THE ISOLATION AND IDENTIFICATION OF DERMATOLOGICALLY ACTIVE CONSTITUENTS OF COAL TAR

A Thesis Submitted

by

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for

the Degree of Doctor of Philosophy

Department of Pharmacy

University of Aston in Birmingham

June 1980

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Doctor of Philosophy
1980

SUMMARY

The production and uses of coal tar are reviewed as are the uses of steroids and cytotoxic agents in the treatment of psoriasis with a review of the condition also.

An attempt was made to improve the efficaciousness and cosmetic acceptability of a low temperature tar, by screening fractions of this tar, derived from a variety of separation procedures. The most efficacious fraction was the highest boiling acid fraction, which is believed to consist mainly of mono- and di-hydric phenols. A time and concentration study showed that the optimum regime was the application of a 10% concentration in 5% wool fat in soft, yellow paraffin daily for 21 days.

The mouse tail skin was selected as an experimental model, to ascertain the efficaciousness of fractions, because of the similarities between this skin and the psoriatic lesion. The activity of a fraction was monitored by the inducement of a granular layer in the mouse tail epidermis.

Because coal tar is not an easy medium to work with, and the active fractions showed no increase in cosmetic acceptability over the parent coal tar, likely coal tar constituents were selected for screening on the basis of phenolic character, and the molecular weight range elucidated by mass spectroscopy.

32 potential anti-psoriatic agents were screened on mouse tail. Two catechols, 3,5-di-t-butyl and 4-t-butyl catechols were active. Other structures showed little or no activity. 24 catechols were screened and two extremely active catechols were discovered, 3-methyl-5-t-octyl and 5-methyl-3-t-octyl catechols.

The screening of catechol-rich coal tar fractions and a coal tar fraction which had had the catechols removed by oxidation, showed that some anti-psoriatic activity was contained in the catechol fraction of coal tar.

Attempts to elucidate the mode of action of these two compounds met with little success, but two modes of action are suggested.

KEY WORDS: Skin, Psoriasis, Coal Tar, Catechol, Mouse Tail Test.

To Kay and

my Parents

ACKNOWLEDGEMENTS

I wish to thank my friend and supervisor, Dr. A. Z. Britten for the opportunity to undertake the research which constitutes this thesis and for his valuable assistance throughout the work. My thanks to Professor D. G. Wibberley and Professor C. B. Ferry of the Department of Pharmacy for allowing me to use the facilities of their department.

I would like to thank Riker Minnesota 3M Laboratories Ltd. for generous financial support throughout the period of this work. My special thanks to Mrs. P. Turnbull for her support, without which this work would not have been possible.

I sincerely thank Miss K. Webb for her patient proof reading and Miss L. Page for her excellent typing of this manuscript.

CONTENTS

The "phenanthrones" referred to in the text have a carbon skeleton comprising of 6,7,8,9,10,14-hexahydro-6-oxo-phenanthrene.

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LIST OF ABBREVIATIONS

WTAF	Whole Tar Acid Fraction
WTPF	Whole Tar Phenolic Fraction
cAMP	Cyclic 3'5' Adenosine Monophosphate
cGMP	Cyclic 3'5' Guanosine Monophosphate
TLC	Thin Layer Chromatography
GLC	Gas-Liquid Chromatography
ETT	Epidermal Turnover Time
DNase	Deoxyribonuclease
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
Rf	Retention Factor
HTT	High Temperature Tar
LTT	Low Temperature Tar
gm	Gram
ml	Millilitre
ltr	Litre
µ	Micron
nm	Nanometre
cm	Centimetre
PUVA	Psoralen - Ultra Violet Light (320 - 400 nm)
UV	Ultra Violet
NCB	National Coal Board
HMSO	Her Majesty's Stationery Office
IARC	International Agency for Research on Cancer
OHE	Office of Health Economics
Psi	Pounds per square inch
W/V	Weight in volume

I. INTRODUCTION

A. Coal Tar

(i) Production

Coal is believed to have been formed from the incomplete decay of plant life (Parks 1963). It has provided the largest source of energy since the Industrial Revolution (Bronowski 1957). Despite competition from nuclear power, oil and natural gas, coal furnishes almost one half of Britain's energy requirements for the 1970's (N.C.B. 1972). Coal reserves are capable of meeting this demand, and will outlast the total reserves of oil and will be cheaper (Clutterbuck 1973).

Coke is the solid fuel residue formed when coal is heated in the absence of air. This coal carbonization produces not only coke but ammonia, gas, benzole and tar.

Coke is required for steel manufacture but was produced as a by-product in the manufacture of coal gas. With the increased use of natural gas a valuable source of coke has disappeared and there was a world shortage of coke. This resulted in the development of smokeless fuels for industrial and domestic uses (Jones 1956). The smokeless fuels were necessary for the implementation of the Clean Air Act of 1956 (H.M.S.O. 1956).

The production of coke is carried out in a high temperature process at about 1200^{o}C, e.g. at the National Coal Board's Avenue Tar Works at Chesterfield, or in a low temperature process at about 500^{o}C, e.g. at the Coalite and Chemical Products Ltd. works again at Chesterfield. In 1961 about 1.5% of the annual production of tar was produced by the low temperature process (Huxtable 1961). This proportion is now much larger due to the increased demand for

coke as both a domestic and industrial fuel. Low temperature coke is largely used as a domestic fuel, as it contains a high proportion of volatiles and therefore burns more readily.

The temperature and blends of coal in the carbonization process determine the nature of the products, especially the tars. The coke from the low temperature process is less dense than that from the high temperature process. The process is especially important for the medical efficacy of tars and will be discussed later.

As previously stated, the four main by-products of the carbonization process are ammonia, benzole, gas and tar.

The ammonia is converted to ammonium sulphate and is utilised as a fertiliser, other ammonia products are converted to fireproof materials.

Crude benzole has many uses including the manufacture of benzene, toluene, xylenes, naphthas and caprolactam. The latter is used for nylon 6 production. Refinement gives rise to products which find their way into such diverse consummables as plastics, photographic materials, aspirin, dyes, perfumes, saccharin, food preservatives, polishes, paints and varnishes.

The use of coal gas has decreased with the advent of natural gas. What coal gas is produced is piped and sold directly to hospitals and industries near the coking ovens (Wrench 1973).

The fourth by-product is coal tar.

(ii) Content and Uses

Up until the mid-nineteenth century, tars were unwanted by-products of coke production (Moore and Hall 1939). The discovery of useful chemicals in coal tar, e.g. benzene, began the search

for useful and economically important chemicals in tar (Robinson 1937).

The largest and most important non-fuel use of coal in present times is the extraction of chemicals from the by-products of carbonization, so much so that tar yields and possible uses are considered before a new method of carbonization is utilised.

Many methods are utilised in carbonization but they are usually divided into the low and high temperature carbonization. The solid product of the low temperature carbonization is the coke which is used domestically. As previously stated this coke is softer, burns more easily, has a lower density and has a higher volatile content than high temperature cokes (Wilson and Clenendin 1963).

Karr (1963) defines the parameters of low temperature carbonization and suggests an upper temperature limit of 500^{o}C as considerable aromatisation occurs at 600^{o}C, often the upper limit quoted by other authors.

Low temperature tars vary with the type of coal, vessel used and residence time in the oven. There are far more qualitative and quantitative differences in low temperature tars than high temperature tars (Pichler et al. 1970). Low temperature tars contain small amounts of a large number of components, these rarely approach 1% of the total mass (Coombes 1944). Coke oven tars (high temperature tars) often contain up to 10% naphthalene.

Most reports on the content of low temperature tars are on the acidic fraction, because the extraction of the phenolic products is of the greatest economical importance (Bristow 1947). The use of coal tar products is summarised in FIG. 1, the main uses of the phenols are resins, plasticizers, wetting agents, inhibitors, insecticides and ore flotation. Phenols also produce disinfectants and antiseptics (Wechsler 1962).

Coalite and Chemical Products Limited

FIG 1. Coal Tar Products.

Internal combustion engine fuels may be made from low temperature tar but when petroleum is plentiful this source of fuel is not utilised. Germany and Japan both used these fuels in World War II (Kusy 1970).

High temperature carbonization increases pitch, naphthalene and the density of tar. Tar acids are decreased and consequently low temperature tars are the most important source of tar acids.

A great deal of work has been carried out on the analysis of high temperature pitch, which is used for road making. There are over 5,000 constituents, of which about 100 have been identified. All are condensed polynuclear ring compounds.

Recovery of individual tar acids has been the subject of many studies (Diericks 1950, Harris et al. 1953, 1956, Wood and Philips 1955, Wood and Wilman 1958) without great success.

Quinoline and isoquinoline are recovered from the base fractions.

The newly formed tar is usually reheated to 300°C to 400°C to distill off the volatile oils leaving pitch. Some of the oils are washed to yield acids and bases. One use of coal tar which does not often occur in the texts is the utilization of coal tar as a topical therapeutic agent. Tar has often been praised for its therapeutic effects (Section IA(iv)) but is not without its drawbacks (Section IA(iii)). The basis of its action has never been elucidated, and is one of the aims of this project.

(iii) Adverse Effects of Coal Tar

To the layman coal tar is more notorious for its ability to cause dermatoses, than its ability to cure them. The incidence of dermatoses and carcinomas amongst tar workers is well documented

(Kennaway 1924, Legge 1910, Wood, 1929, Fisher 1953 & 1965). Fortunately, safety legislation has drastically reduced their occurrence.

One of the many actions of tar is to stimulate epidermal cell division giving rise to many types of tar tumours, the exact opposite of an anti-psoriatic agent!

Kennaway (1924) reviewed the different types of tar with regard to carcinogenicity. He suggested that carcinogenic action was contained in the higher boiling fractions, i.e. 250^{o}C to 500^{o}C. He concluded that, blast furnace tar was not carcinogenic, this tar contained more phenols, but less phenol than gasworks tar. This characteristic is shared with low temperature tars.

Bloch and Widmer (1924) found the carcinogenic factor to have a boiling point greater than 230^{o}C at 1.15 mm Hg pressure. They suggested there was several carcinogenic substances in coal tar, and these were high molecular weight cyclic hydrocarbons.

Coombes (1954) reported the carcinogenic factor to be in the range 230^{o}C to 400^{o}C, the anthracene and heavy oil fraction.

Wood (1929) reviewed the work in this field and concluded that tar warts were caused by high temperature tars, not gasworks tars or low temperature tars. The heavier oils from high temperature tars caused a pustular reaction, but did not cause papillomas.

Fisher in 1953, published a monograph concerning tar ailments in tar distillery workers. Tar affected the hair follicles, causing acute and chronic erythrema, pigmentation, chronic tar dermatoses, warts and epitheliomas. Inflammation of the follicles caused tar acne and a wart often formed round these inflamed follicles. The incidence of warts was strongly related to exposure.

Alleviation of itching by low boiling fractions was reported by Rothman and Shapiro in 1949. Previously Obermayer and Becker

(1935) had reported that the lower boiling fractions caused irritation at high concentrations. At low levels they report these fractions do seem to have anti-pruritic action which they assign to the phenols and cresols.

Hodgson (1973) reported a case of sunburn, increased melanosis, pitch warts and squamous carcinomas on a pitch worker's skin. Saperstein (1979) evaluated four commercially available coal tar ointments by the Ames Salmonella mutagenicity test and found all four to be mutagenic, but not as mutagenic as the parent coal tar. Saperstein goes on to state that many extremely potent carcinogens have been isolated from coal tar. This observation has been verified by the findings of the I.A.R.C. (1973) using animals. Petrozzi and Barton (1979) reported few, if any, proven cases of carcinogenicity caused by coal tar therapy.

Coal tar has a systemic toxicity through skin absorption. Babes (1928) found extensive spleen lesions after painting rabbit skin with tar whilst Davidson (1925) produced liver damage by painting rabbits ears with ethereal coal tar solution. Grigor'ev (1959) reported liver, spleen and kidney lesions which he attributed to systemic absorption of phenols; he was, however, using a benzene solution of coal tar. Benzene is well known to be toxic.

The evidence is overwhelming that coal tar has adverse effects on the skin, yet it is still one of the most useful topical treatments in a variety of skin conditions. It is well known that skin contact with coal tar should be avoided if at all possible. As a result of these two contradictory statements it becomes imperative than an active compound be isolated from coal tar to reap its benefits and to eliminate the undesirable side effects.

(iv) Skin Disorders Treated with Coal Tar

Coal tar has been, and still is, used topically for a variety of skin disorders including psoriasis (Esterley and Soloman 1976, Muller 1978). The first use of coal tar as a topical therapeutic agent was over 2000 years ago (Kerr and Plein 1953). Fischel in 1894 used coal tar for many dermatoses (Coombes 1947) and many reports soon followed (Sack 1896, Leistikow 1900 and Dind 1906). Brocq used neat coal tar in 1909 to treat lichen simplex, and White in 1921 used coal tar for eczemas, pruritus ani et vulvae, neurodermatitis, and a large variety of other skin disorders. Unlike Brocq, White used a 5% coal tar concentration in a zinc oxide and petrolatum base.

It was noticed that most psoriatics experience an improvement with sunlight. Goekerman tested many fluorescent substances on the skin to sensitise the skin to sunlight. These substances included eosin, quinine, rose bengal and sodium chloride. All proved to be useless. Coal tar was the only efficacious material (Goekerman 1931). The coal tar regime consisted of applying the coal tar in Whites ointment for 24 hours. The skin was then wiped clean leaving a thin film of tar and then irradiated with ultra violet light. The remaining debris was cleared with a soap and water, or, if the skin was sensitive, an oatmeal or soda bath. The treatment was repeated daily (Goekerman 1925). Muller and Kierland (1964) have used this treatment for 38 years with no toxicity, and a modified form of this treatment is still used today (Young 1972). Many workers have praised this regime (O'Leary 1943, Perry 1968). Side effects are minimal, those reported being blisters (O'Leary 1943) and folliculitus (Perry 1968). These side effects disappear on interruption of treatment.

Ingram (1953) initiated a modified form of this treatment which involved a coal tar bath and UV (ultra violet) therapy with an ointment consisting of dithranol (1,8,9-trihydroxy-anthracene) in Lassars paste (zinc oxide and salycilic acid in starch and white paraffin). Ingram believes that coal tar potentiates the UV therapy as well as having anti-psoriatic activity.

No consistent explanation has been put forward to explain the co-operation of UV and coal tar. Obermayer and Becker (1935) suggest acridines in the coal tar may be the photosensitiser, but Everett and Miller (1961) state the wavelengths required are out of the range of the hospital lamps. Herrick and Sheard (1928) found that irradiated coal tar shows increased UV transmission, but Muller and Kierland (1964) found that coal tar filtered UV rather than sensitised for it.

With the advent of psoralens (Section I C) it was discovered that UV light could be split into three regions, all with different dermatological properties. These were named UV A, B and C.

Parrish et al. (1978) found that the best results were obtained by using psoralens and UV-A light (320 to 400 nm), this treatment was impracticable at that time because of the lamp intensity which caused long exposure times.

This observation was challenged by Morrison et al. (1978), who seemed to confuse psoralens and coal tar, and claimed that UV-A had no advantages over broad spectrum UV light; surprisingly Parrish was one of the co-workers.

Petrozzi et al (1978), showed that the most efficacious region for light alone was in the UV-B range (290 to 320 nm). This unfortunately is in the eryth emic range. Petrozzi also noted

that only 1% coal tar was needed. Application of tar two hours before irradiation was as good as overnight usage, little difference occurred between vehicles, and the best coal tar product was coal tar itself.

In an earlier comparison study Petrozzi and Barton (1970) showed that in the UV-A range coal tar was inferior to psoralens.

There has been disagreement about whether a tar from a high temperature (HTT), or a low temperature (LTT), carbonization process is the most efficacious. Both tars have been used; Brocq (1909) used a LTT, whilst White used a HTT in 1921. Both claimed successful results.

Obermayer and Becker (1935) suggested that only LTT be used, and pointed out that HTT contains large amounts of pitch and free carbon. These, they claim, make the tar dark, thick and heavy. They also suggest that the higher boiling fractions are used, as the lower boiling fractions are irritant. Downing and Bauer (1948) evaluated many HTT's and LTT's separately on a variety of dermatoses, and concluded that HTT's were useful in the treatment of scaly dermatoses, such as psoriasis, but should not be used in the acute stages of the disease. Coombes (1954) suggested that high tar acid content would cause irritation in eroded parts of the skin. Tars with low tar acid content relieved pruritus and acute vesicular eruptions, whilst tars with high acid content were useful in chronic scaly dermatoses such as psoriasis. Morely (1970) stated that the best treatment for psoriasis was gas works tar, a LTT, whilst Chapman and Finn (1976) found no difference between the two.

Coal tar was found long ago to be irritant and many workers attempted to find a non-irritant therapeutic fraction. Towle

(1921) suggested washing the tar before use whilst the dispenser at Jadossohn and Weisser's clinic was sent to the tar works to collect tar from the bottom of the tank (Coombes 1944, 1954). Presumably this had a smaller proportion of ammonia, liquors, fixed gas and aromatic light oils which would be irritant (Obermayer and Becker 1935). Jaffery's work in 1928 showed that the fractions which contained the highest proportion of tar acids were the most efficacious and that naphthalene was irritant. Coombes (1954) disagreed, and claimed that naphthalenes were non-irritant and beneficial in acute vesicular dermatoses, acting as keratoplastic agents.

Obermayer and Becker (1935) used fractional distillates, solubility products and individual tar components in White's formulation. They concluded that higher boiling fractions were the most efficacious and less irritant, the exception being pitch. Ether soluble and insoluble fractions were equal in effects to tar but were inflammatory. No compound equalled coal tar but catechol, pyrogallol, leningallol and 8-hydroxyquinoline had a decided effect, the latter three were irritant. β-naphthol checked the spread of parakeratosis. The workers themselves interpret the clinical trials they performed with caution, as hospitalisation often causes dramatic improvements. They suggested that future work should concentrate on individual compounds, e.g. catechol and 8-hydroxyquinoline.

Nelson and Osterberg (1927) reported a coal tar steam distillate to be effective in infantile eczema. The preparation was as effective as coal tar and did not cause staining or follicul itus.

In an attempt to reduce irritant effects Carney and Zopf (1955) added a surfactant to reduce the maximum particle size from 100 μ to 3 μ. This was found to be effective. Many other workers used surfactants to produce washable coal tar ointments (Pflag and Zopf 1951, Fanburg 1952, Goldstein 1953) with good results.

Lloyd and King (1959) found alcohol soluble extracts in a water miscible base to be therapeutically and cosmetically acceptable, whilst Thambiah (1938) suggested forming a solution of coal tar in acetone, as it did not cause bad staining.

Wrench and Britten (1975a, b, c) found the higher boiling tar acid fractions of a low temperature tar to be the most efficacious, but the tars were tested on the mouse tail model, and were never clinically tested. If tar acids contain the active therapeutic agent, then LTT must surely be the most efficacious, as LTT's contain 15 to 18% tar acids, compared to 5% in HTT's. Such high boiling tar acids contain many polyhydroxylated phenols (Coal Tar Data Book 1970).

The contribution of tar phenols to therapeutic activity was discussed by Hellier and Whitfield (1967), whilst Thorn (1963) found a mixture of tar acids and bases gave the best results in the treatment of psoriasis.

In a search for a better tar preparation, many workers have combined coal tar with other agents, notably allantoin (Bleiberg 1958, Singer 1962), which seems successful. Combinations with steroids are also available, and useful (Clyman 1957).

Many "synthetic tars" have been formulated in an attempt to provide a safe, non-irritant, therapeutically active preparation

to use on skin disorders. Guy (1939) listed the main constituents of light, middle, heavy and anthracene oils from coke oven tars, from which Guy (1939), Butterworth (1950), Kinmont (1957) and Yarrow and Thorn (1966) formed synthetic tars for use in psoriasis. Kinmonts tar was reported to be superior in every way to crude coal tar. Saunders and Davis (1947) also made a synthetic tar for use on eczema. A synthetic tar was evaluated by Wrench and Britten (1975d) and did not give promising results in the mouse tail test but was not used in a clinical trial.

In a review of anti-psoriatic drugs, Champion (1966) stated that the active constituents of coal tar still had not been found and commercial ointments seemed to differ more in price than in efficacy. Many "new" tars make only one appearance in the literature and are then forgotten. Hodge and Comaish (1977) point out that as there are some 10,000 compounds present in coal tar it is hardly surprising that active constituents have not been isolated, indeed, almost all attempts to refine coal tar seem to provide a preparation less active than the parent coal tar, and reinforces the notion of a "mysterious harmony" between tar constituents mentioned by Rothman and Shapiro (1949).

(v) Disadvantages of Coal Tar as a Therapeutic Agent

The adverse effects of coal tar as a therapeutic agent have been reviewed (Section IA(iii)), other disadvantages of coal tar are due to its very nature. Coal tar is smelly, messy, sticky, immiscible with water and therefore difficult to remove from the skin and clothes (Coombes 1947). It may be irritant (Carney and Zopf 1955).

Many attempts have been made to purify coal tar (Obermayer and Becker 1935, Lloyd and King 1959, Thorne 1963, Wrench and Britten 1975a, b, c), but invariably the purified coal tar is not as efficacious as the parent coal tar (Kerr and Plein 1953).

The purpose of this study is to isolate a compound from coal tar with anti-psoriatic properties as good as the parent coal tar but one that would also be cosmetically acceptable.

B. The Skin and Psoriasis

(i) The Normal Skin

The skin is often regarded by the layman to be the dead protective covering of the body. This could not be farther from the truth, the skin is the largest organ in the body, is a very active tissue and takes part in such diverse functions as physical protection, temperature regulation and the showing of emotion. The skin is not a homogenous tissue, the skin of the scalp is vastly different to the skin of the sole. A broad general outline of the structure and functions of skin helps in the understanding of pathologic conditions such as psoriasis.

FIG. 2 shows a diagrammatic vertical section through the skin showing that it has a stratified structure. The dermis contains sweat glands and capillaries which are essential in temperature regulation, the hairs act as "radiators" to help this function although their part is a minor one. These organs are supported in a network of collagen in which are also found fibroblasts (involved in collagen synthesis), mast cells (important in tissue damage as they release histamine and heparin) and various phagocytosing cells from the blood and recticulo-endothelial system.

FIG. 3 shows a diagrammatic representation of the epidermis. This tissue, unlike the dermis, is composed entirely of cellular tissue which undergoes a slow continuous movement to the surface (Pinkus 1970). The epidermis may be divided into four layers of cells, which are merely the different stages in the formation of keratin. The process is named keratinization and the epidermal cells are often called keratinocytes.

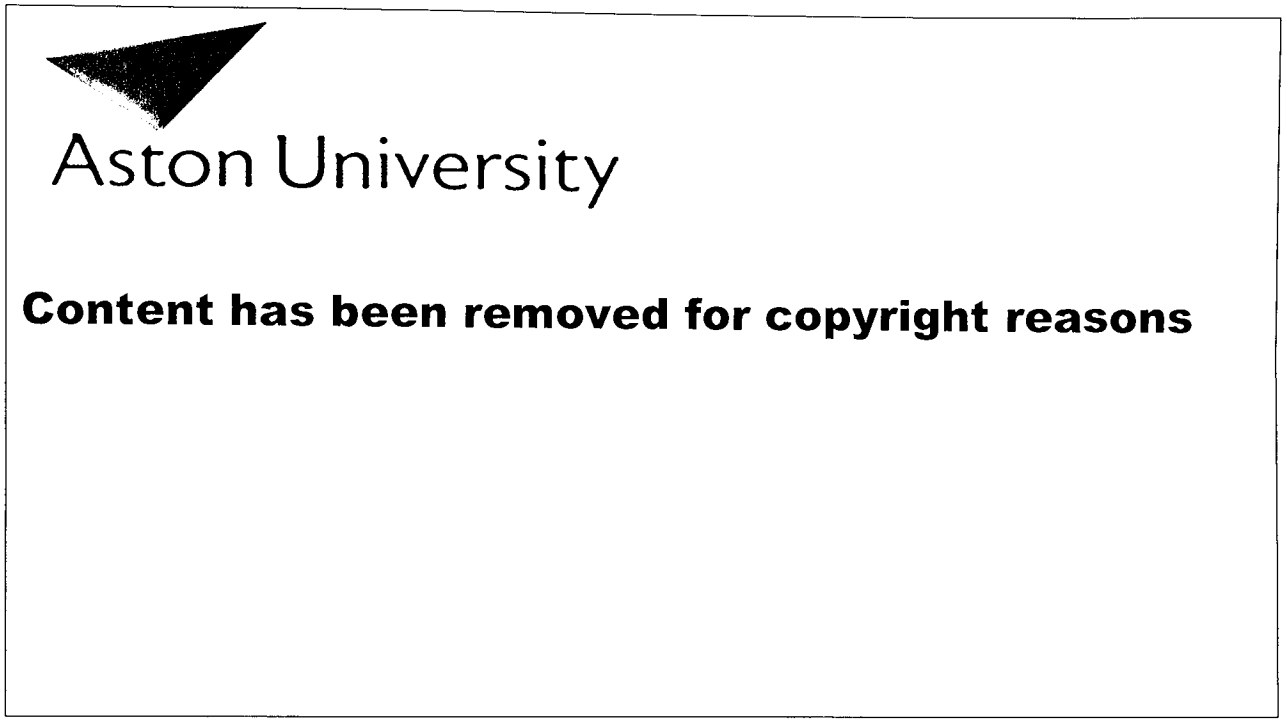


FIG. 2 . Diagrammatic Representation of Normal Human Back Skin (Vertical Section)

FIG. 3. Diagram of the Epidermis

The basal cells ensure cohesion between the dermis and the epidermis and under normal conditions are the main dividing cells of the epidermis.

Above the basal cells are the prickle cells which are daughter cells of the basal cells. They constitute the main part of the epidermis. Above these cells are layers of granular cells known collectively as the stratum granulosum. These cells contain variably sized keratohyalin granules (Montagna and Parakkal 1974). For this reason this layer is known as the granular layer. The final layer, which is being constantly abraded, is known as the horny layer or stratum corneum which consists of dead cornified structures.

Two processes are evident. There is a destruction of cell contents by endogenous enzymes and the synthesis of the fibrous, resistant protein keratin.

There appears to be a delicate balance between the two processes to give the most suitable type of horny layer for the part of the body it has to protect (Jarrett and Spearman 1964).

The cells are far away from the blood supply but the process is not passive (Bern 1952). As cell division occurs at the basal layer, how does a cell from this division differentiate as a basal cell or as a keratinocyte? The answer is unknown; a "clock" mechansism or a diffusion of inducers from the dermis seems unlikely because the dermis is convoluted (Jarrett 1973). The answer probably lies in the stratum corneum or outside it, as this would give a straight level of keratinization, and it is well known that removal or damage of the stratum corneum results in an increased burst of mitotic activity in the basal layer.

There are occasional mitoses in layers other than the basal layer but these always involve basal type cells (Leblond 1964), these migrations and subsequent mitoses are thought to be random (Epstein and Maibach 1965).

The normal mitoses go on continuously, as the stratum corneum is being constantly abraded, although a diurnal rhythm with activity being the greatest during sleep (Bullough and Laurence 1956) is present. Activity is maximal during wound healing.

From studies on mouse skin it was discovered that the mitotic rate was controlled by adrenalin (Bullough 1955, Bullough and Laurence 1964, Marrs and Voorhees 1971), complexing with an "epidermal chalone", a basic glycoprotein of molecular weight 25,000 (Bullough and Laurence 1960, 1964) which appears to be neither species nor class specific (Bullough et al. 1967). The complex breaks down at low adrenalin levels, such as sleep, allowing mitosis to proceed at a higher rate.

There is no disagreement that catecholamines affect mitotic rate, however, these compounds are frequently unable to penetrate the cells (Voorhees and Mier 1974). Voorhees and Duell in 1971 proposed that cyclic adenosine-3'5'-monophosphate (cAMP) mediated the catecholamine induced inhibition of mitosis. It has been shown in vitro that cAMP inhibits epidermal mitosis in a dose dependent manner (Marks and Rebein 1972, Voorhees, Duell and Kelsey 1972, Voorhees et al. 1973).

The catecholamines stimulate adenyl cyclase, the enzyme which catalyses the formation of cAMP from adenosine triphosphate (ATP) The cAMP acts as a second messenger. The ratio of cAMP to cGMP (cyclic guanosine-3'5'-monophosphate) also seems to be important (Voorhees et al. 1973, 1974).

Estimates as to the time to renew the epidermis vary considerably from 7 to 258 days (Epstein and Maibach 1965). Halprin (1972) suggests the time is in the region of 57 to 75 days in his review of the relevant literature. The time for a cell to traverse the horny layer is believed to be 14 days (Rothberg et al. 1961, Baker and Kligman 1967).

The granular layer is a characteristic of mammalian skin. The nature of these granules which give the name to this stratum is obscure (Blank 1952), although they have been claimed to be keratin precursors (Brody 1962), this theory is of dubious acceptability (Bern 1952). These cells, far from degenerating passively, are very active, supporting many degrative enzymes (Jarret and Spearman 1964) and it is thought that the granules may contain the breakdown products of the epidermal cell contents (Huek 1935, Maximow and Bloom 1953).

The keratin formed is given the title "basket weave keratin" due to its histological appearance (Jarrett and Spearman 1964) as opposed to parakeratin formed in psoriasis or mouse tail skin. These keratins are resistant to proteolytic enzymes released from the granular layer because of their rigid nature due to disulphide bonding (Jarrett et al. (1965).

It has been noticed that many of the granular layer enzymes are released from lysosomes and it is possible that a co-ordinated and pre-determined rupturing of these organelles could be the basis of the keratinization process (Jarrett and Spearman 1964).

An alternative theory is that keratinization begins in the basal layers with "tonofibrils" which polymerise on the migration through the epidermis (King 1949, Liss 1965, Jenkins and Tresise

1969). These observations are based on electron microscopy studies and are believed to be artifacts of the fixing process (Jarrett and Spearman 1964).

(ii) The Psoriatic Skin

General and Clinical Features

The typical psoriatic skin consists of lesions of a sharply defined dull red plaque, surmounted with fine silvery scales. The lesion is easily palpable and generally raised. Any part of the body may be affected although elbow and knee involvement is most common. The number of lesions may be one or many. The disease may manifest itself at any age and is generally chronic. The appearance of new lesions and their tendency to increase in size often results in lesions becoming confluent and involving large areas of the body. Although partial or total resolution of lesions is often demonstrated, a permanent remission is uncommon (Farber et al. 1968).

Psoriasis is one of the most common skin diseases, up to 2% of the population suffer from it (Wrench 1973). This is only the proportion which applies for medical help, mild forms of the disease are often treated by the patients themselves and are never reported, skin disease has a long tradition of self medication (OHE 1973).

It is now accepted that psoriasis is genetic in origin (Hodge and Comaish 1977, Kimberling and Dobson 1973) although the mode of transmission has yet to be elucidated. Hoede (1931) determined a familial incidence of 39%. If one parent was affected, the ratio of normal to affected siblings was 8:1; if neither parent was affected, the ratio rose to 21:1. Many later studies have

been undertaken but no recognisable genetic pattern has emerged (Steinberg et al. 1951, Aschner et al. 1957, Watson et al. 1971, Kimberling and Dobson 1973).

The condition is often initiated by stress; Seville (1977) noted that 39% of patients have emotional trouble prior to their first attack; these patients have a better prognosis (Seville 1978).

Psoriasis is linked to arthritis (Bollet and Turner 1958, Bunim et al. 1962), about 10% of psoriatics have some joint involvement (Mier and Cotton 1976).

At present psoriatic arthritis, or arthropathic psoriasis cannot be precisely defined but there is a tendency to accept it as different from other forms of arthritis (Editorial BMJ 1976).

Microscopically, the psoriatic skin is different to normal skin, there are two main abnormalities:-

(i) there is an increase in the rate of cell division in the basal layer strata,

(ii) there is an absence of granular layer.

This results in the production of an excess of keratinocytes which autolyse prior to full keratinization giving rise to parakeratotic scales. These scales have a characteristic silvery-white appearance due to air trapped in them (Burks and Montgomery 1943). The "horny layer" cells retain their nucleii, and the parakeratin produced is of the "horny layer" type rather than the orthokeratotic "basket weave" type.

The capillaries within the lesion become tortuous and dilated, probably due to the lack of vasoconstrictor catecholamines caused by an increase of catechol O-methyl transferase (Bamshad et al. 1970). The effect is reversed by adrenalin (Yamazaki 1963) but capillaries often remain dilated after clearing of the lesion (Illig 1966, Reid and Jarrett 1967).

the rete pegs are grossly elongated and branched. There is a leakage of lymphocytes and polymorphonucleocytes (Jarrett et al. 1966); this cellular infiltration could be due to the large amounts of leucotactic lipids present in the psoriatic epidermis. These cells sometimes collect together to form Munro abscesses which are sterile. In the condition known as pustular psoriasis these abscesses become large and secondarily infected. It has been suggested (Tagami and Ofugii 1976) that this cellular infiltrate is sucked out of the capillaries by leucotactic activity from the psoriatic scales, rather than from lipids in the epidermis.

Psoriasis tends to attack the scalp rather than the hair but hair loss is not uncommon (Schuster 1972). Hair shaft abnormalities associated with psoriasis have been recognised by electron microscopy (Braun-Falco and Rassner 1966, Wyatt et al. 1972). The rate of hair growth is normal (Comaish 1969). Sharad and Marks (1976), from a study on labelled root hairs, concluded that psoriasis was dermal in origin. It was pointed out by Paslin (1976) that hair and epidermis keratinize in different ways and the hypothesis was inaccurate.

Psoriatic nails tend to be thicker, faster growing, pitted, ridged and grooved, all of which are attributable to disease of the nail matrix (Alkiewicks 1949, Dawber 1970). The yellow, greasy look of psoriatic nails may be due to serum glycoproteins deposited under the nail bed (Zaias 1969).

Mucosal psoriasis is controversial, accepted by some (El Zawahry 1973, Wagner et al. 1976), but not by others (Ingram 1954). Oral lesions do occur in generalised pustular psoriasis (Bravermann et al. 1972). These consist of raised grey or yellow

bands which are histologically indistinguishable from normal tongue (O'Keefe et al. 1973, Dawson 1974).

Ophthalmologists report a psoriatic conjunctivitis (Baker 1975).

Epidermal Mitosis and Keratinization in Psoriasis

The mitotic rate of the basal layer, in psoriasis, is raised by a factor of two (Jansen et al. 1974) to seven (Halprin 1972), with a corresponding shortening of epidermal turnover time (ETT). Estimates of ETT range between 8 and 45 days (Goodwin et al. 1974, Halprin 1972, Weinstein et al. 1975, Bergstresser and Taylor 1977). The methodology varies from desquammation rate (Bergstresser and Taylor 1977) to the incorporation of radioactive labels (Weinstein and Van Scott 1965, Porter and Schuster 1968). There seems to be a certain amount of conflict about the duration of the ETT but all the authors on the subject agree that the ETT is shorter because of the increased mitotic rate. Inexplicably the mitotic duration is longer in germinative psoriatic epidermal cells (Fisher and Wells 1968), adrenalin will bring the duration to normal (Fisher 1968). This increase in mitotic duration is disputed by some authors (Goodwin et al. 1974).

It seems that in the region of the granular layer there is an insufficient release of hydrolytic enzymes to metabolise the cell contents during keratinization (Jarrett and Spearman 1964), these workers report many incompletely metabolised cell contents in the horny layer. Steigleder and Raab (1962) found DNase activity to be low, which would explain the retention of nucleii in the horny layer. The lack of hydrolytic enzymes in the granular layer may be explained by deficient lysosome rupture (Jarrett and Spearman 1964, Rees 1967).

The mitotic rate and the state of the granular layer are not easily related. The ETT may be inadequate to allow keratinocytes to form a granular layer (Weinstein and Van Scott 1965, Van Scott 1966). The granular layer is a prerequisite for orthokeratinization (Kaku et al. 1964); however, Fry and McMinn (1968) showed that in healing lesions the granular layer often reformed before a drop in mitotic rate.

Vitamin A will induce a granular layer but will increase the mitotic rate (Jarret and Spearman 1964), this may be explained by the tendency of vitamin A to lyse lysosomes causing an enzyme release. This would cause the reformation of the granular layer. This may cause mitotic stimulation in the germinative layer (Jarret and Spearman 1970, Jarret et al. 1979).

Biochemistry

A good deal of work has been done concerning the biochemistry of psoriasis; diseased skin has been the raw material. Psoriatic skin is different (microscopically and macroscopically) to normal skin, and it is likely that all biochemical parameters are altered. The difficulty is not to find evidence to support this assumption but to integrate the data into a coherent pattern to locate the primary failure.

Psoriatic skin has a much higher metabolic rate than normal skin. This was discovered over 50 years ago by Gans (1923), who demonstrated an increased consumption of oxygen and glucose by psoriatic skin. This and related findings have been reviewed by Ribuffo (1957). Whole pathway studies show that the oxidative pathways of glucose metabolism are increased rather than the anaerobic pathways (Herdenstam 1962). The measurement of individual

glucose metabolites has led to conflicting reports (Ribuffo 1957, Neuman and Blazkovajandova 1963, Halprin and Ohkawara 1966). These findings are hardly surprising and reflect the increased mitotic rate.

There is a four fold increase in the levels of glycogen in the psoriatic lesion (Halprin and Ohkawara 1966, Stankler and Walker 1976). The reason for this is debated. Voorhees and Mier (1974) claim it to be a result of the low levels of cAMP present in the psoriatic epidermis, whilst Halprin and Ohkawara (1966) claim that this accumulation is caused by an enzyme imbalance in carbohydrate metabolism.

Protein metabolism is altered because of both macroscopic changes in structure and a different enzyme system. Workers have shown the psoriatic scale to be rich in arginine and lysine (Zahnd and Citron 1960), aspartic and glutamic acids, glycine and leucine (Liss and Lever 1963), both laboratories report a decrease in sulphur containing amino acids.

It is difficult to see what results this macroscopic amino acid analysis shows in terms of the psoriatic lesion; an amino acid error in a protein will not be revealed by an amino acid analysis of large areas of skin. Mier and Cotton (1976) state "Protein chemists in other fields of research have long been aware that short cuts are doomed to failure; it is sad that this awareness has been so slow in reaching investigative dermatology."

Voorhees et al. (1968) report a histidine rich protein not found in the psoriatic granular layer and different structural proteins in the psoriatic epidermis have been reported by Baden et al (1978).

All varieties of lipid have been shown to be elevated in the psoriatic lesion (Wheatley and Farber 1962, Coon et al. 1963, Okhido et al. 1969, Grimmer et al, 1971, Tsambos et al. 1977), although this is disputed (Cornish et al. 1959). Molokhia and Portnoy (1973) report manganese to be deficient in psoriasis, this element is involved as a co-factor in some of the enzymes concerned with lipid catabolism (Underwood 1971).

As would be expected the DNA content of the psoriatic lesion is elevated (Mier and McCabe 1963). DNA is also present in the psoriatic scales (De Bersaques 1966). RNA is elevated five fold (Mier and McCabe 1963) and all degradative components of nucleic acids are elevated. Hodgson (1962) made a detailed study of this and other parameters involved in psoriasis and concluded that the increased nucleic acid content was due to the increased mitotic rate. Thymidine uptake is approximately double the normal rate in vitro (Goodwin et al. 1973, Marks 1978), folate is also increased (Touraine et al. 1973).

Many other vitamins, co-factors and other cell constituents are present in altered levels, this is thought to be due to the altered metabolism rather than any primary failure. This group includes vitamin A (Hoffman et al. 1950), vitamin B12 (Stankler 1969), vitamin C (Tickner and Basset 1960), copper (Lipkin et al. 1962) and zinc (Voorhees et al. 1969).

These findings have been challenged by several laboratories (Mier and Van Der Hurk 1974, Lipkin et al. 1964, Withers et al. 1968).

(iii) Uninvolved Skin

It was thought that psoriasis was limited to the lesions and

the area around them. Hammar and Hellerstrom (1970) reported that oxygen uptake in uninvolved skin was identical to that of healthy controls. The activity of many enzymes involved in psoriasis have been reported to be unchanged (Comaish 1963, Hammar et al. 1968, Yoshikawa et al. 1975). This is disputed. Herdenstam (1962) claimed oxygen uptake, carbon dioxide output and lactate synthesis were slightly elevated. This was explained by Hammar (1970) to be caused by an area of increased enzyme activity extending beyond the lesion.

There have been many reports of normal phospholipid and free fatty acid in uninvolved skin. Notable exceptions are Scher et al, (1962) and Tsambos et al. (1977). There is controversy about the ability of uninvolved skin to esterify cholesterol, the general opinion being that it cannot, or at least the ability is impaired (Garu et al. 1964, Rothman 1950). This finding has been strongly criticised by Wilkinson and Farber (1967a, b) who believe that this is an artifact caused by casual surface lipids.

Goodwin et al. (1973) found the mitotic rate to be doubled in uninvolved skin. Harper et al.(1978) found that the synthesis of DNA proceeds at the same rate in uninvolved skin and in the psoriatic lesion. Marks (1978) disagrees, he states that, from his studies on thymidine uptake, that uninvolved skin uses 48% more thymidine than normal skin, the lesions use double.

Stankler and Dave (1974) found that application of dithranol around a lesion would slowly improve the lesion and stop it spreading.

The uninvolved skin of a psoriatic patient must differ from that of a normal person, as psoriasis is thought to be a genetic disease (Mier and Cotton 1976). The biochemical investigation of uninvolved skin may offer a direct route to the identification of

the "primary fault" in psoriasis. This would be an easier investigation because of the lack of molecular reorganisation apparent in the psoriatic lesion.

(iv) Theories of the Cause of Psoriasis

The Chalone/cAMP Concept

The question of how a rapidly and constantly renewing tissue, such as epidermis, maintains the correct amount of proliferating cells led to Bullough and Laurence (1960) proposing a feedback mechanism of control in mouse epidermis. This concept was applied to all mammalian epidermis and in 1962, Bullough introduced the word chalone to describe a species non-specific, tissue specific mitotic inhibitor (Bullough et al. 1967). Early attempts to purify the chalone from aqueous extracts of mouse epidermis established adrenalin as a co-factor (Bullough 1953, 1963, Bullough et al. 1964). Bullough and Laurence (1968) associated this chalone with a heat labile glycoprotein.

Many attempts were made to purify this chalone with little success (Marrs and Voorhees 1971, Marks 1973, Chopra et al. 1974). Many attempts were made to identify its site and mode of action (Elgjo and Hennings 1971, Marks 1971). A G1 inhibitor has been found in epidermis (Elgjo et al. 1972) and a G2 inhibitor in the basal layer (Elgjo et al. 1971); the former is believed to be the true chalone.

The chalone has also been implicated in post mitotic cell ageing (Bullough 1973).

The chalones have some similarities to hormones, i.e. control by small amounts. Iverson (1969) suggested that the chalone effects may be mediated by the cAMP system. It was postulated that the

chalone would interact with adenylate cyclase, stimulating the enzyme to produce high levels of cAMP and maintain the cAMP/cGMP ratio within a range that maintains epidermal homeostasis.

A defect in the chalone/cAMP cascade is suggested as a possible cause of psoriasis (Voorhees and Duell 1971, Voorhees et al. 1973). The chalone still remains unidentified, unisolated and without an efficient assay system (Duell et al. 1977).

Cyclic AMP is thought to exert its control by a second messenger system similar to FIG. 4.

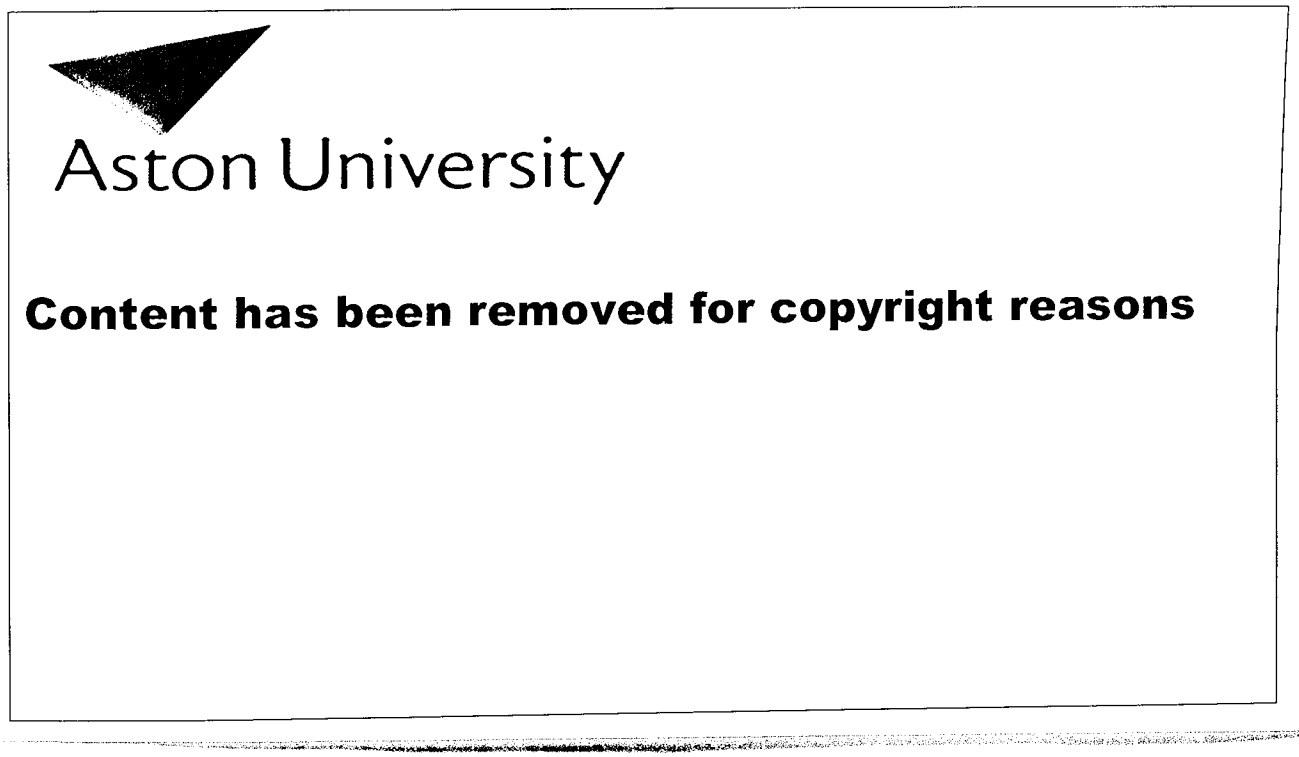


FIG. 4 . The "Second Messenger" System by which many Hormones Exert their Influences over the Metabolism of the Target Cells (Mier and Cotton 1976).

Sutherland and Robinson (1966) showed that the β-adrenergic receptor is functionally identical to adenylate cyclase, which is built into the cell membrane. When it is activated by an external hormone, e.g. adrenalin, the inner surface catalyses the conversion of ATP to cAMP, a cascade mechanism follows and a single external messenger molecule may have its effect amplified a thousand fold.

Cyclic GMP is also implicated, although this is not as well characterised. Evidence suggests that epidermal lysosymes are under the influence of both cAMP and cGMP which stabilise and labilise respectively (Ignaro 1973).

It has been postulated by many authors that cAMP is decreased in the psoriatic plaque (Iverson 1969, Voorhees and Duell 1971, Voorhees et al. 1972, 1973, Gommans et al. 1979). It was also found that cGMP was increased (Voorhees et al. 1973b), this would increase the mitotic rate (Voorhees and Mier 1974). It was pointed out that prostaglandin E1, had lower levels in psoriasis than normal, this would cause a lowering of cAMP levels (Aso et al. 1975) as would a low level of adrenalin.

Yoshikawa et al. (1975) claim that the levels of cAMP are normal in the psoriatic plaque. The evidence against this is overwhelming, although, cAMP is a very difficult compound to assay as any wound, i.e. removal of tissue for assay, causes a rise in cAMP levels (Iizuka et al. 1978).

The following scheme (FIG. 5) for the relationships between the characteristics of the psoriatic lesion has been proposed by Mier and Cotton (1976).

A scheme for the increased levels of glycogen present in the psoriatic lesion has been forwarded by Voorhees and Mier (1974), (FIG. 6).

FIG. 5. Possible Relationships of some of the Changes Characteristic of the Psoriatic Lesion (Mier & Cotton 1976).

FIG. 6. A Possible Scheme by which the Accumulation of Glycogen in the Psoriatic Lesion may Occur (Voorhees and Mier 1974).

Cyclic AMP, cGMP, adrenalin, prostaglandin E1 and the chalone are all implicated in psoriasis. Many questions are still to be answered. Why are the levels altered, and is one of these the primary defect? are the obvious ones. This explanation of the characteristics of psoriasis is the most widely held theory.

The Dendritic Theory of Keratinization

Approximately 10% of human basal cells are melanocytes (Cochrane 1970). These cells synthesise melanin which is passed on to keratinocytes in granules to protect the keratinocytes from UV light (Jarret 1967). The starting point of melanin synthesis is tyrosine. The melanocytes are characterised histochemically by their ability to oxidise dihydroxyphenyl alanine (DOPA), an intermediate in the melanin synthetic pathway, these cells are often called DOPA positive cells.

There are another group of cells, known as the Langerhans cells in the higher levels of the epidermis, these are thought to be effete melanocytes which can no longer oxidise DOPA and are being shed along with other high level keratinocytes. These high level cells possess high enzyme activity (Jarrett and Spearman 1964, Riley 1966, 1966b) and are believed to be involved in the organisation of the keratinization process.

Jarrett and Spearman (1964) and Riley (1966b) suggest that after melanin synthesis these cells move up through the epidermis and influence the type of keratin produced by influencing the high energy reactions taking place in the granular layer. These cells contain high levels of ATPase which are known to be involved in cell motility (Jarrett and Spearman 1964).

It is suggested that these cells augment cytolytic activity in the granular layer by providing lysosomes.

In psoriasis the Langerhans cells are only weakly ATPase positive and are abnormal in structure. They are thought not to influence keratinization.

The suggestion that a number of sufficiently active Langerhans cells is a necessary part of orthokeratinization is supported by a study of these cells in sebhorrheic warts (Molokhia and Portnoy 1971).

The Vascular Theory of Psoriasis

There is no doubt that the capillaries in the psoriatic lesion are tortuous and dilated (Yamasaki 1963). This is thought to be brought about by a lack of vasoconstrictor catecholamines, probably caused by the increase of catechol o-methyl transferase (COMT) (Bamshad et al. 1970) noticed in psoriasis. This enzyme is one of the enzymes which degrade catecholamines, (Axelrod and Tomchick 1958, Axelrod and Senoh 1958) the other main degrative enzyme being mono amine oxidase.

Telner and Fekete (1961) claim that this is the primary lesion in psoriasis. Adrenalin would normally control mitosis (Bamshad et al. 1970, Bullough and Laurence 1964, Voorhees and Mier 1974) but is metabolised by the excess COMT, allowing the increased mitotic rate found in psoriasis.

Unfortunately COMT is more prevalent in the epidermis than the dermis, where the capillaries are situated (Bamshad 1969) and capillaries often remain dilated after clearing of the lesion (Illig 1966, Reid and Jarrett 1967).

Inherent Lysosome Instability

This proposal was made by Reid and Jarrett (1967) on the basis of their studies on vitamin A. This theory has never been put to test.

Inherent Defect of the Capillaries

It has been postulated (Mier and Cotton 1976) that capillaries are abnormal even in clinically unaffected skin. An inherent vasodilation of this sort would result in an exaggerated response to trivial stimuli.

HLA Antigens

A disturbance of histocompatability antigens, which does occur in psoriasis (Russel et al. 1972, White et al. 1972, Rimbaud et al. 1974) would interfere with membrane hormonal receptors (Svejgaard and Ryder 1976); this would interfere with their activities and disturb intracellular metabolism (Marlhe and Orfanos 1977). HLA 13 and 17 are particularly implicated (Svejgaard et al. (1974).

Immunological Phenomena

There are many immunological phenomena associated with psoriasis (Guilhou et al. 1976a, b). They may be summarised as the presence of the following:-

(i) immunoglobulins and complement,

(ii) anti-Ig G factors,

(iii) anti-stratum corneum antibodies,

(iv) anti-nuclear antibodies.

These different factors could account for the structural and functional abnormalities of the psoriatic keratinocyte (Guilhou et al. 1978).

Non-Cycling Cells

Gelfant (1976) suggests that the epidermal cell population can be split into three. One population cycles normally and the other two are blocked at G1 and G2 respectively and are consequently

non-cycling. Gelfant postulates that all three populations cycle in psoriasis giving the increased mitotic rate. He gives no indication of why this should occur.

C. Other Treatments of Psoriasis

There are many other treatments which have been utilised in attempts to cure psoriasis. Some have been successful, some have not. It is pointless to extol the value of coal tar as a therapeutic agent in isolation. This review of other anti-psoriatic treatments shows the value of coal tar by highlighting the poor state of knowledge about the disease and the drugs used to cure it. Most of these drugs, like coal tar, cannot claim to have a specific anti-psoriatic action. These treatments may be divided into either topical or systemic regimes.

(i) Topical Treatments

Dithranol

Dithranol, 1,8,9-trihydroxy-anthracene, is unstable (Fisher and Maibach 1975). It breaks down to a mixture of dithranol, 1,8-dihydroxy-anthroquinone and a dimer; only the former is efficacious. Dithranol has been used successfully in the treatment of psoriasis for the last fifty years (Swanbeck and Lidden 1966), and is possibly the most widely used anti-psoriatic agent in Europe (Ippen 1966).

Dithranol is harmful to the eyes, causes irritation and stains skin. Consequently it can only be used under medical supervision. The activity of dithranol is enhanced by incorporating it into Lassar's paste (Maclennan and Hellier 1961). Lassar's paste is a mixture of zinc oxide and salycilic acid in starch and white paraffin. Penetration and subsequent efficaciousness are also aided by a hydrophobic vehicle (Kammerau *et al.* 1975).

The addition of corticosteroids to dithranol in Lassar's paste, a popular regime, seems to shorten remissions and impair

dithranol tolerance (Seville 1976). Dithranol was found to be safe and free from systemic toxicity in the routine doses used in the treatment of psoriasis (Gay et al. 1972).

Krebs and Schaltegger (1969) show that a hydroxyl group at position 1, a carboxyl or anthrone oxygen at position 9 and two free hydrogens at position 10, are the minimum requirements for anti-psoriatic action. Dithranol structurally resembles acridine, which is known to intercalate with DNA. This led Swanbeck and Thyresson (1965) to postulate that dithranol has a similar mode of action. Swanbeck and Lidden (1966) showed inhibition of DNA synthesis by dithranol in epidermis, and Lidden and Michaelsson (1974) showed the same inhibition in treated psoriatic lesions.

Dithranol is one of the most valuable drugs used in the treatment of psoriasis; it is as beneficial as coal tar. A comparison of the two therapies was conducted by Polano (1973).

Triacetoxy Anthracene

The triacetyl ester of dithranol has been used in the treatment of psoriasis in an attempt to reduce skin staining. The advantage to the patient is that triacetoxy-anthracene is a topical drug which may be self administered. Hellier and Whitefield (1967) claim that it greatly improved, or cleared, 25 out of the 41 cases they prescribed it for. They claim that it is less potent than dithranol, a claim substantiated by many authors (Hodgson and Hell 1970, Farber and Harris 1970, Dahl 1971, Raab 1975).

Triacetoxy-anthracene may be more irritating to the psoriatic plaque than dithranol, and like dithranol will cause acute keratitis if it gets into the eye (Mathalone and Easty 1967).

Hellier and Whitefield (1967) have proposed the following (Fig 7)

FIG. 7 . Proposed Mechanism of the Action of Dithranol (Hellier and Whitefield 1967).

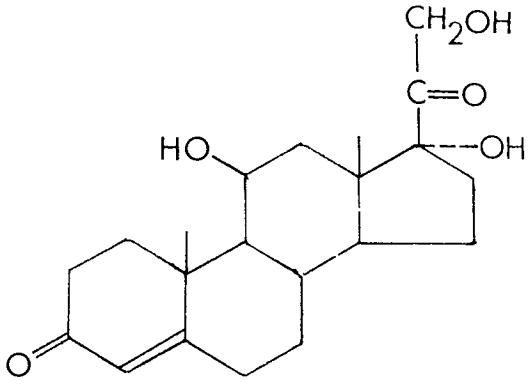
mechanism of action for dithranol, and a rationale for the greater cosmetic acceptance of triacetoxy-anthracene. It has been found that dithranol is more stable as its keto tautomer. The resultant methylene group is highly reducing, abstracting oxygen from the skin at a rate which causes irritation and burning. The anthroquinone produced can react to form a reduced anthroquinone dyestuff, which on oxidation, forms a quinone, which is permanently attached to the skin causing staining. The workers believe that this is the way in which dithranol exerts its anti-mitotic action, by blocking oxygen receptor sites and thereby reducing the energy available for mitosis. It can be seen that the related compound, triacetoxy-anthracene, can only exist in the enolic form, so none of the above reactions can take place. Triacetoxy-anthracene is probably activated at the plaque, where there is increased enzyme activity.

Corticosteroids

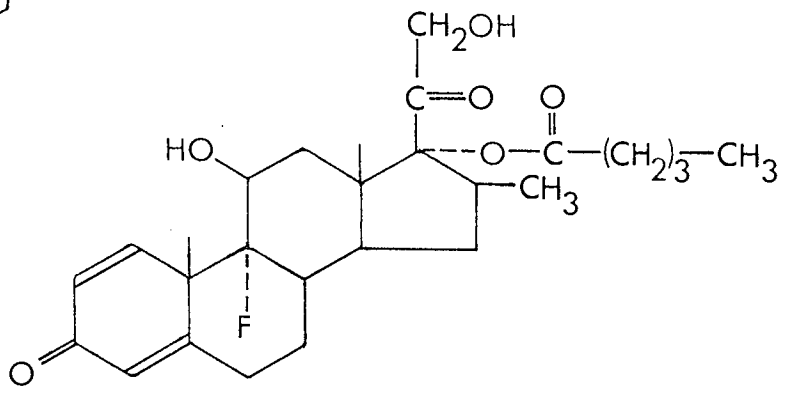
The anti-inflammatory and anti-mitotic properties of steroids are made use of in diseases such as psoriasis, which have an unknown, but non-infective cause.

There is an immense amount of literature on the subject of topical corticosteroid therapy. Topical corticosteroids will clear a psoriatic lesion more quickly than either dithranol or coal tar (Baker 1975b), however, relapses occur more quickly (Hodge and Comaish 1977).

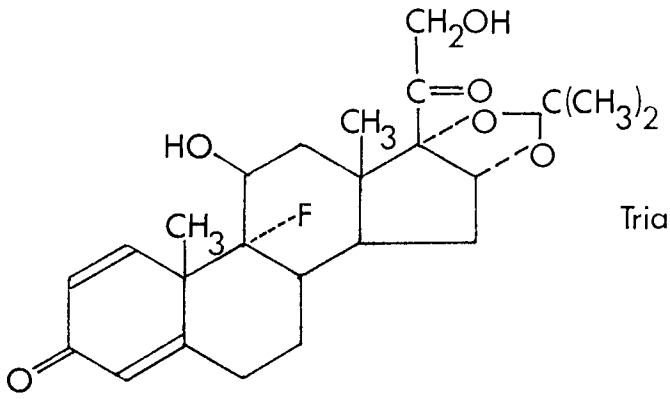
The most potent corticosteroid in use today is clobetasol propionate; this is only used in the United Kingdom (Baker 1975b). Hydrocortisone is much less effective, the reason for this is almost certainly inadequate penetration. A butyrate ester of hydrocortisone, in a 0.1% cream under plastic occlusion, was as



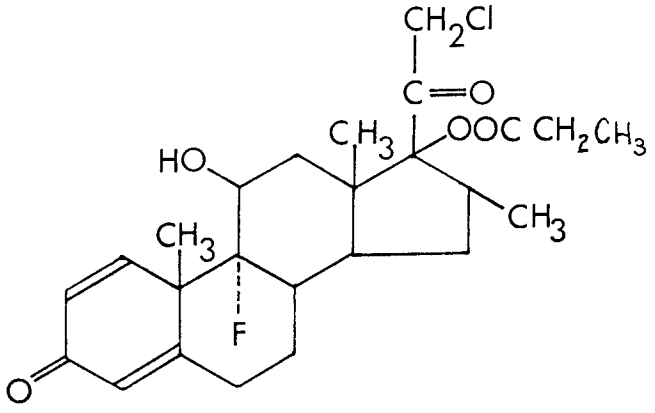
Hydrocortisone



Betamethasone Valerate



Triamcinolone Acetonide



Clobetasol Propionate

Fig. 8 . Some Steroids Used in Psoriasis.

effective in the treatment of psoriasis as 0.1% triamcinolone acetonide (Polono et al. 1970), another potent anti-psoriatic agent (Jarret and Spearman 1964).

Steroids are used in the treatment of generalised pustular psoriasis, but are believed by some authors (Baker and Ryan 1968, Baker 1975a, 1976) to be one of the causes of this condition.

Steroids have been used in nail matrix injections, but relapse rate is high, and thickened nails respond poorly (Peachey et al. 1976, Hodge and Comaish 1977).

It has been discovered that topical steroids, applied a week before PUVA therapy, will greatly enhance this therapy (Gould and Wilson 1978).

Unfortunately, much of the latter day literature dealing with the dermatological use of steroids, is in the form of comparison studies (Goodwin et al. 1973, Corbett 1976, Marriot and Munro 1976). Most of the literature on the mode of action of steroids is over a decade old, and is extensively reviewed by Polano (1973).

Disadvantages of Topical Steroid Therapy

Steroids have been used, in high doses, to suppress inflammation, especially in diseases such as rheumatoid arthritis. They have, however, serious side effects to their ameliorative properties if treatment is prolonged, e.g. in psoriasis. These effects are due to the direct actions of the steroids themselves, and the indirect effects of interfering with endogenous steroids. These systemic effects arise because the drug has to be given in increasing doses, when used in the treatment of a chronic disease such as psoriasis, which gives rise to percutaneous absorption (Morely 1970), especially in children (Feiwel 1969).

The following systemic effects have been noticed after high dose topical steroid therapy:-

(i) excessive protein breakdown leading to muscle weakness and osteoporosis.

(ii) Carbohydrate disturbances which may lead to diabetes mellitus.

(iii) Electrolyte disturbances; the most common is a potassium deficiency, which may be a contributing factor to mental depression, often found in steroid treated patients.

(iv) Adrenocortocoid atrophy, by suppression of adrenal-pituitary feedback by endogenous steroids.

(v) Abolition of the pituitary-adrenal stress response.

(vi) Large topical doses of corticosteroids may cause foetal abnormalities if used on pregnant women.

Interruption of steroid therapy, when it is being used in psoriasis, often causes a rebound condition which is worse than the original condition, and is intractable for some time (Rook 1966). The anti-inflammatory properties of steroids often mask any skin infection, and fungal infections are often impossible to diagnose (Ive and Marks 1968).

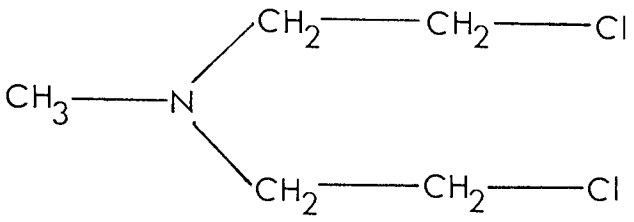
Alkylating Agents

The alkylating agents are thought to react with DNA bases, preventing fissure and replication. They have widespread enzyme inhibiting effects, and are therefore very potent cytotoxic agents. Many have been used topically in the treatment of psoriasis.

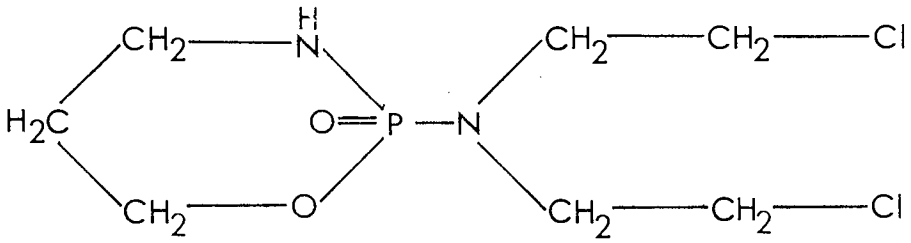
Mechlorethamine

This nitrogen mustard has demonstrated a useful anti-psoriatic

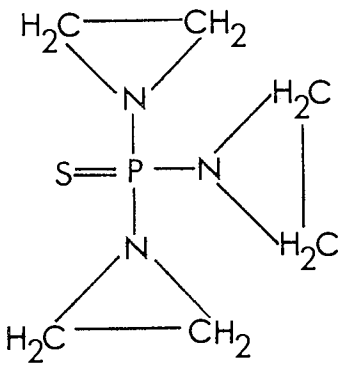
(a) Nitrogen Mustards



Mechlorethamine

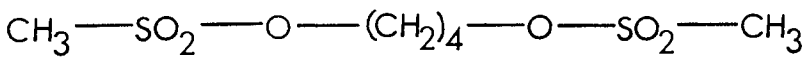


Cyclophosphamide



Triethylenethiophosphamide

(b) Alkyl Sulphonates



Busulphan

FIG. 9. Some Alkylating Agents Used in Psoriasis

ability. The main hazard with its use is contact sensitivity, which has an incidence of up to 80%. This factor precludes the use of mechlorethamine in routine treatments. Liver and kidney function tests prove to be normal.

The usual regime is the application of an aqueous solution (0.01 to 0.05%) daily (Epstein and Ugel 1970, Mandy et al. 1971, Purdy 1973).

Cyclophosphamide

This drug only becomes active when the cyclic group is removed at the P-N linkage by a phosphatase or a phosphamidase. These enzymes occur in high levels in neoplastic cells, such as psoriatic cells. Plasma and liver will activate this drug causing widespread toxicity. Finizi (1969) quoted an 80% success rate with this drug.

Triethylene Thiophosphamide (Thiotepa)

A 0.4% ointment was evaluated, under occlusion, by Heydenreich (1971). 11 out of 14 patients experienced complete clearing of previously intractable plaques. Two cases had to have the treatment terminated prematurely, due to the development of leucopenia. Heydenreich claimed this alkylating agent to be non-irritant.

Lomustine

This nitrosurea alkylating agent was evaluated as a 0.1% ointment by Peck et al. (1972). Undoubted anti-psoriatic activity was complicated by persistent pain. This was due to epidermal separation, irritation of unaffected skin and marrow depression.

Busulphan

Busulphan is thought not to intercalate with base pairs, but to react with thiol groups on proteins (Greenwald 1967). Moller .

and Waldenstrom (1970) reported an improvement in 5 out of 9 cases. Side effects included pulmonary fibrosis, hyperpigmentation and anorexia, which leads to weakness and weight loss.

Retinol and its Derivitives

Retinol (vitamin A) has been used for many years in the treatment of psoriasis. A popular regime is retinol and triamcinolone acetonide (Whittle *et al*. 1961, Jarrett and Spearman 1964). Optimistic claims have been made for the use of retinoic acid (Frost and Weinstein 1969, Fry *et al*. 1970), but irritancy has proved to be a major problem (McDonald and Fry 1972, Peck *et al*. 1973).

Although they are usually applied topically, the retinoids may be taken orally. Orfanos and Runne (1976) report good results using an oral, synthetic, aromatic retinoid. Michaelson *et al*. (1978) observe that an oral retinoid will enhance the effect of topical coal tar and UV-A.

The mode of action of retinoids is unclear; retinoids increase the mitotic rate *in vitro* (Christophers 1974, Chopra and Flaxman 1975), and their *in vivo* action is certainly not by mitotic inhibition (Hodgson and Hell 1976).

Jarrett *et al*. (1978, 1979) show induced phosphodiesterase in the epidermis and increased activity in the dermis after a topical retinoid regime. They believe that dendritic cells may be responsible for the formation of the granular layer by influencing keratinocytes to discharge their lysomal enzymes.

Other Topical Treatments

5-fluorouracil, a pyrimidine anologue, has been used topically in the treatment of psoriasis, with good results, but with considerable skin erosion (Tsuji and Sugai 1972).

Many other topical treatments have been used in an attempt to cure or alleviate psoriasis, some have quite a degree of success, e.g. the cell poisons: arsenic, ammoniated mercury, resorcin and chrysarobin. Their toxicity and side effects far outweigh their usefulness (MacKenna 1959).

(ii) Systemic Treatments

Methotrexate

Methotrexate is a folic acid analogue which interferes with the folic acid pathway by binding to the enzyme dihydrofolic acid reductase, preventing the formation of tetrahydrofolic acid (FIG. 10). This process has been reviewed by Newbold (1972). The main use of methotrexate is in cancer chemotherapy as it kills dividing cells. Methotrexate is used in the treatment of psoriasis because of the rapidly proliferating epidermis. Methotrexate will interfere with several enzyme systems other than the folic acid pathway, making it a very potent cytotoxic agent indeed.

Methotrexate is used when patients are resistant to other forms of therapy (McDonald and Bertino 1969). Its use is standard in the United States of America for the treatment of severe psoriasis (Rees et al. 1967). Many reports tell of excellent results with the use of this drug (Biro et al. 1967, Roenigk et al. 1969, Reese et al. 1974).

The usual dosage is a single 50 milligram injection given at intervals of one or two weeks. The rationale behind this regime is that single large doses seem to be less toxic than a continuous low dose (Condit 1960, Almeyda et al. 1972). Methotrexate can alternatively be given orally.

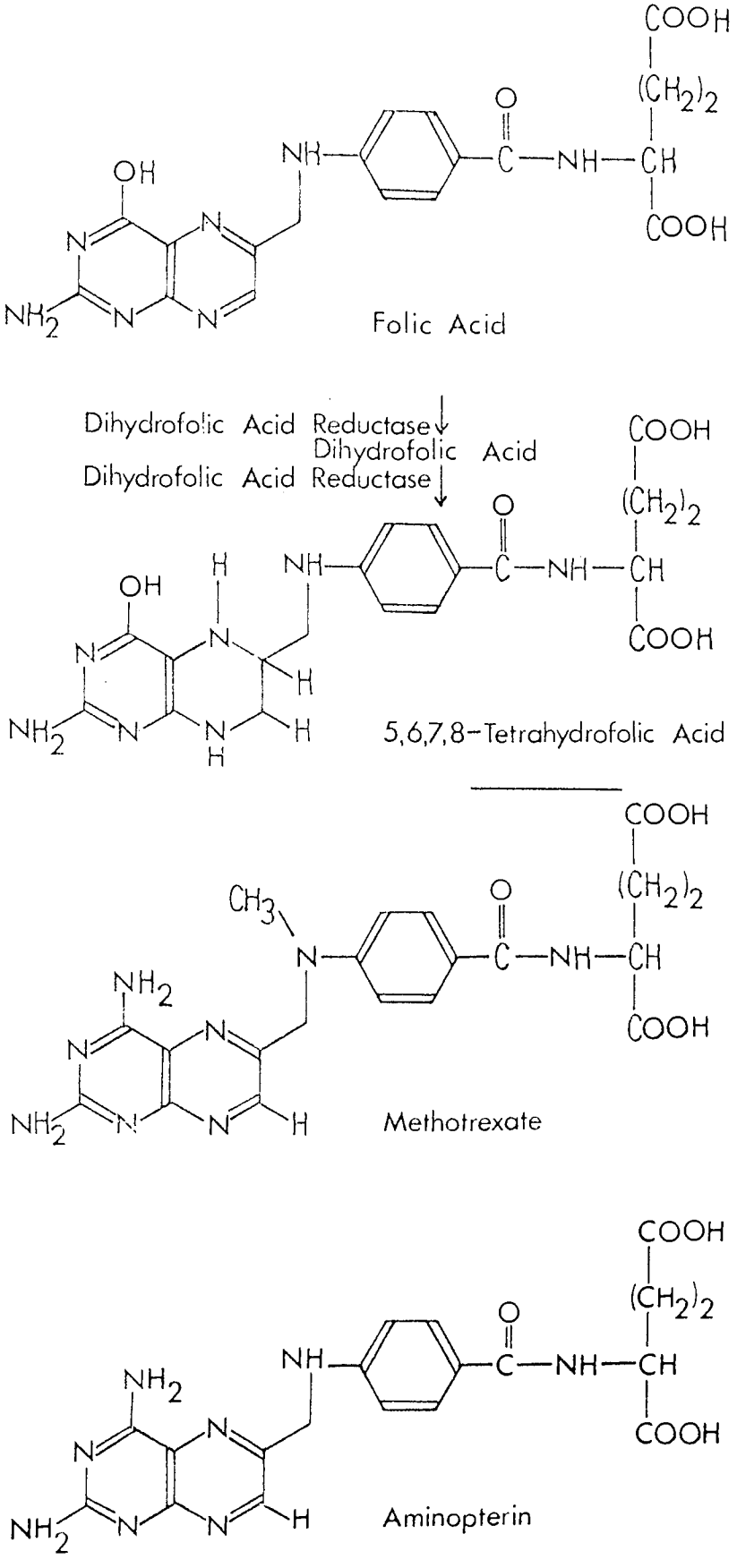


FIG. 10. Folic Acid and its Analogues.

There are many side effects to methotrexate therapy, the most studied is liver damage (Reese et al. 1974, Millward et al. 1974, From 1975). The liver suffers, in 50% of cases (Greaves et al. 1971), from hepatic fibrosis which may lead to cirrhosis. These damaged livers can still give normal liver function tests, liver biopsies are required to monitor any damage (Dubin and Harrel 1970).

Bailin et al. (1975) report that patients undergoing methotrexate therapy are more prone to some types of malignant disease than the healthy populace.

Other side effects of methotrexate therapy are due to the mode of action of the drug. By binding irreversibly to dihydrofolic acid reductase, the drug blocks DNA synthesis and will have a more pronounced effect on rapidly dividing tissue. Unfortunately, there are many tissues in the body which divide rapidly, these tissues are affected as well as the target lesion. These normal, but rapidly dividing cells, include the buccal and gastro-intestinal mucosa and the bone marrow cells. Common side effects are severe anemias, reduction in platelet counts, stomatitis, anorexia, vomiting and gastro-intestinal ulcers. Less common side effects are alopecia, skin rashes, pleuritic chest pain, localised peritonitis and fever (Coe and Bull 1968).

In conclusion, this drug should only be used in the treatment of psoriasis when it is intractable to other safer therapies. It should never be given to women of child bearing age, or to those with inadequate renal clearance, and the drug should never be prescribed if there is any sign of liver malfunction.

Aminopterin

Aminopterin is a derivative of methotrexate. It has been used in the treatment of psoriasis although many workers believe that its toxicity should preclude its use (Rees and Bennett 1959). This drug is rarely used (Hodgson personal communication).

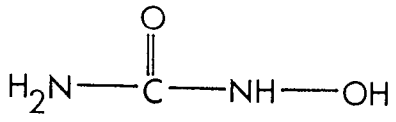
Psoralens and UV-A Light (PUVA)

Psoralens act by binding to pyrimidine bases after photo-activation by light in the UV-A range (320 to 400 nm) (Parrish 1976a). The observed inhibition of various enzymes does not seem to be important, and photoreaction with proteins does not occur (Pathak et al. 1974). UV-A alone is not efficacious (Wolff et al. 1976), the most efficacious light range for light alone is the UV-B range (290 to 320 nm). The two most commonly used psoralens; 8-methoxy-psoralen (methoxalen) and 4,5,8-trimethyl-psoralen (TMP), reach peak blood levels about three hours after ingestion. They are excited by UV-A at this time. Maximum excitation occurs at 365 nm. Excretion and detoxification are rapid, up to 90% is excreted inside twelve hours.

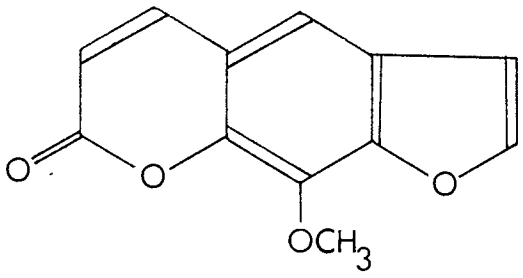
The results are very impressive, only 2 patients out of 91 had less than 90% clearing of lesions. 82 patients out of the same study had a complete clearing of lesions (Parrish et al. 1974, Wolff et al. 1976 collaborative work). A continuing treatment is required to maintain remission.

Hyperpigmentation is an obvious side effect. Nausea after tablet ingestion is the only other well documented one. Hepato-toxicity does not exist (Parrish 1976b).

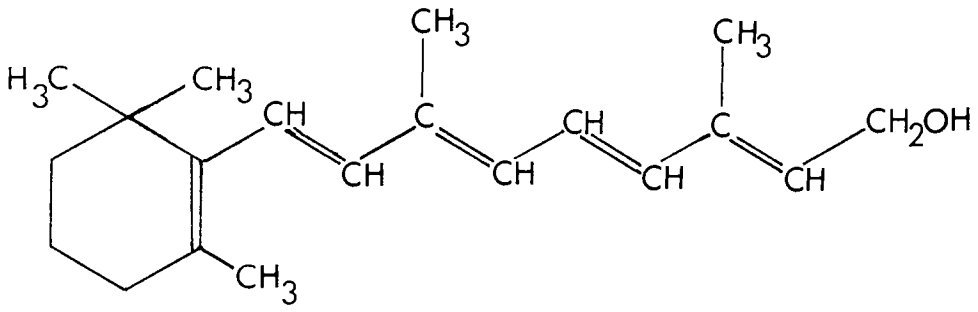
Topical psoralens are not as effective and the ensuing hyperpigmentation is disfiguring if the treatment is confined to the lesions.



Hydroxyurea



Methoxsalen



Vitamin A Alcohol (Retinol)

FIG. 11. Miscellaneous Drugs used in Psoriasis.

Systemic Corticosteroids

Corticosteroids, used systemically, will suppress psoriasis completely in the majority of cases. When these drugs are withdrawn a rebound condition usually occurs.

The view is now widely held that systemic corticosteroids are only justified in certain well-defined situations, as follows:-

(i) In psoriatic erythroderma, causing acute metabolic problems. Later methotrexate may be used, if necessary, to help wean the patient off the steroid. Prednisolone, 30 to 40 milligrams, usually suffices.

(ii) In fulminating generalised pustular psoriasis if methotrexate is contra-indicated. Large doses may be needed initially.

(iii) In rapidly progressing psoriatic arthritis, threatening to destroy useful joint function.

(iv) Occasionally, in severe refractory palmo-plantar pustulosis where very small doses of triamcinolone (2 to 6 mg daily) are often effective.

(Baker 1976b).

Purine Analogues

6-mercapto-purine, and its derivative azathioprine, have been used in the treatment of psoriasis. The drugs interfere with purine biosynthesis and subsequently DNA and RNA synthesis (Baker 1976b). Azathioprine is the less toxic of the two drugs (Finizi 1969), but leukopenia is a side effect. Fredricksson et al. (1967) conclude that these drugs should only be used in resistant cases and under constant control.

Pyrimidine Anologues

Azauridine, and its triacetylated derivative azarabine, interfere with the biosynthesis of uridylic acid, essential in pyrimidine biosynthesis. The acetylated derivative is used at high dose levels in the treatment of psoriasis. This dose is in the order of 125 to 250 mg/kg body weight/day (Milstein et al 1973). Dantzig (1974) had beneficial results with the use of low dose azaribine but they advise its use only in generalised pustular psoriasis or in psoriatic arthritis. This is because of the side effects which include neurotoxicity.

Epilepsy is a contra-indication, as is aspirin. Aspirin will lower the gastric pH, leading to the formation of the neurotoxic 6-azauracil.

Others

Many other drugs have been used as therapies in the treatment of psoriasis. These include hydroxyurea (Leavell and Yarboro 1970), allopurinol (Newbold 1972), actinomycin D (Finizi 1969), dapsone (Peachey 1977), mycophenolic acid (Spatz et al. 1978), vitamin B12 (Edwards and Stillman 1980) and clofazimine (Chauprapaisilp and Diamphongsant 1978).

Most of these drugs are efficacious, but no drug has been found that will clear psoriasis without maintenance therapy. This cannot be given with most of the drugs mentioned in this section.

It is unfortunate that so little is known about the root cause of psoriasis. Because of the lack of knowledge about the disease most of the therapies are cytotoxic therapies which will kill rapidly dividing cells. The rapidly proliferating psoriatic epidermis is not the only tissue which is producing cells at a

rapid rate, consequently these tissues are also attacked and destroyed. This accounts for the drastic side effects noted with almost all forms of systemic therapy.

Practical Treatment

In any review or survey of drugs used in a disease it is possible that a good deal of confusion is generated by the almost bewildering array of therapies which have been used over the years. Psoriasis, as can be seen, is no exception. The most common therapies used to control psoriasis are coal tar, dithranol, PUVA, methotrexate and occasionally topical steroids. The latter is dependent on the skin specialist who is treating the patient; many skin specialists do not like to use topical steroids (Hodgson personal communication).

The other drugs make a few appearances in the literature and are then largely forgotten, except in cases which prove intractable to the more acceptable courses of treatment.

Summary

Psoriasis is an extremely common disease; approximately 2% of the population of this country suffer from the psoriatic condition. In addition to this part of the population who have psoriasis itself, almost all of the inhabitants of this country, and indeed almost all of the world's population who live in a temperate climate, will have suffered at some time from a psoriasiform condition related to psoriasis, dandruff. This is, at worst, an embarrassing inconvenience, but to the psoriatic individual, even when the disease is in its milder forms, the lesions are extremely distressing. In other forms the disease may seriously interfere with the patient's working and social life. Psoriatics understandably will become depressed with the disease and this does not aid recovery.

The tendency towards spontaneous relapses, or towards exacerbating to serious proportions, makes treatment difficult. Therapies for psoriasis, are, at best, not very efficient. Coal tar and dithranol are useful, but fraught with cosmetic difficulties. Steroids, while being very useful at controlling the condition, have many undesirable side effects. Methotrexate and the other cytotoxic agents should be used with care and then only in extreme cases. The most promising therapy at present is PUVA, this treatment needs to be maintained or relapses occur. This factor in itself will disrupt the patient's working life. What is needed is a self-medication which is easy to use, safe and will give a clearing of lesions without the need for maintenance therapy. This may be an impossibility because of the genetic predisposition to the condition.

Coal tar contains an active compound or fraction, if this can be extracted from coal tar it is likely that the irritant and staining constituents will be removed leaving a safe, active and cosmetically acceptable anti-psoriatic agent.

2. METHODS AND DISCUSSIONS

A. An Anti-psoriatic Model: The Mouse Tail Test

All previous attempts to discover the therapeutic constituents of coal tar have lacked unity and met with little success.

Several criticisms have been made:-

(i) There has rarely been a well followed testing routine for fractions and compounds.

(ii) The testing usually consists of clinical observations of the effect of the fraction or compound on psoriatic lesions. The conclusions drawn tend to be subjective.

(iii) Evaluation of drugs is a difficult task due to the high rate of remissions, especially under hospital conditions (Samitz 1959).

(iv) The history of these various attempts is lacking in a histological evaluation of the drugs.

(v) The use of psoriatic lesions for routine screening is not adequate. Psoriasis is a disease which requires different types of therapies at different stages of the disease, it would be difficult to find a drug which will act at the primary site of the disease.

(vi) The use of skin biopsies is distressing to the patient, the numbers of biopsies needed make this method undesirable.

(vii) The moral and practical inadvisability of testing an unknown compound or fraction on man which may have no beneficial effects whatsoever and may be dangerous to the patient.

The testing of coal tar fractions or compounds requires a routine, reproducible screening test. An animal model would allow the standardisation of species, strain, sex, age, weight and diet,

which would be preferable. The animal model chosen was the model suggested by Jarrett and Spearman (1964), they used mouse tail epidermis as a model for psoriatic epidermis.

Jarrett and Spearman pointed out that, unlike the rest of the mouse body skin, the tail skin is histologically and biochemically very similar to the psoriatic skin. Mouse body skin has a similarly flexible, keratinising epidermis to humans, but is, of course, more hairy. The epidermis is thin with a granular layer about 1 cell thick. The tail skin looks scaly, is mainly hairless, and histological examination shows that this horny layer is produced in the absence of a granular layer. Groups of hairs are sparsely scattered among the scales, as may be seen in FIG. 12.

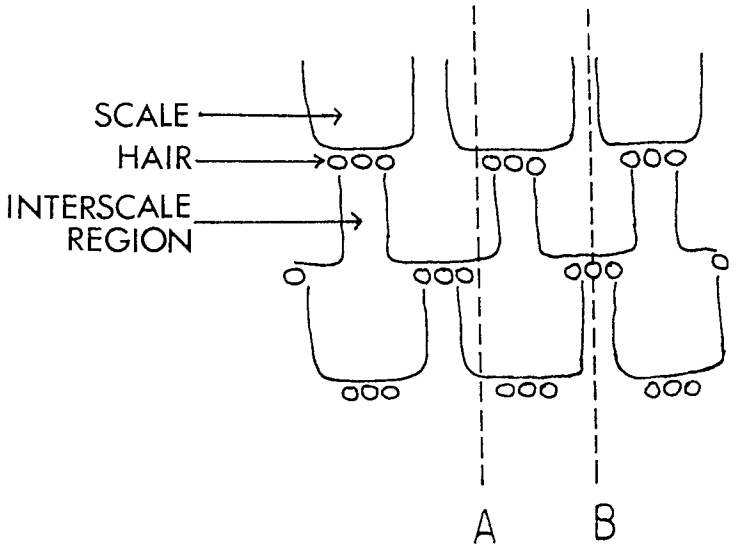


FIG. 12. Diagrammatic Representation of Mouse Tail Skin

Sections along line A were selected for histological examination, the sections along line B were rejected because there is a granular layer present in the interscale regions.

In these areas of hair production, where there is evidence of a granular layer, there is an absence of scale keratin. Thin flakey basket weave keratin is observed. The two types of keratin may be distinguished histochemically by the congo red/thioflavine T fluorochroming method (Jarrett et al. 1956, 1959). Scale keratin will fluoresce blue, whilst follicular, basket weave keratin will fluoresce red.

Jarrett and Spearman (1964) have shown that the two types of keratinising epidermis present in the mouse are similar in many ways to normal human epidermis and the parakeratinising epidermis found in psoriasis.

There are a large number of enzymes present in mouse tail scale horny layer that are found in the parakeratotic horny layer of psoriatic skin. The autolysis of cell contents is incomplete, as it is in psoriatic keratin. This produces a comparatively solid horny layer.

It has already been discussed that a granular layer is a prerequisite for orthokeratinisation (Kaku et al. 1964). This granular layer is absent in both psoriasis and the mouse tail. Both tissues produce a solid, parakeratotic, horny layer. It has been shown that certain substances, e.g. vitamin A, will induce a granular layer in both psoriatic epidermis and mouse tail scales (Laurence and Bern 1958, Jarrett and Spearman 1964).

The occurrence of scale keratin in the mouse tail suggested a model for the screening of potential anti-psoriatic fractions or compounds, for the specific effect of inducing a granular layer in what had been a parakeratotically keratinising tissue. This was thought to be a suitable parameter in looking for a specific anti-psoriatic, for the following reasons:-

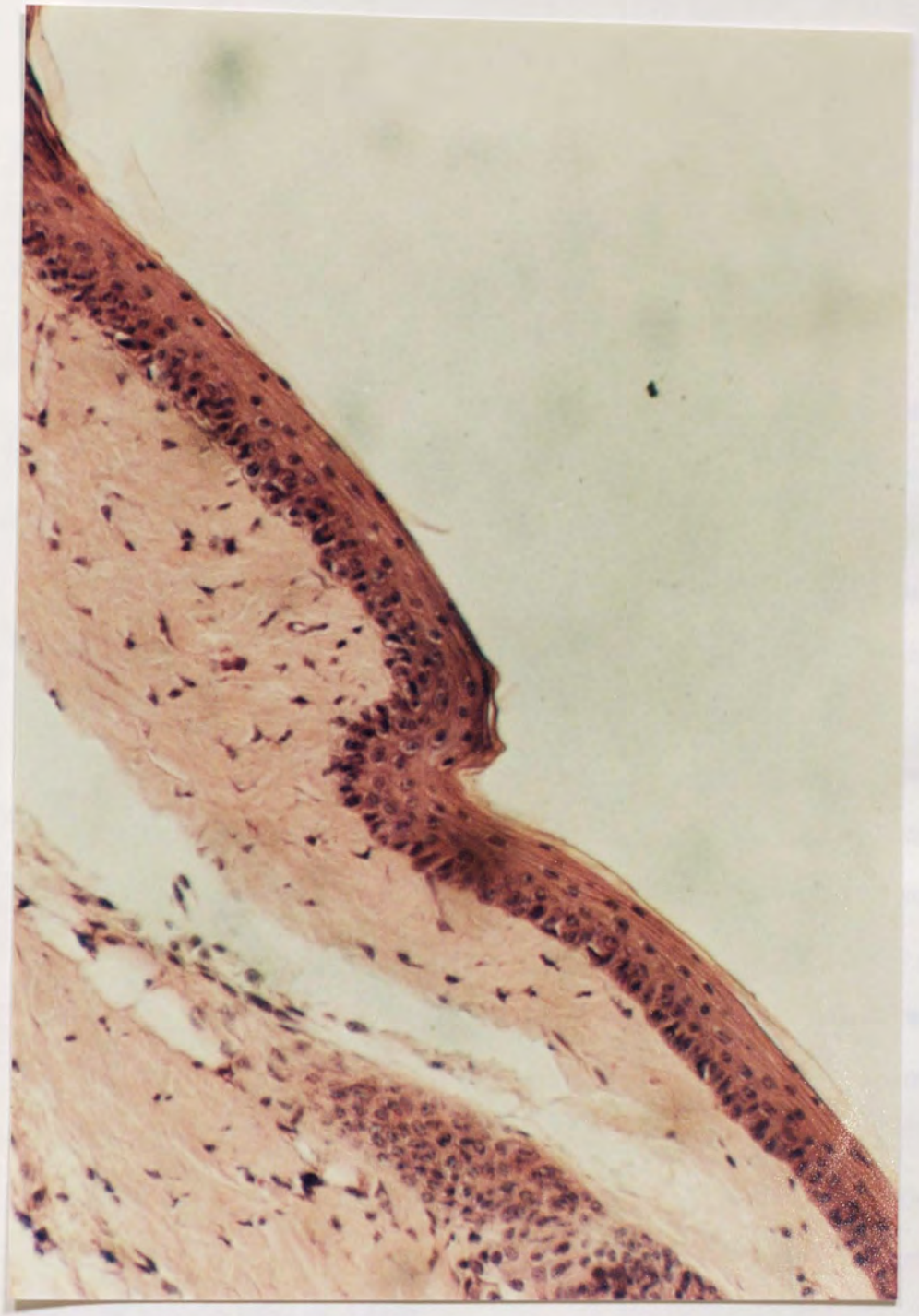


Plate 1 Untreated Mouse Tail

The untreated mouse tail shows no sign of a granular layer in the upper epidermis except round the hair follicle.

(1) There is a disagreement over whether the primary failure in psoriasis is dermal or epidermal in origin (Burks and Montgomery 1943). Many workers believe, from histological studies, that the epidermis is the site of the primary failure (Pinkus and Mehregan 1966, Braun-Falco 1963). It is therefore reasonable to recognise epidermal changes as representing an important feature of the psoriatic lesion.

(2) Induction of a granular layer has been shown to be one of the first events in the healing of a psoriatic lesion, as has been shown by the following drug trials:-

(i) A wide range of anti-psoriatic treatments produced a granular layer as one of the first events (Van Scott and Reinertson 1959).

(ii) Komisurak et al. (1962) felt that the lack of granular layer was important, because it was one of the first histological improvements after intra-lesional injections of triamcinolone.

(iii) Freedman et al. (1963) report that the earliest and most consistent finding in psoriasis treated with corticosteroids was the induction of a granular layer followed by the loss of parakeratosis.

(iv) Within two days of using topical methotrexate, the first sign of histological improvement was the reformation of a granular layer (Fry and McMinn 1967).

(v) Fry and McMinn (1968) also found that when psoriasis was treated with methotrexate and dithranol, there was an improvement in the granular layer before a lowering of the mitotic rate.

(vi) Christophers et al. (1973) noticed that one of the first events in the formation of a new psoriatic lesion was the loss of the granular layer.

(vii) When drugs which are known to have an anti-psoriatic effect are applied to mouse tail, a granular layer will form in the majority of cases (Wrench 1973, Jarrett *et al.* 1978, 1979).

The changes induced in mouse epidermis by anti-psoriatic drug combinations indicated that mouse tail scale skin could provide a guide to the effect of possible anti-psoriatic drugs on the psoriatic lesion.

It was decided that this mouse tail model would provide a test in which a large number of coal tar fractions and possible anti-psoriatic drugs could be topically screened, without putting a patient, or patients, through the discomfort and possible danger of application of an unknown drug.

The aim therefore, was to find which constituent, or fraction, of coal tar produced the most definite granular layer in mouse tail epidermis, with the minimum side effects, e.g. tail thickening, tail peeling, skin staining or any irritant reactions. The would, hopefully, produce a safe and cosmetically acceptable, long term treatment for psoriasiform diseases.

B. Characteristics of the Mouse Tail Test

(i) Method

The choice of vehicle is discussed at a later part of this section. The vehicle which was used was a 5% anhydrous wool fat in soft yellow paraffin base. Wrench (1973) had consistently reproducible results using this base to formulate coal tar ointments. Each fraction was formulated at a known concentration in this base and applied to mouse tail.

These formulations were applied daily, at about 1400 hours, all around the tail, over an area extending about 1 centimetre from the base of the tail, and about 2.5 centimetres along it. This is illustrated in FIG. 13.

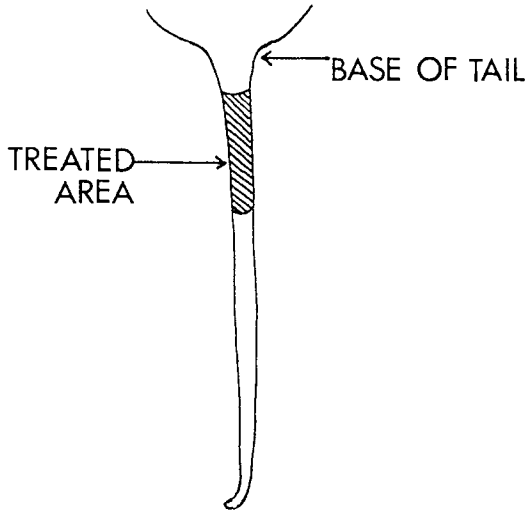


FIG. 13. Area of Mouse Tail Treated

The formulations were applied by cotton wool buds. The duration of the treatment was predetermined at three weeks, unless

otherwise stated. If the animals became distressed or the formulation was obviously harming them in any way the experiment would have been terminated. Fortunately this never occurred.

Termination of the run was made 24 hours after the final application. The animals were killed by stunning and dislocation of the cervical vertebrae.

The tail skin was then cut along the ventral surface, overlaying the ventral vein. After removing the whole tail, the skin was quickly stripped off and flattened, dermal side downwards, on glass in 70% alcohol for 10 minutes. The skin was then trimmed, as shown in FIG. 14, and fixed in 70% alcohol for 18 hours followed by absolute alcohol for 4 hours.

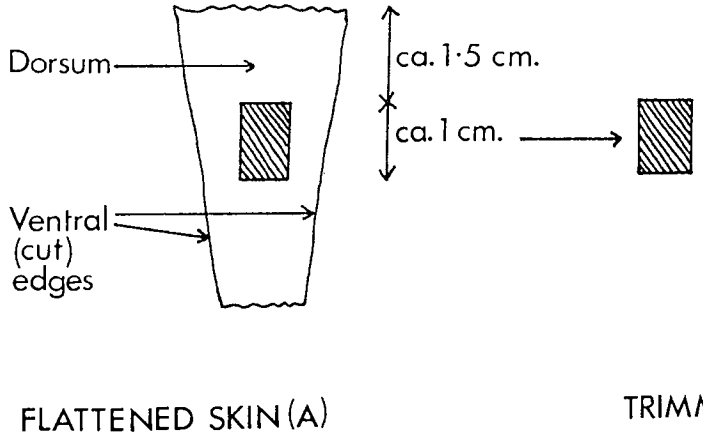


FIG. 14. The Portion of Mouse Tail Trimmed

When the tail is trimmed it is important to exclude skin near the base of the tail, in this area the "soft" keratin of the back

is merging with the "hard" keratin of the tail. Many of the scales have a granular layer (Lawrence and Bern 1958).

The tissue (B) was dehydrated over 9 hours in three changes of absolute alcohol, cleared overnight in cedarwood oil, washed in toluene and impregnated with two changes of "paraffin wax with microcrystalline wax, melting point 56°C" over 4 hours before final embedding in fresh wax.

Sections, 7 μ in thickness, were cut on a MSE Base Sledge Microtome and floated out on distilled water (55°C) onto microscope slides which had been prewashed in 1% acid alcohol. Complete adherence was obtained by subjecting the slides to 60°C for 30 minutes.

Routine haematoxylin and eosin staining followed, this is outlined below.

IMMERSION IN:		FOR:
	Xylene I	2 minutes
	Xylene II	2 "
	Aboslute Alcohol I	2 "
	Absolute Alcohol II	2 "
	70% Alcohol	2 "
	Tap Water rinsing	2 "
	*HAEMATOXYLIN	15 "
	Acid Alcohol 1%	5 seconds
	"Blueing" in tap water	10 minutes
	AQUEOUS EOSIN 1%	5 seconds

The slides were then dehydrated by momentary immersion in 70% alcohol, absolute alcohol and xylene, from which they were then mounted with cover slips in Canada Balsalm.

If the nuclear staining was inadequate the process may be repeated from * then the slides may be dehydrated. The haematoxylin used was Ehrlichs Haematoxylin, later sections were stained with Harris's Haematoxylin which stained the nucleus much faster, an immersion of 4 minutes was adequate.

It was found that the best adherence was attained when the microtome knife was sharp. Ideally the knife should be sharpened every 10 tails. This may be achieved by moving the knife along the chuck.

(ii) The Vehicle

There are many methods for the formulation of coal tars and many different bases (Martindale 1977). Since there are many thousands of constituents in coal tar, they will all have different absorption rates and require different vehicles for optimum penetration. This makes the choice of vehicle difficult. Within this work it was decided to choose one vehicle and to compare the action of different fractions or compounds in this vehicle.

The vehicle chosen was 5% anhydrous wool fat in soft yellow paraffin. This was the vehicle chosen by Wrench (1973) after investigating the properties of several vehicles. This vehicle has the advantage of being easy to prepare.

Since 1973, soft yellow paraffin has been investigated by Comaish and Greener (1976); they discovered that soft paraffin would inhibit the Köbner Response. This response is the triggering of a psoriatic lesion by a physical injury. The lesion is clinically and histologically indistinguishable from a spontaneous lesion, apart from its shape, which closely follows the shape of the injury.

The lesion usually follows 6 to 18 days after the trauma, which may be a scratch, abrasion, sunburn or operation wound. It has been shown that both the dermis and the epidermis must be damaged before this phenomenon occurs (Stankler 1969a). Long chain hydrocarbons have been shown to induce a granular layer by causing the epidermis to be invaded by non-specific esterase containing cells (Jarrett et al. 1979).

Despite these complications this vehicle was used. Controls, consisting of the vehicle only, were run to compare the effect brought about by the fractions under test with that part of the effect, if any, caused by the vehicle. The vehicle did not cause any effect in the experiments performed.

(iii) Examination of Mouse Tail Sections

The mouse tails were examined for any induction of granular layer for the reasons outlined in section 2A. It is important to only examine sections which only contain scale epidermis, i.e. along line "A" in FIG. 12. The reason for this is that it is possible to obtain sections which contain the naturally occurring granular layer found between the scales (Riley 1966, Spearman and Garretts 1966), i.e. along line "B". These granular layers would confuse the search for those induced by the formulation. The sections which included these regions were discarded.

The sections were also examined for an extension of the follicular granular layer as described by Jarrett and Spearman (1964).

(iv) Choice of Mice

The mouse strain used was the [illegible] strain used by Jarrett and Spearman (1964). The source was Bantin and Kingmann Ltd. The

mice were maintained on "Heygates Rat Breeding Pellets", which is a modified version of the MRC's "41 b" diet. The modifications are an increased protein content.

Although in their experiments with vitamin A, Lawrence and Bern (1958) had found no differences in response between the sexes, female mice would have differing hormone levels. Bullough and Laurence (1964) have shown that increased oestrogen levels are associated with increased epidermal activity. Consequently, only male mice were used. Testosterone will increase the mitotic rate in castrated mice, but in intact mice testosterone levels will be constant and therefore of no significance.

(v) Interpretation of Results

Slides were coded and randomised prior to examination to prevent preconceived ideas from clouding judgement.

It is very difficult for an animal model like the mouse tail test to be anything but subjective. The obvious question, "Is this compound efficacious?", must be asked, but note has to be taken of cosmetic acceptability, and any skin damage or irritation occurring. The epidermal thickness was not measured for a variety of reasons:-

(i) It is difficult to align the tissue perpendicularly in the wax block. This would lead to a greater thickness being measured than actuality.

(ii) There is a hypertrophic, as well as a hyperplastic response in many cases, it would be difficult, if not impossible, to separate the two responses.

(iii) Jarrett and Spearman (1964) have shown that triamcinolone will initially decrease the epidermal thickness, but on prolonged treatment the epidermal thickness will return to normal. No other

information is available about the long term treatment of mouse tail. Any response measured may only be a temporary change.

(iv) Wrench (1973) claims that epidermal thickness may vary in any individual mouse. Variation will certainly occur between mice if this is the case.

If any large increase in epidermal thickness did occur the fact was noted.

The granulation of the upper epidermis proceeds in several distinct stages. These stages are a useful index to the efficacy of any preparation, and were used as such. TABLE 1 shows the effects which were used in the evaluation of the fractions and compounds tested in this work.

KEY	RESPONSE
-	No increase in follicular granular layer.
*	Increase in follicular granular layer.
**	Extensive increase in follicular granular layer.
***	Even granular layer 2 to 4 cells thick.
****	Granular layer over 4 cells thick.

TABLE 1. The Key to the Evaluation of the Efficacy of any Anti-psoriatic Formulation

C. The Search for an Active Coal Tar Fraction

(i) Large Scale Separations

The work was based on the finding that the acid fractions in the boiling range 290°C to 340°C (760 mm) obtained from high temperature (coke oven) tar oils, derived from "Creosote" and "Anthracene" oils, were the most active in the mouse tail test as anti-psoriatics (Wrench and Britten 1975a, b, c).

Attention was directed to the evaluation of low temperature tar oils, since these were more readily available commercially, and are believed to have a higher percentage content of acids or hydroxylated aromatic compounds. About 13% of the total constituents of low temperature tar are said to be acids in comparison with 2% in high temperature tar.

Fisher and Eisner (1937) report that simple extraction of tar acids from coal tar with alkali and mineral acids give erroneous results, specifically that extracting with alkali and then mineral acid will give high and low results of tar acids and bases respectively. The reasons for this are twofold:-

(i) The solvent power of alkaline phenolate solutions for amines.

(ii) The absorption of water by the oily layer.

Further problems are caused by the differing acidities of phenols, this is generally weak (pKa phenol = 9.98), but variations in acidity occur from inductive, mesomeric and steric effects of substituents. Picric acid is a very acidic phenol (pKa picric acid = 0.71), but pentamethyl phenol is scarcely soluble in hot, aqueous alkali.

It is possible that not all tar acids are removed by alkali. There are also likely to be basic and neutral contaminants present in this acidic fraction.

Any solvent extraction performed on a mixture as complicated as coal tar must be regarded as an approximation, this is because of the inter-reaction of the many coal tar constituents. These extractions must be used with this limitation in mind when conclusions are drawn from work done on them.

If it can be assumed that these fractions are reproducible, it is worthwhile testing them on mouse tail. Although the extraction method is not ideal, it must be assumed that the majority of the acidic constituents are extracted and it is worthwhile testing these fractions.

When tested on mouse tail, the low temperature whole tar acid fraction (WTAF) induced a granular layer when applied at a 10% concentration in 5% wool fat in soft, yellow paraffin. This granular layer was induced without any epidermal thickening, irritation or tail corrosion. No granular layer induction was associated with the neutral or basic fractions. The WTAF was used for further purification of the active principle.

Coal tar is formed in the absence of oxygen. The WTAF used was from a low temperature tar with an upper temperature limit of 500^{o}C. If this fraction is heated above 300^{o}C in the presence of air a dark, brittle pitch remains after the volatiles have been driven off, only a small amount of tar distills at a temperature greater than 280^{o}C. This phenomenon also occurs if the WTAF is heated above 190^{o}C under 2 mm pressure. If the reaction vessels are flushed with nitrogen prior to heating, and a nitrogen bleed is added to the vacuum distillation apparatus, this problem largely disappears allowing the majority of the tar to be distilled.

The WTAF was distilled in four equal cuts. A commercially extracted acid fraction, XXL, supplied by Coalite Ltd., was split

into four unequal cuts, but with the boundaries between the different cuts at similar temperatures as those for the WTAF.

Again, due to the complex nature of coal tar, the tar will not distill cleanly. Two samples of coal tar, taken from the same extraction, and thus identical, will, on comparison, give slightly different proportions in any given cut. There will be many different factors interfering with the distillation, hydrogen bonding will cause elevated boiling points, co-distillation is bound to occur and the large number of constituents will mean that not all of any constituent will distill at its boiling point.

The only way to achieve any reproducibility is to heat the coal tar very slowly. The problem of reproducibility was solved by distilling a large batch, when this was shown to have anti-psoriatic properties on the mouse tail test it could be assumed that all further separations performed on this fraction would commence with the active principle in the starting material.

In both the WTAF and the XXL tars the active constituent was present in the highest boiling fraction. A comparison between the two tars showed that the WTAF high boiling fraction had a more efficacious content than the XXL high boiling fraction. The WTAF distilled more cleanly than the XXL, the latter left more residue than the former. It was decided that the high boiling WTAF would be used for further isolation and identification of the active constituent of coal tar.

Neither tar had any adverse effects on mouse tail except for bad staining.

The most difficult aspect of recognising reproducibility in an extraction technique, was the lack of an efficient monitoring

technique. The attempted use of gas-liquid chromatography met with little success.

Phenols tend to be too polar for gas-liquid chromatography, and "tail" badly (Supelco bulletin 1974). The most successful method of chromatographing phenols is to chromatograph the trimethyl silyl ethers derived from these phenols.

This may lead to many problems. If silylation is incomplete for any reason, it would be possible to form nine different compounds from one trihydroxylated compound and four from a dihydroxylated compound. There are many reasons for incomplete silylation; steric hindrance, or an excess of starting material, are likely to be the main reasons.

With many classes of compound, it is possible to predict a retention time on the basis of molecular weight when they are chromatographed on the columns used in this work, i.e. OV-1, OV-17 or OV-101. With phenols this is not so because ortho substituted trimethyl silyl ethers, derived from phenols, appear well away from their predicted retention times (Tulleberg et al. 1976).

Similar problems were experienced with acetyl and methyl derivatives. Investigations using these derivatives were abandoned when it was discovered that many phenols, which had been derivatised with these groups, were bound to the column, and could not be removed.

In the opinion of many authors (Bertsch 1976, Brady and Pettitt 1974), conventionally packed columns are not adequate for the separation of phenols or coal tar distillates. Wall coated and packed capillary columns offer an improvement in resolving power. The former are widely used for the analysis of coal tar distillates. The literature on the subject of phenol gas-liquid

chromatography is usually concerned with the separation of simple phenols (Brady and Pettitt 1974), and the literature on the subject of the separation of naturally occurring phenols is sparse (Tyman 1975, Fell and Lee 1976, Vande et al. 1976). It is debatable if any useful improvement could be achieved by the use of capillary columns for the task required, i.e. the monitoring of an extraction.

The problem of reproducibility can be avoided by the bulk preparation of fractions, the process is only performed once and the active principle may be assumed to be in the fraction which gives a positive test. If this bulk fractionation is impossible, as it was in later separations, the samples from different fractionations using the same method must be applied to the mouse tail test. If the mouse tail test gives reproducible results, it may be assumed that, even if the fractionation is not completely reproducible, the active constituent(s) always appear in the same fraction under identical separation conditions.

The mouse tail test takes over a month to complete and the majority of this time is spent applying substances to mouse tail. In an attempt to speed up the screening process, a time and concentration study was undertaken.

High boiling WTAF was applied to groups of 20 mice in concentrations of 10%, 20%, 30% and 40%. Five mice were killed from each group at five day intervals. The tail corrosion which occurred after 9 to 11 days in the 40% and 30% groups respectively, precluded the further use of these concentrations. The induction of granular layer in the 10% and 20% groups took 20 days to achieve maturity. It was concluded that although some effects did take place prior to the 21st day there was no advantage in shortening the therapy. If marginal induction of granular layer did occur

with any fraction it may pass unnoticed on a shortened regime.

The corrosion in the 30% and 40% groups occurred at the same time as granular layer induction. There was an appreciable thickening of the epidermis at this time. Although both conditions occurred, this was a hyperplastic rather than a hypertrophic condition. It is possible that the corrosive peeling caused mitotic stimulation. The suggestion that peeling stimulates mitosis is from observations in sellotape skin stripping experiments, in which the loss of keratinised cells is thought to be the primary stimulus of epidermal cell proliferation (Pinkus 1952, Allenby et al. 1966).

The work discussed dealt with the large scale coal tar extractions and the investigation of their action on mouse tail. The work of Obermayer and Becker (1935) and that of Wrench (1973) was confirmed, viz the nigh boiling tar acids have the greatest anti-psoriatic effect.

The end product of these separations is a high boiling fraction, believed to consist mainly of phenols, which has a bulk of 6% of the original coal tar. Although many compounds have been discarded, there are many still remaining. The remaining fraction still resembles coal tar and no increase in cosmetic acceptability has been achieved. No progress has been made to identifying a discreet compound with anti-psoriatic activity.

(ii) Small Scale Separations

Once the coal tar had been separated into fractions, with respect to acidity and boiling points, spectroscopy was utilised to gain further clues to use in further separations.

Infra red spectroscopy had already identified phenols in the acidic fraction. A carbonyl peak was noticed in a methylated coal tar fraction. If WTAF was extracted with 5% sodium carbonate, and the extract was then methylated, this carbonyl peak became the most intense peak. This suggested that the carbonate wash was extracting carboxylic acids from what had previously been assumed to be a phenolic fraction. A carbonate wash will extract acids but not phenols. The fraction which was not extracted by the carbonate was named whole tar phenolic fraction (WTPF).

When the carbonate extract from methylated WTAF was fractionated on silica and alumina columns, this carbonyl peak was no longer the most intense peak in many of the fractions. This suggests two things:-

(i) Esters may bind to the column under the experimental conditions.

(ii) There may be other compounds present which have been extracted from WTAF by the carbonate wash, these would tend to "dilute" the carbonyl peak.

No speculation has been made about the identity of these "other compounds". The esters derived from carboxylic acids which elute from the columns, do so at a greater polarity than the ethers derived from phenols.

Infra red spectra show that diazomethane will not completely methylate WTAF. The resultant mixture comprises of methyl ethers, methyl esters, partially or unreacted phenols and possibly carboxylic acids. The presence of non-hydrogen bonded hydroxyl at 3630 cm-1 in this methylated fraction, suggests steric hindrance of some hydroxyl groups.

Ultra violet spectroscopy of the column elutants gave very little information. A shoulder appeared in fractions 4, 5 and 6 on both alumina and silica columns, but this was not reproducible synthetically. The mixture of compounds which WTAF is comprised of is too complicated to give any useful resolution and spectra were ill-defined. The lack of any absorbance at wavelengths longer than 300 nm suggests the absence, or very low concentrations of polycyclic structures. This is surprising as it removes phenanthrols, anthranols and naphthols from consideration. These compounds were tested and found to be inactive at a later date.

The fractions prepared from the carbonate wash, column (e), methylated coal tar and methoxy-phenanthrones* were applied to mouse tail with the results shown in section 3D. Only mildly polar phenols are active, and the activity is lost by methylation. This suggests that the hydroxyl is necessary for efficaciousness. The high molecular weight phenanthrones were inactive. In this experiment the lack of activity was almost certainly due to the methoxy groups. The activity of the carbon skeleton was investigated at a later date.

The fractions prepared from column (e) were applied to mouse tail at a 25% concentration for 12 days. There were no adverse effects to the mouse by using this concentration; there was no thick granular layer produced either. All other experiments used the more normal 10% concentration for 21 days regime.

The fractions from these separations, not surprisingly, still resembled coal tar. If an active fraction was isolated that was more efficacious than any previous preparation, it would not be a

* A gift from Dr. A. Z. Britten and A. Amadiffe.

great improvement on existing therapies because, if this fraction still resembled coal tar, all the disadvantages of coal tar would still manifest themselves. It becomes increasingly obvious that a discreet compound with anti-psoriatic activity is required.

Thin layer chromatography was utilised as a means of isolating discreet constituents of coal tar. This was a failure, again because of the complexity of coal tar. The number of constituents still present in the WTPF, which has had other extractions performed on it, is still immense, as can be seen by looking at any of the thin layer chromatography plates. If a component is separated from the WTPF it is done so in very small amounts. Any individual constituent has a mass of less than 1% of the parent coal tar. This would yield 10 grams of any compound from a starting mass of 1 kilogram, almost all of the constituents of coal tar will have a mass which is far less than 1% of the total mass, this will mean that approximately 5 kilograms of tar will have to be processed to isolate any constituent, which may have no activity whatsoever. The use of dry columns, followed by thin layer chromatography, failed for the same reasons.

The suggestion of steric hindrance, supplied by the non-hydrogen bonded hydroxyl peak at 3630 cm-1, suggests that if esters with derivatising groups of varying sizes were synthesised and the esters then hydrolysed back to the parent phenols, a separation on the basis of steric hindrance could be achieved.

WTPF was reacted with acetic anhydride, benzoyl chloride and 2,2-dimethyl propanoyl chloride in order to prepare respectively; the acetates, benzoates and 2,2-dimethyl propionates of the hydroxyaromatic compounds in the fraction. It was hoped that some of the constituents of the fraction would remain inert to these

reagents and could be subsequently extracted from the reaction mixture, thus affording a partial separation of the components. To this end urethane and p-toluenesulphonyl esters were prepared. In all cases, underivatised materials were obtained as well as the derivatives.

The derivatised materials were converted back to their original hydroxylated form by hydrolysis and then formulated into ointments for biological screening. The unreacted materials from the derivatisations were also formulated for screening.

The screen revealed that the components in the fraction which did not form benzoyl or 2,2-dimethyl propionate derivatives were biologically inactive. This is also true of the components which did not form acetate, tosylate or urethane derivatives, although to a lesser extent.

The most active components were those that formed an acetate or benzoate derivative, and to a lesser extent, those that formed a tosyl or urethane derivative.

The active constituents must have a relatively unhindered hydroxyl group, so that the smaller derivatising agents form an ester, which on hydrolysis is biologically active. The bulkier derivatising agents do not derivatise the active constituents. Much of the activity of WTPF is lost in this process.

An attempt to synthesise possible coal tar constituents by the random hydroxylation of likely carbon skeletons, using a sulphonation and alkali fusion technique, met with little success. The skeletons chosen were high molecular weight polyaromatics. The evidence from later mass spectra studies and from existing ultra violet studies, suggested that this class of compounds are either absent in WTPF, or present in very small amounts.

Owing to the paucity of information regarding the identity of the active structures in coal tar, and the difficulty encountered in this work in attempts to isolate single components from such a complex mixture, it was decided to adopt an intuitive approach in further investigations.

D. Deduction of Possible Anti-psoriatic Structures

Because of its messy, complicated and intractable nature, coal tar is not an easy medium to extract potential anti-psoriatics from. Because of the small masses of individual constituents in coal tar, several kilograms of tar have to be processed in order to isolate a sufficient quantity of an individual constituent to allow it to be biologically tested. This assumes that such an isolation is possible. The vast number of coal tar constituents means that this extraction will have to be performed several hundred times, each time with a slight difference to isolate different constituents. The immensity of this task can be appreciated.

It remains a fact that coal tar has a decided anti-psoriatic action (Goekerman 1925, Saperstein and Wheeler 1979), if individual coal tar components cannot be easily or economically extracted from the parent coal tar then a prediction of likely coal tar constituents must be made. These constituents must then be bought or synthesised and then tested for anti-psoriatic action in order to find a useful anti-psoriatic drug.

It is almost certain from the existing evidence that these compounds are phenols.

The mass spectra for the most active fraction (high boiling WTPF), was recorded along with the spectra of the o-methyl, acetyl and benzoyl derivatives of this fraction.

Phenolic compounds give a large parent peak (Beynon 1967).

Mass Spectra of the Phenols

These spectra show the most abundant ions to have molecular masses of 116, 133, 144, 147 and 158. Ions of mass 177 are at only half the intensity of those at 162. There are virtually no

ions present at masses greater than 180. The masses present above 170 are at very low intensities. The active phenols may be summarised to be in the molecular weight range 115 to 215.

Mass Spectra of the O-methyl Ethers of Coal Tar Phenols

These spectra show that this mixture contains very little material beyond the molecular mass of 226; the highest m/e recorded. This corresponds to a monohydric phenol of molecular weight 212, or a dihydric phenol of molecular weight 198.

Mass Spectra of the Acetyl and Benzoyl Esters of Coal Tar Phenols

The mass spectra of these compounds resemble each other and the spectrum of the phenols. The conversion of a hydroxyl group to an acetyl or a benzoyl group would involve an increase in mass of 42 and 104 mass units respectively. Only the benzoyl derivatives are in the range 200 to 300 mass units. These are at very low abundances (less than 10% of the most abundant ion).

It is clear that acetyl and benzoyl groups are readily lost in the mass spectrometer and the spectra of the two derivatives closely resembles that of the parent phenols.

It can be seen from this mass spectral examination that the activity rests in phenols of molecular weight less than 200, unless the activity is extremely high and confined to a high molecular weight compound or compounds present in trace amounts.

It may be deduced from the boiling point of the parent fraction (180^{o}C and over at 2 mm mercury pressure) that monohydroxy alkylated benzenes may be excluded from consideration. Wrench (1973) found that individual xylenols were inactive.

As the active fraction is phenolic, with an upper molecular weight limit of 200 to 250, active structures may be found in

dihydroxy and polyhydroxy alkyl benzenes, (i), or in mono and polyhydroxy bicyclic, (ii) (iii) and tricyclic (iv) (v) (vi) (vii) (viii) benzenoid compounds (FIG. 15).

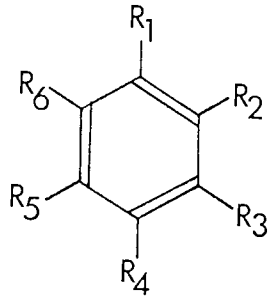
The UV absorption spectra of the active phenols and their derivatives are too ill-defined to draw any firm conclusions from them. It would appear that structures of the type (v) and (vi) are probably absent, or present in very low concentrations. This would also imply the absence of structures of the type (vii) and (viii). These compounds would be easily oxidised into (v) and (vi). It would appear likely that the active compound(s) could be found amongst structures (i) (ii) (iii) and (iv).

Even if structures (v) (vi) (vii) and (viii) are omitted, a formidable number of compounds is possible.

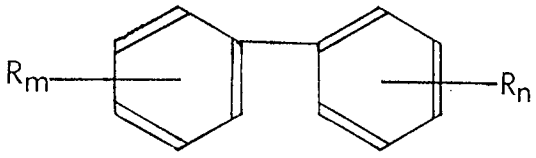
		Molecular Weight of			
		(i)	(ii)	(iii)	(iv)
Unalkylated and	1 x OH	-	170	144	182
" "	2 x OH	110	186	170	198
" "	3 x OH	126	202	186	214
1 Methyl "	1 x OH	-	184	158	196
1 " "	2 x OH	124	200	184	212
1 " "	3 x OH	140	216	200	228
2 Methyl or 1 Ethyl "	1 x OH	-	198	172	210
2 Methyl or 1 Ethyl "	2 x OH	138	214	198	216
2 Methyl or 1 Ethyl "	3 x OH	154	230	214	242
3 Methyl "	1 x OH	-	212	186	224
" "	2 x OH	154	228	212	230
" "	3 x OH	170	244	228	256

Table 2 . The Molecular Weights of Possible Anti-psoriatic Compounds.

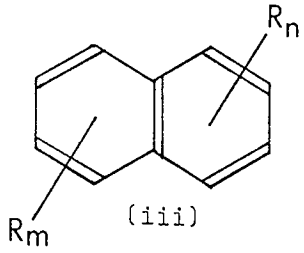
At least Ra = Rb = OH



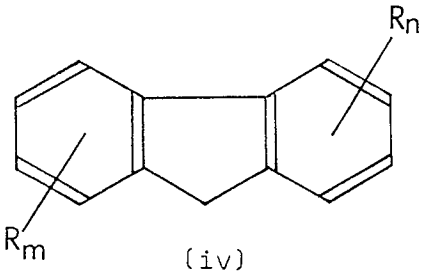
(i)



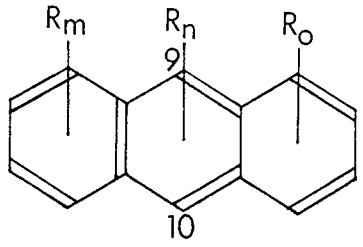
(ii)



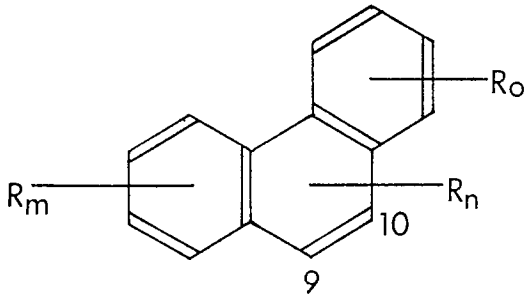
(iii)



(iv)



(v) and (vii) 9,10 Dihydro



(vi) and (viii) 9,10-Dihydro

At least one R=OH, but there may be two or three OH groups with up to three methyl/group equivalents.

FIG. 15. The Possible Anti-psoriatic Structures Elucidated from Spectroscopic Data.

Table 2 shows the range of compounds that are possible anti-psoriatics. The number of isomers that may be present with these simple substituents is large. Any other substituents that are present will make the problem more complex.

The most coherent approach to the problem of finding the active constituent(s) of coal tar, appears not to rest in the isolation of individual coal tar constituents, or in the isolation of an active fraction, but in screening of likely anti-psoriatic structures such as those mentioned above.

E. The Use of Single Compounds as Potential Anti-psoriatics

From the evidence accumulated from mass spectroscopic data and from the conclusions drawn from it, a collection of 31 possible anti-psoriatic agents were purchased. These 31 compounds are listed in section 3D experiment 10.

The 31 potential anti-psoriatics may be categorised into eight groups: the dihydroxy phenols, the trihydroxy phenols, the biphenols, the monohydroxy biphenyl methanes, the dihydroxy biphenyl methanes, the naphthalene diols and two miscellaneous groups. These categories comprised groups (i) (ii) and (iii) but not group (iv).

The groups were applied to mouse tail in a 25% concentration. Individual members of each group had a proportional percentage. Although some of the groups had a different number of compounds comprising them, which led to individual components having different absolute concentrations from components in another group, the range of concentrations only varied from 4 to 8%. This is a doubling of concentration, but it was felt that this concentration would be efficacious as any compound in coal tar would have a concentration of less than 0.1%. If half the constituents of coal tar have an anti-psoriatic action, a very unlikely circumstance, the concentration of active compounds would be 5% in the normal 10% mouse tail-coal tar regime. It would appear that the concentrations used would be adequate.

Group H, one of the miscellaneous groups had only two members, these were both formulated at 5% giving a total concentration of 10% for the group.

Group A, the dihydroxy phenols, had the only efficacious result, this was accompanied by bad tail corrosion and staining.

The mice did not seem to be suffering from this unsightly corrosion, they showed no sign of agitation or burrowing when approached to give further treatment.

This tail corrosion may be the cause of the induced granular layer acting by stimulating mitosis, either by epidermal and horny layer damage and/or by causing lysosome leakage. All of the six compounds in this group are described as irritant. It was pointed out by Obermayer and Becker (1935) that all useful anti-psoriatics are both reducing and irritant. The corrosion would, hopefully, disappear when the constituents of this group were tested individually at 5% concentration.

The other groups had no therapeutic effects and no adverse effects except for group B which caused the tail to be stained a bright purple colour. This may be attributed to pyrogallol which will form this colour on exposure to sunlight.

Many of the compounds in this group of 31 compounds have, or have had, dermatological uses. Resorcinols are used in the treatment of acne vulgaris (Debray 1975, Martindale 1977). Pyrogallol, catechol, phloroglucinol, o-phenyl phenol and 1,2-naphthalenediol have been used in the treatment of psoriasis (Obermayer and Becker 1935), and 4-t-butyl catechol is implicated in the depigmentation of melanocytes (Mansur et al. 1978). Obermayer and Becker claimed that catechol was one of the most efficacious compounds they tested.

When group A constituents were tested individually at a 5% concentration they were inactive, there was no apparent tail corrosion.

Three experiments were performed to elucidate whether the observed granular layer induced by group A was an artefact produced

by non-specific irritation and corrosion, or if these compounds had a therapeutic effect which was not apparent at the concentration used or some synergistic effect was needed.

From the earlier discussion it would seem unlikely that the concentration was inadequate but the members of group A were tested individually at a 10% concentration.

The members of group A were applied collectively at the 25% concentration previously used and at a collective concentration of 10%. It was thought that the lower concentration would not cause tail corrosion, if this experiment was successful and no tail corrosion occurred it would suggest that a synergistic effect was taking place. The individual components were only at 1.66% concentration.

To find any specific synergistic effect, all combinations of any two group A constituents were tested at a collective concentration of 10%, both compounds had the same proportional percentage.

Ethanol, (5%), was added to all the formulations to assist formulation and to aid penetration (Garnier 1971). It is known that ethanol will elevate cAMP levels by activation of adenylate cyclase (Yoshikawa et al. 1976). This is bound to have an effect on mouse tail keratinisation, so these experiments were repeated in the absence of ethanol.

The results of these tests show that at 10% concentration the dihydroxy phenols collectively will induce a granular layer. From the results of the individual screens it becomes apparent that the active principle is in the catechols. Hydroquinones and resorcinols were inactive. The combinations of group A members show that the most efficacious combinations are those which contain

a catechol. None of these tests corroded the mouse tail with the exception of the 25% collective formulation. The 10% collective formulation did not corrode mouse tail but did stain it.

Five interesting observations appear:

(i) When combined with a non-active group A member, an active member will have its effect enhanced, even if the concentration of the active member is insufficient to induce a granular layer if it were tested alone.

(ii) Ethanol will enhance the effect of these compounds.

(iii) Ethanol will increase the corrosion caused by any formulation, this could possibly be because of the dehydrating action of ethanol.

(iv) Ethanol causes an increase in epidermal thickness which is not noticed in the equivalent formulation without ethanol.

(v) The base with ethanol added will induce a granular layer.

This generates a certain amount of confusion. If ethanol will increase cAMP levels, this should lead to a fall in the mitotic rate. The increase in epidermal thickness is partly hypertrophic and partly hyperplastic. Ethanol should cause neither of these conditions. Ethanol is a dehydrating agent and should not cause hypertrophy, it should cause a fall in mitotic rate which certainly should not cause hyperplasia.

The use of ethanol as a formulation aid was discontinued because of the increased corrosion and the thickening of the epidermis.

Rather than investigate the synergistic process, it was decided to investigate different catechols in an attempt to find a highly beneficial drug. It has been shown that the catechols

in group A are capable of inducing a granular layer, to suggest that the two most active catechols are in this group does not seem to be sensible. The most useful course of action would be to apply many different catechols to the mouse tail test in order to find the most efficacious. Synergistic studies could then follow on this highly active catechol.

It is surprising that none of the other compounds have any effect on mouse tail. Several of them have been reported by Obermayer and Becker to be efficacious, many of the remainder have a structure which resembles catechol. 1,2,4-benzene-triol, pyrogallol and 2,3-naphthalenediol all have a 1,2 benzenoid structure. 1,4,9, 10-tetrahydroxy-anthracene and 4,5-dihydroxy-2,7-disulphonic acid disodium salt dihydrate have a structure which partially resembles dithranol by having adjacent hydroxyls on adjacent benzene rings. Wrench and Britten (1975d) have shown that dithranol will not induce a granular layer even though it is a potent anti-psoriatic agent.

The anti-psoriatic effect is unlikely to be related to the catechol structure alone, substituents are likely to have an effect. The more potent of the two catechols tested had a large alkyl side chain in position 4, this structure resembles that of the catecholamines. This interesting observation will be discussed later.

A group of 24 catechols and one methoxy catechol were purchased, synthesised or accepted as a donation. The catechol synthesised was later donated and the commercial product was tested. All these catechols were tested on mouse tail. The catechols may be divided into several groups on the criterion of their substituents.

As the catechols were tested individually this academic exercise would be futile.

The most active catechols were those with an alkyl side chain in the 3 position, i.e. 3-methyl, 3-isopropyl, 3-methyl-6-t-butyl, 3-methyl-5-t-octyl and 5-methyl-3-t-octyl catechols. One of the original, group A, compounds was 3,5-di-t-butyl catechol. The most efficacious compounds were the 3-methyl-5-t-octyl and 5-methyl 3-t-octyl catechols. There is little activity with any other catechol structure tested. There was a hypertrophic increase of epidermal thickness noticed with almost all the active catechols. Guiacol, the methoxy catechol, had the effect of increasing epidermal thickness even though it did not induce a granular layer. It is difficult to see why this hypertrophic increase in epidermal thickening is occurring. The number of cells in any given vertical section through the epidermis is constant so there is little or no hyperplasia. If the cells were affected by lysosome leakage the cells would be shrinking and there is no reason why a hydrophobic vehicle and a water insoluble compound should make the cells collect water. Wrench (1973) suggests that the tar acids, of which catechols are part, cause an induction of granular layer, but when they (or their metabolites) penetrate deeper into the epidermis they are potent mitotic stimulators and/or mediators of cell hypertrophy. The increased basophilia, which occurs when coal tar is applied to mouse tail skin, may be due to increased levels of RNA (Montagna and Parahkal 1974), which will cause hypertrophy.

Having deduced the possibility of a substance in coal tar and demonstrating its efficacy, the compound must be proven to exist in coal tar and the coal tar must be shown to be either less efficacious or inactive with this substance missing from it. To

prove the existence of a particular catechol would lead back to all the problems of isolating individual compounds from coal tar. An alternative is to deal with the class of compound which includes the individual constituent, i.e. the catechols.

Catechol has a molecular weight of 110, addition of methyl groups would raise the molecular weight by 14 mass units per methyl group. This would give masses of 124, 138 and 152 for catechols with 1,2 or 3 methyl groups. Tertiary butyl groups will increase the molecular mass by 57 mass units, giving a mass of 167 for the first compound in the series. These masses all have an abundance of less than 10%. This does not mean that simple catechols do not exist in coal tar; they do, but it shows that they are present in small amounts. This may be because they naturally occur in low concentrations in WTPF, because they have been removed at the tar distillery or because they were oxidised when the acidic components were extracted. If the catechols are the active fraction in WTPF they must be exerting their effect at a very low concentration, which reinforces the notion of a "mysterious harmony" between coal tar constituents postulated by Rothman and Shapiro (1949).

Experimental proof of the action of catechols in coal tar was gained in two ways:

(i) The catechols were removed from coal tar by oxidation at pH 12. Catechols oxidise under very mild conditions to form the corresponding quinones. Other compounds may oxidise under these conditions, but catechols and hydroquinones oxidise the most readily (Coalite Ltd. personal communication to Dr. A. Z. Britten).

When this "decatecholised" coal tar was applied to mouse tail it did induce a granular layer, but by direct comparison to mouse tail that had been treated with WTAF, this effect was diminished.

This suggests that catechols are an efficacious part of coal tar but are probably not the only efficacious compound present.

Catechols are removed from many commercial tars, this may account for the range in activity noticed between different tars. The low concentrations of catechols present in coal tar are doubtless assisted in their actions by the synergistic effect noticed when catechols are mixed with other dihydroxy benzenes, or, possibly, with other structures.

(ii) Dihydroxy benzene rich fractions were supplied by Coalite Ltd. Their composition was as follows:

Catechols Fraction

Approximate Boiling Range - 260°C - 290°C

Composition:	Catechol	60.8%
	4-methyl catechol ..	6.8%
	Resorcinol	0.2%
	3-methyl catechol ..	24.4%
	Others	7.8%

Homocatechol Fraction

Approximate Boiling Range - 265°C - 290°C

Composition:	Catechol	5.0%
	3-methyl catechol ..	6.5%
	4-methyl catechol ..	61.7%
	2-methyl resorcinol ..	2.0%
	4-ethyl catechol ..	3.2%
	Others	21.6%

2-Methyl Resorcinol Fraction

Approximate Boiling Range - 270°C - 300°C

Composition:	Catechol	0.1%
	Resorcinol	1.5%
	2, 4-dimethyl resorcinol	6.5%
	2-methyl resorcinol ..	90.8%
	Others	1.1%

Resorcinols Fraction

Approximate Boiling Range - 260°C - 300°C

Composition:	Catechol	0.8%
	3-methyl catechol ..	2.4%
	Resorcinol	3.17%
	4-methyl resorcinol ..	
	plus 4-ethyl catechol	33.5%
	2-methyl resorcinol ..	12.5%
	Others	19.1%

The boiling ranges of these fractions are similar to those of the high boiling WTPF constituents at atmospheric pressure.

The most active fractions were those which contained the highest percentage of catechols, the highest anti-psoriatic activity was contained in the catechol fraction, this fraction contained the highest percentage of meta alkyl catechols. It may be concluded that the most efficacious anti-psoriatic agents are catechols with an alkyl substituent in the 3 position.

The two original catechols, 4-t-butyl catechol and 3,5-di-t-butyl catechol, were reapplied to mouse tail. After consideration of other screens of catechols, it would be logical to expect the

3,5-di-t-butyl catechol to be the more efficacious of the two. This was not shown in the initial screen of group A compounds and was not shown on this repeat screen. Other catechols with a para substituent did not induce a granular layer of any appreciable thickness, even those with alkyl substituents.

The acetyl esters of these two original catechols were synthesised and applied to mouse tail. The esters would be less damaging because they are less polar and likely to be less toxic. A similar situation exists between dithranol and triacetoxy anthracene. Like these two substances the esters are not active. There are non-specific esterases present in normal human epidermis and psoriatic keratin (Jarrett and Spearman 1964), and mouse tail keratin (Wrench 1973). It would not be unreasonable to expect these esters to be hydrolysed to the active catechols by the non specific esterases. It is reasonable to assume that the lack of activity of these catechol esters is possibly due to lack of penetration.

A group of mice were treated with 10% 3-methyl-5-t-octyl catechol for a period of 21 days. After termination of treatment mice were killed at weekly intervals; in this way it was hoped to gain some knowledge of the change in granular layer with time. After a period of two weeks the granular layer begins to thin; this is probably because the granular layer is eroded away by the turnover of the epidermis. It also suggests that the catechols will induce a granular layer, but will have no permanent effect on keratinisation. After 4 weeks there was still evidence of granular layer but it was slowly disappearing.

It would be too large an extrapolation to apply this observation to human psoriatic skin; psoriasis is a genetic

predisposition leading to abnormal keratinisation, the parakeratotic mouse tail is natural. The effect of withdrawing treatment from human psoriatic skin may only be ascertained by clinical trial.

Both WTPF and the two most active catechols, 5-methyl-3-t-octyl catechol and 3-methyl-5-t-octyl catechol, were shown to be non-mutagenic by the *Ames Salmonella Mutagenicity Test; they were toxic. This toxicity is to be expected as phenols have been used as antiseptics for many years.

*My thanks to Dr. R. W. Middleton for performing this test.

F. The Similarity to Catecholamines

The similarity between the active catechols and the catecholamines did not pass unnoticed. Both groups of compounds have a catechol skeleton and an alkyl side chain. This side chain is substituted in the case of the catecholamines and is in the para position, as opposed to the meta position in the active catechols.

Voorhees et al. (1971) postulated that the adrenalin - cAMP cascade may control the epidermal cell cycle. These observations were made on the basis of in vitro studies with dibutyryl cAMP and isoprenaline on epidermis. Cyclic AMP levels were found to be decreased in the psoriatic lesion compared to uninvolved skin (Voorhees et al. 1972). This observation was challenged by Yoshikawa et al. (1975).

Voorhees et al. (1975) have since proposed that the ratio of cAMP and cGMP may control epidermal proliferation and differentiation. The ratio of cAMP/cGMP was found to be reduced in the psoriatic lesion and this is thought to be the cause of the increased mitotic rate.

An experiment was undertaken to determine the effect of adrenalin, noradrenaline and the β-adrenergic agonist, isoprenaline, on mouse tail.

Isoprenaline had previously been used in the treatment of psoriasis as a 0.1% ointment in white paraffin (Das et al. 1978) with good results. One member of the catecholamine biosynthetic route, DOPA, and a hydroxylated member of the same route, 6-hydroxy tyramine had previously been tested with negative results.

Despite being used at a 10% concentration, two orders of magnitude greater than the clinical trial, isoprenaline, adrenalin and noradrenaline had no positive reaction on the mouse tail test.

There was no damage to the mouse tail, but the animals treated with noradrenaline became hyperactive.

The lack of any activity must be due to inadequate penetration, breakdown of catecholamine or some unspecified process. It is difficult to believe that adrenalin will have no effect on mouse tail, if any adrenalin does penetrate to the β-receptor site, believed by some to be adenylate cyclase (Sutherland and Robinson 1966), it must elevate cAMP levels, which would in turn inhibit mitosis, induce orthokeratinisation and a granular layer.

Another explanation would be that any response may be short lived and not be able to exert a measurable effect. Stimulation of cAMP by exogenous adrenalin is a short-lived phenomenon (Yamazaki 1963, Powell et al. 1971).

If, because of their similarity of structure, the active catechols are eliciting an adrenergic response, their action should be blocked by either an α or β blocking drug.

Both WTPF and 3-methyl-5-t-octyl catechol, had their action partially blocked by both α-and β-blocking drugs. The blocking of action by a β blocker was predictable on the basis of the work done by Voorhees. If the β receptor is adenylate cyclase and the catechols were working as an artificial exogenous catecholamine analogue, their action would be blocked if the receptor site was. The effects of adrenalin and isoprenaline have been shown to be inhibited by propranolol, a β blocking drug, by many authors (Powell et al. 1971, Voorhees et al. 1974b).

The role of the inhibition by an α-blocking drug is something of an enigma. One of the effects of an α-adrenergic response is to increase cGMP levels (Voorhees et al. 1973). There is evidence that cGMP actually engenders the breakdown of cAMP, by activating

the appropriate phosphodiesterase (Jarrett et al. 1978). This is the opposite to what is required in an anti-psoriatic drug. If this situation is occurring, and is not an experimental artefact, it may be that the thickening of the epidermis is a hyperplastic response and not a hypertrophic response as it appears to be.

If an α response is occurring it would be logical to expect an increase in the efficacy of WTPF and the active catechols if this response is blocked, not a decrease, as has been observed.

It is very difficult to rationalise an α-adrenergic response in the light of present knowledge.

3. Experimental

A. Analytical

(i) Gas-Liquid Chromatography

Gas-liquid chromatography was performed on a Perkin Elmer F11 gas-liquid chromatograph fitted with a flame ionisation detector. The column used was a 4 metre 3% OV-1 on Chromosorb W 80 - 100 mesh at 140^{o}C. Gas pressures were 20 psi nitrogen, 15 psi air and 20 psi hydrogen. 2 metre OV-17 and OV-101 columns were also used but with no increase in resolution.

WTAF samples were chromatographed as either the tri-methyl silylated, acetylated or methylated derivatives.

Tri-methyl silylated derivatives were prepared by shaking a mixture of Tri-Sil* with a 1% w/v solution of WTAF in pyridine. Acetylated derivatives were prepared by reacting WTAF with an excess of acetic anhydride under alkaline conditions. The acetylated derivatives were then extracted with diethyl ether. Methylated derivatives were prepared by standing an ethereal solution of WTAF in an ethereal solution of diazomethane overnight. The latter two derivatives were chromatographed as an ethereal solution, the former as a solution in pyridine.

Resolution was good, and was maximal using the tri-methyl silyl derivatives, but was inadequate for the intended task; a monitoring process for fractionation. The chromatograms were too complicated to use either external or internal standards. It is the opinion of many workers that capillary column gas-liquid

*Pierce Chemical Co. Ltd.

chromatography is required for the successful resolution of coal tar samples (Brady and Pettitt 1974, Bertsch 1976).

Acetylated and methylated WTAF constituents bind to OV-type columns, and are therefore not useful derivatives for this type of column.

Chromatographs are reproduced in appendix 1.

(ii) Mass Spectroscopy

Mass spectra were recorded on a VG Micromass 12 mass spectrometer. The results of recording the mass spectra of WTAF, and its acetyl, o-methyl and benzoyl derivatives, show that WTAF is composed of low molecular weight compounds with an upper weight limit of 215. This effectively eliminates polycyclic structures from investigation.

The spectra of the acetyl and benzoyl derivatives are almost identical to that of the parent WTAF and it is clear that these two groups are readily lost in the mass spectrometer.

Mass spectra are tabulated in appendix 1.

(iii) Ultra Violet Spectroscopy

Ultra violet spectra were recorded on a Beckman Acta V Spectrophotometer.

The spectra of the phenolic compounds, which were administered to mouse tail, were compared to observe any correlation between anti-psoriatic activity and absorbance. There were only two active compounds in this group, both were catechols, and both had similar λ max and E max values. This is obviously because the two compounds were catechols, and no relation between activity and absorbance can be postulated.

The absorbances recorded are tabulated in Table 3.

Table 3. The λ max and E max Values of the Potential Anti-psoriatic Agents Applied to Mouse Tail in Experiment 10

Compound	λ max nm (S) = shoulder (E max) average of 3	λ max nm alkali (S) = shoulder
4-t-butyl catechol	280 (3075)	237.5, 255, 300, 311, 350 (S)
3,5-di-t-butyl catechol	279 (2550)	300 (S), 312, 358 (S)
4-n-dodecyl resorcinol	281 (3010)	292
4,6-di-t-butyl resorcinol	225 (S) (3825), 292 (4240)	252
Trimethyl hydroquinone	290 (3080)	252, 309 (S)
2,5-di-t-butyl hydroquinone	225 (S) (3377), 248 (692), 294 (4267)	252, 259 (S)
1,2,4-benzene-triol	290 (3110)	225, 332
Phloroglucinol dihydrate	254 (3934), 272 (2079), 287 (1401), 306 (840)	252, 345
Pyrogallol	267 (807)	243 (S), 364
O(Benzyloxy)phenol	276 (3124)	290
OO'-biphenol	285 (6932), 244 (S) (10063)	310
PP'-biphenol	265 (20306)	289
44'-isopropylidene diphenol	279 (3852), 284 (3361) 225 (15,139)	243, 294
2-hydroxydiphenyl methane	277 (2370)	294
4-hydroxydiphenyl methane	225 (S) (13828), 270 (S) (1996), 279 (1532)	242, 296
2,2-bis(4-hydroxy phenol)butane	229 (12962), 279 (3783), 284 (3279)	254, 294
Bis(2-hydroxy phenol)methane	275 (4683), 279 (4505)	293
Bis(4-hydroxy phenol)methane	230 (17697), 280 (3645) 287 (S) (3015)	245, 294

Table 3 (cont'd.)

Compound	λ max nm (S) = shoulder (E max) average of 3	λ max nm alkali (S) = shoulder
4-cumyl phenol	225 (9182), 278 (1958), 285 (S) (1617)	243, 294
22',44'-tetrahydroxy benzophenone	243 (7270), 253 (S) (6509), 287 (9864), 351 (15114)	272, 320, 386
Nordihydro--guaiaretic acid	283 (6375)	262
1,4,9,10-tetrahydroxy anthracene	236 (26207), 252 (S) (22230), 375 (19175), 284 (S) (16548), 380 (7525), 397 (10951), 414 (10806), 420 (S) (9817),	242, 332
1,3-dihydroxy naphthalene	211 (31824), 236 (45638), 287 (4224), 297 (4035), 325 (2241), 335 (2283)	318
1,5-dihydroxy naphthalene	226 (66595), 289 (S) (6563), 299 (8575), 310 (S) (6369), 317 (7308), 331 (7615)	252 (S), 343 (S)
1,7-dihydroxy naphthalene	240.5 (30185), 286 (4291), 297 (3819), 326 (2180), 338 (2336)	258
2,3-dihydroxy naphthalene	230 (118,398), 273 (S) (7604), 281 (7934), 292 (S) (6065), 305 (3272), 312 (5278) 318 (4815), 326 (8210)	246, 284, 336
2,7-dihydroxy naphthalene	233 (77015), 277 (S) (3117), 286 (3455), 292 (S) (3095), 298 (S) (2861), 307 (S), (2534) 314 (2764), 321 (2063), 329 (3252)	252, 294, 328 (S), 337 (S), 346
2,6-dihydroxy naphthalene dihydrate	229 (88626), 260 (5836), 269 (6002), 280 (3240), 340 (3637), 351 (3952)	267 (S), 279, 286, 359
4,5-dihydroxy-naphthalene-2,7-disulphonic acid disodium salt dihydrate	237 (6186), 331 (695), 347 (1029)	238 (S), 362

Routine spectra were recorded of all column elutants in an attempt to ascertain any changes in composition. A shoulder appears in fractions 4, 5 and 6, in both alumina and silica columns at 260 nm (Section 3B(v)). This could not be reproduced by an artificial mixture of phenols or their derivatives.

There is an absence of any measurable absorbance above 300 nm. This suggests the absence of polycyclic structures and confirms the findings of mass spectroscopy.

A confirmation of phenolic structure was ascertained by a bathochromic shift.

(iv) Infra Red Spectroscopy

Infra red spectra were run on a Pye Unicam SP 200 infra red spectrometer. Coal tar samples were run as a thin film between sodium chloride discs.

Infra red spectra of WTAF showed the presence of a carbonyl group. This was due to carboxylic acids in the tar and were removed by a 5% sodium carbonate wash. The resulting spectra suggest that the acid fractions of coal tar, after being subjected to this wash, were phenolic. Many workers have reported an absence of carbonyl structures in coal tar (Fair and Freidrich 1955, Karr et al. 1960).

Infra red analysis was used to determine the success of derivatisation, and subsequent extractions, when derivatisation was used as a mode of separation. The non-hydrogen bonded hydroxyl groups at 3630 cm-1 suggested that this method of separation may be viable.

B. Fractionation

(i) Partition

(a) Acid, base and neutral

After preliminary heating, stirring and filtering, 2 litres of 10% sodium hydroxide was added to 500 gm whole coal tar dissolved in 500 ml toluene : diethyl ether 1 : 1. The mixture was shaken thoroughly and allowed to separate. The aqueous layer was washed with 200 ml diethyl ether and then acidified with concentrated hydrochloric acid. The tar acids were extracted by 2 x 250 ml toluene : diethyl ether 1 : 1, dried over anhydrous magnesium sulphate and the solvents were removed under reduced pressure to leave the TAR ACIDS. Yield: 24%.

The basic and neutral organic layer from the above fractionation was shaken with 2 litres of 1 molar hydrochloric acid. The organic layer was washed with tap water until the washings were neutral to litmus, dried and the solvent removed under reduced pressure to leave the NEUTRAL fraction. Yield: 29%.

The acidic aqueous layer was washed with 200 ml diethyl ether and the protonated bases were reconstituted by the addition of 10% sodium hydroxide until the solution was alkaline. The solution was extracted by 2 x 250 ml toluene : diethyl ether 1 : 1, dried and the solvent removed under reduced pressure to leave the BASES. Yield: 33%.

Total yield: 86%.

(b) Carbonate wash

20 gm of WTAF was dissolved in chloroform (500 ml) and shaken for 5 minutes with 500 ml of 5% sodium carbonate. The

aqueous layer was acidified with concentrated hydrochloric acid and extracted sequentially with 200 ml of:-

(a) N-pentane

(b) chloroform

(c) ethyl acetate

These three extractions gave weight yields of (a) 1%, (b) 5% and (c) 5%.

The carbonate insoluble fraction gave a yield of 84%, this gives a total recovery of 95%.

All of the separated fractions from both fractionations were tested on mouse tail.

(ii) Distillation

Two tar acids were distilled, "XXL" which is a "phenolic" fraction and the acid fraction of whole tar (WTAF), a low temperature tar. Both tars were donated by Coalite and Chemical Products Ltd.

Atmospheric distillation met with little success due to the formation of a brittle, honeycombed pitch, with volatile components boiling at a maximum of 290^{o}C. Vacuum distillation at 2 torr met with the same problem. Vacuum distillation at 2 torr, with a nitrogen bleed and prior flushing of the distillation apparatus with nitrogen, allowed a clean separation with minimum pitch formation.

The WTAF was distilled into 4 equal cuts and the "XXL" was distilled into cuts at similar temperatures. The WTAF had a greater number of high boiling components, but an upper limit of 230^{o}C was observed, because of the danger of a higher incidence of carcinogenic components above this temperature (Bloch and Widmer 1926).

Fractions collected (2 torr)

WTAF	XXL
76°C to 113°C	76°C to 120°C
114°C to 130°C	121°C to 140°C
131°C to 180°C	141°C to 180°C
181°C to 230°C	181°C to 200°C

(iii) Derivatisation

An attempt was made to separate WTPF by derivatisation. The rationale behind this mode of separation is that, with a given derivatising agent and identical conditions, the proportion of WTPF which would not derivatise because of steric hindrance would be constant. Hydrolysis would free the derivatised fraction as free phenols and enable them to be applied to mouse tail.

Five different derivatives and their subsequent hydrolysis were achieved. Yields are expressed as a percentage of original sample by weight

(a) Acetylation

Acetic anhydride (20 ml) was added to 10 gm of high boiling WTPF, dissolved in 80 ml of 10% sodium hydroxide solution and standing in 80 gm of crushed ice. The mixture was shaken for five minutes and 30 ml of carbon tetrachloride was added. This organic layer was separated and extracted with 100 ml of 10% sodium hydroxide solution. The carbon tetrachloride contained the coal tar acetates, which were recovered by removing the solvent under reduced pressure.

The aqueous layers were bulked, acidified with concentrated hydrochloric acid and extracted with 250 ml of diethyl ether. The ethereal layer was washed with distilled water until the washings were neutral to litmus and the diethyl ether was removed under

reduced pressure. This removal of solvent also removed any remaining acetic anhydride or acetic acid. This yielded the underivatised coal tar. Yield (average): 20%.

Hydrolysis

The carbon tetrachloride was removed under reduced pressure. The acetylated coal tar was refluxed for two hours with 200 ml of 20% sodium hydroxide solution. The reaction mixture was then acidified and the hydrolysed acetates were extracted with 250 ml of diethyl ether. The solvent was then removed under reduced pressure, this also removes any acetic acid which may be present. Yield (average): 60%.

(b) Benzoylation

High boiling WTPF was benzoylated by a Schotten-Baumann reaction.

Freshly distilled benzoyl chloride (10 ml) was added dropwise, into 10 gm of high boiling WTPF, dissolved in 200 ml of 10% sodium hydroxide solution over a period of fifteen minutes. The reaction was constantly cooled in an ice bath. The benzoylated derivatives were extracted with 50 ml of carbon tetrachloride and washed with 200 ml of 10% sodium hydroxide solution.

The bulked aqueous extracts were acidified and extracted with 200 ml of diethyl ether. The products were heavily contaminated by benzoic acid. This was removed by washing the residue, after the solvent had been removed, five times with 300 ml of boiling water. Yield (average): 15%.

Hydrolysis

The benzoylates were hydrolysed by the same method as the

acetates. The product had to be washed five times with boiling water to remove benzoic acid. Yield (average): 55%.

(c) Tosylates

10 gm of high boiling WTPF was dissolved in 25 ml of pyridine. This mixture was heated on a water bath with 10 gm of tosyl chloride for thirty minutes. When the reaction had cooled, 50 ml of water and 25 ml of concentrated hydrochloric acid were added to remove the pyridine. Both the reacted and unreacted phenols were extracted with 200 ml of diethyl ether. The unreacted phenols were removed by 200 ml of 10% sodium hydroxide solution which was acidified, extracted by 100 ml of diethyl ether, washed twice by 100 ml of 5% sodium carbonate solution and recovered by the removal of solvent under reduced pressure.

Hydrolysis

The tosylates were subjected to alkaline hydrolysis in the same way as the acetates and benzoates after removal of diethyl ether. An additional wash by 100 ml of 5% sodium carbonate removed any remaining reaction impurities.

(d) Diphenylurethanes

10 gm of high boiling WTPF was dissolved in 50 ml pyridine and heated on a water bath for one hour in the presence of 10 gm of diphenyl carbamyl chloride. The extraction procedure was identical to that of the tosylates.

Hydrolysis

The hydrolysis procedure was identical to that of the tosylates. The diphenylurethane esters could not be isolated, but the hydrolysis products were.

(e) 2,2-di-methyl propionates

These esters were prepared by a Schotten-Baumann reaction with freshly distilled 2,2-di-methyl propionyl chloride. The procedure was similar to that of the benzoates with the exception of the boiling water extraction. A 5% sodium carbonate wash was substituted. Yield (average): 15%.

Hydrolysis

The derivatives were hydrolysed by alkaline hydrolysis. The extraction procedure was similar to that of the acetates with the addition of a 5% sodium carbonate wash. Yield (average): 70%.

The tosylates and diphenylurethanes did not show constant yields; they would vary by up to 25%.

The purity of the phenols or the esters was determined by infra red spectroscopy. An ester will show a carbonyl at about 1700 cm-1 and an absence of hydroxyl at 3300 cm-1. A phenol will show the opposite.

(iv) Thin Layer Chromatography

Phenols were chromatographed as their methyl ethers to reduce polarity and to prevent "tailing". Methyl ethers were prepared by standing an ethereal solution of high boiling WTPF in an ethereal solution of diazomethane overnight. The volume was reduced by evaporation at reduced pressure. The sample was applied to the thin layer chromatography plate as this concentrated ethereal solution.

20 cm x 20 cm glass plates were coated with an 0.25 mm thickness of Kieselguhr Gf 254 nach stahl and activated by heating to 120°C for thirty minutes. For preparative work the support thickness was 1 mm. Spot "development" was by UV light at 254 nm.

The solvent system used was glacial acetic acid (3% to 15%) in chloroform. All solvents were distilled prior to use and the thin layer chromatography plates were run in ethyl acetate and dried prior to their use. A chromatogram was run in 3% glacial acetic acid in chloroform, the bottom 2 cm was scraped off, extracted by diethyl ether, and run in 6% glacial acetic acid in chloroform, the process repeated until the whole coal tar was separated.

Two highly fluorescing bands at Rf's of 0.1 and 0.3 in 15% glacial acetic acid in chloroform were observed. These bands were collected on preparative thin layer chromatography plates from 10 gm of high boiling WTPF. On the basis of IR and UV evidence the structures were tentatively identified as naphthalene methyl ethers derived from naphthols. Further analysis was not possible because of the small amounts of material present (4.6 and 5.3 mg respectively).

Other solvent systems gave poor resolution.

Other solvent systems tried

Acetone

Ethanol

Acetone/ethanol (varying proportions)

Toluene

Chloroform

Methanol

Ethanol/toluene (varying proportions)

Acetone/toluene (" ")

Chloroform/methanol (varying proportions)

Ethanol/petroleum ether 100/120 (varying proportions)

Glacial acetic acid/toluene (" ")

N- pentane/ethyl acetate (" ")

Benzene/methanol 95 : 5*

Toluene/piperidine 5 : 2*

Benzene/isopropanol/ammonia 3 : 1 : 1*

Benzene/ethyl acetate/glacial acetic acid 18 : 1 : 1*

*Smith et al. (1974).

(v) Column Chromatography

Six types of chromatography column were used to separate high boiling WTAF, WTPF and the other constituents of WTAF.

(a) Alumina/Methylated Derivatives

A 9" x 1" neutral alumina 100 - 120 mesh chromatographic grade column was packed in 40 - 60 petroleum ether. 1 gm of high boiling WTPF methyl ethers were applied to the top.

The column was eluted with:-

Fraction		
1	40 - 60 petroleum ether	150 ml
2	5% diethyl ether in petroleum ether	100 ml
3	10% diethyl ether in petroleum ether	100 ml
4	20% diethyl ether in petroleum ether	100 ml
5	50% diethyl ether in petroleum ether	100 ml
6	Diethyl ether	100 ml
7	10% ethanol in diethyl ether	100 ml
8	20% ethanol in diethyl ether	100 ml
9	50% ethanol in diethyl ether	100 ml
10	Ethanol	100 ml
11	Ethanol flood	300 ml

The solvents were removed under reduced pressure and the fractions weighed. The recovery was in the order of 90%.

(b) Silica/Methylated Derivatives

The use of silica columns with the same elutants gave an equivalent separation and no increase in recovery.

(c) Alumina/Methylated Carbonate Extraction Products

A column identical to (a) was covered by 1 gm of the bulked,

methylated extracts from the WTAF carbonate wash and eluted as column (a). Recovery was 62%.

(d) Silica/Methylated Carbonate Extraction Products

An experiment identical to the previous one, except that a silica column was used instead of an alumina column. The silica column behaved in the same way as the alumina column. Recovery was 57%.

(e) Alumina/WTAF

An identical column to (a) had 1 gm of WTAF applied to it. The column was eluted with:-

(i)	Diethyl ether	200 ml
(ii)	Ethanol	200 ml
(iii)	Ethanol/10% 880 ammonia	200 ml

The column was then broken up and extracted with 200 ml of ethanol/10% sodium hydroxide solution (10% w/v). 78% of the initial sample was recovered.

The four fractions were applied to mouse tail (Experiment 5).

(f) Dry Columns

A 9" x 1" alumina dry column was packed (Loev and Goodman 1967) and 1 gm of WTPF was eluted with chloroform. The column was stopped when the chloroform reached the bottom of the column and a pink band of Rf 0.3 was removed from the destroyed column. Further thin layer chromatography work on this band enabled the extraction of two discreet components (8.7 and 4.7 mg) which had Rf's of 0.7 and 0.8 respectively on a 12% glacial acetic acid in chloroform solvent system on Kieselguhr Gf 254.

These two samples were not analysed because of the small amounts which had been extracted.

C. Synthetic

(i) Synthesis of Poly-hydroxylated Poly-aromatics

Poly-hydroxylated derivatives of fluorene, anthracene, phenanthrene, 2-and 4-phenyl phenol were prepared by the method of Fieser (1929). This preparation gives a mixture of mono and di-hydroxy derivatives. There was no attempt made to isolate individual compounds, as the rationale behind testing these substances on mouse tail was to ascertain the activity of the carbon skeleton. The procedure was identical for each parent compound.

50 gm of the parent compound was stirred for 4 hours with 50 gm of concentrated sulphuric acid in an oil bath at 125^{o}C. Initially, the reaction formed a thick viscous liquid, which crystallised and after 4 hours was a thick paste. This paste was dissolved in 400 ml of water and neutralised with 100 gm of potassium hydroxide dissolved in minimum water. Concentrated sulphuric acid was added until the solution was just acidic. This solution was boiled down to a paste and then dried overnight in an oven.

The dry cake was ground and the resultant finely powdered mixture of potassium sulphate and mono-and di-sulphonates were fused at 300^{o}C with 50 gm of potassium hydroxide. The thick brown melt from this reaction was cooled and dissolved in iced water. The greater part of the alkali was neutralised, but the solution was kept alkaline. After filtration and acidification the poly-hydroxyl derivatives precipitated out as a grey powder which was used for the animal experiments.

Thin layer chromatography of the products was carried out on 10 cm × 10 cm × 0.25 mm Kieselguhr Gf 254 plates with a solvent system of toluene : methanol : ethyl acetate 60 : 25 : 15. This showed 4 to 8 spots to each parent compound when the plates were placed under a UV light source of 254 nm. The Rf values were in the range 0.3 to 0.45.

Infra red and ultra violet spectroscopy show a hydroxyl peak, at 3300 cm-1, and a bathochromic shift respectively, which indicates a phenolic structure.

The boiling ranges of these derivatives at 2 torr varied from 160°C to 215°C.

(ii) Acetylation of Catechols

Catechols were acetylated by the method described by Vogel (1964).

2 gm of catechol was refluxed with 10 ml of acetic anhydride in the presence of two drops of concentrated sulphuric acid for 15 minutes. When cool the reaction was tipped into 25 ml of iced water and the product filtered off, washed with iced water and recrystallised from dilute ethanol. Homogenity of the products were ascertained by gas-liquid chromatography.

Yields were in the order of 90%.

(iii) Attempted Synthesis of 3-methyl-catechol

Kablaoui (1975) describes the synthesis of alkyl catechols from the corresponding α-alkyl cyclohexanones.

Aromatisation of 2-methylcyclohexanone

Into a 250 ml three-neck flask, equipped with a magnetic stirrer, a gas sparger, a condenser and a thermometer, were charged

5 gm of 2-methylcyclohexanone and 60 ml of acetic anhydride. Concentrated sulphuric acid (9 gm) was slowly added at room temperature to the mixture. The reaction mixture was then heated to reflux for one hour while dry nitrogen was passed through at a rate of 140 ml/minute. The work-up of the reaction mixture was done by quenching in 150 ml of iced water and stirring for 30 minutes to decompose all the acetic anhydride followed by extraction by diethyl ether (4 x 50 ml). The combined diethyl ether extracts were washed once by 50 ml of saturated sodium bicarbonate solution and once with saturated sodium chloride solution, dried, and the solvent removed under reduced pressure.

The product, which should have been a mixture of O-cresyl acetate and 3-methyl-catechol diacetate, was present in very small quantities.

The oxidation of cyclic ketones with ferric chloride is known (Nakatani and Matsui 1968) and proceeds via an α-chloro derivative which is then hydrolysed to a dione. This may be aromatised by concentrated sulphuric acid in acetic anhydride to form the corresponding catechol.

Oxidation-Aromatisation of 2-Methylcyclohexanone by Ferric Chloride

Anhydrous ferric chloride (25 gm) was dissolved in 30 ml of distilled water. The solution was filtered into a three necked flask containing 3.8 gm of 2-methylcyclohexanone in 30 ml of acetic acid. The mixture was refluxed for 2 hours under nitrogen. The mixture was cooled and the organic salts were filtered off. The solution was then extracted by ether, dried, and the solvent removed under reduced pressure.

Aromatisation of 2-Methylcyclohexanone in the presence of Ferric Chloride

2-Methylcyclohexanone (5 gm) was aromatised with acetic anhydride (60 ml) and concentrated sulphuric acid (10 gm), in the presence of benzene (60 ml) and catalytic amounts of ferric chloride (1 gm).

Both of these reactions yielded an intractable, black paste because the ferric chloride was soluble in both diethyl ether and water.

(iv) Oxidation of Catechols in Coal Tar

Catechols will oxidise more readily in alkaline conditions than phenols.

50 gm of high boiling WTPF was dissolved in 250 ml of 10% sodium hydroxide and the pH was adjusted to the range pH 9 - 12 by the addition of 5 M hydrochloric acid. Air was bubbled through this solution at a rate of 150 ml/minute, the solution was constantly stirred. Samples were taken every 5 minutes and tested for the presence of catechols by the following procedure.

10 ml of the reaction mixture was neutralised and partitioned between 50 ml of distilled water and 50 ml of petroleum ether 40-60. To the aqueous solution 1 ml of 20% (w/v) lead acetate and 1 ml of 5% (w/v) ammonium acetate was added with stirring. Catechols gave a white precipitate.

Under these conditions all catechols in the high boiling WTPF were oxidised in 35 minutes. The remaining "decatecholised" high boiling WTPF was recovered by acidification, diethyl ether extraction and removal of solvent under reduced pressure.

(v) Synthesis of Methylated Coal Tar Derivatives

Methyl derivatives of phenols were prepared by two methods.

(1) Dimethyl Sulphate

The method is modified from that of Vogel (1964) for the preparation of anisole from phenol. An excess of dimethyl sulphate is used to allow for the possibility of there being more than one hydroxy or carboxy group per molecule.

A solution of 10.5 gm of sodium hydroxide in 100 ml of water was used to dissolve 15 gm of WTAF. 31.5 gm (23.5 ml) of dimethyl sulphate was added dropwise over a period of 30 minutes whilst ensuring that the temperature did not rise over $10^{o}C$.

The mixture was then refluxed for 2 hours to complete the reaction. When cool the mixture was washed with water, washed twice with dilute sulphuric acid and then with water until the washings were neutral to litmus. The product was extracted by 50 ml of 10% sodium hydroxide to remove any unreacted phenols, dried and weighed.

For the methylation of phenol to anisole the yield is only 60%, the yield for the methylation of WTAF was even lower (30%). This method is not very efficient for methylating phenols.

(2) Diazomethane

Diazomethane was prepared as described in Vogel (1964) using p-tolylsulphonylmethylnitrosamide as a starting material.

The ethereal solution of diazomethane was mixed with 12 gm of WTAF dissolved in 25 ml of diethyl ether and left overnight in a fume cupboard to methylate.

The diethyl ether and any remaining diazomethane was removed at reduced pressure in a rotary evaporator at ROOM TEMPERATURE

using an ice bath to cool the receiver. The receiver contained 25 ml of glacial acetic acid to break down any diazomethane which co-distilled over with the diethyl ether.

Yield (average): 90%.

D. Mouse Tail Tests

The sectioning and staining of mouse tails was performed as has been described in section 2B. The standard assessment tabulated in section 2B(iv) was applied. A blank square indicates that the mouse did not survive the experiment or that the mouse was killed because of injuries inflicted by other mice. NM signifies that the tissue was damaged during handling and could not be assessed.

Experiment 1

Whole Tar Acid, Base and Neutral Fractions 10%

The fractions obtained by the partition of whole tar between acid and base were applied to mouse tail at a 10% concentration in soft, yellow paraffin : wool fat 95 : 5 for 21 days. The control consisted of the vehicle only.

Results

Fraction	Mouse				
	1	2	3	4	5
Acid	*	*	**	**	**
Base	-	-	-	-	-
Neutral	-	-	*	*	-
Control	*	-	-	-	-

Table 4. The granular layer inducement of acidic, basic and neutral fractions of coal tar when applied to mouse tail

Observations

The fractions all stained mouse tail. There were no effects of irritability or corrosion at this concentration with these formulations. The number of cells in an epidermal thickness was unaltered between the control and any fraction.

Experiment 2

Coal Tar Distillates

The fractions obtained from the distillation of XXL and WTAF were applied to mouse tail at a 10% concentration in yellow, soft paraffin : wool fat 95 : 5 for 21 days. The control was the same as experiment 1.

Results

Fraction		Mouse 1	2	3	4	5
XXL	76°C to 120°C	-	-	-	-	**
	121°C to 140°C	-	-	-	-	-
	141°C to 180°C	*	*	-	-	*
	181°C to 200°C	*	*	*	*	NM
WTAF	76°C to 113°C	*	NM	-	*	*
	114°C to 130°C	*	-	**	*	*
	131°C to 180°C	**	*	**	*	*
	181°C to 230°C	**	*	**	**	
Control		*	-	-	-	-

Table 5 . The granular layer inducement of coal tar distillates when applied to mouse tail

Observations

All of these fractions stained mouse tail but no corrosion or irritation took place at this concentration.

WTAF stained the least and gave the best granular layer induction and was used for the remaining coal tar work, except where the purified form, WTPF, was used. The XXL fraction was abandoned because of its inferior granular layer induction and for reasons given in section 2C.

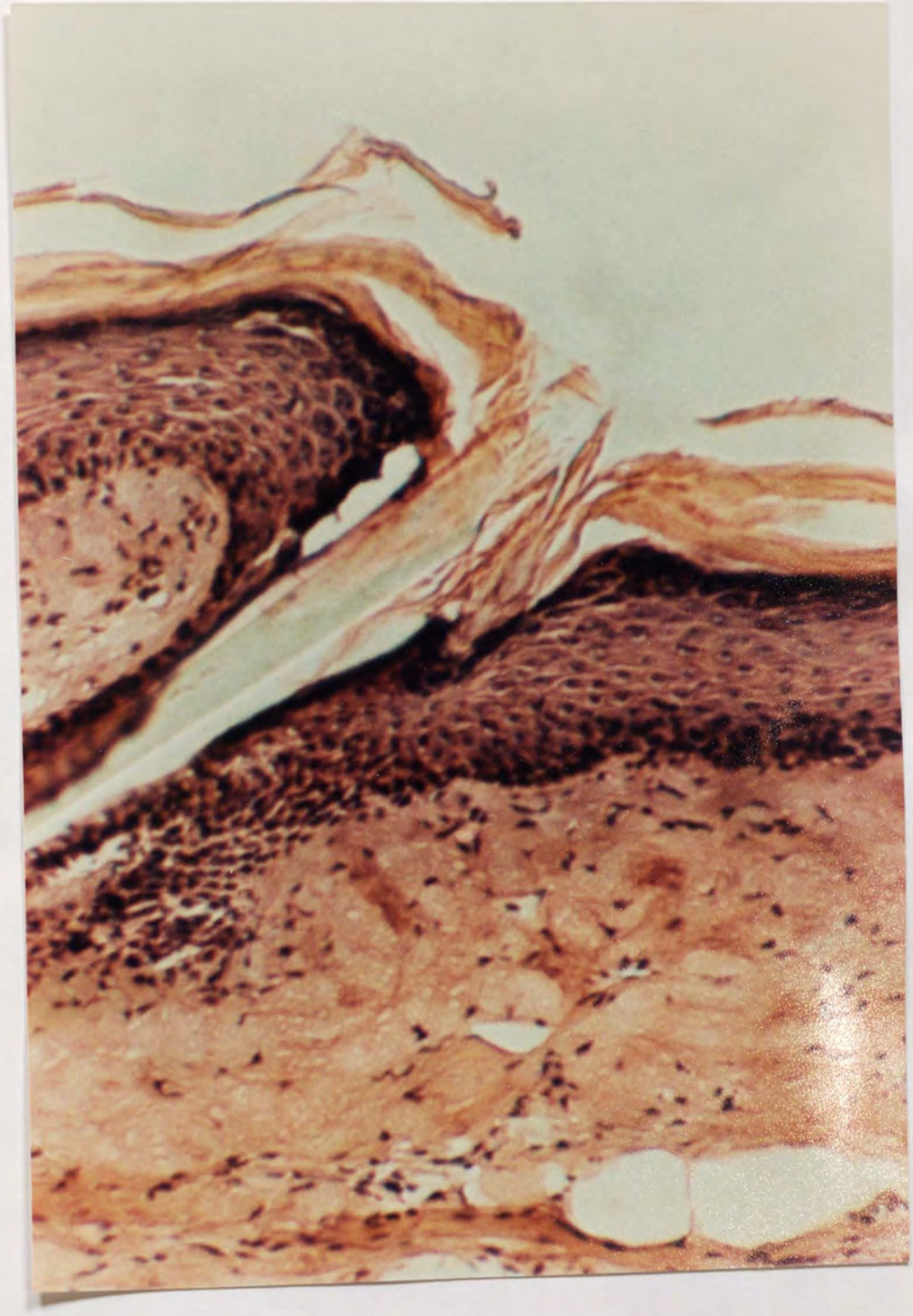


Plate 2. Mouse Tail Hair Follicle after 12 Treatments of WTAF 10%

The granular layer around the hair follicle is thickening and spreading.

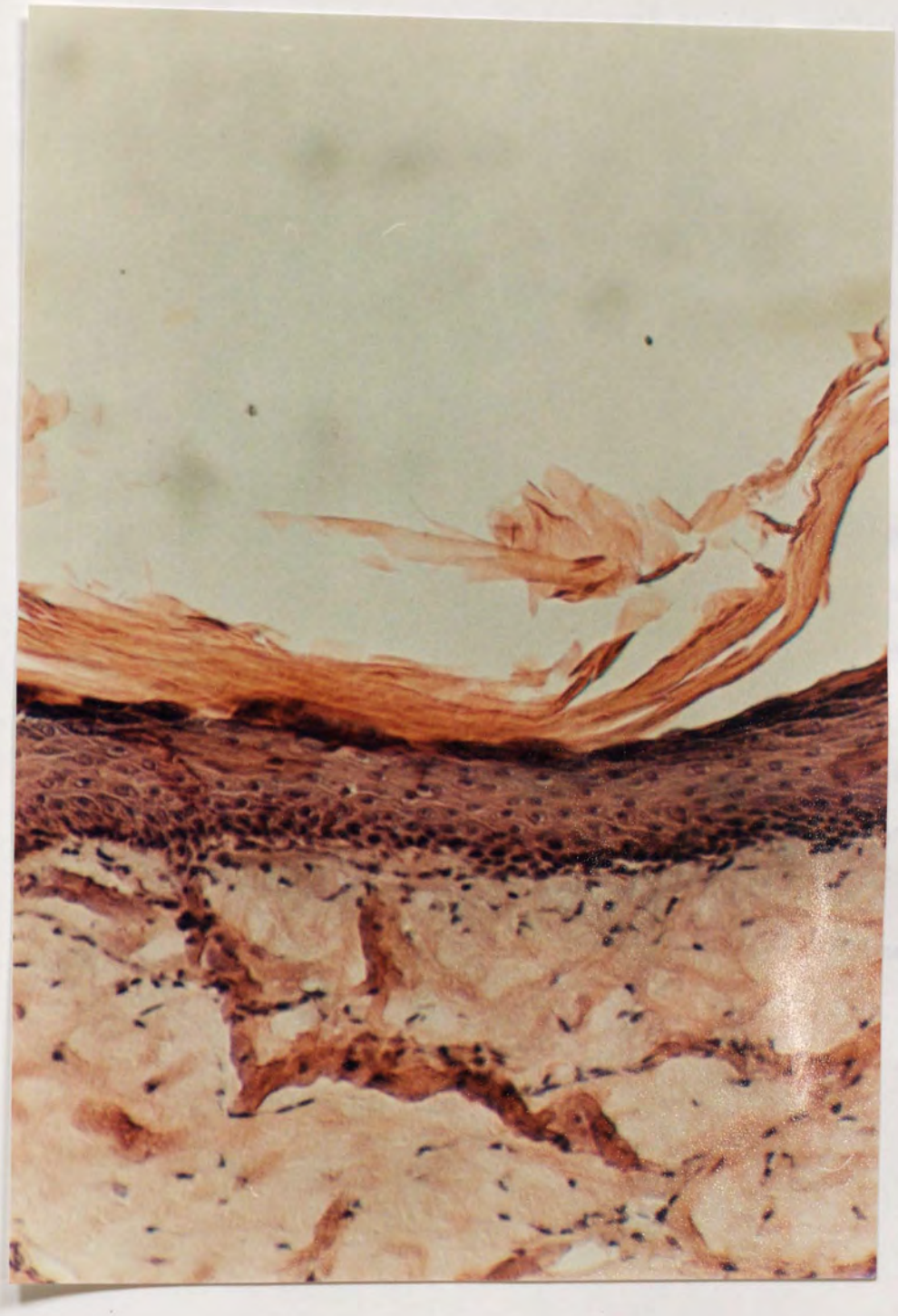


Plate 3. Mouse Tail Treated for 21 Days with 10% WTAF

The granular layer has spread over the whole scale and is about 3 cells thick.

Experiment 3

Time and Concentration Studies

In an attempt to discover an optimum dose and time for the animal screen, varying concentrations of the high boiling WTAF ($180^{o}C$ to $230^{o}C$) were applied to mouse tail in soft, yellow paraffin : wool fat 95 : 5 daily for varying times. The control used in experiment 1 was used.

Observations

Extensive staining of the tail was noticed after only 4 days when the 30% and 40% formulations were applied to mouse tail. After 8 days epidermal peeling was evident in the 40% group, the 30% group showed the same peeling 2 days later. The onset of this peeling was rapid and coincided with granular layer formation. The epidermis thickened appreciably at the same time. To the naked eye the tail skin had a texture similar to that of inflammed human skin when the corrosion was removed.

Despite the ugly appearance of the tail skin, the mice were unaffected by it and did not show signs of irritation, even when approached for subsequent applications.

The 10% and 20% groups exhibited staining but no damage was apparent to the naked eye or microscopically.

The results of this experiment are tabulated on the following page.

Results (Experiment 3)

Time and Fraction	Mouse				
	1	2	3	4	5
10% high boiling WTAF					
5 Day	-	-	-	-	-
10 Day	*	*	*	*	NM
15 Day	*	*	NM	*	*
20 Day	NM	*	**	**	*
20% high boiling WTAF					
5 Day	-	-	-	-	-
10 Day	*	*	*	**	NM
15 Day	*	NM	**	*	**
20 Day	**	**	**	***	**
30% high boiling WTAF					
5 Day	*	-	-	*	-
10 Day	**	***	**	**	*
15 Day	**	**	**	**	**
20 Day	**	***	**	***	**
40% high boiling WTAF					
5 Day	*	*	*	*	*
10 Day	NM	NM	NM	NM	*
15 Day	**	***	**	**	***
20 Day	***	***	***	***	**

Table 6 . The granular layer inducement by varying the time and concentration of high boiling WTAF when applied to mouse tail

Experiment 4

Sodium Carbonate Extracted Fractions

The four fractions from the 5% carbonate wash of high boiling WTAF (section 3B(i)(b)) were applied daily to mouse tail at a 10% concentration in soft, yellow paraffin : wool fat 95 : 5 for 21 days. The control consisted of the vehicle only.

The fraction which was not extracted by the 5% sodium carbonate solution is thought to consist of phenolic structures and was re-named whole tar phenolic fraction (WTPF).

Results

Fraction	Mouse				
	1	2	3	4	5
WTPF	**	*	*	**	**
Pentane extract	-	-	-	-	NM
Chloroform extract	NM	-	-	-	-
Ethyl Acetate extract	NM	-	-	*	-
Control	-	-	-	-	-

Table 7. The granular layer inducement of the fractions from the carbonate wash when applied to mouse tail

Observations

Tail staining was evident in the mice which had been treated with WTPF. The mice treated with the fractions which had been extracted by the 5% sodium carbonate, exhibited no tail staining but showed no granular layer inducement. There was no irritation or corrosion in any of the groups at this concentration.

Experiment 5

Column Eluates

The four fractions obtained from the alumina column (column (e)) were applied daily to mouse tail at a 25% concentration in soft, yellow paraffin : wool fat 95 : 5 for 12 days. This regime was attempted on the basis of the results from experiment 3. The control consisted of vehicle only.

Results

Eluent	Mouse				
	1	2	3	4	5
Diethyl ether	**	*	**	*	*
Absolute Ethanol	*	-	*	-	*
Ethanol/10% Ammonia	-	-	-	*	-
Ethanol/10% Sodium Hydroxide 10% w/v	-	-	-	-	-
Control	-	-	-	-	-

Table 8. The granular layer inducement of the eluates from column (e) when applied to mouse tail

Observations

Bad tail staining occurred on the animals treated by the ethanol/ammonia and the ethanol/alkali fractions. No corrosion or epidermal thickening was apparent at this concentration, but the original regime was reverted to, for reasons given in the discussion .

Experiment 6

Hydrolysed WTPF Derivatives and Non-derivatised Fractions

The hydrolysates and residues from the derivatisations described in section 3B(iii), with the exception of the methyl ethers, were applied at a 25% concentration to mouse tail for 12 days in soft, yellow paraffin : wool fat 95 : 5. The control from experiment 5 was used, as the two experiments were run simultaneously. A second control was run, which consisted of all the likely by-products from the reactions which were used to achieve these separations formulated in the vehicle. The concentration of these by-products was 5% which is an order of magnitude greater than the expected concentrations. These by-products were sodium chloride, sodium acetate, sodium benzoate, sodium tosylate, sodium 2,2-dimethyl-propionate and the sodium salt of diphenyl carbamyl chloride.

Observations

There was no irritation or corrosion of mouse tail in the course of this experiment. Tail staining was universal. Epidermal thickness was unaltered in all fractions.

The induced granular layers were in a state of incomplete development. This may be due to the reduced span of the trial and was one of the factors for changing back to the original regime of 21 days at a 10% concentration.

The results are tabulated overleaf.

Key to Abbreviations in Table 9.

Non- unreacted phenols.

Hyd- phenols obtained from the derivative after its hydrolysis.

2,2 DMP - 2,2-di-methyl-propanoyl.

Results (Experiment 6)

Fraction	Mouse				
	1	2	3	4	5
Non-acetylated WTPF	-	*	-	*	*
Hyd-acetylated WTPF	*	**	**	*	**
Non-benzoylated WTPF	-	-	-	-	-
Hyd-benzoylated WTPF	**	**	**	*	**
Non-tosylated WTPF	*	-	*	*	-
Hyd-tosylated WTPF	*	*	*	**	*
Non-urethane WTPF	*	*	-	-	*
Hyd-urethane WTPF	*	*	-	**	-
Non-2,2-DMP WTPF	*	-	-	-	-
Hyd-2,2-DMP WTPF	*	*	-	*	*
Control	-	-	-	-	-
By-product control	-	-	-	-	-

Table 9. The granular layer inducement by the hydrolysed derivatives and non-derivatised fractions when applied to mouse tail

Experiment 7

Poly-hydroxylated Poly-aromatics

The products of the sulphonation and alkali fusion described in section 3C(i) were applied to mouse tail at a 10% concentration in soft, yellow paraffin : wool fat 95 : 5 daily for 21 days. Each product contains a number of compounds, all of which contain the same carbon skeleton, but with differing numbers of hydroxyl groups in differing positions.

A control consisted of the vehicle only and a by-product control consisted of the vehicle formulated with a 1% collective concentration of sodium chloride, potassium chloride, sodium sulphate, potassium sulphate and ethanol.

Results

Carbon Skeleton	Mouse				
	1	2	3	4	5
2-phenyl-phenol	-	-	-	-	-
4-phenyl-phenol	-	*	-	-	-
Fluorene	-	-	*	*	-
Anthracene	-	-	-	-	-
Phenanthrene	-	-	-	-	-
Control	-	-	*	*	-
By-product control	-	*	-	-	-

Table 10. The granular layer inducement of some synthetic poly-hydroxylated poly-aromatics when applied to mouse tail

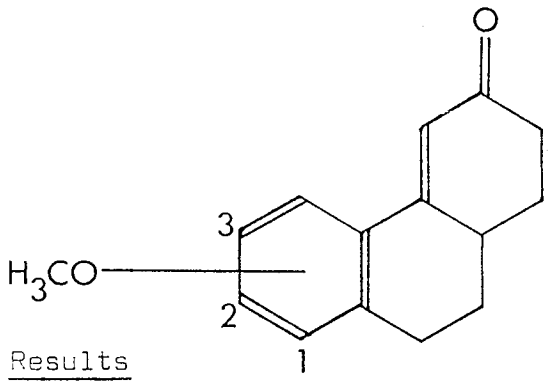
Observations

Although there were no unwanted side-effects, there was no anti-psoriatic activity in these compounds.

Experiments 8 and 9

WTPF Methyl Ethers and Methyl Phenanthrones

Methylated WTPF and three methyl phenanthrones were applied to mouse tail at a 10% concentration in soft, yellow paraffin : wool fat 95 : 5 daily for 21 days. A control was applied to mouse tail which consisted of the vehicle only.



The Phenanthrone Skeleton

Results

Compound or Fraction	Mouse				
	1	2	3	4	5
Methylated WTPF	-	-	-	-	-
1-methoxy-phenanthrone	-	-	-	-	-
2-methoxy-phenanthrone	-	-	-	-	-
3-methoxy-phenanthrone	-	-	-	-	-
Control	-	-	-	-	-

Table 11. The granular layer inducement of methylated WTPF and some methoxy-phenanthrones when applied to mouse tail

Observations

Although there were no unwanted side-effects, there was no anti-psoriatic activity in these compounds.

Experiment 10

Screen of Phenolic Structures

From the evidence collected by mass spectroscopy, a group of 32 compounds with a possible anti-psoriatic action were purchased. These compounds were arranged into 8 groups which are shown below.

Group	Definition	Compounds
A	Dihydroxy Phenols	4-t-butyl catechol
		3, 5-di-t-butyl catechol
		4-n-dodecyl resorcinol
		4, 6-di-t-butyl resorcinol
		tri-methyl hydroquinone
		2, 5-di-t-butyl hydroquinone
B	Tri hydroxy phenols	1, 2, 4-Benzenetriol
		Phloroglucinol dihydrate
		pyrogallol
C	Biphenols	OO'- Biphenol
		PP'- Biphenol
		2-hydroxy-phenyl phenol
		4-hydroxy-phenyl phenol
D	Biphenyl Methanes monohydroxy	O-(Benzyloxy) phenol
		2-hydroxy-diphenyl methane
		4-hydroxy-diphenyl methane
		4-Cumyl phenol
E	Biphenyl methanes dihydroxy	2, 2-bis-(4-hydroxyphenyl) butane
		Bis-(2-hydroxyphenyl) methane
		Bis-(4-hydroxyphenyl) methane
		4, 4 - Isopropylidene diphenol

Group	Definition	Compounds
F	Others	2,2'4,4'-tetra-hydroxy benzophonone
		Nordihydro-guaiaretic acid
		1,4,9,10-tetra-hydroxy anthracene
G	Naphthalene diols	1,3-dihydroxy naphthalene
		1,5- " "
		1,7- " "
		2,3- " "
		2,7- " "
		2,6- " " dihydrate
H	Others (2)	2,4,5,7-tetra-hydroxy naphthalene
		3,4-bis-(p-hydroxy-phenol)3,4-hexanediol

The groups were applied at a 25% collective concentration for 21 days in soft, yellow paraffin : wool fat 95 : 5. The individual members of each group had a proportional percentage.

The control consisted of the vehicle only.

Results

Group	Mouse				
	1	2	3	4	5
A	***	***	****	***	***
B	-	*	*	-	-
C	-	*	-	*	-
D	**	*	*	**	*
E	-	-	*	-	-
F	*	-	-	-	-
G	*	-	*	*	-
H	-	-	-	-	-
Control	*	-	-	-	-

Table 12. The granular layer inducement of some phenols when applied to mouse tail

Observations (Experiment 10)

All of the groups roughened the mouse tail skin. The epidermal thickness was increased in the animals treated with group A. This thickening was mainly due to an increase in cell size.

Tail corrosion occurred in group A and the tail was stained purple when treated with group B.

Experiment 11

Group A. 5% Individually

The six compounds which comprised group A were tested individually at a 5% concentration for 21 days in soft, yellow paraffin : wool fat 95 : 5. The control consisted of the vehicle only.

Results

Compound	Mouse				
	1	2	3	4	5
4,6-di-t-butyl resocinol	*	*	-	*	-
4-n-dodecyl resorcinol	-	*	-	-	-
3,5-di-t-butyl catechol	*	*	*	*	*
4-t-butyl catechol	-	*	-	*	*
Trimethyl hydroquinone	*	-	-	-	-
2,5-di-t-butyl hydroquinone	-	-	-	-	*
Control	-	-	-	-	*

Table 13. The granular layer induction of the compounds comprising group A when applied to mouse tail.

Observations

Although the compounds in this group have a collective anti-psoriatic effect at a 25% concentration, individually at a 5% concentration, they have no effect.

There was no evidence of tail staining, irritancy or corrosion at this concentration.

Experiment 12

Group A. 10% and 25% Collective Concentrations and the Effect of Ethanol

Group A was applied collectively at concentrations of 10% and 25% in soft, yellow paraffin : wool fat 95 : 5 for 21 days.

Ethanol (5%) was added to assist the formulation. It has been pointed out by Yoshikawa (1976) that ethanol will increase cAMP levels and cause a lowering of mitotic rate. The experiment was performed with 5% ethanol in the formulation medium (+), and without (-).

Two controls were run, one consisted of the vehicle only, control (-), and one of the vehicle containing 5% ethanol, control (+).

Results

Formulation	Mouse				
	1	2	3	4	5
Group A 25% (-)	***	****	***	****	
Group A 25% (+)	****	****	***	***	****
Group A 10% (-)	**	**	***	**	
Group A 10% (+)	***	***	***	***	***
Control (-)	-	-	-	-	-
Control (+)	**	-	**	*	-

Table 14. The granular layer inducement of group A and the effects of ethanol on the activity of this group when applied to mouse tail.

Observations

Tail corrosion was readily apparent in the animals treated with the 25% groups, tail staining was apparent in all groups. The corrosion is accelerated by the addition of ethanol. Ethanol caused epidermal thickening but increased efficacy. The vehicle and ethanol will induce granular layer.

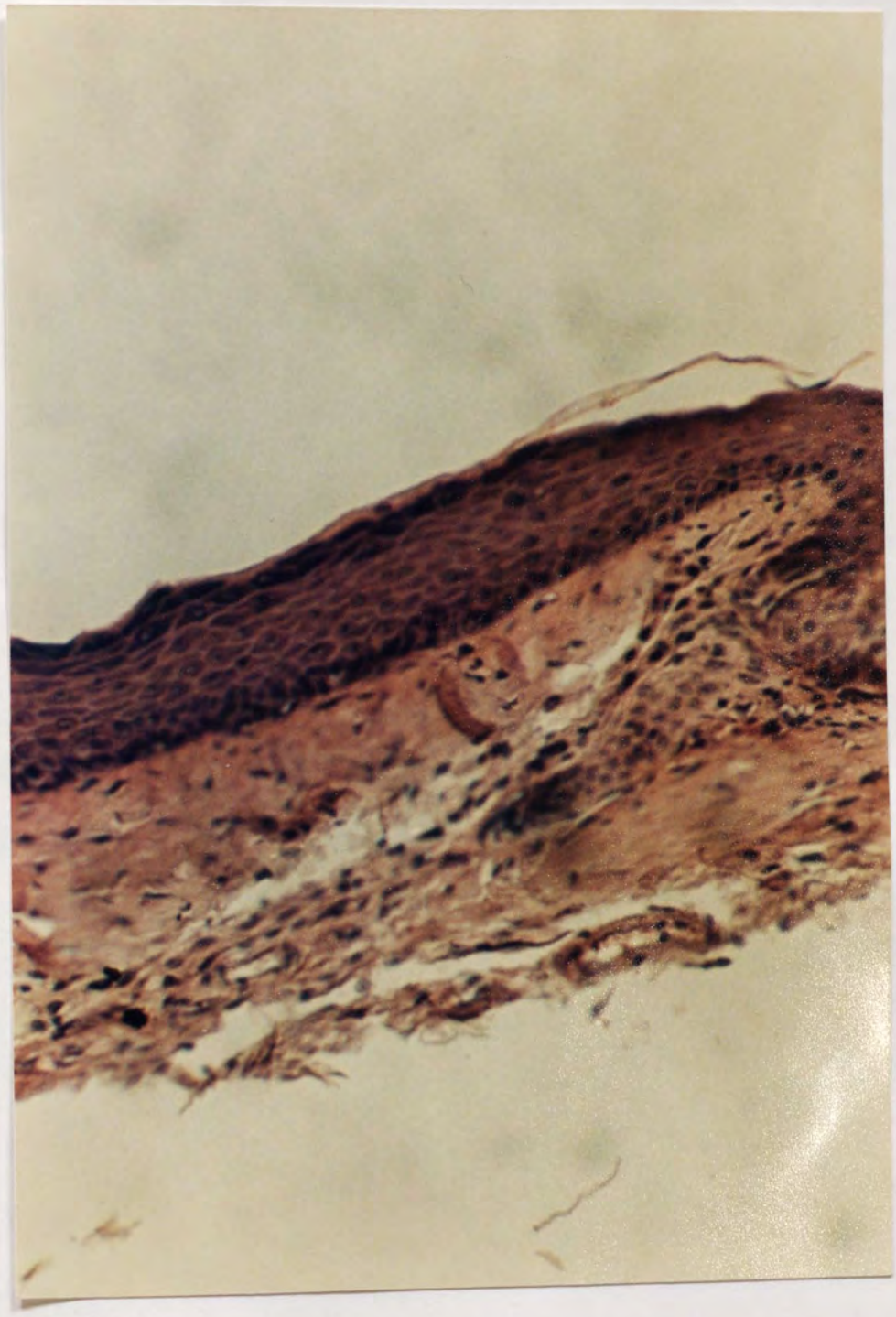


Plate 4. Mouse Tail Treated with 21 Treatments of 10% Group A in the Absence of Ethanol

There is a thick granular layer formed all over the tail scale, the epidermal thickness is not appreciably thicker than normal.

Experiment 13

Individual Constituents of Group A at 10% Concentration and the Effect of Ethanol

The components of group A were individually applied at a 10% concentration in soft, yellow paraffin : wool fat 95 : 5 for 21 days. The experiment was repeated in the presence of 5% ethanol. The control from experiment 12 was used as the two experiments ran simultaneously. A (+) denotes that 5% ethanol was present in the vehicle, a (-) that it was not.

Results

Compound and Formulation	Mouse				
	1	2	3	4	5
(1) 4-n-dodecyl resorcinol (-)	*	-	*	-	-
(2) 4,6-di-t-butyl resorcinol (-)	-	-	-	*	-
(3) 3,5-di-t-butyl catechol (-)	*	*	*	**	-
(4) 4-t-butyl catechol (-)	***	***	**	***	***
(5) Trimethyl hydroquinone (-)	-	-	*	-	-
(6) 2,5-di-t-butyl hydroquinone (-)	-	-	-	-	-
(1) (+)	*	-	*	*	-
(2) (+)	**	*	**	*	*
(3) (+)	***	***	***	**	***
(4) (+)	**	**	**	**	***
(5) (+)	*	*	-	-	-
(6) (+)	-	-	*	-	-

Table 15. The granular layer inducement of the components of group A when applied individually to mouse tail at a 10% concentration and the effects of ethanol.

Observations

No tail corrosion occurred in any animal in this trial, the only tail staining occurred in the animals treated with the hydroquinones. Ethanol had the effect of increasing the epidermal thickness.

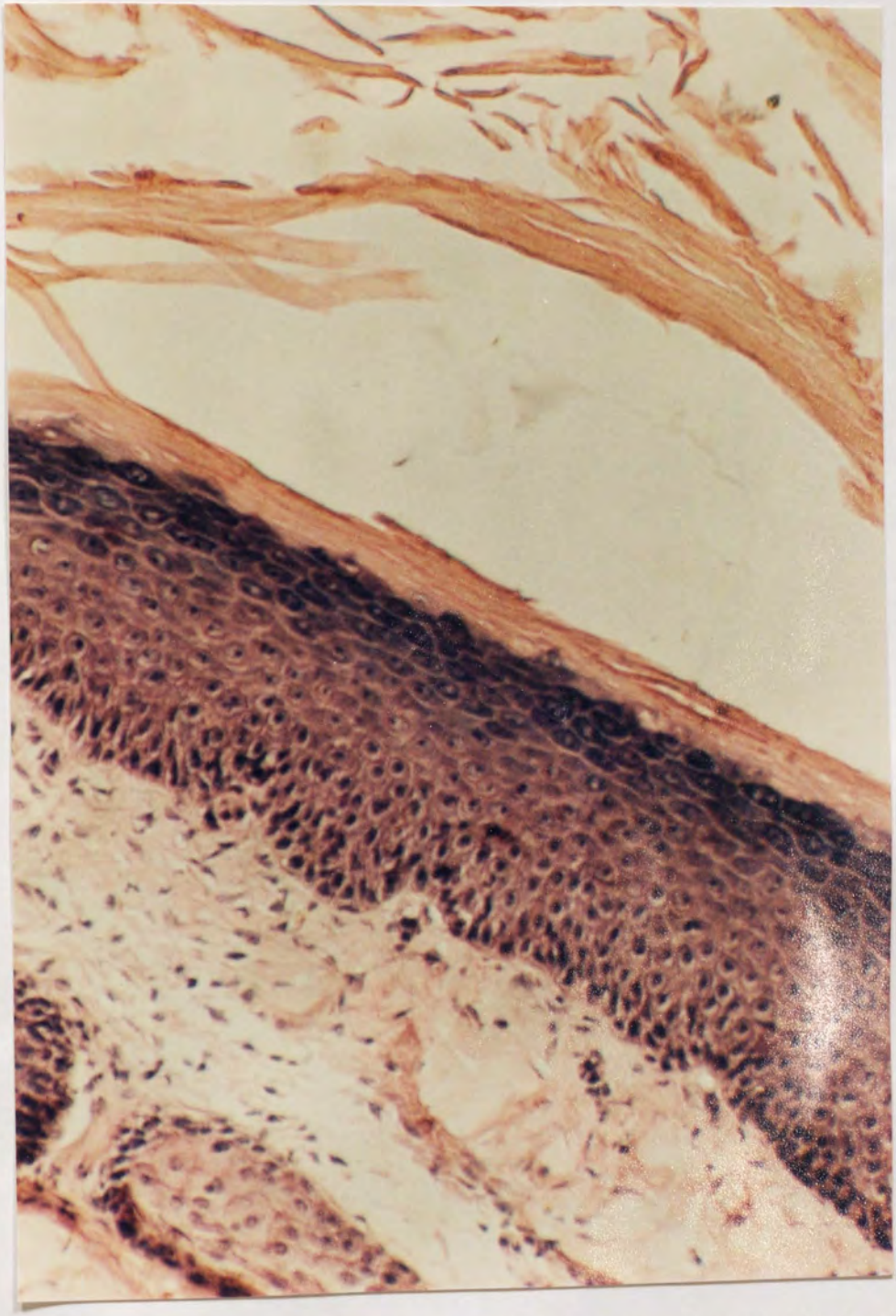


Plate 5. Mouse Tail Treated with 21 Treatments of 10% Group A in the Presence of Ethanol

The ethanol used in conjunction with group A induces a thicker granular layer but there is an increase in epidermal thickness.

Experiment 14

Group A Combinations and the Effect of Ethanol

Whilst the compounds in group A were effective at 10% concentration, they were not at 5% concentration. Group A is effective at both 10% and 25%. In the former, individual components have a concentration of approximately 4%, and in the latter, approximately 1.5%. Combinations of compounds from group A were studied to find if any synergistic effect was taking place.

Combinations of two compounds from group A were tested, each at a 5% concentration by application to mouse tail in the soft, yellow paraffin : wool fat 95 : 5 vehicle. The effect of ethanol was also studied. The controls from experiment 12 were used.

The components are numbered in the same way as experiment 13.

Results

(i) Formulations in the absence of ethanol

Combination	Mouse				
	1	2	3	4	5
(1) & (2)	-	-	-	*	-
(1) & (3)	*	**	*	*	-
(1) & (4)	**	*	**	**	*
(1) & (5)	-	*	*	-	-
(1) & (6)	-	*	-	**	-
(2) & (3)	***	***	***	***	***
(2) & (4)	**	**	*	***	**
(2) & (5)	*	**	*	-	*
(2) & (6)	*	*	-	*	-
(3) & (4)	***	**	**	***	****
(3) & (5)	**	**	**	**	**
(3) & (6)	**	**	***	**	**
(4) & (5)	**	**	*	**	***
(4) & (6)	**	**	**	**	**
(5) & (6)	-	*	*	-	-

Table 16. The granular layer inducement of combinations of the components of group A when applied to mouse tail.

(ii) Formulations in the Presence of Ethanol

Combination	Mouse				
	1	2	3	4	5
(1) & (2)	*	**	*	*	**
(1) & (3)	**	**	*	**	**
(1) & (4)	**	**	**	**	*
(1) & (5)	-	*	-	*	-
(1) & (6)	*	-	*	*	*
(2) & (3)	**	**	**	**	**
(2) & (4)	*	**	**	*	**
(2) & (5)	***	-	*	**	-
(2) & (6)	**	*	**	**	*
(3) & (4)	***	***	***	**	**
(3) & (5)	**	*	**	*	**
(3) & (6)	***	**	**	**	**
(4) & (5)	***	**	***	**	*
(4) & (6)	**	**	*	**	*
(5) & (6)	*	*	-	*	*

Table 17. The granular layer inducement of combinations of the components of group A in the presence of ethanol when applied to mouse tail.

Observations

There was no corrosion or bad staining on the tails of any of the animals used in this experiment.

Experiment 15

Catechols

A collection of 24 catechols and a catechol methyl ether, guiacol, were tested at a 10% concentration in soft, yellow paraffin : wool fat 95 : 5 for 21 days. The control consisted of the vehicle only.

Results

Catechol	Mouse				
	1	2	3	4	5
3,4-dihydroxy benzaldehyde	*	*	**	**	
Tiron	*	*	*	**	*
2,3-dihydroxy benzoic acid	*	-	-	-	-
3,4-dihydroxy phenyl acetic acid	*	*	*	-	-
4-nitro catechol	*	**	*	*	
DL Dopa	*	*	**	**	
Trihydroxy-acetophenone	*	**	**	**	*
3,4-dihydroxy benzoic acid	-	*	*	-	-
2,3-dihydroxy benzaldehyde	*	*	-	*	*
3-isopropyl catechol	***	**	**	***	***
3-methyl catechol	**	**	***	*	**
Guiacol	-	*	*	*	**
3,4-dihydroxy cinnamic acid	*	**	*	*	
3-methoxy catechol	-	*	*	*	*
Catechol	**	*	**	**	**
4-methyl catechol	*	*	**	**	
3-hydroxy tyramine HBr	-	*	-	*	-
6t-butyl-3-methyl catechol	***	***	**	***	**
ethyl-3,4-di-hydroxy benzoate	**	*	*	**	*
4(p-nitro-phenyl-azo) catechol	*	**	**	*	
3,4-di-hydroxy-isopropyl-benzyl alcohol	*	*	*	*	**
3-methyl-5t-octyl catechol	****	****	****	****	****
5-methyl-3t-octyl catechol	****	****	***	****	****
4t-octyl catechol	*	*	*	**	*
Adrenolone HCl	*	*	*		
Control	-	-	-	*	-

Table 18. The granular layer inducement of some catechols when applied to mouse tail.

Observations (Experiment 15)

The most effective compounds gave an increase in epidermal thickness. This increase in thickness is mainly caused by cells swelling. It was not possible to deduce if there was an increase in cell number.

Tail corrosion did not occur with any catechol, but bad staining did occur on the tails of the animals which had been treated with 4-nitro catechol. The staining was bad enough to discolour the complete fur of three of the animals in this group bright yellow. The mouse fur was discoloured by the animals rubbing against each other rather than by systemic absorption: the fur roots were white.

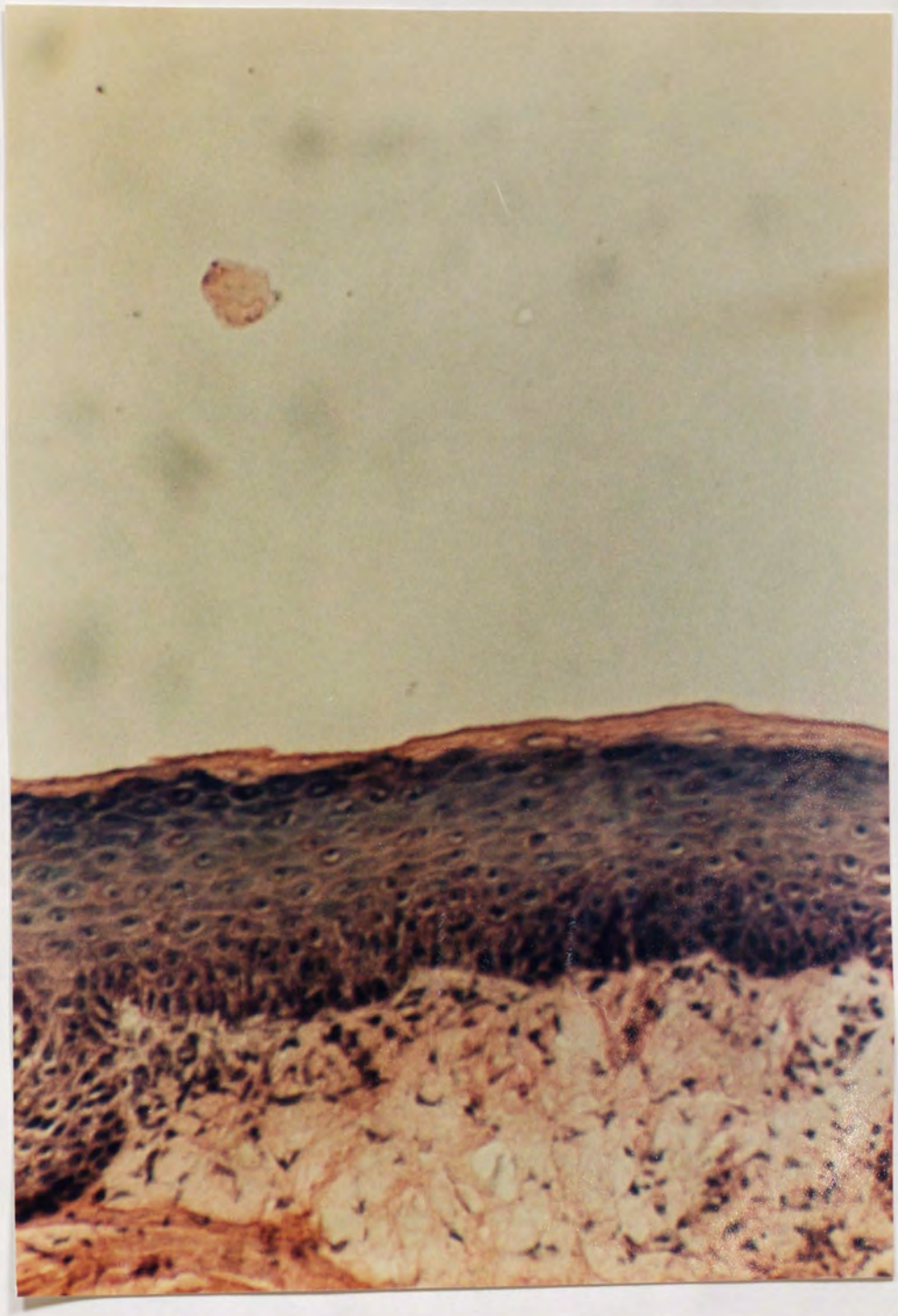


Plate 6. Mouse Tail Treated with 21 Treatments of 10% 3-methyl 5-t-octyl Catechol

There is a very thick induced granular layer, the epidermis has thickened substantially.

Experiment 16

Coal Tar Fractions

The catechol and resorcinol fractions*, described in section 2E, and the "decatecholised" coal tar, described in section 3C(iv), were applied to mouse tail for 21 days at a 10% concentration in soft, yellow paraffin : wool fat 95 : 5. The control consisted of the vehicle only.

Results

Fraction	Mouse				
	1	2	3	4	5
Catechol fraction	****	***	**	***	****
2-Methyl resorcinol fraction	*	-	-	**	-
Homo-catechol fraction	**	**	***	*	**
Resorcinol fraction	**	**	***	***	**
"Decatecholised" coal tar	**	*	**	**	*
Control	-	-	-	-	-

Table 19. The granular layer inducement of dihydroxy benzene rich fractions and "decatecholised" coal tar when applied to mouse tail.

Observations

No tail corrosion or staining occurred in the animals treated with the dihydroxy benzene fractions. Slight staining of the tail occurred in the animals treated with the "decatecholised" coal tar fraction. The epidermal thickness increased in the animals treated with the catechol fraction.

*Kindly donated by Coalite Chemical Products Ltd.

Experiment 17

Acetyl Esters of Catechols

Acetyl esters of the two catechols in group A were synthesised. The two esters were applied to mouse tail at a 10% concentration for 21 days in soft, yellow paraffin : wool fat 95 : 5. The control consisted of the vehicle only.

Results

Ester	Mouse				
	1	2	3	4	5
1,2-diacetyl-3,5-di-t-butyl benzene	*	*	*	*	-
1,2-diacetyl-4-t-butyl benzene	-	-	*	*	-
Control	-	-	-	-	-

Table 20. The granular layer inducement of two catechol acetyl esters when applied to mouse tail.

Observations

There was no evidence of staining or corrosion on the tails of any of the animals used in this experiment.

Experiment 18

3-Methyl-5-t-octyl Catechol Time Study

3-Methyl-5-t-octyl catechol, the most potent granular layer inducer found in this set of experiments, was applied to mouse tail at a 10% concentration for 21 days in soft, yellow paraffin : wool fat 95 : 5. 15 mice were treated, 3 mice were killed at weekly intervals after the completion of the treatment.

The control was the same as used in Experiment 17.

Results

Week after completion of treatment	Mouse				
	1	2	3	4	5
0	****	****	****		
1	****	****	****		
2	***	***	****		
3	**	**	**		
4	**	***	**		
Control	-	-	-	-	-

Table 21. The loss of granular layer in mouse tail on termination of treatment.

Observations

The mouse tail exhibited a thickening of the epidermis which reduced with time. There was no staining or corrosion of the tails of any of the animals treated in this experiment.

Experiment 19

Adrenalin, Noradrenaline and Isoprenaline

The two naturally occurring catecholamines, adrenalin and noradrenaline, and the β-adrenergic agonist, isoprenaline, were applied to mouse tail at a 10% concentration for 21 days in soft, yellow paraffin : wool fat 95 : 5. The control was the control used in experiment 17.

Results

Compound	Mouse				
	1	2	3	4	5
Adrenalin	*	-	-	-	
Noradrenaline	*	*	*	*	
Isoprenaline	-	*	*	-	
Control	-	-	-	-	-

Table 22. The granular layer inducement of adrenalin, noradrenaline and isoprenaline when applied to mouse tail.

Observations

There was no apparent staining or corrosion of mouse tail in the course of this experiment. The mice treated with noradrenaline became hyperactive.

Experiment 20

α-and β-Adrenergic Blockers

Two β-adrenergic blocking drugs, practolol and propranolol, and two α-adrenergic blocking drugs, phentolamine and phenoxybenzamine mesylate were formulated with coal tar or with 3-methyl 5-t-octyl catechol in soft, yellow paraffin : wool fat 95 : 5. The blocking drugs were at a 5% concentration and the anti-psoriatic agents were at a 10% concentration. The formulations were applied daily for 21 days. The control consisted of the vehicle only.

Results

Formulation	Mouse			
	1	2	3	4
Coal tar and:				
Practolol	*	*	**	*
Propranolol	*	*	*	*
Phentolamine	**	*	*	**
Phenoxybenzamine mesylate	**	**	*	**
3-methyl-5-t-octyl catechol and:				
Practolol	*	*	*	*
Propranolol	*	*	*	*
Phentolamine	*	*	**	*
Phenoxybenzamine mesylate	*	**	**	*
Control	-	-	-	-

Table 23. The effect of α-and β-adrenergic blocking drugs on the efficiency of the inducement of a granular layer in mouse tail by coal tar and 3-methyl-5-t-octyl catechol.

Observations

There were no side-effects on the tails of the animals in this experiment.

Experiment 21

Group A Catechols

Because all of the most active catechols have an alkyl group in position 3, except 4-t-butyl catechol. This catechol was re-tested along with the other catechol in group A, 3,5-di-t-butyl catechol, in the same way as before.

Results

Compound	Mouse			
	1	2	3	4
3,5-di-t-butyl catechol	**	**	**	*
4-t-butyl catechol	***	***	***	***

Table 24. The granular layer inducement of the catechols from group A when applied to mouse tail.

Observations

There were no adverse effects to the animals which took part in this experiment.

4. Criticisms, Conclusions and Hypotheses

A. Criticisms of Experiments

(i) The Mouse Tail Test

The use of an animal model to simulate a human pathological condition has many disadvantages. The results which are obtained from such a model can only be interpreted as a guideline to the effect that the drug used will have on the human condition.

The tail skin of a mouse may be regarded as normal for the mouse, for a patient genetically predisposed to psoriasis what can be called the normal skin? Is parakeratin more normal than orthokeratin? If the parakeratotic mouse tail skin is more different to orthokeratotic skin than the psoriatic plaque, the mouse tail test may be regarded as more specific. It will take a more potent drug to effect a change in keratinisation in mouse tail than in a psoriatic lesion.

It has been shown (Jarrett and Spearman 1964) that there are many similarities between the keratinisation of mouse tail and keratinisation of the human psoriatic plaque, they also point out the similarities in structure and the similarities in the enzyme systems operating.

Mouse tail is obviously not an ideal model of the psoriatic condition, there are no dermal abnormalities and no inflammatory reactions in the mouse tail, which are very much apparent in the psoriatic condition.

Because of the difficulties, both moral and logistic, of testing potential anti-psoriatic drugs on human beings, either by direct testing on the lesions or by inhibiting the Köbner response, the mouse tail is probably the most realistic and useful screen available for the testing of possible anti-psoriatic agents.

(ii) The Vehicle

Only one vehicle was used in this work, this was a greasy, occlusive, hydrophobic ointment consisting of 5% wool fat in soft, yellow paraffin. Soft paraffin is known to inhibit the Köbner response (Comaish and Greener 1976), which may be due to one or more of several reasons:

(a) Epidermal Hydration

This is one of the effects of polythene occlusion (Anderson et al. 1973) and decreases the mitotic rate. It is reasonable to assume that soft paraffin would have the same effect. Tree and Marks (1974) showed that the mitotic rate is decreased by many ointment bases.

(b) Lack of Oxygen

A layer of paraffin might prevent ready access of molecular oxygen to a rapidly metabolising tissue (Hammar and Hellerstrom 1958). This assumes a requirement for, and an ability to use, atmospheric oxygen by epidermis.

(c) Retention of Mitotic Inhibitors

The sealing effect of soft paraffin may prevent the loss of chalone or other mitotic inhibitors. The absorption of these substances back into the epidermis would be increased by the epidermal hydration observed in (a) above.

(d) Specific Chemical Effect

There may be a direct effect of the soft paraffin on some psoriatic process.

It would have been advantageous to use many different vehicles in order to elucidate any vehicle enhancement and to find the optimum vehicle for penetration. The formation of a granular layer

as the first change in parakeratotic skin when treated by an anti-psoriatic agent, suggests that the primary effect is achieved by minimum penetration.

The object of this work was to find one or more anti-psoriatic agents which are present in coal tar, and the best way of screening potential anti-psoriatic agents was to choose a vehicle and use it for the screen of all of these potential agents. In this way it is possible to directly compare the effects of these compounds to each other.

(iii) Interpretation of the Animal Tests

The estimation of the activity of compounds on the mouse tail tests is at best crude. The granular layer was used as a marker of activity for reasons which have been given in section 2A.

The use of epidermal thickness measurements was not considered because of the difficulty of distinguishing between a hypertrophic and a hyperplastic response, the variation of epidermal thickness between different mice and the variation in epidermal thickness in any given mouse. The sectioning, embedding and the dehydration and rehydration of the tissue will cause deviations from the actual epidermal thickness by compaction, cutting at an angle and performing processes on non-viable tissue respectively.

Direct observation of the state of the newly formed granular layer was considered to be the best method for the estimation of the activity present in the compound or fraction under test. The slides were coded and randomised prior to the estimation of anti-psoriatic activity to prevent any pre-conceived ideas clouding the judgement of the observer. Direct comparisons of some fractions were made for two reasons:

(a) To compare the difference between two fractions thus finding the most effective should they have a similar "score", e.g. to find if there was a difference between high boiling WTPF and the "decatecholised" coal tar.

(b) to ensure a uniform assessment throughout the period of this work.

(iv) Oxidation of Coal Tar Samples

It was noted by Obermayer and Becker (1935) that coal tar distillates darken on exposure to air; the use of any solvent has a similar effect. This darkening is almost certainly due to an oxidation of some of the coal tar constituents. It was noted that the dihydroxy benzene rich fractions also darkened over a period of a month.

This oxidation is important because the compounds which are thought to give coal tar its beneficial effects, the catechols, are very easily oxidised.

All attempts to stop this darkening by refrigeration and the use of air-tight containers did not have any effect. The samples used in the mouse tail screens were contaminated by this darker coal tar and still gave beneficial results, as did the dihydroxy benzene rich fractions. One explanation for this is that a small amount of oxidised material may cause a large colour change suggesting a greater amount than the actual amount altered.

(v) Fractionation of Coal Tar

Coal tar is not an easy medium to separate on a reproducible basis. The large numbers of compounds, many of them almost identical, make binding of compounds, co-distillation and co-solubility important factors in any separation. The greatest activity was always in the

one fraction so although the fractionation may not be always completely reproducible, the active agent(s) were.

B. Suggested Modes of Action of Active Catechols

Any suggested mode of action of the catechols, which have been found to be active on mouse tail, must be an attempt to rationalise the work which has been reported in the literature, because no experiments have been performed to ascribe specific actions.

There are two ways in which these active catechols may be expected to influence the psoriatic skin because of similarity of structure to molecules actively engaged in controlling keratinisation and the mitotic rate.

(i) The Adrenalin Theory

Psoriasis may be broadly described as an increase in the mitotic rate of the basal cells, accompanied by a loss of granular layer, which results in the formation of an excess of epidermal cells and abnormal keratinisation.

It has been suggested that vascular changes are the initial lesion in psoriasis (Telner and Fekete 1961). There is an increase of catechol o-methyl transferase (COMT), which causes vasodilation by metabolism of vasoconstrictor catecholamines (Bamshad et al. 1970); this effect may be reversed by adrenalin (Yamazaki 1963).

Adrenalin may act in a control mechanism in mitosis of the cells of the basal layer (Bullough 1955). The actual mechanism is a subject of controversy and may be mediated by a chalone (Bullough and Laurence 1964, Iverson et al. 1965, Marrs and Voorhees 1971), cAMP levels (Voorhees and Duell 1971) abnormal cAMP/cGMP ratios (Goldberg et al. 1976) or more probably, a contribution is made by all three (Voorhees et al. 1975); the three mediators are certainly interlinked.

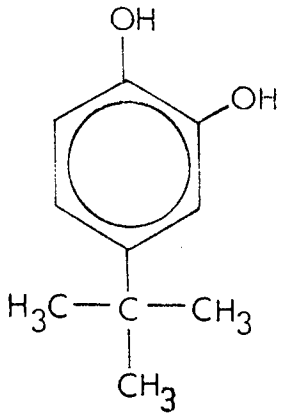
If an excess of COMT is present this would metabolise adrenalin to metanepherine (Axelrod et al. 1958, Axelrod and Tomchick 1958) at a greater rate than normal, causing low adrenalin levels and a subsequent increase in mitotic rate.

As psoriasis is often brought about by stress (Seville 1977, 1978) excess adrenalin levels in stress would induce excess COMT levels.

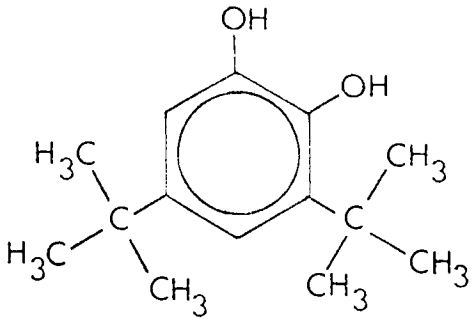
Certain catechols will induce a granular layer in mouse tail; it has yet to be determined if these catechols cause a slowing of the mitotic rate, but it is reasonable to assume that a possible mode of action of these catechols is to competitively inhibit COMT, allowing adrenalin to participate in its actions with regard to the control of the mitotic rate, before it is detoxified.

The question remains, why is the level of COMT so high in psoriatic skin? It is possible that the primary genetic lesion in psoriasis is the lack of control of COMT. If high levels of this enzyme are induced, possibly by an increase of adrenalin in stress, and they are not "switched off" when the levels of adrenalin fall, because of a fault in the gene control, the levels of COMT would remain high. These high levels of COMT are observed in psoriasis and may be the cause of the increased mitotic rate.

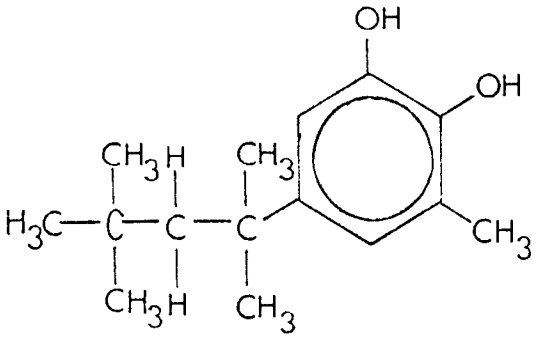
This lack of control of mitosis could result because of low adrenalin levels, causing a low level of adrenalin/chalone complex, which would agree with Bulloughs work. The low levels of this complex would not have the concentration to adequately stimulate adenylate cyclase, which would result in low levels of cAMP, this is the basis of the work of Voorhees and co-workers. The low levels of cAMP would result in the abnormal cAMP/cGMP ratio, which is the basis of the Yin-Yang Hypothesis (Goldberg et al. 1976).



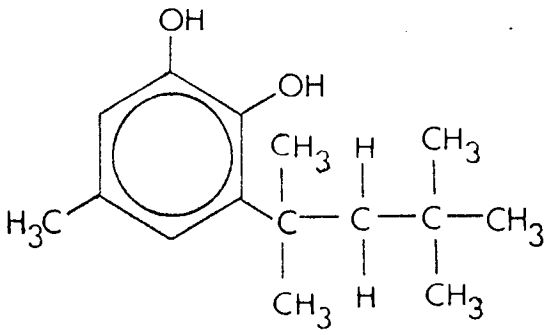
4-t-Butyl Catechol



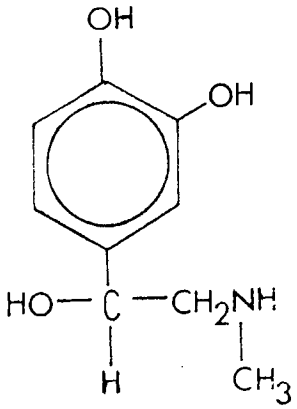
3,5 Di-t-Butyl Catechol



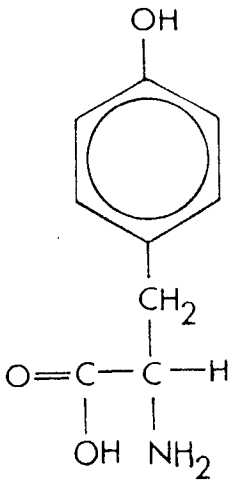
3-Methyl 5-t-Octyl Catechol



5-Methyl 3-t-Octyl Catechol



Adrenalin



Tyrosine

FIG. 16. The Active Catechols and their Possible Biological Analogues.

These theories are interlinked, mainly by the workers themselves. It is hoped that this hypothesis is another link in the chain.

This hypothesis suggests a reason why the mitotic rate is high in psoriatic skin, it does not necessarily follow that a high mitotic rate is the cause of parakeratinisation. The following theory which was suggested by Wrench (1973) attempts to explain how high boiling tar acids, including the catechols, may alter the type of keratin produced by the skin.

(ii) The Dendritic Cell - Tyrosinase Theory

Riley (1966a) cites evidence for the suggestion that the Melanocyte/Langerhans cell transformation (section 1B iv) takes place by the inhibition of tyrosinase, thus terminating the melanocyte phase of the cell. This would mean that there is relatively more ATPase activity in the dendritic cells. Reid and Jarret (1967) have suggested the possibility of dendritic cells having altered levels of ATPase and DOPA +ve activity, depending on conditions.

The mouse dendritic cells do not possess ATPase activity, and are not present in the scale regions, occurring only in the follicular regions. Riley (1966) correlated the occurrence of orthokeratin with the presence of underlying high level dendritic cells.

It has been found that some germicides which contained high boiling tar acids caused de-pigmentation in human skin (McGuire and Hendee 1971). These workers found that the alkyl phenols, e.g. p-tertiary butyl phenol, inhibited tyrosinase activity. They suggested that this was the reason for the observed de-pigmentation. The work compared the phenolic structures with the natural substrates

for tyrosinase, tyrosine and DOPA, and suggested that there had been competitive inhibition of tyrosinase. 4-t-butyl catechol also causes de-pigmentation (Mansur et al. 1978) and 4-isopropyl catechol has been used to treat hypermelanosis (Bleehen 1976). The structure of these catechols is similar to that of the natural substrates for tyrosinase, and the possibility that they are competitively inhibiting tyrosinase is likely.

It is possible that alkyl catechols and alkyl phenols will inhibit tyrosinase in the dendritic cells leading to an increase in their activity which would result in an increase in the degree of ortho-keratinisation.

C. Future Work Required

Future work must be split into three distinctly different directions.

(i) An attempt must be made to elucidate the mode of action of 3-methyl 5-t-octyl and 3-t-octyl 5-methyl catechols. It is suggested that the two modes of action forwarded in the last section be investigated. Structurally, the two compounds resemble the two sets of biological molecules outlined, which play a part in the train of events leading to the formation of normal skin.

It is suggested that at the present time it would be futile to conduct a large scale screen of other catechol structures to find a catechol with optimum activity. This is a process which may be conducted in the future. It is unlikely that the catechol with the highest activity has been found by a screen of 24 randomly chosen catechols, but at present more useful work can be done by finding the mode of action of these compounds to enable a better structured search, based on a structure-activity relationship in the future.

(ii) In the course of this work, all of the fractions and compounds which have been tested have been done in the soft, yellow paraffin : wool fat base. Again it is unlikely that the base with the optimum penetration characteristics has been stumbled upon by chance. The rationale behind using only one base is that the fractions or compounds could be compared with each other, no attempt has been made to find the base with the characteristics required.

It is suggested that a study is undertaken to find the optimum base, using the two catechols which have been shown to have a high level of anti-psoriatic activity.

(iii) It is well known that phenolic structures are toxic. Martindale (1977) states that severe and fatal poisoning can occur from percutaneous absorption of phenols, especially if the skin is damaged. Ingestion of 1 gram of phenol has proven fatal. Joseph Lister in 1864 pioneered the use of antiseptics in surgery. He used phenol as the antiseptic. In present times phenolic structures are widely used as disinfectants.

It is suggested that the toxicity of catechols are investigated in order to assist in the development of a catechol with optimum activity and minimum side-effects.

D. Conclusions

All acidic tar formulations had an effect on mouse tail skin. The first effect, when the tar was applied at a 10% concentration for 21 days, was an induction of granular layers. At concentrations higher than 10% the coal tar formulation caused corrosion and peeling of the mouse tail. Although this corrosion was unsightly, the mice did not appear to be suffering from it and did not try to evade further treatments. The only fractions which were applied at a concentration high enough to cause corrosion or peeling, were high boiling WTAF in the time and concentration study and group A when applied at a 25% concentration. Almost all coal tar fractions stained the mouse tail skin to some degree, many of the individual compounds which were formulated did so also.

The anti-psoriatic activity of coal tar; if the mouse tail test is assumed to be valid, resides in the highest boiling acidic fraction of whole tar, this fraction induces the thickest granular layer but unfortunately exhibits the worst staining and corrosive side effects.

The neutral and basic fractions of coal tar did not induce a granular layer. They were not tested at a high enough concentration to produce any epidermal damage, but staining and scaliness of the mouse tail skin suggested that they were not as corrosive as the acidic fraction. Because of the lack of activity these fractions were not investigated further.

XXL, a commercially extracted coal tar fraction which was composed of the acidic fraction of coal tar, was compared to the acidic fraction of whole tar, WTAF. The WTAF was superior in every way, it was more potent, easier to work with and did not stain the mouse tail skin as badly. The use of XXL was discontinued.

A time and concentration trial using WTAF showed that the optimum regime was a 10% formulation applied daily for 21 days. If the regime was shortened the granular layer development appeared to be incomplete, if the concentration was raised the epidermis was damaged. This regime was used in all of the experiments except for experiments 5 and 6.

The WTAF was further separated from evidence which had accumulated from spectroscopic data. The active principle was shown to be phenolic, not highly polar and not sterically hindered. Consequently, the active principle was not extracted by 5% sodium carbonate solution, eluted off a column at low solvent polarities and formed esters readily. Distillation under a nitrogen atmosphere at reduced pressure showed that this active principle was in a boiling range of 180^{o}C to 230^{o}C at 2 torr. This corresponds to a boiling range of 300^{o}C to 360^{o}C at atmospheric pressure.

Methylation of these active phenols resulted in a loss of activity, suggesting that a hydroxyl function was necessary for activity.

Because of the paucity of information of the constituents of coal tar, the difficulty of working with this mixture and the low concentrations of individual coal tar constituents, it was decided to synthesise or purchase likely coal tar constituents and screen them on the mouse tail test.

Poly-hydroxylated poly-aromatics were prepared from 2 and 4 phenyl phenol, anthracene, phenanthrene and fluorene. The hydroxyl functions were randomly situated by sulphonation and alkali fusion. The individual compounds were not separated, but the compounds were applied to mouse tail in a group derived from the parent molecule. All of these compounds were inactive.

Mass spectral data showed that the molecular weight of the phenolic coal tar constituents was low; the highest m/e ratio was in the range of 170 to 180. Theoretical constituents, based on the data available, were purchased and screened in groups of similar structures.

Anti-psoriatic activity was present in only one of these groups; the dihydroxy benzenes. These compounds were tested individually, and the most potent granular layer inducing agents, and thus the best potential anti-psoriatic agents, were the two catechols, 4-t-butyl and 3,5-di-t-butyl catechols, present in this group.

These catechols exhibited a synergistic effect when they were mixed with other dihydroxy benzenes, this may explain how very low concentrations of an active constituent of coal tar can have a pronounced effect.

All of the other compounds which were tested in this screen were either totally or relatively inactive, even though many of them had similarities in structure with either catechol or dithranol.

Ethanol was shown to assist active compounds in producing a granular layer in mouse tail, the enhancement was very good. Unfortunately ethanol thickens the epidermis appreciably and its use was discontinued.

A large variety of catechols were purchased and screened on mouse tail with a wide range of results. The most potent granular layer inducers were catechols which had an alkyl side chain at position 3, the bulkier the side chain, the more effective the compound was at inducing a granular layer.

The most potent potential anti-psoriatic agents were 3-methyl 5-t-octyl and 5-methyl 3-t-octyl catechols. The structure of these two compounds is such that they are similar in structure to the

catecholamines, a group of compounds of which one, adrenalin, is implicated in mitotic control.

In order to show that catechols are the active principle in coal tar, a sample of coal tar had the catechols removed from it by oxidation. The resulting "decatecholised" coal tar was tested on mouse tail and was shown to be less active in inducing a granular layer than the parent coal tar. Commercial dihydroxy benzene rich fractions of coal tar were shown to have a marked granular layer inducing ability, the most active of these fractions were the fractions with the highest percentage of 3-alkyl catechols. The evidence from these fractions, and from the "decatecholised" coal tar, suggest that a large proportion of the anti-psoriatic activity in coal tar is contained in the catechol fraction.

Acetyl esters of the active catechols were synthesised to try to find an active compound without the difficulties of phenol toxicity. These compounds were inactive.

It was demonstrated in mouse tail, that if a regime was terminated the induced granular layer was eroded over a period of several weeks and not replaced. It is not possible to extrapolate this result to gain any insight to the effects of withdrawing treatment in the condition of psoriasis.

Adrenalin is implicated in the control of mitosis, as the levels of adrenalin in psoriatic tissue are low because of the elevated level of COMT, it was decided to study the effect of exogenous catecholamines. Two catecholamines, adrenalin and noradrenaline as well as the β-adrenergic agonist, isoprenaline, were screened on mouse tail. None of these compounds induced a granular layer. It is known that exogenous adrenalin will have effects on cAMP levels and this lack of activity was unexpected, the problem of this unexpected lack of

activity may be explained by inadequate penetration, breakdown of adrenalin, a short-lived action or any unspecified cause.

It is now accepted that a β-adrenergic response is implicated in psoriasis, it has been postulated that the β-adrenergic receptor site in skin is adenylate cyclase (Sutherland and Robinson 1966). This enzyme is involved in mitotic control. Two α-and two β-adrenergic blocking drugs were applied to mouse tail along with either an active catechol or coal tar. Both the α-and the β-adrenergic blocking drugs caused a drop in efficiency of both of these active formulations. The β-adrenergic blocking drug should cause this drop in activity if a β-adrenergic response is implicated in psoriasis, the action of the α-adrenergic blocking drugs is something of an enigma.

The two catechols selected as those which have the most potent granular layer inducing effect, were used as substrates for the Ames Salmonella Mutagenicity Test, both 3-methyl-5-t-octyl and 5-methyl-3-t-octyl catechols gave a negative mutagenic response; both compounds were shown to be toxic. This toxicity is almost certainly due to the antiseptic properties of phenolic structures.

Two suggested modes of action of these catechols have been forwarded, both theories use the relationship between these molecules and naturally occurring compounds in the skin and suggest that the catechols exert their activity by competitively inhibiting enzymes present in the skin. The two enzymes in question being tyrosinase and catechol o-methyl transferase.

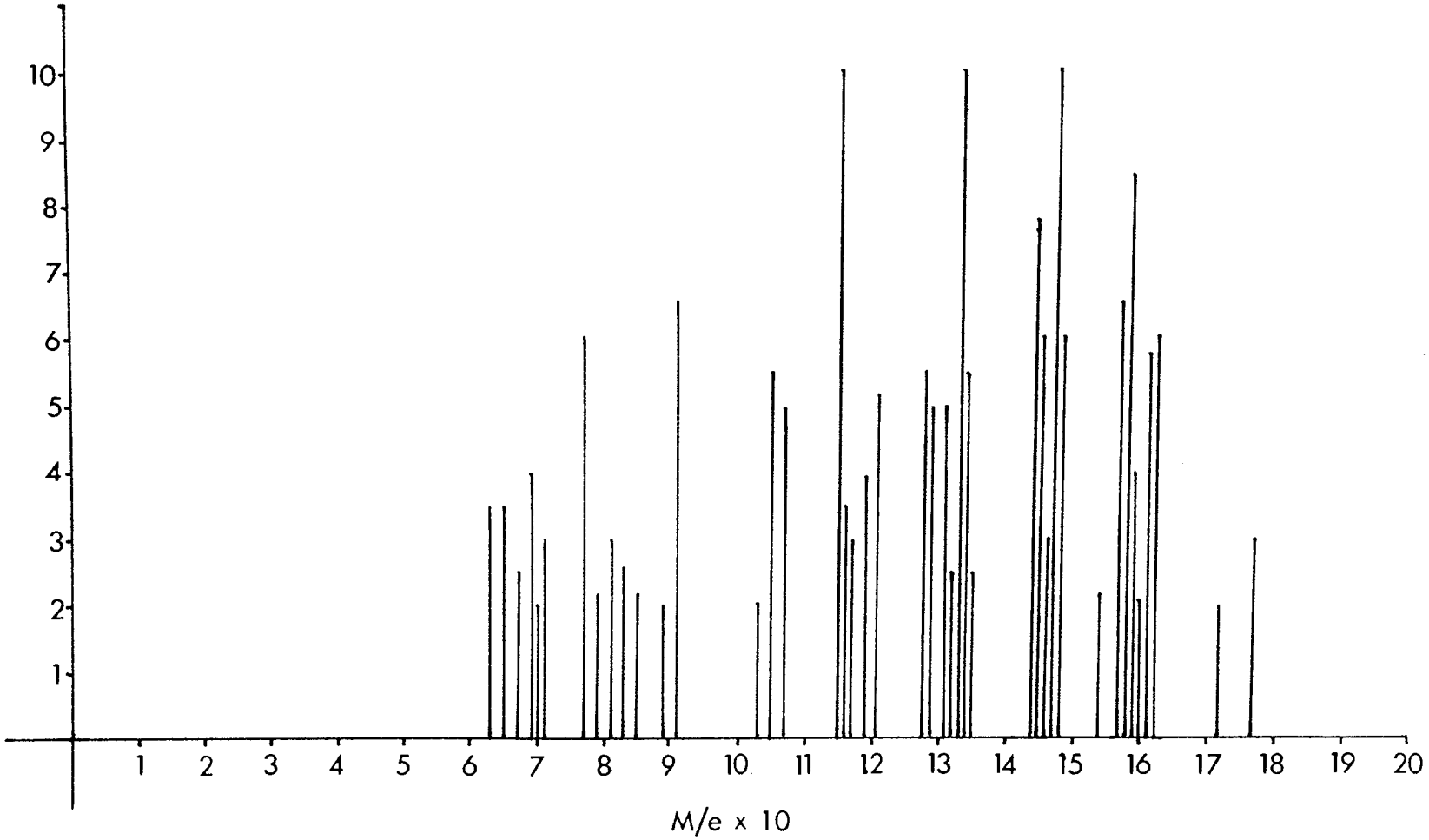
The work has been concerned with isolating a compound which has potential anti-psoriatic activity and is cosmetically acceptable. To this end the work has been successful. There has been no experimental

attempt to rationalise the biochemical effects that the active catechols have on either the mouse tail skin, or on human psoriatic skin. It is possible that the inducement of a granular layer in mouse tail skin is a non-specific effect caused by irritation or it is possible that the active catechols are correcting a defect in keratinisation either directly or indirectly. It is hoped that the answer to this problem can be elucidated.

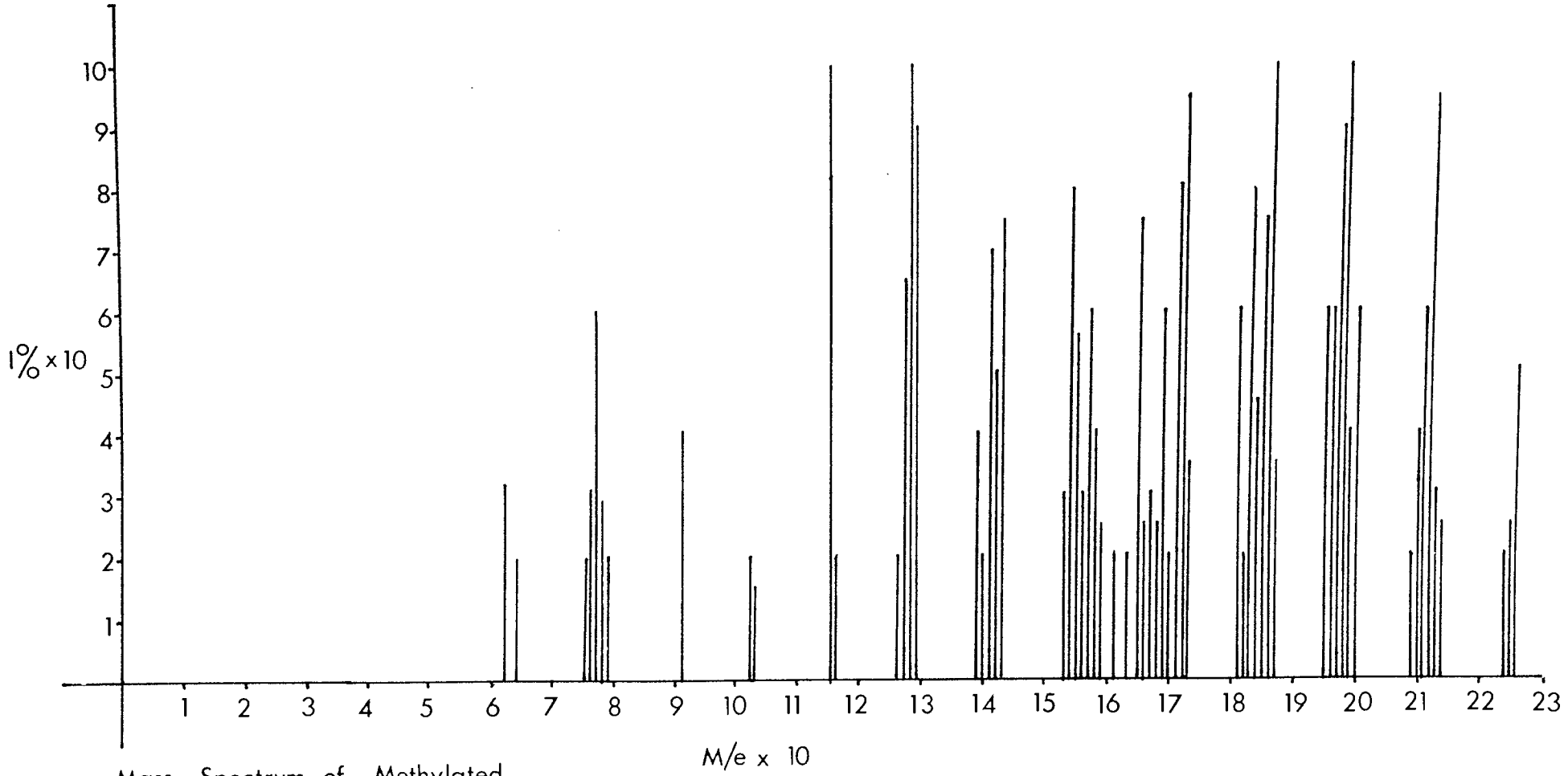
The use of coal tar as a starting material for the isolation of an active compound is extremely difficult as many workers have discovered. The coal tar is not cosmetically acceptable and has a wide range of constituents, of which many have never been isolated or tested for safety.

The two catechols, 3-methyl-5-t-octyl and 5-methyl-3-t-octyl catechols, have been shown to possess potential anti-psoriatic activity, are cosmetically acceptable but have yet not been tested for safety. It is hoped that these two compounds will prove to be safe and be useful in alleviating, if not curing, the section of the population which is predisposed to psoriasis.

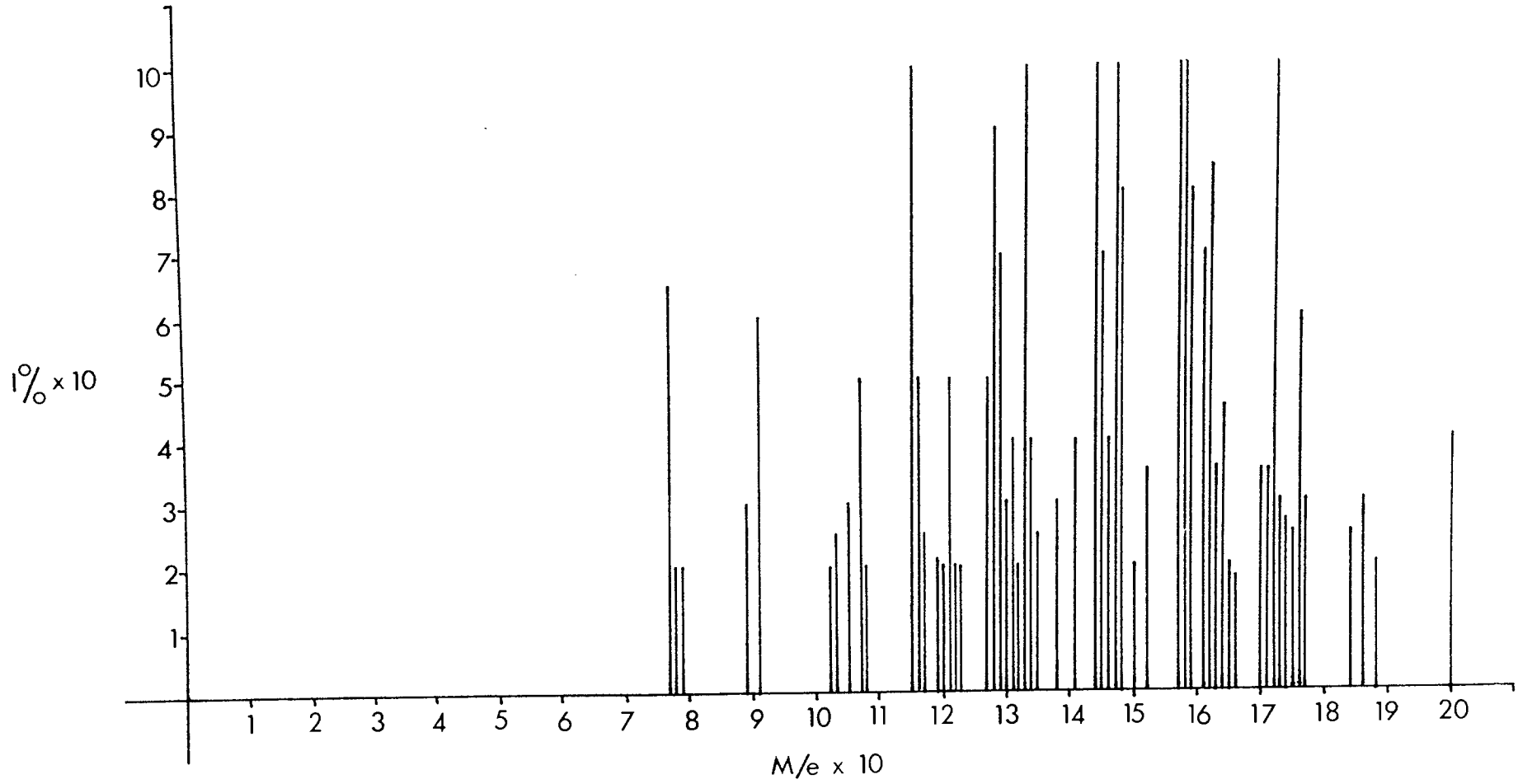
APPENDIX.



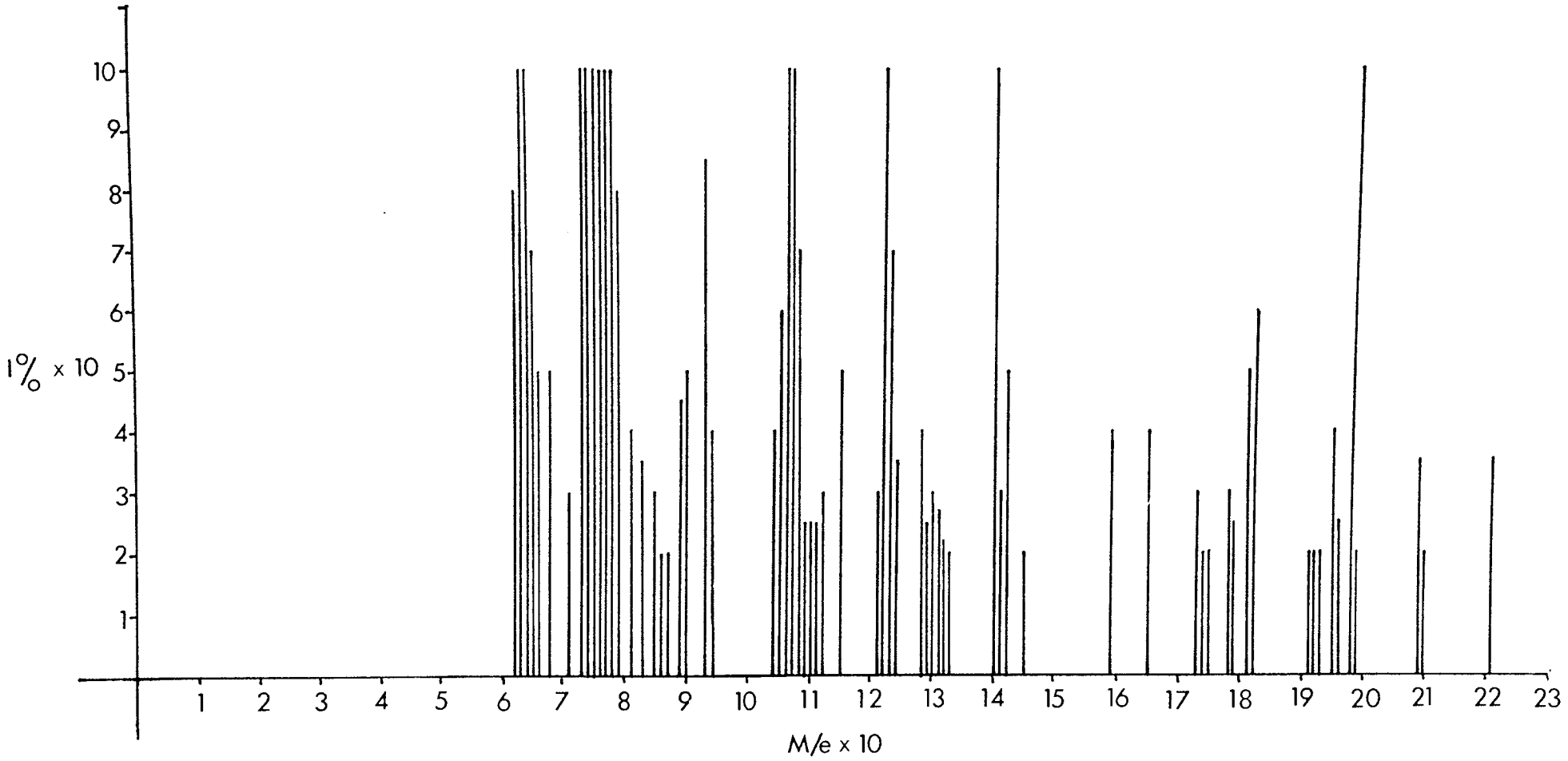
Mass Spectrum of WTPF B.Pt. 180°c – 230°c



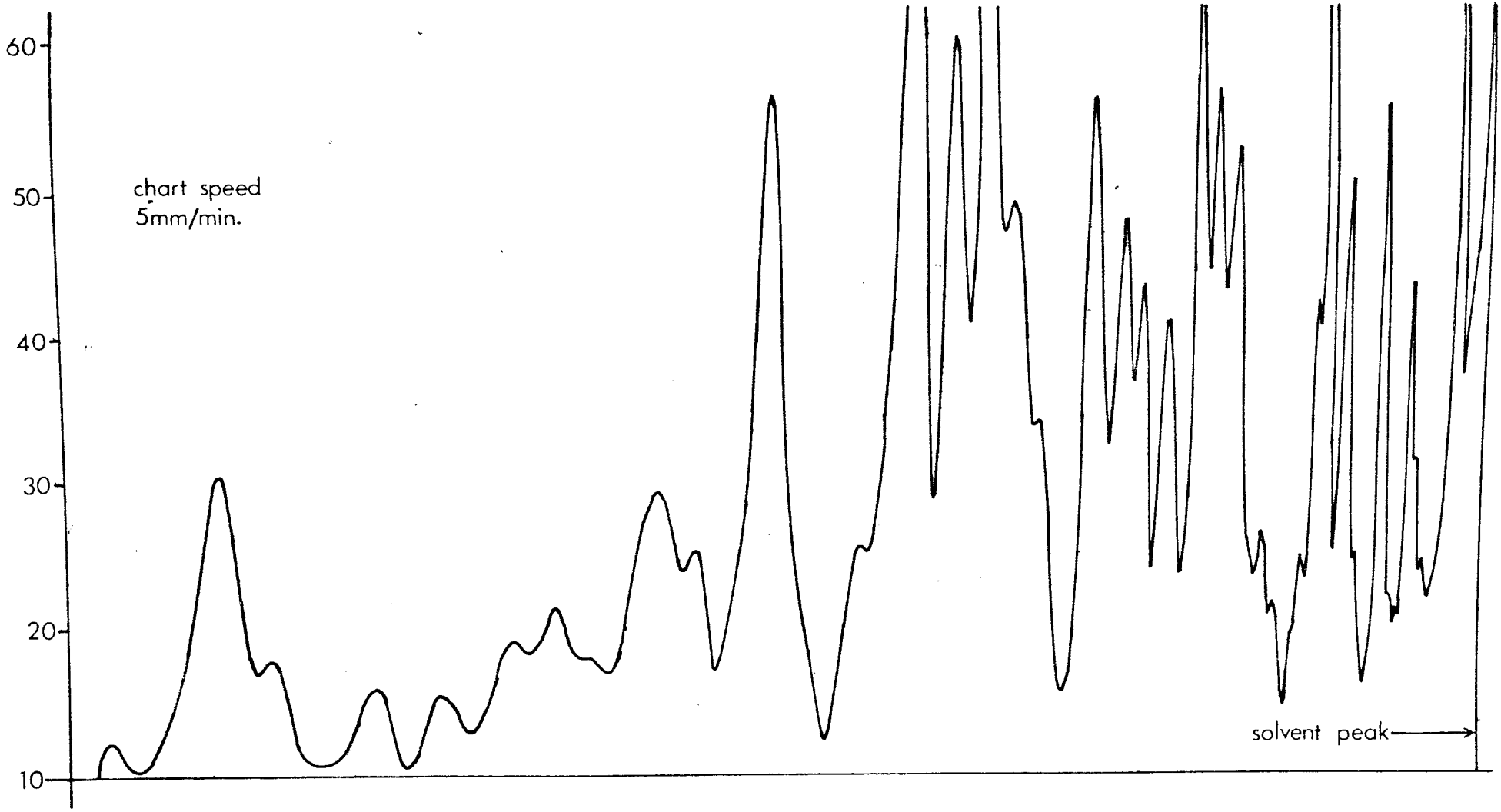
Mass Spectrum of Methylated
WTPF B.Pt 180°c-230°c



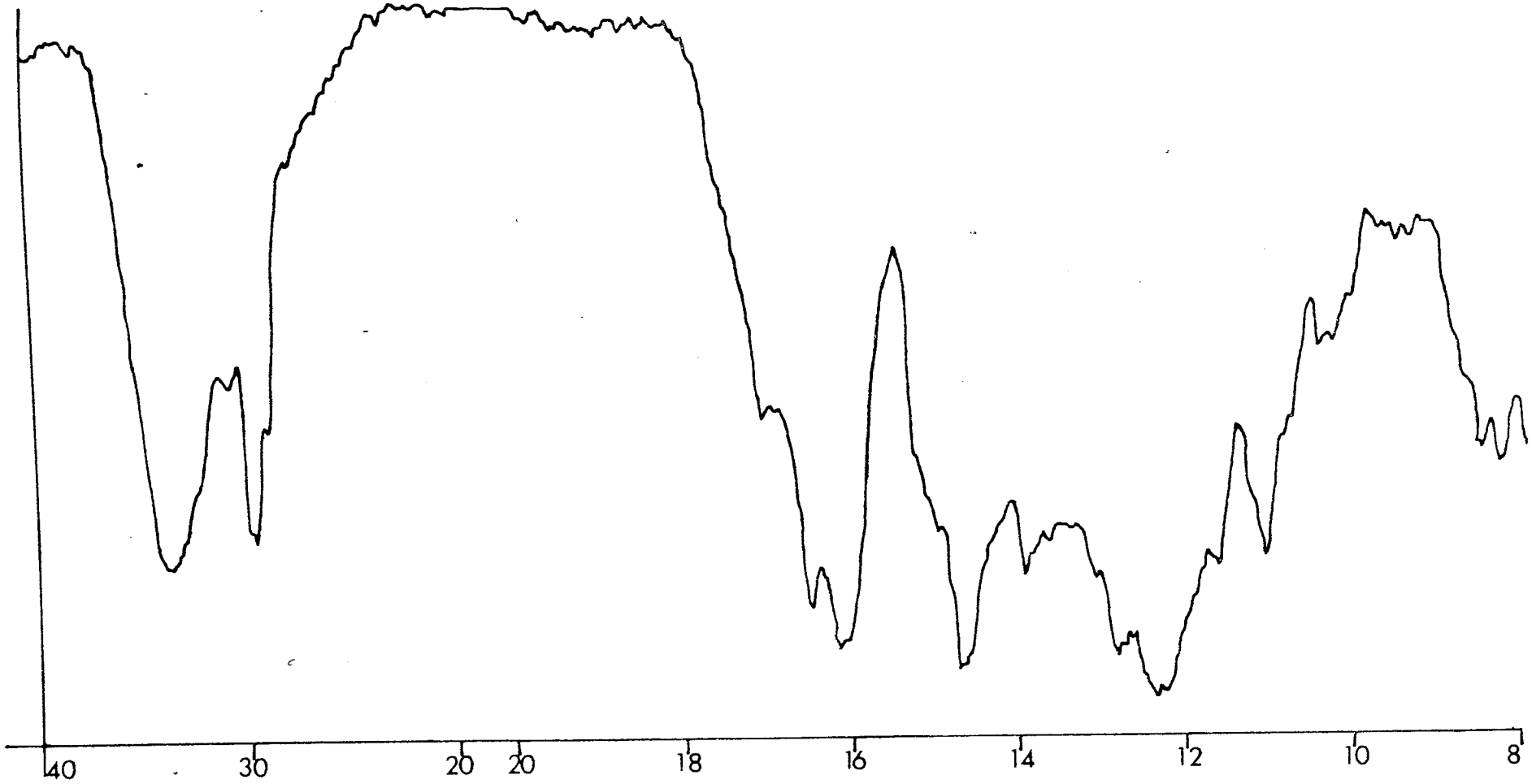
Mass Spectrum of Acetylated WTPF 180°c–230°c



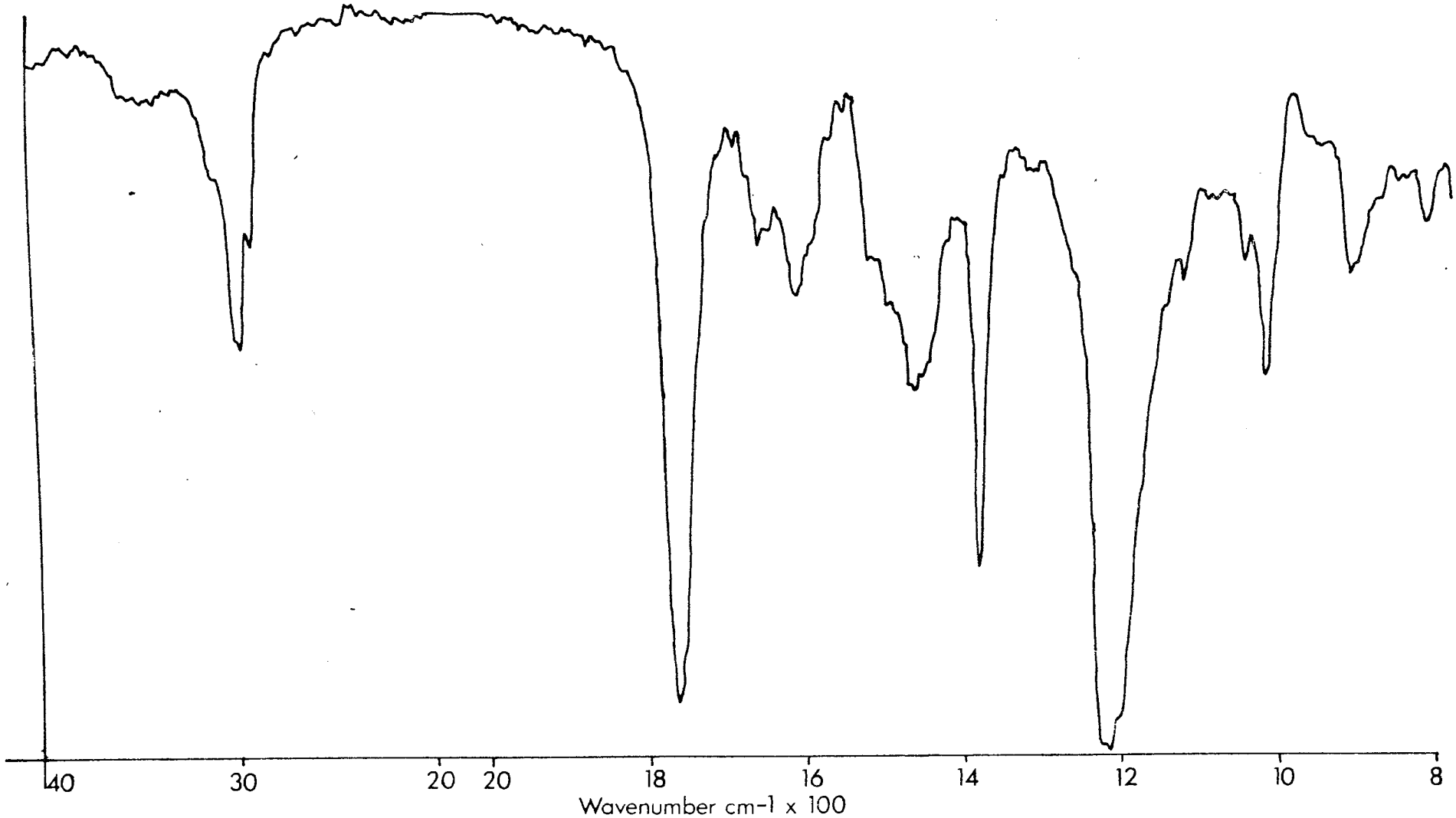
Mass Spectrum of Benzoylated WTPF B.Pt 180°c-230°c



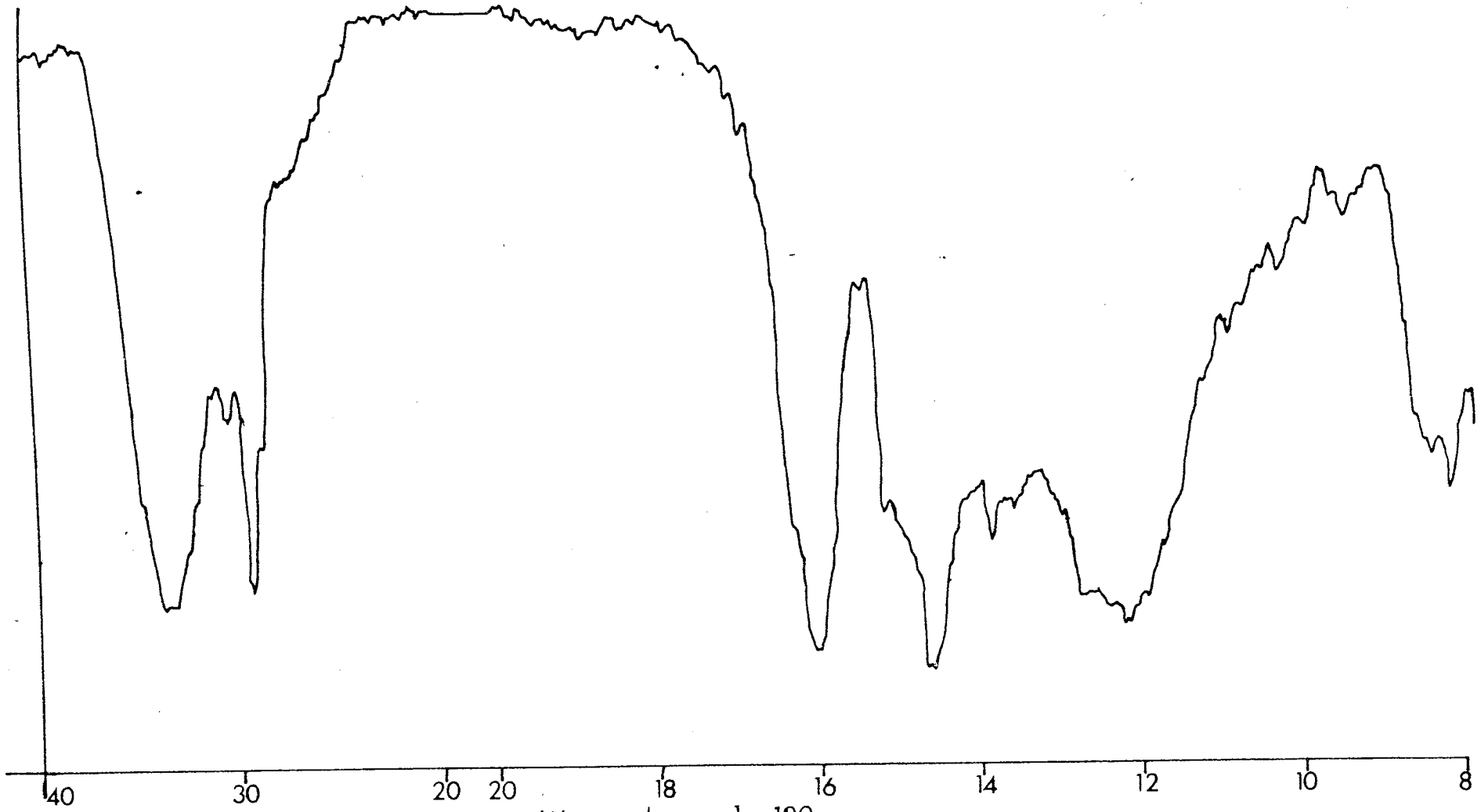
Full Scale Reproduction of a Partial Gas Liquid Chromatogram of WTAF B.Pt 180°c - 230°c



Reproduction of the Infra Red Spectrum of
WTAF B.Pt 180°c- 230°c



Reproduction of the Infra Red Spectrum
of the Acetylated WTPF B.Pt 180°c-230°c



Reproduction of the Infra Red Spectrum of the Hydrolysate of Acetylated WTPF B.Pt 180°c–230°c

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