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The Effect of Fetal Age on Skin Permeability in Guinea Pigs

Benita Stiles Whitted

Abstract

THE EFFECT OF FETAL AGE ON SKIN PERMEABILITY IN GUINEA PIGS

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Preliminary investigation has indicated large decreases in fetal skin permeability during the latter half of gestation (Parmley and Seeds, 1970; Treager, 1966). The purpose of this study was to demonstrate the degree of skin permeability in fetal guinea pigs at various gestational stages. The test hypothesis was that these skins would show a significant decrease in permeability to tritiated water when tested at time increments throughout the latter half of gestation.

In vitro skin permeability to tritiated water was measured in samples from 22 guinea pig fetuses. Gestational age ranged from 30 to 65 days in increments of five, as well as 67-day (term) samples. A significant decrease ($p=.05$) in obtained permeability coefficients was seen between 45 and 50 days gestation, or near the beginning of the third trimester of pregnancy.

The results of this study give scientifically-based reason for further specific research regarding skin-related care of the small, immature neonate. Areas with significant nursing implications in this patient population include topical application of pharmaceutical agents, heat regulation, fluid evaporation, and maintenance of skin integrity.

LOMA LINDA UNIVERSITY

Graduate School

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by

Benita Stiles Whitted

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Each person whose signature appears below certifies that this thesis in his/her opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.

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Benita Stiles Whitted

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Chapter 1

THE PROBLEM

Introduction and Need for the Study

Although clinical care of the immature newborn still presents many challenging problems, advances in technology and clinical practice, based on research, have significantly decreased infant mortality. Basic to increased knowledge in this area is research in the field of perinatal physiology. Many changes have been brought about in infant care by findings which show the physiologic dissimilarities between infants and older people.

This research explored basic developmental characteristics of 22 fetal guinea pig skins as related to permeability. Previous research suggests that similarities exist between human and guinea pig skin in permeability to water (Treager, 1966), and in chemical composition (Widdowson, 1950). A more thorough understanding of this developmental progression may aid in delivering optimal skin care to preterm and immature infants, and in management of other related phenomena such as maintenance of body heat, prevention of percutaneous dehydration, and prevention of absorption of harmful topical agents, in these neonates. Data such as these become more important as the age of neonatal viability decreases.

Background of the Problem

Neonatal nurses generally manage and treat a majority of the integumentary related problems of their patients. Significant areas

of concern include maintenance of skin integrity, thermal regulation, percutaneous water loss, and percutaneous absorption of chemicals (Klaus and Fanaroff, 1979; Curley, 1971; Shuman, 1974; Kopelman, 1973). Infants born earlier in the gestational period and infants who are small-for-gestational-age manifest these problems in a more pronounced manner.

Empirical observation, clinical research and technological advances have made strides in successfully dealing with some of these clinical problems; nevertheless, much baseline research still needs to be done. Development of skin impermeability, and its protective qualities, during the gestational period remains a largely unexplored area. Findings related to the development of mature levels of skin impermeability may have a bearing upon the understanding and management of the previously stated areas of concern.

Statement of the Problem

The overall objective of this research was to gain basic descriptive data from animal studies which might relate to humans, especially immature and preterm neonates. Very little basic research pertaining to skin permeability of the developing fetus or neonate has been published. This knowledge is important in the delivery of informed clinical care to neonates. Due to governmental regulations and ethical considerations, direct study of human fetuses is difficult. Because of reported similarities between the guinea pig and human, guinea pig fetuses were chosen for investigation. If protective skin qualities are not fully developed until quite near term, or fetal maturity, this information may be especially crucial to care of the immature or preterm infant.

Objective

The objective was to investigate the developmental process of fetal guinea pig skin from the relative permeability of the single cell state, to the relative impermeability demonstrated by fully-developed skin. The basic question was, "What is the degree of permeability at each stage of development?"

Theoretical Framework

The skin is one of the largest of organs, and performs multiple functions. Rook and others (1972) listed these as: prevention of penetration of foreign materials and radiation, resistance against mechanical shocks, regulation of heat loss, and mediation of sensation. Each of these conveys the sense of the skin as being a barrier from the outside, and a protection for the inside of its boundaries.

The embryo begins with a single cell which is dependent upon its permeability for continued survival. This cell eventually develops into a viable fetus through the process of multiplication and differentiation. As growth proceeds, the fetus develops skin, which at some point in gestation reaches the relative impermeable status of the normal, mature newborn.

As clinical population changes occur, pertinent areas of related knowledge must also be sought out. Clinical sophistication in obstetrical and neonatal care has decreased the age of viability for preterm babies, and increased the incidence of survival in these infants to 75 percent (Blackburn, 1982). The largest segment of the neonatal population to experience reductions in mortality rates in the past 15 years

has been those small-for-gestational age infants (Shapiro, 1981; Williams and Chen, 1982).

Basic research data describing a developmental curve for human fetal skin permeability is scarce. In relation to fetal and neonatal research, the 1978 Federal Register bans experimentation on live, viable fetuses, defined as those being over 20 weeks gestational age or over 500 grams in weight. Due to governmental regulations and ethical considerations, direct study of human fetuses is not a viable research option in many situations. Physiological research has depended heavily on laboratory animals as models. Guinea pigs were chosen as experimental animals for this study due to their availability, and because of the relatively large fetal size, in comparison to other small laboratory animals. In addition, significant similarities have been demonstrated between the skin and tissues of guinea pigs and humans. Treager (1966) discussed the relationship of skin permeabilities of various species. On his scale of skin permeabilities to water, the guinea pig is the species most similar to the human. In comparing the chemical composition of newly-born mammals, Widdowson (1950) noted that guinea pigs and humans had very similar water and fat concentrations. Holt and Perks (1975) found that comparative values for water permeability through human and guinea pig amnion tissues agreed well.

Some research on fetal skin development has been done. One study measured permeability of human fetal skin to isotopic water. It was found that permeability decreased significantly between 12 and 24 weeks gestation (Parmley and Seeds, 1970). If further study lends validity to this finding that impermeability occurs at about the halfway mark

of gestational development, implications for care of immature infants may be clarified.

Statement of Hypothesis

Fetal guinea pig skin samples will show a significant decrease in permeability to tritiated water when tested at time increments throughout the latter half of gestation.

Definition of Terms

Tritiated water. Plain water labeled with tritium as the radioactive material, and in this study obtained from New England Nuclear.

Amniotic saline solution. Artificially prepared perfusion solution, approximating human amniotic saline, and prepared according to Holt and Perks (1975).

Permeability. Operationally defined here as the number of tritium counts per second, crossing a square centimeter of skin, as calculated by use of a modified Ficks equation (Behl and Barrett, 1981).

Fetal guinea pigs. This study used 22 New Zealand guinea pig fetuses, not necessarily from different litters, and ranging in gestational age from 35 to 67 days, or term.

Description of Method

The study design used was that of association-testing (Diers, 1979). Two previous studies have indicated a significant observable decrease in skin permeability near the halfway point of gestation (Parmley and Seeds,

1970; Treager, 1966). The intent of this study was to further test these tentative findings.

This study investigated the rate of crossover of tritiated water in the fetal guinea pig skin. This was expressed as a permeability coefficient and was calculated using a modified Ficks equation. Experiments were conducted using a perfusion chamber which circulated tritiated amniotic saline solution past the epidermal side of the skin, and plain amniotic saline solution past the dermal side. The sample size was 22 skins, and fetuses between 35 and 67 days gestation were used.

Methodological Assumptions

Methods used in this study were similar to those used by Parmley and Seeds (1970) in their study of permeability of human fetal skin to labeled water. Pilot studies were run to determine and correct potential problems. Specific testing was done relating to the problems of edge leaking and unstirred layer effects. Neither of these problems was found to be significant in affecting permeability coefficients obtained. Viability of excised skins was retained by careful control of oxygen, electrolytes, glucose, pH, and bacteria in bathing solution. Skins showed comparable permeability ratios up to three days post-excision, when tested.

Chapter 2

REVIEW OF LITERATURE

Introduction

Areas of literature review pertaining to skin are presented in the following order: (1) anatomy and physiology of the skin; (2) permeability of the skin as related to historical perspectives, the skin barrier layer, fetal skin development, and comparisons of guinea pigs and humans; and (3) nursing management considerations related to immature skin.

Anatomy and Physiology of the Skin

The topmost layer of skin is called the epidermis, and is further subdivided into several sections. The outer layer, horny layer, or stratum corneum, is composed of stacked, desquamating, dead cells. Under this lies the Malpighian layer which contains variable thicknesses of stratum granulosum, stratum spinosum and stratum germinativum. The stratum corneum is continuously replenished by the slow upward migration of cells from the germinative basal layer by a process of dehydration and polymerization of the intracellular material.

The dermis lies under the stratum germinativium of the epidermis, and over the subcutaneous layer of fat. It consists of loose connective tissue, contains blood vessels, lymph vessels, nerves, hair, and sweat follicles. The dermis provides support and structure for the epidermis,

facilitating skin conformation to the body parts with its tough and elastic collagen bundles. Below the dermis are subcutaneous fat cells, by which the superficial skin is linked to the underlying anatomy (Montagna and Parakkal, 1974; Rook, Wilkinson, and Ebling, 1972; Scheuplein and Blank, 1971).

Permeability of the Skin

Historical Perspectives

A brief, but thorough overview of the history of scientific interest and work on skin permeability is presented by Scheuplein and Blank (1971). Prior to the twentieth century, the scientific community generally thought of whole skin as being an impermeable barrier, as presented in 1877 in Fleischer's review. By the turn of the century enough work had been done to enable Schwenkenbacker to present the facts that skin is more permeable to lipid-soluble materials, and is relatively impermeable to water and electrolytes. Over the next 30 years, Collander and Barlund further developed and established the significance of skin permeability to lipid solubles (Scheuplein, 1977). This work eventually aided in the development of topical medications.

A related question of considerable interest and significance had to do with the location of the barrier to permeability in skin. In 1853 and 1856 two different investigators observed that the process of blister formation indicated differences in the permeability of various skin layers (Scheuplein and Blank, 1971). Controversy over where the barrier layer resided in the skin was complicated for many years by observations of the very permeable, although hypertrophic horny layer or stratum

corneum of patients with ichthyosis vulgaris. It was not for many years that definitive experimentation pointed out that the stratum corneum layer was the barrier layer to free permeability. Scheuplein and Blank further report that in 1945 Mackee found that dye was blocked from traversing through this area, and Berenson and Burch found that the horny layer was the barrier for water permeability of skin in 1951, as did Blank in 1953.

The Skin Barrier Layer

Although various theories regarding the mechanism of skin permeability have been advanced, it is generally agreed that the stratum corneum layer of the epidermis forms the barrier layer (Blank, 1953; Mali, 1955; Rothman, 1959; Sizahall, 1951; Treager, 1966). This is the area where keratinization or differentiation from the aquaous epidermal cell to the relatively dry and solid epidermal membrane occurs (Montagna and Parakkal, 1974; Scheuplein and Blank, 1971; Treager, 1966). This conclusion was reached only after years of debate and findings from more definitive methods of investigation.

One of the first experiments to yield currently-accepted information on the barrier layer was performed by Berenson and Burch in 1951. Passive diffusion of water under various environmental atmospheric conditions was observed in blistered skin, and separate preparations of dermis and epidermis. The conclusion reached was that water transfer inhibition occurred in the superficial layer of cells of the epidermis. The specifics remained unidentified, even though alluded to.

In 1953, Blank performed an experiment using a technique previously

introduced by Wolfe in 1939 (Scheuplein and Blank, 1971). The skin layers were successively stripped from the specimen as measurements of the diffusion rate of water were taken. No significant changes were noted until the base of the stratum corneum was reached. The abrupt increase of water diffusion through the specimen at that point seemed indicative that the stratum corneum was indeed the barrier layer.

Later experiments confirmed these data (Blank, 1953; Monash and Blank, 1958), and have shown by means of isotopic tracers that the top portions of the stratum corneum are involved in barrier activity, as well as the lower portions demonstrated by Blank (Blank and Gould, 1962; Fredrikkwon, 1962; Matoltsy, and Others, 1963).

Fetal Development of Skin

Research evidence to the present point indicates that the human fetus does not begin skin keratinization and the formation of a stratum corneum layer until the 16th to 17th week of gestation (Cheek, 1975; Dunham, 1961; Hashimoto, 1966; Rook, Wilkinson, Ebling, 1972; Serri and Montagna, 1961). It is presumed that the early periderm is open to amniotic fluid components as a means of survival. As the fetus develops, amniotic fluid components change, and fetal cell function becomes more sophisticated (Seeds, 1965). The healthy newborn is born with relatively impermeable skin. This characteristic becomes more pronounced as the child grows toward adulthood, and the epidermis becomes thicker (Hashimoto, and Others, 1966; Montagna and Parakkal, 1974; Serri and Montagna, 1961; Treager, 1966). These observations would tend to indicate keratin production as the crucial factor involved in the general impermeability of skin.

A study by Parmley and Seeds in 1970 showed that skin permeability decreases as keratinization increases. Fourteen human fetal skins between the ages of 12 and 24 weeks gestation were tested for permeability to isotopic water. Skin displaying partial and full keratinization by histologic examination showed little or no permeability.

Comparison of Guinea Pig and Human Skin

Historically, the guinea pig has been used for a bulk of the standard laboratory experimentations. Many changes in clinical practice have occurred due to information gained from basic research using guinea pigs.

Studies of the effects of detergents on the percutaneous absorption of guinea pigs have produced results similar to the same sort of experiments utilizing human skin (Bettley, 1961; Bettley, 1963; Gisslen and Magnusson, 1966; Wahlberg, 1968).

In 1950, Widdowson noted that at term the total water content in the newborn human and guinea pig is similar, and lower than in other newborn animals. In addition to the human and guinea pig, the pig, the cat, rabbit, rat, and mouse were tested. The similarities in human and guinea pig components were attributed to an increased total body fat deposition. This similarity may or may not be significant. Treager presented comparative values of the relationship of skin permeabilities of various species. The guinea pig is the species most similar to the human on his scale of skin permeabilities to water (Treager, 1966).

Holt and Perks (1975) found that comparative values for water permeability through human and guinea pig amnion tissues agreed well. At term, the amnion has histologic resemblance to skin (Pritchard and MacDonald, 1981), and may tentatively be used in this comparison.

Nursing Management Considerations Related to Immature Skin

Clinical observation of the newborn has revealed specific characteristics and peculiarities of neonatal skin. The term baby of normal weight presents with soft, wrinkled, velvety skin, covered with a greasy layer of vernix caseosa. The preterm and/or small-for-gestational-age infant exhibits more transparent, gelatinous skin, which tends to be loose, and free of wrinkles. Approximately two weeks after birth a dry scaling of parchment-like skin may produce a physiologic desquamation (Morselli, and Others, 1980; Solomon and Esterly, 1970).

Due to lower rates of melanin production and less pigmentation, newborn skin is more sensitive to sunlight. In contrast to adults, infants also have an increased tendency to blister. This is attributed to a less adherent attachment of the epidermis to the dermis. The stratum corneum is also thought to be more permeable to chemical agents during this period (Morselli, and Others, 1980; Solomon and Esterly, 1970).

Several conditions having to do with incomplete skin development are seen in the preterm or low-birth-weight infant. These include "collodian baby," or lamellar exfolliation of the newborn, nonbullous congenital icthyosiform erythroderma, pseudomonas aeruginosa and staphylococcus aureus infections (Easterly and Solomon, 1970; Somerville, 1969).

In order to preserve and restore skin integrity and to prevent infection, various chemical and pharmaceutical agents are applied to the infant's skin. Skin uptake characteristics of these babies have not been established for the majority of topical agents used. Scientific

research of optimal methods for preservation of skin integrity of the immature infant remains scarce (Morselli, and Others, 1980; West, and Others, 1981).

Some investigation of effects of topical absorption of various substances has taken place. Phenol derivatives have been shown to cause percutaneous toxicity. Armstrong reported 20 cases of poisoning which were traced to laundry powder concentrations on nursery linens (Armstrong, and Others, 1969). Aniline dyes and topically administered corticosteroids have also been implicated in nursery deaths (Kagan, and Others, 1949; Feinblatt, 1966). Several studies regarding dermal absorption of hexachlorophene via soap powders and antiseptic solutions showed especially significant levels in serum of low-birth-weight babies (Aggett, and Others, 1981; Curley, and Others, 1971; Kopelman, 1973; Shuman, and Others, 1975; West, and Others, 1981).

Nachman and Esterly (1971) report evaluation of skin permeability by skin blanching in response to topically applied Neo-Synephrine. Infants 28 to 34 weeks gestational age had a rapid and prolonged blanching response. This was in contrast with the infants of 38 to 42 weeks gestational age, who generally failed to show any response.

Preterm and small-for-gestational-age newborns are in a very precarious state in relation to heat regulation and loss of fluid by evaporation. The margins of heat control are narrow, and due to the limited ability to increase metabolic rate, insensible water losses are great when a neutral thermal environment is not maintained (Klause and Fanaroff, 1979). Experimental support for use of monitored radiant warmers and double-walled incubators in heat maintenance and control of

metabolic and water losses has been presented (Baumgart, and Others, 1980; Marks, and Others, 1980; Yeh, and Others, 1980). Predisposing factors in the immature neonate relating to these problems include large surface-to-volume ratios, and increased water content, as well as a thinner epidermis and possibly increased permeability (Fanaroff, and Others, 1972; Wilson and Maibach, 1980).

In 1978 Versmold and others noted high surface oxygen levels on the unheated skin of premature infants of less than 1500 grams, who were receiving transcutaneous monitoring. This was positively related to arterial oxygen levels. They conjectured that immature cutaneous circulation and low resistance of the immature skin determined the high oxygen permeability of skin.

Summary

Basic research related to skin permeability has thus far pertained mainly to the adult. Although some preliminary data are available, further investigation is needed to develop a model of human development of skin impermeability, and its relation to clinical management, especially of the preterm and small-for-gestational-age neonate. Due to difficulties associated with research on human beings, laboratory animals became the choice of subjects for basic research. The guinea pig was determined the most suitable animal for purposes of this study.

Chapter 3

FETAL DEVELOPMENT OF SKIN IMPERMEABILITY IN GUINEA PIGS*

Introduction

Adult skin has been extensively studied to discover its characteristics of permeability in relation to many types of penetrants (Montagna, W., Ed., 1972; Sheuplin, R. J. and Blank, I. H., 1971). Data relating to skin permeability of the fetus is comparatively scarce.

Fetal skin permeability is reported in the following two studies. Treager and Hunt showed sharp decreases in permeability to 5% triethyl phosphate from day 16 to 20 in the fetal rat (Treager, R. T., 1966). Parmley and Seeds found that the permeability to tritiated water fell about 20-fold from the 19th to 24th week of gestation for the human fetus (Parmley, T. H. and Seeds, A. E., 1970).

Available data seem to indicate that keratinization and skin impermeability seem to occur at about the same time in the developmental process (Montagna, W. and Parakkel, P. G., 1974; Parmley and Seeds, 1970; Sheuplin, R. J. and Blank, I. H., 1971; Treager, R. T., 1966). This, of course, would imply that before fetal skin begins keratinization processes, it would maintain certain amounts of permeability, reminiscent of the single cell ability to transfer measurable amounts of permeants and water.

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*This chapter consists of an article submitted to the Department of Perinatal Biology, Loma Linda University Medical Center, with intent for future publication.

The data presented here are intended to investigate the development process of fetal skin from the relative permeability of the single cell state, to the relative impermeability demonstrated by fully developed skin. The youngest possible fetuses were used as available skin area would permit, in order to follow changes through as large a portion of the gestational period as physically possible.

Methods

Sample Description

Skin samples from guinea pig fetuses were used ranging in gestational age from 30 to 65 days in increments of five, as well as 67-day samples.

Skin Preparation

Maternal guinea pigs and their fetuses were sacrificed at various gestational stages. After removal from the mother, skin was excised from the trunk of the fetus, using a midline abdominal incision. The skin was immediately placed in a container of continuously aerated amniotic saline solution (Holt, W. F. and Perks, A. M., 1975), together with 100,000 U. of penicillin to inhibit bacterial growth. The skins remained in this solution until experimentation (from 1 to 72 hours). Older skins did not require added support; however, skins of 35 and 40 days gestation were supported in the perfusion chamber between single screens of nylon mesh.

Apparatus

Fetal skins were placed between greased O-rings and mounted between the two conical plexiglas hemichambers. The two halves of the chamber were placed together lightly but firmly, and held by a screw vice mounted between two metal rods. Amniotic fluid solution was prepared according to Holt and Perks (1975). This was initially perfused via syringes through both hemichambers simultaneously, taking care to maintain equal pressures on each side of the skin. The maternal (fetal epidermal) side of the chamber was then connected to a 60 cc. syringe in a Harvard infusion pump. Solution introduced to the maternal side contained tritiated water (NEN) which had been added to amniotic saline solution in concentration of 3,000 to 200,000 cpm/ml. Increased concentrations were used when studying more mature and less permeable skins, in order to maximize the sensitivity of the measurement.

The fetal (fetal dermal) hemichamber was perfused via a peristaltic pump, through a closed loop circulating 39 cc. of unlabeled amniotic saline solution. Flow rate of both pumps was maintained at 1 cc/minute.

All experiments were conducted with solutions and skins at room temperature, which ranged from 23.0 to 24.5 degrees Celsius.

Sampling Procedure

From the fetal loop 100 µl were taken every 5, 10, or 15 minutes, depending upon the experiment, with a Gilson pipette. Pipette samples of 100 µl were also taken from the maternal runoff at 30-minute intervals.

The samples were placed in glass scintillation vials along with a

mixture of toluene (J. T. Baker) and liquiflour (NEN) in a 20:1 ratio. Samples were counted for 10-minute intervals in a Packard scintillation counter.

Each experiment was preceded by a stabilization period of at least 20 minutes, during which time labeled fluid was given the opportunity to saturate fetal skin, and flux of solution across skin might approach a steady state. Following this initial period, collections were taken for approximately three hours.

Data Analysis

The permeability coefficient for water crossing the membrane in a unilateral direction from the maternal to the fetal side was calculated from the following derivation of Fick's equation (Behl, C. R. and Barrett, M., 1981).

$$P = \frac{V}{A} \frac{(d\Delta C/dt)}{C} \qquad \text{(Eq. 1)}$$

P = permeability coefficient (cm/sec.)

A = diffusional area (1.77 sq. cm.)

ΔC = concentration difference across the membrane, which was taken to be equal to the donor concentration (cpm)

V = half cell volume and tubing (39 ml.)

$d\Delta C/dt$ = steady state slope (cpm/cubic cm/hr.)

Least mean square regression slopes were fitted to data points, relating the build-up in fetal concentration over time. The slope in the interval from 20 minutes to 3 hours was used as the best estimate of $d\Delta C/dt$ in

Eq. 1. Data are given as mean (± 1 SE). The statistical significance of the change in permeability with time was assessed using the one-tailed t test.

Results

Figure 1 shows the time course of tritium build-up on the fetal side for a representative 35-day-old skin. After an initial stabilization period, a nearly constant flux of tritium was recorded during the ensuing two-and-one-half-hour duration of the experiment. The slope during this time was linear, and its average value, using the least-square techniques, was the basis for calculations of permeability according to Eq. 1.

Study results are summarized in Figure 2. The figure shows one data point for each fetal skin, ranging in age from 35 to 67 days of gestation. A precipitous fall in permeability is seen between 45 and 50 days. This indicates the period of greatest decrease of permeability in fetal guinea pig skins tested. After 55 days gestation practically no flux of tritium across the skin could be detected.

Discussion

Critique of Methodology

Methods used in this study were similar to those used by Holt and Perks (1975) in their experiments with water movement through guinea pig amniotic membrane, and those of Parmley and Seeds (1970) in their experiments on human fetal skin permeability to isotopic water.

Guinea pigs were chosen as experimental animals for this study due

to ready availability, and because of their relatively large fetal size. In addition, significant similarities have been shown between the skin and tissues of guinea pigs and humans. Treager (1966) shows a comparison between the relationship of skin permeabilities of various species. On his scale of skin permeabilities to water, the guinea pig is the species most similar to the human. In a comparison of the chemical composition of newly-born mammals, Widdowson (1950) noted that guinea pigs and humans had very similar water and fat concentrations. Holt and Perks (1975) found that comparative values for water permeability through human and guinea pig amnion tissues agreed well.

Several potential problems relating to the experimental method were recognized and dealt with as follows. Edge effects may be produced by leaking of labeled fluid through injured tissue. To avert this effect, rubber O-rings were partially embedded in the outside perimeter of the chamber openings, on either side of the skin membrane. Vice screws held the two chamber halves together only to the point of snugness. No leaking injuries were assumed to have occurred, as older skins did not show measurable amounts of tritium crossover from maternal to fetal hemichamber.

The problem of unstirred layers was investigated by increasing flow rates through a four-fold range. It was reasoned that if unstirred layers were a significant problem, their importance would diminish at higher flow rates. Lowest tested speeds (1 cc/min.) showed a permeability component of 98 percent of highest tested speeds (4 cc/min.). At flow rates of 2 cc/minute, the permeability component was 93 percent

of highest speeds tested. In view of these values, unstirred layer effect was considered insignificant, and obtained results reliable and valid.

Comparative Studies

Although research relating to development of fetal skin impermeability is not plentiful, work by Hunt and Treager (1966) contributes data on the rat skin. Permeability of fetal rat skin to 5% triethyl phosphate decreased sharply between gestational days 16 and 20. Continuation of the fall was seen after birth until full development of the stratified squamous layer was reached. These findings compare agreeably with the data presented here, taking into consideration the developmental differences between rats and guinea pigs.

A study by Parmley and Seeds in 1970, found that sharp decreases in skin permeability of the human fetus coincided with development of keratinization, as determined by histologic examination (Table 1).

Physiologic Significance

Histologic study of the human embryo has revealed approximate timing of appearance of epidermal skin layers. From the seventeenth week on, a gradual replacement of the periderm with the keratinizing stratum corneum occurs. Latest areas to show keratinization are the back, abdomen and limbs at about 25 weeks gestation (Breathnach, C. B., 1965; Hashimoto, K., et al.. 1966; Montagna. W. and Parakkel. P. F.. 1974).

If it is true that skin becomes less permeable as keratinization progresses, and if histologic observation of embryonic skin layer development is correct, we may anticipate a decrease of fetal skin permeability between the halfway and three-quarter marks of gestation.

This did indeed seem to be the case in the studies previously presented by Treager and Hunt, and Parmley and Seeds, as well as the data presented here.

Changes in permeability of fetal skin are likely to affect not only fetal fluid balances, but also changes in amniotic fluid. As pregnancy progresses, the volume of amniotic fluid increases. Compiled mean volumes of human amniotic fluid by Seeds (1965) and Wagner and Fuchs (1962) show sharp increases after the fifteenth week of gestation. Fluid volume continues to grow until term.

As gestation proceeds, changes in composition of amniotic fluid are also seen. The plasma-like ionic concentration and osmolality of early pregnancy becomes decidedly hypotonic in later stages, after fetal skin keratinization has occurred. Seeds (1975) records a high concentration of 291 mOsm./L between two and four months, and a low of 251 mOsm./L at nine months gestation, in the human amniotic fluid.

The data presented in Figure 2 suggest a sharp decrease between the relative permeability of 35 days, and the lack of permeability after 55 days gestation. The importance of skin amniotic fluid exchange at 35 days may be calculated by using the average obtained permeability coefficient of .000005 ml/sq. cm. of skin and an estimated total fetal skin area of 5 sq. cm. The permeability rate of fetal skin to an approximate volume of 10 cc. of amniotic fluid at 35 days gestation would be .36% of the amniotic volume over a 24-hour period. Greater amounts of crossover may possibly be seen in earlier and more permeable stages of skin development.

Further study and more information is needed regarding the development of permeability of fetal and newborn skin, and the consequent considerations involved. This knowledge is important to the continuing development of the scientific model of human formation and growth.

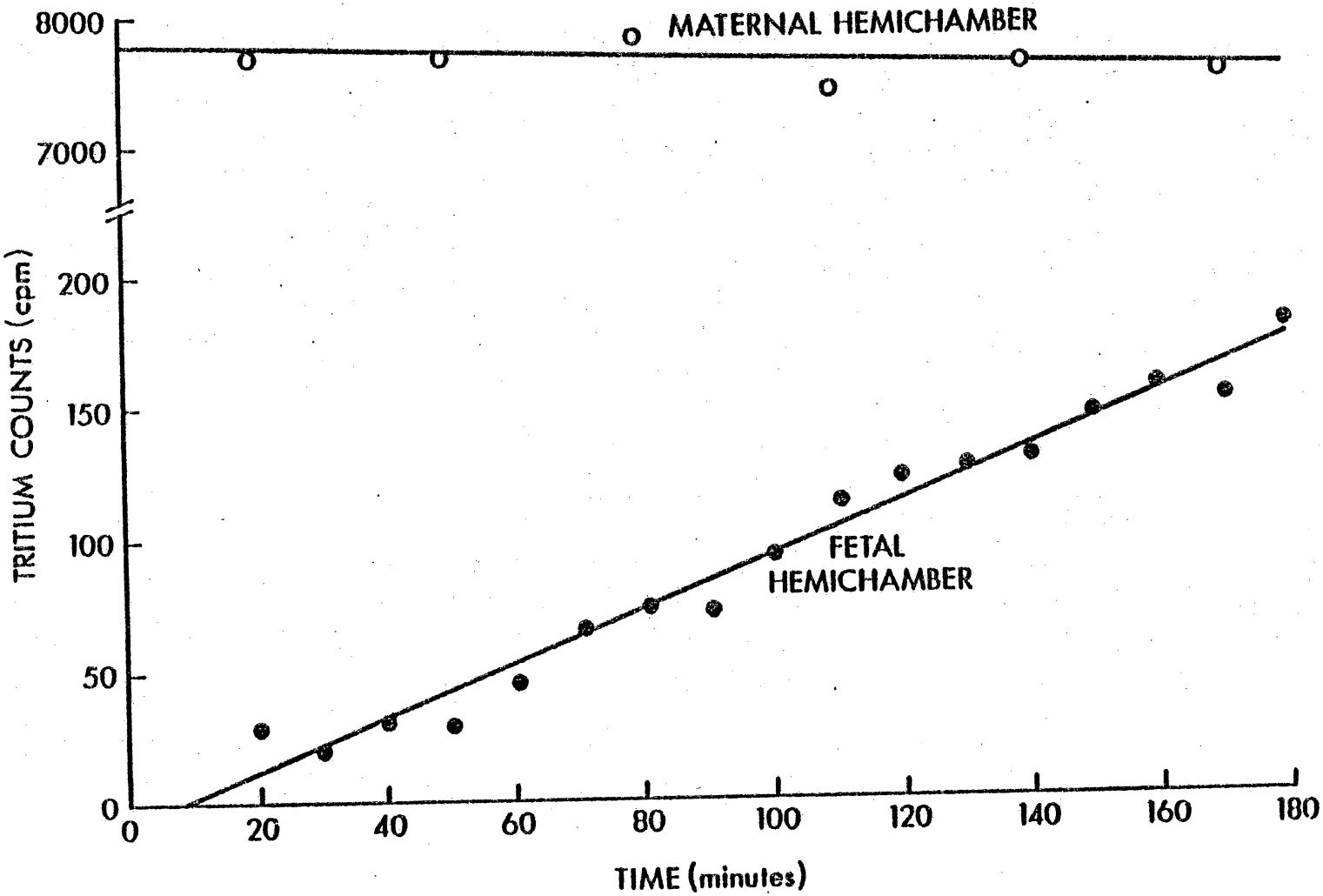

Figure 1

Representative Sample of Tritium Crossover Related to Time in a Guinea Pig Skin of 35 Gestational Days

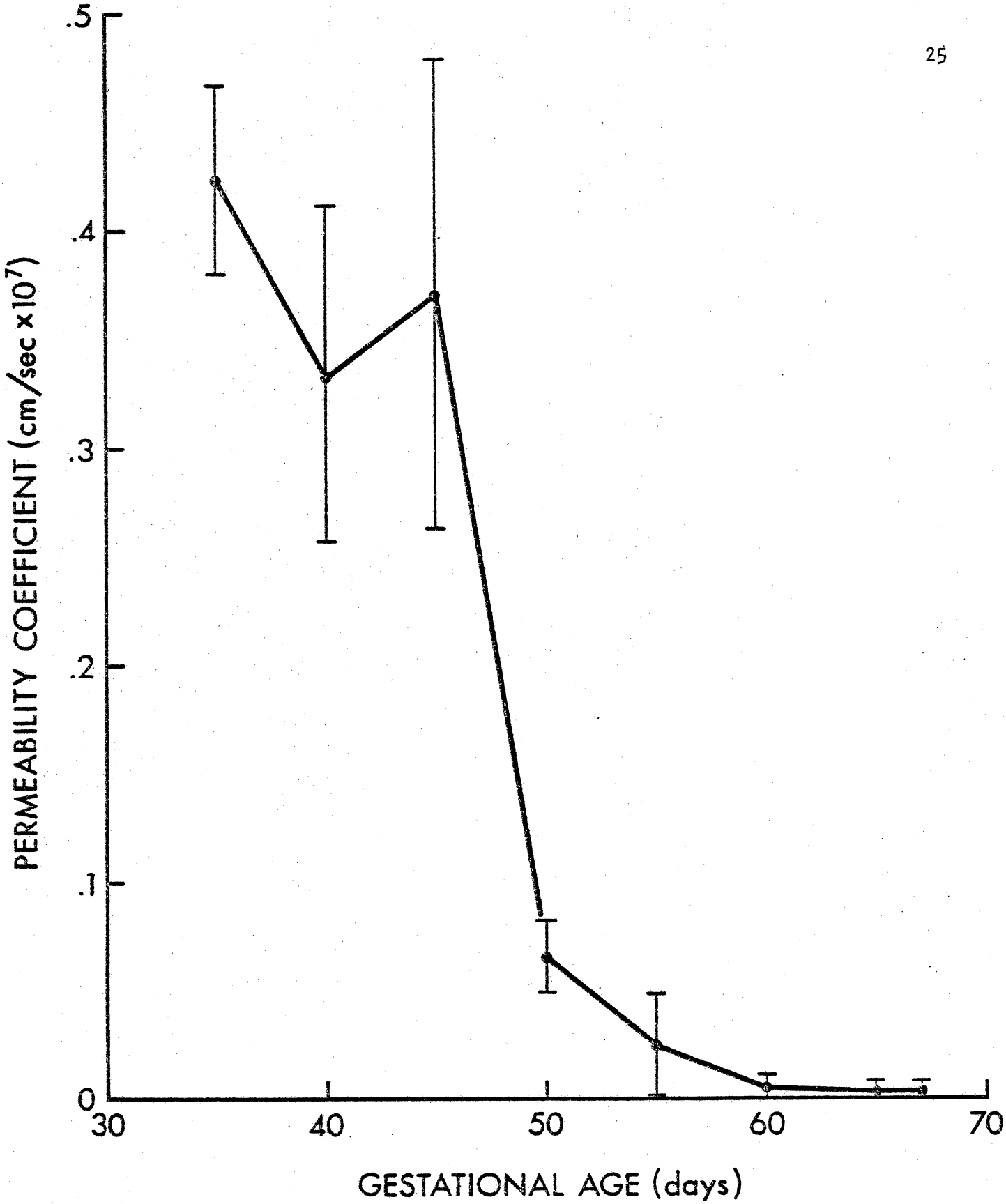

Figure 2

Data Results Comparing Obtained Skin Permeability Coefficient Means with Gestational Age, Along with Ranges of Standard Error

Table 1

Permeability Coefficients

Guinea Pigs (Present Study)		Human Fetuses (Parmley and Seeds, 1970)	
Day of Gestation	P (cps) x 10^7	Week of Gestation	P (cps) x 10^4
35	.4706	14	.881
35	.4649	17	.732
35	.3361	18	.711
40	.2598	18	.855
40	.2892	19	.716
40	.2259	19	.453
40	.5620	24	.020
45	.4774		
45	.2644		
50	.0810		
50	.0497		
55	.0949		
55	.0018		
55	.0002		
55	.00075		
60	.0056		
60	.0005		
60	.0065		
65	.0018		
67	.0007		
67	.0065		
67	.0000		

Chapter 4

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary and Conclusions

The purpose of this study was to determine at what point in gestation the fetal skin gains relative impermeability and specifically to measure the degree of permeability at each stage tested. Fetal guinea pigs were studied in the latter half of gestation, using an association-testing design. Fetal skins were tested for permeability to tritium labeled water between 35 and 65 days gestation, in increments of five days as well as at 67 days. A conical perfusion chamber was used to measure crossover of labeled water from the epidermal to dermal side of the skin. Study results showed a precipitous fall in permeability coefficients (as calculated by a modified Fick's equation) between 45 and 50 days gestation. After 55 days gestation practically no flux of tritium across the skin could be detected.

A review of literature covered three main relevant areas. These included anatomy and physiology of the skin, permeability of the skin, and nursing management considerations related to immature skin. Although research has provided information regarding characteristics of permeability in adult skin, testing of fetal and infantile skin remains scarce. A developmental model of skin impermeability needs to be developed as a basis for more scientific clinical management of the preterm and small-for-gestational-age infant.

The material presented in Chapter 3 was submitted to the Perinatal Biology Research Department of Loma Linda University with intention of submission for publication. The methods and procedures of data collection were detailed and a description of calculations and study results were given. Permeability coefficients of tested guinea pig skins to tritiated water were plotted against fetal gestational ages to determine validity of the hypothesis that a significant decrease in permeability would be seen in the latter half of gestation. This study did indicate a significant decrease ($p=.05$) in fetal guinea pig skin permeability in the last part of the second trimester, between 45 and 50 days gestation. The decrease in skin permeability at about 45 days of gestational age was statistically significant. Comparing pooled data from skins before and after 45 days using a nonpaired t test there was less than one chance in a thousand that the decrease would be explained by chance. The statistical significance of the change in permeability with time was assessed using the one-tailed t test. These results agreed well with a small study of skin permeability to tritiated water of 13 human fetuses between the gestational ages of 12 and 19 weeks.

Implications for Nursing

Although findings from animal studies cannot be generalized to humans, basic animal research is an important and accepted beginning point for physiological findings. Information regarding fetal skin development becomes more important to nurses and increasingly sophisticated technology makes less mature and smaller infants viable. Areas

of concern related to skin of preterm and small-for-gestational-age infants include preservation of integumentary integrity, heat regulation, prevention of undue body fluid evaporation, and percutaneous uptake of topically applied pharmaceutical agents.

The results of this study indicate that skin permeability does indeed show a significant decrease in permeability near the end of the second trimester of gestation in the guinea pig. This agreed with findings of Parmley and Seeds (1970) in the human fetus, and Treager (1966) in the rat fetus. General nursing implications from these data would indicate caution in application of topicals, and scientifically-based reasons for further specific research regarding skin-related care of the small, immature neonate.

Recommendations for Further Research

Further study in this area would optimally be conducted using human fetal skin. However, this continues difficult to accomplish due to government regulations and ethical considerations. Therefore, further study will probably be confined to guinea pigs, at least in the near future.

1. Repetition of this study for added external validity that significant skin permeability decreases do indeed occur near the same gestational stages that are currently considered compatible with life.

2. Repetition of this study testing water movement in the opposite direction (fetal to maternal, or epidermal to dermal) to validate the conjecture of two-way flux in the very young fetus.

3. Further testing of skin permeability correlating obtained permeability coefficients with measured skin thickness, or weight.

4. Further investigation of development patterns of keratinization in the fetus, and its relationship to development of regional skin impermeability.

5. Systematic clinical studies on agents and techniques most practical and safe for optimal preservation of the skin of high-risk infants.

6. Further investigation of percutaneous uptake of various pharmaceutical agents commonly used in the nursery.

7. Further investigation of percutaneous uptake of oxygen in the preterm and small-for-gestational-age infant, with refined equipment now available, such as transcutaneous oxygen monitoring.

8. Further investigation of the relationship of degree of hydration and amount of percutaneous absorption in high-risk neonates.

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APPENDIX A

Figure 3

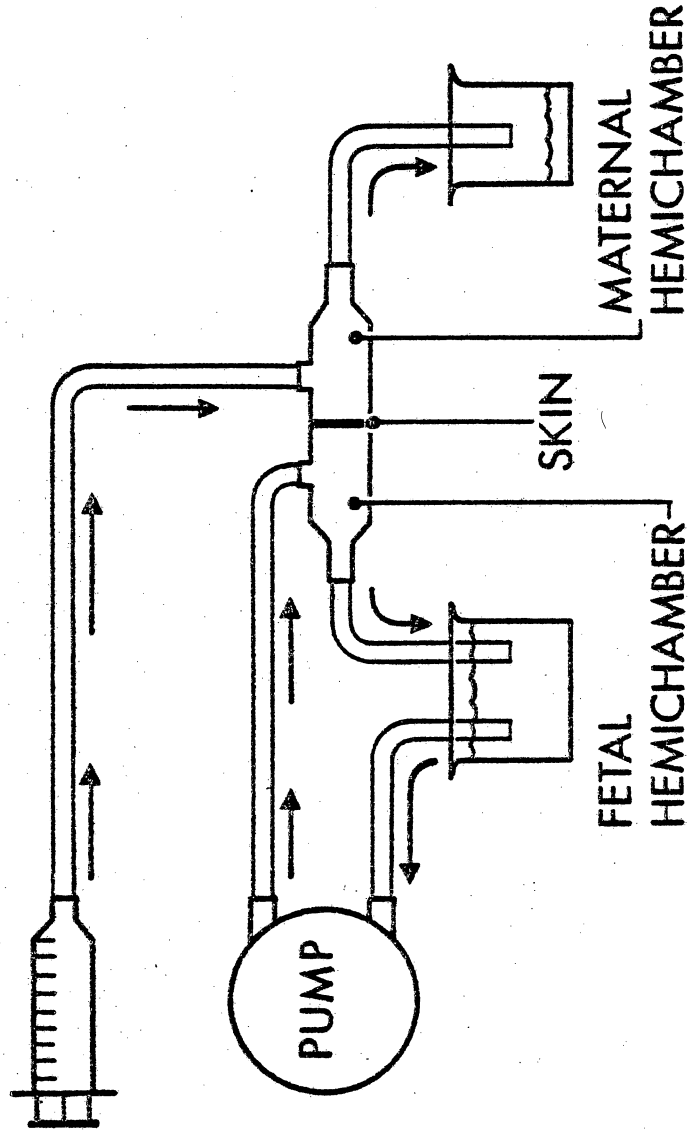

Figure 3

Experimental Apparatus Setup Used in This Study for Measurement of Skin Permeability