 300 | * | 0 |
| | (10) | 120 | 120 | * | 0 | 600 | 0 | * | 0 |
| | (11) | 0 | 0 | 0 | | 240 | 0 | * | 0 |
| | (12) | 420 | * | * | 0 | 0 | 0 | 720 | 0 |
| | Mean | 247 | 98 | 140 | 7 | 276 | 25 | 120 | 49 |

*Information unavailable due to histological artifact

| Animal | Controls (Day) | | | | $PGE_2$ Tested (Day) | | | |
|---|---|---|---|---|---|---|---|---|
| | 1C | 2C | 3C | 4C | 1T | 2T | 3T | 4T |
| Vascularity of Perforation (1) | 1 | 3 | 4 | 4 | 4 | 4 | 4 | 4 |
| (2) | 4 | 4 | 3 | 4 | 4 | 3 | 1 | 2 |
| (3) | 3 | 4 | 4 | 4 | 4 | 1 | 3 | * |
| (4) | * | 4 | 2 | 2 | * | 4 | 3 | 2 |
| (5) | 2 | 1 | 4 | 2 | 3 | 4 | 3 | 2 |
| (6) | 4 | 4 | 4 | 2 | 4 | 4 | 2 | 4 |
| (7) | * | 3 | 3 | * | 4 | 4 | 3 | 2 |
| (8) | 4 | 2 | 4 | * | 3 | 3 | 2 | 3 |
| (9) | 4 | 3 | 4 | 4 | 4 | 4 | * | 3 |
| (10) | 3 | 1 | * | 3 | 3 | 4 | 4 | 4 |
| (11) | 4 | 3 | 1 | * | 4 | 4 | * | 3 |
| (12) | 3 | * | * | 3 | 4 | 3 | 4 | 2 |
| Mean | 3.2 | 2.9 | 3.3 | 3.1 | 3.7 | 3.5 | 2.9 | 2.8 |
| Collagen Regrowth at Perforation | | | | | | | | |
| (1) | * | 2 | 2 | 2 | 2 | 2 | * | 2 |
| (2) | 1 | 1 | 2 | 2 | 3 | 2 | 2 | 2 |
| (3) | * | 1 | * | 2 | 1 | 1 | 4 | * |
| (4) | * | 2 | 3 | 2 | * | 1 | 2 | 2 |
| (5) | 2 | 2 | 2 | 3 | 2 | 4 | 1 | 3 |
| (6) | 1 | 1 | 2 | 2 | 1 | 3 | 2 | 2 |
| (7) | * | 2 | 4 | * | 1 | 1 | 4 | 2 |
| (8) | 1 | 1 | 2 | * | * | 4 | 4 | 4 |
| (9) | 1 | 2 | 2 | 4 | 1 | 2 | * | 2 |
| (10) | 1 | 2 | * | 2 | 2 | 2 | * | 2 |
| (11) | 2 | 2 | 2 | * | 2 | 2 | * | 2 |
| (12) | 2 | * | * | 2 | 1 | 2 | 2 | 2 |
| Mean | 1.4 | 1.6 | 2.3 | 2.3 | 1.6 | 2.2 | 2.6 | 2.3 |

*Information unavailable due to histological artifact

| | Animal | Controls (Day) | | | | $PGE_2$ Tested (Day) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Granulocyte Infil- | | 1C | 2C | 3C | 4C | 1T | 2T | 3T | 4T |
| tration at | (1) | 2 | 2 | 0 | 2 | 4 | 1 | 3 | 3 |
| Perforation | (2) | 0 | 1 | 0 | 2 | 3 | 0 | 0 | 2 |
| | (3) | 0 | 4 | 0 | 3 | | 3 | 0 | * |
| | (4) | * | 3 | 2 | 0 | 4 | 3 | 3 | 1 |
| | (5) | 1 | 0 | 2 | 0 | 4 | 4 | 0 | 4 |
| | (6) | 0 | 0 | 0 | 1 | 1 | 3 | 0 | 4 |
| | (7) | * | 0 | 2 | 3 | 4 | 4 | 0 | 0 |
| | (8) | 4 | 0 | 0 | * | 4 | 2 | 4 | 0 |
| | (9) | 4 | 3 | 1 | * | 3 | 0 | 0 | 0 |
| | (10) | 3 | 2 | * | 1 | 0 | 0 | * | 2 |
| | (11) | 0 | 0 | 1 | 0 | 4 | 0 | 0 | 1 |
| | (12) | 2 | * | * | * | 2 | 0 | * | 0 |
| | Mean | 1.6 | 1.4 | 0.8 | 1.3 | 3.0 | 1.7 | 1.0 | 1.5 |
| Fibroblast Infiltration at Perforation | | | | | | | | | |
| | (1) | 4 | 2 | 3 | 2 | 3 | 3 | 2 | 3 |
| | (2) | 4 | 3 | 2 | 4 | 3 | 4 | 2 | 3 |
| | (3) | 3 | 4 | 4 | 3 | * | 2 | 1 | * |
| | (4) | * | 2 | 2 | 3 | 2 | 2 | 1 | 2 |
| | (5) | 2 | 1 | 2 | 4 | 4 | 2 | 2 | 3 |
| | (6) | 3 | 4 | 0 | 2 | 2 | 3 | 2 | 4 |
| | (7) | * | 3 | 2 | 2 | 2 | 2 | 2 | 1 |
| | (8) | 2 | 3 | 4 | * | 0 | 2 | 3 | 2 |
| | (9) | 4 | 2 | 1 | * | 2 | 2 | 3 | 4 |
| | (10) | 2 | 2 | * | 0 | 2 | 1 | * | 3 |
| | (11) | 2 | 4 | 1 | 1 | 2 | 3 | 2 | 1 |
| | (12) | 1 | * | * | * | 2 | 1 | * | 2 |
| | Mean | 2.7 | 2.7 | 2.1 | 2.3 | 2.2 | 2.3 | 2.0 | 2.5 |

*Information unavailable due to histological artifact

| Animal | Controls (Day) | | | | $PGE_2$ Tested (Day) | | | |
|---|---|---|---|---|---|---|---|---|
| Blood Clot Quantity | 1C | 2C | 3C | 4C | 1T | 2T | 3T | 4T |
| (1) | 4 | 3 | 3 | 3 | 4 | 4 | 4 | 3 |
| (2) | 4 | 3 | 2 | 1 | 4 | 4 | 2 | 4 |
| (3) | 4 | 4 | 0 | 3 | 2 | 2 | 1 | * |
| (4) | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 2 |
| (5) | 1 | 1 | 4 | 1 | 4 | 4 | 3 | 4 |
| (6) | 2 | 1 | 1 | 4 | 4 | 4 | 0 | 4 |
| (7) | * | 2 | 4 | 3 | 4 | 4 | 0 | 3 |
| (8) | 4 | 1 | 4 | * | 4 | 3 | 4 | 4 |
| (9) | 4 | 4 | 4 | * | 3 | 4 | 3 | 3 |
| (10) | 4 | 4 | * | 0 | 3 | 4 | * | 4 |
| (11) | 4 | 4 | 1 | 0 | 2 | 4 | 1 | 3 |
| (12) | 4 | * | * | * | 4 | 4 | * | 1 |
| Mean | 3.4 | 2.8 | 2.7 | 2.1 | 3.5 | 3.8 | 2.2 | 3.2 |

*Information unavailable due to histological artifact

# APPENDIX C

## Statistical Analysis of Data

Two-Tailed Student T Test

$$T = \frac{(M_1 - M_2) - d_o}{\sqrt{\frac{(n_1 S_1^2 + n_2 S_2^2)(n_1 + n_2)}{(n_1 + n_2 - 2)(n_1 n_2)}}}$$

$v = n_1 + n_2 - 2$

$\alpha = 0.05$

Where:

$M_1$ = mean of control data

$M_2$ = mean of test data

$d_o$ = 0 (hypothesis that there is no difference between the means)

$S_1^2$ = variance of sample control data

$S_2^2$ = variance of sample test data

$n_1$ = sample size of control data

$n_2$ = sample size of test data

$v$ = degrees of freedom

| T value at | 24 hours | 48 hours | 72 hours | 96 hours |
|---|---|---|---|---|
| Exudate Quantity | -0.364 | 0.323 | -1.352 | 0.832 |
| Epithelium Thickness of Regrowing Perforation | -0.228 | 0.459 | 0.227 | 2.003 |
| Relative Thickness of Regrowing Perforation | -0.261 | 0.339 | 1.497 | -0.827 |
| Diameter of Remaining Gap in Micrometers | -0.210 | 1.556 | 0.164 | -0.933 |
| Vascularity of Perforation | -1.454 | -1.324 | -0.826 | 0.688 |
| Collagen Regrowth at Perforation | -0.715 | -1.479 | -0.587 | -0.188 |
| Granulocyte Infiltration at Perforation | -1.993 | -0.436 | -0.320 | -0.314 |
| Fibroblast Infiltration at Perforation | 1.106 | 1.165 | 0.207 | -0.381 |

| T value at | 24 hours | 48 hours | 72 hours | 96 hours |
|---|---|---|---|---|
| Blood Clot Quantity | -0.322 | -2.087 | 0.666 | -1.731 |

| p value at | 24 hours | 48 hours | 72 hours | 96 hours |
|---|---|---|---|---|
| Exudate Quantity | .60-.75 | .60-.75 | .90-.95 | .75-.80 |
| Epithelium Thickness of Regrowing Perforation | .60-.75 | .60-.75 | <.60 | .95-.975 |
| Relative Thickness of Regrowing Perforation | .60-.75 | .60-.75 | .90-.95 | .75-.80 |
| Diameter of Remaining Gap in Micrometers | <.60 | .90-.95 | <.60 | .75-.80 |
| Vascularity of Perforation | .90-.95 | .90-.95 | .75-.80 | .75 |
| Collagen Regrowth at Perforation | .75-.80 | .90-.95 | .60-.75 | <.60 |
| Granulocyte Infiltration at Perforation | .95-.975 | .60-.75 | .60-.75 | .60-.75 |
| Fibroblast Infiltration at Perforation | .85-.90 | .85-.90 | < .60 | .60-.75 |
| Blood Clot Quantity | .60-.75 | .975-.99 | .60-.75 | .90-.95 |

# APPENDIX D

## Glossary

Arachidonic Acid: a lipid compound derived from membrane phospholipids.

Autacoid: an organic substance made in one organ in the body and carried to other organs where the substance acts.

Cholesteatoma: a cyst-like mass of keratinized epithelium occurring commonly in the middle ear area.

Cyclic AMP: cyclic adenosine monophosphate, a chemical which directs many cell functions.

External Acoustic Meatus: the outer ear canal.

Fibrous Annulus: the fibrocartilaginous ring surrounding the tympanic membrane.

Malleus: one of the auditory ossicles and the ossicle which is attached to the tympanic membrane.

Manubrium: the longest process of the malleus.

Myringoplasty: to surgically close a perforated tympanic membrane with a graft.

Otitis Media: inflamation of the middle ear.

Pars Flaccida: small portion of the tympanic membrane at the margin and in the area of the lateral process of the malleus.

Pars Tensa: the large area of the tympanic membrane excluding the area of the pars flaccida.

Petrous Bone: a part of the temporal bone.

$PGE_2$: a prostaglandin with oxygen double bound to the ninth carbon and two double bonds in the carbon chain.

Prostaglandin: a group of naturally occurring, long chain hydroxy fatty acids that are derived from arachidonic acid.

Tympanic Membrane: a thin partition between the external auditory canal and the middle ear.

Tympanic Rings: the fibrocartilagenous rings to which the tympanic membrane attaches.

Umbo: the tip of the manubrium at the center of the tympanic membrane.

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