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An investigation of the release of 5-fluorouracil from ointment bases

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AN INVESTIGATION OF THE RELEASE OF 5-FLUOROURACIL FROM OINTMENT BASES

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P R E F A C E

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V. L. G.

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INTRODUCTION

Chronic sun exposure produces many alterations in the skin. A sequence of events beginning with a gradual and progressive actinic damage to the sun exposed areas of the skin, followed by the development of actinic (senile) keratoses and terminating with the formation of squamous and basal cell carcinomas, is responsible for the most common form of human malignancy. Actinic keratoses, for the most part, are flat, intraepidermal lesions. Basal and squamous cell carcinomas are slow to metastasize and with appropriate treatment, complete cures are expected. If neglected, however, basal cell carcinomas, as well as squamous cell carcinomas may metastasize, with fatal results (1).

Skin cancers are many times more common in warm and sunny areas of the southwestern states than in northern climates. Altitude is also important, in that damaging ultraviolet rays which are removed by atmospheric filtration are more intense at higher elevations. The complexion and occupation of a given individual are also factors in determining his likelihood of accumulating actinic damage of sufficient degree to produce malignant degeneration.

When these lesions are distributed over a wide area of the face, it is a significant cosmetic defect. The usual means of local destruction for widespread lesions include electrosurgery, cryotherapy, and dermabrasive surgery. When scores of lesions are to be treated in an individual, all of these procedures have certain disadvantages.

Since 1956, 5-Fluorouracil (herein referred to as 5-FU) has been used extensively in the therapy of human malignancies. During this time, it has been found to be most effective in adenocarcinomas of the colon and breast. It also appears to have some effect in carcinomas of the bladder, ovary, stomach and undifferentiated carcinomas (2).

In the past eight years, there has been increasing interest in topical chemotherapy of cutaneous malignancies. Klein (3,4), Goldman (5) and Dillaha (6) were the first to become interested in this field and worked primarily with the antimetabolites, methotrexate, mercaptopurine, 5-fluorouracil, demecoline and N-desacetyl thiocolchicine. Of all the compounds tested, 5-FU is currently the best method available for treating widespread multiple actinic keratoses; it appears clinically to selectively affect the actinic keratoses. Destruction of keratoses with relative sparing of normal skin can be regularly anticipated.

During the several years in which this therapy has been developed and evaluated, a standard dermatological preparation has not been available. The objective of the present work was to study the release of 5-FU from various ointment bases and to attempt to determine the type of base which would release the compound most satisfactorily.

The methods chosen were designed to determine the release and penetration of 5-FU both by *in vitro* and *in vivo* methods. A modified agar plate method was chosen as the *in vitro* test for measuring release and subsequent diffusion into the agar. The penetration of the compound from various bases was determined by applying the medicated bases to the skin of guinea pigs and subsequently analyzing a biopsy of the innuncted skin, using quantitative spectrophotometry.

Survey of the Literature

5-Fluorouracil was synthesized by Charles Heidelberger in 1957 (7). He had observed the biological effects often obtained when fluorine is substituted for hydrogen in several classes of compounds. Adding this to his knowledge of the effectiveness of various nucleic acid analogues, he felt that a fluorine substituted purine or pyrimidine might display tumor inhibiting activity. His synthesis of 5-FU and further studies

established the effectiveness of 5-FU especially in carcinomas of the breast and digestive tract (8).

Falkson and Schultz (9) investigated the skin changes following systemic 5-FU therapy and found that 31 of 85 patients were sensitive to sunlight. In their study of skin changes, they observed a patient with light complexion and red hair who had multiple keratoses on the face, arms, and hands. After one exposure to sunlight during parenteral 5-FU administration, she developed erythema on the exposed parts, most marked around the senile keratoses. The erythema disappeared three weeks after stopping treatment, by which time the senile keratoses had disappeared as well. However, these began to reappear a year later. In several other patients, keratoses and senile ichthyosiform roughening of the skin were seen to disappear during therapy without preceding erythema.

It is interesting to note that these investigators were not specifically interested in actinic keratoses or skin cancers of any type. However, their recorded observations have opened up several new areas of study in the therapy of skin cancer.

The earliest attempts to apply 5-FU topically were made by Klein (3). His first reported case involved a 63 year old white male, with a diagnosis of primary adenocarcinoma of the colon. The patient developed metastatic lesions to the

skin at other sites. A highly vascular lesion on the tip of the right index finger was selected for study. Biopsy showed typical metastatic adenocarcinoma. A concentration of 20 percent 5-FU in an unidentified hydrophilic base was applied daily, except weekends, directly to the lesion. Within 48 hours, a necrotic layer was formed on the surface of the tumor, while the skin of the non-involved area appeared normal. The vascularity of the tumor was markedly reduced. Within eight days, demarcation of the tumor from the underlying areas was apparent. A biopsy taken at this time showed no tumor cells; necrosis with heavy leukocytic infiltration was observed.

Continued topical application of 20 percent 5-FU in hydrophilic base resulted in progressive necrosis of the surface of the tumor and demarcation of the malignant lesion from the underlying, non-involved area. This was accompanied by reduction in the size of the tumor and in continued reduction of its vascularity.

Another reported case involved a 35 year old white male with a diagnosis of inoperable adenocarcinoma of the colon. The patient developed extensive malignant infiltration of the anterior abdominal wall exhibiting two protuberant masses. Preparation of 20 percent 5-FU in an unidentified hydrophilic base was applied topically at daily intervals to the medial lesion, while unmedicated base was applied to the lateral

protuberance as a control. Within 72 hours, necrosis and decreased vascularity at the surface of the treated lesion was observed. On continued topical application of 5-FU in hydrophilic base, progressive necrosis and devascularization of the treated areas were observed. No changes in the normal surrounding skin could be determined. Although neither of these two early cases involved carcinomas of cutaneous origin, they served to show that 5-FU was topically effective and that it displayed little, if any, effect on normal tissue.

Encouraged by the results of these early trials, Klein treated several cases of keratoacanthoma (4). A lesion of keratocanthoma on the left ear was treated with local injections of 0.1 ml. of a five percent solution of 5-FU in saline. These injections were administered directly into the base to the tumor twice daily for 17 days. After 48 hours, the size of the lesion had decreased considerably. The depressed center of the lesion had undergone necrosis. By the 16th day, the tumor could not be seen. Biopsy failed to demonstrate tumor cells and showed normal structures of the skin had remained undamaged.

A lesion located posterior to the left ear was treated with local applications of an ointment containing 20 percent 5-FU in a hydrophilic base. The ointment was applied five times daily to the affected area for 17 days. Biopsy at

the end of this period showed no tumor cells present and no damage to normal epithelium and connective tissue of the skin.

The third lesion of keratocanthoma was located on the right ala of the nose. This lesion was treated with local injections of 0.1 ml. of a five percent solution of 5-FU which were administered every second day directly to the base of the tumor. After the seventh injection, 15 days after the first injection, no clinical or pathological evidence of residual tumor could be demonstrated. The author concluded that 5-FU causes rapid regression of keratocanthoma, a cutaneous neoplasm of presumably viral origin.

In 1963, Nurse (10) studied the effects of some antimetabolites on normal epidermal structure. Included in the study was 5-FU. His preparation consisted of incorporating 5 ml. of the commercially available ampule[a] (500 mg. 5-FU per 10 ml. ampule, pH 9) into 10 Gm. of Aquaphor[b]. This ointment was applied to psoriatic plaques without cover under Saran Wrap[c] secured by Tubegauze[d]. Significant changes were seen

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[a]Available as 5-Fluorouracil from Roche Laboratories, Nutley, New Jersey.

[b]Duke Laboratories, Inc., South Norwalk, Conn.

[c]The Dow Chemical Company, Midland, Michigan.

[d]The Scholl Manufacturing Company, Inc., New York, N. Y.

in the plaques treated as described on the previous page. In four of six patients, the plagues became flatter, erythema was observed and a rim of erosion developed at the periphery. Normal surrounding skin was unaffected, but the patients experienced some discomfort and irritation. Healing left normal but pigmented skin and, when relapse occurred in one case, the peripheral areas which had been eroded remained normal and did not relapse.

Nurse also studied the effect of the ointment on normal epithelial cells. The 5-FU ointment was applied daily or each second day to 5 X 5 cm. areas of skin, with occlusion by 'Saran Wrap' secured by tape. In three of the four volunteers, specific changes were seen after application of 5-FU differing markedly from the control patch. In these three subjects, erythema developed in areas where removal of the tape had caused partial skin stripping and around the follicles. In these same areas, punctate erosions developed after eight to fourteen days, which healed rapidly with residual pigmentation persisting for many weeks.

Microscopically, biopsy specimens taken at the stage of erosion showed edema of the epidermis with loss of cell detail and, in places, epidermal destruction. Many vasculated cells were present in the basal layers of the epidermis, although their relationship to melanocytes could not be determined.

There was little inflammatory activity in the dermis, and no damage to sebaceous or sweat glands was apparent.

Van Scott and Reinertson (11) had earlier reported that no effects could be observed on lesions of psoriasis or on uninvolved skin when 5-FU was topically applied in a concentration of 10 mg. per 2 ml. of water.

Goldman (5) reported on the response of skin cancer to topical therapy with 5-FU. His report showed that the drug had some cytotoxic effect in some skin malignancies. The topical preparations included five percent 5-FU in Neobase[a], (an oil in water ointment base) five percent 5-FU with five percent salicylic acid in Neobase, and 20 percent 5-FU with five percent salicylic acid in Neobase. Salicylic acid was used to help penetrate keratotic lesions. Intralesional injections of 5-FU solutions were made, with the solution taken directly from the ampule, 1 ml. containing 50 mg. of 5-FU. Control materials for topical application, especially in the wart studies, included the Neobase, and five percent salicylic acid in Neobase. No control materials were used for local injections. Also, to help penetration, adhesive covering was often used after the application of the cream. The creams were applied in a thin layer over the tumor twice daily for periods ranging from three weeks to eight weeks.

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[a]Burroughs Wellcome and Co., Tuckahoe, N. Y.

In a later work (12), Goldman studied the effect of 5-FU upon various warts. In this study, he used 5-FU in a concentration of five percent in Neobase and intralesional injections from ampules. He reported the drug to be effective in periungual and plantar types only if used under occlusive dressings.

The use of 5-FU in actinic keratoses was studied in detail by Dillaha, et al (6). In this work, the authors first attempted to repeat the observations of Falkson and Schulz (9). They administered intravenous 5-FU in the usual dosage schedule to a single patient with extensive actinic keratoses and squamous and basal cell carcinomas of the face, neck, and dorsum of the hands. Within 72 hours, they observed that numerous keratoses were undergoing selective inflammation and areas of squamous carcinoma were beginning to shrink, while several basal cell carcinomas remained unchanged. None of the lesions completely disappeared, and within two months all were as they had been before therapy.

Following these results, these authors topically applied 20 percent 5-FU in Hydrophilic Petrolatum U.S.P. to the skin of patients with extensive actinic keratoses of the face and neck, for a period of four weeks. All areas were treated twice daily without occlusive dressing. Approximately

60 Gm. of 20 percent 5-FU ointment was applied to the entire face and neck during a four week period. An inflammatory reaction was noticed within 48 to 72 hours in the keratotic areas, without detectable alteration in the normal skin. During the following two weeks, the reaction became more brisk, and erosions formed at the sites of the keratoses. In some of the patients, the inflammatory process continued until the medication was stopped, while in others epithelization began to cover the eroded areas during the last two weeks of treatment. Healing proceeded rapidly in all patients at the end of therapy and was usually completed in two to four weeks, resulting in a slightly depressed smooth pink scar. No evidence of hair loss or suppression of hair growth was apparent, even though the epidermal response was marked.

Dillaha and coworkers also treated 19 cases of squamous and basal cell carcinomas in this study. These responded similarly to the 5-FU ointment and rapidly became eroded and ulcerated. Histological studies affirmed that several carcinomas disappeared completely. Deeper and less accessible lesions responded slowly and incompletely with best results following the use of an occlusive dressing.

Reported toxicities in the above study included transient conjunctival irritation in four patients with corneal erosion in one of these. This was eliminated by avoiding

application of the ointment to the eyelids. Also, they found erythema and crusting in skin folds and creases in some patients; erosion of the lower lip was common, resembling the change produced by systemic 5-FU. No hair loss was observed. Three patients noticed a phototoxic erythema at the treatment sites when exposed to the sun. This phototoxicity was diffuse, involving normal skin, and subsided when the sun was avoided or medication discontinued. One patient developed a profound seborrheic dermatitis of the face and scalp during the two week period immediately following therapy. The hair seemed unaltered.

In a later study, Dillaha et al. (13) continued their work with 5-FU by altering the concentration of the drug. Actinic keratoses were treated with ointments containing one percent, 2.5 percent, or five percent concentration of 5-FU in Hydrophilic Ointment U.S.P. The authors reported that observation of patients treated with the five percent ointment indicated that the results were comparable to those obtained with the 20 percent ointment. Although clearing was initially complete, early recurrence was noted in all patients treated with the one percent and 2.5 percent ointments. Based on these observations, they recommended that the five percent ointment, applied twice daily, could be used as an effective outpatient treatment in the person with numerous actinic keratoses of the face and neck.

In addition to the effect on keratoses, the authors observed that the normal skin folds and creases, particularly the nasolabial and retroauricular areas, responded with inflammation and erosion. They recommended that the patient avoid application near the eye-lid margins and mucocutaneous junctions. They felt that the inflammatory response, which makes numerous indistinct areas of keratosis in sun damaged skin apparent, could be used to advantage by the clinician.

Topical application of 5-FU labeled with carbon-14 in five patients, indicated that approximately six percent 5-FU is absorbed systemically. These data along with repeated hematologic studies suggest that 5-FU ointment applied to limited skin areas is not absorbed in a degree that would produce general toxicity.

In a more recent work, Stoll, Klein, and Case (14) also studied the effects of varying the concentration of locally administered 5-FU on basal cell carcinomas. From previous studies, they had noted that the variables that appeared to influence the effects of 5-FU upon the carcinomas included: concentration of the agent, use of occlusive dressings, duration of application, and nature of the vehicle containing the agent.

In that report, the effects of different concentrations of 5-FU concurrently administered by inunction to mul-

tiple, superficial, basal cell carcinomas in the same patient were compared. The concentrations selected for study were 20 percent, five percent, 0.5 percent, and 0.05 percent 5-FU in hydrophilic base (Aquaphor). The 5-FU preparations were applied concurrently to different lesions on the same patient. In one subject, the concentration or 5-FU was subsequently lowered to 0.005 percent because the 0.05 percent concentration had produced a marked reaction.

All preparations were applied once daily for one month. Application of 0.05 percent 5-FU for one week resulted in no gross changes or slight erythema of the tumors. Minimal erythema usually appeared by the end of the second week, with a slight erythema persisting for a few weeks after the applications were discontinued. No additional changes were seen with this low concentration in three subjects. Histological examination following cessation of the application of 0.05 percent 5-FU showed persistent tumor in three instances and no tumor in two others.

The patient who had shown marked gross changes and tumor resolution with the 0.05 percent 5-FU was further studied with a 0.005 percent 5-FU preparation in an attempt to determine the minimum concentration of 5-FU that would not produce gross changes or tumor resolution. Two weeks after applying

0.005 percent 5-FU, the tumor showed minimal erythema which persisted for some weeks after cessation of drug administration. Histological examination showed no persistent tumor following cessation of the application of this low concentration of 5-FU. The site showed no recurrence of tumor during the year of observation. All the higher concentrations of 5-FU studied in this patient had also produced tumor resolution.

The 0.5 percent concentration produced more rapid and pronounced changes than those following application of 0.05 percent 5-FU. Histological examination one month following cessation of 5-FU application showed persistent tumor in two instances and absence of tumor in three instances.

Application of five and 20 percent concentrations produced gross changes that were more rapid in onset and more marked than those following application of the lower concentrations. Erythema, increase in height of the tumor site and/or surface denudation appeared with the first week of application. Ulceration of the tumor was evident during the second week of application. During the third and fourth weeks, there was progressive increase in the degree and extent of erythema and in the area of necrosis. Erythema and ulceration extended beyond the area of clinically evident tumor in all five lesions to which the 20 percent 5-FU preparation had been applied.

With the five percent 5-FU preparation, erythema and ulceration were usually limited to the area of gross tumor involvement. After cessation of application of five percent and 20 percent 5-FU, histological examination revealed persistent tumor in one instance and no tumor in the other nine instances.

In a double-blind study on the effects of local administration of anti-tumor agents in basal cell carcinoma, Klein et al. (15) administered 20 percent 5-FU in Acid Mantle Cream[2], using the Acid Mantle Cream base as a control. The study included six basal cell carcinomas. In most of the tumors, erythema became evident within 24 to 48 hours. Continued daily application of 5-FU under occlusive dressing produced increased erythema. Denudation of the epithelium and ulceration through the epithelium appeared in portions of the tumor and usually terminated by involving the whole surface of the tumor. The base of the ulceration was soft, presumably because of necrosis of the tumor the was microscopically evident at that time. Five of the six tumors studied were no longer evident following administration of the agent. All showed smooth slightly depressed erythematous sites.

The response of Bowenoid conditions of the skin to 5-FU was reported by Jansen, Dillaha, and Honeycutt (16).

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[2]Dome Laboratories, New York, N. Y.

A five percent or 20 percent 5-FU in Hydrophilic Ointment U.S.P. was applied twice daily without occlusion to the involved areas for a four week period. Within a week, a brisk erythema and erosion of the involved skin occurred. This reaction progressed until an ulcerated denuded dissolution of the lesion resulted. In a two to four week period following the application, a flat, smooth, slightly depressed, healed area of skin had replaced the ulceration. Lesions in nine of thirteen patients responded promptly after one month of treatment with clearing. In three instances small recurrent areas required reapplication of the ointment, but once again a good resolution followed.

These early studies served to establish the usefulness of 5-FU in topical therapy. The various concentrations used established that strengths as low as five percent and perhaps lower were clinically effective. The variety of ointment bases used serves to emphasize the need for studies to determine an optimum topical formulation for this therapeutic agent.

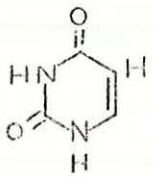
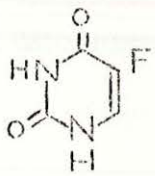
Theoretical Considerations

5-Fluorouracil is a white crystalline, odorless compound which is stable when exposed to air and which melts between 281° and 283° C. One Gm. dissolves in 80 ml. of water,

170 ml. of ethanol, and 55 ml. of methanol. It is practically insoluble in chloroform, ether, and benzene. The solubility in aqueous solutions increases with increasing pH of the solution (17). Structures and physical properties of uracil and 5-FU are shown in Table I (18).

T A B L E I

Structures and Physical Properties of Uracil and 5-Fluorouracil

	Uracil	5-Fluorouracil
Structure		
λ_{H^+}	259 mμ	265 mμ
pK_a	9.45	8.15

5-Fluorouracil is a congener of uracil which acts both as a surrogate and an antimetabolite of that nucleotide. It has been shown that 5-FU is converted in a living cell into the ribonucleoside mono-, di-, and triphosphates and into RNA,

and is also converted into 5-fluoro-2'-deoxy-uridine-5'mono-phosphate. The latter compound prevents DNA synthesis by blocking the conversion of uridine monophosphate to thymidine monophosphate (19). Thus net result is thought to be a fluorinated RNA molecule and a thymidine deficient DNA molecule.

Histopathological studies (6) verify the clinical observations of the reaction and selective effect. In tissue taken after treatment with 5-FU, there is enlargement of the epithelial cells, bizarre nuclear distortion, considerable acantholysis, edema, intensification of the mononuclear inflammatory reaction and eventual sloughing away of the reaction area.

Percutaneous Absorption

The consideration of the mechanisms involved in the passage of medicinal substances through the skin is of primary concern in the study of ointment bases and their subsequent release of medication. Intelligent formulation of dermatological preparations depends on a thorough understanding of percutaneous absorption; the penetration of substances from the outside into the skin and through the skin into the blood stream (25).

The direct topical application of medication is often the preferred route of administration, as in the case of antihistamine, corticosteroids, antibiotics, and antifungal prep-

arations. It can be assumed that by this route of administration of a drug in the local tissue can be reached with minimum amounts and the possibility of untoward toxic reactions may be minimized (20). Often, depending upon the particular skin condition under consideration and the drug of choice, topical application is the only route possible or reasonable. For these and many other reasons, the study of the release of medication from ointment bases and its subsequent penetration into the skin is of practical and scientific interest. It is, therefore, important that the efficiency of the vehicle in allowing or promoting the diffusion, penetration, and absorption of agents incorporated in it be determined (21). Although dermatological vehicles, themselves, may not penetrate the skin to any extent nor actually carry the medication through the epidermal barrier, it is known that the clinical effectiveness of a drug may vary when it is incorporated in different vehicles (22). The choice of an optimum vehicle for a particular medicament depends on the physical and chemical properties of the drug, alone and in the vehicle, as well as the nature of the skin condition being treated (23).

Ointments are defined as semi-solid preparations for external application and of such a consistency that they may be applied to the skin by inunction. The ointment base has the function of carrying a drug to the skin and maintaining it

there. As pointed out above, the nature of the base will also influence the penetration of the drug into the skin.

The medicinal agent must be brought into intimate contact with the skin so that the incorporated medicament is available locally at the site of application where its action is desired. In a broad sense, an ointment base may be considered the 'solvent' into which an active ingredient is incorporated for ease of application. In the past, the base has been regarded as a completely passive carrier, the choice of which was left completely to personal preference. However, the ointment base must permit the active constituent to exert its full therapeutic effect. The medicament of proven therapeutic usage cannot be incorporated into any convenient base, because the medication may exhibit little or no activity due to a restricting influence of that base (24).

Theories on the basic mechanism which is largely responsible for percutaneous absorption through intact skin are still subject to a great deal of controversy. There is general agreement that there are potentially three distinct routes of penetration through the skin: the follicular openings, the sweat ducts, and the unbroken stratum corneum between these appendages. There is some division of opinion as to which of the above is the principle route of penetration. One group favors the theory of percutaneous absorption primarily by dif-

fusion via sweat ducts and hair follicles, the other holding that diffusion through the unbroken stratum corneum is the principle pathway (26). It is likely that all these avenues take part in the transfer of substances through the skin. Under the appropriate conditions, each one of the three contending routes of penetration may be, in turn, overwhelmingly dominant.

As the epidermis presents a surface area 1000 times greater than the sebaceous glands or sweat glands, it must, of necessity, be given consideration. The film on the surface of the skin is composed of sebum, sweat, and desquamating horny layer, and has a complex chemical composition. The character and extent of its interaction with the penetrating agent are unpredictable (27).

The horny layer, which is 20 to 40 microns in thickness, is composed largely of keratin, a protein containing sulfhydryl groups, which absorbs a large amount of water and other polar compounds. The horny layer also contains surface lipid which may spread along the channels between cells and absorb lipid-soluble material. It may thus act as a sponge, becoming a reservoir for the penetrating agent and maintaining a maximum concentration gradient just above the barrier layer, thus possibly hindering further penetration.

The 'barrier layer' is proposed to be located between the horny layer and granular layer. It is stated to be an

electronegatively charged barrier which repels anions and attracts and holds cations from further penetration (23). The barrier layer is also said to check the transfer of water across the skin. This layer is about 10 microns thick and is thought to prevent the penetration of molecules having molecular weights greater than 200 or 300 (28); although the diameter of the pores in the intercellular spaces in the barrier layer is much larger than the largest penetrating molecule. Thus the restraining force may be the molecular interaction between the penetrant and pore contents, in addition to electrostatic forces. If the penetrating agent has a high electrostatic charge, e.g., ions, the electrostatic force is so great that no penetration occurs. If the agent has a water-lipid partition coefficient of approximately 1, it has the highest penetration. The barrier must then have both polar and nonpolar groups in the pore contents (27). It thus appears that the ratio of the solubilities in water and in lipids is an important factor in the absorption of substances through the skin.

Absorption through appendages, e.g., the hair follicles, is believed to be a major avenue for percutaneous absorption. The pilosebaceous apparatus holds a prominent role in percutaneous absorption. This concept takes into consideration the solubility of the drug in sebum.

In the upper portion of the follicular canal, the hair shaft does not adhere to the follicular wall, and, there-

fore, a space is formed which is filled with horny scale and air. This interspace is continuous with the duct of the sebaceous gland, and the sebum from this duct eventually empties into the interspace. Therefore, any medication possessing a solubility in sebum may penetrate this space and reach inside of the sebaceous gland whose membrane is more permeable than the epidermal barrier. From the sebaceous gland and hair follicles, medicaments may penetrate downward into the corium and from there into the blood, thus by-passing the barrier layer.

Any abnormal condition of the skin may alter normal absorption patterns. If the barrier is destroyed by trauma, as in cuts, chapping, ruptured blisters, or eczema, all substances may pass freely into the dermis (27). Higuchi (29) has studied the importance of the thermodynamic properties of a medicament as it relates to its percutaneous absorption. These properties include the thermodynamic activity of the drug in the vehicle and in the skin barrier phase, and the diffusion coefficient of the drug in the vehicle and skin barrier phase. In his work, Higuchi set up an equation for an idealized system which showed the main characteristics of the penetrating agent which determine its rate of entry through the skin to be its effective distribution coefficient and diffusion in the barrier phase of the skin. The product of these two is referred to as the 'permeability constant.' Higuchi's equation follows:

$$\frac{dq}{dt} = (P.C) \frac{(\text{concentration of drug})\ DA}{L}$$

where(P.C.) is the effective distribution coefficient of the penetrating medicament between the vehicle and the barrier of the skin; (concentration of drug), the concentration of the medicament in the vehicle; D, the effective average diffusion of the medicament in the barrier phase; A, the effective cross section area; and L, the effective thickness of the barrier phase.

The important variable in the permeability constant of Higuchi is the effective distribution coefficient factor since the diffusion of a substance of similar molecular weight and shape usually differs only slightly. The distribution coefficient, on the other hand, seems to be an extremely sensitive function of molecular structure and size (29).

Although the skin is relatively impermeable to water, its presence in the vehicle and in the skin has an influence on the penetration of substances and on percutaneous absorption. The hydration of the stratum corneum appears to increase the rate of passage of all substances which penetrate the skin. There is not only a physical alteration of the tissue due to hydration but also at high water activities there will be changes in both the diffusion coefficient and activity coefficients of the penetrating agent (29). Wurster and Kramer (30), investigating the absorption of three salicylate esters, demonstrated that a large increase in their absorption rates

was produced by increased moisture conditioning. They assumed that percutaneous absorption involves a diffusional process, i.e., the spontaneous movement of a substance from an area of high concentration to an area of low concentration in the tissue fluids. They concluded that the magnitude of the effect of moisture on percutaneous diffusional rates appears to be proportional to the oil/water distribution coefficient and the water solubility of the test substance. The greatest increase of the penetration rate by moisture was found in the penetrant with the smallest oil/water distribution coefficient.

In view of the above observations, the effect of vehicles on percutaneous absorption must be considered. Not only may the vehicle alter the degree of hydration of the skin, but it may also influence absorption and penetration in other ways. Greases and oils are the most occlusive vehicles and induce the greatest hydration through sweat accumulation at the skin-vehicle interface. Emulsions of the water-in-oil type are somewhat less occlusive than greases. Oil-in-water emulsions tend to invert as the outer aqueous phase evaporates, and the final state may be considered to be a continuous oil film containing other dissolved or suspended substances. Water soluble vehicles produce the least change in hydration of the stratum corneum. All powders probably tend to interfere with

the continuity of oil films and, in this manner, probably decrease the occlusive effect of any vehicle in which they are used. It should be noted that if an ointment is covered with a bandage, there will be a tendency to hold perspiration and increased hydration may occur (31). Further, the thickness of the applied film of topical preparations will also directly affect hydration of the stratum corneum.

Absorption of medicaments is frequently found to be better from animal and vegetable oils than from mineral oils because the former readily penetrate the skin (23). Higuchi (29) stated that the application of many solvents, other than water, appears to cause marked alteration in the resistance of the skin barrier toward penetration and that this phenomenon is possibly caused by marked changes produced by such solvents in the activity coefficient and diffusion constant of the penetrating agent in the skin barrier.

Another factor that may influence percutaneous absorption is the presence of a surfactant in the base. These components offer possibilities of improving topical vehicles and promoting a more thorough diffusion of the medicament from the vehicle, thus influencing therapeutic performance (32). As the concentration of the surfactant is increased up to a certain percentage, the absorption and penetration of the medicament is generally increased.

A complete delineation of the various factors which may affect the absorption characteristics of a topical preparation might also include considerations of the concentration of medicament, pH of vehicles, areas of application, and the effective thickness of the skin barrier phase (29).

C H A P T E R II

EXPERIMENTAL

General Objectives

The general objectives for the experimental work were to study the release of 5-FU from the various ointment bases and to attempt to determine the type of ointment base which might release the drug most satisfactorily.

The methods chosen were designed to determine the release and penetration of 5-FU both by *in vitro* and *in vivo* techniques. A modified agar plate method was selected to measure the *in vitro* release, whereas the *in vivo* penetration of the drug was determined by applying the medicated ointment bases to the skin of guinea pigs and subsequently analyzing the biopsy of the inuncted area by spectrophotometry.

Formulation of Ointment Bases

Ointment bases for the study were selected from those used in the studies of topical 5-FU previously discussed, and from other formulations which might result in better release of the drug. In the case of Hydrophilic Ointment, U.S.P., the formulation was modified by using sodium lauryl sulfate and

emulsifiers from the Miranol[a] and Brij[b] group of surfactants in concentrations of one to three percent as given in Table II. Only those formulations showing acceptable physical properties were used in the study. Included in Table II are the commercial bases which were tested in this work.

An experimental base was prepared with the following formulation.

Base No. 11 Experimental Base

Polyethylene Glycol-400 Monostearate . .	15 Gm.
Propylene Glycol Monostearate	35 Gm.
Glycerin	5 Gm.
Water	45 Gm.

The 5-Fluorouracil,[c] as received, was in crystalline form and was reduced to a fine powder before incorporation into the ointment bases. In the agar plate studies it was found necessary to use a maximum concentration of 0.01 percent 5-FU to obtain zones of measurable diameters. A standard solution containing 2 mg. of 5-FU per milliliter of water was prepared and 1 ml. of this solution was incorporated into 20 Gm. of each ointment base studied.

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[a]The Miranol Chemical Company, Inc., Irvington, N. J.

[b]The Atlas Powder Company, Wilmington, Delaware

[c]Supplied by W. E. Scott, Hoffman La Roche Laboratories, Nutley, New Jersey.

TABLE II

General Evaluation of Test Formulations

Base No.	Formulation	Emulsifier	Conc. %	Evaluation
1a	Hydrophilic Oint.	Sod. Lauryl Sulf.	1	Excellent
1b	"	"	2	"
1c	"	"	3	"
2a	"	Miranol DM	1	Good
2b	"	"	2	Excellent
2c	"	"	3	"
3a	"	Miranol SM Conc.	1	Semi-liquid
3b	"	"	2	Granular
3c	"	"	3	Good
4a	"	Miranol HM Conc.	1	Excellent
4b	"	"	2	"
4c	"	"	3	"
5a	"	Miranol OM-SF Conc.	1	Stiff
5b	"	"	2	Very Good
5c	"	"	3	Very Good
6a	"	Miranol C2M Conc.	1	Good
6b	"	"	2	Good
6c	"	"	3	Good
7a	"	Brij 30	1	Granular
7b	"	"	2	Good
7c	"	"	3	Excellent
8a	"	Brij 35	1	Excellent
8b	"	"	2	Excellent
8c	"	"	3	Excellent
9a	"	Brij 97	1	Granular
9b	"	"	2	Good
9c	"	"	3	Excellent
10a	"	Brij 99	1	Stiff
10b	"	"	2	Fair
10c	"	"	3	Excellent
11	Experimental Base	-	-	Excellent
12	Aquaphor	-	-	"
13	Hydrophilic Petrolatum	-	-	"
14	White Petrolatum	-	-	"
15	Cetaphil	-	-	"
16	Unibase	-	-	"
17	HEB Base	-	-	"
18	Neobase	-	-	"

In the *in vivo* studies, the finely reduced powder was incorporated directly into the ointment bases using standard pharmaceutical methods. A concentration of five percent 5-FU was used and the ointments were each passed through an ointment mill twice. The finished ointments were stored in ordinary opal ointment jars at room temperature throughout the investigative period.

In Vitro Evaluation of Drug Release

A widely accepted method of ointment base evaluation has been the agar plate or agar cup-plate procedures or their modifications. These procedures were first described by Ruehle and Brewer (34) of the U. S. Department of Agriculture and were used in this study with a few modifications.

Based on the suggested assay of 5-FU in body fluids, as described in the Roche Product Reference (35), *Sarcina lutea*, A.T.C.C. # 9341, was selected as the test organism. The culture was maintained on nutrient agar, being transferred daily into nutrient broth for at least five consecutive transfers before the tests were performed.

Porcelain penicylinders were placed in the center of sterile 100 x 20 mm. Petri dishes with glass covers. Twenty ml. of sterile nutrient agar, which had been seeded with 0.1 ml. of a 24-hour culture of the test organism, was poured into

each dish around the penicylinder. When the agar had cooled, the penicylinder was removed and the resulting cup filled with exactly 0.2 ml. of ointment, dispensed by disposable plastic syringes of 2.5 ml. capacity. Only one ointment could be placed in each Petri dish because of the large diameter of the resulting zone of inhibition. A set of observations consisted of four plates containing the 5-FU ointment and one control plate with the corresponding ointment base. All plates were incubated at 37° for 24 hours and the extent of release of the drug was determined by measuring the diameter of the zone of inhibition from the edge of the ointment to the edge of the zone. The averages of the zones of inhibition from the four plates, for each 5-FU ointment, are tabulated in Table III.

In Vivo Evaluation of Drug Penetration

White guinea pigs, weighing approximately 200 to 600 Gm. were selected, without regard to sex, for use in this study. The backs of the animals were clipped, under light ether anesthesia, with a Toastmaster hair clipper and shaved with a Sunbeam electric shaver. On the day following the clipping, again under light ether anesthesia, an area 4 by 4 cm. was marked off on the shaven back of each of four animals. Exactly 2 Gm. of the ointment to be tested was spread over the

T A B L E III

Inhibition of *Sarcina lutea* by 5-FU from Test Ointments

Ointment Base No.	Base Type	Ave. Zone of Inhibition mm.	Control
1a	Hydrophilic Oint.	24	0
1b	"	24	0
1c	"	24	0
2a	"	24	0
2b	"	25	0
2c	"	25	0
3b	"	22	0
4c	"	22	0
5c	"	24	0
6a	"	25	0
6b	"	25	0
6c	"	25	0
7b	"	25	0
7c	"	25	0
8b	"	23	2
8c	"	23	2
9c	"	21	0
10c	"	22	0
11	Experimental Base	21	3
12	Aquaphor	0	0
13	Hydrophilic Petrolatum	8	0
14	White Petrolatum	9	0
15	Cetaphil	19	0
16	Unibase	20	0
17	H E B Base	22	0
18	Neobase	22	0

area, with minimum friction and pressure, using a warm spatula. Without covering the inuncted site, the animals were returned to individual cages for four hours, the time interval in which maximum absorption was reported by Stolar, et al. (36). At the end of that period, each animal was administered deep ether anesthesia, and the excess ointment removed thoroughly with a spatula and clean, dry gauze sponges. The animal was then sacrificed with a bludgeon, the entire section of medicated skin removed, stretched to original size and shape, placed in aluminum foil and quickly frozen at -20°.

Standard skin plus 5-FU curves were prepared by placing known weights of skin sections in 50 ml. glass stoppered erlenmeyer flasks and adding known volumes of a standard 5-FU solution (100 mg. of 5-FU dissolved in 100 ml. of water) and sufficient water to bring that volume to 2 ml. After adding 0.5 ml. of 6 N HCL, each flask was placed on a steam bath for 30 minutes. At the end of the digestion period, the flask was shaken vigorously for 60 seconds to break up the tissue and cooled for five minutes. Exactly 2 ml. of a saturated solution of ammonium sulfate were added before a final shaking of 30 seconds. Finally, the mixture was brought to a volume of 29.5 ml. by the addition of 25 ml. of water. The entire mixture was transferred to a thick-walled centrifuge tube and centrifuged at 1500 r.p.m. for 5 minutes. Following filtration

of the solution through Whatman No. 44 filter paper, the absorbance of the solution was determined on a Bausch and Lomb Spectronic 600 spectrophotometer at 265 millimicrons. All readings taken in this study were against a blank of 2 ml. of water and 0.5 ml. of 6 N HCL, processed as described above. The standard curve was constructed on linear graph paper with absorbance plotted against milligrams of 5-FU.

A standard absorbance curve for 5-FU alone was also prepared, by combining known volumes of a standard 5-Fu solution with sufficient water to bring that volume to 2 ml. adding 0.5 ml. 6 N HCL and proceeding as under the standard skin plus 5-FU curve. The standard curve was plotted with absorbance against milligrams of 5-FU.

A standard skin curve was obtained by placing known weights of skin in 50 ml. glass stoppered erlenmeyer flasks, adding a volume of 2 ml. of water and 0.5 ml. of 6 N HCL and proceeding as under the standard skin plus 5-FU curve. The standard curve was constructed with absorbance plotted against milligrams of skin.

Preliminary spectrophotometric investigations showed that untreated guinea pig skin, taken through the analytical procedure, gave a maximum absorbance in the range of approximately 269 millimicrons, which interferred with the analysis of 5-FU at 265 millimicrons. However, when the absorbances of

samples, read at 265 millimicrons, were plotted against the weights of skin, a straight line plot was obtained, which originated at zero. This plot is given in Graph 1. Using these values, the absorbance per 100 mg. of skin weight was calculated to be 0.75 (Table IV), indicating that for a given weight of skin a constant absorbance could be expected. Using Graph 1, the absorbance corresponding to any given skin weight could be determined. In order to further test this observation, the absorbance from samples of approximately 100 mg. each of skin, plus known varied amounts of 5-FU, was plotted against milligrams of 5-FU. Graph 2A shows that this curve was a straight line originating at 0.75. From these two observations, it was concluded that if the skin absorbance values from Graph 1 were subtracted from the absorbance values in Graph 2A, the remainder should yield absorbances for the corresponding amounts of 5-FU alone. These calculations are given in Table IV. Graph 2B shows the coincidence of values so calculated, and those recorded for the 5-FU standard curve, supporting the above conclusion.

Using this approach, it was possible to demonstrate that the absorbance of a known weight of skin in the biopsy samples studied could be deducted from the recorded total absorbance of the sample, thus giving the absorbance value representing the 5-FU. It must be pointed out that no attempt

was made in this investigation to determine absolute drug content in the tissues, but rather, the relative concentrations attained from the bases employed were determined, and the apparent differences, if any, were attributed to the vehicle. In the actual analysis procedure standard skin curves were prepared from skin taken from the control guinea pigs to which the ointment base under consideration had been applied. This was necessary to account for any slight absorbance of light by the ointment base itself. These curves are given in Graphs 3, 4, 5, and 6.

Quantities of 5-FU in the skin of the test animals were determined by cutting four samples, 2 cm. by 2 cm., from the inuncted area of the frozen skin, weighing each sample rapidly, and digesting it in 0.5 ml. of 6 N HCL and 2 ml. of water on a steam bath for 30 minutes, in a glass stoppered erlenmeyer flask. The digested tissue was shaken vigorously for 60 seconds to break up the tissue and the assay continued as under preparation of the standard skin plus 5-FU curve. The quantities of 5-FU found were calculated to milligrams per gram of skin sample. Results of these assays are given in Tables V, VI, VII, and VIII.

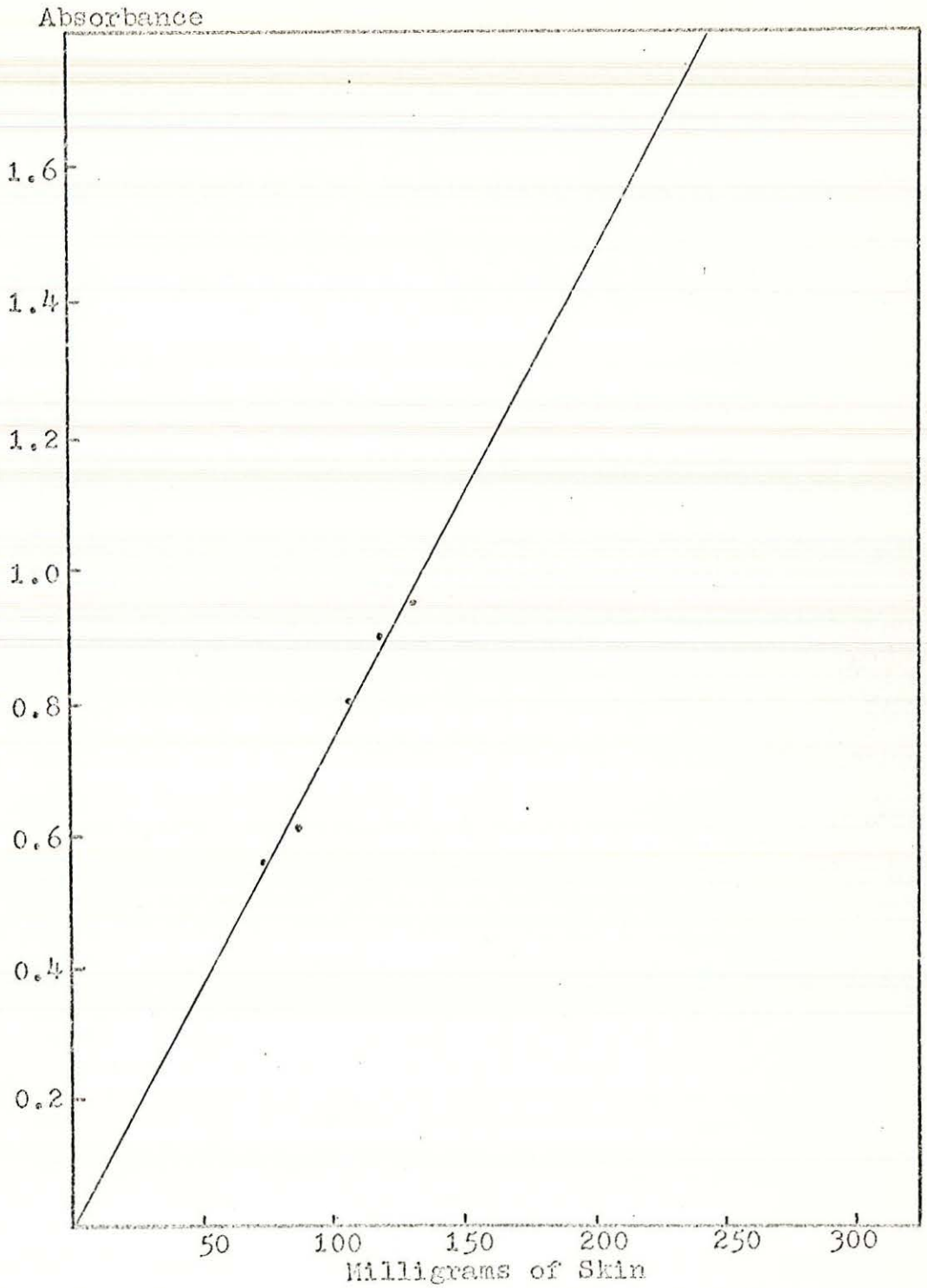
T A B L E IV

Absorbance Values for 5-FU and Guinea Pig Skin Samples

Weight of Skin (mg.)	Absorbance	Calculated Absorbance of 100 mg. of Skin
74.6	0.56	0.75
88.4	0.62	0.71
95.7	0.81	0.85
110.0	0.80	0.73
120.2	0.90	0.75
132.6	0.95	0.72
	Average	0.75

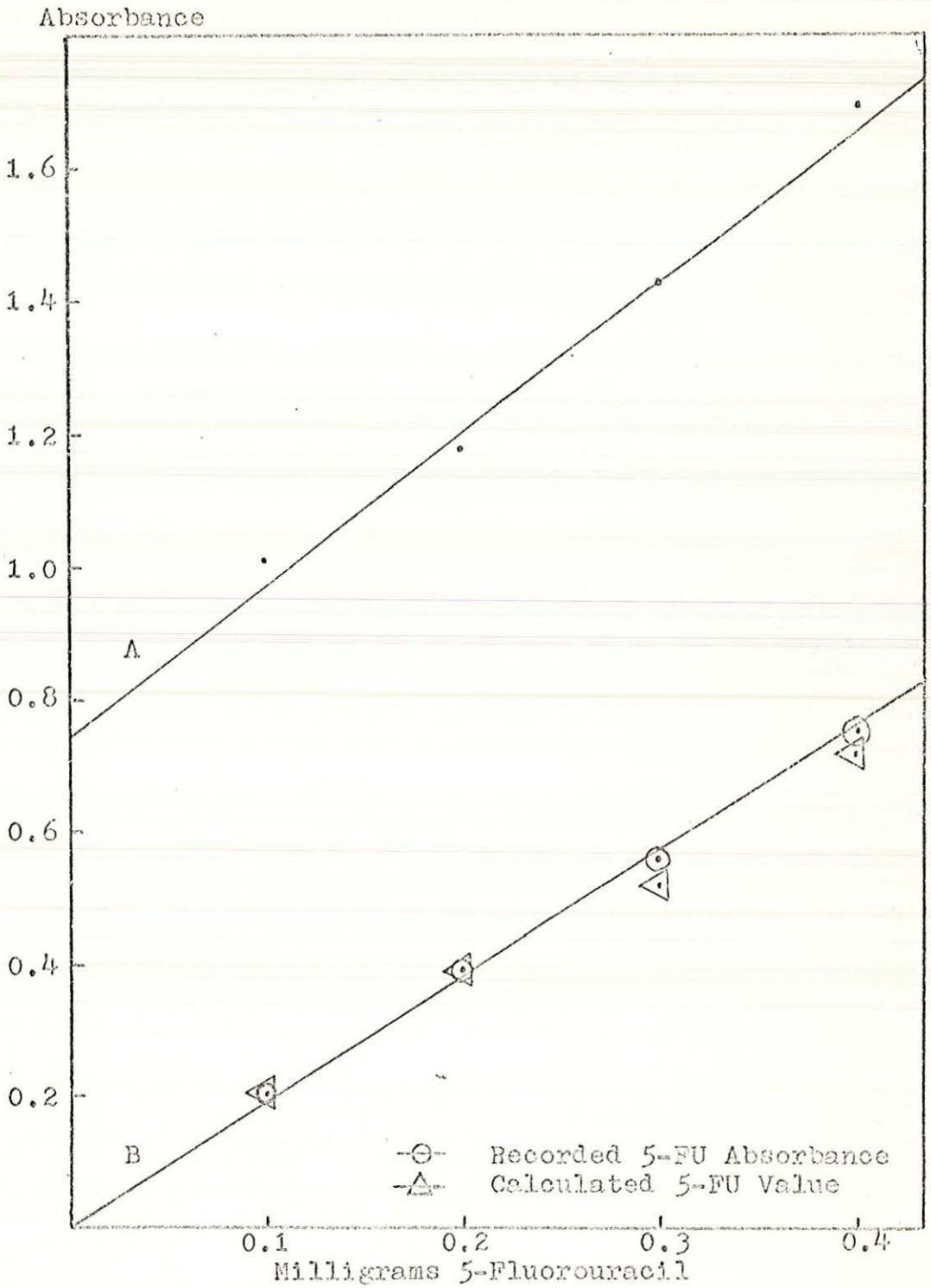
Comparison of Calculated Values of 5-FU with Recorded Values

Weight of Skin (mg.)	Amount of 5-FU (mg.)	Recorded Absorbance	Calculated Absorbance 5-FU	Recorded Absorbance 5-FU
106.2 +	0.1	1.02	0.20	0.20
104.0 +	0.2	1.18	0.38	0.38
113.8 +	0.3	1.43	0.51	0.55
118.2 +	0.4	1.70	0.69	0.74



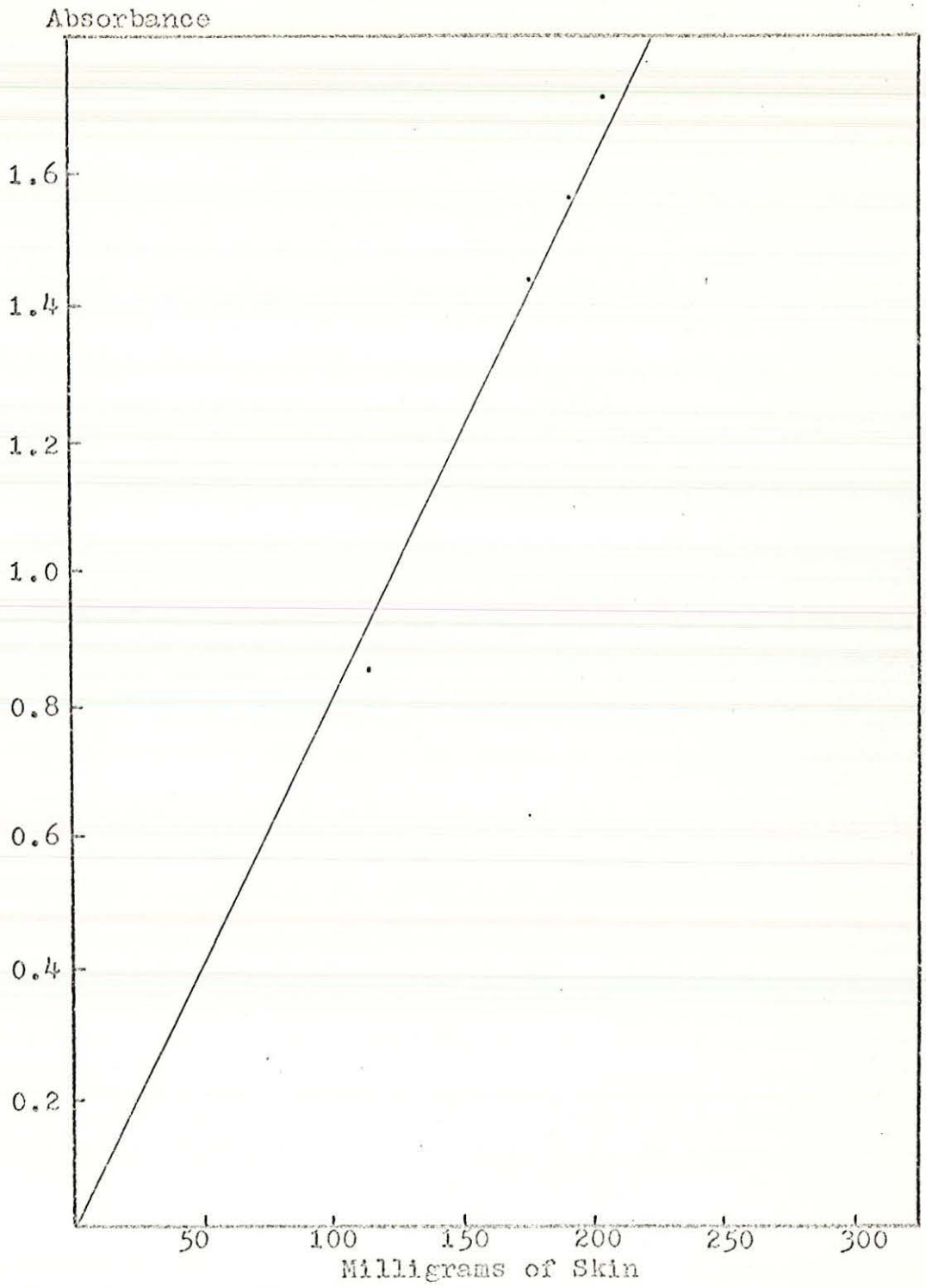
Graph 1

Standard Curve for Guinea Pig Skin Samples



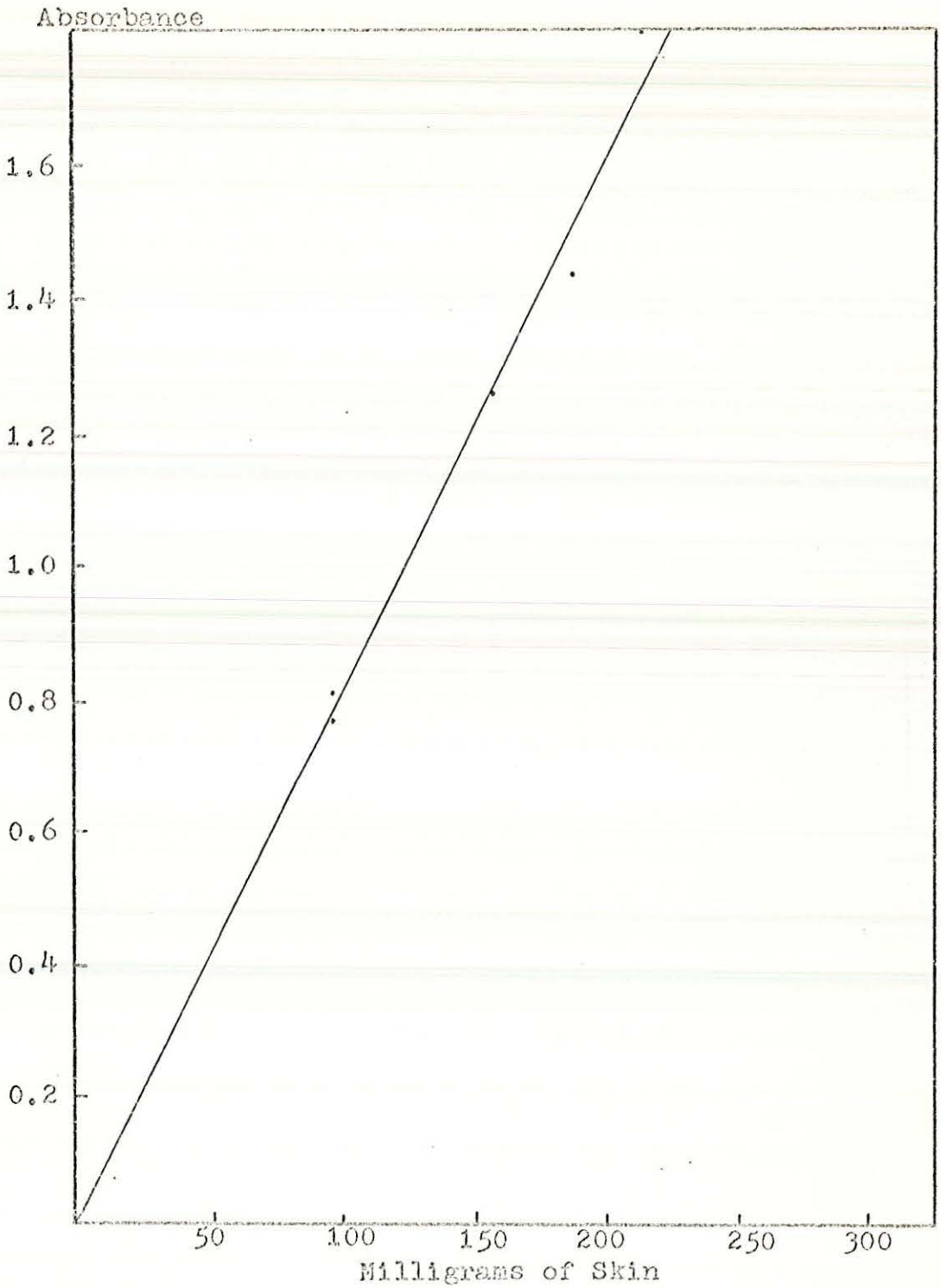
Graph 2

A. Skin Plus 5-Fluorouracil Cruve
B. 5-Fluorouracil Curve



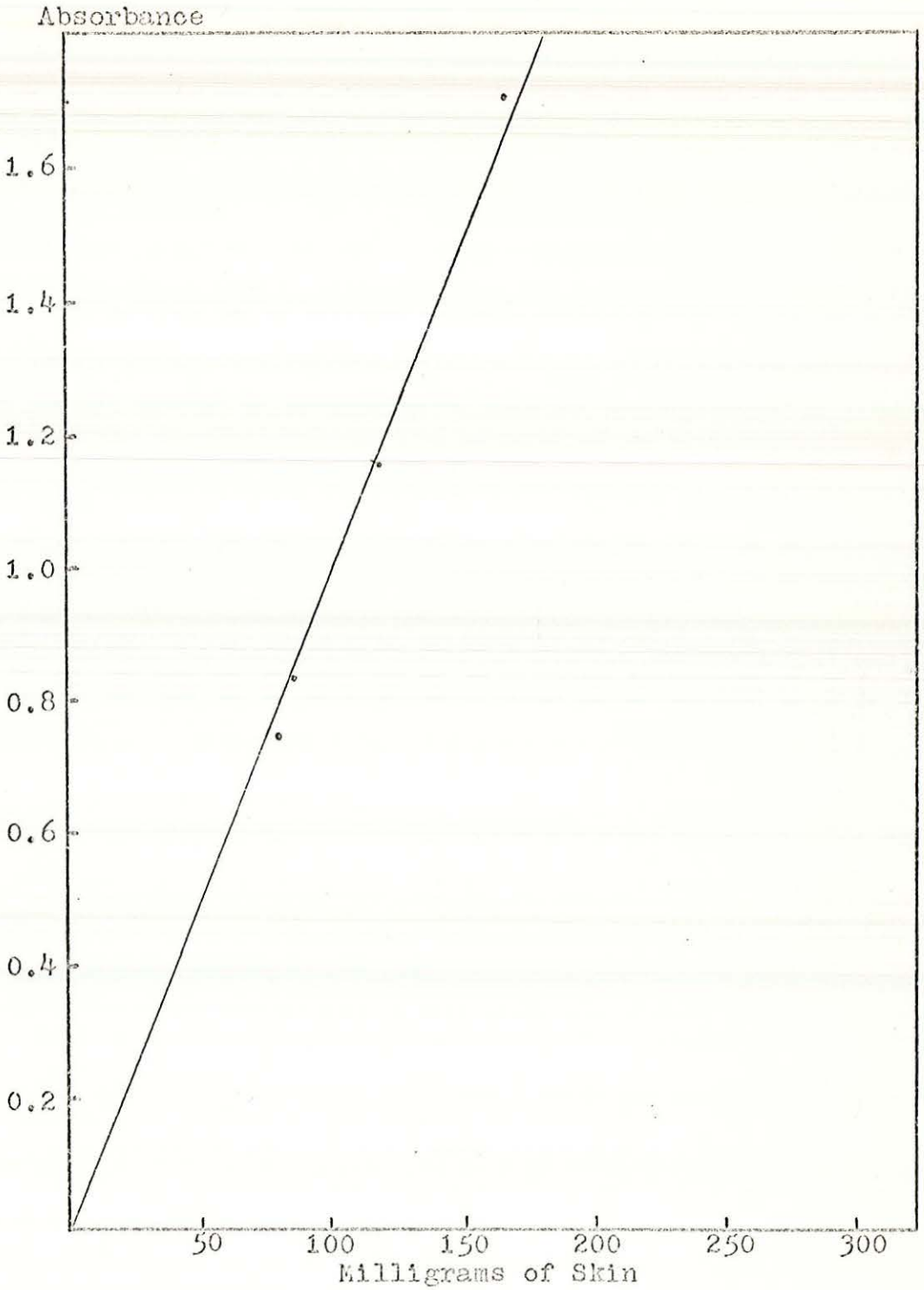
Graph 3

Standard Curve of Skin with Hydrophilic Ointment



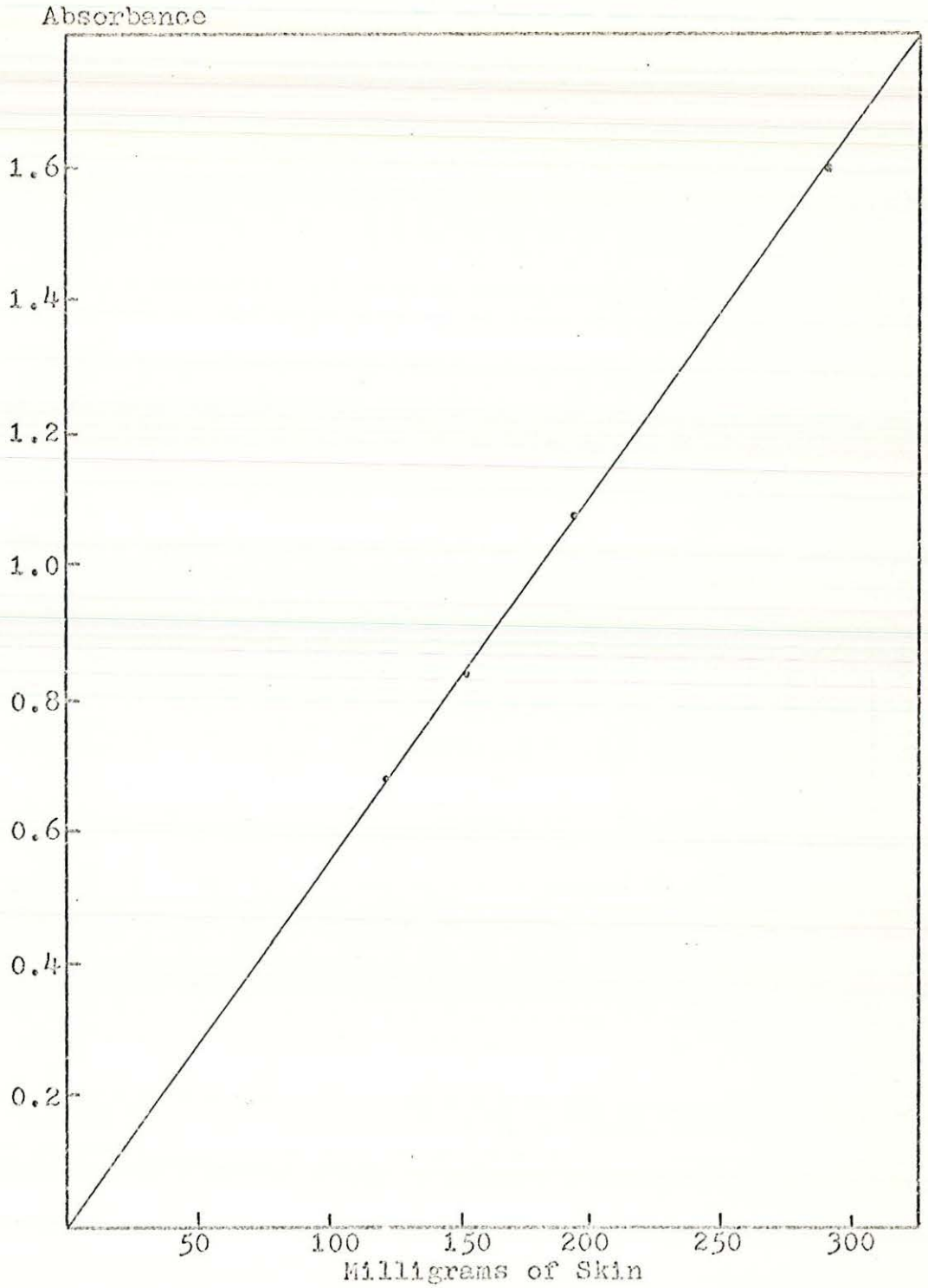
Graph 4

Standard Curve of Skin with Aquaphor



Graph 5

Standard Curve of Skin with Hydrophilic Petrolatum



Graph 6

Standard Curve of Skin with Experimental Base

T A B L E V

Penetration of 5-FU from
Hydrophilic Ointment U.S.P. XVII

Animal	Weight (Gm.) of Skin Sample	mg. of 5-FU Per Gm. Skin
1	0.0993	1.66
	0.1048	0.72
	0.1287	3.11
	0.1041	0.72
2	0.1541	0.72
	0.1145	1.44
	0.1604	0.81
	0.0987	0.81
3	0.1168	1.80
	0.0979	1.43
	0.1120	1.12
	0.1017	2.66
4	0.0996	0.45
	0.0984	0.66
	0.1105	0.45
	0.1009	0.44
	Average	1.19

T A B L E VI

Penetration of 5-FU from Aquaphor

Animal	Weight (Gm.) of Skin Sample	mg. of 5-FU Per Gm. Skin
5	0.0855	
	0.1095	none detected
	0.0847	
	0.1121	
6	0.1139	
	0.1145	none detected
	0.1259	
	0.1029	
7	0.0946	
	0.1112	none detected
	0.1042	
	0.0898	
8	0.1161	0
	0.1062	0.29
	0.1032	0
	0.0962	0.31
	Average	0.04

T A B L E VII

Penetration of 5-FU from
Hydrophilic Petrolatum U.S.P. XVII

Animal	Weight (Gm.) of Skin Sample	mg. of 5-FU Per Gm. Skin
9	0.1498	
	0.1641	none detected
	0.1579	
	0.1562	
10	0.0759	0
	0.0732	0
	0.851	0.17
	0.0704	0.14
11	0.1056	
	0.1163	none detected
	0.1058	
	0.1215	
12	0.0952	
	0.0859	none detected
	0.1248	
	0.1205	
	Average	0.02

T A B L E VIII

Penetration of 5-FU from
Experimental Base 11

Animal	Weight (Gm.) of Skin Sample	mg. of 5-FU Per Gm. Skin
13	0.2113	
	0.2385	none detected
	0.2070	
	0.1812	
14	0.2268	0.42
	0.2302	0
	0.2663	0
	0.2520	0
15	0.2452	0
	0.3090	0
	0.2222	0
	0.2470	0.53
16	0.1941	0.77
	0.2259	0
	0.1881	0.02
	0.1954	0.41
	Average	0.13

C H A P T E R III

DISCUSSION

Ointments are semi-solid preparations used primarily as vehicles or bases for the topical application of active medicinal substances. They should be of such consistency that they may be applied to the skin by inunction. Ideally, an ointment base should be stable, smooth and pliable, non-irritating, non-sensitizing and able to readily release its incorporated medication. Further, it should be able to absorb water and other liquid preparations.

As no single ointment base possesses all of the above characteristics it is necessary to have a knowledge of the individual characteristics of various bases in order that an acceptable formulation can be suggested.

Oleaginous, absorption and emulsion ointment bases were included in this study. Each of these types of bases has certain advantages and disadvantages under a given situation. The oleaginous base, such as white petrolatum has a low water absorbing capacity, and is not washable, but normally is very compatible with the skin. The absorption ointment bases generally have a high index of compatibility toward the majority of medicaments used topically. These bases are anhydrous, but will absorb large amounts of water and, like the oleaginous bases, still possess the property of greasiness, but are more readily removable from the skin. Emulsion ointment bases or

washable ointment bases as they are often called, are actually solid emulsions. Bases of this type will permit the incorporation of small amounts of water without reducing the consistency of the base below that of a soft cream. These bases are generally smooth, white semi-solid preparations with good cosmetic acceptance. The surfactants which these bases contain are known to enhance release and penetration of many medicaments, while they may inhibit the release of others. The water in-oil emulsion base can be removed from the skin and clothing with water. The present trend seems to be toward the use of emulsion ointments or creams, whenever compatability with the active ingredient allows the use of such a vehicle.

The experimental base, consisting of polyethylene glycol 400 monostearate, propylene glycol monostearate, glycerin and water may be classed a hydrous glycol ointment base. This hydrous base is a glossy, white semi-solid, which has good cosmetic characteristics. The formulation originally did not include glycerin. However, preliminary work indicated that the addition of glycerin made the base smoother and more pliable. It will incorporate liquids up to ten percent of the total weight of the product without reducing the consistency below that of a soft cream.

Release and penetration probably occurs from all ointment bases, although in some instances the release and penetration may be so slight as to be insignificant. This appears to

be the case with 5-FU in oleaginous and absorption type bases. In general, the degree of release and penetration depends on many factors: (1) the physico-chemical relationship between the medicament and vehicle and/or components, (2) the physico-chemical relationship between the medicament and tissue, (3) the condition of the skin, i.e. whether it is intact or abraded, (4) the degree of hydration of the skin, (5) the degree of inunction, and (6) the concentration of the medicament. The problem lies in knowing what degree of release and penetration will be achieved from a particular combination.

From the above discussion and the observations reviewed on percutaneous absorption in the introduction, it appears that, at present, the most acceptable approach to ointment base evaluation is to estimate the release of each active ingredient on an individual basis, because of the myriad of factors which ultimately affect the overall performance of various formulations. The researcher can then make appropriate decisions, based on the agent's individual chemical and physical properties, its intended use, and the condition of the patient's skin.

The selection of a topical vehicle for 5-FU, thus far, has been influenced largely by the form available to the investigator. When the agent was available to him only in the commercially available ampule, the investigator was forced to select a base which would incorporate the volume of liquid.

When the crystalline form was available, the ointment base usually selected has been an emulsion type with more acceptable cosmetic characteristics. Thus, the several different types of ointment bases used in the studies of topical 5-FU has made comparative evaluation of the results difficult. In several studies, high concentrations appeared to require long periods of application for clinically acceptable results. In those cases, it is possible that the ointment base selected may have been responsible for inhibiting the release of 5-FU.

In the present work the release of 5-FU from a variety of experimental and commercially available ointment bases was studied by *in vitro* and *in vivo* methods. In the *in vitro* studies, various emulsifiers were employed in the hydrophilic ointment formulation in an attempt to determine if the type of emulsifier or its concentration might influence the release of the agent from the base. The emulsifiers selected represented anionic (sodium lauryl sulfate), nonionic (Brij group) and amphoteric (Miranol group) types of emulsifiers. Results obtained in the agar cup-plate studies appeared to indicate that the release of 5-FU was not significantly changed by the type of emulsifier used. This does not imply that the presence of an emulsifier in the formulation did not enhance the release of 5-FU from the base. As can be seen in Table III, the release of the agent from emulsion type bases appears to be superior to that from oleaginous bases.

The experimental base provided release of the drug comparable to that of Hydrophilic Ointment U.S.P. In the case of the hydrophilic bases, i.e. Hydrophilic Petrolatum U.S.P. and Aquaphor, repeated testing of the 5-FU ointments confirmed the relative inefficiency of those bases in releasing the active ingredient. This apparent inhibition of release may aid in understanding why rather high concentrations were used in the early studies of topical 5-FU.

The differences in levels of 5-FU in guinea pig skin samples from the ointments used appears to correlate with the agar cup-plate studies. Higher levels were obtained from the Hydrophilic Ointment U.S.P., than from the experimental base or the oleaginous bases. As the literature indicated a five percent concentration to be clinically effective, this concentration was selected for the in vivo work. When these ointments, and the corresponding control bases, were applied to guinea pig skin, no statistically significant skin levels of 5-FU could be determined for; Aquaphor ($P < 0.9 > 0.7$), Hydrophilic Petrolatum U.S.P. ($P < 0.9 > 0.7$), or the experimental base ($P < 0.2 > 0.1$). The Hydrophilic Ointment U.S.P. demonstrated good release and penetration at this concentration, with statistical evaluation showing significant 5-FU levels ($P < 0.001$) when compared with the control ointment.

C H A P T E R IV

SUMMARY AND CONCLUSION

The release of 5-Fluorouracil from a selected variety of experimental and commercially available ointment bases was studied by *in vitro* and *in vivo* mehtods. The study was undertaken in an attempt to determine the type of ointment base which would release this agent most satisfactorily.

Investigators studying topical 5-FU have thus far placed the agent in a variety of types of ointment bases, making any comparative evaluation of release difficult.

The agar cup-plate method indicated a better release of this medicinal agent from emulsion type bases. The various emulsifiers used in these formulations did not appear to alter the rate of release of the medication significantly. The experimental formulation presented appears to release the agent in a manner comparable to Hydrophilic Ointment, U.S.P., while the oleaginous bases showed a generally inferior release of the drug.

Data resulting from the *in vivo* studies appeared to correlate well with the *in vitro* results, again inicating the oleaginous formulations to be inferior to Hydrophilic Ointment U.S.P., in the release and subsequent penetration of 5-FU *in vivo*.

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