STUDIES IN PERCUTANEOUS ABSORPTION IN THE RABBIT

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PREFACE

Ointments, creams and other topical preparations are applied to the broken or to the intact skin. This thesis is a study of the absorption of drugs through the intact skin of rabbits, since a survey of the literature has shown that the results and conclusions of many workers are both confusing and conflicting. The degree of penetration and absorption of drugs through broken skin has not been considered.

In general the methods used to assess or evaluate the efficiency of topical applications fall into two categories, namely *in vivo* and *in vitro* techniques. The *in vivo* techniques include histological methods, which follow the path of the applied drug into and through the skin, and blood or urine assay methods, which estimate the amount of drug which penetrates and is absorbed into or excreted from the blood stream. The *in vitro* techniques, on the other hand, usually simulate the conditions met with when preparations are applied to the broken skin and they assess the rate at which the incorporated drug diffuses or is liberated from its vehicle or base.

An *in vivo* test has been designed to estimate the blood levels of several drugs when these are incorporated in a number of selected vehicles and bases and applied to the

clipped intact skin of rabbits over a period of time. The test devised is considered to be a reasonable method of assessing the degree of percutaneous absorption encountered when drugs, easily and accurately assayed, are used in this way.

INTRODUCTION

Topical preparations may be formulated with two different purposes in view depending upon whether a local or systemic effect of the incorporated drug is desired. In each of these cases there is a need for suitable methods which evaluate drug release from the vehicles or bases used. The number of vehicles and bases in use for the presentation of medicinal substances to the skin is large and the recent advances in formulation with the modern synthetics such as the silicones and the propylene glycols and related compounds have led to the introduction of new types and forms.

The degree of penetration and absorption of a drug from a topical application is influenced by the properties and structure of the skin. The skin consists of a non-vascular external covering of epithelium, the epidermis, and a layer of vascular connective tissue, the corium or dermis. On the outer surface of the epidermis is a thick non-nucleated layer, the stratum corneum. Beneath this is the stratum lucidum at the base of which is the stratum granulosum. The innermost layer of the epidermis is the stratum Malpighii or stratum germinatuvum, a layer of actively dividing cells. The inner corium or dermis is composed

mainly of connective tissue and is well supplied with blood capillaries.

The outer epidermis is rich in lipoids and cholesterol esters presenting a greasy layer which delays penetration of aqueous solutions into the keratinised layers of the stratum corneum. It is electrically polarised and behaves like a membrane with a negative charge on the outside. Theoretically this membrane should be permeable to cations and impermeable to anions. This accounts for the general impermeability of the skin to electrolytes.[1]

The appendages of the skin are the hair follicles, the sebaceous glands and the sweat glands which penetrate to the dermal layer. The greater part of percutaneous penetration occurs by way of the appendages and when penetration has taken place the only barrier to systemic absorption is the sebum, a secretion of glycerides, fatty acids and cholesterol.

The presentation of medicaments in the form of ointments, creams, lotions, etc., may be considered from three main aspects. Firstly, the penetration of the outer epidermis of the skin by the drug incorporated in a vehicle or base, secondly, the liberation of the drug from the

vehicle or base, and thirdly, the passage of the drug to the dermal capillaries and its absorption into the bloodstream. The conditions governing each of these aspects vary. Among the factors influencing drug penetration and absorption through the intact skin are the mode of application, the vehicle or base employed, the lipoid solubility of the drug, and the partition coefficient of the drug between its vehicle or base and the dermal secretions such as sweat, sebum, tissue lipoids and the extracellular fluids.

Penetration and absorption readily occur through broken skin because the protective barrier of the outer layers has been removed and only the partition coefficient of the drug between the base and the wound exudate need be considered.[2-8] Following the application of sulphanilamide to wounds in rabbits Legroux[9] found a maximum blood concentration of 4 - 5 mg./100 ml. A study in the systemic absorption of minute quantities of neutral soluble strontium-89 through the skin of the rat showed that fifty per cent of the amount applied reaches the body interior when the continuity of the outer skin layers is broken.[10] Repeated applications of a five per cent sulphathiazole ointment over half the body surface of infants under treatment for skin infections were shown to give blood levels of 2-4 mg./100 ml.[11]

As early as 1809 the systemic absorption of drugs through the skin was proved by the detection in the urine of substances which had been previously applied to the skin.[12] In 1871 and 1880, histological methods indicated that the avenues for the entrance of mercury applied to the skin in an ointment were the hair follicles and sebaceous glands.[13,14] It has been observed that pretreatment of the skin with such substances as chloroform, ether or alcohol enhances the penetration of water soluble alkaloidal salts, since the hygroscopic properties of the skin surface and of the walls of the hair follicles and sebaceous glands had been increased with the removal of the natural water repelling lipoid materials.[15] This work was later confirmed and chloroform, ether and alcohols were shown to have penetrated the skin.[16] An early review (1901) emphasised that many medicinal agents applied to the unbroken skin would produce therapeutic effects.[17] Mention was made of the constitutional effects of belladonna from plasters containing the drug; salivation from mercury inunction, systemic effects from cutaneous applications of pilocarpine mixed with lard, and the finding in the urine of iodine, salicylic acid, turpentine, guaiacol, creosote and phenol as a result of percutaneous absorption. The usefulness of inunction with cod liver oil

in scrofulous conditions was also noted.[17] In 1908 the dyes fuschin and scarlet red were added to a number of fats and oils in order to trace their paths into the skin after inunction. In no instance was dyed fat ever observed to pass directly into or through the skin from which it was assumed that the skin was impermeable to fat.[18] Penetration, however, occurred in the appendages. An approximate evaluation of the degree of penetration of these common oils and fats showed that the penetration of mineral oils and paraffins was poor while the penetration of animal fats such as goose grease was excellent.[19] By adopting a method often referred to as the "analysis by difference" technique, Wild in 1911 obtained quantitative results for a range of ointment bases containing mercurials. The experiments were made by rubbing a carefully weighed quantity of the ointment into a definite area of skin for a fixed time. The ointment was then scraped off by a dulled, tared razor blade which was weighed. The loss of weight represented the amount of ointment absorbed. The concentration of mercury or mercurial salt in the ointment before and after application gave the amount of mercury absorbed. The results showed that greatest absorption of mercury took place from a lard base but showed also that hydrous woolfat was absorbed to a greater extent

than lard although the absorption of mercury was actually less. Of the different mercurials tested the oxide was the most readily absorbed.[20] Burgi adapted this method by cementing to the skin a bell-shaped glass vessel filled with the substance to be studied. Aliquot portions were removed and assayed.[21] The weakness of the technique lies in the assumption that when a substance disappears from the surface of the skin it has passed through the skin and entered the organism. Some material alleged to have "penetrated" may have been lodged in the skin appendages, and must be judged outside the body.

Discussing nutrients for external applications to the body Latzel and Stejskal[22] prepared emulsions from substances such as hog fat, butter, olive oil and vitamins and claimed that it was possible to feed patients by daily inunction with 150 - 200 grammes olive oil. Winternitz and Nauman[23] rejected this claim and found on the basis of iodised oil injections that approximately one fifteenth-hundreth of this amount penetrated.

The dangers associated with the absorption of drugs through the skin has been noted from time to time.[24-26] A fatality resulted from the spilling of an arsenical liquid over the legs and feet.[27] Death has resulted from the absorption

of phenol through the broken skin and renal damage has followed the application of phenol dressings.[28] Fatalities have been reported due to the external application of salicylic acid[29,30] and cases of poisoning have resulted from the use of five to ten per cent salicylic acid ointments.[31]

The purpose for which a topical application is intended must be considered in the design of any test for the evaluation of its efficiency. The rate of liberation of a medicament from a base may be conveniently determined by *in vitro* tests such as diffusion techniques. Degree of penetration may be estimated by *in vivo* tests making use of histological or histochemical methods. Absorption into the bloodstream can be assessed by *in vivo* or by clinical tests involving the determination of the drug in the blood or urine or by other methods.

REVIEW OF METHODS

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Several reviews have dealt fully with the absorption of drugs. A survey and evaluation of the wide variety of methods which have been devised to determine the rate of release, degree of penetration and absorption of the applied medicament from the vehicle is given. Chemical, physical, pharmacological, toxicological, histological and microbiological methods have been used. In this wide range of tests and techniques some have concerned themselves with liberation, some with penetration, and some with absorption. To rationalise the range of methods the following classification has been adopted.

CLASSIFICATION OF THE METHODS USED FOR THE EVALUATION OF DRUG RELEASE FROM TOPICAL APPLICATIONS

1. In vitro Methods
   - (a) Diffusion Methods
     - (i) Chemical and Physical Estimations
     - (ii) Microbiological Assay
   - (b) Membrane Methods
     - (i) Chemical and Physical Estimations
     - (ii) Microbiological Assay

2. In vivo Methods
   - (a) Blood, Urine and Faeces Analysis
   - (b) Organ Analysis
   - (c) Characteristic Reactions
   - (d) Biopsy Examination
   - (e) Histological Methods
   - (f) Miscellaneous Methods

3. Clinical Methods

4. Radio-active Tracer Methods

1. In Vitro Methods

The in vitro methods which have been used are of limited value but they usually give an indication of the ability of the vehicle or base to liberate the incorporated medicament under the conditions of the particular test.

(a) Diffusion Methods

The usefulness of these methods lie in the fact that a definite estimate of the rate of diffusion of a drug from its vehicle can be made, a valuable result where the surface activity is the purpose of the application.

(i) Chemical and Physical Estimations

The assumption in the tests under this heading is that the distribution of the medicament between the vehicle or base and the area of skin under treatment will be similar to the distribution occurring between the vehicle or base and the medium of the test. This distribution will, however, be controlled by the partition coefficient, which is approximately equal to the ratio of the solubility of the medicament in the vehicle or base to its solubility in the medium of the test. The amount of phenol liberated from a 5 per cent solution of phenol in oil was shown to be almost negligible,[42] and it has been pointed out that the vehicle must be able to part with the drug and not "hold" it, this

fact being emphasised by reference to the diffusion of salicylic and boric acids from different bases.[43] Salicylic acid diffused rapidly from lard and slowly from paraffin into a supernatant aqueous solution of ferric chloride while there was practically no diffusion of the boric acid incorporated in a paraffin ointment. The diffusion of sulphonamides from various ointment bases into both saline and serum at 37°C has been used for their evaluation.[44,45] Fuller, Hawking and Partridge[46] carried out experiments to determine the rate of diffusion into water of sulphonamides incorporated in a solid gel of agar or gelatin and oily bases. They concluded that only a small proportion of the total sulphonamide passed into the water from an ointment base and the diffusion took place from the surface layers, the preparations miscible with water liberated the drug much more readily than preparations immiscible with water and that increase in particle size of the drug and preferential wetting with oil retarded diffusion. The speed of diffusion of salicylates from twelve bases into water, gelatin blocks and olive oil has also been compared.[47]

(ii) Microbiological Assay Methods

In very many cases topical applications are intended to be antiseptic in action and a number of methods and

techniques have been devised which depend upon a form of microbiological assay. The tests assess the antiseptic value and give fundamentally an indication of the rate and degree of release of the medicament from the vehicle.

Robert Koch[48] stated that phenol when dissolved in oil did not exhibit any antiseptic properties. In his experiments silk fibres were impregnated with the spores of Bacillus anthracis and immersed in phenol solution and he was able to demonstrate that the spores were not killed after immersion in a 5 per cent phenol in oil solution for 110 days. This partition of phenol in an oily solution has already been noted.[42] It has been shown that a phenol ointment failed to inhibit growth when samples were inoculated with Serratia marcescens and Pseudomonas fluorescens and transfers made to nutrient gelatin.[49] Under the same conditions an ointment of mercuric chloride inhibited growth. A method was devised in 1895 whereby platelets of glass were inoculated with a broth of Serratia marcescens and dried. The platelets were then introduced into the ointment under test for a definite time, washed with ether to remove the ointment, transferred to sterile broth and observed for growth. The conclusions arrived at in the

series of tests were that cold cream and lanolin were the best bases for antiseptics.[50] A variation of this method was used where antiseptic ointments were spread on cover slips which were then laid under agar in a petri dish. The surface of the agar was now inoculated with M. pyogenes var aureus and incubated. Growth was observed and of the vehicles used lanolin gave the best results while hydrocarbons gave the poorest.[51]

Reddish[52] devised the most widely used test for assessing the effectiveness of antiseptics included in ointment bases and this test with certain modifications was eventually adopted by the United States Food and Drugs Administration.[53] It is the only officially recognised test for ointment efficiency. In the original test plates were inoculated with a culture of M. pyogenes var. aureus and spread with the ointment under test melted at 37°C. Controls using the base without medicament were also set up. After incubation the width of the zone of inhibition was taken by Reddish to be an indication of antiseptic value. He suggested that the nutrient agar of the petri dish simulated the conditions met with in wounds and skin and stated, "it is permeable, semisolid, and isotonic and constitutes a

valuable laboratory means of approximating the conditions found in human and animal tissue". This medium, however, is hydrophilic and uniformly permeable whereas animal skin is hydrophobic and permeable only by way of its appendages.

Reddish and Wales[54] and other workers[55-61] using this and similar tests have shown the much greater efficiency of emulsified bases over fat, oil and wax bases as carriers of antiseptics. Reddish's test was modified by the use of weighed quantities of ointment spread over a definite area and it was shown that antiseptic efficiency was diminished or entirely absent with fat, oil or wax bases, while oil-in-water emulsion bases gave a greatly enhanced germicidal value.[62] Gershenfeld and Brillhart[63] found that the more water a base contained the greater was its zone of inhibition. In measuring the hydrogen ion concentration in hydrophilic ointments and lotions agar cup plate tests indicated that in general the greater the hydrogen ion concentration the greater the germicidal or bacteriostatic properties of the preparations. Straksoch and Olsen[64] demonstrated that the maximum bacteriostatic effect of sodium sulphathiazole was obtained by a five per cent ointment and nothing could be

gained by a higher concentration. Similar methods have been used by other workers.[65-73] Silica gels have been investigated in this way as carriers for antiseptics[74] and it has been found that the antiseptic value of certain ointments is improved when the water content is increased in the presence of oxy-cholesterol.[75] Increased effectiveness was also noted when sulphated hydrogenated castor oil was used as a base with 40 per cent water[76] although certain claims for water containing bases were not fully supported. The plate method has also been used to investigate the effect of particle size and the addition of a wetting agent on antiseptic activity.[77] A unique method of measuring the antiseptic activity of ointments involved scrubbing the hands according to a standard technique for a given period of time. The number of bacteria removed was determined by plating a sample of the final rinsings. Ointment was then applied for a stated time and after removal and washing the number of bacteria was again determined, comparison of diminution in count being the criterion of efficiency.[78]

(b) Membrane Methods

Membrane methods are used in an attempt to simulate in vitro the barrier which is presented by the skin to a topical application. Both artificial and natural membranes

have been used and it is assumed that the process of penetration taking place in the skin is similar to the quantitative diffusion of a drug from the vehicle through the permeable membrane.

(i) Chemical and Physical Estimations

Two methods may be noted. First the estimation of the sodium chloride released from various ointment bases after diffusion through a cellophane membrane into distilled water,[79] and second, the comparison of the exosmosis of potassium iodide, phenol and resorcinol from paraffin, lard, and lanolin bases all of which had been suspended in sheeps' bladders[80] immersed in water at 37°C.

(ii) Microbiological Assay Methods

The diffusion of penicillin and sulphonamides from various bases has been determined by placing the ointments to be examined in cellophane bags and immersing them in saline at 37°C for a fixed time, the amount of penicillin or sulphonamide diffusing into the saline being estimated by the cup plate method.[81,82] Samples of different ointments containing antiseptics have been applied to cellophane squares which were then placed on the surface of inoculated media. After incubation the time required to produce inhibition of growth was taken as a measure of drug release. No diffusion

of active agent could be detected from scarlet red, icthammol, coal tar and salicylic acid ointments when tested by this method.[83] Other workers have examined the diffusibility of antibiotics from various ointments and creams by placing them in dialysers and taking frequent samples for assay.[84]

2. In Vivo Methods

Numerous methods using test animals have been devised to determine both penetration and absorption of the medicament incorporated in a vehicle or base and it has been found that although differences in permeability between human and animal skin exist the results of animal experiments may justifiably be extended to problems concerning human beings.

(a) Blood, Urine and Faeces Analysis

Following its cutaneous application the detection of a substance in the bloodstream, urine or faeces offers the most conclusive proof of its absorption. Blood levels have been taken as a measure of the absorption of topically applied sulphonamides.[85] Woodward and others[86,87] determined sulphathiazole levels in blood samples from rabbits after the application of an ointment to a definite area of clipped skin. Various ointments were tested comparatively using suitably designed experiments and sufficient animals to ensure that the

differences reported were statistically significant. Bases containing propylene glycol were superior to fat or oil bases as regards the penetration of sulphathiazole. The addition of certain surface active agents, notably sodium lauryl sulphate or an aryl alkyl poly-ether alcohol, improved penetration while others did not. These substances increased the irritant properties of the bases. The authors stated that absorption from a 5 per cent sulphathiazole ointment equalled that from a 20 per cent ointment and confirmed in vivo the results of certain in vitro tests. Tests have been carried out to compare ointment bases using phenolsulphothalein and potassium iodide. The ointments were applied to rats. The time taken for the first coloration of the urine with phenolsulphothalein was noted and a chemical analysis was made for potassium iodide. The results were correlated with the findings obtained from the agar plate method and it was found that the addition of a wetting agent did little to increase absorption, while the addition of 2 per cent silica or 5 per cent cetyl alcohol markedly reduced absorption.[88] Fuller, Hawking and Partridge[46] estimated the excretion in the urine of various sulphonamides after application to surface wounds in rabbits and observed the rates of absorption of sulphanilamide, sulphapyridene and sulphathiazole.[89] Lund studied the absorption of calcium

and sodium penicillins from various ointment bases by assaying the drug in urine samples. Mercury, applied percutaneously, has been estimated in the body fluids, tissues and excreta by routine chemical analysis.[90,91]

(b) Organ Analysis

There is a tendency for the animal body to store in particular organs the drug being applied and in these cases the determination of the drug in the blood, urine or faeces may not give a true picture of the rate of absorption. In confirmation of this it has been shown that after inunction with a mercurial ointment, one hundred times as much mercury was found in the kidney as was found in the rest of the body and urine.[92] The absorption of lead from different vehicles applied to rats has been determined in the kidney, liver, muscle and lung.[93]

(c) Characteristic Reactions

Many workers have adopted pharmacological or physiological end-points to determine the time taken for the passage of a drug through the skin and to produce a systemic effect. The actions of various ointment bases on the penetration of alkaloids has been studied using strychnine. Tonic spasm was taken as the end-point in tests on rats and

mice and the results seemed to indicate that the use of vehicles did little to improve and may even have hindered penetration.[94] A number of volatile oils were successfully used as vehicles to introduce into the body various potent alkaloids and other drugs. A direct technique has been used to demonstrate the percutaneous absorption of antigen by first sensitizing passively a skin site to the antigen and then proving absorption by the production of an urticarial reaction at another site 24-48 hours later.[95]

The effect of the passage of hormones through the skin has been the subject of wide research. In 1929 Zondek demonstrated the physiological effects resulting from the passage of sex hormones through the skin[96] and other workers confirmed Zondek's findings.[97-101] It is worth noting that Moore[102] reporting on the effects androgens and oestrogens had on the skin of guinea pigs stated that "there is yet a lack of appreciation of the readiness with which substances are taken up by the skin and are effective in the body".[103] Zondek making use of alcohol, ether and benzene as vehicles for the application of oestrogen was able to detect only little difference between the cutaneous and subcutaneous administration of the drug. Using the weight increase in

the prostate and seminal vesicles of the rat as the criterion of measurement Nelson[104] and others found testosterone more effective in alcohol than in oil, lanolin or ointment, and more efficient percutaneously in alcohol than subcutaneously in oil. It was, however, less efficient percutaneously in oil, lanolin or ointment than subcutaneously in oil, a finding which emphasises the superior penetrant properties of alcohol. This finding is in agreement with that of Emmens[105] who showed that the volatile organic solvents ether, benzene and 96 per cent alcohol are more effective as carriers of androgens, oestrogens and progesterone from the skin surface than is oil or lanolin. In tests with rabbits, inunction with benzene but not with ether was superior to their injection in oil. A slight but consistent superiority when dealing with oestrogens was found with ether as a medium and when dealing with androgens and progesterone with benzene as a medium. The effects of the sex hormones following their percutaneous application may be explained by the extremely small amounts required in the bloodstream to produce their characteristic effects and these amounts are usually within the concentrations attained by substances applied percutaneously.

Several authors have claimed that insulin will penetrate the skin[106-110] while others have reported that absorption can be increased by pretreatment of the skin with saponins[111,112] and organic solvents[113] which pathologically increase its permeability. The action of a heparin cream was studied on rabbits and humans and caused a significant increase in the coagulation time of the blood of animals and humans.[114]

The passage of vitamins through the skin has been shown to occur and as early as 1927 experiments illustrated that the application of irradiated cholesterol to the undamaged skin prevented rickets[115] but varying the vehicle had little or no influence on the absorption of vitamin D.[116] The penetration and local effects have been noted when vitamin A was applied to the skin of guinea pigs.[117]

A comparative analysis of ointments has been carried out by studying a pharmacological reaction following the release of local anaesthetics. A pain threshold method was adopted and ointments containing local anaesthetics were applied to the blackened tails of rats. A test stimulus was given by means of radiant heat and control ointments containing no anaesthetics served as a check.[118] A similar technique was used by means of the

Harding-Wolff-Goodall[119] pain threshold apparatus. The tail of an albino rat was coated with Indian ink and subjected to 400 millicalories of heat until a positive reaction was noted. Ointment bases containing local anaesthetics were then applied and the reaction time noted.

The protective effect of topically applied chemotherapeutic agents has been used to demonstrate absorption and could be used to evaluate vehicles. Zondek[120] has studied the use of halogenated phenols, particularly p-chloroxylenol, as external disinfectants in protecting rats infected with streptococci and pneumococci, a protective effect which has been utilised in clinical trials (vide infra). The quantity of chloroxylenol excreted after rubbing in the form of an ointment on the shaved back of a rabbit was found to be approximately 15 per cent. Percutaneously applied in man in the form of a 30 per cent ointment, a chemotherapeutic effect was noted. The urine excreted remained sterile and while organisms inoculated into the contents of cantharide blisters in human subjects multiply rapidly, strong antibacterial effects are possessed by the same contents

in patients treated with p-chloroxylenol ointments. The results obtained encouraged Zondek and others[87] to continue this work using sulphonamides and they showed that these compounds were absorbed by the skin and were present in the blood after cutaneous application. Using rabbits it was found that the application of ointments gave less satisfactory results than the use of organic solvents in the administration of a sulphonamide when applied to the shaved skin of the backs of the animals. The estimations were carried out on blood and it was noted that percutaneous absorption was augmented by the addition of soap and glycerin. They stated that the concentration of sulphanilamide in rabbit blood following its percutaneous administration was as high as that obtained after its oral administration but inferior to that following subcutaneous or intramuscular injections. Although the blood concentration of sulphanilamide following its percutaneous administration in men is lower than that after oral administration the authors state that the percutaneous use of sulphanilamide may serve as an auxiliary method of chemotherapeutic treatment in certain cases. Investigation of the activity of various sulphanilamide creams has been carried out by

inoculating a group of mice intramuscularly with Staphlycoccus pyogenes. The creams were then injected subcutaneously and the survival times of the mice compared with the survival times of untreated infected controls.[121]

Irritation studies have been conducted on ointment bases and the results of patch tests on intact and abraded skin of animals have been correlated with patch tests on man.[122] It was shown that the addition of surface active agents increased the irritation of the ointments.[123]

The keratolytic action of different ointments may influence the absorption of medicaments and the effects of sulphur, salicylic acid and resorcinol in different bases have been noted.[124-127] Biopsies were performed at intervals and the time to produce keratolysis was taken as a measure of efficiency.[128]

(d) Biopsies

The use of biopsies entails the removal of tissue from the animal under test and extracting and estimating the drug in the tissue by suitable means.[129] Eller and Wolff applied materials to the shaved skin of albino rats, biopsies were taken, and examination showed that animal fats

were the most efficient and mineral fats the least efficient. Volatile substances such as alcohol, ether and benzene were vehicles with a much higher absorption rate than fats. Optimum penetration was observed after four to six hours and wetting agents were shown to assist this process.[130] The penetration of sulphonamides through the intact skin of rats, rabbits and humans was measured by means of tissue analysis and the results obtained from the application of wet dressings, iontophoresis and ointments have been compared. Although individual variations were large, tissue concentrations tended to plateau after two to three hours. Penetration from an ointment was inferior to that from wet dressings or iontophoresis.[131] The rate of penetration of sulphanilamide into the intact skin of guinea pigs from water-in-oil and oil-in-water emulsions has been determined by direct chemical analysis, but no difference in efficiency between the emulsions was found. Increasing the concentration of sulphanilamide in the bases above one per cent had little effect on the tissue levels reached whereas increasing the time of application consistently increased these levels. The addition of a surface active agent did not give improved results but injured skin took up greater amounts of sulphanilamide than intact skin. Sulphanilamide, sulphathiazole and sulphadiazine gave comparable tissue levels but after a

3 day application time sodium sulphacetamide gave greater penetration than these three compounds.[132,133]

As a result of tissue analysis, the use of polyethylene glycols has been recommended for the sulphonamides.[134]

(e) Histological Methods

The histological methods adopted for the evaluation of drug release are concerned with the degree or depth of penetration of the vehicle and the incorporated drug into the skin.[135-142] Fluorescent microscopy has been used to detect chemotherapeutic substances in sections of tissue.[143,144] The fluorescence of many common drugs in ultra-violet light can be demonstrated in very low concentrations. The organs and pieces of tissue to be studied are removed and immediately frozen in liquid air. They are dried over phosphorus pentoxide in vacuo at about -40°C. and sectioned. Drugs of which the fluorescence colour is other than blue, for example Prontosil rubrum, can be demonstrated directly in the tissue. Drugs with a blue fluorescence (the natural fluorescence of the tissues) can be made to change their fluorescence colour by heating to a different temperature for different periods of time. By this method it is possible to demonstrate

sulphathiazole, sulphanilamide, sulphapyridene, papaverine, inulin and penicillin in tissues.[145] Other histological methods use a specific dye for the oil or drug applied to the tissues.

(f) Miscellaneous Methods

An unusual method of estimation measured the release and penetration of drugs by the use of chick embryos. The chorio-allantoic membrane served as the medium for penetration, the ointment being injected into the natural air sac. Toxicity was determined by the number of embryos remaining alive after varying times. Permeability was determined by the incorporation of antibiotics in the ointments and assaying aliquot portions of the allantoic fluid gave an assessment of release.[146]

3. Clinical Methods

Clinical methods have amply demonstrated the systemic absorption of medicaments.[147] Daily inunction with an arsenic ointment led to an excretion of the element as high as 10 mg. daily,[148] and arsenic has been detected in the urine after exposure of the feet to a hot bath of dilute arsenical solution.[149] Work has been carried out on the permeability of the human skin to lead oleate and

progressive permeation of this compound has been shown on prolonged contact.[150,151] The penetration of copper oleate from a lanolin-paraffin base has been examined and it was noted that the amount of copper excreted increased if the skin was damaged.[152] The "analysis by difference" previously referred to[21,153] was continued by Wild and Roberts[154] who tested various bases and found that the greatest absorption took place from lard. They stated that inunction with four grammes of a mercurial ointment for two minutes gave an absorption into the body not exceeding 0.12 grammes of mercury, and after 10 minutes an absorption not exceeding 0.17 grammes.

Evidence has been supplied by many workers[155-166] for the lipoid theory of absorption which holds that lipoid soluble drugs will be absorbed more readily than lipoid insoluble drugs. The presence of boric acid was demonstrated in the urine after immersion of the feet in alcoholic boric acid solution but no boron could be detected when the sodium salt was used.[167,168] It has been claimed that the sugar content of the blood and urine of both diabetics and normal persons has been increased after the application of oils containing sugars.[169]

Moncorps[170] carried out the first clinical trials which could be considered comparative when he measured the amount of salicylic acid eliminated after its cutaneous application in various bases. Much has been published on the absorption of salicylates and iodides.[171-177] [178-189] Brown and Scott[190] studied the absorption of salicylates from aqueous solutions and considered the influence which different oils and ointment bases had on absorption. In their experiments, the hands served as the area of application and the excretion of salicylate in the urine was determined. No essential difference between olive oil and lard was noted but the absorption from liquid paraffin and anhydrous lanoline was higher. Temperature proved to be an important factor; a rise from 26-28°C to 43-44°C resulted in a two-fold increase in the absorption of salicylate. Zondek[120] in his work on p-chloroxylenol showed that the blood of patients who had received percutaneous treatment with the disinfectant was able to inhibit the growth of staphylococci. Treatment with p-chloroxylenol was far more effective than that with sulphanilamide, sulphapyridene or sulphathiazole. Nadkarni and others[191] applied phenolsulphothalein to a group of patients

and estimated colorimetrically the concentration of dye excreted in the urine. On normal human skin they concluded that the use of a water soluble vehicle promoted greater absorption of the dye than did an oleagineous base and found polyethylene glycols more efficient as vehicles for inflamed skin than paraffin bases.[192] Shelley and Melton confirmed that vehicles containing propylene glycol and surface active agents facilitated the passage of acetylcholine chloride, pilocarpine nitrate, atropine sulphate, hyoscine, ephedrine and histamine phosphate through human skin. Inunction with histamine resulted in perifollicular wheals and diffuse erythema which indicated that the significant route of penetration was that by way of the hair follicles and sebaceous glands. It was also shown by these experiments that greatest absorption occurred in the most hirsute areas of the body while no evidence of absorption was noted when the drugs incorporated in penetrating types of vehicles were applied to the palms of the hands and the soles of the feet, areas where hair follicles are normally absent. The formulation and examination of new penetrant vehicles containing propylene glycol has been carried out by Hermann

and others.[193] Metals and sulphanilamide were shown to have penetrated by estimations on blood and urine and maximum penetration was reported within one-half to three hours after application.

An unusual method for the determination of absorption from ointments and creams involved the mixing of Sudan Red with the preparations tested. Filter paper is laid upon the circle of skin treated with ointment and pressure applied. The procedure is repeated with fresh paper until no colour comes off the skin. A photometric value of the skin is then taken and compared with that for untreated skin. This value, taken to be a measure of penetration, was obtained for each of the preparations tested.[194]

4. Radio-active Tracer Methods

Radio-active tracer techniques have been increasingly used in evaluating drug release from topical applications. Completely negative penetrant values have been obtained for cholesterol, vaseline and the saponifiable components of ointments. Labelled sodium chloride has been incorporated in lanoline, paraffin and lard applied to

human skin. The urine of all the patients was found to be radio-active and the greatest absorption was observed from lard.[195] Radon has been applied to skin in a lanoline base and its exhalation through the lungs noted. Significantly greater absorption was found when the ointment was applied to wounds.[196] The absorption of radio-active sodium iodide from different bases has been measured and it was found that no appreciable difference in absorption took place when the vehicle was an oil-in-water or a water-in-oil emulsion. By a similar method, lard, woolfat and paraffin have been compared as bases, the entire thyroid gland being used to determine the amount of sodium iodide absorbed. The amount of radio-active iodine absorbed by the gland was small but the absorption from paraffin was greater than that from woolfat or lard.[197,198] The efficiency of liquid preparations and creams have been examined by comparing the rate of diffusion of their labelled ingredients across isolated frog membranes.[199,200] By measuring the radio-activity of the urine, absorption from water miscible bases containing zinc sulphate was higher than that from greasy bases although the efficiency of the latter was increased by the addition of soap.[201] Autoradiographic findings have

shown that radiothorium which scarcely penetrates into the horny layer preferably permeates into certain structures such as the hair follicles and the excretory ducts of the sweat glands in human skin[202] and this has been confirmed by the examination of biopsies of tissue treated with Thorium X in various vehicles.[203,204] With an isotopic technique no deuterium oxide could be demonstrated in the products of combustion of the body fats after application of a salve made of hydrated oil containing deuterium oxide.[205,206]

EXPERIMENTAL

As has been shown in the review section a large number of *in vitro* methods has been used to investigate the diffusion of drugs from various vehicles and bases and an equally large number of *in vivo* tests has been used to determine the degree of percutaneous penetration and absorption of drugs from topical applications. It is clear that penetration and absorption is best investigated by means of *in vivo* techniques since these give results of practical importance and throw some light on the factors influencing percutaneous absorption.

In the first stages of the experimental work it was decided to investigate the properties of isolated rat skin as a barrier to the passage of drugs. This investigation proved of little value and the remainder of the work involved estimating the concentration of drug, following its topical application, in the blood of rabbits. Two aspects of percutaneous absorption were studied. In the first instance the absorption of a single drug, namely sulphanilamide, from a range of vehicles and bases was studied, and in the second, a range of drugs from a limited number of vehicles and bases was examined.

1. The Passage of Sulphanilamide through Isolated Intact Rat Skin

The factors which exert the greatest influence on absorption are the properties of intact skin and the effects these have on the passage of bases and drugs. An attempt to examine these properties was made by suspending sections of living skin in oxygenated mammalian Ringer solution. The passage of a drug applied to this could then be followed simply by estimating the concentration of drug in the Ringer solution.

Experimental Details.

The backs and sides of six, healthy, mature male albino rats were closely shaved by means of an electric shaver on the day prior to the experiment. The rats were then sacrificed and the skin of the dorsal-thoracico lumbar region quickly removed. The isolated skin was laid on a wad of filter paper moistened with mammalian Ringer warmed to 37°C, the interior surface of the skin being in contact with the Ringer. On an area of skin one inch in diameter, approximately two grammes of an ointment containing ten per cent sulphanilamide were gently applied, and the skin placed around one end of a piece of glass tubing, five inches long, one millimetre thick and one inch in diameter, in a manner

such that the exterior surface of the skin was in contact with the glass. The tissue was suspended in 100 ml. oxygenated mammalian Ringer solution at a temperature of 37°C, this temperature being regulated to within 0.1°C by means of a controlled temperature water bath. At intervals of thirty minutes, samples of Ringer solution were withdrawn by pipette and assayed for sulphanilamide content. The tissue was suspended usually for eight hours but on no occasion was any sulphanilamide detected in the Ringer solution even when the tissue was suspended for twenty-four hours. Liquid preparations containing the drug were placed on the area of skin after it had been strapped around the glass tubing and suspended in the Ringer solution.

In all cases negative results for the detection of sulphanilamide were obtained. The preparations tested were lard, woolfat and soft paraffin; the liquid preparations, saturated with sulphanilamide, were liquid paraffin, glycerin, propylene glycol and water.

2. The Absorption of Sulphanilamide through the Intact Skin of the Rabbit from various vehicles and bases

In the light of the foregoing experiments it was decided to conduct experiments utilising suitable *in vivo* techniques since no artificial or natural membrane exists which simulates the properties of intact healthy skin.

The most promising method for this work was considered to be a technique whereby sulphanilamide, following its cutaneous application, was detected in the bloodstream of animals. This method provided conclusive proof of its absorption since sulphanilamide applied in a suitable vehicle must necessarily penetrate the skin and enter the bloodstream in order to be detected and simple penetration or lodgement within the appendages may be discounted. The blood levels of sulphanilamide estimated at suitable time intervals would allow a comparison of the efficiency of various vehicles and ointment bases as "carriers" for the drug. Since the study of blood concentrations has been a feature of sulphonamide therapy and the sulphonamide drugs are uniformly distributed in the animal body, this seemed the

logical approach. Furthermore, the passage of a drug through the skin is not dissimilar to a form of continuous drip-feed and it is therefore important to have an overall estimate of the systemic concentration attained over a period of time from a topically applied drug. The estimation of drug content in the urine was rejected on the grounds that it cannot be assumed that the rate of excretion of a drug is directly proportional to its rate of entry into the animal body. This method also excludes the possibility of preferential absorption, such as iodine by the thyroid, or the destruction or storage of the drug in the liver or kidney. While the total blood volume of the animals cannot be known accurately, the relative changes in absorption which can be obtained from blood estimations are of value in that they yield results likely to be met with in practice.

Design of Test

In this field previous workers, with the exception of Woodward,[86] have generally provided insufficient results for complete statistical examination. The series of experiments undertaken were designed with a view to obtaining sufficient results to make such an analysis.

All tests were made on six rabbits from the same litter, since litter-mates usually yield results less variable than those obtained from animals selected at random from an animal population. Litters from three separate breeds of rabbit were used, these breeds being Copenhagen White, Dutch and an inbred litter of Beveren rabbits. With experience it was found that the Copenhagen Whites gave results uniform within the limits of the test and this breed of rabbit was used in all subsequent tests. The variation between litter-mates and non litter-mates in this breed could be compared in the later stages of the work by using six litter-mates and six rabbits drawn at random from the normal population. Rabbits used in the tests consisted of males and females between 2.5 and 3.5 Kg. in weight. The design of the tests involved the application of the three vehicles or bases under examination, A, B, and C, to six rabbits in random order, thus avoiding any cumulative effect which might have occurred. By applying a base twice an estimate was obtained of the error incurred in duplicating an experiment. The design of the duplicated tests on the three bases may be represented diagrammatically.

No. of rabbits	Base	No. of tests on each base
6	A	2
6	B	2
6	C	2

Experimental Details

Six rabbits, litter-mates, weighed and sexed, were used in each test. Food, but not water, was withheld for eighteen hours before the test since it was found that by this procedure the animals seldom if ever urinated or defaecated while the test was in progress. On the day before the test the fur was removed from the skin of the back and sides of each animal with electric clippers, taking care not to damage the skin. The area under test was marked by a dermograph pencil using a 6 x 4 inch template placed on the dorsal thoacico-lumbar region of the rabbit. The rabbits were then placed in restraining boxes which consisted merely of two sides and a floor and were fitted only with neck-stocks. The distance between the floor and the hole in the neck-stock could be adjusted to suit the size of the rabbit required in the experiment. Details of the boxes have been described by Wylie.[207] The animals were thus rendered immobile but comfortable and, being held gently but firmly in the neck-stock, were prevented from

absorbing orally the applied preparation. A piece of cardboard was fixed to the cross-bars of the boxes to prevent the rabbit flicking its ears behind the cross-bar on to the applied ointment. This ensured that all absorption taking place was percutaneous and eliminated the possibility of accidental contamination. Figure I shows a restraining box containing a rabbit whose area of application is covered with ointment.

A blood sample was withdrawn from the marginal ear vein of each animal as a blank for assay purposes. About 30 grammes of vehicle or base, containing the drug, were applied to the marked area and rubbed in for a period of three minutes. Liquid preparations were applied with a soft hair brush. Reapplications, in both instances, were at fifteen minute intervals. At intervals of thirty minutes after the initial application, blood samples were withdrawn over a period of eight hours.

In the early stages of the work a six hour period was considered adequate but this was extended to eight hours to ensure that the period of maximal absorption had been passed and also to determine whether the fluctuations in the systemic concentrations tended to disappear.

Thus each completed test involved the withdrawal of sixteen blood samples from the ear of each of the six rabbits

Figure I

Test Animal in Restraining Box

giving a total of ninety-six samples for one base, and for the complete experiment on three bases, each applied twice, a total of five hundred and seventy-six blood samples. On completion of a single test, the rabbits had the application removed and the anointed area thoroughly washed with warm, soapy water. After several washings, their backs were rinsed and dried. In order to facilitate the withdrawal of blood, the ears of the rabbits were vigorously massaged or, in the case of sluggish circulation, treated with xylol which was washed off with alcohol immediately it had exerted its effect. On the completion of the test, the ears of the rabbits were smeared with white soft paraffin which prevented any undesirable effects such as hardening or desquamation. Before a further test, the rabbits were allowed at least one week's rest and their blood was then tested for absence of drug.

Choice of Drug.

A drug was selected to demonstrate a wide range of pharmacological and chemical properties but the choice was limited to one readily and accurately determined in small quantities in blood. The drug chosen was sulphanilamide, one of the more rapidly absorbed sulphonamides.

Sulphanilamide, p-aminobenzenesulphonamide, has a solubility in water of 400 mg./100 ml. at 15°, and 1,500 mg./100 ml. at 37°, and a solubility in serum of 1,970 mg./100 ml. It is soluble in ethanol, acetone, glycerol, hydrochloric acid and sodium and potassium hydroxide. It is insoluble in ether and benzene and soluble in chloroform to the extent of 25 mg./100 ml. and in propylene glycol to the extent of 10,000 mg./100 ml. The acid dissociation constant (pKa) is 10.43.

Choice of Vehicles

The vehicles and bases in which sulphanilamide was incorporated included those in wide use. Seven common constituents of ointment bases were tested for their ability to promote the absorption of sulphanilamide and their respective efficiencies were compared. The vehicles chosen were liquid paraffin, white soft paraffin, lard, woolfat, propylene glycol, ethyl oleate and water in the form of a five per cent carboxymethyl cellulose gel.

Four bases were tested. They were Hydrous Ointment B.P., a water-in-oil emulsion, Emulsifying Ointment B.P., and two oil-in-water emulsions, Hydrous Emulsifying Ointment B.P. and a cetomacrogol emulsifying wax base.

To ensure standard preparations the drug was incorporated into the vehicles and bases by hand and subsequently passed through an ointment mill. The carboxymethyl cellulose gel was prepared by adding 10 grammes of carboxymethyl cellulose to 190 ml. distilled water in a bowl into which were immersed electrically driven stirrers. By suitably adjusting the speed of rotation a smooth gel was obtained. The requisite concentration of drug was then added and thoroughly stirred in.

Concentration of Incorporated Drug

The concentration of sulphanilamide used in the vehicles and bases was ten per cent weight-in-weight, one which had been recommended as a maximum useful concentration.[133]

Estimation of Sulphanilamide

Before being excreted by the body, sulphanilamide is conjugated with acetic acid to form N-acetyl-sulphanilamide and since the chemical assay depends on the presence of a free amino group in the sulphanilamide molecule, the conjugated complex must be broken down into free sulphanilamide and acetic acid by one hour's hydrolysis with 1N hydrochloric acid.

The total amount of circulating sulphanilamide, both free and conjugated, was thus determined in the assay since it cannot be assumed that the degree of acetylation in the rabbits was uniform and constant.

The assay adopted was King's micro-modification of the method of Bratton and Marshall.[208]

To 3.2 ml. water was added 0.2 ml. blood followed by 0.6 ml. of 25 per cent trichloracetic acid. The precipitated proteins were filtered off. To 2 ml. of the filtrate in a 5 ml. volumetric flask were added 0.5 ml. 1N hydrochloric acid and this solution was heated at 98-100°C for one hour. On cooling, one drop of a freshly prepared solution of 0.5 per cent sodium nitrite was added and the solution allowed to stand for three minutes; this was followed by 1 ml. of a 0.5 per cent solution of ammonium sulphamate to remove excess nitrite and the solution allowed to stand for two minutes. 1 ml. of a 0.1 per cent solution of N-(1-naphthyl) ethylenediamine dihydrochloride in water was added and the solution finally diluted to 5 ml. with water. The intensities of the colours formed in the different samples were measured at 540 mu, the wavelength of maximal absorption for sulphanilamide solutions.[209] Reagent blanks were prepared with distilled water.

The Standard Solution was made by dissolving 100 mg. sulphanilamide in 10 ml. 0.05N sodium hydroxide and diluting to one litre with distilled water. To 5 ml. of this solution was added 20 ml. of 15 per cent trichloracetic acid and 75 ml. water. The extinction of this dilution which contained the equivalent of 0.005 mg. sulphanilamide per ml. was measured at 540 mu.

Comparison of Four Modes of Administration of Sulphanilamide

The efficiency of percutaneous applications with other modes of administration was compared by administering sulphanilamide to the rabbits orally and by subcutaneous and intravenous injections. The experiments were then carried out as before, the blood samples being withdrawn at half-hourly intervals after administration.

3. The Absorption of various drugs through the Intact Skin of the Rabbit from three vehicles and bases.

The results obtained from these experiments showed that differences in efficiency existed between bases as "carriers" of sulphanilamide. It was then decided to investigate whether the efficiencies of vehicles and bases occurred in the same order for drugs other than sulphanilamide. Besides allowing a study of the differences in efficiency between the vehicles

and bases chosen, the results would also yield comparative values showing the effects of different drugs on absorption.

Design of Test and Experimental Details

The design of test and the experimental details were similar to those for sulphanilamide.

Choice of Drugs

The drugs for further examination were chosen because they exhibited a wide range of properties and could be accurately assayed in blood with relative simplicity. The drugs chosen were salicylic acid, copper acetyl-acetonate and copper sulphate.

Salicylic acid, o-hydroxybenzoic acid, an organic compound has a solubility in water of 180 mg/100 ml. at 20°C and 6,670 mg./100 ml. at 100°C. It is soluble to the extent of 3,330 mg./100 ml. in alcohol, 2,220 mg./100 ml. in chloroform, 3,330 mg./100 ml. in ether and 740 mg./100 ml. in benzene. The solubility of salicylic acid in fixed oils is about 1 in 40.

Copper acetyl-acetonate is a non-ionic compound of copper chelated with acetyl acetone. This complex is insoluble in water and ether but soluble in alcohol and chloroform. Copper acetyl-acetonate is stable in solutions of sulphuric,

nitric and hydrochloric acids but is unstable in solutions of alkali, precipating copper salts.

Copper sulphate is an ionic compound of copper. The sulphate of copper has a solubility in water of 3,330 mg./100 ml. at 15°C while 200 grammes will dissolve in 100 ml. water at 100°C. It is soluble in alcohol to the extent of 200 mg./100 ml. and is very slowly soluble in glycerin to the extent of 3,330 mg./100 ml. Copper sulphate is insoluble in fixed oils.

Choice of Vehicles

Three vehicles and bases were chosen on the basis of the results obtained with sulphanilamide. They were lard, which was shown to have the highest efficiency value, and water, as a five per cent carboxymethyl cellulose gel, which was shown to have the lowest efficiency value. Emulsifying ointment was included since it had an efficiency value intermediate between those for lard and water, as a five per cent carboxymethyl cellulose gel; it had a further interest of being an ointment commonly used in pharmaceutical preparations.

Concentration of Incorporated Drugs

The concentrations of drugs used in the vehicles and bases was ten per cent weight-in-weight, which had been

recommended as a useful concentration.[133] In the case of salicylic acid, however, the concentration was lowered to five per cent in order to reduce the local irritant and keratolytic effect. It was felt that this decrease in concentration would not invalidate comparative blood level studies since it had been reported[86] that an increase of over five per cent in the concentration of the incorporated drug did not lead to an increase in absorption.

Estimation of Incorporated Drugs

The following assays were adopted.

Salicylic acid

A modification of Smith and Talbot's colorimetric method was used.[210]

0.2 ml. blood was added to 3.2 ml. distilled water followed by 0.6 ml. of a 25 per cent solution of trichloracetic acid. To 2 ml. of the filtrate, 1 ml. 1.5N sodium hydroxide was added. 0.5 ml. Folin-Ciocalteu's reagent, diluted one to four with distilled water, was then added and the volume adjusted to 5 ml. with 1.5N sodium hydroxide. After standing the solutions were filtered to remove any phospho-molybdate complex formed and the extinctions measured against a reagent blank at a wavelength of 690 mu.

To correct for non-specific plasma phenols which are included in this estimation, the average reading of thirty-six normal blood samples treated as above was subtracted from the observed values before referring to a calibration curve constructed by similarly treating known dilutions of sodium salicylate in distilled water. The concentration of reductable material in blood estimated by this method gave a value of 10.41 mg./100 ml.

Copper acetyl-acetonate and copper sulphate

Both copper acetyl-acetonate and copper sulphate could be estimated in blood by the same method.

Copper, in ammoniacal solution, was estimated colorimetrically with sodium diethyl dithiocarbamate which is capable of detecting one part of copper in fifty million parts of solution. The colour produced is stable for several hours.[211]

0.2 ml. blood was added to 3.2 ml. distilled water followed by 0.6 ml. of a 25 per cent aqueous solution of trichloracetic acid. To 2 ml. of the filtrate was added 0.5 ml. of a 10 per cent solution of ammonia. Three drops of a 0.1 per cent solution of sodium diethyl dithiocarbamate were then added and the final volume adjusted to 5 ml. with

distilled water. The extinctions of the solutions were measured against reagent blanks at a wavelength of 455 mu. Estimated by this method, the concentration of copper present in normal circulating rabbit blood, taking the average of twenty-four blood samples, was 0.18 mg./100 ml.

The Standard Solutions were prepared by adding known concentrations of copper to freshly drawn rabbit blood and the procedure carried out as described. The estimation of copper was accurate to within one per cent so that under the conditions of the assay any complexes formed by the metal and the blood proteins appear to have broken down, the copper remaining in solution as the stable trichloracetate.

The effect of an irritant and an astringent on the percutaneous absorption of sulphanilamide

The irritant keratolytic effect of salicylic acid[125,126] on the absorption of sulphanilamide was studied by incorporating five per cent salicylic acid in lard containing ten per cent sulphanilamide. The astringent action of copper sulphate was observed by incorporating ten per cent copper sulphate in lard containing ten per cent sulphanilamide. The vehicles were applied to rabbits and the blood levels of sulphanilamide attained were determined as before.

RESULTS

The results are recorded in three sections. The negative results obtained for the experiment testing percutaneous absorption through isolated rat skin, the results obtained for the bases tested with sulphanilamide and the results obtained with salicylic acid, sulphanilamide, copper acetyl-acetonate and copper sulphate in selected vehicles and bases.

Also included are the results of analyses of variance carried out on the data from three breeds of rabbits, each tested with the same ointment bases.

The results of the entire series of experiments are fully recorded in Appendix One.

1. The Passage of Sulphanilamide through Isolated Intact Rat Skin.

No sulphanilamide was ever detected in the mammalian Ringer solution except in the case of accidental contamination.

From these experiments it may be concluded that intact rat skin, isolated in oxygenated saline at 37°C, is impermeable to sulphanilamide contained in solid and liquid preparations; it therefore presents an impenetrable barrier to preparations tested in this way.

The failure of the drug to penetrate the skin may be explained on the grounds that the blood supply to the dermal tissues had been severed by isolating the tissues. Having penetrated to the base of a follicle, no means therefore existed to transport the drug through the remaining layers of the skin to the Ringer solution. The penetration of sulphanilamide through isolated intact skin would have necessitated the physical permeation of the drug through the epidermis, the dermis and the deeper subcutaneous fatty tissue, conditions which were not to be expected from histological findings.

2. The Absorption of Sulphanilamide through the Intact Skin of the Rabbit from various vehicles and bases.

The results of all experiments are shown in tables. The weight, sex and number of each animal and the blood level of the drug in mg./100 ml. at half-hourly intervals following the application of the topical preparation are noted. These values, added together, give an estimate of the response of the six rabbits to the base under examination for the duration of the experiment. The average response of the animals to the vehicle or base is determined by dividing the sum total observed by the number of blood samples withdrawn.

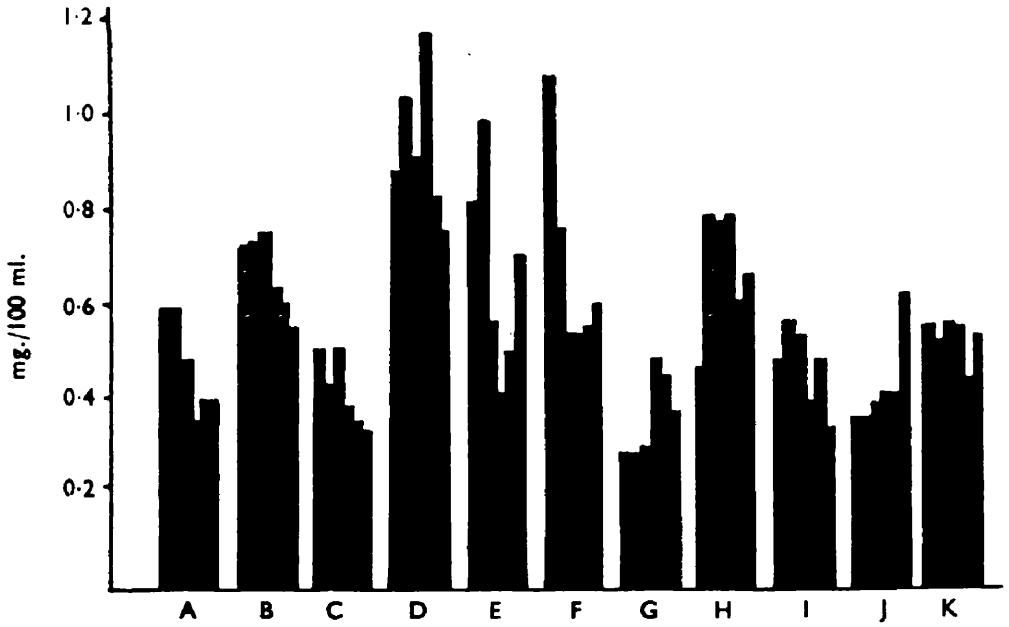
The results of all tests are summarised in Table I. The base applied, the drug and rabbit litter used, and the sum totals are given. The experiments were not duplicated in every case but only where necessary for statistical purposes. Where experiments were duplicated the average of the two results obtained are entered.

A summary of the results is given in Tables II - IV. The response of individual rabbits to the same base varies considerably, this being represented diagrammatically in in Figure II. The "scatter" of results within one rabbit, seen in the tables of experimental results, in Appendix One

Figure II

The response of individual rabbits to selected vehicles and bases containing sulphanilamide

A Liquid paraffin.
B White soft paraffin.
C Woolfat.
D Lard.
E Ethyl oleate.
F Propylene glycol.
G 5 Per cent methyl cellulose gel in water.
H Hydrous Ointment B.P.
I Emulsifying Ointment B.P.
J Hydrous Emulsifying Ointment B.P.
K Cetomacrogol emulsifying wax base.



The blood level of sulphanilamide observed in individual rabbits following the application of the selected vehicles and bases. Six animals were used with each base.

is wide. Duplicated tests also show differences in results.

The summarised results obtained for sulphanilamide are shown in Table II. These are given for six individual rabbits; the values obtained in each of the sixteen blood samples withdrawn over the eight hours were added together and this sum entered in the Table. The individual totals were then added and the response of the six rabbits to the to the base under examination thus found.

The average blood levels observed are given in Table III which was calculated from Table II. The sum totals of the individual rabbits over eight hours was divided by sixteen, the number of blood samples withdrawn, and the mean blood level for the duration of the test obtained. The average of the blood levels found for the individual rabbits was then taken for each vehicle or base and this figure used as a practical index of its efficiency. Therapeutically, this is the value most likely to be attained by the base when applied under standardised conditions.

From this value a table of efficiencies was constructed for the vehicles and bases tested with sulphanilamide. The vehicle or base with the lowest mean blood level, water as a five per cent carboxymethyl cellulose gel, was taken as unity

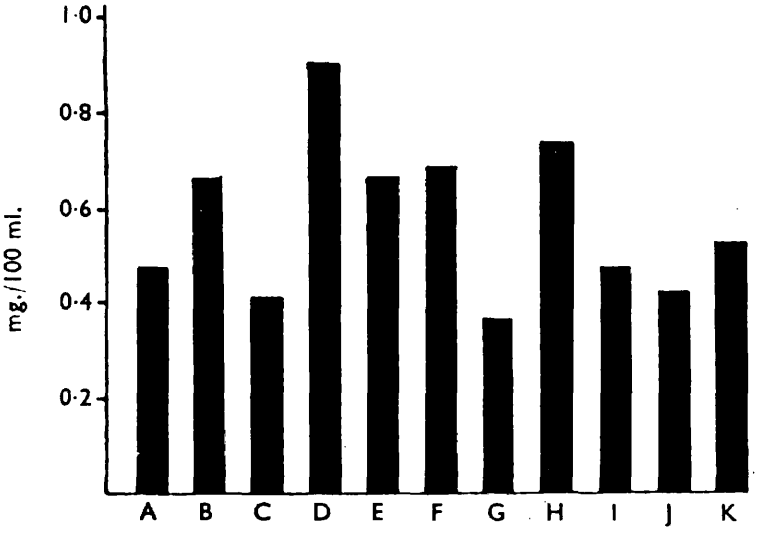
and the other values calculated accordingly. In Table IV lard was the most efficient vehicle or base tested with sulphanilamide, being approximately two-and-a-half times more efficient than water as a carboxymethyl cellulose gel. Propylene glycol, hydrous ointment, white soft paraffin and ethyl oleate gave roughly the same efficiency value and were almost twice as efficient as the aqueous gel. Emulsifying ointment, liquid paraffin, hydrous emulsifying ointment and woolfat were only slightly more efficient than the gel. These values are represented diagrammatically in Figure III.

Comparison of four modes of administration of sulphanilamide

Four methods of administering sulphanilamide were examined by comparing the blood levels attained following the oral administration of a sulphanilamide tablet, an intravenous injection and a subcutaneous injection with the average of the blood levels attained from emulsifying ointment which contained ten per cent sulphanilamide. While the blood levels attained orally and by injection could be varied by altering the concentration of sulphanilamide administered, an estimate of the rapidity with which the drug was removed from the circulation was obtained. The blood levels from emulsifying ointment were chosen since their values lie between those for the most efficient and the least efficient

Figure III

The mean response of rabbits to selected vehicles and bases containing sulphanilamide



The mean blood level of sulphanilamide observed in rabbits following the application of the selected vehicles and bases.

A Liquid paraffin.
B White soft paraffin.
C Woolfat.
D Lard.
E Ethyl oleate.
F Propylene glycol.
G 5 Per cent methyl cellulose gel in water.
H Hydrous Ointment B.P.
I Emulsifying Ointment B.P.
J Hydrous Emulsifying Ointment B.P.
K Cetomacrogol emulsifying wax base.

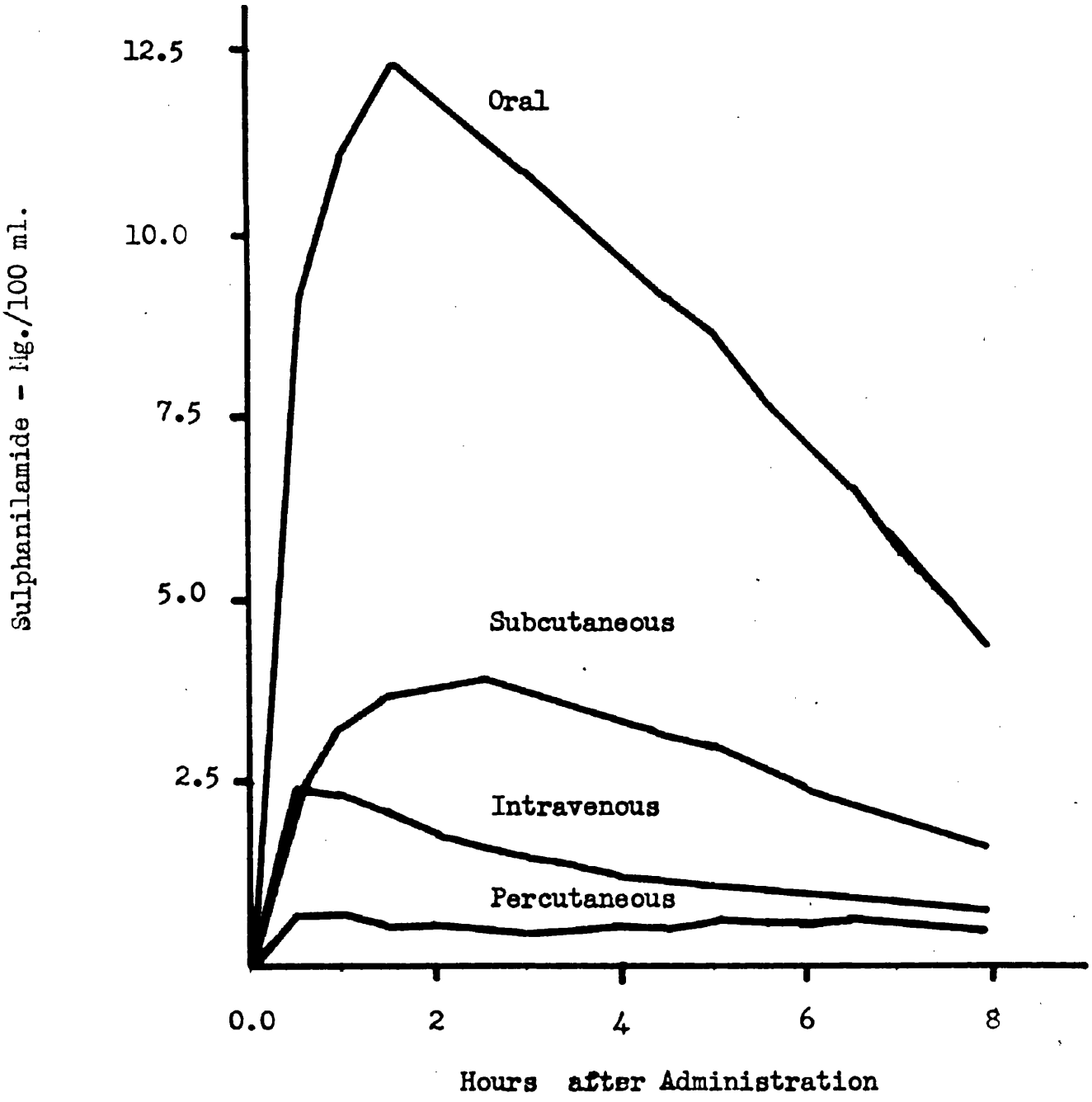
vehicle or base and they can therefore be cited as average for the topical preparations tested with sulphanilamide.

The blood levels attained by these methods are illustrated in Figure IV. Those for the orally administered sulphanilamide were the highest of the four modes examined. Each point on the graph was the average of the values obtained from six rabbits. The blood level of sulphanilamide reached a value of 12 mg./100 ml. after one-and-a-half hours and dropped to 4 mg./100 ml. after eight hours. The "depot" effect of the subcutaneous injection can be observed. The level of sulphanilamide rises to 2 mg./100 ml. after thirty minutes, rises further to 3 mg./100 ml. at which it remains for four hours, and slowly falls to 1.5 mg./100 ml. after eight hours. The high initial values for the intravenous injection last for two hours and then fall throughout the remainder of the eight hours to 0.7 mg./100 ml.

The blood levels plotted for the percutaneous absorption of sulphanilamide from emulsifying ointment are the average of eighteen individual samples. The blood concentration remains fairly constant and fluctuates around a value of 0.5 mg./100 ml. This is to be expected since the amount of drug passing to the dermal capillaries will be proportional to the number of hair follicles in the area of

Figure IV

The blood levels of Sulphanilamide observed in rabbits following four modes of administration



application. When the ointment has penetrated to the base of a follicle the transfer of sulphanilamide to the capillaries will occur at a constant rate. As the ointment fills each follicle in the anointed area, the overall rate of transfer to the bloodstream will gradually reach a limiting value. The concentration of drug in the blood will rise to a maximum about which fluctuations occur.

The blood levels attained by oral, intravenous and subcutaneous administration fell to zero within forty-eight hours. Following percutaneous administration only traces of the drug could be detected after five days although estimable levels remained for a longer period than the other modes of administration, presumably since the ointment in the hair follicles could not be removed by washing.

3. The Absorption of various drugs through the Intact Skin of the Rabbit from three vehicles and bases.

The results of these experiments are shown in tables similar to those for sulphanilamide. The results of all tests are summarised in Table V. The base applied, the drug and rabbit litter used, and the sum totals are given. Since the experiments were duplicated, the average of two results is entered. Similarly, tables were constructed to show the order of efficiency of the three bases, lard, emulsifying ointment and water as a five per cent carboxymethyl cellulose gel, containing salicylic acid, sulphanilamide, copper acetyl-acetonate and copper sulphate.

The individual total of each drug absorbed by the rabbits over eight hours from the three vehicles and bases under examination is noted in Table VI and the mean blood levels attained by these different drugs in these vehicles and bases are shown in Table VII. The efficiency of the three vehicles and bases for each of the four drugs are shown in Table VIII. In this table, water as a five per cent carboxymethyl cellulose gel was taken as unity for the group of applications containing a drug since it always gave the lowest blood levels, regardless of the drug incorporated.

The comparative efficiencies of the applications tested with the four drugs are shown in Table IX. The blood level from copper sulphate in the carboxymethyl cellulose gel, the lowest recorded in the complete series of tests, was taken as unity. For all four drugs, lard was the most efficient vehicle or base tested and water as a five per cent carboxymethyl cellulose gel the least efficient. The degree of absorption of the incorporated drug can also be seen. Salicylic acid was absorbed more readily than sulphanilamide while, by comparison, the acetyl-acetonate and the sulphate of copper were poorly absorbed.

The effect of an irritant and an astringent on the percutaneous absorption of sulphanilamide

Salicylic acid and copper sulphate were incorporated into separate ointments of lard containing ten per cent sulphanilamide and the blood concentration of sulphanilamide noted.

The results are summarised in Table X. The small decrease in the amount of sulphanilamide absorbed from lard when salicylic acid was also incorporated lay well within the limits of error found between replications and it may be concluded that the incorporation of salicylic acid with sulphanilamide in lard neither hinders nor promotes absorption of the latter. The incorporation of copper sulphate, however, decreases the absorption of sulphanilamide in lard by twenty-five per cent and the astringent action of the sulphate appears to exert a significant hindering effect.

TABLE I.

Total Amount of Sulphanilamide Observed in the blood of Six Rabbits from various vehicles and bases.

The Average of Duplicated Experiments Entered

Mg./100 ml.

Vehicle or Base	Litter	Result	Average
Water as 5 per cent carboxy methyl cellulose Subcutaneous injection	96-101	34.36	34.36
	102-107	268.57	268.57
Intravenous injection	102-107	127.29	127.29
Oral Administration	102-107	837.21	837.21
Hydrous ointment	48-53	92.51 : 103.74	98.12
Hydrous ointment	96-101	64.90	64.90
Hydrous ointment	73-78	94.67 : 45.33	70.00
Emulsif. ointment	48-53	73.30 : 74.60	73.95
Emulsif. ointment	96-101	44.70	44.70
Emulsif. ointment	73-78	41.39 : 55.02	48.21
Hydrous emulsif. ointment	48-53	42.30 : 41.85	42.08
Hydrous emulsif. ointment	96-101	40.71	40.71
Hydrous emulsif. ointment	73-78	36.17 : 45.35	40.76
Woolfat	96-101	32.36 : 47.29	39.83
Lard	96-101	85.82 : 86.02	85.92
White soft paraffin	96-101	63.23 : 64.45	63.84
Liquid Paraffin	96-101	44.73 : 39.11	41.92
Propylene glycol	48-53	60.95 : 69.12	65.03
Ethyl oleate	48-53	63.04	63.04
Cetomacrogol emulsifying wax	96-101	51.94	51.94

TABLE II

Comparison of Percutaneous Absorption of Sulphanilamide in the Rabbit from different vehicles and bases

Base	Total Amount Observed in the blood of 6 individual rabbits (each value is the sum of 16 samples) Mg./100 ml. Rabbit Number						Total Amount Observed in six rabbits over eight hours Mg./100 ml.
	1	2	3	4	5	6	
Liquid paraffin	9.39	9.53	7.71	5.53	6.21	6.32	44.69
White soft paraffin	11.57	11.72	12.02	10.02	9.65	8.85	63.83
Woolfat	8.08	6.84	8.00	6.06	5.61	5.24	39.83
Lard	14.14	16.05	14.54	16.24	13.03	11.92	85.92
Ethyl oleate	12.98	15.60	8.92	6.53	7.89	11.12	63.04
Propylene glycol	17.17	12.19	8.64	8.69	8.80	9.55	65.04
Water as 5 per cent carboxy methyl cellulose	4.46	4.47	4.64	7.67	7.19	5.93	34.36
Hydrous ointment	7.39	12.54	12.29	12.44	9.68	10.56	64.90
Emulsifying ointment	7.62	9.08	8.45	6.27	7.71	5.57	44.70
Hydrous emulsifying ointment	5.69	5.72	6.28	6.56	6.58	9.88	40.71
Cetamacrogol emulsifying wax base	8.82	8.36	9.01	8.76	6.97	8.43	50.29

TABLE III

Comparison of Percutaneous Absorption of Sulphanilamide in the Rabbit from different vehicles and bases

	Mean Blood Level Observed in six individual rabbits over eight hours Mg./100 ml. Rabbit Number						Mean Blood Level Observed in six rabbits over eight hours Mg./100 ml.
	1	2	3	4	5	6	
Liquid paraffin	0.59	0.59	0.48	0.35	0.39	0.39	0.47
White soft paraffin	0.72	0.73	0.75	0.63	0.60	0.55	0.66
Woolfat	0.50	0.43	0.50	0.38	0.35	0.33	0.41
Lard	0.88	1.03	0.91	1.02	0.82	0.75	0.89
Ethyl oleate	0.81	0.98	0.56	0.41	0.49	0.70	0.66
Propylene glycol	1.07	0.76	0.54	0.54	0.55	0.60	0.68
Water as 5 per cent carboxy methyl cellulose	0.28	0.28	0.29	0.48	0.44	0.37	0.36
Hydrous ointment	0.46	0.78	0.77	0.78	0.60	0.66	0.68
Emulsifying ointment	0.48	0.56	0.53	0.39	0.48	0.35	0.47
Hydrous emulsifying ointment	0.36	0.36	0.39	0.41	0.41	0.62	0.42
Cetomacrogol emulsifying wax base	0.55	0.52	0.56	0.55	0.44	0.53	0.52

TABLE IV

Comparison of Percutaneous Absorption of Sulphanilamide from different vehicles and bases in the Rabbit

Table of Efficiencies

Base	Mean Blood Level Observed in Six Rabbits over eight hours Mg./100 ml.	Efficiency Value
Lard	0.89	2.48
Propylene glycol	0.68	1.89
Hydrous ointment	0.68	1.89
White soft paraffin	0.66	1.83
Ethyl oleate	0.66	1.83
Cetomacrogol emulsifying wax base	0.52	1.44
Emulsifying ointment	0.47	1.31
Liquid paraffin	0.47	1.31
Hydrous emulsifying ointment	0.42	1.17
Woolfat	0.41	1.14
Water as 5 per cent carboxy methyl cellulose	0.36	1.00

TABLE V

Total Amount of three drugs Observed in the blood of Six Rabbits from various vehicles and bases

The Average of Duplicated Experiments Entered

Mg./100 ml.

Vehicle or Base	Drug	Litter	Result	Average
Lard	Salicylic Acid	G-L	107.50 : 115.93	111.72
White soft paraffin	Salicylic acid	102-107	98.50 : 105.02	101.76
Hydrous ointment	Salicylic acid	102-107	116.11 : 108.20	112.16
Propylene glycol.	Salicylic acid	102-107	129.70 ; 107.92	118.81
Water as 5 per cent carboxy methyl cellulose	Salicylic acid	G-L	79.91 : 89.26	84.59
Emulsifying ointment	Salicylic acid	A-F	86.47 : 101.14	93.81
Water as 5 per cent carboxy methyl cellulose.	Copper sulphate	102-107	16.93 : 18.00	17.47
Emulsifying ointment	Copper sulphate	102-107	20.24 : 19.86	20.05
Lard	Copper sulphate	102-107	21.64 : 20.39	21.02
Lard	Copper acetyl-acetonate	A-F	23.59 : 27.45	26.52
Lard	Copper acetyl-acetonate	G-L	25.34 : 24.21	24.78
Water as 5 per cent carboxy methyl cellulose.	Copper acetyl-acetonate	A-F	17.22 : 18.15	17.68
Emulsifying ointment	Copper acetyl-acetonate	G-L	20.75 : 22.85	21.80
Lard	Sulphanilamide + Copper sulphate	G-L	64.77	64.77
Lard	Sulphanilamide + Salicylic acid	A-F	82.94	82.94

TABLE VI

Comparison of Percutaneous Absorption of four drugs from selected vehicles and bases in the Rabbit.

Drug	Base	Total Amount observed in the blood of 6 individual rabbits over 8 hours (each value is the sum of 16 samples) Mg./100 ml. Rabbit Number.						Total Amount observed in 6 rabbits over 8 hours Mg./100 ml.
		1	2	3	4	5	6	
Salicylic acid	Lard	17.56	18.04	19.80	19.42	18.15	18.72	111.69
	Emulsifying Ointment	13.71	13.47	16.18	15.86	17.33	17.27	93.82
	Water as 5 per cent carboxy methyl cellulose	14.45	13.65	13.02	16.42	14.78	11.78	84.10
Sulphanilamide	Lard	14.14	16.05	14.54	16.24	13.03	11.92	85.92
	Emulsifying Ointment	7.62	9.08	8.45	6.27	7.71	5.57	44.70
	Water as 5 per cent carboxy methyl cellulose	4.46	4.47	4.64	7.67	7.19	5.93	34.36
Copper acetyl-acetonate	Lard	3.96	4.05	4.54	4.19	3.85	4.24	24.83
	Emulsifying Ointment	3.71	3.67	3.78	3.42	3.48	3.74	21.80
	Water as 5 per cent carboxy methyl cellulose	3.03	2.97	2.99	2.94	2.74	3.00	17.67
Copper sulphate	Lard	3.40	3.37	3.47	3.18	3.78	3.83	21.03
	Emulsifying Ointment	3.27	3.41	3.35	3.21	3.17	3.65	20.06
	Water as 5 per cent carboxy methyl cellulose	2.99	2.75	2.77	2.94	2.89	3.21	17.55

TABLE V11

Comparison of Percutaneous Absorption of four drugs from selected vehicles and bases in the Rabbit.

Drug	Base	Mean Blood Level observed in the blood of 6 individual rabbits over 8 hours Mg./100 ml. Rabbit Number 1	2	3	4	5	6	Mean Blood Level observed in 6 rabbits over 8 hrs. Mg./100 ml.
Salicylic acid	Lard	1.09	1.13	1.24	1.21	1.13	1.17	1.16
	Emulsifying Ointment	0.86	0.84	1.01	0.99	1.08	1.09	0.98
	Water as 5 per cent carboxy methyl cellulose	0.90	0.85	0.81	1.03	0.92	0.74	0.88
Sulph-anilamide	Lard	0.89	1.03	0.91	1.02	0.82	0.75	0.89
	Emulsifying Ointment	0.48	0.56	0.53	0.39	0.48	0.35	0.47
	Water as 5 per cent carboxy methyl cellulose	0.28	0.28	0.29	0.48	0.44	0.37	0.36
Copper acetyl-acetonate	Lard	0.25	0.25	0.28	0.26	0.24	0.26	0.26
	Emulsifying Ointment	0.23	0.23	0.24	0.21	0.22	0.23	0.23
	Water as 5 per cent carboxy methyl cellulose	0.19	0.19	0.19	0.18	0.17	0.19	0.19
Copper sulphate	Lard	0.21	0.21	0.22	0.19	0.24	0.24	0.22
	Emulsifying Ointment	0.20	0.21	0.21	0.20	0.20	0.23	0.21
	Water as 5 per cent carboxy methyl cellulose	0.19	0.17	0.17	0.18	0.18	0.20	0.18

TABLE V111

Comparison of Percutaneous Absorption of three drugs from selected vehicles and bases in the Rabbit

Water as a five per cent carboxymethyl cellulose gel, the least efficient vehicle, is taken as unity for all drugs

Drug	Base	Mean Blood Level Mg./100 ml.	Efficiency Value
Salicylic acid	Propylene glycol	1.24	1.41
	Hydrous Ointment	1.16	1.32
	Lard	1.16	1.32
	White soft paraffin	1.05	1.20
	Emulsifying ointment	0.98	1.11
	Water as five per cent carboxymethyl cellulose	0.88	1.00
Copper adetyl-acetonate	Lard	0.26	1.37
	Emulsifying Ointment	0.23	1.21
	Water as five per cent carboxymethyl cellulose	0.19	1.00
Copper sulphate	Lard	0.22	1.22
	Emulsifying Ointment	0.21	1.17
	Water as five per cent carboxymethyl cellulose	0.18	1.00

TABLE 1X.

Table of Comparative Efficiencies

The Percutaneous Absorption of four drugs from three vehicles of bases in the Rabbit

The blood level for Copper sulphate in water as five per cent carboxymethyl cellulose is taken as unity

	Lard	Emulsifying ointment	Water as five per cent carboxymethyl cellulose
Salicylic acid	6.32	5.32	4.75
Sulphanilamide	4.85	2.54	1.95
Copper acetyl-acetonate	1.42	1.23	1.01
Copper sulphate	1.19	1.14	1.00

TABLE X.

The Effect of an irritant and an astringent on the Percutaneous Absorption of Sulphanilamide

Mg./100 ml.

	Total Amount Sulphanilamide Observed in the blood of Six Rabbits over eight hours	Percentage Decrease in Absorption
Lard containing ten per cent sulphanilamide	85.92	-
Lard containing ten per cent sulphanilamide and five per cent salicylic acid	82.94	3.5
Lard containing ten per cent sulphanilamide and ten per cent copper sulphate	64.77	24.6

The Analysis of Variance

The results obtained were statistically analysed to examine as far as possible the factors involved in percutaneous absorption.It is a valuable property of variance that if a process has a number of factors each making a contribution to the variance of the final result, then this total variance is equal to the sum of the component variances. This property is called additivness of variance makes possible the technique known as the analysis of variance whereby the total variance of a process can be analysed into its component factors, the relative importance of which can then be assessed.[212]

Analysing the components in these experiments consisted of examining a set of results to determine the variance between bases, between individual rabbits, between blood samples drawn at different times, between male and female rabbits, between rabbits of different weights and between rabits of different breeds. Several of these factors may interact with one another; for instance, the values for a rabbit-base, a base-rabbit or a rabbit-time interaction factor, if shown to be significant, may play an important part in the passage of drugs through the skin. When allowance has been made for the various factors, a component known as the residual variance is still required to constitute the total variance. The residual may be

interpreted as an estimate of experimental error; the smaller the value of the residual error the more sensitive the method.

Three breeds of rabbit, a Beveren litter, a Dutch litter and a Copenhagen White litter, were tested with three bases, Hydrous Ointment B.P., Emulsifying Ointment B.P. and Hydrous Emulsifying Ointment B.P., each containing 10 per cne sulphanilamide. Litter-mates were chosen since it has been found that the biological variation is less in families than in animals drawn at random from a population. A complete statistical analysis of variance was carried out on the results obtained from each litter. For comparison with other vehicles and bases the results from the Copenhagen White litter were quoted. Due to the deaths of two of the test animals while the tests were being carried out it proved impossible to replicate the determinations for sulphanilamide on the litter of Copenhagen White rabbits. Since each base had only been tested once the statistical treatment of the results were complicated when these were compared with those from the Dutch and Beveren litters. The mean blood levels and the efficiencies of these ointments tested on each of the litters are given in Table XI.

As the work progressed it was found that the residual

variance dropped in value until it became constant. This implied that the experimental error decreased until reaching a constant value. Unfortunately, this complicated the interpretation of the results obtained from rabbits of different breeds. Accordingly it cannot be stated with certainty that a significant difference exists in the response of rabbits from different breeds since the results also warrant the conclusion that the variation may be partly due to the large difference in the residual variances for each breed. The fact that the residual for the Beveren litter, 0.35, is more than twice that for the Dutch, 0.15, may be explained by the fact that when carried out on the Beveren, the experimental technique had not reached the stage of refinement attained later in the tests conducted on the Dutch and Copenhagen White litters. Nevertheless, the general conclusions emerging from the analysis on the Beveren are still of value and confirm those from the other litters.

The full analysis of variance for each breed and the treatment of the results required for this analysis are shown in Appendix Two. A summarised table of the analyses is given in Table XII.

TABLE XI

Comparison of Percutaneous Absorption of Sulphanilamide from three ointments in Rabbits of different breeds.

Base	Hydrous Ointment	Emulsifying Ointment	Hydrous Emulsifying Ointment
		Mean Blood Level Mg./100 ml.	
Litter			
Beveren	1.36	1.03	0.58
Dutch	0.97	0.67	0.57
Copenhagen White	0.68	0.47	0.42
		Order of Efficiency	
Litter			
Beveren	2.34	1.78	1.00
Dutch	1.70	1.18	1.00
Copenhagen White	1.62	1.12	1.00

TABLE XII

A summary of the analysis of variance carried out on the results obtained with sulphanilamide from three breeds of rabbit.

Litter	Beveren	Dutch	Copenhagen
Scource of Variance		Mean Squares	
Between bases	21.9539**	4.8105**	1.7519**
Between rabbits	0.3500*	0.2507	0.1258
Between times	1.2851	0.2179	0.1011**
Base-Time	1.0929***	0.1428	0.0380
Base-Rabbit	2.4917**	0.4088**	0.1985**
Rabbit-Time	0.3558	0.0694**	0.0434
Base x Time x Rabbit	0.4086	0.1207	0.0508
Residual	0.3489	0.1559	-

The sources of variance which are asterisked are those which give significant values and which play an important part in the percutaneous absorption of sulphanilamide.

The source of variance between bases is significant in all three analyses and it can therefore be said that the vehicle or base exerts an important effect on the blood level of sulphanilamide attained. This result establishes that

vehicles and bases vary in their efficiency as "carriers" of sulphanilamide and that these differences are large enough to be detected by the method used. The source of variance between rabbits and between times are shown to be significant in the Beveren and Copenhagen White Litters respectively. The response between rabbits in the Beveren litter is small, being significant only at the five per cent level. Since the conclusion was never confirmed the significance of this negative correlation may be ignored and is almost certainly due to errors inherent in the early stages of an experimental technique. The source of variance between times in the Copenhagen White litter shows that the response of the rabbits at different times varied significantly and is due to the fluctuation in blood concentrations. The fluctuations might be due to an irregular rate of removal of sulphanilamide from the blood to the liver but at the present time the phenomenon cannot be adequately interpreted.

The significance of the interaction factors probably have several causes. The significance of the Base-Time factor in the Beveren litter means that the time at which the concentration of drug reaches a specific blood level is a function of the base. This finding was not repeated but where sharp differences in efficiency are observed between

bases this interaction factor may be expected since the blood levels at corresponding times will also differ sharply. The differences in efficiency values of the three bases, shown in Table XI,were most clearly marked with the Beveren litter.

The rabbit-base interaction is one which has been repeatedly shown to be significant and the source of variance due to this factor is as important as that due to the source between bases. This finding illustrates the variation found in animals and shows clearly that individual animals respond differently to the same base. These responses are represented in Figure II. Despite this difference in response, the difference in efficiency between the vehicles and bases applied can still be detected by the animals and the method may be considered valid as an assay.

The rabbit-time interaction is smaller than the residual in all three analyses and in the case of the Dutch litter the ratio of one to the other is highly significant. This is readily explained by a difference in time of response to the different bases and leads to negative correlation. The results from the Dutch litter show that where the blood level in one rabbit rises over a period of time, the blood level in another rabbit falls in the same period of time.

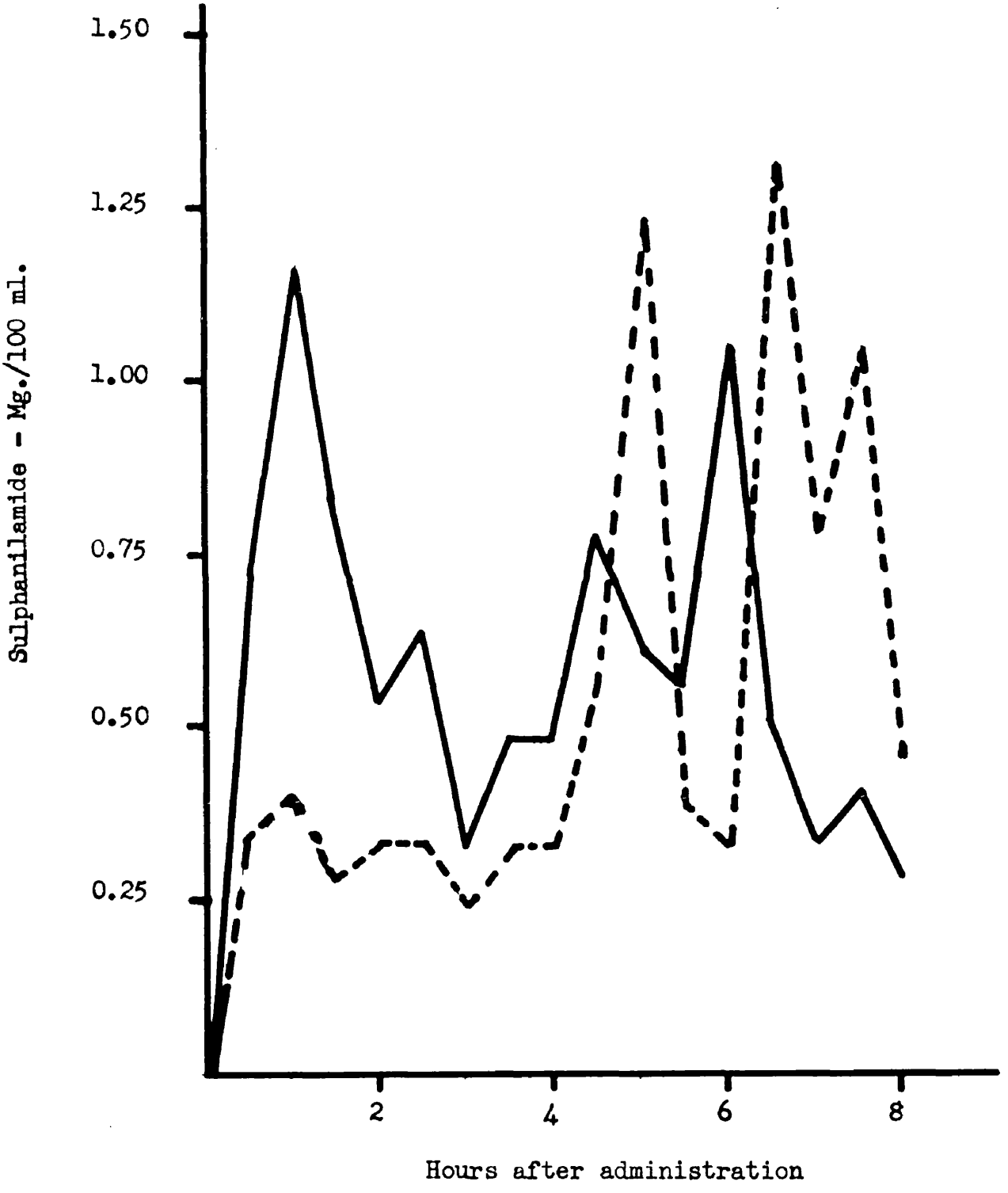
Figure V, the blood levels of sulphanilamide obtained on rabbits 74 and 75 following the application of emulsifying ointment, illustrates this point. The fluctuations are "out of step" and negative correlation results since the increase in concentration in one rabbit is greater than the decrease in the other rabbit at the same time. This factor again emphasises the fluctuation in blood levels observed for drugs applied percutaneously.

Table XIII shows the analysis of variance carried out on the results of the series of experiments on sulphanilamide as shown in Table II. Only the significant source of variance has been shown. The Standard Deviation corresponding to the residual variance of 3.8969 is 1.97 giving a standard deviation for means of samples of six equal to 0.81. This value may be used as a rough index for significant differences between the vehicles and bases tested with sulphanilamide. A difference between the vehicles and bases in the mean individual rabbit response of less than 0.81 is unlikely to be significant. A difference to this response of more than 0.81 is likely to be significant.

A separate analysis of variance was carried out on the results obtained from the series of experiments with salicylic acid, copper acetyl-acetonate and copper sulphate

Figure V

The blood levels observed in two Rabbits following the application of Emulsifying Ointment B.P. containing ten per cent sulphanilamide



in lard, emulsifying ointment and the five per cent aqueous carboxymethyl cellulose gel. This analysis is given in Table XIV. It should be noted that the wide variation in the level of reaction to the different drugs and the wide variation in the variances makes the result of an analysis of variance rather more difficult to interpret than in the orthodox case. Since the determinations for sulphanilamide were not replicated on water as a methyl cellulose gel these results were excluded from the analysis which thus became a two factor analysis with replication. The differences between drugs was very highly significant although the difference between the two compounds of copper as tested by the t-test was not significant. This implied that there was a very large difference between the vehicles and bases containing salicylic acid and those containing copper. The drug-base interaction factor was significant at a level between $P=0.01$ and $P=0.05$. Examination showed that this was almost entirely due to the unexpectedly high efficiency of the salicylic acid-lard interaction. The apparently large differences between the bases was found to be due almost entirely to this interaction and when allowance was made for this, the differences between the bases was found to be insignificant.

Incorporation of the sulphanilamide non-replicated results modified these conclusions as follows:

(i) There were significant differences between salicylic acid, sulphanilamide and the two compounds of copper (although these did not differ significantly from each other)

(ii) Lard is particularly effective as a "carrier" for both salicylic acid and sulphanilamide.

(iii) The experiments have not been sufficiently sensitive to give differences between vehicles and bases large enough to produce significant results in the analysis. Nevertheless, when account is taken of the manner in which differences between the bases occur (lard was always shown to be more efficient than emulsifying ointment which was always more efficient than the five per cent acqueous carboxymethyl cellulose gel) it is highly probable that more extensive and refined experiments would show such differences between bases to be significant.

TABLE XIII

The Analysis of Variance carried out on the results obtained with sulphanilamide in various vehicles and bases.

Source of Variance	Sum of Squares	Degree of Freedom	Variance
Between bases	397.834	10	39.7834
Residual	214.332	55	3.8969

$$F = \frac{39.7834}{3.8969} = 10.21$$

F = 3.7 corresponds to P = 0.001

The Standard Deviation corresponding to the residual variance of 3.8969 is 1.97

This gives a Standard Deviation for means of samples of six equal to 0.81

$$\frac{1.97}{\sqrt{6}} = 0.8059 = 0.81$$

TABLE XIV

The Analysis of Variance carried out on the results obtained with various drugs in selected vehicles and bases

Source of Variance	Sum of Squares	Degree of Freedom	Mean Square	F	Remarks
Between drugs	21351	2	11576	116	F=61.3 for P=0.001
Between bases	489.8	2	224.9	2.72	F=6.9 for P=0.05
Drug : Base interaction	359.6	4	89.89	4.42	F=3.6 for P=0.05 F=6.4 for P=0.01
Residual	182.8	9	20.31		

DISCUSSION

Salicylic acid, sulphanilamide, copper acetylacetonate and copper sulphate, when incorporated in various vehicles and bases penetrate the intact healthy skin of rabbits in varying amounts and circulate in the bloodstream in measurable quantities. In all cases the amount of drug circulating in the bloodstream is small. The highest average blood level in any one rabbit was observed with salicylic acid in propylene glycol as a vehicle and gave a value of 1.39 mg. salicylate per 100 ml.; the lowest value was that observed with copper sulphate in water as a five per cent carboxymethyl cellulose gel, and gave a value of 0.18 mg. copper per 100 ml.

Histological work has shown that the principle route of percutaneous absorption is by way of the hair follicles and sebaceous glands[129]. In the upper half of the follicular canal the hair shaft does not adhere to the follicular wall, the space between being loosely filled with greasy horny scales and air; the duct of the sebaceous gland empties the sebum, a mixture of triglycerrides and fatty acids, into this passage.

The application of vehicles and bases to intact skin allows penetration of the incorporated drug to the

dermis by way of the side walls of the follicles and the sebaceous glands. Systemic absorption can thus occur without rupture of the epidermis. The effect of the vehicle, the effect of the drug, and the interaction between the two are the three aspects of penetration and absorption which will be considered.

The effect of the vehicle or base is shown by the results obtained for sulphanilamide which was incorporated in eleven vehicles and bases. Theoretically, a vehicle promotes absorption through the follicles by diminishing the tension between the base and the follicular pore, allowing a continuous surface layer to be maintained, and by mechanically bringing the drug into contact with the follicle or pressing it into the follicular canal. Drugs are thus brought more rapidly and in larger amounts to the absorbing surface of the follicular walls and sebaceous glands.[213] This effect is best achieved with vehicles and bases which are, or become, liquid at body temperatures. The results in Table IV support this view. Lard, propylene glycol, hydrous ointment and ethyl oleate from which mean blood levels of sulphanilamide varying between 0.66 and 0.89 mg./100 ml. were obtained had high efficiency values. These applications were either liquid or non-viscous and

permeated the outer surface of the skin readily. An unexpected finding was the relatively high efficiency of white soft paraffin, a result which has been confirmed by Hadgraft, Somers and Williams[214] using diiodofluorescein. It has been generally assumed that the inert hydrocarbons serve merely as a protective coat but the blood levels of drugs attained following their application in this vehicle may be explained by the fact that this substance is liquid at body temperature and consequently aids the transport of the drug to the site of absorption. On the other hand, woolfat, a thick viscous vehicle which does not spread easily, has a low efficiency value as a carrier despite the fact that its chemical composition is not dissimilar to sebum. Presumably woolfat does not promote absorption because of its viscosity which may hinder both its penetration of the follicles and the release of the drug. Rothman[213] has shown that absorption from bases containing cholesterol, a constituent of woolfat, is poorer than that from bases containing no cholesterol. Liquid paraffin gave a mean blood level of 0.47 mg./100 ml. and showed comparatively low efficiency as a carrier although it was a smooth easily applied application. This low value may be explained by assuming that a layer of moisture, formed by insensible

perspiration on the back of the rabbit, forms a thin liquid film between the paraffin and the skin. Since the skin is water repellent, penetration and absorption of sulphanilamide would be hindered by this additional barrier. It is also possible that the rate at which the drug separates from the vehicle in the follicle has a significant effect on the degree of absorption.

A difference in efficiency was observed between the two emulsion bases as carriers. The absorption from hydrous ointment, a water-in-oil emulsion, gave a mean level of 0.68 mg./100 ml., a value approximately one-and-a-half times greater than that for hydrous emulsifying ointment, an oil-in-water emulsion, which gave a mean blood level of 0.42 mg./100 ml. A similar conclusion has also been reported by Hadgraft, Somers and Williams[214], who found hydrous ointment more than twice as efficient as hydrous emulsifying ointment when labelled diiodofluorescein was the incorporated drug. This suggests that the absorption of drugs is enhanced when they are presented to the intact skin in an oil phase. Since the skin surface is covered with a greasy layer of waxes impervious to water and aqueous solutions, the absorption of sulphanilamide occuring from the aqueous phase must be due to the partitioning of the drug between

the aqueous phase and the fats of the sebaceous glands and follicles through which the continuous oil phase of hydrous ointment will penetrate with greater ease. Estimating sulphanilamide in skin biopsies, Strakosch[132,133] found no significant difference between oil-in-water and water-in-oil emulsions but his method merely measured the "depot" of each emulsion lodged in the skin and took no account of the rate of drug release into the blood stream. The view that the absorption of sulphanilamide is enhanced when presented to the skin in an oil phase is supported by the amount absorbed from water as a carboxymethyl cellulose gel, which as a "carrier" of sulphanilamide was the least effective vehicle tested, having a mean blood level of 0.36 mg./100 ml. This may be regarded as the absorption of sulphanilamide from water since the methyl cellulose served merely as a convenient method of applying water as a vehicle. It therefore appears that a continuous water phase hinders the absorption of sulphanilamide through the intact skin.

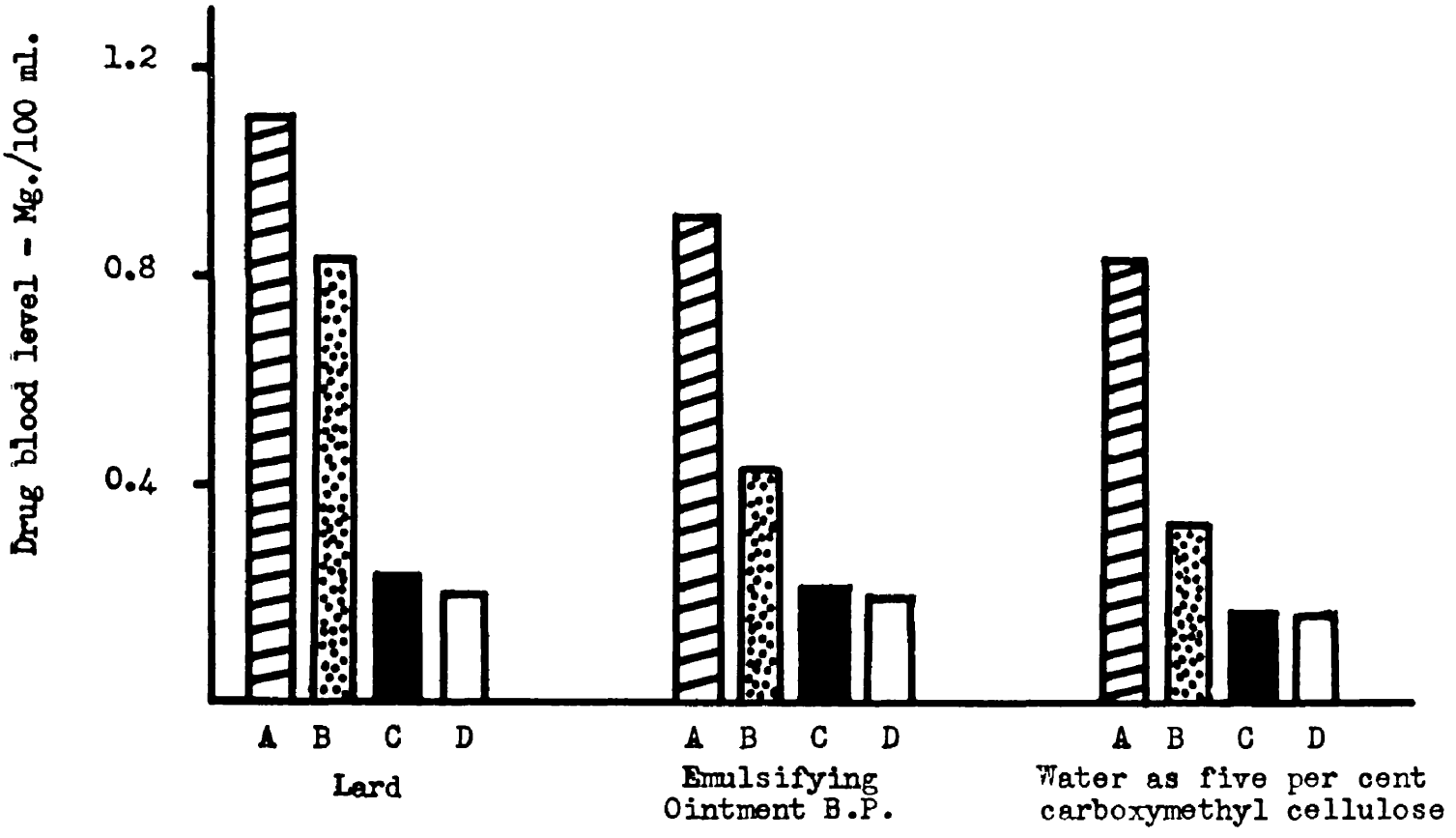
The effect of the drug was estimated by comparing the results obtained when four different drugs, salicylic acid, sulphanilamide, copper acetyl-acetonate and copper sulphate, were incorporated in three different vehicles

and bases, lard, Emulsifying Ointment and water as a five per cent carboxymethyl cellulose gel. From Tables VI and VII it is seen that salicylic acid is absorbed in greater amounts than the other drugs. The mean blood level for the preparations tested with salicylic acid range from 0.88 to 1.24 mg./100 ml.; those for sulphanilamide from 0.36 to 0.89 mg./100 ml.; those for copper acetyl-acetonate from 0.19 to 0.26 mg./100 ml., and those for copper sulphate from 0.18 to 0.22 mg./100 ml. Table IX, a summary of the comparative efficiencies of the drugs and vehicles and bases, show roughly six times as much salicylic acid, three times as much sulphanilamide, one-and-a-quarter times as much copper acetyl-acetonate absorbed as copper sulphate. Each of the copper compounds was estimated as copper. The differences in efficiency are illustrated in Figures VI and VII.

The molecular weights of salicylic acid, sulphanilamide, copper acetyl-acetonate and copper sulphate are 138.13, 172.21, 261.78 and 249.70 respectively. It can be seen that were the blood levels calculated on a molar basis the relative rates of absorption of the four compounds would occur in the same order.

Figure VI

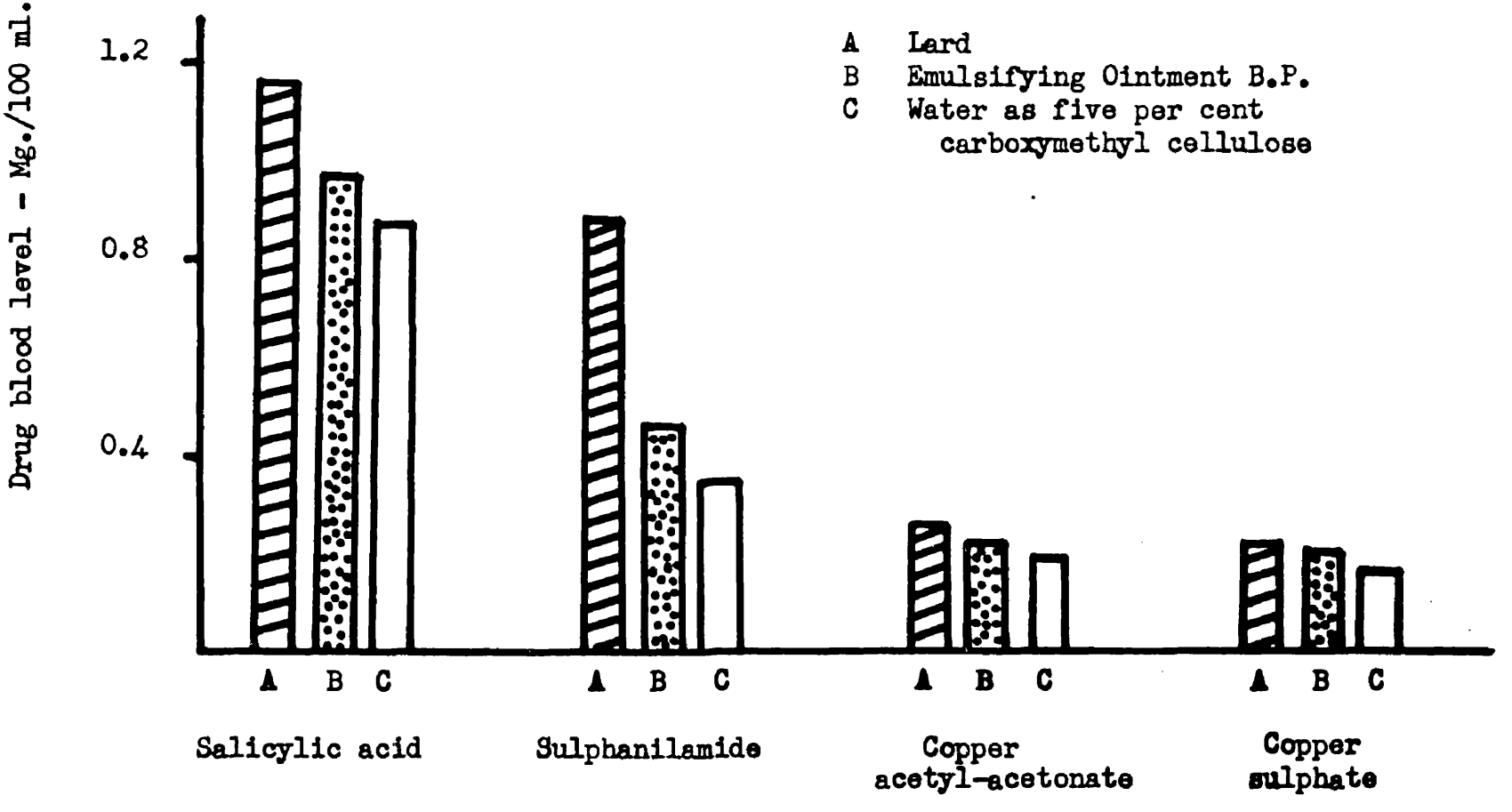
Comparison of the percutaneous absorption of four drugs from selected vehicles and bases



A Salicylic acid
B Sulphanilamide
C Copper acetyl-acetonate
D Copper sulphate

Figure VII

Comparison of the percutaneous absorption of four drugs from selected vehicles and bases



It is possible to offer an explanation of the differences in the amounts of the four drugs absorbed from the three bases in terms of their local action and lipid solubilities. A relationship between the drug and the living cell was first postulated in 1902 by Meyer and Overton who claimed that the cell membrane, which a drug must permeate before being absorbed systemically, was lipid in nature since only those substances soluble in lipids penetrated the cell wall. Their degree of penetration appeared to depend on their degree of solubility in lipids. Based mainly on the absorption of alcohols, aldehydes, ketones and lipid soluble narcotics, the theory was found to have many exceptions and the cell membrance was then thought to be a mosaic of lipid and protein particles. Although this theory failed to explain many experimental results it seems to be firmly established that the ratio of the solubility of a substance in water to that in lipids plays an important part in its passage through the cell and consequently through the follicular walls and

sebaceous glands.

Compared with the other drugs tested, the high blood levels obtained for salicylic acid may well be due to its lipid solubility, shown by the readiness with which the acid dissolves in alcohol, ether, glycerol and chloroform. This property will ease the passage of the acid through the fats and waxes of the sebum and follicular walls. Further evidence for this view was advanced by Kahlenberg and Barwasser[168] who noted that sodium salicylate was never observed to penetrate intact skin. Describing the penetration of topical agents into dead human skin, Flesch, Satanove and Brown[216] found salicylic acid to penetrate most rapidly while no passage of the sodium salt was observed. Moreover, the irritant and keratolytic action of the acid on the skin and its destruction of the epithelial cells which soften and desquamate following the acid's application are factors which will tend to increase systemic concentrations.[125,126] Damage by salicylic acid to the outer layer of the skin may allow greater penetration of the epidermis while the irritant effect produces dermal hyperaemia and alters the transfer gradient in favour of greater drug passage when the acid has penetrated to the vascular bed. Localised vasodilatation was observed after removal of the

salicylic acid preparations from the backs of the animals. Sulphanilamide, although lipid soluble, is neutral and appears to exert neither an irritant nor an astringent effect when applied topically and probably produced no reactions which would increase or decrease percutaneous absorption. The lower blood levels of sulphanilamide obtained in comparison with those of salicylic acid may be explained on this basis. In contrast, copper is astringent and is used locally for its precipitant action on protein. This action affects the surface cells and consequently reduces permeability. The low amounts of copper absorbed into the blood stream 0.18 to 0.26 mg./100 ml., tend to confirm this effect. The slightly higher systemic levels of copper acetyl-acetonate, 0.19 to 0.26 mg./100 ml., might be due to the non-ionic character of the copper which is chelated with acetyl-acetone molecules to increase its lipid solubility in contrast to the ionic character and lipid insolubility of the sulphate which gave blood levels of 0.18 to 0.22 mg./100 ml. Apart from the lipid insolubility of the sulphate, the electrically negative charge on the outer surface of the skin would tend to repel the polarised sulphate and reduce even further the amount of copper absorbed.

It should be stated that the estimation of copper and salicylic acid involve assaying substances normally present in the body. While large numbers of blood blanks were assayed to allow for this, the absorption of small quantities may remain undetected because of the fluctuation of these substances in the animal circulation. Consequently, the assay of both salicylic acid and copper lack the specificity of that for sulphanilamide, a factor to be considered in the evaluation of the results. Nevertheless, that individual drugs differ greatly in their ability to penetrate intact skin is clear, and this difference is more marked than the differences in efficiency between the vehicles or bases as "carriers" in which the drugs are incorporated. This finding confirms the conclusion of Bliss[217] who stated that "the properties and powers of the drug itself rather than the ointment vehicle are the major determining factors in absorption from the skin" and of Plein and Plein[218] who noted that "drugs vary greatly in their ability to penetrate through normal intact skin".

A result of great interest was the interaction between drug and base. From a set of observed results it is possible to construct a table of theoretical values and, on comparing these with the observed values, plot a table

showing the positive and negative percentage deviation of the observed from the theoretical. If an observed value differs significantly from its theoretical value then an interaction factor is said to be functioning. Significantly positive deviation was noted for the salicylic acid:lard, sulphanilamide:lard and copper sulphate:water, as a carboxymethyl cellulose gel, interactions, this meaning that the interaction of these drugs with these bases promoted their percutaneous absorption in amounts greater than that which would have been expected on theoretical grounds. Significantly negative deviation was noted for the copper sulphate:lard and the salicylic acid: water, as a carboxymethyl cellulose gel, interactions, this meaning that less was absorbed than would have been theoretically expected. These conclusions imply that while the properties of the drug and the base are of great importance, the interaction between the two also determines the blood levels subsequently attained. In these experiments, the interactions occurred positively when the incorporated drug was soluble in the base, and negatively when the incorporated drug was insoluble in the base. Coper sulphate, freely soluble in water, is readily soluble in the methyl cellulose gel and insoluble in lard. Similarly salicylic acid, being lipid soluble, dissolves in lard and remains insoluble in water as a carboxymethyl cellulose gel. This strongly

suggests that a lipid soluble drug incorporated in a lipid base will be absorbed in greater quantities than a lipid insoluble drug in a lipid base. On this ground, the interaction of the drug with the base must be considered a factor of importance in percutaneous absorption through intact skin.

When compared with oral, subcutaneous and intravenous administration, percutaneous therapy must be considered inefficient and variable on the basis of the results quoted. As shown in Figure IV, the oral method was sixteen times and the subcutaneous and intravenous methods six and five times respectively more efficient than the percutaneous route. Furthermore, these differences could be increased simply by injecting a concentrated solution of soluble sulphonamides or by increasing the oral dose. The levels attained percutaneously from these tests varied between 0.18 and 1.24 mg./100 ml. for all drugs and bases, and unless the outer layers of the skin were damaged the levels of drug obtained percutaneously is unlikely to rise above 2 or 3 mg./100 ml. Blood levels which have been reported over 3 mg./100 ml. following the topical application of substances to intact skin should be viewed with caution but where the skin is wounded, burned or diseased, systemic concentrations of this order and higher may be expected. It is interesting to note that for prophylaxis the blood level of sulphanilamide

considered adequate is 2 to 3 mg./100 ml.[219], four to six times higher than the average level observed for this drug when applied in eleven separate vehicles.

However, a drug exerting a pharmacological action at low systemic levels, may find limited use in percutaneous therapy.[96-105] Several workers have observed pharmacological effects following the percutaneous application of sex hormones. To exert their characteristic action, these hormones, the majority of which are lipoid soluble, require a blood level as low as, or even lower than 0.5 mg./100 ml. Judging by the results obtained in the above experiments, it is more than probable that such a level was attained with soft paraffin, ether and benzene, the vehicles and solvents in which these workers incorporated the hormones used. Valette and Etcheverry[220] have recently noted the analgesic effect of morphine and pethidine when applied in p-cymene, and organic solvent, to the intact skin of mice. The blood levels required to produce such a pharmacological response are less than 0.5 mg./100 ml., again one which appears to be easily attained with organic solvents. There is general agreement that the blood levels obtained from drugs applied to intact skin in organic solvents are higher than those obtained following the use of conventional ointment bases.

For systemic concentrations greater than approximately 1 mg./100 ml., percutaneous medication through intact skin, on the basis of the results obtained in these experiments, must be considered of dubious value, the more so since it is almost impossible to control quantitatively. The conclusions of Zondek, Bromberg and Shapiro[87], that the "concentration of sulphanilamide in the blood of rabbits following its percutaneous administration is as high as that obtained after its oral administration" and that "the percutaneous use of sulphanilamide may serve as an auxiliary method of chemotherapeutic treatment in certain cases" cannot be substantiated from the results quoted here. These workers found a maximum sulphanilamide blood level as high as 8 mg./100 ml. after applying the drug in an acetone-soap solvent. When allowance is made for the greater efficiency of organic solvents, such levels still seem remarkably high.

Owing to the variation in technique used, it is difficult to compare strictly the results from the experiments described with those of other workers in the field of percutaneous medication. Systemic absorption has been studied by estimating the concentration of applied drug either in the blood or the urine. It has also been expressed

as a percentage of the amount topically applied, especially in experiments using radio-active tracers.[214] The major disadvantage of this calculation lies in the fact that the amount applied topically is in excess of the amount absorbed. While this excess may be varied the amount penetrating remains approximately constant and consequently this variation in the excess applied affects any quantity expressed as a percentage.[86] Woodward and others, for instance, showed that for sulphanilamide no increase in blood levels resulted from increasing the strength of the ointment from five per cent to twenty per cent. Expressing the amount absorbed as a percentage, the results will show a four-fold difference for a systemic concentration which is the same for both ointments. This method of expression, cautiously interpreted, is useful in that it illustrates the inefficiency of percutaneous medication as a mode of administration.

Table XV shows the blood levels observed by other workers following the percutaneous application of certain drugs. Since the drug exerts an effect greater than the vehicle in which it is applied, the bases used have been omitted and the range of blood levels entered.

TABLE XV

The Blood Levels obtained following the application of certain Drugs to Intact Skin.

Workers	Drug used	Blood Level Observed Mg./100 ml.
Zondek et al.[87]	Sulphanilamide	1 - 8
Plein and Plein[218]	Salicylic acid	3 - 7
Gemmell and Morrison	Salicylic acid	0.88 - 1.24
	Sulphanilamide	0.36 - 0.89
	Copper	0.18 - 0.26
Borelli[221]	Chloramphenicol	0.01 - 0.05

Table XVI shows the order of efficiency obtained by two groups of workers from various vehicles and bases in which different drugs were incorporated. The least efficient base in both sets of results, hydrous emulsifying ointment, was taken as unity and although the experimental techniques were not strictly comparable the results are of some value for general comparative purposes.

TABLE XVI

Comparison of Percutaneous Absorption of two drugs from the same vehicles and bases

Workers	Hadgraft, 214 Somers & Williams	Gemmell and Morrison
Drug	Diiodofluorescein	Sulphanilamide
Base		
Lard	1.82	2.10
Cetomacrogol	2.78	1.27
Hydrous ointment	2.27	1.59
Soft paraffin	1.23	1.51
Hydrous emulsifying ointment	1.00	1.00

The order of efficiency of the same vehicles and bases tested with various drugs by several workers are shown in Table XVII

TABLE XVII

The Comparative Efficiency of three bases tested with various drugs by several workers.

Workers	217 Bliss	154 Wild and Roberts	Gemmell and Morrison	190 Brown and Scott		176 Sauerland
Drug	Methyl sal.	Mercuric oxide	Sulphanil-amide	Methyl salicylate	Methyl sal.	Spiro-sol
Base						
Lard	1.09	3.47	2.15	0.95	1.46	0.11
Soft paraffin	1.04	1.17	1.60	0.92	1.37	0.80
Lanolin	1.00	1.00	1.00	1.00	1.00	1.00

In Table XVII, the results between workers vary considerably, this being due to the drug and the experimental method used. It may be stated that lard was generally found to be more efficient than soft paraffin. The results found for the efficiency of lanolin are more conflicting. The findings of Bliss, Brown and Scott and Sauerland, illustrate the variation encountered when the same drug, methyl salicylate, is tested in the same vehicles by different workers. Despite this, the difference in the ability of individual drugs to penetrate intact skin can be seen to be considerable.

A further source of error in the results of experiments reporting the efficiency of vehicles and bases lies in the fact that only one or a few blood samples were withdrawn from the test animals. The variation within individual animals is wide and reliable estimates of the efficiency of topical preparations can be made only if large numbers of samples are assayed. Hitherto workers appear to have evaluated vehicles and bases on a few blood samples but never in numbers large enough to allow for the variation occurring. Since the systemic concentrations have been observed to fluctuate and show between one and six hundred per cent differences in corresponding samples, any evaluation of the efficiency of a vehicle or base estimated on a few blood

samples must be judged meaningless.

The results obtained for salicylic acid, sulphanilamide, copper acetyl-acetonate and copper sulphate, when incorporated in various vehicles and bases, emphasise that the intact healthy skin of the rabbit presents an effective and almost impermeable barrier to the passage of drugs applied to it, especially when it is considered that the area exposed to the applications, 24 square inches, approximates to one-fifth of the body area of the animals under test.

Conclusions

Three main conclusions emerge from this work.

Firstly, it can be seen that the degree of percutaneous absorption of drugs is influenced by the vehicle or base in which the drug is presented to the skin. Nevertheless, the relative efficiencies of vehicles and bases as carriers of drugs through intact skin must not be overestimated since the amount of drug absorbed, even under the most suitable conditions, is so small that any differences in efficiency are of little practical value. In selecting vehicles or bases to promote or delay absorption of medicaments through intact skin, the choice may be determined by convenience and elegance.

Secondly, there is proof that the ability of different

drugs to penetrate intact skin and be absorbed systemically varies considerably. The choice of drugs selected in this work was inevitably limited by the ease and accuracy with which they could be detected in minute quantities in the bloodstream, but it is probable that greater differences than those observed between salicylic acid, sulphanilamide, copper acetyl-acetonate and copper sulphate, will be detected with other drugs where a suitable assay exists.

Thirdly, there is evidence that the interaction of a drug with the base or vehicle in which it is incorporated may either hinder or promote the percutaneous absorption of that drug. This interaction appears to be related to the solubility of the drug in the base. Where a lipid soluble drug, for example salicylic acid, is incorporated in a lipid soluble vehicle such as lard, the two tend to promote absorption and their properties may be said to be additive. Where both drug and base are lipid insoluble, for example copper sulphate and water as a five per cent carboxymethyl cellulose gel, the two properties tend to hinder absorption and low blood levels consequently result. Where the lipid solubility of the drug is the opposite of that for the base, for example copper sulphate in lard, the amount of drug absorbed percutaneously appears to be decreased.

SUMMARY

1. A review of the methods used to estimate the release of medicinal substances from topical applications has been carried out.

2. An *in vivo* test has been designed to estimate the efficiency of topical applications as "carriers" of drugs through intact skin. The blood level attained by the applied drug was taken as the criterion of efficiency.

3. Sulphanilamide was tested in eleven vehicles and bases and, taking the least efficient vehicle as unity, the following comparative values obtained: lard (2.48), propylene glycol (1.89), Hydrous Ointment B.P. (1.89), white soft paraffin (1.83), ethyl oleate (1.83), cetomacrogol emulsifying wax base (1.44), Emulsifying Ointment B.P. (1.31), liquid paraffin (1.31), Hydrous Emulsifying Ointment B.P. (1.17), woolfat (1.14), water as a five per cent carboxymethyl cellulose gel (1.00).

4. Salicylic acid, sulphanilamide, copper acetyl-acetonate and copper sulphate were respectively incorporated in lard, Emulsifying Ointment B.P. and water as a five per cent carbodymethyl cellulose gel, and the blood levels of the

drugs noted. Salicylic acid and sulphanilamide were respectively five and four times more efficient than the two compounds of copper. The results of all experiments were statistically analysed and the significant sources of variance noted.

5. The vehicle or base influences the blood level attained by the incorporated drug but since the amount absorbed through intact skin is so small, these differences in efficiency are of little importance. The ability of different drugs to penetrate intact skin and be absorbed systemically varies considerably. The interaction of a drug with the vehicle or base in which it is incorporated may either hinder or promote the percutaneous absorption of that drug.

PUBLICATIONS

From this work, the following papers have been published in conjunction with Mr. D.H.O. Gemmell.

The review of methods of assay of topical applications was presented as "The Release of Medicinal Substances from Topical Applications and their passage through the Skin". (J. Pharm. Pharmacol. 9, 641, 1957).

The results obtained with sulphanilamide were presented as "The Percutaneous Absorption of Sulphanilamide". (J. Pharm. Pharmacol. 10, 167, 1958).

The results for salicylic acid, copper acetyl-acetonate and copper sulphate were presented as "Comparative Studies in Percutaneous Absorption", (J. Pharm. Pharmacol. 10, 553, 1958).

A short paper entitled "Some Factors influencing Percutaneous Absorption" has been compiled and accepted for reading at the British Pharmaceutical Conference to be held at Llandudno, September, 1958.

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APPENDIX ONE.

TABLES OF

EXPERIMENTAL RESULTS

Base: Water as 5 per cent carboxy methyl cellulose containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	96	97	98	99	100	101	
Weight (Kg.)	2.450	2.900	2.650	3.700	3.450	2.925	Row
Sex	Female	Female	Female	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.41	0.45	0.60	0.31	0.41	0.38	2.56
1.0	0.10	0.46	0.55	0.61	0.52	0.29	2.53
1.5	0.21	0.35	0.43	0.49	0.50	0.30	2.28
2.0	0.30	0.23	0.15	0.51	0.61	0.29	2.09
2.5	0.28	0.26	0.24	0.56	0.71	0.45	2.50
3.0	0.10	0.25	0.51	0.31	0.58	0.50	2.25
3.5	0.31	0.31	0.38	0.49	0.50	0.46	2.45
4.0	0.25	0.30	0.21	0.68	0.38	0.52	2.34
4.5	0.21	0.14	0.23	0.49	0.41	0.31	1.79
5.0	0.35	0.11	0.00	0.37	0.37	0.47	1.67
5.5	0.15	0.31	0.14	0.42	0.40	0.33	1.75
6.0	0.47	0.36	0.40	0.50	0.45	0.49	2.67
6.5	0.40	0.31	0.13	0.43	0.30	0.45	2.02
7.0	0.21	0.15	0.31	0.59	0.41	0.25	1.92
7.5	0.31	0.18	0.21	0.41	0.38	0.24	1.73
8.0	0.40	0.30	0.15	0.50	0.26	0.20	1.81
Column Totals	4.46	4.47	4.64	7.67	7.19	5.93	34.36

Intravenous Injection of Sulphanilamide (5 mg./ml.)

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	102	103	104	105	106	107	
Weight (Kg.)	3.200	3.150	2.600	3.400	3.350	2.650	
Sex	Female	Female	Female	Female	Male	Male	Row Totals
Hours after Injection	80 mg. injected	80 mg. injected	65 mg. injected	85 mg. injected	85 mg. injected	65 mg. injected	
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	2.95	2.70	2.76	2.31	2.06	2.12	14.90
1.0	2.63	2.69	2.53	1.93	2.06	1.76	13.60
1.5	2.46	2.33	2.23	1.81	1.73	1.53	12.09
2.0	1.98	2.10	2.06	1.55	1.36	1.48	10.53
2.5	1.76	1.79	1.78	1.31	1.33	1.26	9.23
3.0	1.66	1.65	1.63	1.26	1.30	1.20	8.70
3.5	1.60	1.60	1.45	1.18	1.18	1.08	8.09
4.0	1.23	1.50	1.30	1.06	1.15	0.93	7.17
4.5	1.20	1.30	1.20	1.03	1.15	0.95	6.83
5.0	1.11	1.20	1.15	1.03	1.03	0.90	6.42
5.5	0.98	1.16	1.08	1.00	0.85	0.85	5.92
6.0	0.93	1.08	0.98	0.95	0.70	0.75	5.39
6.5	0.90	1.06	0.96	0.81	0.68	0.68	5.09
7.0	0.90	1.00	0.91	0.78	0.61	0.61	4.81
7.5	0.87	0.96	0.71	0.73	0.57	0.60	4.44
8.0	0.81	0.80	0.63	0.71	0.57	0.56	4.08
Column Totals	23.97	24.92	23.36	19.45	18.33	17.26	127.29

Subcutaneous Injection of Sulphanilamide (8 mgns./ml.)

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	102	103	104	105	106	107	
Weight (Kg.)	3.150	3.150	2.650	3.300	3.300	2.650	
Sex	Female	Female	Female	Female	Male	Male	Row Totals
Hours after Injection	130 mg. injected	130 mg. injected	110 mg. injected	135 mg. injected	135 mg. injected	110 mg. injected	
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	2.85	2.56	2.20	1.56	2.31	2.20	13.68
1.0	4.50	3.36	2.86	2.46	3.91	2.25	19.34
1.5	4.48	3.75	3.40	3.08	3.73	2.93	21.37
2.0	4.50	4.10	3.18	3.35	3.46	3.60	22.19
2.5	3.98	3.85	3.18	4.16	3.53	4.13	22.83
3.0	4.33	3.88	2.61	3.71	3.33	3.25	21.11
3.5	3.93	3.89	2.70	3.36	3.20	3.36	20.44
4.0	3.70	3.70	2.65	3.48	2.85	2.78	19.16
4.5	3.75	3.85	2.60	2.93	2.66	2.78	18.57
5.0	3.13	2.75	2.53	2.98	2.75	3.18	17.32
5.5	3.28	2.46	2.50	2.36	2.00	2.20	14.80
6.0	2.70	2.38	2.35	2.30	1.78	2.30	13.81
6.5	2.46	2.45	1.76	1.93	1.63	2.75	12.98
7.0	2.38	2.20	1.71	1.81	1.48	1.66	11.24
7.5	1.90	2.05	1.58	1.73	1.30	1.60	10.16
8.0	1.88	1.85	1.56	1.80	1.25	1.23	9.57
Column Totals	53.75	49.08	39.37	43.00	41.17	42.20	268.57

Oral Absorption of Sulphanilamide Concentration

500 mg. Tablet of Sulphanilamide per rabbit

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	102	103	104	105	106	107	
Weight (Kg.)	3.250	3.100	2.650	3.425	3.350	2.650	Row
Sex	Female	Female	Female	Female	Male	Male	Totals
Hours after Administration							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	9.89	11.50	11.19	8.02	6.61	7.21	54.42
1.0	14.46	13.13	11.90	10.10	9.03	8.46	67.08
1.5	12.83	14.56	10.35	12.01	10.66	13.05	73.46
2.0	12.35	13.03	10.56	13.14	8.68	10.83	68.59
2.5	11.90	13.53	9.21	11.96	9.31	11.16	67.07
3.0	12.30	11.70	9.15	11.66	8.88	10.96	64.65
3.5	11.53	11.20	8.83	11.50	7.56	9.70	60.32
4.0	10.33	11.60	8.63	10.96	8.33	10.51	60.36
4.5	9.89	10.91	7.96	9.78	7.75	7.43	53.72
5.0	9.67	10.98	7.81	8.66	7.25	6.66	51.03
5.5	8.55	9.65	7.83	8.01	6.36	5.55	45.95
6.0	7.43	9.66	7.10	7.96	5.44	5.41	43.00
6.5	6.53	7.66	6.70	7.50	5.60	4.68	38.67
7.0	6.33	6.53	5.83	6.25	4.56	4.03	33.53
7.5	5.10	6.35	4.51	5.73	4.40	3.48	29.57
8.0	4.47	5.21	4.18	5.00	3.82	3.11	25.79
Column Total	153.56	167.20	131.74	148.24	114.24	122.23	837.21

Base: Hydrous Ointment B.P. containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	48	49	50	51	52	53	
Weight (Kg.)	2.400	2.650	2.600	3.150	3.250	2.950	
Sex	Male	Male	Male	Female	Female	Female	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	1.44	1.07	1.68	1.47	1.57	1.58	8.81
1.0	2.75	1.65	0.81	1.92	0.74	1.79	9.66
1.5	1.70	0.45	1.40	0.86	0.89	0.86	6.16
2.0	0.25	0.23	1.05	2.63	0.85	0.86	5.87
2.5	1.75	0.12	0.00	0.53	0.65	0.46	3.51
3.0	1.39	1.21	0.93	0.35	0.65	0.56	5.09
3.5	0.43	1.82	1.06	2.92	1.26	0.93	8.42
4.0	3.48	0.40	0.61	1.01	1.30	0.90	7.70
4.5	1.02	0.41	2.78	1.21	1.29	1.04	7.75
5.0	0.95	0.56	0.94	1.51	0.95	0.79	5.70
5.5	1.68	0.31	1.59	2.54	2.04	1.52	9.68
6.0	3.37	0.40	2.58	3.62	2.46	1.73	14.16
Column Totals	20.21	8.63	15.43	20.57	14.65	13.02	92.51

Base: Hydrous Ointment B.P. containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration.

Mg./100 ml.

Rabbit No.	48	49	50	51	52	53	
Weight (Kg.)	2.475	2.525	2.900	2.850	3.500	2.925	Row
Sex	Male	Male	Male	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	2.86	3.58	2.57	0.60	0.46	0.96	11.03
1.0	2.04	5.56	0.42	3.10	1.55	2.96	15.63
1.5	2.38	1.08	1.09	2.80	1.14	0.85	9.34
2.0	2.94	0.71	0.64	1.64	1.90	1.22	9.05
2.5	0.91	0.51	1.49	0.54	0.40	1.02	4.87
3.0	2.13	0.76	1.41	1.18	0.65	0.94	7.07
3.5	0.82	1.02	0.54	0.55	0.75	1.25	4.93
4.0	1.33	0.73	1.44	1.36	0.71	1.41	6.98
4.5	1.32	0.60	0.72	0.72	0.39	0.75	4.50
5.0	1.88	0.82	1.24	0.72	0.83	0.75	6.24
5.5	1.80	1.07	3.55	1.80	0.84	1.73	10.79
6.0	3.37	0.68	1.36	4.50	2.46	0.94	13.31
Column Totals	23.78	17.12	16.47	19.51	12.08	14.78	103.74

Base: Hydrous Ointment containing 10 per cent Sulphanilamide

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	96	97	98	99	100	101	
Weight (Kg.)	2.425	2.850	2.600	3.700	3.425	2.900	
Sex	Female	Female	Female	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.78	0.70	0.86	0.58	0.56	0.68	4.16
1.0	0.56	0.71	1.03	0.58	0.65	0.49	4.02
1.5	0.40	0.55	1.08	0.90	0.49	0.63	4.05
2.0	0.43	1.31	0.90	1.15	0.49	0.80	5.08
2.5	0.40	0.75	0.70	1.00	0.80	1.03	4.68
3.0	0.33	0.65	0.83	1.10	1.05	0.70	4.66
3.5	0.41	0.99	1.00	0.70	0.55	0.80	4.45
4.0	0.51	0.63	0.66	0.66	1.17	0.45	4.08
4.5	0.33	0.56	0.63	0.80	0.33	0.76	3.41
5.0	0.40	0.61	1.00	1.45	0.59	0.53	4.58
5.5	0.45	0.80	0.71	0.63	0.56	1.21	4.36
6.0	0.31	1.44	0.65	0.60	0.33	0.40	3.73
6.5	0.98	0.80	0.56	0.66	0.40	0.55	3.95
7.0	0.60	0.55	0.56	0.33	0.60	0.73	3.37
7.5	0.25	0.50	0.63	0.80	0.45	0.40	3.03
8.0	0.25	0.99	0.49	0.50	0.66	0.40	3.29
Column Totals	7.39	12.54	12.29	12.44	9.68	10.56	64.90

Base: Hydrous Ointment B.P. containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration.

Mg./100 ml.

Rabbit No.	73	74	75	76	77	78	
Weight (Kg.)	2.900	2.875	2.875	3.000	2.950	2.925	Row
Sex	Male	Male	Male	Female	Female	Male	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.68	0.31	1.83	0.94	0.00	1.00	4.76
1.0	0.51	0.89	1.65	1.02	0.86	0.72	5.65
1.5	0.26	0.40	2.05	0.97	0.63	1.40	5.71
2.0	0.40	0.46	0.84	0.43	1.01	0.69	3.83
2.5	0.94	1.12	1.35	1.09	0.63	0.36	5.49
3.0	0.93	0.79	1.03	0.91	0.91	1.20	5.77
3.5	0.26	0.73	1.09	1.10	0.60	0.79	4.57
4.0	0.60	0.94	1.09	1.25	1.00	1.07	5.95
4.5	1.17	0.95	1.09	2.38	1.14	0.55	7.28
5.0	1.36	1.39	1.84	1.03	0.83	0.70	7.15
5.5	1.54	0.50	1.32	1.20	1.11	0.96	6.63
6.0	1.01	0.20	1.44	0.83	0.89	0.83	5.20
6.5	1.02	1.06	1.16	1.30	1.02	1.17	6.73
7.0	0.89	0.79	1.44	0.88	1.62	1.35	6.97
7.5	1.00	1.14	3.40	1.47	1.14	0.68	8.83
8.0	0.56	0.45	0.88	0.53	1.20	0.53	4.15
Column Totals	12.13	12.12	23.50	17.33	14.59	14.00	94.67

Base: Hydrous Ointment B.P. containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	73	74	75	76	77	78	
Weight (Kg.)	2.975	2.975	2.950	2.650	2.825	2.775	
Sex	Male	Male	Male	Female	Female	Male	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.89	0.36	0.46	0.12	0.43	0.51	2.77
1.0	0.25	0.22	0.26	0.24	0.40	1.87	3.24
1.5	0.43	0.35	0.23	0.40	0.35	0.33	2.09
2.0	0.31	0.46	0.43	0.27	0.10	0.48	2.05
2.5	0.30	0.26	0.43	0.56	0.03	0.47	2.05
3.0	0.31	0.22	0.61	0.48	0.23	0.35	2.20
3.5	0.25	0.45	0.53	0.55	0.36	0.45	2.59
4.0	0.63	0.26	0.15	0.38	0.23	0.50	2.15
4.5	0.28	0.45	2.60	0.76	0.45	0.60	5.14
5.0	0.36	0.30	0.36	0.20	0.38	0.20	1.80
5.5	0.48	0.53	0.26	0.23	0.53	0.48	2.51
6.0	0.53	0.40	0.12	0.20	0.56	0.35	2.16
6.5	0.48	0.37	0.60	0.36	0.83	0.71	3.35
7.0	0.41	0.61	0.40	1.29	0.17	0.94	3.82
7.5	0.40	0.46	0.36	0.30	1.00	0.65	3.17
8.0	0.61	0.51	0.50	0.93	0.50	1.19	4.24
Column Totals	6.92	6.21	8.30	7.27	6.55	10.08	45.33

Base: Emulsifying Ointment B.P. containing 10 per cent Sulphanilamide

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	48	49	50	51	52	53	
Weight (Kg.)	2.450	2.750	2.600	3.025	3.150	2.775	Row
Sex	Male	Male	Male	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.63	0.98	0.84	0.71	1.40	0.95	5.51
1.0	0.88	1.55	1.64	0.07	2.48	1.25	7.87
1.5	0.00	1.29	0.58	0.23	1.63	0.23	3.96
2.0	0.91	0.00	0.87	0.47	1.41	0.23	3.89
2.5	0.39	0.62	0.12	0.47	1.24	0.23	3.07
3.0	0.78	0.98	0.26	0.78	2.66	0.43	5.89
3.5	0.79	1.88	0.38	0.60	1.61	0.39	5.65
4.0	1.16	2.04	0.37	1.17	2.81	0.26	7.81
4.5	1.13	2.40	2.86	0.55	1.83	0.10	8.87
5.0	1.55	1.58	1.32	0.66	1.83	0.24	7.18
5.5	0.46	0.91	1.40	0.88	2.41	0.21	6.27
6.0	0.71	2.53	1.02	1.02	1.40	0.65	7.33
Column Totals	9.39	16.76	11.66	7.61	22.71	5.17	73.30

Base: Emulsifying Ointment B.P. containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration.

Mg./100 ml.

Rabbit No.	48	49	50	51	52	53	
Weight (Kg.)	2.475	2.500	2.850	2.850	3.500	2.925	Row
Sex	Male	Male	Male	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	1.05	1.00	2.34	0.11	1.36	0.61	6.47
1.0	0.62	0.83	1.14	1.33	0.83	1.33	6.08
1.5	0.41	0.44	1.36	0.19	2.05	2.05	6.50
2.0	0.47	1.55	1.68	0.83	0.57	1.07	6.17
2.5	0.44	1.03	1.21	0.44	0.03	0.19	3.34
3.0	1.15	0.80	2.10	2.14	2.37	1.00	9.56
3.5	0.98	0.74	1.66	1.31	1.38	2.44	8.51
4.0	0.66	0.61	0.63	1.47	0.50	0.82	4.69
4.5	0.33	0.80	3.08	1.22	1.25	1.24	7.92
5.0	0.47	0.66	0.82	0.87	0.62	0.97	4.41
5.5	0.37	0.43	0.90	0.72	1.04	0.80	4.26
6.0	0.19	1.18	1.58	1.00	1.08	1.66	6.69
Column Totals	7.14	10.07	18.50	11.63	13.08	14.18	74.60

Base: Emulsifying Ointment B.P. containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	96	97	98	99	100	101	
Weight (Kg.)	2.350	2.800	2.600	3.600	3.400	2.750	
Sex	Female	Female	Female	Female	Female	Female	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.80	0.61	0.68	0.50	1.40	0.35	4.34
1.0	0.66	0.85	0.65	0.43	0.30	0.25	3.14
1.5	0.61	0.99	0.30	0.33	0.83	0.30	3.36
2.0	0.85	0.66	0.43	0.30	0.23	0.40	2.87
2.5	0.27	0.50	0.41	0.56	0.26	0.24	2.24
3.0	0.50	0.40	0.46	0.30	0.30	0.21	2.17
3.5	0.38	0.56	0.36	0.41	0.51	0.71	2.93
4.0	0.68	0.78	0.38	0.51	0.31	0.20	2.86
4.5	0.26	0.46	0.30	0.41	0.60	0.23	2.26
5.0	0.51	0.40	0.76	0.36	0.60	0.23	2.86
5.5	0.45	0.48	0.60	0.56	0.65	0.56	3.30
6.0	0.28	0.71	1.25	0.48	0.33	0.63	3.68
6.5	0.33	0.36	0.86	0.21	0.45	0.30	2.51
7.0	0.56	0.36	0.35	0.30	0.43	0.25	2.25
7.5	0.20	0.63	0.30	0.20	0.25	0.45	2.03
8.0	0.28	0.33	0.36	0.41	0.26	0.26	1.90
Column Totals	7.62	9.08	8.45	6.27	7.71	5.57	44.70

Base: Emulsifying Ointment B.P. containing 10 per cent Sulphanilamide

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	73	74	75	76	77	78	
Weight (Kg.)	2.950	2.925	2.875	2.900	3.000	2.975	Row
Sex	Male	Male	Male	Female	Female	Male	Totals
Hours After Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.00	0.26	0.00	0.30	0.65	0.33	1.54
1.0	0.30	0.98	0.56	0.74	0.48	0.50	3.56
1.5	0.00	0.60	0.26	0.45	0.47	0.23	2.01
2.0	0.51	0.05	1.26	0.42	0.60	0.20	3.04
2.5	0.61	0.00	0.73	0.28	0.43	0.53	2.58
3.0	0.25	0.38	0.40	0.33	0.46	0.30	2.12
3.5	0.69	0.20	1.44	0.12	0.41	0.33	3.19
4.0	0.86	0.00	0.20	0.74	0.28	0.61	2.69
4.5	0.31	0.69	0.19	0.33	0.46	0.30	2.28
5.0	0.15	0.45	0.25	0.26	0.19	0.81	2.11
5.5	0.86	0.30	0.20	0.23	0.41	0.46	2.46
6.0	0.10	0.46	0.12	0.13	0.40	0.35	1.56
6.5	0.51	0.50	0.00	1.00	0.17	0.65	2.83
7.0	0.43	0.30	0.30	0.38	0.45	1.45	3.31
7.5	0.46	0.50	0.00	0.23	0.30	0.84	2.33
8.0	2.56	0.40	0.15	0.22	0.15	0.30	3.78
Column Totals	8.60	6.07	6.06	6.16	6.31	8.19	41.39

Base: Emulsifying Ointment B.P. containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	73	74	75	76	77	78	
Weight (Kg.)	2.800	2.800	2.750	2.550	2.700	2.575	
Sex	Male	Male	Male	Female	Female	Male	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.69	0.73	0.33	1.83	0.46	1.19	5.23
1.0	1.32	1.17	0.40	1.20	0.56	0.41	5.06
1.5	0.70	0.83	0.27	0.63	0.23	0.58	3.24
2.0	0.61	0.53	0.33	0.94	0.28	0.31	3.00
2.5	0.72	0.64	0.33	1.21	0.34	0.53	3.77
3.0	0.45	0.32	0.24	0.74	0.29	0.28	2.32
3.5	0.51	0.48	0.32	0.58	0.10	0.25	2.24
4.0	0.31	0.47	0.33	0.84	0.45	0.26	2.66
4.5	0.81	0.78	0.55	0.33	0.84	0.25	3.56
5.0	0.35	0.61	1.24	1.34	0.46	0.56	4.56
5.5	0.64	0.56	0.40	0.68	0.45	0.29	3.02
6.0	0.64	1.06	0.33	0.83	0.51	0.22	3.59
6.5	0.63	0.50	1.32	0.69	0.38	0.65	4.17
7.0	0.23	0.33	0.78	1.04	0.68	0.23	3.29
7.5	0.30	0.41	1.06	0.91	0.54	0.64	3.86
8.0	0.22	0.28	0.41	0.46	0.00	0.08	1.45
Column Totals	9.13	9.70	8.64	14.25	6.57	6.73	55.02

Base: Hydrous Emulsifying Ointment B.P. containing 10 per cent Sulphanilamide

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	48	49	50	51	52	53	
Weight (Kg.)	2.550	2.900	2.750	3.175	3.275	2.950	Row
Sex	Male	Male	Male	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.69	0.50	0.20	0.28	0.68	0.98	3.33
1.0	0.94	1.12	0.74	0.48	0.36	0.84	4.48
1.5	0.33	1.44	0.00	0.33	0.50	0.66	3.26
2.0	0.71	1.17	1.49	0.36	0.79	0.55	5.07
2.5	0.43	0.66	0.00	0.12	1.12	0.40	2.73
3.0	0.63	0.58	0.35	0.68	0.00	0.48	2.72
3.5	0.26	0.41	0.53	0.31	0.15	0.78	2.44
4.0	0.78	0.40	1.16	0.36	0.98	0.91	4.59
4.5	0.97	0.30	0.21	0.96	0.84	0.66	3.94
5.0	0.61	0.78	1.22	0.40	0.91	0.58	4.50
5.5	0.36	0.20	0.41	0.18	0.38	0.61	2.14
6.0	0.60	0.50	0.89	0.58	0.25	0.28	3.10
Column Totals	7.31	8.06	7.20	5.04	6.96	7.73	42.30

Base: Hydreus Emulsifying Ointment B.P. containing 10 per cent Sulphanilamide

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	48	49	50	51	52	53	
Weight (Kg.)	2.400	2.650	2.600	3.150	3.250	2.950	Row
Sex	Male	Male	Male	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.50	1.50	0.51	0.86	0.78	0.71	4.86
1.0	0.96	0.99	0.51	1.12	0.78	0.55	4.91
1.5	0.53	1.78	0.00	0.89	0.46	1.68	5.34
2.0	0.10	2.08	0.23	0.36	1.06	0.63	4.46
2.5	0.78	0.91	0.60	0.00	0.53	0.51	3.33
3.0	0.41	0.74	0.79	0.53	0.69	0.61	3.77
3.5	0.40	0.17	0.23	0.00	0.61	0.58	1.99
4.0	0.49	0.00	0.20	0.50	0.86	0.45	2.50
4.5	0.25	0.16	0.38	0.38	0.56	0.63	2.36
5.0	0.66	0.45	0.18	0.43	0.78	0.61	3.11
5.5	0.20	0.91	0.41	0.18	0.41	0.50	2.61
6.0	0.02	1.32	0.03	0.35	0.66	0.23	2.61
Column Totals	5.30	11.01	4.07	5.60	8.18	7.69	41.85

Base: Hydrous Emulsifying Ointment containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	96	97	98	99	100	101	
Weight (Kg.)	2.400	2.800	2.600	3.600	3.400	2.950	
Sex	Female	Female	Female	Female	Female	Female	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.85	0.41	0.35	0.71	0.88	0.73	3.93
1.0	0.30	0.57	0.47	0.53	0.24	0.64	2.75
1.5	0.31	0.30	0.81	0.68	0.23	0.53	2.86
2.0	0.28	0.22	0.31	0.31	1.09	0.47	2.68
2.5	0.10	0.27	0.27	0.44	0.18	0.48	1.74
3.0	0.29	0.38	0.16	0.29	0.35	1.17	2.64
3.5	0.20	0.47	0.48	0.36	0.41	0.50	2.42
4.0	0.25	0.29	0.55	0.60	0.20	0.60	2.49
4.5	0.35	0.31	0.26	0.40	0.46	1.06	2.84
5.0	0.51	0.44	0.37	0.26	0.50	0.41	2.49
5.5	0.43	0.29	0.74	0.47	0.33	0.78	3.04
6.0	0.29	0.33	0.42	0.35	0.20	0.33	1.92
6.5	0.58	0.40	0.27	0.21	0.31	0.43	2.20
7.0	0.31	0.51	0.38	0.31	0.58	0.64	2.73
7.5	0.39	0.21	0.16	0.29	0.29	0.83	2.17
8.0	0.25	0.32	0.28	0.35	0.33	0.28	1.81
Column Totals	5.69	5.72	6.28	6.56	6.58	9.88	40.71

Base: Hydrous Emulsifying Ointment B.P. containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	73	74	75	76	77	78	
Weight (Kg.)	2.950	2.975	2.875	2.850	2.900	2.925	Row
Sex	Male	Male	Male	Female	Female	Male	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.58	0.21	0.73	0.46	0.64	0.89	3.51
1.0	0.20	0.35	0.31	0.48	0.48	0.64	2.46
1.5	0.35	0.31	0.48	0.73	0.38	0.30	2.55
2.0	0.10	0.18	0.23	0.35	0.33	0.33	1.52
2.5	0.41	0.55	0.69	0.46	0.25	0.28	2.64
3.0	0.26	0.10	0.20	0.35	0.58	0.38	1.87
3.5	0.40	0.51	0.46	0.35	0.35	0.33	2.40
4.0	0.10	0.30	0.25	0.21	0.13	0.33	1.32
4.5	0.36	1.08	0.74	0.40	0.30	0.35	3.23
5.0	0.40	0.29	0.26	0.26	0.29	0.46	1.96
5.5	0.30	0.58	0.40	0.30	0.35	0.64	2.57
6.0	0.29	0.30	0.12	0.36	0.12	0.55	1.74
6.5	0.23	0.21	0.26	0.35	0.46	0.73	2.24
7.0	0.20	0.29	0.25	0.38	0.50	0.40	2.02
7.5	0.30	0.43	0.35	0.41	0.45	0.40	2.34
8.0	0.10	0.23	0.17	0.20	0.44	0.66	1.80
Column Totals	4.58	5.92	5.90	6.05	6.05	7.67	36.17

Base: Hydrous Emulsifying Ointment B.P. containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	73	74	75	76	77	78	
Weight (Kg.)	3.000	2.950	2.875	2.800	2.900	2.825	Row
Sex	Male	Male	Male	Female	Female	Male	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	1.40	0.33	0.64	0.88	1.17	0.48	4.90
1.0	0.74	0.81	0.74	1.09	0.00	0.21	3.59
1.5	0.38	1.82	0.53	0.91	0.33	0.94	4.91
2.0	0.26	0.43	0.68	0.23	0.12	0.58	2.30
2.5	0.33	0.36	0.33	0.24	1.23	0.28	2.77
3.0	0.18	0.46	0.43	0.10	0.43	0.35	1.95
3.5	0.17	0.61	0.21	0.23	0.50	0.36	2.08
4.0	0.43	0.58	0.44	0.18	0.40	0.23	2.26
4.5	0.15	0.71	0.30	0.23	0.39	0.86	2.64
5.0	0.33	0.61	0.66	0.31	0.26	0.20	2.37
5.5	0.22	1.82	0.43	0.28	0.48	0.20	3.43
6.0	0.60	0.37	0.58	0.28	0.21	0.19	2.23
6.5	0.15	0.99	0.36	0.10	0.22	0.23	2.05
7.0	0.61	0.33	0.50	0.35	0.61	0.20	2.60
7.5	0.73	0.41	0.53	0.20	0.35	0.20	2.42
8.0	0.36	0.89	0.56	0.41	0.33	0.30	2.85
Column Totals	7.04	11.53	7.92	6.02	7.03	5.81	45.35

Base: Woolfat containing 10 per cent Sulphanilamide

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	96	97	98	99	100	101	
Weight (Kg.)	2.500	3.400	2.450	2.800	2.450	2.500	Row
Sex	Female	Female	Female	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	1.05	0.45	0.31	0.31	0.40	0.26	2.78
1.0	0.58	0.46	0.71	0.28	0.21	0.26	2.50
1.5	1.15	0.30	0.33	0.30	0.33	0.21	2.62
2.0	0.63	0.43	0.23	0.20	0.25	0.35	2.09
2.5	0.28	0.31	0.25	0.20	0.31	0.35	1.42
3.0	0.35	0.26	0.51	0.18	0.20	0.25	1.75
3.5	0.30	0.93	0.21	0.16	0.26	0.21	2.07
4.0	0.20	0.26	0.26	0.50	0.31	0.25	1.78
4.5	0.40	0.36	0.38	0.25	0.75	0.28	2.42
5.0	0.46	0.56	0.25	0.36	0.28	0.20	2.11
5.5	0.20	0.55	0.93	0.21	0.30	0.15	2.34
6.0	0.66	0.20	0.28	0.23	0.30	0.18	1.85
6.5	0.31	0.30	0.16	0.00	0.28	0.30	1.35
7.0	0.23	0.31	0.16	0.10	0.23	0.16	1.19
7.5	0.71	0.21	0.20	0.31	0.35	0.40	2.18
8.0	0.30	0.30	0.26	0.31	0.23	0.23	1.63
Column Totals	7.81	6.19	5.43	3.90	4.99	4.04	32.36

Base: Woolfat containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	96	97	98	99	100	101	
Weight (Kg.)	2.550	3.425	2.475	2.850	2.450	2.600	
Sex	Female	Female	Female	Female	Female	Female	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.78	0.45	0.48	0.66	0.68	0.38	3.43
1.0	0.83	0.36	1.01	0.63	0.45	0.38	3.66
1.5	0.23	0.33	0.95	0.62	0.30	0.45	2.88
2.0	0.63	0.35	0.73	0.40	0.28	0.41	2.80
2.5	0.67	0.35	0.83	0.62	0.36	0.35	3.18
3.0	0.30	0.35	0.45	0.41	0.41	0.80	2.72
3.5	1.25	0.50	0.37	0.38	0.96	0.36	3.82
4.0	0.40	0.22	0.40	0.33	0.51	0.48	2.34
4.5	0.38	0.23	0.28	0.38	0.25	0.28	1.80
5.0	0.15	0.62	0.55	0.62	0.40	0.31	2.65
5.5	0.48	0.45	1.09	1.02	0.28	0.50	3.82
6.0	0.45	0.66	0.50	0.56	0.25	0.30	2.72
6.5	0.45	0.53	0.41	0.50	0.41	0.40	2.70
7.0	0.30	0.50	0.98	0.31	0.21	0.35	2.65
7.5	0.75	0.88	0.63	0.55	0.28	0.30	3.39
8.0	0.30	0.71	0.91	0.23	0.20	0.38	2.73
Column Totals	8.35	7.49	10.57	8.22	6.23	6.43	47.29

Base: Lard containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	96	97	98	99	100	101	
Weight (Kg.)	2.475	3.300	2.525	2.950	2.500	2.550	
Sex	Female	Female	Female	Female	Female	Female	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	1.08	0.78	0.85	0.50	1.10	0.38	4.69
1.0	0.87	1.43	1.05	0.83	1.30	0.55	6.03
1.5	0.15	0.60	0.78	0.97	0.60	0.31	3.41
2.0	0.60	0.77	0.77	0.98	0.61	0.28	4.01
2.5	0.77	1.31	0.69	0.51	0.53	0.63	4.44
3.0	1.48	0.99	1.05	1.30	0.73	0.53	6.08
3.5	1.41	0.87	1.28	0.70	1.18	1.20	6.64
4.0	1.02	0.71	1.11	0.90	1.25	1.35	6.34
4.5	1.14	0.35	0.98	0.93	0.35	0.94	4.70
5.0	1.27	0.67	0.89	1.35	0.82	0.46	5.46
5.5	1.30	1.08	1.03	1.03	1.25	1.31	7.00
6.0	1.17	0.70	0.71	0.88	0.76	0.99	5.21
6.5	1.09	1.10	1.15	1.13	0.00	1.00	5.47
7.0	1.21	0.67	0.92	1.30	0.86	1.21	6.17
7.5	1.04	0.63	0.51	0.95	0.76	1.10	4.99
8.0	1.00	0.95	0.53	1.13	0.76	0.81	5.18
Column Totals	16.60	13.61	14.30	15.39	12.86	13.06	85.82

Base: Lard containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	96	97	98	99	100	101	
Weight (Kg.)	2.550	3.300	2.500	2.875	2.550	2.550	Row
Sex	Female	Female	Female	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	1.00	1.41	0.90	1.41	1.31	0.76	6.79
1.0	0.67	0.65	0.83	1.15	0.63	0.73	4.66
1.5	0.70	0.83	1.11	1.40	0.73	0.51	5.28
2.0	0.40	1.23	0.47	0.93	1.23	0.40	4.66
2.5	0.55	1.05	0.85	1.08	0.58	0.40	4.51
3.0	0.40	1.51	0.70	0.35	0.76	0.58	4.30
3.5	0.55	1.25	0.83	0.96	1.21	1.08	5.88
4.0	0.83	1.13	0.51	1.31	0.96	1.25	5.99
4.5	0.49	1.25	0.80	1.45	0.73	0.99	5.71
5.0	1.20	1.50	0.63	1.33	0.89	0.80	6.35
5.5	1.49	1.35	1.14	1.18	1.43	0.80	7.39
6.0	0.78	1.15	1.55	0.93	0.53	0.80	5.74
6.5	0.73	0.78	1.45	0.83	0.45	0.43	4.67
7.0	0.72	1.02	1.51	0.50	0.51	0.40	4.66
7.5	0.40	1.23	0.53	1.38	0.71	0.40	4.65
8.0	0.77	1.15	0.96	0.90	0.55	o.45	4.78
Column Totals	11.68	18.49	14.77	17.09	13.21	10.78	86.02

Base: White Soft Paraffin containing 10 per cent Sulphanilamide

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	96	97	98	99	100	101	
Weight (Kg.)	2.575	3.275	2.500	2.800	2.525	2.500	Row
Sex	Female	Female	Female	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	1.43	1.21	1.30	1.63	1.35	1.46	8.38
1.0	0.56	0.71	0.91	0.61	0.95	0.51	4.25
1.5	0.57	0.84	0.83	0.70	0.55	0.41	3.90
2.0	0.72	0.63	0.84	0.50	0.53	0.48	3.70
2.5	0.15	0.60	0.58	0.61	0.81	0.35	3.10
3.0	0.75	0.51	0.70	0.58	0.75	0.43	3.72
3.5	0.93	0.38	0.51	0.63	0.53	0.45	3.43
4.0	0.70	0.43	1.15	0.49	0.41	0.75	3.93
4.5	0.68	0.80	1.00	0.55	0.33	1.00	4.36
5.0	0.81	0.72	0.79	0.70	0.50	0.40	3.92
5.5	0.70	0.81	0.68	0.76	0.41	0.60	3.96
6.0	0.55	0.75	0.80	0.46	0.45	0.46	3.47
6.5	0.64	0.53	0.60	0.71	0.56	0.30	3.34
7.0	0.68	0.51	0.42	0.71	0.30	0.41	3.03
7.5	0.30	0.58	0.72	0.51	0.61	0.38	3.10
8.0	0.82	0.50	0.71	0.50	0.64	0.47	3.64
Column Totals	10.99	10.51	12.54	10.65	9.68	8.86	63.23

Base: White Soft Paraffin containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	96	97	98	99	100	101	
Weight (Kg.)	2.500	3.400	2.525	2.850	2.600	2.550	
Sex	Female	Female	Female	Female	Female	Female	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	1.31	0.90	0.69	0.78	0.88	0.60	5.16
1.0	1.00	1.08	0.76	0.75	0.73	0.60	4.92
1.5	0.68	0.58	0.45	1.10	0.70	0.86	4.37
2.0	0.65	0.70	0.75	0.51	0.40	0.60	3.61
2.5	0.60	0.63	0.55	0.46	0.61	0.40	3.25
3.0	0.49	0.81	1.01	0.45	0.40	0.25	3.41
3.5	0.83	0.75	0.90	0.68	0.61	0.29	4.06
4.0	0.73	0.80	0.60	0.37	0.51	0.53	3.54
4.5	0.70	0.90	0.75	0.40	0.83	0.50	4.08
5.0	0.83	0.93	0.56	0.55	0.61	0.58	4.06
5.5	0.65	1.12	0.80	0.43	0.41	0.73	4.14
6.0	0.91	0.87	0.71	0.60	0.79	0.56	4.44
6.5	0.60	0.86	0.83	0.56	0.36	0.63	3.84
7.0	0.85	0.73	0.60	0.37	0.71	0.40	3.66
7.5	0.66	0.68	0.60	0.65	0.40	0.58	3.57
8.0	0.66	0.59	0.95	0.73	0.68	0.73	4.34
Column Totals	12.15	12.93	11.51	9.39	9.63	8.84	64.45

Base: Liquid Paraffin containing 10 per cent Sulphanilamide

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	96	97	98	99	100	101	
Weight (Kg.)	2.425	2.925	2.650	3.800	3.500	2.950	Row
Sex	Female	Female	Female	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	1.40	1.52	1.45	0.68	0.48	0.36	5.89
1.0	0.25	0.89	0.35	0.40	0.10	0.41	2.40
1.5	0.61	0.63	0.10	0.10	0.60	0.30	2.34
2.0	0.25	1.62	0.40	0.23	0.25	0.31	3.06
2.5	0.69	0.48	0.30	0.27	0.10	0.73	2.57
3.0	1.10	0.56	0.10	0.10	0.46	0.56	2.88
3.5	0.48	1.77	0.41	0.76	0.20	0.26	3.88
4.0	0.36	0.91	0.55	0.61	0.60	0.36	3.39
4.5	0.41	0.10	0.30	0.28	0.26	0.26	1.61
5.0	0.10	0.45	0.23	0.40	0.28	0.23	1.69
5.5	0.31	0.18	0.30	0.15	0.26	0.53	1.73
6.0	0.45	0.43	0.23	0.28	0.25	0.15	1.79
6.5	1.51	0.33	1.12	0.20	0.45	0.30	3.91
7.0	1.82	0.18	0.25	0.30	0.10	0.20	2.85
7.5	1.44	0.33	0.35	0.21	0.00	0.10	2.43
8.0	0.58	0.20	0.51	0.56	0.20	0.26	2.31
Column Totals	11.76	10.58	6.95	5.53	4.59	5.32	44.73

Base: Liquid Paraffin containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	96	97	98	99	100	101	
Weight (Kg.)	2.500	2.475	2.425		3.025	2.775	Row
Sex	Female	Female	Female		Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00		0.00	0.00	0.00
0.5	1.00	0.41	0.50		0.60	0.26	2.77
1.0	0.38	0.36	0.15		0.61	0.43	1.93
1.5	0.43	0.26	0.63		0.46	0.46	2.24
2.0	0.30	0.46	0.53		0.21	0.23	1.73
2.5	0.30	0.45	0.28		0.35	0.26	1.64
3.0	0.33	0.46	0.35		0.55	0.56	2.25
3.5	0.32	0.41	0.73		0.81	1.06	3.33
4.0	0.75	0.65	0.50		0.50	0.81	3.21
4.5	0.33	0.50	0.31		0.68	0.41	2.23
5.0	0.53	0.00	0.43		0.50	0.43	1.89
5.5	0.25	0.30	0.33		0.28	0.38	1.54
6.0	0.26	0.46	0.48		0.53	0.26	1.99
6.5	0.26	0.91	0.51		0.41	0.30	2.39
7.0	1.11	1.16	0.75		0.53	0.28	3.83
7.5	0.21	0.86	1.31		0.31	0.43	3.12
8.0	0.26	0.83	0.68		0.50	0.75	3.02
Column Totals	7.02	8.48	8.47		7.83	7.31	39.11

Base: Propylene Glycol containing 10 per cent Sulphanilamide

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	48	49	50	51	52	53	
Weight (Kg.)	2.475	2.950	2.625	3.325	3.475	3.075	Row
Sex	Male	Male	Male	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.96	1.29	0.58	0.55	0.40	0.46	4.24
1.0	0.83	0.75	0.45	0.80	0.26	0.23	3.32
1.5	1.79	0.61	0.30	0.51	0.41	0.31	3.93
2.0	1.56	0.78	0.36	0.53	0.47	0.53	4.23
2.5	1.34	0.71	0.33	0.47	0.56	0.85	4.26
3.0	0.99	0.40	0.31	0.25	0.25	0.66	2.86
3.5	1.12	0.43	0.28	0.47	1.40	0.65	4.35
4.0	1.59	0.93	1.87	1.32	0.45	1.11	7.27
4.5	1.04	1.01	0.30	0.29	0.50	0.73	3.87
5.0	0.93	0.96	0.25	0.30	0.40	0.35	3.19
5.5	0.55	0.41	0.33	0.51	0.41	0.41	2.62
6.0	0.63	0.59	0.33	0.22	0.80	0.41	2.98
6.5	1.27	0.96	0.18	0.43	0.32	0.15	3.31
7.0	0.68	1.50	0.56	1.01	0.26	0.23	4.24
7.5	0.43	0.40	1.00	1.54	0.36	0.23	3.96
8.0	0.60	0.26	0.26	0.36	0.61	0.23	2.32
Column Totals	16.31	11.99	7.69	9.56	7.86	7.54	60.95

Base: Propylene Glycol containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration

Mg/100 ml.

Rabbit No.	48	49	50	51	52	53	
Weight (Kg.)	2.525	2.750	2.450	3.300	3.375	3.025	Row
Sex	Male	Male	Male	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	1.17	0.40	0.63	0.66	0.75	0.69	4.30
1.0	0.64	1.21	2.06	0.55	1.70	0.45	6.61
1.5	0.73	0.91	0.76	0.41	0.51	1.22	4.54
2.0	1.16	0.23	0.73	0.44	0.53	0.58	3.67
2.5	0.51	0.57	0.40	0.41	0.45	0.61	2.95
3.0	1.32	0.41	0.51	0.26	0.45	0.51	3.46
3.5	2.21	0.46	0.52	0.66	0.58	1.55	5.98
4.0	2.38	0.76	0.60	0.36	0.45	0.83	5.38
4.5	0.91	0.69	0.33	1.20	0.66	0.51	4.30
5.0	0.81	1.55	0.64	0.67	0.80	0.48	4.95
5.5	1.27	1.02	0.52	0.53	0.40	0.58	4.32
6.0	2.23	0.98	0.79	0.40	0.50	0.73	5.63
6.5	0.53	0.76	0.10	0.31	0.58	0.76	3.04
7.0	0.71	1.24	0.40	0.25	0.45	0.58	3.63
7.5	0.88	0.56	0.10	0.50	0.45	0.89	3.38
8.0	0.58	0.63	0.51	0.20	0.48	0.58	2.98
Column Totals	18.04	12.38	9.60	7.81	9.74	11.55	69.12

Base: Ethyl Oleate containing 10 per cent Sulphanilamide.

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	48	49	50	51	52	53	
Weight (Kg.)	2.450	2.925	2.700	3.350	3.475	3.050	
Sex	Male	Male	Male	Female	Female	Female	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.81	0.69	0.56	0.33	0.66	0.55	3.60
1.0	0.66	0.98	0.63	0.25	1.34	0.66	4.52
1.5	0.71	0.89	0.63	0.40	0.73	0.94	4.30
2.0	0.93	0.93	0.43	0.36	0.51	0.56	3.72
2.5	0.63	0.25	0.56	0.56	0.96	1.38	4.34
3.0	0.47	0.65	0.61	0.50	0.31	0.56	3.10
3.5	0.94	0.55	0.63	0.31	0.50	0.25	3.18
4.0	0.51	0.91	0.56	0.65	0.31	0.89	3.83
4.5	0.73	1.59	0.33	0.28	0.33	0.25	3.48
5.0	0.89	1.43	0.41	0.36	0.40	0.65	4.14
5.5	0.80	1.40	0.58	0.30	0.35	0.45	3.88
6.0	1.03	1.27	0.63	0.40	0.45	0.66	4.44
6.5	1.11	1.03	0.62	0.56	0.15	0.80	4.27
7.0	1.19	0.98	0.60	0.51	0.28	1.26	4.82
7.5	0.89	1.20	0.63	0.33	0.28	0.75	4.08
8.0	0.68	0.88	0.51	0.43	0.33	0.51	3.34
Column Totals	12.98	15.60	8.92	6.53	7.89	11.12	63.04

Base: Cetomacrogol Emulsifying Wax containing 10 per cent Sulphanilamide

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	96	97	98	99	100	101	
Weight (Kg.)	2.450	2.800	2.650	3.650	3.300	2.850	Row
Sex	Female	Female	Female	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.70	0.73	0.51	0.45	0.43	0.40	3.22
1.0	0.81	0.80	0.45	0.53	0.66	0.80	4.05
1.5	0.66	1.00	0.58	0.61	0.90	0.55	4.30
2.0	0.58	0.61	0.61	0.66	0.63	0.78	3.87
2.5	0.53	0.23	0.78	0.81	0.53	0.58	3.46
3.0	0.61	0.33	0.83	0.70	0.10	0.55	3.12
3.5	0.38	0.45	0.61	0.60	0.40	0.80	3.24
4.0	0.40	0.56	0.79	0.45	0.50	0.80	3.50
4.5	0.48	0.76	0.66	0.55	0.45	0.85	3.75
5.0	0.53	0.55	0.70	0.66	0.46	0.80	3.70
5.5	0.88	0.68	0.50	0.46	0.15	0.74	3.41
6.0	0.51	0.50	0.61	0.50	0.36	0.51	2.99
6.5	0.53	0.36	0.46	0.33	0.41	0.53	2.62
7.0	0.33	0.28	0.43	0.41	0.38	0.71	2.54
7.5	0.48	0.18	0.28	0.36	0.28	0.40	1.98
8.0	0.41	0.28	0.21	0.68	0.33	0.28	2.19
Column Totals	8.82	8.30	9.01	8.76	6.97	10.08	51.94

Base: Lard containing 5 per cent salicylic acid.

Salicylate Concentration

Mg./100 ml.

Rabbit No.	G	H	I	J	K	L	
Weight (Kg.)	3.350	3.400	3.300	3.650	3.450	3.500	
Sex	Male	Male	Male	Female	Female	Female	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	1.31	1.20	1.22	1.01	1.51	1.35	7.60
1.0	1.10	1.11	1.31	0.90	1.29	1.36	7.07
1.5	0.91	1.32	1.27	0.60	0.80	0.91	5.81
2.0	0.72	0.84	1.18	1.52	0.75	1.24	6.25
2.5	0.83	1.27	0.71	1.31	0.74	1.24	6.10
3.0	1.46	0.77	0.75	1.48	1.17	0.84	6.47
3.5	0.41	1.26	0.99	1.10	0.89	0.85	5.50
4.0	0.99	1.44	1.34	0.92	1.34	0.93	6.96
4.5	1.21	0.91	0.91	0.81	0.79	0.89	5.52
5.0	0.72	1.13	0.80	1.35	0.80	1.41	6.21
5.5	0.83	1.24	1.43	1.82	1.41	1.63	8.36
6.0	0.85	0.71	1.56	1.64	1.41	1.42	7.59
6.5	1.01	0.93	1.78	1.65	1.62	1.77	8.76
7.0	0.92	1.44	1.04	1.41	1.43	1.50	7.74
7.5	1.32	0.88	0.92	0.83	0.91	0.95	5.81
8.0	0.69	1.03	1.18	0.88	0.74	1.23	5.75
Column Totals	15.28	17.48	18.39	19.23	17.60	19.52	107.50

Base: Lard containing 5 per cent salicylic acid.

Salicylate Concentration

Mg./100 ml.

Rabbit No.	A	B	C	D	E	F	
Weight (Kg.)	3.425	3.550	3.500	3.475	3.250	3.475	Row
Sex	Female	Female	Female	Female	Male	Male	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.71	1.23	1.00	1.07	1.56	0.81	6.38
1.0	1.43	0.90	1.43	1.51	1.71	1.42	8.40
1.5	0.92	1.01	1.10	1.67	1.21	1.63	7.54
2.0	1.03	1.13	1.21	1.77	1.31	1.51	7.96
2.5	1.17	1.64	1.10	2.03	0.81	1.82	8.57
3.0	1.23	0.70	1.11	1.21	0.71	0.78	5.74
3.5	1.09	0.96	1.42	1.34	1.43	0.69	6.93
4.0	0.98	1.39	1.00	1.29	0.69	0.96	6.31
4.5	1.44	1.19	1.67	1.31	0.91	1.15	7.67
5.0	1.39	1.54	1.72	1.04	1.43	1.05	8.17
5.5	1.52	1.09	1.83	0.74	1.51	1.38	8.07
6.0	1.09	1.03	1.20	0.98	1.43	0.97	6.70
6.5	1.51	1.07	1.28	1.21	1.02	1.12	7.21
7.0	1.10	1.51	1.80	0.63	1.16	0.78	6.98
7.5	1.83	1.19	0.71	0.78	0.92	1.04	6.47
8.0	1.40	1.01	1.63	1.03	0.88	0.88	6.83
Column Totals	19.84	18.59	21.21	19.61	18.69	17.99	115.93

Base: White Soft Paraffin containing 5 per cent salicylic acid

Salicylate Concentration

Mg./100 ml.

Rabbit No.	102	103	104	105	106	107	
Weight (Kg.)	3.250	3.200	2.650	3.400	3.300	2.700	Row
Sex	Female	Female	Female	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	1.89	1.41	1.44	1.47	1.50	1.59	9.30
1.0	1.51	0.80	1.39	1.81	1.41	0.90	7.82
1.5	0.95	0.91	0.76	1.93	1.48	1.00	7.03
2.0	1.64	1.47	1.44	1.33	1.61	0.71	8.20
2.5	1.17	0.81	0.94	1.78	1.77	1.67	8.14
3.0	1.22	0.88	0.93	1.47	1.42	1.34	7.26
3.5	1.00	1.51	0.71	1.10	1.01	0.92	6.25
4.0	0.90	0.51	0.96	1.54	0.52	0.71	5.14
4.5	0.90	0.74	1.72	0.81	0.73	0.94	5.84
5.0	1.24	1.21	1.24	1.31	0.91	0.50	6.41
5.5	0.96	0.84	0.42	0.49	0.96	0.51	4.18
6.0	0.96	0.77	0.55	0.41	0.98	0.20	3.87
6.5	0.84	0.50	0.53	0.53	1.11	0.51	4.02
7.0	1.04	0.82	0.53	0.31	1.31	0.67	4.68
7.5	0.62	0.61	0.38	0.72	0.63	0.74	3.70
8.0	0.89	1.36	1.61	0.91	1.28	0.61	6.66
Column Totals	17.73	15.15	15.55	17.92	18.63	13.52	98.50

Base: White Soft Paraffin containing 5 per cent salicylic acid.

Salicylate Concentration

Mg./100 ml.

Rabbit No.	102	103	104	105	106	107	
Weight (Kg.)	3.100	3.050	2.600	3.300	3.300	2.600	Row
Sex	Female	Female	Female	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.80	1.00	0.56	1.25	1.27	0.64	5.52
1.0	1.89	1.29	0.58	0.88	1.38	0.41	6.43
1.5	2.14	0.46	0.33	1.33	1.64	0.62	6.52
2.0	2.32	0.20	0.97	0.95	1.50	1.64	7.58
2.5	1.76	1.25	1.00	1.26	1.66	1.30	8.23
3.0	1.50	1.56	0.67	0.94	1.28	1.52	7.47
3.5	1.29	1.35	0.56	1.62	1.28	1.50	7.60
4.0	1.38	0.50	0.57	0.45	1.72	0.95	5.57
4.5	1.70	0.47	0.65	0.66	0.91	1.25	5.64
5.0	2.05	1.42	1.02	0.51	1.30	0.95	7.25
5.5	1.31	0.40	1.45	0.98	1.17	1.10	6.41
6.0	1.50	0.97	0.75	0.50	1.02	1.44	6.18
6.5	0.70	1.31	0.94	0.90	0.99	0.96	5.80
7.0	0.49	1.52	1.01	1.32	1.53	0.73	6.60
7.5	0.91	0.91	0.90	1.02	0.95	0.61	5.30
8.0	0.89	0.82	1.21	1.40	1.33	1.27	6.92
Column Totals	22.63	15.43	13.17	15.97	20.93	16.89	105.02

Base: Hydrous Ointment B.P. containing 5 per cent salicylic acid.

Salicylate Concentration

Mg./100 ml.

Rabbit No.	102	103	104	105	106	107	
Weight (Kg.)	3.300	3.200	2.600	3.300	3.300	2.700	
Sex	Female	Female	Female	Female	Female	Female	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.91	1.36	1.81	1.64	1.84	1.71	9.27
1.0	1.33	1.49	1.78	1.35	1.52	1.36	8.83
1.5	0.61	1.71	1.17	1.43	1.39	1.07	7.38
2.0	1.41	1.67	1.21	1.07	0.48	1.36	7.20
2.5	0.51	1.34	0.87	1.56	0.22	1.33	5.83
3.0	0.65	1.19	1.64	1.29	0.69	1.21	6.67
3.5	0.71	0.87	1.39	1.47	0.91	1.49	6.84
4.0	1.31	0.67	1.45	1.88	1.43	0.63	7.37
4.5	0.47	1.04	1.15	1.00	1.37	1.22	6.25
5.0	1.08	1.23	0.92	1.57	0.52	1.01	6.33
5.5	1.31	1.31	1.04	1.44	1.43	1.29	7.82
6.0	0.67	1.45	1.27	1.30	1.07	1.38	7.14
6.5	0.93	1.62	1.51	1.41	1.24	1.51	8.22
7.0	0.91	1.30	1.51	1.97	1.19	1.24	8.12
7.5	0.98	0.90	1.20	1.17	0.99	1.30	6.54
8.0	1.41	0.87	1.43	0.71	1.18	0.70	6.30
Column Totals	15.20	20.02	21.35	22.26	17.47	19.81	116.11

Base: Hydrous Ointment B.P. containg 5 per cent salicylic acid

Salicylate Concentration

Mg./100 ml.

Rabbit No.	102	103	104	105	106	107	
Weight (Kg.)	3.200	3.150	2.600	3.300	3.250	2.700	Row
Sex	Female	Female	Female	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	1.79	1.71	1.73	1.68	1.35	1.28	9.54
1.0	1.36	1.29	1.39	1.30	1.25	1.41	8.00
1.5	1.12	1.07	1.14	1.32	1.43	0.60	6.68
2.0	1.09	1.00	1.24	1.33	1.39	0.58	6.63
2.5	1.54	1.50	1.09	1.17	1.45	1.20	7.95
3.0	1.21	0.93	0.51	0.92	1.43	0.79	5.79
3.5	1.29	1.26	1.08	1.41	1.62	0.51	7.17
4.0	1.29	0.76	1.13	1.04	1.58	0.63	6.43
4.5	0.81	1.56	0.62	1.67	1.33	0.59	6.58
5.0	1.18	1.17	1.20	1.35	0.83	0.55	6.28
5.5	1.37	1.31	1.08	1.22	1.08	0.70	6.76
6.0	1.28	1.31	1.36	1.41	1.22	1.02	7.60
6.5	1.05	0.59	1.07	1.03	1.49	0.21	5.44
7.0	1.21	1.19	0.63	1.48	0.80	0.60	5.91
7.5	0.75	0.81	0.90	1.02	1.59	0.71	5.78
8.0	0.73	0.71	0.67	1.38	0.71	1.46	5.66
Column Totals	19.07	18.17	16.84	20.73	20.55	12.84	108.20

Base: Propylene Glycol containing 5 per cent salicylic acid.

Salicylate Concentration

Mg./100 ml.

Rabbit No.	102	103	104	105	106	107	
Weight (Kg.)	3.100	3.000	2.700	3.450	3.300	2.600	
Sex	Female	Female	Female	Female	Female	Female	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	1.97	1.81	1.50	1.35	1.29	1.67	9.59
1.0	1.61	1.31	1.54	1.04	1.37	1.41	8.28
1.5	1.63	1.47	1.81	1.70	0.54	1.69	8.84
2.0	1.82	0.71	1.75	1.41	1.35	1.71	8.75
2.5	1.31	1.69	1.54	1.86	1.48	0.92	8.80
3.0	1.07	0.74	1.20	1.86	1.68	1.71	8.26
3.5	1.22	0.53	1.46	1.39	1.70	1.67	7.97
4.0	0.84	0.78	1.54	0.98	1.70	1.51	7.35
4.5	0.91	1.01	1.71	0.76	1.86	0.81	7.06
5.0	0.99	1.51	1.91	0.80	1.51	0.83	7.55
5.5	1.07	1.31	1.52	1.82	0.41	1.69	7.82
6.0	1.74	0.72	1.85	1.42	1.58	1.54	8.85
6.5	1.54	0.61	1.30	1.47	1.61	1.50	8.03
7.0	1.34	0.64	1.29	0.79	1.57	1.99	7.62
7.5	1.00	1.50	0.58	1.51	1.72	1.53	7.84
8.0	0.89	1.27	0.81	0.96	1.39	1.77	7.09
Column Totals	20.95	17.61	23.31	21.12	22.76	23.95	129.70

Base: Propylene Glycol containing 5 per cent salicylic acid.

Salicylate Concentration

Mg./100 ml.

Rabbit No.	102	103	104	105	106	107	
Weight (Kg.)	3.200	3.150	2.650	3.350	3.350	2.600	
Sex	Female	Female	Female	Female	Female	Female	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.91	1.70	1.72	1.43	1.33	1.12	8.21
1.0	1.47	0.81	1.70	1.92	1.90	1.84	9.64
1.5	1.91	0.57	1.60	1.44	1.40	1.26	8.18
2.0	0.50	0.46	1.64	1.71	0.87	1.87	7.05
2.5	0.71	0.75	1.57	1.51	0.80	1.01	6.35
3.0	0.43	0.77	0.90	1.19	0.94	0.94	5.17
3.5	0.49	0.50	0.46	1.18	1.65	0.76	5.04
4.0	1.38	0.81	1.02	1.80	0.90	0.89	6.80
4.5	0.61	1.21	0.87	1.48	0.83	0.98	5.98
5.0	1.35	1.37	0.44	1.11	0.96	1.76	6.99
5.5	1.57	0.99	1.21	0.87	1.17	1.11	6.92
6.0	0.68	1.44	1.50	1.17	1.10	1.38	7.27
6.5	0.50	0.84	0.67	1.34	0.71	1.14	5.20
7.0	0.55	0.69	0.89	1.56	0.66	1.23	5.58
7.5	1.00	1.31	0.87	1.44	0.83	1.55	7.00
8.0	0.97	0.92	1.60	0.88	0.54	1.64	6.55
Column Totals	15.03	15.14	18.66	22.03	16.59	20.48	107.92

Base: Water as 5 per cent carboxy methyl cellulose containing 5 per cent salicylic acid

Salicylate Concentration

Mg./100 ml.

Rabbit No.	G	H	I	J	K	L	
Weight (Kg.)	3.400	3.425	3.350	3.600	3.500	3.450	Row
Sex	Male	Male	Male	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.91	0.86	1.14	0.60	0.63	1.18	5.32
1.0	0.61	1.01	0.83	0.83	1.11	0.71	5.10
1.5	0.76	0.73	0.71	0.77	0.94	0.99	4.90
2.0	1.02	0.99	0.51	0.91	0.90	0.99	5.32
2.5	0.81	0.71	0.63	1.13	1.02	0.81	5.11
3.0	0.61	0.65	0.75	0.88	1.14	0.43	4.46
3.5	0.42	0.69	0.71	1.26	1.07	0.60	4.75
4.0	1.04	0.61	0.63	0.94	1.04	0.30	4.56
4.5	1.09	1.06	0.48	0.68	0.67	0.26	4.24
5.0	0.74	0.77	0.89	0.92	1.55	0.88	5.75
5.5	0.41	0.77	0.98	1.24	0.98	0.81	5.19
6.0	1.03	0.67	0.91	0.88	1.09	0.90	5.48
6.5	0.62	1.14	0.99	0.72	1.54	0.70	5.71
7.0	0.70	0.99	0.62	1.00	0.81	0.61	4.73
7.5	0.78	0.96	0.67	0.41	0.91	0.99	4.72
8.0	0.91	1.04	0.54	0.57	0.84	0.67	4.57
Column Totals	12.46	13.65	11.99	13.74	16.24	11.83	79.91

Base: Water as 5 per cent carboxy methyl cellulose containing 5 per cent salicylic acid.

Salicylate Concentration

Mg./100 ml.

Rabbit No.	G	H	I	J	K	L	
Weight (Kg.)	3.375	3.400	3.350	3.550	3.450	3.425	Row
Sex	Male	Male	Male	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	1.07	0.91	0.94	0.81	1.26	0.89	5.88
1.0	1.06	1.09	0.66	1.60	0.95	1.18	6.54
1.5	0.72	0.52	0.77	1.19	0.73	0.91	4.84
2.0	1.62	1.69	1.05	0.64	0.59	0.85	6.44
2.5	1.10	1.07	0.92	0.90	0.62	1.08	5.69
3.0	1.61	0.83	0.51	0.83	0.41	1.04	5.23
3.5	1.32	1.00	0.61	1.38	0.69	0.71	5.71
4.0	1.33	0.64	1.04	1.62	0.83	0.39	5.85
4.5	0.91	0.72	1.33	1.49	1.08	0.85	6.38
5.0	0.54	0.91	0.98	1.08	1.18	0.61	5.30
5.5	1.19	0.77	0.81	0.92	0.83	0.65	5.17
6.0	0.80	0.97	0.70	1.42	0.58	0.89	5.36
6.5	0.51	0.69	1.27	1.88	0.67	0.59	5.61
7.0	0.60	0.89	0.92	1.72	0.71	0.22	5.06
7.5	0.92	1.11	0.80	1.00	1.13	0.41	5.37
8.0	1.14	0.83	0.75	0.61	1.05	0.45	4.83
Column Totals	16.44	14.64	14.06	19.09	13.31	11.72	89.26

Base: Emulsifying Ointment B.P. containing 5 per cent salicylic acid.

Salicylate Concentration

Mg./100 ml.

Rabbit No.	A	B	C	D	E	F	
Weight (Kg.)	3.425	3.500	3.450	3.450	3.300	3.400	
Sex	Female	Female	Female	Female	Male	Male	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.62	0.76	0.95	0.79	0.55	1.42	5.09
1.0	0.85	0.72	1.58	0.79	0.70	0.95	5.59
1.5	0.69	0.74	0.71	1.06	0.94	0.93	5.07
2.0	0.68	0.65	0.95	1.03	1.42	0.51	5.24
2.5	0.52	0.57	0.64	0.74	1.60	0.96	5.03
3.0	0.77	0.58	0.88	1.36	1.39	1.30	6.28
3.5	0.74	0.58	1.31	0.99	1.37	1.54	6.53
4.0	0.69	0.54	1.47	1.32	1.37	1.34	6.73
4.5	0.86	0.74	1.53	1.46	0.89	0.97	6.45
5.0	0.78	0.65	0.91	1.56	0.83	1.21	5.94
5.5	0.98	0.67	0.91	0.70	0.61	1.27	5.14
6.0	0.86	0.80	0.69	1.49	0.35	0.81	5.00
6.5	0.75	0.47	0.75	0.95	0.94	0.76	4.62
7.0	0.51	0.56	0.75	0.86	0.90	0.53	4.11
7.5	0.64	0.57	0.86	0.75	0.61	0.91	4.34
8.0	0.59	0.64	1.09	1.22	0.75	1.02	5.31
Column Totals	11.53	10.24	15.98	17.07	15.22	16.43	86.47

Base: Emulsifying Ointment B.P. containing 5 per cent salicylic acid

Salicylate Concentration

Mg./100 ml.

Rabbit No.	A	B	C	D	E	F	
Weight (Kg.)	3.400	3.450	3.500	3.525	3.350	3.425	Row
Sex	Female	Female	Female	Female	Male	Male	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.71	0.82	1.50	0.74	0.72	0.91	5.40
1.0	0.51	1.04	1.41	1.12	0.94	1.42	6.44
1.5	1.03	1.23	0.84	0.91	1.23	1.02	6.26
2.0	1.20	0.90	0.73	0.88	1.20	1.40	6.31
2.5	0.81	1.21	0.75	0.61	1.31	1.33	6.02
3.0	1.20	1.01	0.84	0.74	1.43	1.23	6.45
3.5	0.94	0.73	0.71	1.31	1.43	1.14	6.26
4.0	0.81	0.99	1.21	0.94	1.04	0.73	5.72
4.5	1.20	1.18	1.18	0.86	1.74	0.60	6.76
5.0	1.30	1.39	0.91	0.86	1.90	1.05	7.41
5.5	0.72	0.91	1.34	1.03	1.82	1.17	6.99
6.0	0.83	1.62	0.81	0.93	0.86	1.28	6.33
6.5	1.33	0.93	1.12	0.82	1.09	1.26	6.55
7.0	1.20	0.42	0.97	1.20	0.90	1.32	6.01
7.5	1.17	1.11	0.95	0.71	0.73	1.31	5.98
8.0	0.92	1.20	1.11	0.99	1.10	0.93	6.25
Column Totals	15.88	16.69	16.38	14.65	19.44	18.10	101.14

Base: Water as 5 per cent carboxy methyl cellulose containing 10 per cent copper sulphate.

Copper Concentration

Mg./100 ml.

Rabbit No.	102	103	104	105	106	107	
Weight (Kg.)	3.000	3.100	2.500	3.250	3.200	2.550	Row
Sex	Female	Female	Female	Female	Male	Male	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.19	0.22	0.26	0.16	0.17	0.23	1.23
1.0	0.22	0.27	0.26	0.21	0.22	0.16	1.34
1.5	0.14	0.16	0.16	0.13	0.18	0.29	1.06
2.0	0.09	0.14	0.09	0.22	0.18	0.21	0.93
2.5	0.12	0.07	0.16	0.18	0.23	0.18	0.94
3.0	0.23	0.11	0.21	0.19	0.16	0.14	1.04
3.5	0.16	0.00	0.14	0.27	0.18	0.17	0.92
4.0	0.15	0.16	0.12	0.30	0.23	0.19	1.15
4.5	0.27	0.22	0.12	0.21	0.27	0.21	1.30
5.0	0.20	0.34	0.18	0.16	0.14	0.34	1.36
5.5	0.14	0.16	0.21	0.15	0.15	0.21	1.02
6.0	0.13	0.11	0.14	0.11	0.09	0.10	0.68
6.5	0.00	0.21	0.22	0.21	0.21	0.26	1.11
7.0	0.12	0.17	0.22	0.23	0.16	0.17	1.07
7.5	0.19	0.21	0.11	0.16	0.13	0.00	0.80
8.0	0.23	0.14	0.15	0.16	0.17	0.13	0.98
Column Totals	2.58	2.69	2.75	3.05	2.87	2.99	16.93

Base: Water as 5 per cent carboxy methyl cellulose in 10 per cent copper sulphate.

Copper Concentration

Mg./100 ml.

Rabbit No.	102	103	104	105	106	107	
Weight (Kg.)	3.200	3.100	2.600	3.350	3.300	2.650	
Sex	Female	Female	Female	Female	Male	Male	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.29	0.19	0.23	0.26	0.18	0.21	1.36
1.0	0.32	0.21	0.21	0.35	0.22	0.18	1.49
1.5	0.16	0.33	0.19	0.14	0.22	0.27	1.31
2.0	0.15	0.21	0.27	0.11	0.17	0.14	1.05
2.5	0.11	0.18	0.25	0.21	0.25	0.18	1.18
3.0	0.22	0.19	0.16	0.31	0.23	0.21	1.32
3.5	0.23	0.22	0.22	0.16	0.22	0.31	1.36
4.0	0.14	0.00	0.14	0.22	0.16	0.26	0.92
4.5	0.26	0.14	0.13	0.14	0.18	0.18	1.03
5.0	0.19	0.11	0.10	0.09	0.14	0.14	0.77
5.5	0.17	0.22	0.14	0.00	0.21	0.22	0.96
6.0	0.23	0.19	0.21	0.15	0.11	0.33	1.22
6.5	0.18	0.13	0.13	0.18	0.20	0.20	1.02
7.0	0.23	0.20	0.00	0.21	0.14	0.11	0.89
7.5	0.30	0.13	0.19	0.16	0.10	0.16	1.04
8.0	0.21	0.16	0.21	0.14	0.17	0.19	1.08
Column Totals	3.39	2.81	2.78	2.83	2.90	3.29	18.00

Base: Emulsifying Ointment B.P. containing 10 per cent copper sulphate

Copper Concentration

Mg./100 ml.

Rabbit No.	102	103	104	105	106	107	
Weight (Kg.)	3.250	3.200	2.700	3.450	3.450	2.750	Row
Sex	Female	Female	Female	Female	Male	Male	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.23	0.20	0.30	0.19	0.26	0.36	1.54
1.0	0.27	0.35	0.25	0.23	0.27	0.21	1.58
1.5	0.25	0.29	0.17	0.21	0.16	0.22	1.30
2.0	0.24	0.16	0.20	0.22	0.21	0.24	1.27
2.5	0.18	0.19	0.16	0.34	0.26	0.17	1.30
3.0	0.11	0.16	0.22	0.19	0.24	0.16	1.08
3.5	0.19	0.16	0.24	0.18	0.25	0.19	1.21
4.0	0.14	0.25	0.27	0.25	0.13	0.27	1.31
4.5	0.26	0.29	0.19	0.22	0.19	0.31	1.46
5.0	0.20	0.23	0.11	0.18	0.14	0.14	1.00
5.5	0.17	0.22	0.09	0.17	0.15	0.17	0.97
6.0	0.24	0.19	0.21	0.26	0.17	0.28	1.35
6.5	0.23	0.17	0.25	0.24	0.24	0.15	1.28
7.0	0.19	0.20	0.17	0.19	0.26	0.31	1.32
7.5	0.15	0.20	0.22	0.23	0.11	0.20	1.11
8.0	0.27	0.15	0.14	0.20	0.21	0.19	1.16
Column Totals	3.32	3.41	3.19	3.50	3.25	3.57	20.24

Base: Emulsifying Ointment B.P. containing 10 per cent copper sulphate.

Copper Concentration

Mg./100 ml.

Rabbit No.	102	103	104	105	106	107	
Weight (Kg.)	3.200	3.100	2.650	3.350	3.300	2.700	Row
Sex	Female	Female	Female	Female	Male	Male	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.26	0.31	0.33	0.30	0.19	0.23	1.62
1.0	0.20	0.24	0.36	0.25	0.21	0.19	1.45
1.5	0.18	0.20	0.22	0.24	0.28	0.22	1.34
2.0	0.14	0.26	0.14	0.19	0.16	0.27	1.16
2.5	0.23	0.18	0.20	0.16	0.22	0.14	1.13
3.0	0.10	0.13	0.16	0.20	0.25	0.23	1.07
3.5	0.22	0.20	0.23	0.10	0.14	0.34	1.23
4.0	0.19	0.26	0.20	0.00	0.14	0.21	1.00
4.5	0.16	0.18	0.16	0.13	0.19	0.19	1.01
5.0	0.20	0.19	0.24	0.21	0.23	0.26	1.33
5.5	0.22	0.21	0.17	0.31	0.22	0.21	1.34
6.0	0.25	0.17	0.22	0.21	0.11	0.18	1.14
6.5	0.27	0.21	0.26	0.16	0.13	0.33	1.36
7.0	0.23	0.28	0.24	0.13	0.20	0.27	1.35
7.5	0.20	0.19	0.21	0.20	0.19	0.25	1.24
8.0	0.16	0.19	0.17	0.12	0.23	0.22	1.09
Column Totals	3.21	3.40	3.51	2.91	3.09	3.74	19.86

Base: Lard containing 10 per cent copper sulphate.

Copper Concentration

Mg./100 ml.

Rabbit No.	102	103	104	105	106	107	
Weight (Kg.)	3.100	3.100	2.600	3.300	3.250	2.650	
Sex	Female	Female	Female	Female	Male	Male	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.35	0.31	0.33	0.27	0.19	0.35	1.80
1.0	0.22	0.26	0.29	0.30	0.24	0.24	1.55
1.5	0.25	0.29	0.14	0.22	0.18	0.19	1.27
2.0	0.19	0.15	0.16	0.14	0.22	0.25	1.11
2.5	0.15	0.21	0.21	0.11	0.27	0.15	1.10
3.0	0.19	0.16	0.27	0.16	0.33	0.17	1.28
3.5	0.31	0.21	0.28	0.19	0.26	0.26	1.51
4.0	0.14	0.11	0.20	0.27	0.24	0.31	1.27
4.5	0.21	0.16	0.31	0.14	0.17	0.22	1.21
5.0	0.22	0.20	0.33	0.26	0.23	0.23	1.47
5.5	0.14	0.23	0.19	0.22	0.25	0.27	1.30
6.0	0.22	0.18	0.27	0.17	0.29	0.34	1.47
6.5	0.16	0.21	0.25	0.17	0.11	0.23	1.13
7.0	0.17	0.26	0.18	0.24	0.21	0.30	1.36
7.5	0.24	0.22	0.22	0.27	0.30	0.19	1.44
8.0	0.27	0.14	0.22	0.23	0.19	0.32	1.37
Column Totals	3.43	3.30	3.85	3.36	3.68	4.02	21.64

Base: Lard containing 10 per cent copper sulphate

Copper Concentration

Mg./100 ml.

Rabbit No.	102	103	104	105	106	107	
Weight (Kg.)	3.100	3.100	2.550	3.300	3.300	2.650	Row
Sex	Female	Female	Female	Female	Male	Male	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.39	0.40	0.30	0.21	0.33	0.35	1.98
1.0	0.32	0.30	0.15	0.22	0.39	0.22	1.60
1.5	0.21	0.18	0.31	0.16	0.22	0.17	1.25
2.0	0.14	0.27	0.22	0.20	0.16	0.19	1.18
2.5	0.17	0.23	0.16	0.21	0.20	0.24	1.21
3.0	0.21	0.24	0.18	0.15	0.14	0.16	1.08
3.5	0.22	0.16	0.20	0.10	0.23	0.27	1.18
4.0	0.11	0.16	0.17	0.19	0.25	0.26	1.14
4.5	0.19	0.22	0.14	0.21	0.17	0.22	1.15
5.0	0.24	0.09	0.16	0.21	0.14	0.20	1.04
5.5	0.25	0.23	0.10	0.18	0.27	0.14	1.17
6.0	0.12	0.21	0.27	0.21	0.32	0.18	1.31
6.5	0.25	0.13	0.18	0.25	0.26	0.22	1.29
7.0	0.18	0.15	0.14	0.20	0.27	0.31	1.25
7.5	0.22	0.28	0.13	0.18	0.26	0.26	1.33
8.0	0.15	0.18	0.28	0.11	0.27	0.24	1.23
Column Totals	3.37	3.43	3.09	2.99	3.88	3.63	20.39

Base: Lard containing 10 per cent copper acetyl-acetonate.

Copper Concentration

Mg./100 ml.

Rabbit No.	A	B	C	D	E	F	
Weight (Kg.)	3.450	3.500	3.350	3.400	3.350	3.525	Row
Sex	Female	Female	Female	Female	Male	Male	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.36	0.28	0.36	0.31	0.23	0.30	1.84
1.0	0.24	0.20	0.23	0.26	0.19	0.29	1.41
1.5	0.30	0.19	0.24	0.21	0.29	0.18	1.41
2.0	0.33	0.27	0.26	0.27	0.20	0.22	1.55
2.5	0.24	0.26	0.22	0.25	0.28	0.30	1.55
3.0	0.32	0.23	0.25	0.30	0.27	0.22	1.59
3.5	0.35	0.27	0.28	0.27	0.32	0.20	1.69
4.0	0.22	0.24	0.33	0.20	0.19	0.24	1.42
4.5	0.36	0.21	0.25	0.25	0.24	0.33	1.64
5.0	0.32	0.27	0.27	0.23	0.23	0.15	1.47
5.5	0.18	0.21	0.28	0.25	0.21	0.30	1.43
6.0	0.17	0.18	0.20	0.27	0.23	0.20	1.25
6.5	0.24	0.27	0.29	0.28	0.21	0.26	1.55
7.0	0.14	0.22	0.18	0.18	0.20	0.22	1.14
7.5	0.19	0.25	0.23	0.26	0.24	0.25	1.42
8.0	0.29	0.18	0.18	0.22	0.15	0.21	1.23
Column Totals	4.25	3.73	4.05	4.01	3.68	3.87	23.59

Base: Lard containing 10 per cent copper acetyl-acetonate.

Copper Concentration

Mg./100 ml.

Rabbit No.	A	B	C	D	E	F	
Weight (Kg.)	3.500	3.550	3.350	3.375	3.300	3.500	
Sex	Female	Female	Female	Female	Male	Male	Row Totals
Hours after Application							
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.27	0.51	0.54	0.25	0.14	0.60	2.31
1.0	0.18	0.15	0.27	0.28	0.54	0.19	1.61
1.5	0.31	0.19	0.18	0.51	0.24	0.39	1.82
2.0	0.19	0.42	0.40	0.21	0.18	0.24	1.64
2.5	0.16	0.21	0.31	0.46	0.51	0.50	2.15
3.0	0.47	0.27	0.18	0.30	0.30	0.26	1.78
3.5	0.15	0.44	0.00	0.21	0.54	0.40	1.74
4.0	0.19	0.20	0.16	0.48	0.12	0.24	1.39
4.5	0.40	0.21	0.09	0.21	0.17	0.17	1.25
5.0	0.25	0.39	0.14	0.18	0.53	0.20	1.69
5.5	0.26	0.21	0.46	0.15	0.14	0.31	1.53
6.0	0.47	0.19	0.15	0.41	0.36	0.09	1.67
6.5	0.26	0.46	0.36	0.19	0.60	0.33	2.20
7.0	0.58	0.15	0.47	0.09	0.29	0.45	2.03
7.5	0.23	0.13	0.29	0.22	0.17	0.18	1.22
8.0	0.25	0.19	0.16	0.19	0.38	0.25	1.42
Column Totals	4.62	4.32	4.16	4.34	5.21	4.80	27.45

Base: Lard containing 10 per cent copper acetyl-acetonate

Copper Concentration

Mg./100 ml.

Rabbit No.	G	H	I	J	K	L	
Weight (Kg.)	3.325	3.375	3.300	3.750	3.425	3.600	Row
Sex	Male	Male	Male	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.28	0.42	0.33	0.20	0.27	0.39	1.89
1.0	0.33	0.19	0.23	0.33	0.21	0.31	1.60
1.5	0.32	0.28	0.18	0.20	0.24	0.25	1.47
2.0	0.18	0.27	0.29	0.25	0.38	0.30	1.67
2.5	0.31	0.29	0.33	0.27	0.21	0.26	1.67
3.0	0.28	0.22	0.24	0.22	0.20	0.28	1.44
3.5	0.37	0.27	0.35	0.23	0.28	0.19	1.69
4.0	0.27	0.24	0.25	0.32	0.31	0.30	1.69
4.5	0.20	0.22	0.32	0.26	0.24	0.20	1.44
5.0	0.14	0.26	0.24	0.21	0.26	0.32	1.43
5.5	0.20	0.21	0.37	0.31	0.24	0.19	1.52
6.0	0.26	0.26	0.20	0.22	0.30	0.30	1.54
6.5	0.24	0.32	0.52	0.19	0.24	0.27	1.78
7.0	0.16	0.17	0.32	0.30	0.21	0.20	1.36
7.5	0.27	0.22	0.24	0.25	0.37	0.21	1.56
8.0	0.33	0.22	0.31	0.21	0.23	0.29	1.59
Column Totals	4.14	4.06	4.72	3.97	4.19	4.26	25.34

Base: Lard containing 10 per cent copper acetyl-acetonate.

Copper Concentration

Mg./100 ml.

Rabbit No.	G	H	I	J	K	L	
Weight (Kg.)	3.300	3.350	3.250	3.800	3.400	3.700	Row
Sex	Male	Male	Male	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.23	0.31	0.36	0.27	0.24	0.33	1.74
1.0	0.29	0.27	0.32	0.38	0.31	0.29	1.86
1.5	0.33	0.33	0.21	0.25	0.19	0.22	1.53
2.0	0.18	0.22	0.41	0.19	0.22	0.23	1.45
2.5	0.26	0.27	0.22	0.23	0.27	0.29	1.54
3.0	0.22	0.19	0.29	0.37	0.23	0.17	1.47
3.5	0.31	0.21	0.25	0.24	0.19	0.24	1.44
4.0	0.24	0.26	0.26	0.23	0.24	0.31	1.54
4.5	0.17	0.34	0.16	0.25	0.19	0.22	1.33
5.0	0.29	0.26	0.37	0.36	0.14	0.40	1.82
5.5	0.29	0.27	0.29	0.29	0.28	0.21	1.63
6.0	0.19	0.23	0.27	0.30	0.25	0.36	1.60
6.5	0.21	0.20	0.31	0.31	0.21	0.23	1.47
7.0	0.20	0.19	0.18	0.27	0.11	0.14	1.09
7.5	0.14	0.27	0.28	0.31	0.18	0.27	1.45
8.0	0.23	0.22	0.17	0.16	0.26	0.21	1.25
Column Totals	3.78	4.04	4.35	4.41	3.51	4.12	24.21

Base: Water as 5 per cent carboxy methyl cellulose containing 10 per cent copper acetyl-acetonate

Copper Concentration

Mg./100 ml.

Rabbit No.	A	B	C	D	E	F	
Weight (Kg.)	3.500	3.550	3.425	3.350	3.200	3.450	Row
Sex	Female	Female	Female	Female	Male	Male	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.30	0.27	0.31	0.22	0.31	0.20	1.61
1.0	0.28	0.16	0.21	0.22	0.11	0.18	1.16
1.5	0.26	0.18	0.00	0.14	0.21	0.27	1.06
2.0	0.20	0.23	0.17	0.15	0.18	0.16	1.09
2.5	0.22	0.20	0.14	0.20	0.16	0.15	1.07
3.0	0.21	0.12	0.20	0.19	0.19	0.18	1.09
3.5	0.19	0.27	0.13	0.23	0.27	0.20	1.29
4.0	0.14	0.27	0.17	0.24	0.24	0.15	1.21
4.5	0.22	0.20	0.15	0.16	0.15	0.11	0.99
5.0	0.16	0.17	0.10	0.14	0.12	0.17	0.86
5.5	0.14	0.14	0.22	0.12	0.00	0.24	0.86
6.0	0.13	0.21	0.15	0.21	0.09	0.13	0.92
6.5	0.19	0.12	0.16	0.20	0.17	0.17	1.01
7.0	0.00	0.13	0.21	0.18	0.22	0.21	0.95
7.5	0.10	0.19	0.18	0.11	0.14	0.24	0.96
8.0	0.15	0.17	0.18	0.18	0.25	0.16	1.09
Column Totals	2.89	3.03	2.68	2.89	2.81	2.92	17.22

Base: Water as 5 per cent carboxy methyl cellulose containing 10 per cent copper acetyl-acetonate.

Copper Concentration

Mg./100 ml.

Rabbit No.	A	B	C	D	E	F	
Weight (Kg.)	3.550	3.575	3.500	3.350	3.200	3.400	
Sex	Female	Female	Female	Female	Male	Male	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.30	0.30	0.33	0.27	0.26	0.34	1.80
1.0	0.31	0.21	0.24	0.24	0.21	0.21	1.42
1.5	0.24	0.25	0.22	0.22	0.26	0.24	1.43
2.0	0.20	0.17	0.23	0.23	0.23	0.19	1.25
2.5	0.18	0.15	0.19	0.23	0.19	0.17	1.11
3.0	0.19	0.22	0.24	0.22	0.00	0.14	1.01
3.5	0.17	0.13	0.15	0.22	0.14	0.14	0.95
4.0	0.12	0.19	0.17	0.11	0.18	0.11	0.88
4.5	0.17	0.21	0.17	0.00	0.15	0.21	0.91
5.0	0.13	0.15	0.21	0.15	0.16	0.23	1.03
5.5	0.15	0.18	0.18	0.17	0.19	0.16	1.03
6.0	0.20	0.24	0.20	0.13	0.11	0.14	1.02
6.5	0.18	0.00	0.19	0.22	0.08	0.21	0.88
7.0	0.19	0.15	0.16	0.18	0.17	0.30	1.15
7.5	0.27	0.20	0.22	0.16	0.23	0.18	1.26
8.0	0.17	0.15	0.20	0.23	0.16	0.11	1.02
Column Totals	3.17	2.90	3.30	2.98	2.72	3.08	18.15

Base: Emulsifying Ointment B.P. containing 10 per cent copper acetyl-acetonate.

Copper Concentration

Mg./100 ml.

Rabbit No.	G	H	I	J	K	L	
Weight (Kg.)	3.250	3.325	3.250	3.800	3.450	3.900	Row
Sex	Male	Male	Male	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.27	0.29	0.16	0.26	0.25	0.19	1.42
1.0	0.31	0.29	0.19	0.24	0.19	0.27	1.49
1.5	0.24	0.18	0.51	0.21	0.19	0.24	1.57
2.0	0.21	0.27	0.25	0.22	0.15	0.23	1.33
2.5	0.17	0.26	0.23	0.39	0.21	0.10	1.36
3.0	0.26	0.17	0.14	0.22	0.16	0.19	1.14
3.5	0.21	0.22	0.23	0.19	0.17	0.28	1.30
4.0	0.25	0.21	0.21	0.20	0.20	0.18	1.25
4.5	0.26	0.11	0.17	0.28	0.14	0.26	1.22
5.0	0.23	0.20	0.23	0.26	0.19	0.23	1.34
5.5	0.25	0.18	0.26	0.22	0.13	0.20	1.24
6.0	0.14	0.13	0.24	0.21	0.27	0.34	1.33
6.5	0.23	0.25	0.17	0.18	0.16	0.19	1.18
7.0	0.26	0.24	0.12	0.15	0.17	0.18	1.12
7.5	0.17	0.20	0.14	0.22	0.28	0.26	1.27
8.0	0.17	0.18	0.21	0.25	0.20	0.18	1.19
Column Totals	3.63	3.38	3.46	3.70	3.06	3.52	20.75

Base: Emulsifying Ointment B.P. containing 10 per cent copper acetyl-acetonate

Copper Concentration

Mg./100 ml.

Rabbit No.	G	H	I	J	K	L	
Weight (Kg.)	3.350	3.250	3.275	3.500	3.475	3.475	Row
Sex	Male	Male	Male	Female	Female	Female	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.32	0.24	0.33	0.18	0.23	0.29	1.59
1.0	0.27	0.29	0.21	0.30	0.22	0.28	1.57
1.5	0.18	0.40	0.24	0.20	0.27	0.20	1.49
2.0	0.29	0.20	0.23	0.23	0.25	0.20	1.40
2.5	0.29	0.23	0.25	0.25	0.33	0.22	1.57
3.0	0.21	0.23	0.31	0.17	0.21	0.24	1.37
3.5	0.23	0.24	0.18	0.23	0.23	0.24	1.35
4.0	0.21	0.26	0.25	0.13	0.20	0.32	1.37
4.5	0.20	0.23	0.27	0.20	0.23	0.18	1.31
5.0	0.24	0.15	0.16	0.21	0.33	0.24	1.33
5.5	0.30	0.21	0.34	0.19	0.22	0.24	1.50
6.0	0.23	0.34	0.28	0.16	0.25	0.23	1.49
6.5	0.24	0.27	0.22	0.19	0.23	0.29	1.44
7.0	0.16	0.21	0.29	0.19	0.26	0.34	1.45
7.5	0.22	0.21	0.32	0.14	0.15	0.24	1.28
8.0	0.19	0.24	0.22	0.17	0.29	0.23	1.34
Column Totals	3.78	3.95	4.10	3.14	3.90	3.98	22.85

Base: Lard containing 10 per cent sulphanilamide and 10 per cent copper sulphate.

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	G	H	I	J	K	L	
Weight (Kg.)	3.500	3.550	3.450	3.700	3.550	3.600	
Sex	Male	Male	Male	Female	Female	Female	Row Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.60	0.95	0.68	1.14	0.68	0.65	4.70
1.0	0.61	1.03	0.85	0.70	0.69	0.80	4.68
1.5	0.54	0.72	0.67	0.53	0.74	0.83	4.03
2.0	0.43	0.84	0.61	0.67	1.07	0.72	4.34
2.5	0.63	0.79	0.55	0.31	0.84	0.94	4.06
3.0	0.84	0.81	0.65	0.91	0.67	0.90	4.78
3.5	0.50	0.74	0.75	0.88	0.99	0.54	4.40
4.0	0.35	0.62	0.93	0.60	0.80	0.79	4.09
4.5	0.53	0.81	1.02	0.42	0.85	0.81	4.44
5.0	0.20	0.51	0.70	0.63	0.63	0.72	3.39
5.5	0.29	0.70	0.89	0.81	0.44	0.34	3.47
6.0	0.15	0.61	0.32	0.75	0.67	0.65	3.15
6.5	0.71	0.83	0.46	0.77	0.82	0.41	4.00
7.0	0.62	0.72	0.69	0.51	0.91	0.38	3.83
7.5	0.66	0.50	0.80	0.34	0.88	0.84	4.02
8.0	0.53	0.64	0.51	0.47	0.78	0.46	3.39
Column Totals	8.19	11.82	11.08	10.44	12.46	10.78	64.77

Base: Lard containing 10 per cent sulphanilamide and 5 per cent salicylic acid

Sulphanilamide Concentration

Mg./100 ml.

Rabbit No.	A	B	C	D	E	F	
Weight (Kg.)	3.550	3.575	3.400	3.425	3.400	3.525	Row
Sex	Female	Female	Female	Female	Male	Male	Totals
Hours after Application							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.54	0.95	0.71	1.21	0.72	0.67	4.80
1.0	0.78	0.72	0.89	1.09	1.17	0.81	5.46
1.5	1.23	0.64	1.14	0.83	0.91	1.16	5.91
2.0	0.99	0.70	1.02	1.12	0.50	0.73	5.06
2.5	0.46	1.18	0.87	0.77	1.21	0.91	5.40
3.0	1.15	0.61	0.91	0.43	1.31	0.81	5.22
3.5	0.95	0.61	0.47	0.91	0.81	1.03	4.78
4.0	1.13	1.15	0.92	1.20	1.08	1.15	6.63
4.5	0.72	0.52	1.12	0.94	1.28	0.83	5.41
5.0	0.80	0.78	0.84	0.33	1.23	0.95	4.93
5.5	0.91	1.03	0.77	0.56	1.14	0.43	4.84
6.0	1.10	0.89	0.92	0.90	1.00	0.67	5.48
6.5	1.02	1.12	0.83	1.07	0.56	0.31	4.91
7.0	0.91	0.71	0.41	1.11	0.72	0.40	4.26
7.5	1.27	0.83	0.91	1.03	0.66	0.65	5.35
8.0	0.75	0.57	1.04	0.88	0.93	0.33	4.50
Column Totals	14.71	13.01	13.77	14.38	15.23	11.84	82.94

APPENDIX TWO.

THE STATISTICAL ANALYSES OF VARIANCE.

The Analysis of Variance

Three complete analyses of variance were carried out on the results obtained from Hydrous Ointment B.P., Emulsifying Ointment B.P. and Hydrous Emulsifying Ointment B.P., on a Dutch, Beveren and Copenhagen White litter of rabbits.

From the results obtained in each litter, three tables were constructed. The first compared the response of each of the six rabbits to each of the three bases, the second compared the response of the six rabbits at corresponding times, and the third compared the amount absorbed from each of the three bases at corresponding times.

On each of these three tables an analysis of variance into components due to Rows, Columns and a Residual was carried out. These three two-factor analyses were drawn together and combined in a three factor analysis and each term tested for significance. The three factor analyses obtained for the Dutch, Beveren and Copenhagen White breeds are given and the sources of variance shown to be significant are marked.

The implication and possible cause of their significance are discussed in the results.

The Analyses of Variance on the results obtained from Hydrous Ointment B.P., Emulsifying Ointment B.P., and Hydrous Emulsifying Ointment B.P., containing ten per cent sulphanilamide, applied to six Dutch rabbits

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F
Between bases	2	9.6210	4.8105	Significant
Between rabbits	5	1.2539	0.2507	Not significant
Between times	15	3.2698	0.2179	Not significant
Base x Time	30	4.2827	0.1428	Not significant
Base x Rabbit	10	4.0881	0.4088	Significant
Time x Rabbit	75	5.2016	0.0694	Significant
Base x Time x Rabbit	150	18.1288	0.1207	Not significant
Residual	288	45.3252	0.1574	
Total	575	136.4963		

The Analyses of Variance on the results obtained from Hydrous Ointment B.P., Emulsifying Ointment B.P., and Hydrous Emulsifying Ointment B.P., containing ten per cent sulphanilamide, applied to six Copenhagen White rabbits

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F
Between bases	2	3.5308	1.7519	Significant
Between rabbits	5	0.6288	0.1258	Not Significant
Between times	15	1.5172	0.1011	Significant
Base x Time	30	1.1396	0.0380	Not Significant
Base x Rabbit	10	1.9874	0.1985	Significant
Rabbit x Time	75	3.2520	0.0434	Not Significant
Base x Time x Rabbit	150	7.6205	0.0508	Not Significant
Total	287	19.6466		

Since the results of this experiment could not be duplicated the Base x Time x Rabbit interaction factor is the best estimate of a Residual which can be made.

The Analyses of Variance on the results obtained from Hydrous Ointment B.P., Emulsifying Ointment B.P., and Hydrous Emulsifying Ointment B.P., containing ten per cent sulphanilamide, applied to six Beveren rabbits

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F
Between bases	2	43.9078	21.9539	Significant
Between rabbits	5	1.7501	0.3500	Not Significant
Between times	11	16.1366	1.2851	Not Significant
Base x Time	22	24.0431	1.0929	Significant
Base x Rabbit	10	24.1965	2.4197	Significant
Rabbit x Time	55	19.5705	0.3558	Not Significant
Base x Time x Rabbit	110	44.9525	0.4086	Not Significant
Residual	216	75.0986	0.3489	
Total	431	249.6557		

Comparison of the Means of Male and Female Rabbits By Student's t-test

This was undertaken on the rabbits of the Dutch litter

$$\text{Mean result for the six rabbits} = \frac{317.93}{576} = 0.5520$$

$$\text{Mean result for male rabbits} = \frac{213.75}{384} = 0.5568$$

$$\text{Mean result for female rabbits} = \frac{104.18}{} = 0.5426$$

Residual Variance for the tests = 0.1574

$$t = \frac{0.5569 - 0.5426}{\sqrt{0.1574}} \times \sqrt{\frac{384 \times 192}{384 + 192}}$$

$$= \frac{0.0142}{0.3968} \times 11.31$$

$$= \frac{0.1423}{0.3968} \times 11.31$$

$$= 0.405$$

This value is not significant at the 5 per cent level.

It may therefore be concluded that there is no significant difference in the percutaneous absorption of sulphanilamide by male and female rabbits.

Since no large differences between the sexes were ever apparent in subsequent tests, this conclusion may justifiably be extended to a general principle.

The Comparison of results from the Dutch and Copenhagen litters

$$\text{Mean response of Dutch litter} = \frac{317.93}{576} = 0.5520$$

$$\text{Mean response of Copenhagen litter} = \frac{150.31}{288} = 0.5219$$

$$\text{Difference between means} = 0.5520 - 0.5219 = 0.0301$$

$$\text{Variance} = \frac{13.9996 + 0.7432}{862} = \frac{14.7428}{862} = 0.0171$$

$$\text{To allow for numbers in samples, divide by 192} = \frac{0.0171}{192}$$

$$= 0.000089$$

$$\text{Therefore, the standard deviation} = \sqrt{0.000089} = 0.009438$$

$$\text{Therefore, the difference between means} = \frac{0.0301}{0.009438} = 3.1892$$

This value is significant and it may be concluded that there is a significant difference in the response of rabbits of different breeds. For accuracy it is therefore desirable that all experiments be conducted on rabbits of the same breed. As a result of this finding all subsequent tests were carried out on Copenhagen White rabbits.

STUDIES IN PERCUTANEOUS ABSORPTION

Summary of Thesis by

John C. Morrison

1. A review of the methods used to estimate the release of medicinal substances from topical applications has been carried out.

2. An *in vivo* test has been designed to estimate the efficiency of topical applications as "carriers" of drugs through intact skin. The blood level attained by the applied drug was taken as the criterion of efficiency.

3. Sulphanilamide was tested in eleven vehicles and bases and, taking the least efficient vehicle as unity, the following comparative values obtained: lard (2.48), propylene glycol (1.89), Hydrous Ointment B.P. (1.89), white soft paraffin (1.83), ethyl oleate (1.83), cetomacrogol emulsifying wax base (1.44), Emulsifying Ointment B.P. (1.31), liquid paraffin (1.31), Hydrous Emulsifying Ointment B.P. (1.17), woolfat (1.14), water as a five per cent carboxymethyl cellulose gel (1.00).

4. Salicylic acid, sulphanilamide, copper acetyl-acetonate and copper sulphate were respectively incorporated in lard, Emulsifying Ointment B.P. and water as a five per cent carboxymethyl cellulose gel, and the blood levels of the drugs noted. Salicylic acid and sulphanilamide were respectively five and four times more efficient than the two compounds of copper. The results of all experiments were statistically analysed and the significant sources of variance noted.

5. The vehicle or base influences the blood level attained by the incorporated drug but since the amount absorbed through the intact skin is so small, these differences in efficiency are of little importance. The ability of different drugs to penetrate intact skin and be absorbed systemically varies considerably. The interaction of a drug with the vehicle or base in which it is incorporated may either hinder or promote the percutaneous absorption of that drug.