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The effect of moisture on re-epithelization of cutaneous wounds

Laurence W. Levinger
Yale University

THE EFFECTS OF MOISTURE ON THE RE-EPITHELIZATION OF CUTANEOUS WOUNDS

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A Thesis

In Partial Fulfillment of the Requirements for the Degree

Doctor of Medicine

Presented to the Department of Surgery of Yale University

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Acknowledgment

I wish to extend my thanks to Dr. C. Francis Roe, without whose guidance this study would have been impossible. His many suggestions and untiring support made this an invaluable experience.

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Introduction

For centuries, physicians have searched for materials or methods which would accelerate the healing of wounds. In years past, various substances, including egg whites, herbs, salts, and boiling oils, have been applied to open lesions. One of the first scientific observations concerning wound healing is credited to Paré in about 1535. At that time, according to the story (1), he was traveling with the French army; and as was the practice of the day, he treated gunshot wounds with boiling oil. On one occassion, however, his supply of oil ran out, and he could only dress the wounds. He noted that these wounds healed better than those treated with oil, and he therefore advocated that the use of oil be discontinued.

Following Paré, observations were made by many physicians concerning the contraction of wounds, the formation of granulation tissue, and the inflammation associated with healing and infection. With the advent of the microscope, the cellular reaction to wounding, and the processes of healing were studied. The cleansing of wounds became routine, and the use of sterile dressings was initiated.

During the twentieth century, great technological advances have been made in almost every area of scientific endeavor. Natural and synthetic antibiotics were developed which have enabled the control of most wound infections. Even with the

progress of recent years, however, much remains to be learned about the healing of wounds and the factors which regulate tissue repair.

In almost every current text dealing with wound healing, the following points are made:

1. Upon wounding, a scab of blood elements forms over the surface of the lesion.

2. After a lag period, granulation tissue, composed of fibrocytes and capillary buds, grows in from the floor of the lesion and covers the wound.

3. The epithelial cells at the margin of the wound become hyperplastic, multiply rapidly, and migrate over the surface of the granulation tissue as a monocellular sheet.

4. Collagen fibers are formed in the scar tissue below the new epithelium; and as the wound matures, the scar contracts.

5. The tensile strength of the wound depends initially upon the adhesive properties of the clot; and as collagen is formed during the process of healing, the strength of the wound is greatly increased.

While there is some truth in this description, Gillman et al. in 1955 (2), and Ordman and Gillman in 1966 (3), have found several discrepancies. They note that the epithelium begins to migrate over the surface of the wound within 24 hours. This migration occurs below the surface of the scab, and before a bed of granulation tissue has formed. The migrating epithelium lacks mitotic figures, and cellular differentiation does not occur until the cells have ended their migration. At this time, mitoses become frequent and differentiation occurs.

The newly formed epithelium, when hyperplastic, often sends spurs into the dermis below. These "pseudo rete pegs" cause a foreign body reaction in the dermis. By 10-15 days the spurs have separated from the surface to form epithelial "pearls" which may remain for years or degenerate entirely.

As the epithelium begins to migrate from the skin margin over the wound, a lip of necrotic epidermis and dermis is undermined by the migrating sheet and becomes incorporated into the scab.

These findings of Gillman were substantiated by Lindsay and Birch (4) in 1964.

To determine the sequence of events in the healing of wounds, and to determine if the migration of epithelium is dependent upon a bed of granulation tissue, Boulas (5) devised an excellent experiment. He made standard lesions on the backs of rabbits, and fixed the wound margins with a metal ring to prevent contraction. Half of the wounds were covered with a hydrocortisone solution, and half were treated with the solvent without hydrocortisone. While the latter formed a bed of granulation tissue upon which the epithelium migrated, the test wounds formed no granulation tissue, yet the epithelium migrated over the panniculus carnosus to cover the wound.

In a related experiment, Billingham and Reynolds (6) found that epithelial sheets, treated with trypsin to remove all adherent dermis, were able to grow on wounds where all epidermis and dermis had been removed. They noted that the graft became firmly attached to the bed of the wound before

any granulation tissue had formed. Thus it seems that the migration and attachment of epithelium is not dependent upon the granulation tissue that is usually present in the base of a wound.

Gibbins (7), while studying the histology of migrating epithelial cells in rats made several discoveries. He noted that the migrating epithelial cells contained lysosomes rich in acid phosphatase, lipid bodies, and large accumulations of glycogen; whereas these inclusions were not seen in stationary cells. McMinn (8) also found that hypertrophic epithelial cells contained large stores of glycogen. He noted, however, that these stores rapidly disappeared when the cells began to differentiate and form keratin.

Gibbins also observed that when thorotrast was injected intravenously before cutaneous wounds were made in rats, the marker was incorporated into the scab with the other blood elements. As the epithelial cells began to migrate, they were found to contain particles of thorotrast, implying that they were able, through phagocytosis, to ingest their way through the clot.

Similarly, Platt (9) found that epithelial cells in the foot pads of mice were able to ingest, not only injected carbon particles, but also extravasated erythrocytes.

Several other investigators have made interesting contributions to the study of wound healing. Riley and Peacock (10) found that human epithelium often contains a collagenase, and that all samples of wound epithelium contained this substance.

Wells and Babcock (11) were also able to extract a proteolytic enzyme from epithelium, and suggested that migrating epithelial cells might digest their way through a clot.

The coverage of wounds, according to Van Winkle (12), depends upon the migration of cells of the prickle cell layer of the marginal epithelium, and from cells of the skin appendages, the hair follicles and sebaceous glands. Johnson (13) notes that the migrating cells become extremely flattened, even though the cells were originally cuboidal or polyhedral. In this way a much larger area is covered than would have been possible previously.

Although it seems that current research is beginning to unravel some of the mysteries of wound healing, the clinical treatment of lesions has changed very little.

In 1962, however, Winter (14) made an important discovery. He noted that cutaneous wounds on the backs of domestic pigs healed almost twice as quickly when covered with a polythene film as did his control wounds which were uncovered. Hinman and Maibach (15) made the same observation in 1963 using human volunteers. Thus, after centuries of work, someone had finally discovered a method to hasten the healing of wounds.

These authors noted that a scab was not formed over the wounds covered with the membrane, and attributed the healing property of the film to its ability to retain moisture. Winter and Scales (16) found that scabs were thicker and re-epithelization of wounds slower when warm air was blown over cutaneous lesions. They theorized that the epithelial cells could migrate much

more quickly over a layer of moisture (retained by the plastic films) than they could between the scab and the base of the wound. Thus, when the depth of the scab was increased by air drying the wounds, migration was further retarded.

Gimble and Farris (17) in 1966 studied the effects of temperature on the epithelization of wounds. They noted an increase in epithelization with an increase in temperature from 26° C. to 40° C. Below 26° C. there was little, if any, epithelial growth, and above 40° C. the rate of growth decreased until the cellular thermal death point was reached at 44° C.

Since 539.6 calories are lost each time one ml. of water evaporates from the surface of a wound, a plastic film, by retaining moisture, would allow the epithelium to migrate on the wound surface unhindered by a scab, and it would conserve heat, permitting a more rapid growth of epithelium.

Theoretically, an impermeable film would provide an excellent covering for a wound. It would retain all moisture, thus conserving heat maximally. The fluid that such a membrane would collect, however, being rich in protein, and warm, would serve as an excellent growth medium for bacteria.

Perhaps by employing a membrane with a low water vapor permeability, one could limit the loss of heat from the surface of the wound while allowing the epithelium a moist surface upon which to migrate. The film, ideally, would permit enough water to evaporate that a massive accumulation of fluid under the film would be prevented.

Although other investigators have tested the effects of

various films on the healing of wounds (18-23), to date, no study has quantitated the effects of moisture on the epithelization of wounds. Thus, this project was designed to test films of different water vapor permeabilities on cutaneous wounds to determine if an "ideal environment for healing" could be found. If any single membrane significantly accelerated the healing of wounds, it might be used in the therapy of a variety of lesions including burns, traumatic wounds, and the ulcers of vascular disease and diabetes mellitus.

Materials and Methods

To determine the effect of moisture on the healing of wounds, it was decided to cover a series of cutaneous lesions with films of different water vapor permeabilities, and to measure the growth of new epithelium under each membrane. Thus, the effect of moisture retention on the re-epithelization of wounds could be quantitated.

The experimental animal chosen for this project was the domestic pig. The back of this animal provides enough space for multiple lesions, and the skin is much like that of man. Montagna and Yun (24) note that while there are histochemical differences between the skins of the pig and man, that grossly they are similar. Furthermore, the skin of the pig, like that of man, overlies a thick panniculus adiposus, Marcarian and Calhoun (25). Finally, unlike many animals, the healing of cutaneous wounds in man and in the pig depends primarily upon tissue growth, and wound contraction plays a small role in the closure of lesions.

The membranes selected for the study consisted of copolymers of L - leucine and D L - methionine.* This film is colorless, clear, and flexible. The water vapor permeability of the film can be varied, during synthesis, by oxidation of the pendant methyl-thioethyl groups, and therefore, membranes of similar composition and different permeabilities may be obtained, Martin, May, and McMahon (26).

*Gulf South Research Institute, New Orleans, Louisiana

The water vapor permeabilities of the membranes were tested (see Appendix I) in order that a wide range of permeabilities be represented in the project. Because a very low permeability was not possible with the amino acid films, a polyethylene film was included in the study (see Appendix I).

The experimental animals (40 kg. domestic pigs) were anesthetized (see Appendix II), their backs were shaved and prepped with benzalkonium chloride and isopropyl alcohol, and 64 lesions were made with a biopsy punch, (see Figure 1.). Each lesion was 8mm. in diameter and 5mm. in depth. Many hair follicles were removed with the punch, and the bases of the wounds consisted of dermal tissue, and adipose tissue of the panniculus adiposus. By removing hair follicles, growth from these appendages, Eisen (27), rarely contributed to the covering of the wounds; and thus, the growth of epithelium could be measured from the margins of the wounds.

Bleeding from the lesions was controlled with direct pressure, using a sterile sponge. The lesions were then covered with patches of film, cut to overlap the normal skin, (see Figure 2.). Sixteen wounds were covered with each of three test films. Two of these membranes were of the poly-amino acid variety, with water vapor permeabilities of 1267 grams/M^2/24 hours, and 793 grams/M^2/24 hours at 35° C. The third film, a polyethylene, had a permeability of 6.1 grams/M^2/24 hours at 35° C. The remaining sixteen lesions were not covered and served as controls.

As each patch was sutured in place with 000 silk, moisture began to collect on the inner surface of the film, (see figures 3. and 4.). Collodion was applied along the margins of the film to insure a tight seal. The margins of the control wounds were also sutured and covered with a layer of collodion.

Four lesions covered by each of the membranes and four control wounds were biopsied by total excision on days 4, 6, and 8. The specimens were placed in 10% formalin for 48 hours. They were then embedded, sectioned, stained and mounted (see Appendices III and IV). Three slides were made of each specimen.

Using a microscope with an ocular scale, the growth of new epithelium was measured for each lesion, and the results tabulated.

Results

As shown on the following pages (Table I and Graphs 1-5) there was little difference in the growth of new epithelium over the wounds covered by each of the test films and the control lesions. While the controls demonstrated a growth pattern which was almost a linear function of time, each of the test lesions showed an increase in the rate of epithelization after the sixth day of the experiment. The range of values recorded, for each lesion, on each day of biopsy, was large, however, and there was no statistical difference in the re-epithelization of the various lesions ($p < .70$).

To determine if the test films significantly reduced the evaporative heat loss from the wounds, temperatures of the lesions, covered and uncovered, were measured with a radiometer.* The normal skin temperature over the animal's back was found to be 35.0 ± 0.6 degrees Centigrade, while the temperatures of open lesions were approximately two degrees below that of the skin. When the open lesions were covered by any of the test membranes or by a scab, the temperatures returned to near normal (Table II).

In order to clarify the results of the experiment, the permeabilities of several four and six day old scabs was determined. They were tested with the Payne Cup fitted with a special rubber gasket. This gasket had a central hole, of known area, over which the scabs were fixed with collodion.

*Infrared Thermometer, Barnes Engineering Company, Stamford, Connecticut.

The various scabs were found to permit the evaporation of 3-5 liters of water/M^2/24 hours at 35° Centigrade.

While examining the microscopic slides prepared from the test lesions, several histological observations were made that are of interest. First, it was noted that a scab (Figures 5,7, and 10.) formed over every lesion (although several were lost during the fixing and staining of the specimens). Furthermore, there was no difference in the thickness of the scabs taken from the test lesions and the controls.

The new epithelium was noted to migrate, in the majority of cases, in a multicellular sheet (Figure 6.) rather than in a monocellular layer, and the migrating cells retained their original polyhedral shape. Keratin was not formed by the cells until their migration had ceased.

The terminal portion of collagen fibers extending from the dermis to the base of the wounds often seemed to stain differently than the underlying dermal collagen, and was presumed to be necrotic. As the epithelial sheet migrated, it cleaved these collagen strands leaving the normal portion of the fibers below, and forcing the necrotic collagen upward into the scab (Figures 7. and 8.).

Although on microscopic examination, leukocytes were observed along the layer of necrotic tissue in the base of the wounds, none of the lesions were found to have large collections of these cells, nor were any of the wounds infected on gross inspection.

By the sixth or eighth day of the experiment, the wound epithelium frequently became hyperplastic and sent processes (Figures 9. and 10.) into the tissues below. These processes were unlike true rete pegs in that they were long, narrow, and very numerous. They therefore resembled the "pseudo rete pegs" described by Ordman and Gillman in 1966.

Table I — Growth of New Epithelium*

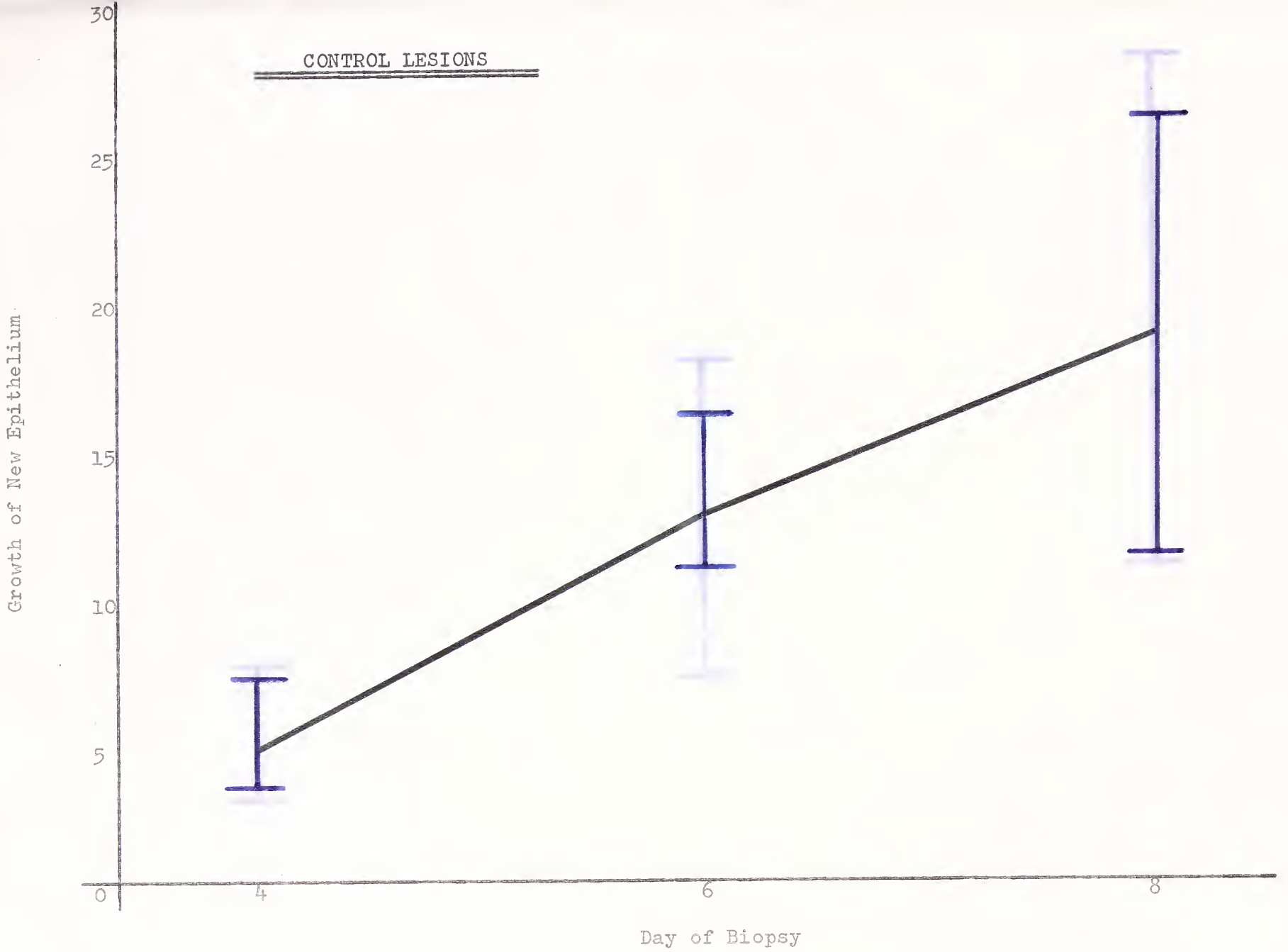
Biopsy		Slide #1**	Slide #2	Slide #3	Mean Growth Range
	1.	4.3	5.7	3.9	
Day 4	2.	4.1	4.3	4.3	5.2
Control	3.	4.5	4.7	5.2	3.9 - 7.4
	4.	6.5	7.3	7.4	
	5.	6.0	7.4	8.0	
Day 4	6.	5.4	4.6	3.3	6.9
Film 1	7.	7.3	7.9	5.5	3.3 - 8.0
	8.	5.6	6.0	6.1	
	9.	5.8	5.3	5.9	
Day 4	10.	4.8	4.8	4.3	5.8
Film 2	11.	8.0	7.3	7.2	4.3 - 8.0
	12.	5.4	5.8	5.5	
	13.	5.5	5.7	6.3	
Day 4	14.	6.8	7.4	6.3	5.7
Film 3	15.	6.0	4.6	4.9	3.7 - 7.4
	16.	3.7	5.5	---	
	17.	---	---	---	
Day 6	18.	14.6	12.4	16.3	13.1
Control	19.	13.1	11.4	11.0	11.4 -- 16.3
	20.	13.1	11.8	14.1	
	21.	8.0	7.3	8.1	
Day 6	22.	10.8	8.5	10.5	10.9
Film 1	23.	10.2	7.3	8.9	7.3 -- 18.0
	24.	17.6	18.0	15.9	
	25.	7.2	8.3	7.9	
Day 6	26.	8.6	9.2	8.6	8.8
Film 2	27.	10.6	10.5	8.9	6.0 - 10.6
	28.	9.6	6.0	10.3	
	29.	8.4	5.5	8.4	
Day 6	30.	7.0	---	5.9	7.4
Film 3	31.	8.0	4.8	6.3	4.8 - 12.6
	32.	10.8	12.6	11.2	
	33.	14.1	11.9	13.5	
Day 8	34.	26.8	26.2	25.9	18.1
Control	35.	13.5	16.5	17.1	11.9 -- 26.8
	36.	20.0	18.6	13.0	
	37.	24.5	24.0	---	
Day 8	38.	11.5	10.8	11.2	20.8
Film 1	39.	21.5	22.0	19.2	11.2 -- 28.6
	40.	28.6	28.2	27.2	
	41.	14.5	17.2	14.2	
Day 8	42.	16.0	19.0	16.5	15.6
Film 2	43.	16.5	17.2	17.4	11.8 -- 19.0
	44.	11.8	12.8	14.6	
	45.	22.8	24.3	18.5	
Day 8	46.	12.0	10.6	11.3	16.4
Film 3	47.	22.8	22.7	21.6	7.8 -- 24.3
	48.	7.8	11.1	11.1	

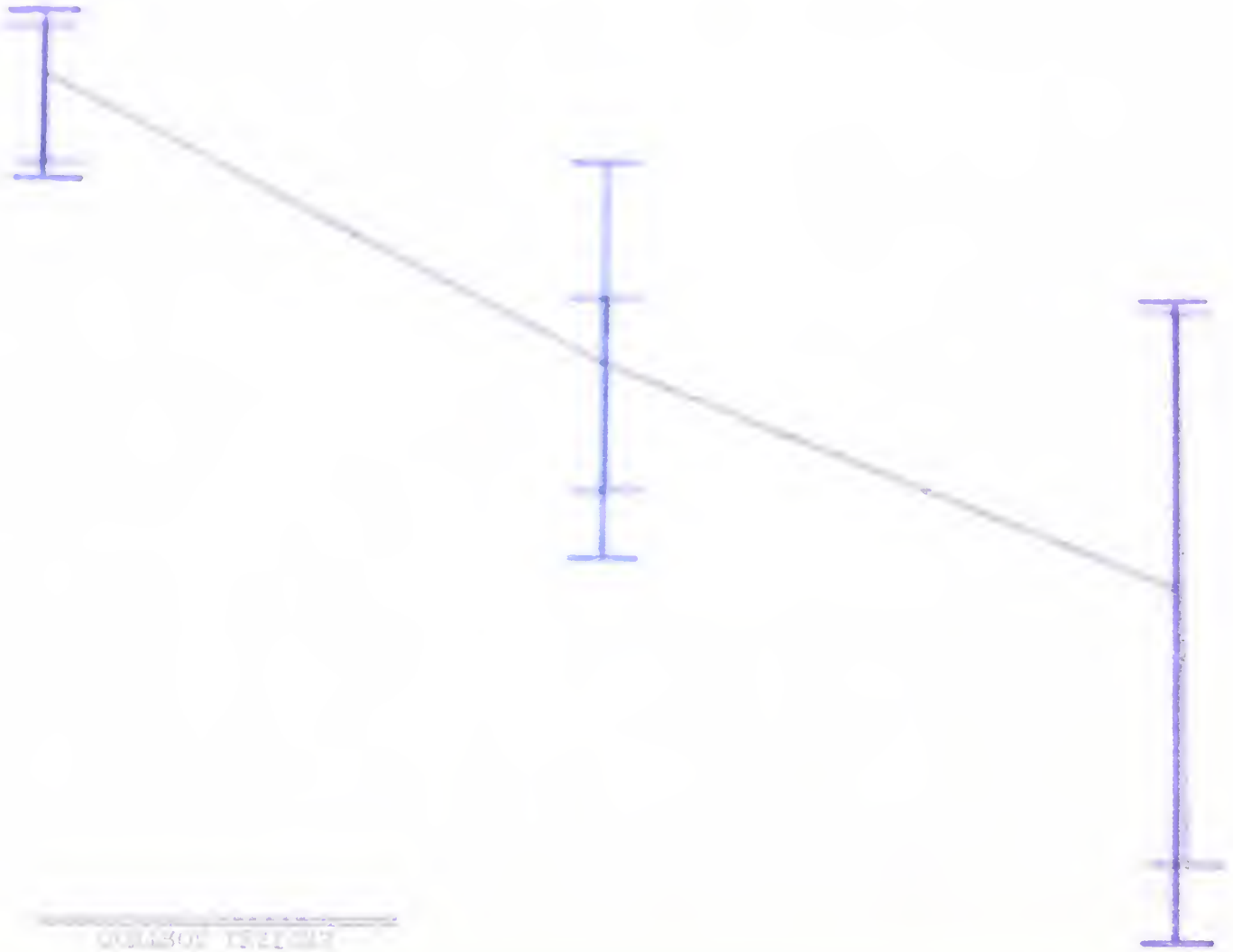
* Ocular Scale Units, where 1mm. = 3 Units

** Three slides were prepared from each specimen.

Table II Radiometric Temperature Determinations

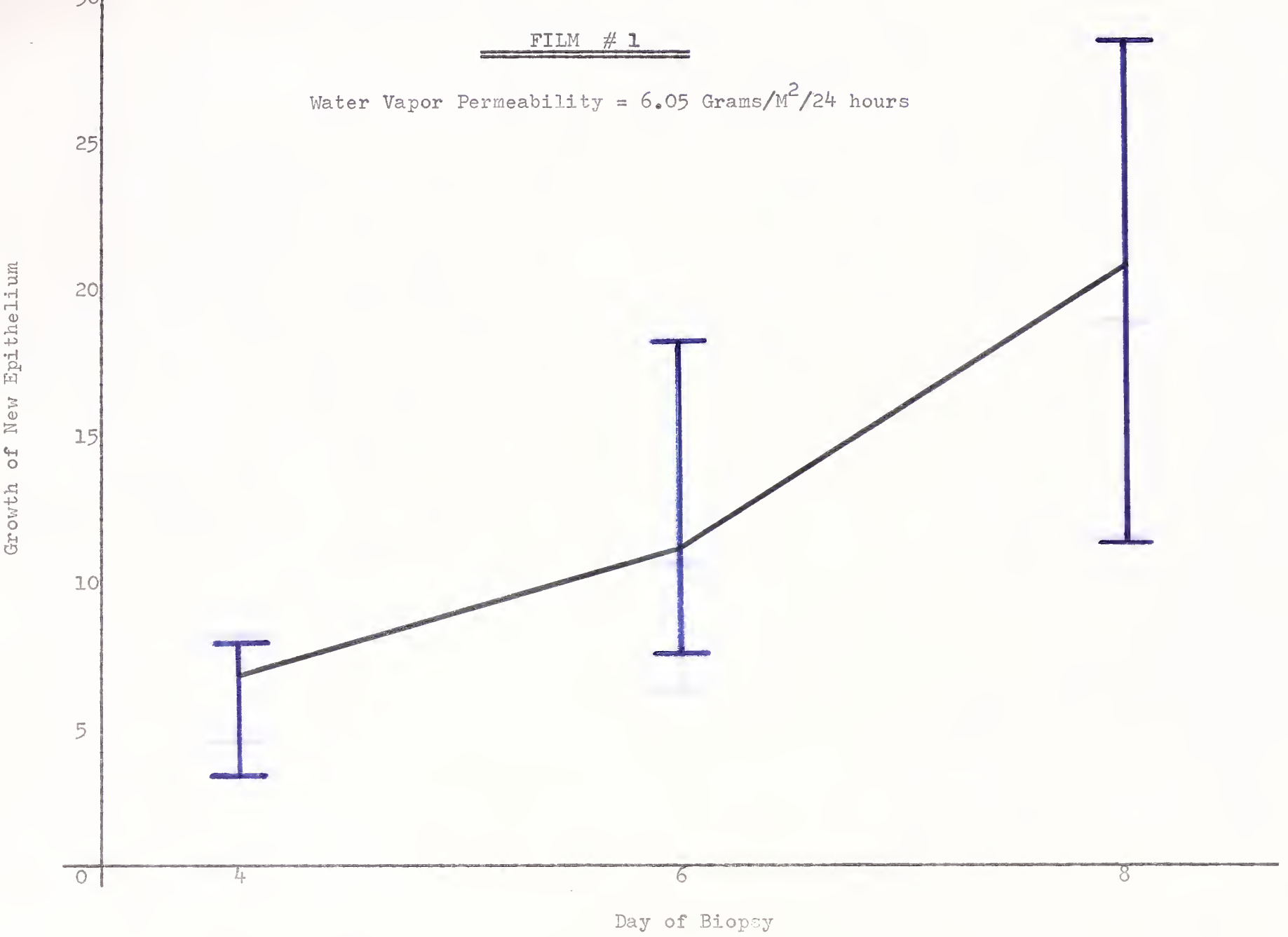
Area	Temperature	Mean	Range
Normal Skin	35.1 34.9 34.4 34.7 35.5 35.2	35.0	34.4-35.5
Uncovered Wound 1st Hour	33.2 32.6 32.9 33.0 32.4 32.8	32.8	32.4-33.2
Uncovered Wound 96th Hour	34.9 34.6 34.7 34.8 34.2 34.9	34.7	34.2-34.9
Plastic #1	35.2 34.7 34.6 34.8 34.5 34.9	34.8	34.5-35.2
Plastic #2	35.0 35.3 34.9 35.1 35.3 35.3	35.2	34.9-35.3
Plastic #3	34.5 34.7 35.2 35.0 34.7 35.2	34.9	34.5-35.2

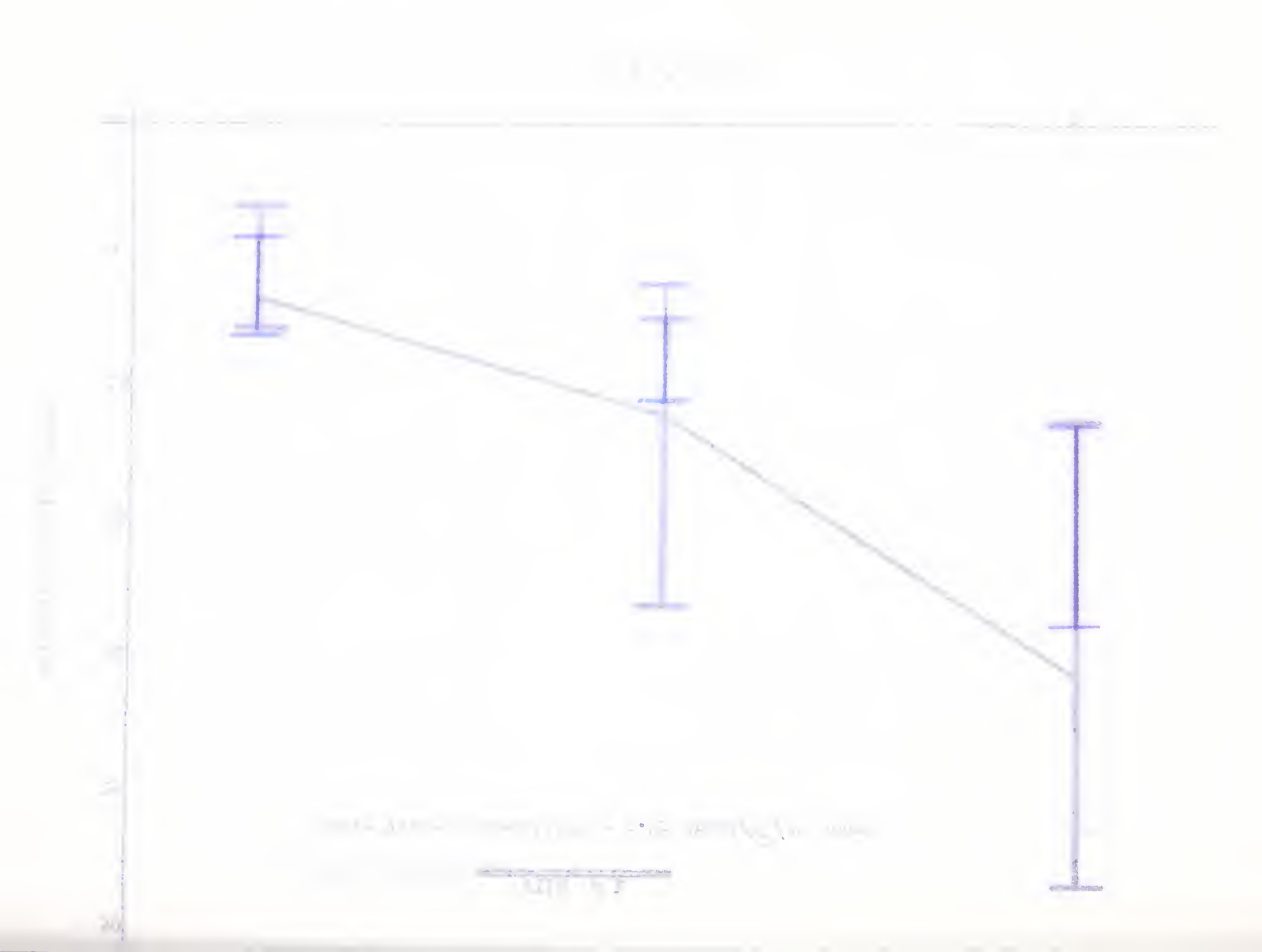

CONTROL LESIONS
Growth of New Epithelium
30
25
20
15
10
5
0
4
6
8
Day of Biopsy



FILM # 1

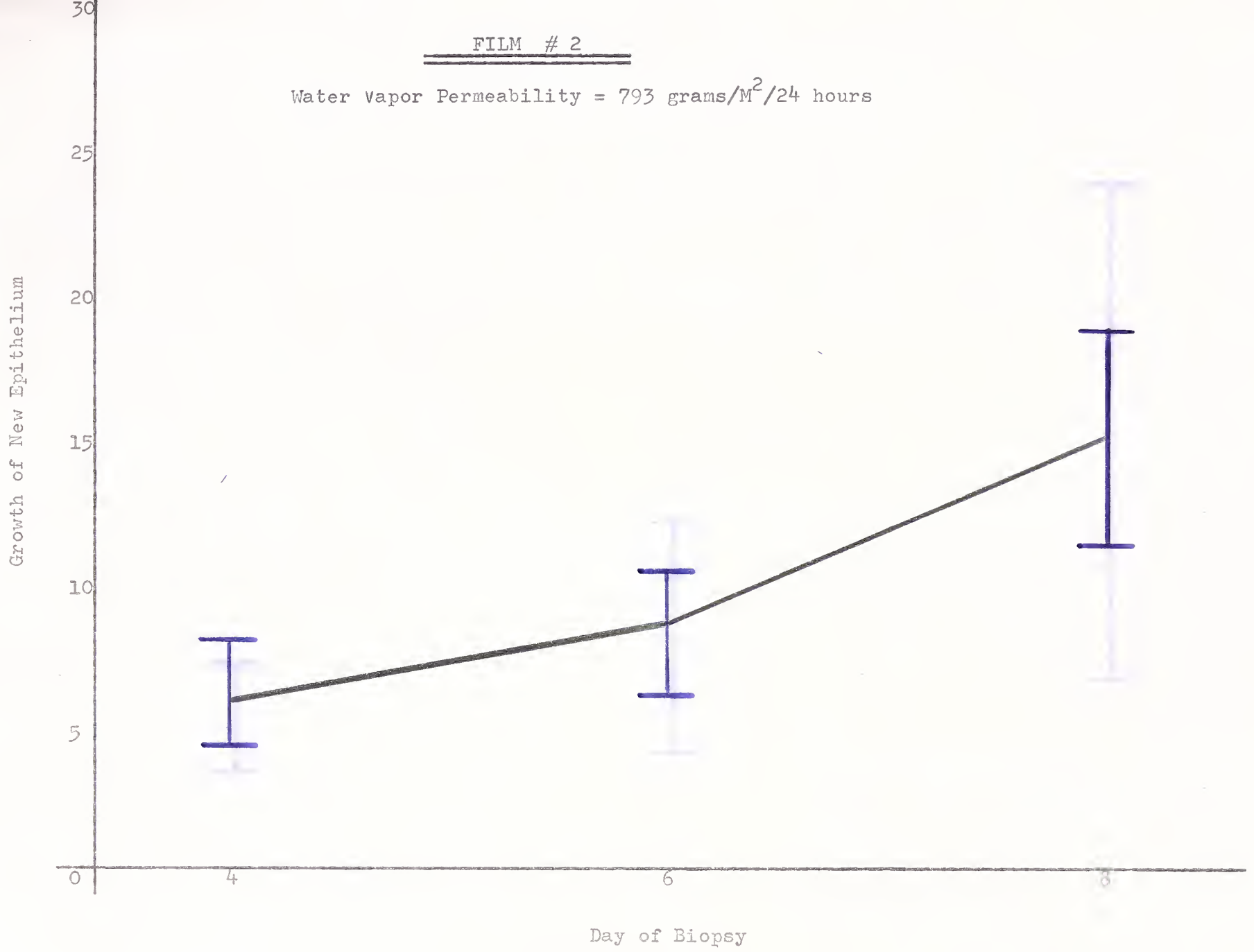
Water Vapor Permeability = 6.05 Grams/M^2/24 hours

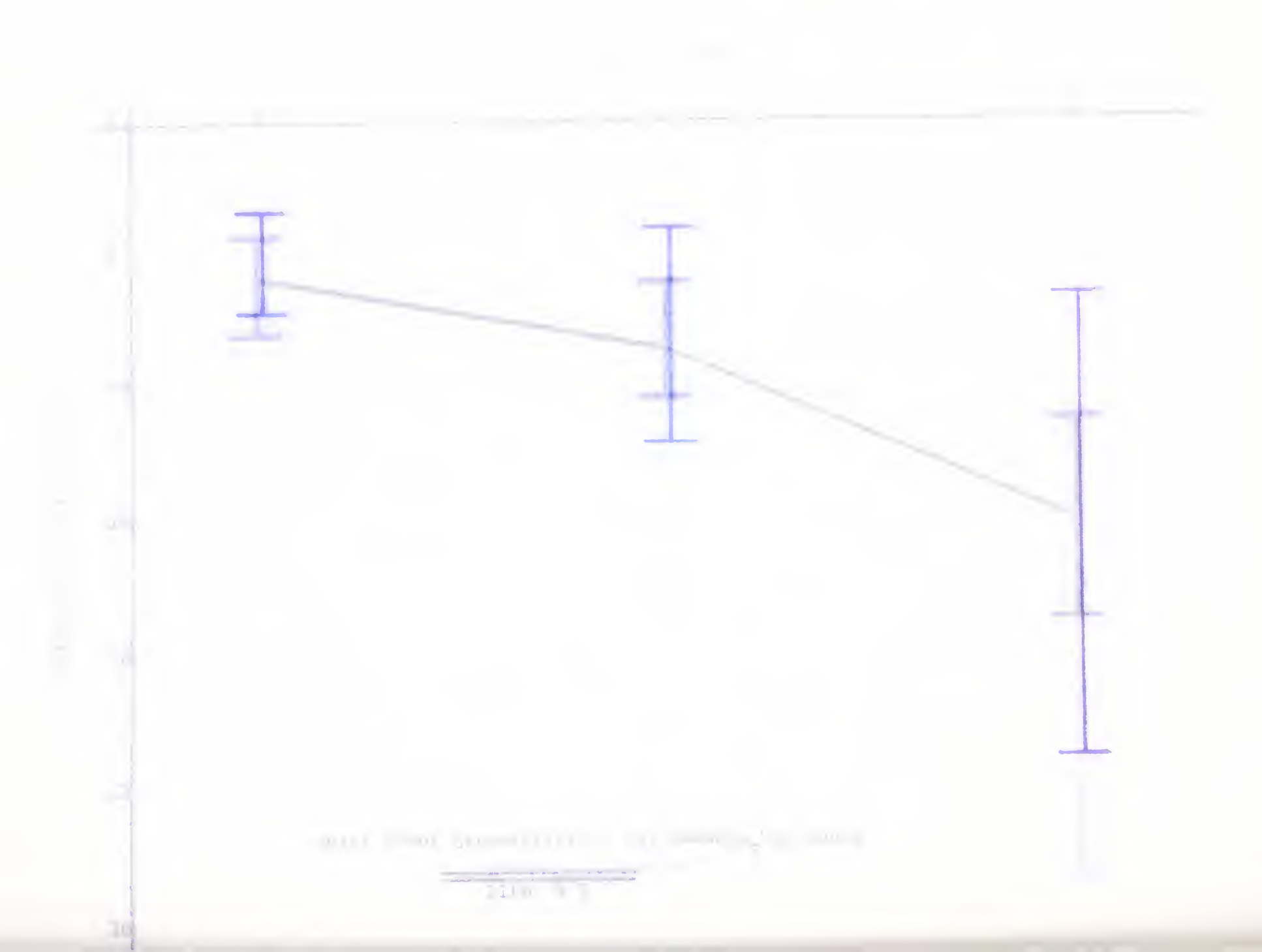




FILM # 2

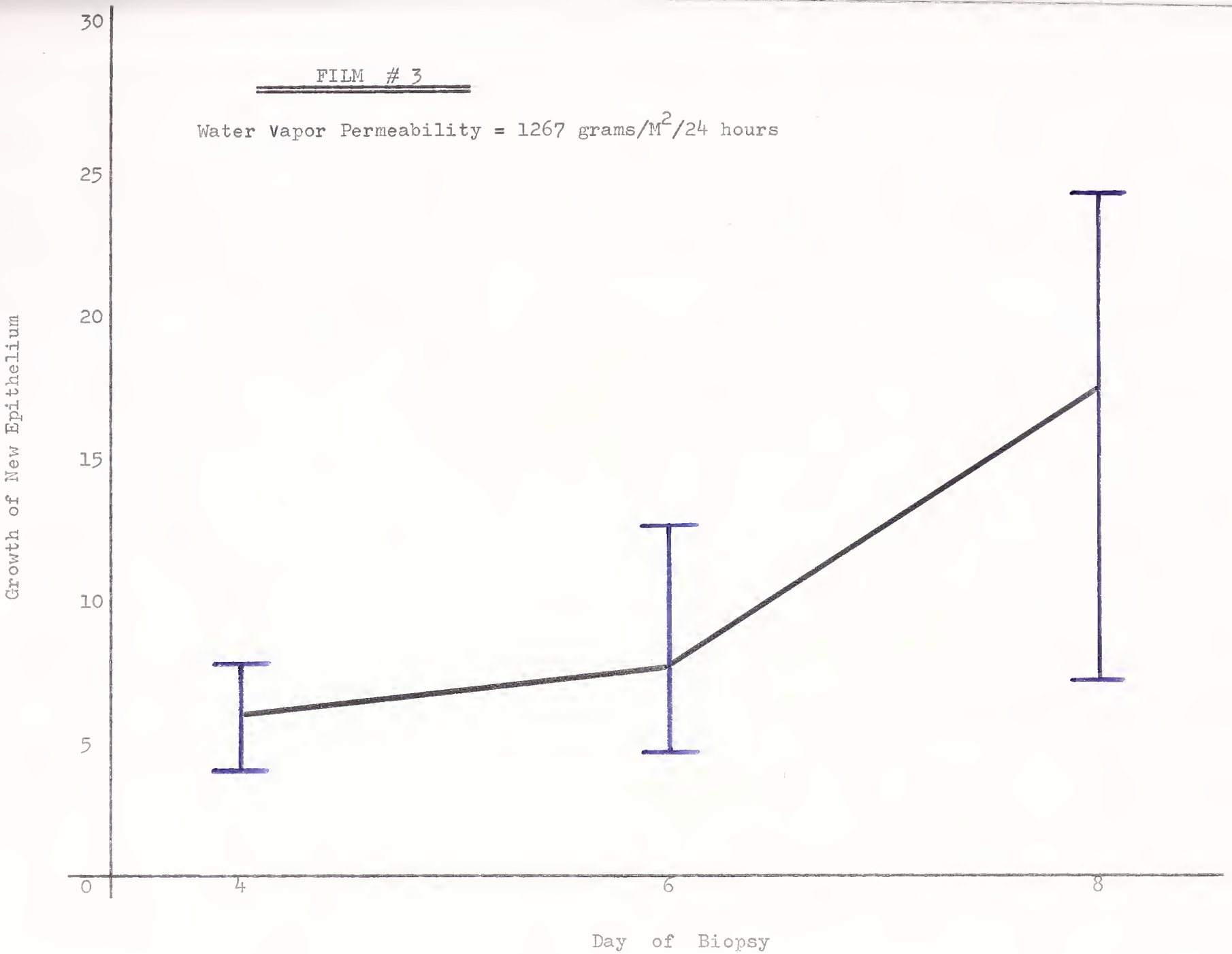
Water Vapor Permeability = 793 grams/M^2/24 hours

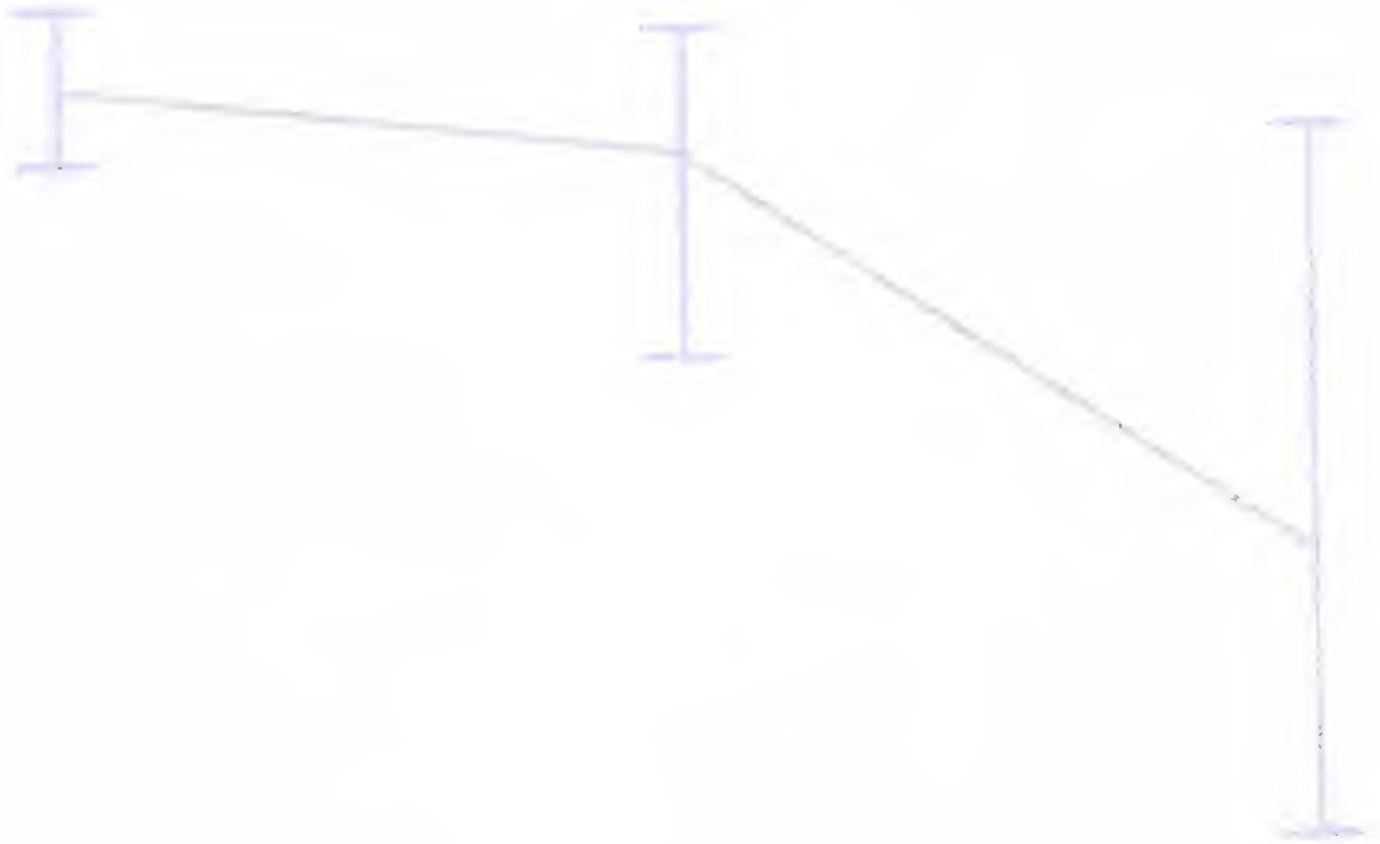




FILM # 3

Water Vapor Permeability = 1267 grams/M^2/24 hours





COMPARISON OF GROWTH CURVES

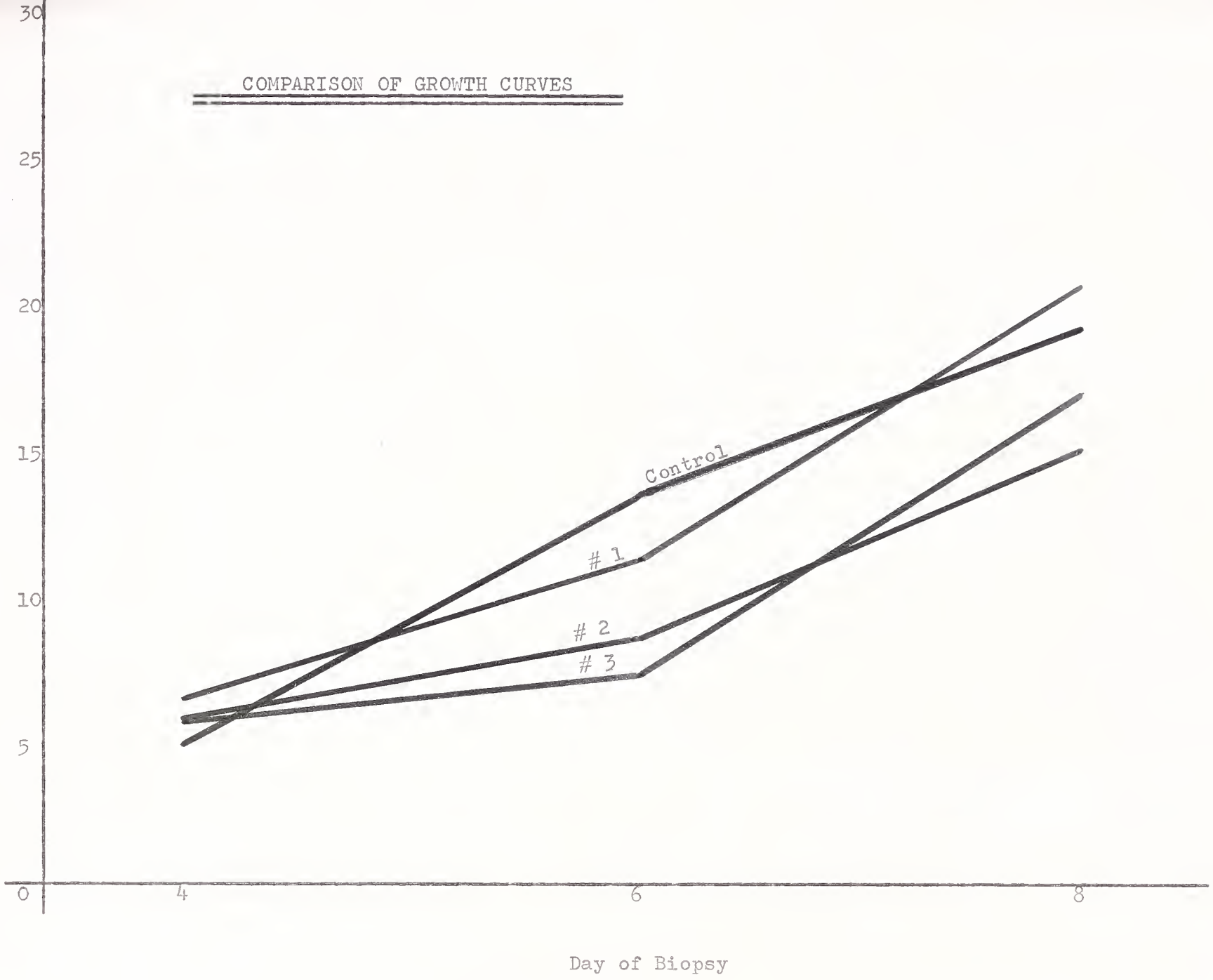


Figure 1. Lesions on the Back of an Experimental Animal

Figure 2. Plastic Patches in Position

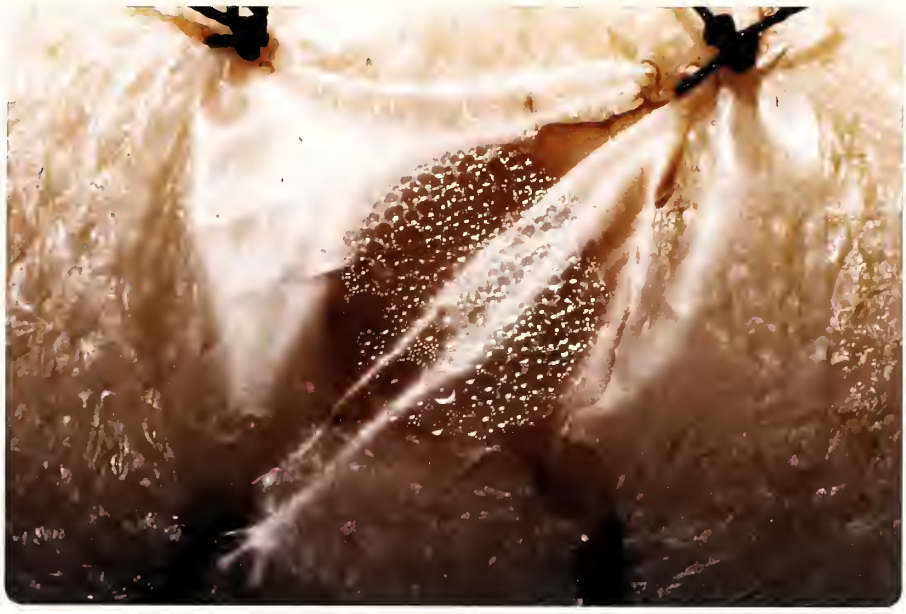


Figure 3. Moisture Under the Polyethylene Film

Figure 4. Moisture Under the Amino Acid Film

Figure 3. Moisture Under the Polyethylene Film

Figure 4. Moisture Under the Amino Acid Film

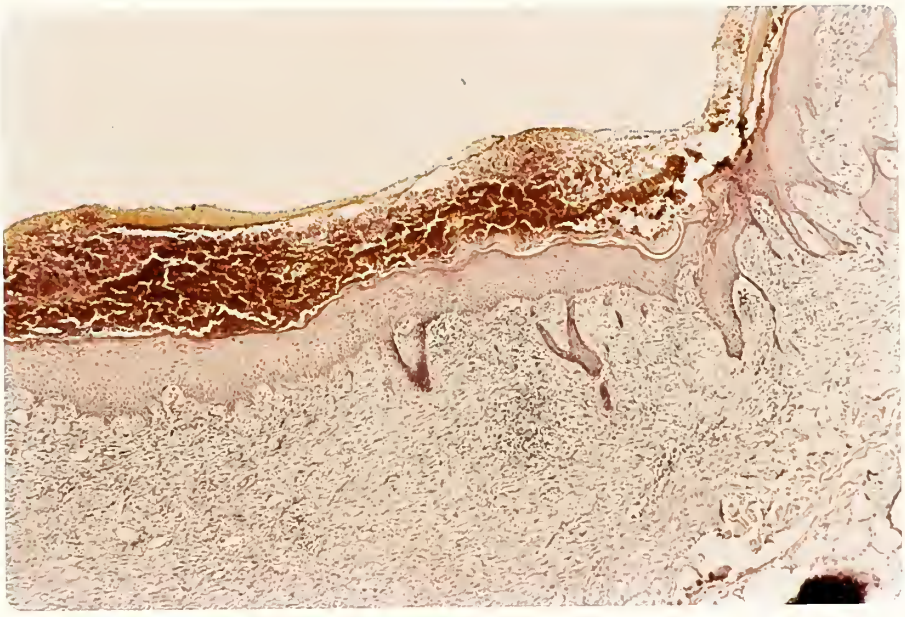


Figure 5. Epithelium Growing Under a Scab

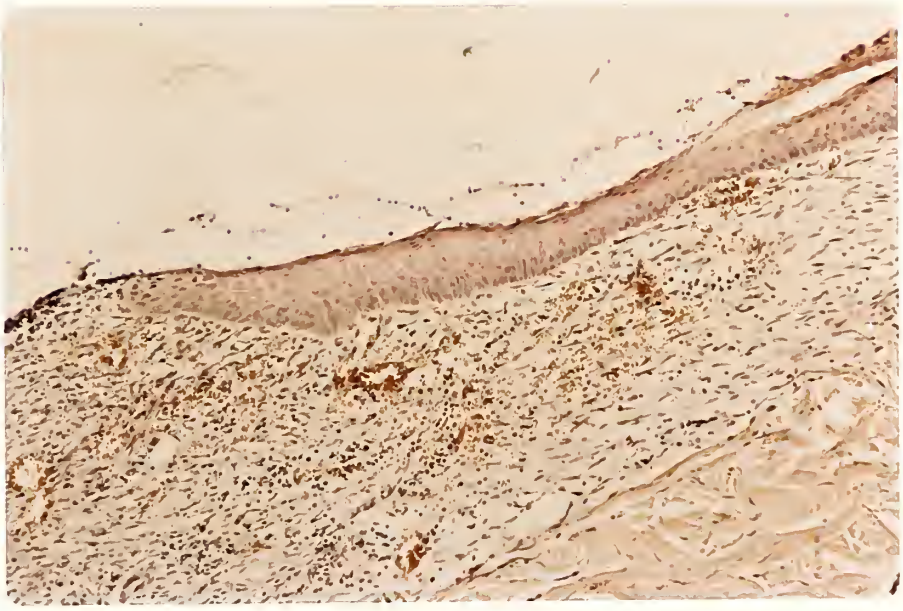


Figure 6. Multicellular Layer Migrating

Figure 5. Epithelium Growing Under a Scab

Figure 6. Multicellular Layer Migrating

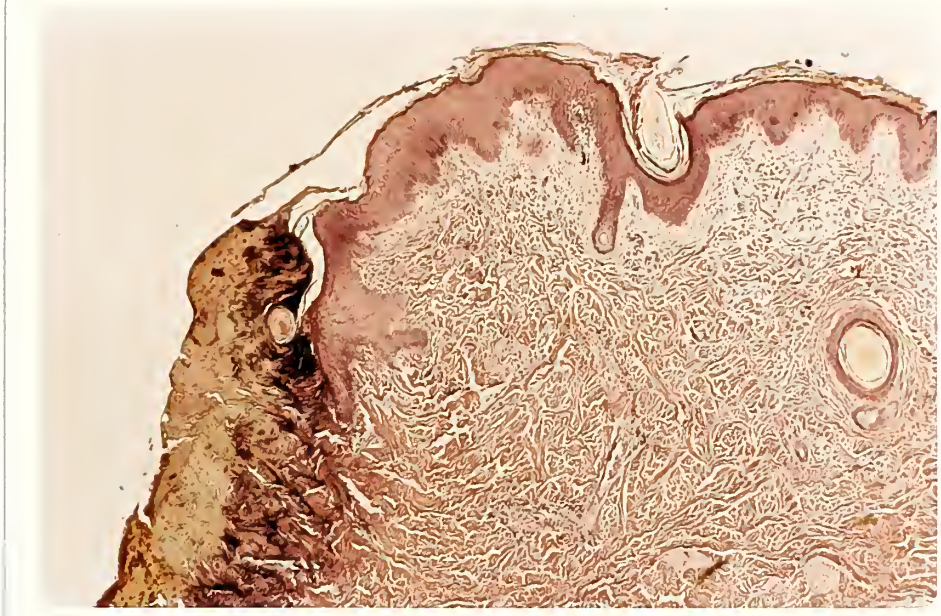


Figure 7. Epithelium Cleaving Dermal Collagen

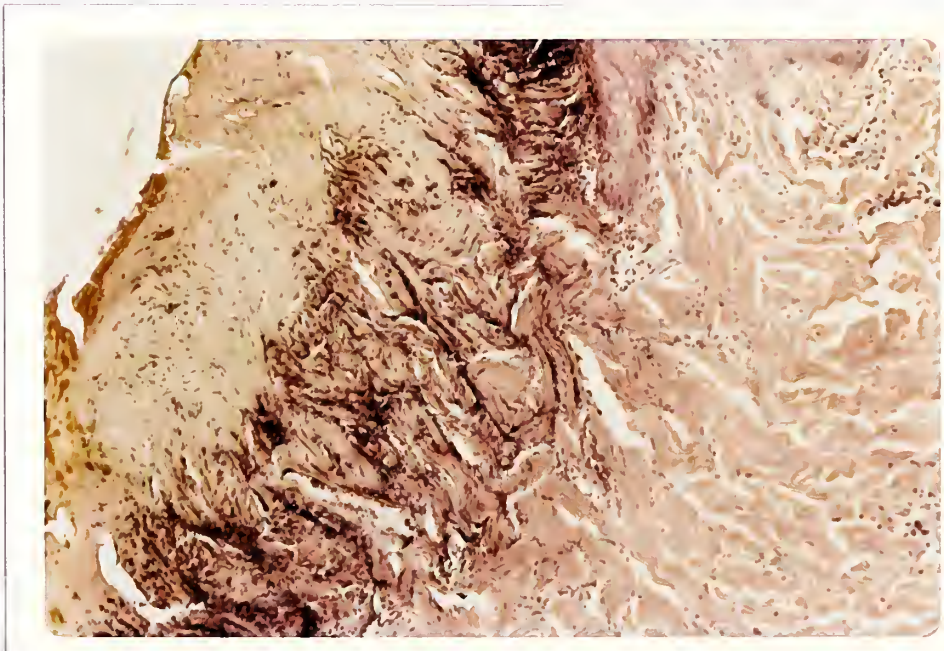


Figure 8. Collagen Strands in Scab
(an enlargement from Figure 7.)

Figure 7. Epithelium Cleaving Dermal Collagen

Figure 8. Collagen Strands in Scab

(an enlargement from Figure 7.)

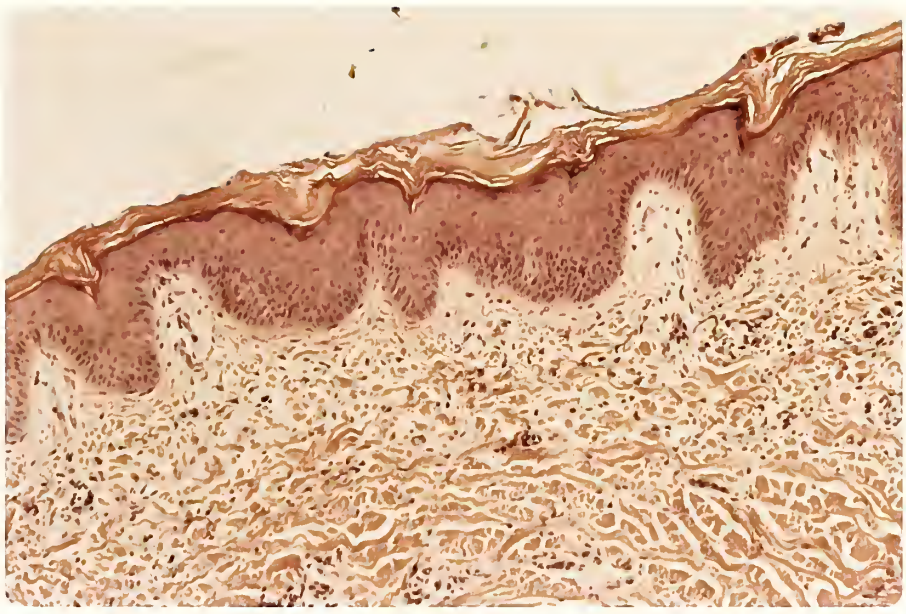


Figure 9. Normal Porcine Skin (note rete pegs)

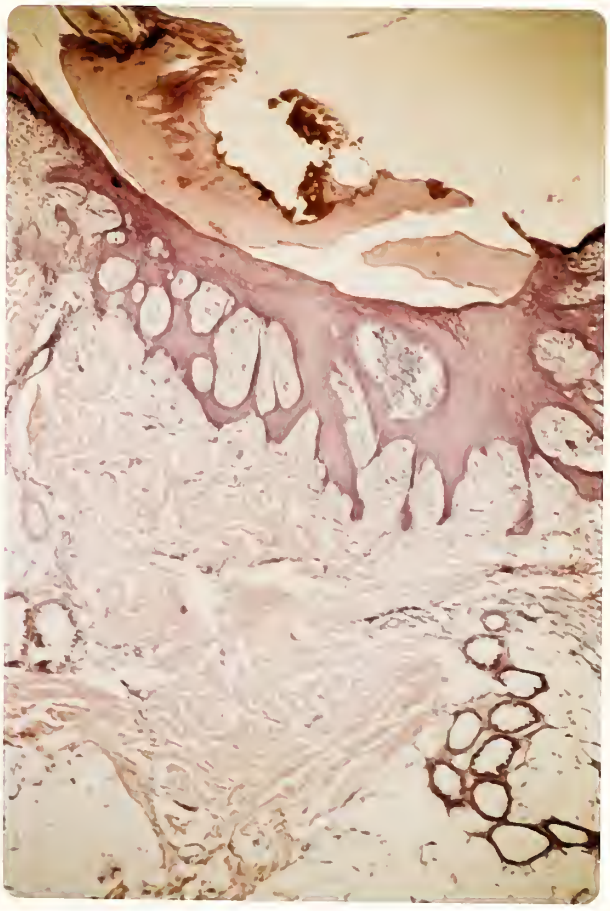


Figure 10.

"Pseudo Rete Pegs"

Figure 9. Normal Porcine Skin (note rete pegs)

Figure 11. The Payne Cup Disassembled

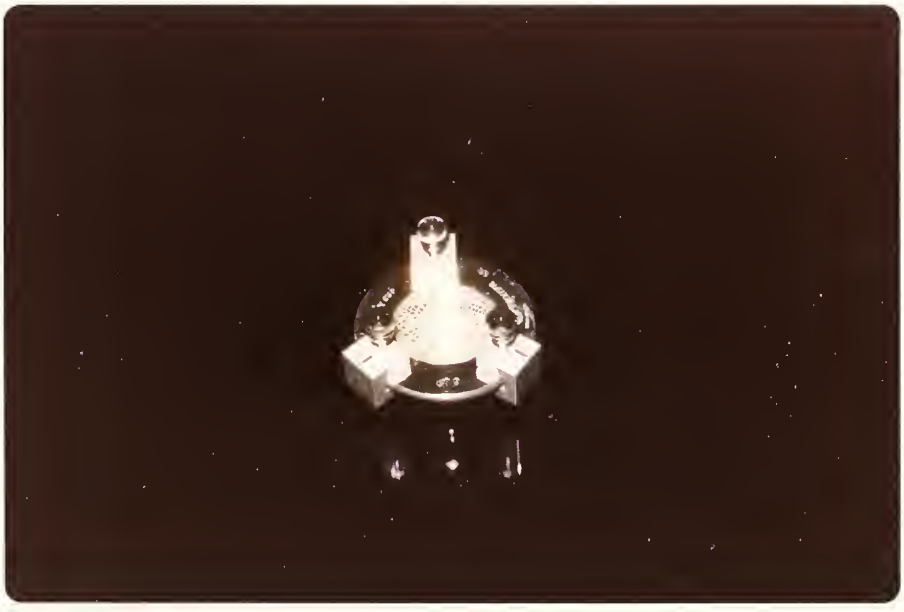


Figure 12. The Payne Cup and Amino Acid Film

Figure 11. The Payne Cup Disassembled

Figure 12. The Payne Cup and Amino Acid Film

Discussion

The results of this study indicate that moisture retained over a wound by an impermeable film has little, if any, effect on the rate of re-epithelization of the lesion. The membranes employed in the experiment had water vapor permeabilities of 6.1, 793, and 1267 grams/M^2/24 hours at 35°C. Furthermore, the scabs covering the control lesions permitted the evaporation of 3-5 liters/M^2/24 hours at 35° C. Thus a wide range of permeabilities were evaluated, yet there was no statistical difference in the rate at which the wounds healed.

The large range of epithelial growth recorded for each lesion was initially attributed to differences in temperature over the backs of the experimental animals. This was disproved, however, with the radiometric measurements. As an alternate explanation, differences in blood supply to the backs of the experimental animals was suggested. Because adjacent lesions covered with the same film often demonstrated widely different amounts of epithelial growth, it seems unlikely that this factor was responsible, but it remains as a possibility.

Since all of the lesions were of nearly the same temperature by the fourth day, one might speculate that the evaporative heat loss was the same from each lesion, or that the lesions covered with the more permeable films had a greater blood supply than the other lesions, thus compensating for the increased evaporative heat loss. While either of these explanations is

possible, it would be strange if the permeable films allowed the same amount of evaporation as the impermeable films. Also improbable is an increased blood supply to the more permeable films. This would implicate either evaporative heat loss or dessication in the stimulation of blood supply. Most likely, however, is a uniformly large blood supply to each of the lesions. This would maintain the temperature of each lesion at approximately the same temperature, that of the cutaneous blood. Since the scabs were the most permeable wound coverings, and since they allowed only 151 calories of evaporative heat loss every 24 hours, at an absolute maximum, this minuscule amount of heat could easily escape without changing the measured temperature of the wounds.

Scab Permeability = maximum of 5 liters/M^2/24 hours

= .5 cc./ cm^2./ 24 hours

Scab Area = πr^2 = 3.14 (0.4 cm.)^2

= 0.56 cm^2.

Heat of Vaporization = 539.6 calories/ cc. water

$$\text{Heat loss/24 hours} = \frac{.5\text{cc}}{\text{cm}^2} \times 0.56\text{cm}^2 \times \frac{539.6 \text{ calories}}{\text{cc.}}$$

= 151 calories

It is interesting that Winter found impermeable films to hasten the epithelization of cutaneous wounds. Furthermore, he observed that scabs did not form over lesions that were covered with such films. This would indicate that either moisture increased the epithelization of wounds, or that moisture prevented the formation of scabs and that scabs retarded healing. Winter in a later paper stated that by increasing the thichness

of scabs by blowing air over cutaneous wounds, he was able to increase the time required for healing. Thus it seems that the thickness of a scab is indirectly proportional to the time required for epithelization of an underlying lesion. However, that an impermeable film over a wound could prevent the formation of a scab seems impossible. Upon wounding, blood pours forth into the lesion, bringing the various clotting factors and cells. if this blood were removed and hemostasis obtained by direct pressure over the wound before the film was applied, the blood elements might be prevented from entering the wound directly. However, once the pressure was removed, serous fliud would collect below the film, and leukocytes would infiltrate the margin of the wound and collect in the fluid. With time, a scab would form from clotting factors which leaked into the fluid, from cellular debris formed by the degeneration of leukocytes, and from necrotic tissue along the margin of the wound.

Since a scab formed over every lesion in this study, it seems unlikely that an impermeable film alone could prevent the formation of a scab. Furthermore, there was no significant difference in the thickness of the scabs which formed over the test lesions and the control wounds. How Winter was able to prevent the formation of scabs, and increase the rate of healing of cutaneous wounds using impermeable polythene films as dressings remains unexplained.

Since the epithelium is often described as moving in a monocellular layer composed of flattened cells, it is noteworthy that most lesions in this study were covered by the migration of a multicellular layer. The cells of this layer were cuboidal or

polyhedral in configuration. While the epithelium might migrate, under some circumstances, in a thin, monocellular layer, and by doing so it would cover a greater area with fewer cells, this study indicates that the cells may also migrate as a multicellular layer.

It was observed that the "pseudo rete pegs" described by Ordman and Gillman were formed by the hyperplastic wound epithelium. Because the study was limited to eight days, the formation of epithelial pearls, and the degeneration and foreign body reaction that these processes initiated were not seen.

Thus the results of this study indicate that the use of impermeable membranes as wound covers to accelerate the healing of cutaneous lesions is not feasible. Not only is there no increase in the rate of healing, but the films, by retaining moisture, might increase the chance of wound infections.

Although none of the test lesions in this study became infected, sterile techniques were used throughout the experiment. In clinical practice, aseptic conditions are the exception rather than the rule, and thus the probability of infection is normally very high. With a reservoir of fluid over a wound, retained by an impermeable membrane, many more lesions would likely become infected. Therefore, it seems that wounds should be covered with sterile dressings designed to permit the evaporation of water. With this method, not only would healing be as fast as with less permeable dressings, but the chance of wound infections would be reduced.

Summary

To determine the effects of moisture on the re-epithelization of cutaneous wounds, sixty four lesions were made on the backs of 40 kg. domestic pigs under general anesthesia. Sixteen of these lesions were covered with each of three synthetic membranes of different water vapor permeabilities, and sixteen lesions remained uncovered to serve as controls. On days 4, 6, and 8, four lesions of each type were biopsied, fixed, sectioned, stained, and mounted, and the growth of new epithelium measured.

No statistical difference was found between the growth of new epithelium over the test lesions and the controls. Since the similarity of values would be expected if the scabs retained moisture like the membranes, several scabs were obtained and their water vapor permeabilities tested. The scabs were found to permit 3-5 liters/M^2/24 hours to evaporate at 35° C. Since the most permeable membrane allowed only 1.267 liters/M^2/24 hours to evaporate, it seems that moisture retention has little effect on the healing of cutaneous wounds.

A large range of values was recorded which could not be explained by a difference in temperatures over the backs of the experimental animals, and which was probably unrelated to differences in blood supply to the test area.

Several histological observations were made while examining the slides prepared from the biopsy specimens. These were:

1. All wounds formed scabs whether they were covered with the impermeable films or uncovered.

2. The epithelium migrated, in most cases, as a multicellular sheet over the wounds, rather than as a monocellular layer which is often described.

3. The migrating epithelium undermined the scabs, separating the scabs from the underlying tissues.

4. Fibers of collagen were often seen continuing from the dermis into the scab. These were cleaved by the epithelium as it migrated under the scab.

5. The epithelium became hyperplastic after it migrated, and often formed the "pseudo rete pegs" described by Ordman and Gillman.

Thus, while several interesting observations concerning wound healing were made, the results of this study indicate that the clinical use of impermeable membranes to accelerate the healing of cutaneous wounds is entirely without experimental support; and due to the increased probability of infection with the use of such films (due to moisture retention) wounds should be covered with dressings designed to permit the evaporation of water.

Appendix I Water Vapor permeability of Membranes (28)

To determine the water vapor permeability of the various membranes used in this project, a Payne Cup* was employed. This device consists of a small metal cup with a wide lip, a lock ring, and three "C" clamps. After filling the cup with water, the test material was positioned over the mouth of the cup. A rubber gasket** was then placed on the lip of the cup, covering the edge of the membrane. The lock ring was placed on the gasket and clamped in position. The cup was weighed at six hour intervals, and the weight of evaporated water was calculated for each period of time. Once these weights became constant, the permeability of the membrane was easily calculated using the evaporative surface area of the membrane, (see Figures 11. and 12.).

The testing was done in room air with a relative humidity which ranged from 50% - 80%. Each membrane was tested over a wide range of temperatures to determine the effect of this factor on the water vapor permeability.

*Fisher Scientific Company, Silver Springs, Maryland

** For precise measurements, a rubber gasket should be cut to fit the lip of the cup.

Samples of the Test Membranes

1. The Poly Amino Acid Film (with gauze backing)

2. The Polyethylene Film

Appendix II Anesthesia in the Experimental Animal

Several combinations of agents were tried before a satisfactory method of anesthesia was obtained. Surital* was selected as our primary agent. Wright and Hall (29) note that this agent is more potent than sodium pentothal; however, it causes more airway secretions and thus necessitates the use of atropine as a premedication. The agent was administered intravenously. A "butterfly" needle was placed in the ear vein of the experimental animal and connected through a three-way stopcock to a bottle of 5% dextrose in water. A slow infusion was maintained between injections of the agent to keep the intravenous line open. Ten ml. of a 5% solution of Surital was injected initially. This was followed by 1-2 ml. of the solution when the animal became "light". A surgical level of anesthesia was reached when the animal would not blink with a light tap near the orbit, and the tail uncoiled. Because respiratory depression was a hazard with an excess of agent, the level of anesthesia was titrated to the point that the blink reflex just disappeared. As Surital is soluble in fat, the initial injection provided anesthesia for only ten to fifteen minutes. At that time, the blink reflex would return, and additional agent was injected. After approximately 40 minutes, the fat stores seemed to become saturated, however, as 1 ml. injections provided anesthesia for 15-20 minutes.

* Sodium Thiamylal, Parke Davis & Co., Detroit, Michigan

Because the placement of an intravenous line in the ear vein was traumatic to the experimental animal, Metofane* was used for induction. This was administered by the "open drop" method, and anesthesia was produced in approximatley two minutes. The intravenous line was then inserted with ease.

Booth (30) suggests that sedation may be obtained in the pig by injecting chlorpromazine (1.0 mg./kg. I.M.) 45 minutes prior to induction. We found, however, that this provided little, if any, sedation, and therefore this drug was not used.

To prevent the accumulation of secretions in the airways, 0.04 mg./kg. atropine sulfate was injected intramuscularly thirty minutes prior to induction.

Anesthetic Agents

Premedication	:	0.04 mg./kg. atropine sulfate, I.M. 30 minutes before induction
Induction	:	Metofane, open drop
Anesthesia	:	Surital, 10cc. of 5% solution initially, followed by 1-2 cc. as needed

With this combination of agents, the animal was awake in 20-30 minutes after the last injection. Because the large fat deposits were saturated with Surital, however, the animal was unstable on his feet for 24 hours. For this reason, food and water were withheld on the day of the operation and on the following day.

*Methoxyflurane, Pitman-Moore, Inc., Ft. Washington, Pennsylvania.

Because the taking of biopsies required only 10-15 minutes of anesthesia, Metofane alone was used for these procedures. Once the agent was withdrawn, the animal was awake in ten minutes, and could take food and water later in the day.

Because the testing of disorder requires only a few minutes of anesthesia. Before-and-after tests of these procedures. Once the agent was eliminated, the patient would awake in ten minutes, and could take food and drink in the day.

Appendix III Preparation of Microscopic Slides

I. Fixing - Tissue placed in 10% formalin - 48 hours

II. Drying - Using Technicon Tissue Processor* :

95% ethanol - 1 hour
95% ethanol - 1 hour
95% ethanol - 3 hours
100% ethanol - 2 hours
100% ethanol - 2 hours
50% (100% ethanol) 50% toluene - 2 hours
50% (100% ethanol) 50% toluene - 2 hours
100% toluene - 1 hour
100% toluene - 1 hour
100% toluene - 2 hours
Paraplast** 60°C. - 1 hour

III. Hardening - Paraplast bath in vacuum oven - 1 hour

-15 lbs./in^2 60° C.

IV. Imbedding - Tissue placed in mold, covered with Paraplast

V. Sectioning - Tissue blocks sectioned in microtome, 8 micron sections placed on slide with thin film of albumin and dried in vacuum oven - 1 hour

VI. Staining -

100% toluene - 5 minutes
100% toluene - 5 minutes
100% ethanol - 3 minutes
100% ethanol - 3 minutes
95% ethanol - 3 minutes
wash with water
Hematolylin - 5 minutes
wash with water
Acid Alcohol - Until tissue pink
wash with water
wash with ammonia water
wash with water
Eosin - 10 seconds
wash with water
95% ethanol - 5 minutes
100% ethanol - 5 minutes
100% toluene - 5 minutes
100% toluene - 5 minutes
Air dry, add drop balsam and cover slip

* The Technicon Company, Chauncey, New York

** Arthur H. Thomas Company, Philadelphia, Pennsylvania

Appendix IV. Staining Solutions*

1. Harris' Hematoxylin

Dissolve: 1 gm. hematoxylin in 10 cc. absolute alcohol
20 gm. ammonia alum in 200 cc. water
Mix solutions
Bring to boil and add 0.5 gm. mercuric oxide
Boil for three minutes then cool rapidly
Add 10 cc. acetic acid

2. Acid Alcohol

70 cc. - 100% ethanol
29 cc. - water
1 cc. - hydrochloric acid

3. Ammonia Water

5 cc. spirits of ammonia in 1 L. water

4. Eosin

Add to 40 cc. water

0.5 gm. eosin
0.25 gm. potassium dichromate
5 cc. saturated solution trinitrophenol
5 cc. (100%) ethanol

* From Guyer (31)

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