STUDIES ON SOME MYCOTIC INFECTIONS OF DOMESTIC ANIMALS

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STUDIES ON SOME MYCOTIC INFECTIONS OF DOMESTIC ANIMALS

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INTRODUCTION

Ringworm has been recognised in Britain for over a century, and although the causal dermatophytes have been treated with numerous drugs, the disease remains prevalent in both man and the domestic animals. However, the eventual elimination of the human type dermatophyte is envisaged in the Report of the Medical Mycology Committee of the Medical Research Council (1956); nevertheless the fact that all animal type dermatophytes are infectious to man emphasises the importance of the disease in the animal population as a reservoir of human infection. The above M.R.C. Committee and the Technical Development Committee of the British Veterinary Association, working in collaboration, have suggested that animal infection may be a crucial factor in the eradication of the disease from man. For example, recent surveys of human dermatomycosis by Walker (1950) and Carlier (1954) indicate the importance of animal type dermatophytes found on domestic /

domestic animals as a persistent source of infection for humans. More recently La Touche (1956) drew attention to the interruption in the education of children which occurs from M. canis infection originating from infected cats. The same author pointed out the economic importance T. discoides infection from cattle assumes when school children or wage earners require hospital treatment. Furthermore, in man T. discoides can cause a great deal of suffering and often leaves a patient scarred (La Touche 1956) (Rook and Frain-Bell 1954).

Stock owners generally are indifferent to the presence of ringworm in their cattle, and often it is only when the farm workers, the farmer's wife or his children become affected that any action is taken. The disease, however, may assume economic importance when stock destined for sale become involved, and in the case of pedigreed animals this may result in export to foreign countries being interfered with. Grossly affected young stock may suffer a retarded growth rate, but the loss of body weight and of milk yield in adult cattle as a result of infection, observed in Canada (Blank 1953), has not been recorded in this country.

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From the foregoing it would appear that this reservoir of animal infection merits study. Ainsworth (1954) noted the dearth of reliable information concerning the dermatophytes affecting animals in this country, and Rook and Frain-Bell (1954) could find no reliable records of the incidence of cattle ringworm in Britain. More recently, however, Mortimer (1955) in a survey restricted to cattle on infected premises in East Anglia found 15% of approximately 2,000 animals to be infected. This was regarded in the M.R.C. Report (1956) as "probably not unrepresentative of the incidence of the infection in cattle throughout the country". While this may be true of the state of affairs on infected premises, it gives little indication of the incidence in the total cattle population in any locality. Mortimer's findings suggest that young stock are very susceptible to the disease. On the other hand the earlier data obtained by Ainsworth and Austwick (1955) did not indicate a greater incidence of ringworm in young or housed stock and they concluded that the epidemiology of cattle ringworm was still very obscure. The evidence was therefore conflicting and clearly the available fundamental data was insufficient for a study of the incidence of cattle ringworm.

A survey of the cattle population in several counties in the North of Britain was therefore carried out during a period embracing all seasons of the year. The chief objects of this survey were to determine the nature of the dermatophyte causing ringworm in cattle, to obtain evidence of the incidence of the disease in the cattle population, and to study the effect on this of the age of the animals, the season of the year, the type of herd, and the part of the country in which they were kept. The effect of certain other factors incidental to cattle keeping were noted during the survey and an attempt was made to assess the incidence of ringworm of cattle origin in human contacts.

A long-standing belief exists that cattle recover spontaneously from the disease when put to grass in Spring, due either to the better nutrition or to the action of ultra-violet light (Rook and Frain-Bell 1954) known to be lethal to fungi on the surface of bakery products (Smith 1946) and used therapeutically on human cases. Some observations were therefore made on the nutritional state of affected animals, and the effect of ultra-violet light was studied experimentally. When control

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or eradication measures are contemplated the time for which infected premises remain infective is of great importance. The survival of spores in infected skin scrapings was therefore studied experimentally. Walker (1956) found that twenty-nine out of fifty samples of T. discoides infected hair contained viable spores after fifteen months' storage in a dry state. This author and Solomon (1954) recorded circumstantial evidence of the survival of that dermatophyte for two years, and one and a half years respectively on cattle enclosures. Using skin scrapings from affected cattle, horses, dogs and cats, the survival time was determined of three Trichophyton species and one Microsporum species.

In the course of the work described above the need for reliable fungicides for disinfecting purposes became evident. The absence of precise information concerning these and the unsatisfactory position regarding therapy in cattle has been pointed out in the M.R.C. Report (1956). Ormsby and Montgomery (1948) reviewed the medical literature and concluded that no one drug was the answer to the ringworm problem. Seventy-eight different treatments for ringworm in animals have been described in various text-books (Jones 1956). The great need is for a remedy, which is /

is both cheap, reliable and easily applied, thus allowing the treatment of large numbers of animals, even extending to whole herds, with a view to eradication of the disease. To this end 'in vitro' experimental studies of a large number of different types of compounds were carried out. An initial screening technique was followed by a more critical study of a few, notably the surface active detergents. Since, in the past, high hopes have been held for the usefulness of compounds subsequently found worthless clinically, the need for 'in vivo' experimental study was obvious (Smith 1956). Ormsby and Montgomery (1948) summarising the work of several experimentalists, concluded that the guinea pig gave the most constant response to inoculation with a virulent dermatophyte. The disease process resulting when guinea pigs were infected with a number of strains of T. discoides was studied to assess the possibilities of using infected guinea pigs for the experimental evaluation of antimycotic drugs and to find a suitable strain of T. discoides for the purpose. On such infected animals an attempt was made to assess the relative clinical potentialities of five quaternary ammonium compounds.

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Two of these proved promising, and the final testing, "under working conditions" (Smith 1946), was initiated by clinical trial on cattle. The work to be described is in four main sections as follows:

I. A Survey of the Incidence of Ringworm in Domestic Animals with Special Reference to Cattle.

II. The Effect of Time on the Viability of Arthrospores Stored in Dried Skin Scrapings.

III. Experimental Studies on the Action of ultra-violet Light 'In Vitro' and 'In Vivo' on T. discoides.

IV. Studies on the Antimycotic Activity of Some Chemicals

(a) 'In Vitro'

(b) 'In Vivo'

(i) On Experimental Infections.

(ii) By Clinical Trial.

I.

A SURVEY OF THE INCIDENCE OF RINGWORM IN DOMESTIC ANIMALS WITH SPECIAL REFERENCE TO CATTLE

Cattle

Survey Plan

To enable a significant number of animals to be inspected in a limited period the co-operation was requested and readily obtained of Veterinary Surgeons working in the Counties of Cumberland, Westmorland, Durham, Dumfries, Peebles, Midlothian, Stirling, Fife, Perth and Kinross, Inverness, Sutherland, The Outer Hebrides and Orkney. All herds in the areas were subject to compulsory tuberculin testing and so far as ringworm was concerned the appearance of a herd on the individual veterinarian's monthly list was a matter of chance. Each observer during any period recorded data on a consecutive number of herds, e.g. first twelve or twenty herds tested from any starting date. The selection was therefore for all practical purposes a random one. For each herd a report was sent and from /

from infected herds a mixed sample of skin scraping or several samples, if unusual or doubtful cases were seen, were submitted for determination of the dermatophyte.

Primary cultures were made on the Nutrient Agar containing Penicillin and Streptomycin devised by Austwick (1954). The inclusion of 'Actiodione' in the medium was found to be of little advantage as it failed to inhibit the growth of one of the most common contaminants, a species of Absidia. The morphological features were checked microscopically and subcultures were made on Sabouraud's Glucose Agar to check the identity of the isolate since some strains of T. mentagrophytes when grown on Nutrient Agar resemble T. discoides. At the conclusion of the survey the data was assembled and the results recorded in Appendix A are described below. For the purpose of this survey bulls were too few in number to merit separate classification. They were therefore added to the cows to give a total of adults in each herd and in the Tables. All females after the first calving, approximately $2\frac{1}{2}$ to $2\frac{3}{4}$ years of age, were considered to be adult.

All /

All animals under ten months of age were classed as calves. The remainder comprised heifers from ten months to $2\frac{1}{2}$ to 3 years and bullocks (neuter males) of approximately the same age group. These latter two categories were considered separately to determine if any difference in susceptibility existed in view of the well-known fact that after puberty humans are less susceptible to M. audouini infection.

Results:

Effect of Age on Incidence of Ringworm

Of the total of 30,766 cattle observed, 888 (or 2.89%) were affected with ringworm; the incidence increasing to 8.46% when infected herds only were considered. Age appears to have a marked effect on the relative incidence, calves under ten months old being much more susceptible than more mature cattle. The results are summarised in Table (1).

Calculation of Chi-square, 729.26 and 589.28 respectively for the total cattle and for the total cattle in infected herds only, each with three degrees of freedom, gives a value of P of less than 0.01 showing a highly significant association between age and the incidence of ringworm. This is /

is ascribable mainly to the relatively low incidence in adult stock and the high incidence in calves. In the two categories bullocks and heifers of equal age, the former appears to be somewhat less prone to the disease than the latter, and this difference may be judged statistically significant.

($X^2 = 17.07$ $n = 1$ $P < 0.01$ for total cattle

$X^2 = 12.49$ $n = 1$ $P < 0.01$ for total cattle in infected herds only)

These findings agree with those of Mortimer (1955) and Sellars (1956) and the conclusion of Rook and Frain-Bell (1954).

At the age of ten months to $2\frac{3}{4}$ years approximately, the slightly higher incidence in heifers which were mainly of the dairy type is probably associated with differences in herd management. This difference in management of stock is also reflected by the increased incidence of ringworm in bullocks (3.9%) kept in dairy herds compared with the total incidence in bullocks (1.98%). This may be due to the longer period of housing of young stock practised in herds producing mainly dairy stock as opposed to beef cattle.

Effect of Season of the Year on the Incidence of Ringworm

The effect of season of the year on incidence

as /

as shown in Table 2 is of some interest especially since after analysis of the present data one is unable to subscribe to the commonly held view that the condition usually clears up when cattle go out to grass in the Spring.

For the total cattle the changes in overall incidence with season are not statistically significant; the percentage of infection showing no trend to a decreased incidence in the Spring and Summer months. In infected herds only there is a significant association between season and degree of infection, which appears to be due mainly to the relatively high incidence in the April to August period.

Since seasonal changes in the proportions of the various age groups of stock may mask the effect of season on incidence, the percentage incidence for the bi-monthly periods are shown in Table 3.

It is apparent from this Table that the present data do not indicate a spontaneous remission of the condition in cattle during the Spring and Summer months. This point is further illustration by the lack of a consistent trend in the percentage incidence of affected herds at the different seasons. These /

These were:

	Infected Herds.	Total Herds.	% Infected.
October and November	42	148	28.4
December and January	30	123	24.4
February and March	18	62	29.0
April and May	25	104	24.0
July and August	18	81	22.2

Influence of Type of Herd on the Incidence of Ringworm

In this survey the herds were classified as Dairy, Beef or Rearing and the relative incidence in each is shown in Table 4.

The significant association between the type of herd and the overall incidence is due mainly to the relatively high level of infection found in rearing herds. This effect may be ascribable in part to the greater proportion of calves to total stock in the rearing herds coupled with the greater susceptibility of stock of this age. The percentage distribution of total cattle in the three types of herds is:

	Percentage Distribution		
	Calves	Others	Total
Dairy	10.74	45.01	55.75
Beef	4.10	17.84	21.94
Rearing	6.23	16.08	22.30
Total	21.07	78.93	100.0

CATTLE RINGWORM SURVEY

The Effect of Age on Incidence

TABLE 1

	TOTAL CATTLE					INFECTED HERDS ONLY				
	Affected		Not Affected			Affected		Not Affected		
Category	Obs.	Exp.	Obs.	Exp.	Total	Obs.	Exp.	Obs.	Exp.	Total
Adults	47	312.56	10782	10516.44	10829	47	293.43	3422	3175.57	3469
Bullocks	102	148.79	5053	5006.21	5155	102	138.89	1540	1503.11	1642
Heifers	263	239.56	8037	8060.44	8300	263	241.84	2596	2617.16	2859
Calves	476	187.09	6006	6294.91	6482	476	213.84	2052	2314.16	2528
Total	888		29878		30766	888		9610		10498

$X^2 = 729.26$ $n = 3$ $P < 0.01$ $X^2 = 589.28$ $n = 3$ $P < 0.01$

PERCENTAGE INFECTED

	Total Cattle	In Infected Herds Only
Adults	0.43	1.35
Bullocks	1.98	6.21
Heifers	3.17	9.20
Calves	7.34	18.83
All Cattle	2.89	8.46

n = Degrees of Freedom.

CATTLE RINGWORM SURVEY

The Effect of Season of the Year on Incidence

TABLE 2

	TOTAL CATTLE					INFECTED HERDS ONLY				
	Affected		Not Affected			Affected		Not Affected		
SEASON	Obs.	Exp.	Obs.	Exp.	Total	Obs.	Exp.	Obs.	Exp.	Total
Oct.-Nov.	239	272.58	9205	9171.42	9444	239	302.83	3341	3277.17	3580
Dec.-Jan.	186	180.71	6075	6080.29	6261	186	158.52	1688	1715.48	1874
Feb.-Mar.	126	111.90	3751	3765.10	3877	126	164.62	1820	1781.38	1946
Apl.-May	183	185.91	6258	6255.09	6441	183	152.17	1616	1646.83	7799
July-Aug.	154	136.90	4589	4606.10	4743	154	109.88	1145	1189.12	1299
Total	888		29878		30766	888		9610		10498

$X^2 = 8.49$ n = 4 $P > 0.50$

$X^2 = 51.01$ n = 4 $P < 0.01$

PERCENTAGE INFECTED

	Total Cattle	Infected Herds Only
Oct.-Nov.	2.53	6.68
Dec.-Jan.	2.97	9.93
Feb.-Mar.	3.35	6.47
Apl.-May	2.92	10.17
July-Aug.	3.36	11.86
Total	2.89	8.46

n = Degrees of Freedom.

CATTLE RINGWORM SURVEY

Percentage Infected. Classified according to Season and Category of Cattle.

TABLE 3

Season	TOTAL CATTLE				INFECTED HERDS ONLY			
	Adult	Bullocks	Heifers	Calves	Adult	Bullocks	Heifers	Calves
Oct.-Nov.	0.43	1.53	1.33	8.88	1.10	5.16	3.99	18.29
Dec.-Jan.	0.89	3.60	3.40	5.02	4.34	6.84	12.01	18.37
Feb.-Mar.	0.62	2.11	6.14	5.39	1.21	8.82	11.11	9.51
Apl.-May	0.00	0.25	2.59	9.78	0.00	1.07	8.07	26.91
July-Aug.	0.20	2.97	4.65	6.04	0.78	12.44	16.04	19.75

CATTLE RINGWORM SURVEY

The Influence Type of Herd on the Incidence

TABLE 4

	TOTAL CATTLE					INFECTED HERDS ONLY				
	Affected		Not Affected			Affected		Not affected		
	Obs.	Expt.	Obs.	Expt.	Total	Obs.	Expt.	Obs.	Expt.	Total
Dairy	426	495.09	16727	16657.91	17153	426	482.32	5276	5219.68	5702
Beef	197	194.85	6554	6556.15	6751	197	201.74	2188	2183.26	2385
Rearing	265	198.06	6597	6663.94	6862	265	203.94	2146	2207.06	2411
Total	888		29878		30766	888		9610		10498

$X^2 = 25.81$ n = 2 P $<$ 0.01

$X^2 = 25.78$ n = 2 P $<$ 0.01

PERCENTAGE AFFECTED

	Total Cattle	Infected Herds Only
Dairy	2.48	7.47
Beef	2.92	8.26
Rearing	3.86	10.99

n = Degrees of Freedom.

While in dairy and beef herds the 'calves to others' ratios are 1 to 4.2 and 1 to 4.5 respectively the ratio in rearing herds is 1 to 2.6.

To decide if, in the individual classes of stock (calves, for example) susceptibilities differ according to the type of herd, Chi-square was calculated for each class (Appendix A Table 2) and is as follows:

	Chi-square	Degrees of Freedom	P
Adults	17.04	2	< 0.01
Bullocks	18.89	2	< 0.01
Heifers	13.25	2	< 0.01
Calves	24.52	2	< 0.01

Each class shows a significant association. In adults and calves the significant effect is due to a relatively greater incidence of disease in the Rearing Herds, while bullocks kept on dairy farms, as noted previously, appear to be affected more often than those on the other two types of farms. The numbers of affected animals are somewhat too small to give a precise picture of the effect of the type of farm on incidence.

Incidence of Cattle Ringworm in Different Areas of the Country

The incidence is often said to vary in different parts of the country, as stated, for example, by Rook and /

and Frain-Bell (1954). From the data of this survey the overall percentages of affected cattle for each County were calculated and are as follows:

County.	Percentage of Cattle Affected with Ringworm.
Cumberland	3.73
Dumfries	2.74
Durham	1.41
Inverness	3.89
Fife	3.33
Midlothian	2.18
Orkney	7.18
Stirling	1.26
Peebles	2.69
Perth	4.26
Westmorland	3.38
Sutherland	0.0

For the Counties of Inverness, Orkney, Stirling and Sutherland, the cattle examined were too few to give a clear picture of the position in these areas. In the other Counties the percentage incidence is relatively constant. The overall incidence of 8.46% affected cattle in the affected herds (Table 1) compared with Mortimer's (1955) 15% affected cattle on infected premises in East Anglia indicates a difference between the areas surveyed. Mortimer's sample, however, contained a high proportion of young stock - the ratio 'Cattle under one year to others' being 1:2.4 is comparable to the 1:2.6 ratio of calves to others in Rearing Herds in this present survey. In affected Rearing Herds the overall percentage of affected cattle /

cattle was 10.99%. The difference in incidence between the Northern Counties surveyed and the area of East Anglia surveyed by Mortimer appears for the most part to be associated with the higher proportion of young stock in the latter area.

Influence of Some Miscellaneous Factors on the Incidence of Ringworm

The influence of a number of miscellaneous factors on the incidence of cattle ringworm was also noted. The herds were classified as above average; average; or poor in relation to stock husbandry. The percentage of affected herds in each class is:

Husbandry Above Average.	Husbandry Average.	Husbandry Poor.
27.3%	27.5%	19.0%

In pedigree herds the overall percentage affected was 27.2% compared with 25.7% for all herds.

The classification of a herd as average or higher implied that the state of nutrition of the animals was satisfactory, and in most pedigree herds the nutrition is maintained at a high level to achieve maximum production. In Groups of Cattle on a high nutritional plane the disease was often seen to spread and seventeen of sixty-eight unweaned calves were recorded /

recorded affected. This evidence therefore does not support the commonly held view that animals in a good nutritional state are less frequently affected by ringworm.

Dark, damp cowsheds are said to favour spread of infection (Rook and Frain-Bell 1954). In this survey during the months of October to May inclusive, the percentages of infected herds, where the buildings were well or badly lighted were:

Well Lighted: 21.7% Badly Lighted: 23.7%

This does not support the view that there is an association between dark buildings and the incidence of ringworm.

Lice have been considered to aid the spread of ringworm (Brander 1956). In this survey the bi-monthly percentages of the infected herds also infested with lice were as follows:

October and November:	38%
December and January:	64%
February and March :	50%
April and May :	50%
July and August :	33.3%

In view of the marked increase in the lice population in the winter months and the lack of evidence of a seasonal /

seasonal increase in the incidence of ringworm, it appears that lice have little effect on the incidence of ringworm.

Incidence of Ringworm in Human Contacts to Affected Cattle

During this survey twenty-eight humans, either farm workers, farmers' wives, or children with access to the infected farms, had active ringworm lesions. These were not typed but all the circumstances suggested it was of cattle origin. Thus twenty-eight humans were infected by association with 888 affected cattle or 30,766 total cattle. If this be a true indication of the relative incidence in Britain, and as there are 9776 thousand cattle in the country, the importance attached to the cattle reservoir of ringworm infection noted by the M.R.C. Report (1956) is well founded.

The Nature of the Dermatophyte responsible for Bovine Ringworm

From each herd a mixed sample of material was received, but where any clinically unusual feature was noted an individual sample was taken.

Isolates were obtained from 166 of the 168 specimens received from the 148 affected herds. In two cases, though they were microscopically positive, no growth resulted from one, while the other was overgrown on every occasion by a contaminant of the Absidia species.

Results:

All /

All were classified as Trichophyton verrucosum var discoides. The colony character on Sabouraud's Glucose Agar varied from white to a deep ochre in a few cases, but most were of an intermediate shade. The contour of the colonies also varied slightly. Most were typical but in a few cases a less corrugated effect was evident.

Incidence of Ringworm in Domestic Animals other than Cattle

The Table below records the number of specimens of ringworm material examined for diagnostic purposes during a period of four years.

Confirmed Ringworm Specimens 1/7/52 to 1/7/56

Species of Host	Number of Specimens	M. canis	T. mentagrophytes	T. discoides	T. equinum
Horse	5	0	0	2	3
Pig	1	0	1	0	0
Cat	20	19	1	0	0
Dog	8	2	6	0	0

These findings are similar to those of Ainsworth and Austwick (1955).

T. discoides infection of the horse in Britain was first recorded by Ainsworth and Austwick (1955). Neither of the two cases here recorded include the case reported by other workers and were unrelated outbreaks. The infection of pigs by T. mentagrophytes was /

was recorded for the first time in Britain (McPherson 1956). A copy of the paper is attached in Appendix C.

M. canis was most frequently isolated from the cat and the experience of La Touche that examination of suspected animals under Wood's Light is essential, was repeated in many of these cases. On the two dogs the lesions were circumscribed. T. mentagrophytes produced well defined circumscribed lesions in both cat and dog, the reaction of the host being more inflammatory in nature than when these animals are affected by M. canis.

From the nineteen cases of M. canis recorded in cats, four adults and fifteen children are known to have been affected. This is a lower ratio than found by La Touche (1956) but still contrasts with the ratio of one human case of presumed bovine origin to approximately thirty-one cattle cases found in this survey. The difference is in all probability due to the closer association of humans with cats or household articles contaminated by the infected cats.

II

THE EFFECT OF TIME ON THE VIABILITY OF ARTHROSPORES IN STORED DRIED SKIN SCRAPINGS

Method: From skin scrapings stored dry in small cardboard boxes at laboratory temperature cultures were made on Nutrient Agar (Austwick 1954) for the Trichophyton species, and on Sabouraud's Glucose Agar for Microsporum canis. Incubation was at 37°C for the Trichophyton species and at room temperature for M. Canis. The serial numbers are the specimen number in the routine laboratory records. All samples sufficiently large enough for this work were included.

Results: These are shown in Fig. 1.

All samples of Trichophyton verrucosum var discoides material and two of the three T. equinum specimens were still viable when last tested, the oldest samples of each having survived for over four years. The other dermatophytes died out within the period of investigation. The longest survival recorded for M. canis and for T. mentagrophytes was one and a half years. In the recoveries of the Trichophyta discoides and equinum after two and a half years' /

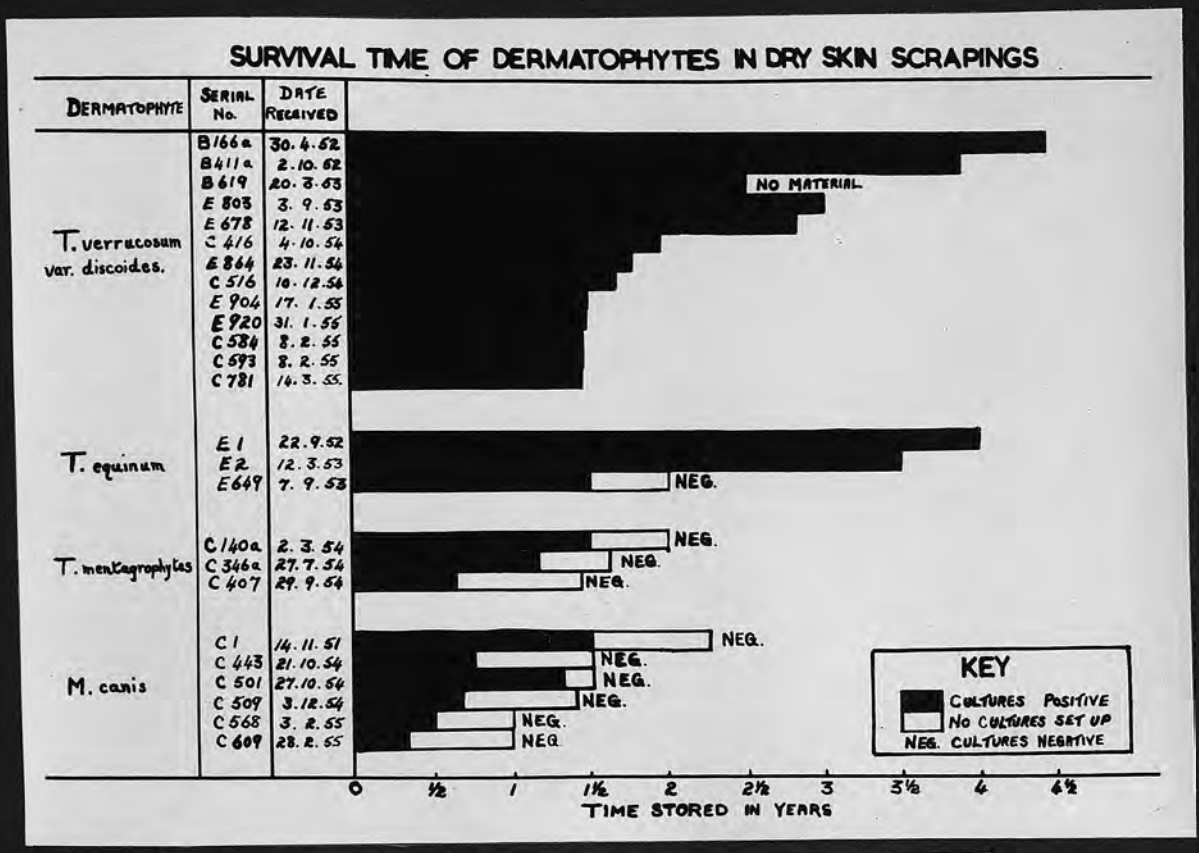

Fig. 1 Survival time of Dermatophytes in dry skin scrapings.

Fig. 2. Lesions on bovine 21 days after inoculation with T. discoides. (Strains B/166a and E.678).

years' storage the viable spores were less numerous but colonies could still be readily obtained by the routine method.

Infectivity of Stored Material for Cattle

Samples B.166a and E.678 after three years and five months' and two years and five months' storage respectively infected a two year old Jersey heifer when rubbed into scarified skin. From the seventeenth day lesions developed as described by Hoerlein (1945). On the twenty-first day photographs were taken (Fig. 2).

Discussion:

The survival of Trichophyton discoides for over four years in a dried state confirmed the belief that animal houses, equipment, fence posts, trees, etc. can remain infective for periods of years. That the same is true in relation to Trichophyton equinum was apparent. The use of a fungicide to free premises and equipment from infection appears to be essential in controlling or eradicating disease due to these dermatophytes. Trichophyton mentagrophytes and M. canis chiefly found in dogs and cats in Britain (so far as domestic animals are involved) survived off a host for upwards of one year in the case of two specimens of each.

This /

This clearly is of considerable importance from the public health viewpoint. La Touche (1956) regarded the major factor in the contraction of human ringworm from cats as the opportunity of contact with infected animals, to which might be added "or materials contaminated by such animals", provided the spores are still infective for mammals, a matter which requires investigation.

III

EXPERIMENTAL STUDIES ON THE ACTION OF ULTRA-VIOLET LIGHT 'IN VITRO' AND 'IN VIVO' ON T. VERRUCOSUM VAR DISCOIDES.

Experimental Study

To determine the result of exposure of Trichophyton discoides to ultra-violet light 'in vitro' and on a host several experiments were carried out.

1. The result of exposure 'in vitro' of arthrospores from skin scrapings and of cultures was recorded.
2. The protective action of hair and scab was then tested.
3. The effect of exposure of lesions on the relatively hairless skin of human volunteers (naturally infected Trichophyton discoides) was recorded.
4. The effect of exposure of infected and contact cattle to summer sunshine was noted.

Source and Intensity of Ultra-violet Light

All exposures were at 24" from the tube of

a /

a Model II Hanovia lamp. At that distance the irradiation was about 720 micro-watts per cm^2. One minute and forty-five seconds' exposure to this was equivalent to twenty minutes of mid-day, mid-summer, mid-latitude sunshine and produced on normal human skin a minimum of erythema (Hanovia Ltd. 1955).

1. Effect of exposure of arthrospores and cultures of T. discoides to ultra-violet light

Materials

Arthrospores: Skin crust from a badly affected yearling heifer (Case F) was very heavily laden with arthrospores, contained no contaminant fungi and very few bacteria.

Culture Suspensions: A ten day culture on Nutrient Agar (Austwick 1954) of the same strain "F" provided chlamydospores, mixed with hyphae. Ten day old cultures of a further five strains of Trichophyton discoides were also tested, C516, E.678, E.803, E.904 and E.920.

Methods: The skin crust and the colonies removed by scalpel from the medium were each reduced to a fine suspension in sterile distilled water by use of a Griffith Tube. The spores were counted on a haemocytometer and each suspension adjusted to contain approximately /

approximately 294,000,000 spores per ml. From each, Nutrient Agar antibiotic treated plates (Austwick 1954) were sown by pasteur pipette with an excess of suspension. After removing the latter the surface moisture was dried off in the incubator. The glass petri dish lids were then replaced by aluminium lids which had been cut into quarters. By removal or retention of the lid over a sector during exposure of the plate to ultra-violet light the time of exposure was regulated. Each minute one quarter plate was exposed until a series of exposures up to fifteen minutes had been made, with one quarter plate left unexposed as a control. Each experiment was in triplicate.

Results: The Table below and the Figures 3 to 12 inclusive record the results.

STRAIN	MEAN MINIMUM TIME TO KILL ALL SPORES	
	Culture Spores (Chlamydospores)	Skin Scab Suspension (Arthrospores)
F	6 minutes	7.6 minutes
C516	6.3 minutes	-
E678	5 minutes	-
E803	6 minutes	-
E904	5.3 minutes	-
E920	6 minutes	-

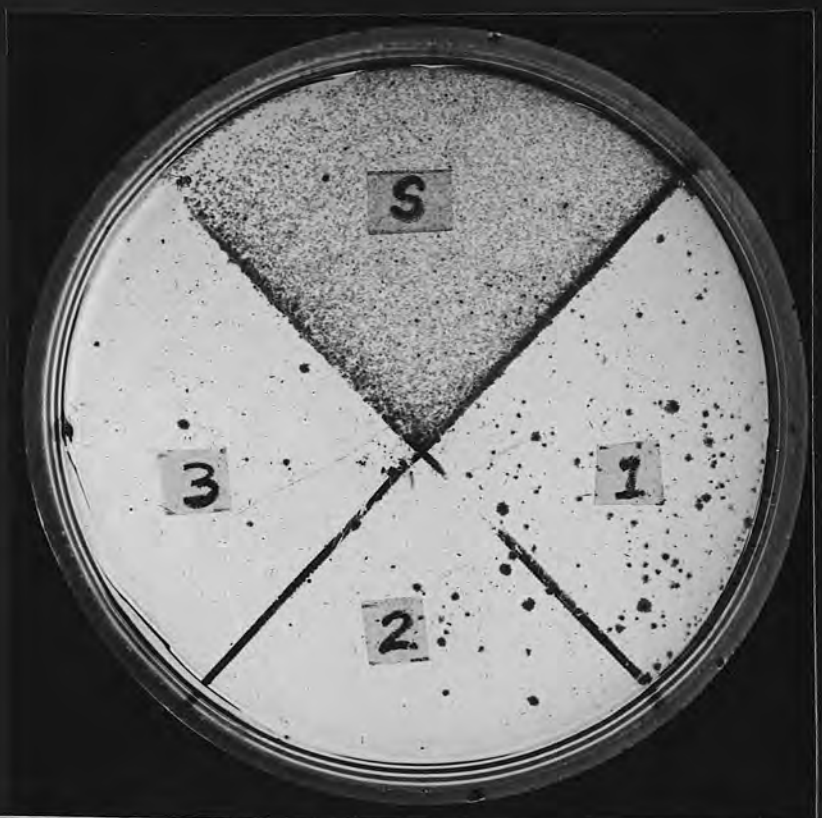

Fig. 3. The effect of Ultra-violet light on T. discoides (arthrospores suspension) Numerals indicate exposure time in minutes. 'S' indicates unexposed sector.

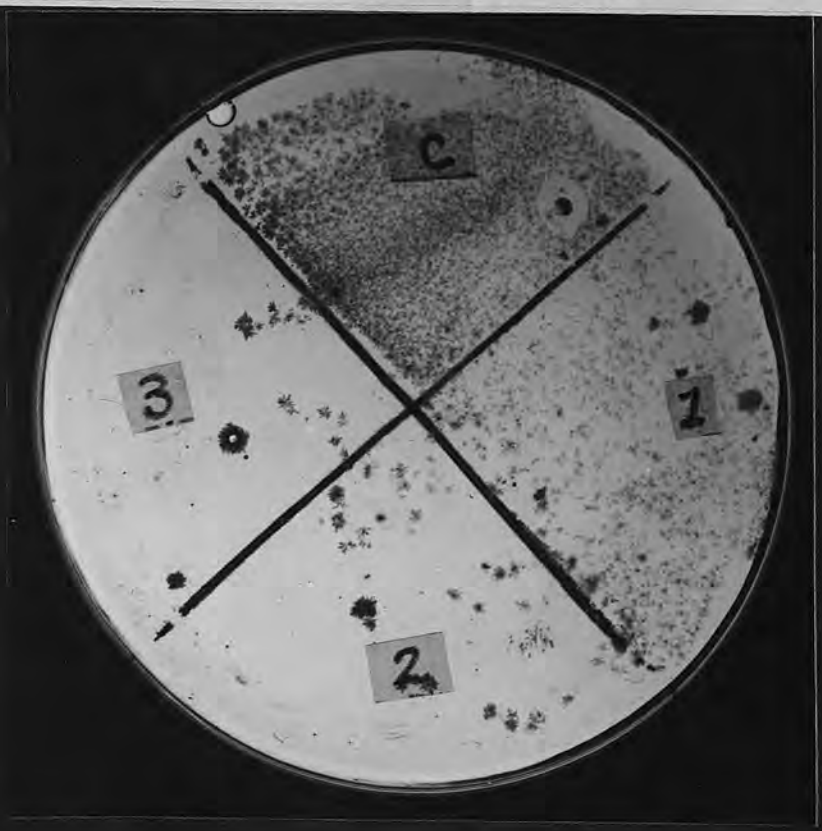

Fig. 4. The effect of Ultra-violet light on T. discoides (culture suspension) Numerals indicate exposure time in minutes. 'C' indicates unexposed sector.

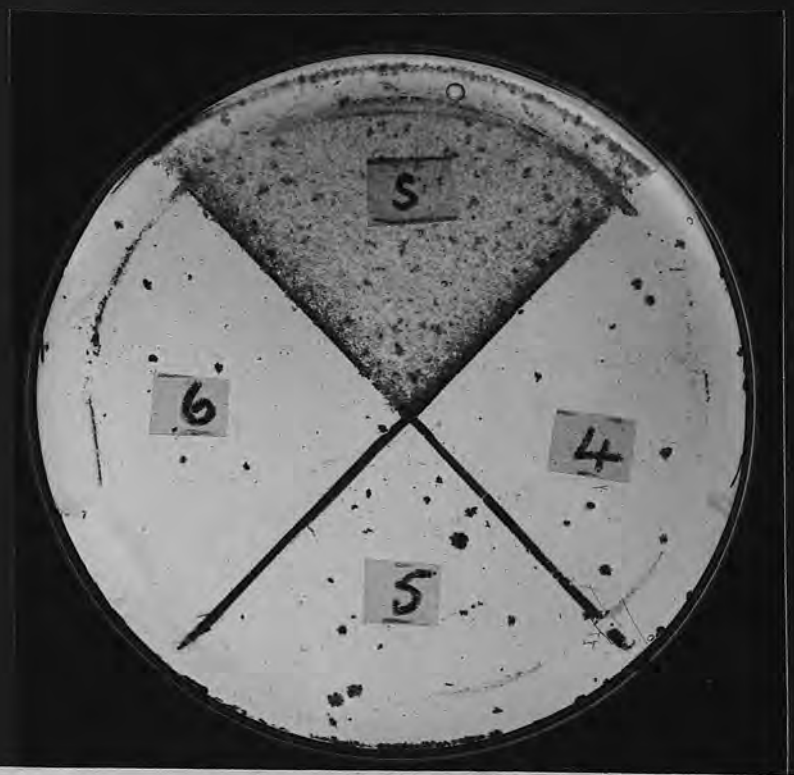

Fig. 5. The effect of Ultra-violet light on T. discoides (arthrospores suspension) Numerals indicate exposure time in minutes. 'S' indicates unexposed sector.

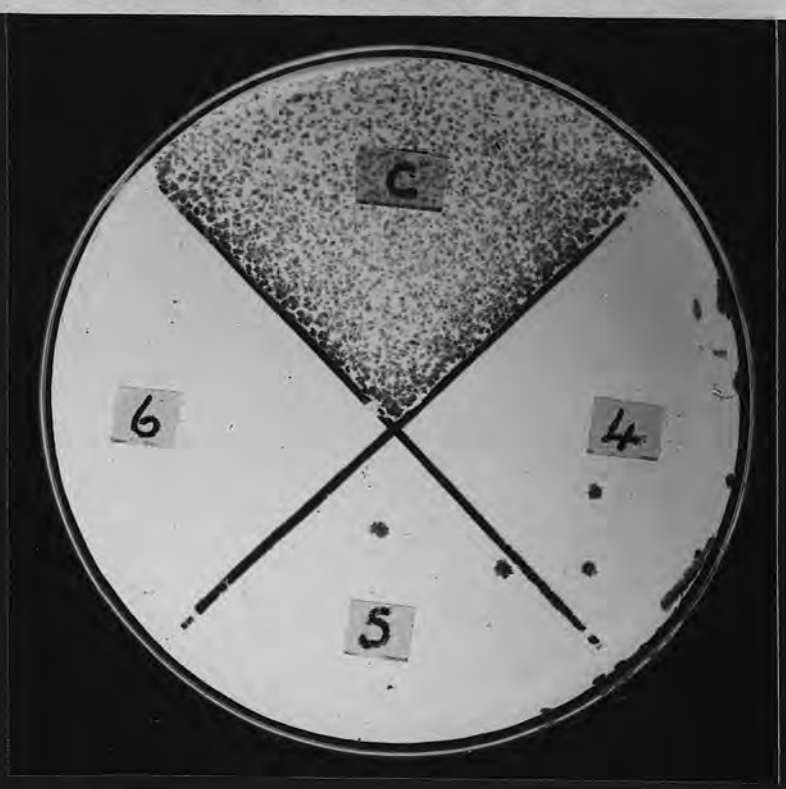

Fig. 6. The effect of Ultra-violet light on T. discoides (culture suspension) Numerals indicate exposure time in minutes. 'C' indicates unexposed sector.

Fig. 7. The effect of Ultra-violet light on T. discoides (arthrospores suspension) Numerals indicate exposure time in minutes. 'S' indicates unexposed sector.

Fig. 8. The effect of Ultra-violet light on T. discoides (culture suspension) Numerals indicate exposure time in minutes. 'C' indicates unexposed sector.

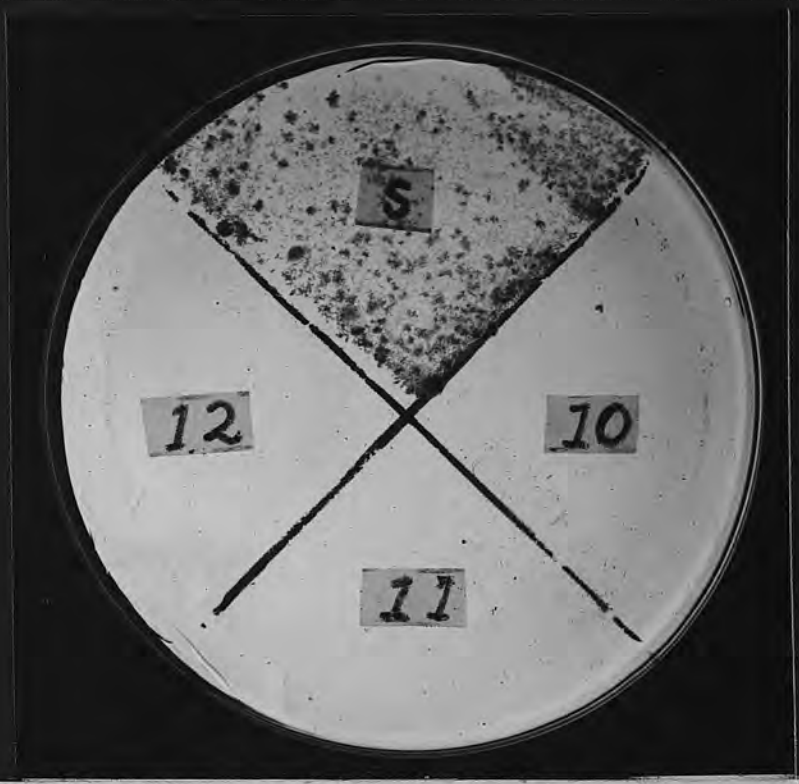

Fig. 9. The effect of Ultra-violet light on T. discoides (arthrospores suspension) Numerals indicate exposure time in minutes. 'S' indicates unexposed sector.

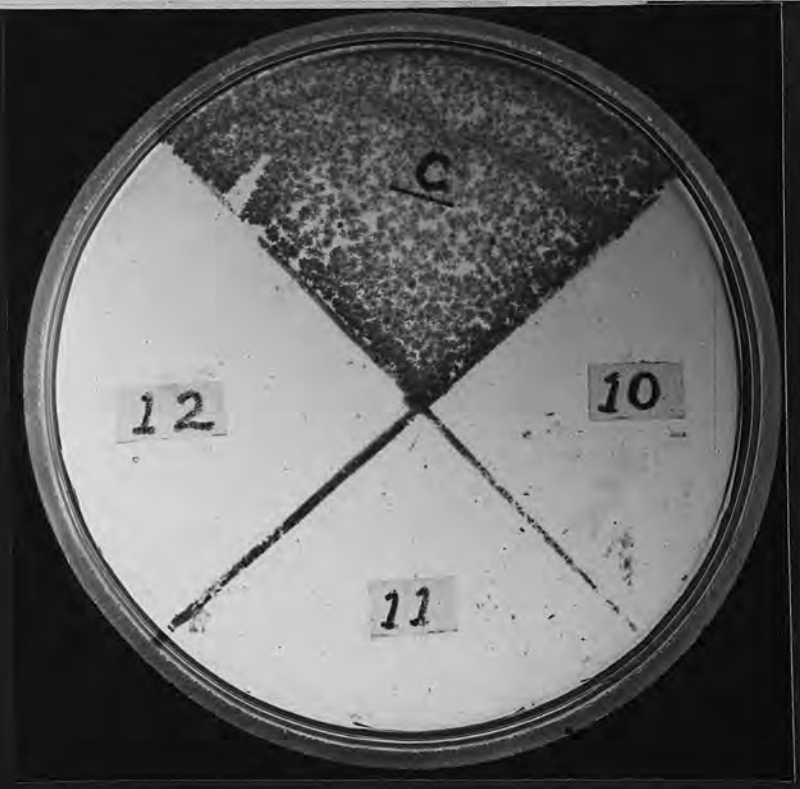

Fig. 10. The effect of Ultra-violet light on T. discoides (culture suspension) Numerals indicate exposure time in minutes. 'C' indicates unexposed sector.

Fig. 11. The effect of Ultra-violet light on T. discoides (arthrospores suspension) Numerals indicate exposure time in minutes. 'S' indicates unexposed sector.

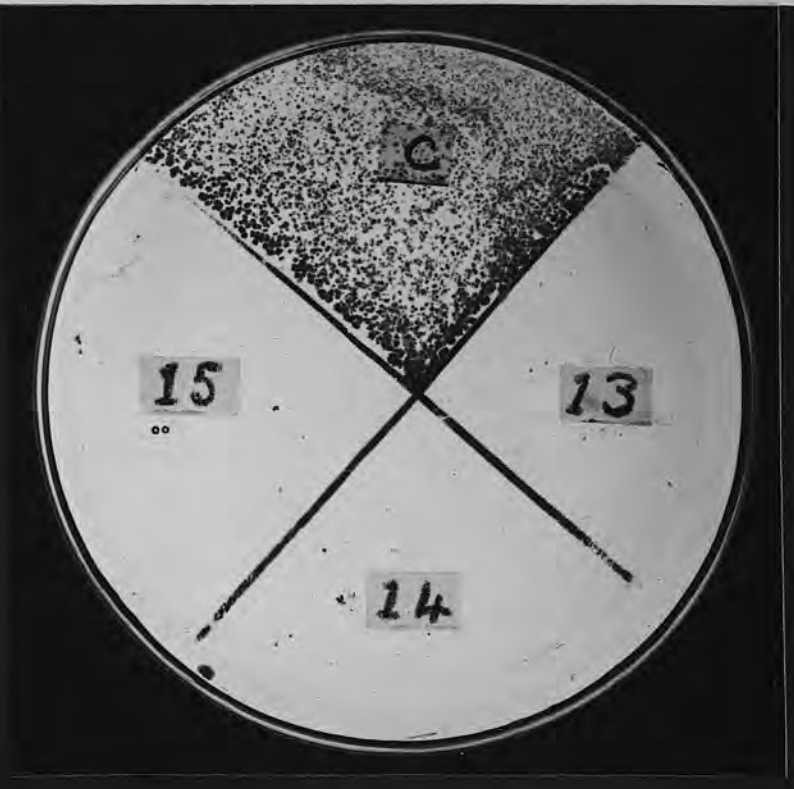

Fig. 12. The effect of Ultra-violet light on T. discoides (culture suspension) Numerals indicate exposure time in minutes. 'C' indicates unexposed sector.

Fig. 13. The effect of Ultra-violet light on T. discoides as in Fig. 11 but sown with a coarse suspension of arthrospores.

Fig. 14. Protective action of hair on T. discoides exposed to Ultra-violet light. Numerals indicate time of exposure in minutes. 'U' indicates unexposed sector.

Fig. 15. Protective action of hair on T. discoides exposed to Ultra-violet light. Numerals indicate time of exposure in minutes. 'U' indicates unexposed sector.

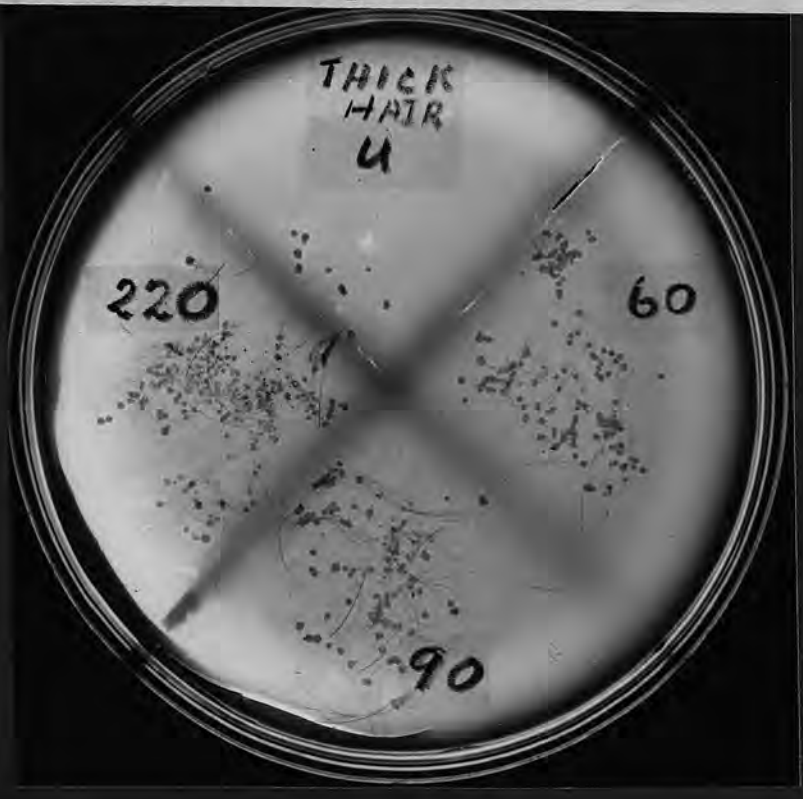

Fig. 16. Protective action of hair on T. discoides exposed to Ultra-violet light. Numerals indicate time of exposure in minutes. 'U' indicates unexposed sector.

In the Figures, Strain "F" is shown. The plates S and C were shown with arthrospores and culture suspension respectively. The lethal effect of the ultra-violet light was evident after only one minute's exposure. After three minutes' exposure only odd colonies appeared in both series, but were more persistent in the case of the skin scab suspension which was more difficult to reduce to a fine suspension in the Griffith Tube. In Fig. 7 a multiple colony appeared after seven minutes indicating a thick piece of a material. After fifteen minutes' exposure plates sown with a coarse suspension still contained viable spores (Fig. 13).

CONCLUSION:

The arthrospores of the strain "F" and the culture suspensions of the same strain and of five other strains of T. discoides were all killed by exposure to ultra-violet light for a few minutes. The shelter given by large particles in the suspension gave considerable protection to the spores.

2. Protective action of bovine hair and skin crust on arthrospores exposed to ultra-violet light

(a) Hair

Materials: The same arthrospore suspension "F" as in /

in the previous experiments was used. White bovine hair was sterilised in universal containers.

Method: Plates were prepared as in the previous experiment but before replacing the glass cover with the aluminium quarter covers, the hair was placed on the plates. Some had a thinner coating of hair than is found on a normal bovine, while the plate designed "thick" hair in the Figures had a coating representing the normal covering provided to bovine skin. This latter was arranged in roughly circular areas. The time of exposure was extended and the steps between quarters of the plate increased.

Results: The Figs. 14-16 inclusive show the results.

Under the thick covering of hair, spores were still viable after 220 minutes' exposure, though the colonies were small, smooth and cream coloured. The control quarter on which, in this case, no hair had been placed, failed to show growth. All three of the triplicate plates showed this feature and investigation showed that the white hair reflected light rays on to the undersurface of the aluminium cover. A second reflection partially illuminated the shaded area. With the long exposure sufficient rays penetrated to kill off the spores. When black hair was employed and the /

the control section was also covered with hair, no such lethal action took place on the control quadrant.

CONCLUSION:

Bovine hair protected the arthrospores of Trichophyton discoides from the lethal action of ultra-violet light for longer than the equivalent of forty-two hours continuous mid-day and summer, mid-latitude sunshine. It appeared therefore that the normal coat of cattle protected T. discoides on the skin surface from a very considerable exposure to strong sunshine.

(b) Scab from a Bovine Lesion

Material: A piece of scab from the case "F" measuring 15 x 6 mm. x 1.5 mm. thick was used.

Method: The scab was exposed, outer side uppermost, in a petri dish on a dark surface. Cultures were set up at intervals during the exposure which extended over twelve days.

Results: Protection afforded to T. discoides by dry scab is illustrated below.

	Times during Exposure of Scab to Ultra-violet Light at which cultures were made. (Minutes)									
	70	170	325	600	820	875	950	1630	2295	2445
Result of Cultures	+	+	+	+	+	+	+	+	+	-

+ = Growth obtained; - = No growth obtained.

After 2295 minutes colonies were smooth, cream coloured and compact, as occurred after 220 minutes in the previous experiment with hair; nevertheless a profuse growth occurred. The scab was inverted at the next exposure after which no further growth could be obtained.

CONCLUSION:

A relatively thin piece of scab from a Trichophyton discoides lesion on a heifer harboured viable spores after exposure to ultra-violet light for the equivalent of 437 hours of mid-day, mid-summer, and mid-latitude sunshine which was a more severe test than natural exposure over the 122 days of May, June, July and August in an average year would have been, as the average total sunshine near Edinburgh over the years 1954, 1955 and 1956 was only 624 hours, including many hours of weak morning and evening light.

3. Effect of exposure of lesions, on the relatively hairless skin of human volunteers, to ultra-violet light

Materials: Six adult humans (four males and two females) victims of natural infection with different strains of Trichophyton discoides volunteered.

Method: Diagnosis was based on microscopical examination of scrapings and on recovery of Trichophyton discoides in cultures on Nutrient Agar. Exposures were made /

made for various times producing over some lesions no erythema and on others a deliberate "sunburn".

Case I

This man had initially five lesions on his forearm and four on his back. (Fig.17). A fresh lesion developed later on the forearm and a second on his chin.

Treatment: Thirty-two minutes' exposure was given to nine lesions over a period of ten days, commencing with two minutes and finishing with six minutes' exposure. Chemotherapy was then used on the dry but still pink lesions. The fresh forearm and chin lesions were deliberately burned by eighteen minutes' exposure over three days and nine minutes over two days respectively, a mask being used to protect the normal skin. No other treatment was used on these areas.

Result - Case I : A slow improvement took place on the areas exposed for a gradually increased time to ultra-violet light. The lesions deliberately "burned" were freed of infection rapidly.

Case II:

This man had initially two lesions 15 mm. and 5 mm. diameter on his wrist.

Treatment /

Treatment: After five minutes' exposure this volunteer went on holiday but returned after a week for thirteen minutes' exposure over three days before again going away for twelve days, during which time he kept the area covered with a bandage. The lesions dried but on returning from holiday the large lesions were 15 mm. and 20 mm. in diameter with a marked central crust and a very active proliferating edge not well shown on the photograph. (Fig. 18). There was also a fresh lesion on the wrist. (Fig. 18 inset). Six minutes' exposure produced a blistering of the skin in twenty-four hours, but the patient took a further two minutes at that time on each lesion. At the seventy-second hour most of the large scabs were removed and five minutes' exposure was given to each lesion. No further treatment other than antibiotic cream for the pus pockets in the centre of one large lesion was used for the next three days during which great improvement took place. A final six minutes which did not blister the patient was given. Fourteen days after the commencement of uninterrupted treatment the skin was healed and rapidly returned to normal texture. (Figs. 19 and inset).

Case III

This male had an initial lesion of 10 mm. (Fig. 20) on /

on his wrist from which the colonies illustrated in Fig.21 were obtained by direct seeding of the Nutrient Agar plate containing penicillin and streptomycin.

Treatment: Over three days, fifteen minutes ultra-violet light were given without even erythema resulting. The lesion subsided, but after eleven days was 25 mm. in diameter and very active. Seventy-five minutes' exposure was given over seven days but no erythema resulted even though the skin had been covered for the previous fourteen days and the initial exposure was for seven minutes. The lesion dried, became scaly, and the colour returned to normal. Six days later, however, an active lesion of 35 mm. diameter from Trichophyton discoides was present. Chemotherapy cured the case in fourteen days. (Castellani's Paint).

Case IV

A 12 mm. lesion on the forearm of this male was cured by fifteen minutes' exposure, over three days, which produced a marked "sunburn" over the lesion exposed through an opening in a cloth mask.

Case V

One exposure of six minutes produced a marked local "sunburn" effect on this female. No further treatment was necessary.

Case /

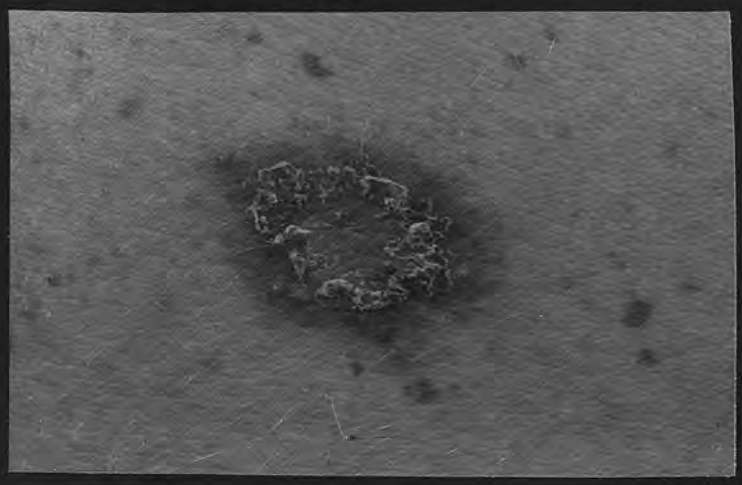

Fig. 17 Case I. Man. T. discoides. Initial lesion.

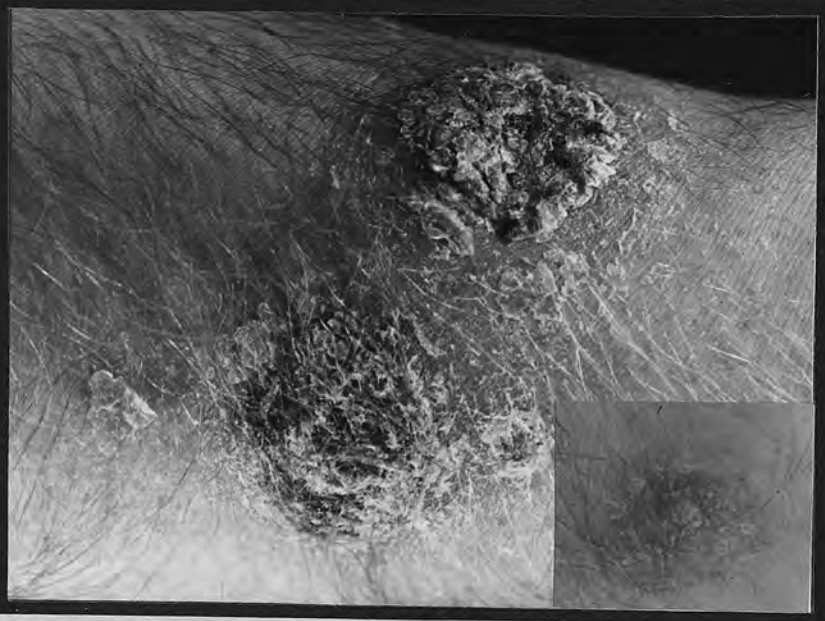

Fig. 18 and Inset. Case II. Man. T. discoides. Lesion at maximum development.

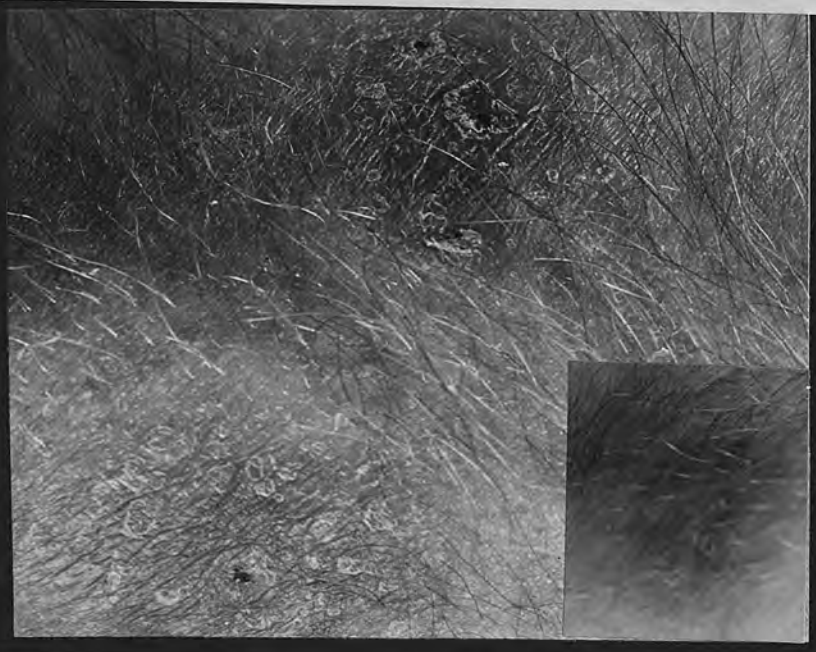

Fig. 19 and Inset. Case II. As in Fig. 18, but fourteen days later.

Fig. 20 Case III. Man. T. discoides. Initial lesion

Fig. 21 Case III. Primary Cultures on Nutrient Agar.

Case VI

Adult female with a single lesion ¾" diameter. Three daily exposures totalling thirteen minutes produced a marked "sunburn" effect and cured the case without further treatment.

CONCLUSION: That provided skin is not damaged sufficiently to provoke a marked inflammatory reaction the comparatively hairless human and presumably animal skin affords Trichophyton discoides in the deeper epidermis considerable protection from the lethal action of ultra-violet light.

4. Effect of Exposure of Infected Cattle to Summer Sunshine

In August 1953 all of forty-one calves at grass since the previous April in a field without shelter from the sun were found affected with Trichophyton discoides. In August 1955 a group of twenty-four calves on the same field were all affected by the first of August, despite 668 hours of sunshine in the previous three months. In the latter group disease was present on several animals when they were put to grass on 1st May and by 1st July twelve had visible lesions. During July all became affected.

Discussion on effect of sunshine on bovine ringworm

Summer outbreaks of ringworm in cattle at grass were recorded by Ainsworth and Austwick (1955) and have been observed by Staugaard (1956) in Denmark. During the Survey already discussed extensive summer outbreaks of ringworm were encountered. In view of this, and the histories of the two groups of cattle here reported, it is apparent that summer sunshine does not prevent the spread of T. discoides infection in cattle.

General conclusion on the action of ultra-violet light on T. discoides

While ultra-violet light was rapidly lethal to arthrospores in single layers, bovine hair, scabs from lesions and skin itself combined to provide an effective protective barrier. Summer sunshine has in all probability not influenced the incidence of ringworm in cattle in Britain. This gives support to the findings during the survey that season of the year did not affect the incidence of the disease in cattle.

For efficient therapeutic action the lethal action of ultra-violet light appeared to have been aided by the production of a skin weal followed by rapid exfoliation of the epidermis though the cases were too few for definite conclusions.

IV

STUDIES ON THE ANTIMYCOTIC ACTIVITY OF SOME CHEMICALS

Plan for the investigation of antimycotic agents

Trichophyton discoides grows relatively fast and characteristically on Nutrient Agar (Austwick 1954) and in Nutrient Broth (No_2 Oxo). The disease this dermatophyte causes in cattle was known to be widespread, and to offer better opportunity for therapeutic trial than any other dermatophyte infection of man or animals (Sellars 1956). In these circumstances T. discoides was used as a test organism for 'in vitro' work on antimycotic agents (a role not hitherto given it, except on limited tests by Collier et alia (1955) and Babbs et alia (1956)) and for 'in vivo' evaluation of drugs experimentally and by therapeutic trial. As there existed no one drug with a universal application in the treatment of ringworm a large number of chemicals were first examined by a screening technique. For this purpose an adaptation of the paper disc technique in common use in microbiological assay of antibiotics was used, after the manner of Kligmann and Rosenzweig (1948). This was by preliminary experiment found to be a method whereby the screening /

screening process was achieved rapidly and accurately except in the case of very volatile substances which present a special problem as noted by Byrde (1956). Thereafter selected chemicals were more critically tested for fungicidal and fungistatic activity in liquid medium and a few 'in vivo' experimentally and by clinical trial.

Preliminary screening of substances for antimycotic activity

The paper disc technique was employed essentially for screening. The test chemicals or preparations were used in the form in which they were readily available, no attempt being made to test at comparable concentrations of each and by this method a large number of chemicals were quickly eliminated as inactive while others were shown to have but weak antimycotic activity.

Method: Chemicals were "A.R." or B.P. standard where appropriate. The seeded plates of media were prepared as in the ultra-violet light experiments. After the completion of drying stage 'Fordiscs' (B.D.H.) completely impregnated with the test solution or in some cases with one side of a water-moistened disc carrying solid powdered chemical or dry discs lightly smeared with ointments were applied to the surface of the medium. Four discs placed around the perimeter of the plates were used initially.

Experience /

Experience determined whether the number of discs per plate or the concentration of the drug had to be reduced to obtain a reading. After incubation at 37°C for fourteen days, readings were made in millimetres of the distance radially from the edge of the disc to the nearest colony of T. discoides and a mean determined.

Preliminary experiments showed that both the arthrospores and the culture suspensions "F" gave the same readings with some twelve substances. With the other five culture strains triplicate plates varied by less than 2.0 mm. from the mean for the six strains. Thereafter the suspension C.516 was used exclusively. The Tables below show the mean radial measurement of the zone of inhibition (to the nearest millimeter) for at least triplicate plates.

Results: The results are illustrated in Figs. 22 and 23, and listed in Appendix B.

Discussion of the results of the preliminary screening

In the case of some chemicals the development of colonies was delayed two to three days either over the whole of an initial zone which disappeared or just beyond the perimeter of a final zone. In a few cases recorded by the insertion of the note (incomplete) or ('hazy zone') very /

Chemical on Disc	Width Radially of Zone of Inhibition. mm.
'Avis' (45% Sulphathiazole Sodium)	Nil
'Omo' (Powder on Disc)	16
'Merth. 1/1000th' (.1% Thiomersal)	45
'Roc 10%' (Benzalkonium Chloride 10%)	25
'Cet 20%' (Cetrimide 20%)	27

See Figs. 22 and 23 opposite.

Fig. 22 Screening of Antimycotic Agents by Paper Disc Method. (see table opposite).

Fig. 23. Screening of Antimycotic Agents by Paper Disc Method. (see table opposite).

very small colonies were present over the whole of a zone at the end of the incubation period. This phenomenon may be an indication of true fungistatic activity while the clear zone indicates fungicidal action when the drug and dermatophytes are incubated in nutrient medium, as postulated by Collier (1955) when discussing the action of his bisquaternary ammonium compounds when used in incubated liquid nutrient medium.

Chemicals which did not inhibit the growth of T. discoides

Appendix B. Table 1

Ammonia was too volatile for this type of test but as it has been used to kill the rather resistent oöcysts of coccidia, tests by a more suitable technique were carried out and are reported later. The antibiotics and sulphonamides were not unexpectedly classified in this group. Some Salts of certain metals or organic compounds containing these were inactive against T. discoides, e.g. Barium, Lead, Uranium and Zinc Salts, and two arsenical preparations used clinically, one as a vermifuge and the other as a skin stimulant and tonic. The inactivity of Alcohols, Benzole, Pyridine, Carbon tetrachloride, and Tetrachlorethylene was noteworthy. Advantage was taken of this feature of Ethyl and Methyl Alcohol to reducing the bacterial flora in skin material, prior /

prior to primary cultivation for dermatophytes before the advent of penicillin and streptomycin. The use of Carbon tetrachloride to clean clothing would not kill T. discoides in the process. 'Prostigmin' nor its antagonist 'Atropine' had any effect on the dermatophyte. The same was true of 'Piperazine' and drugs containing it.

Of the inactive dyes, 0.1% Proflavine hemisulphate was in contrast to the activity of pure Acriflavine recorded later. Dapsone, a bacteriostatic agent, had no antimycotic activity. Neither the ester of natural oestrogenic hormones, Stilboestrol nor Vitamin E had any effect on the growth of the fungus. Salicylic acid and its allied compounds were inactive, suggesting that in ointments these drugs act by their keratolytic action. Urea, lately advocated as a keratin degrading agent, and its allied compound Urethane, had no effect on the growth of the colonies. Keratolytic action facilitates penetration of fungicides but alone aids the dermatophytes (Barlow and Chataway 1955). The inactivity of Sodium propionate was at variance with the statement of Ormsby and Montgomery (1948) that its activity 'in vitro' was undisputed. Sodium Carbonate at the concentration tested has frequently been used to remove scabs from lesions but does not protect from spread. The growth of T. discoides was uninfluenced by the presence of Potassium permanganate. Mineral oil (waste engine) commonly /

commonly used by farmers on ringworm affected cattle had no antimycotic activity 'in vitro'. Methyl benzoate was inactive in contrast to Benzyl benzoate which exhibited moderate antimycotic activity. Amongst the remaining inactive compounds 5% Phenol, Santonin and Phenolphthalein were noted.

Chemicals producing a zone of inhibition 1-5 mm. in Radius

Appendix B. Table 2

From the veterinarian's viewpoint the weak action of gamma-benzene hexachloride, 20% Dettol, 'Hibitane', 0.2% Red Iodide of Mercury, Liq. Iod. Mitis and Phenothiazine are noteworthy, though the test was perhaps unsuitable for Iodine. That Sod. hypochlorite was also too volatile for the technique was clear as later it was shown to be an efficient fungicide for T. discoides. Hypochlorites have been extensively used on dairy farms, and Wedderburn (1955) observed that the feet of men badly affected with athlete's foot rapidly became healthy after these soldiers commenced work on water chlorination plants in the Middle East.
'Dibrogan Cream' found very useful clinically in inflamed conditions of the skin exhibited a weak but possibly useful antimycotic activity.

Chemicals producing a zone of inhibition 6-10 mm. in Radius

Appendix B. Table 3.

The chemicals used in a pure state on the test plates and appearing in this Table did not appear to be potentially useful as antimycotic agents. Basic Fuschin, present at 10% concentration in Castellani's Paint, produced at the 3% concentration a 9 mm. zone which equalled the effect of 5% Lysol and Phenol Liq. B.P. under the test conditions. 'Cuprinol' used as a wood dressing did as expected inhibit the dermatophyte and could probably on woodwork be relied on to deal with T. discoides, though Creosote as noted elsewhere proved much more efficient as a fungicide for the test dermatophyte.

Chemicals producing inhibition zones greater than 25 mm. in Radius

Appendix B. Table 7.

Of the chemicals producing inhibitory zones exceeding 25 mm. in radius the mercurials are all well known fungicides, but are too toxic for clinical purposes except mercuric iodide and Thiomersal. Creosote, Thymol, Hexachlorethane and Trinitrophenol all inhibited complete plates. Creosote has been used diluted in oil as a cure for ringworm in cattle but not with universal success.

Discs /

Discs, impregnated with Creosote, one part, mineral one part oil, produced no inhibitory zone. As a treatment for infected woodwork it would appear to be efficient against rot fungi and probably is fungicidal, though proof is needed. (M.R.C. Report 1956). Thymol might merit more extensive use in veterinary therapeutics. Hexachlorethane and Picric Acid might be useful, though the former is rather unpleasant to handle. 25% Terpineol has not, in clinical experience, proved as efficient as the 62 mm. zone suggested. Monosulfiram 25% solution has been described as a potent fungicide in the "British Veterinary Codex" though cattle require prolonged treatment. Further investigation seemed desirable as in T. mentagrophytes infection in dogs the drug has been found efficient. Dettol when diluted to 20% (an economical use strength) only inhibited a zone of 1 mm, suggesting a weak activity on dilution. 'Captan' which has been used in the U.S.A. for some time, and latterly in Britain on ringworm cases was chosen for more critical tests in comparison with other chemicals.

Chemicals producing inhibition zones between 20 and 25 mm. in Radius

Appendix B. Table 6.

The eight substances producing inhibitory zones /

zones between 21 and 25 mm. were interesting. Promezathine hydrochloride did not completely inhibit and clinically it failed to cure T. equinum infection (Jarvie 1953). Boric Acid, a known fungicide used commercially on Citrus fruits (Smith 1946) has not been used extensively in veterinary practice, though in man every text-book on dermatology notes the usefulness of wet Boric Acid dressings in acute cases of ringworm. The two quaternary ammonium compounds, Cetrimide B.P. and Benzalkonium Chloride, were found on dilution to retain their antimycotic activity and are discussed below. Klarmann (1948) found this type of compound fungistatic in low dilutions, but not fungicidal. Though 2% Dichlorophen was reported on favourably by Brander (1956) his cases recovered slowly. The zone produced by Potassium Iodide suggested the use of this agent, but the intravenous administration to affected cattle of Sodium Iodide which pharmacologically produces a similar action (British Veterinary Codex 1953) has proved unsatisfactory (Boddie 1956). That 'Lysol' was useful as a fongicide merited confirmation especially as it gave an 8 mm. zone at 5% concentration.

Chemicals and Preparations producing inhibition zones of 11-20 mm. in Radius.

The substances producing zones between 11 and 20 mm. are all of some interest. Of two antimycotic ointments /

ointments tested, one "Asterol' did not completely inhibit growth but gave a hazy 20 mm. area, while the other containing Undecylenates produced a 20 mm. zone of complete inhibition. The latter, however, have (according to Ormsby and Montgomery 1948) not proved an unlimited success clinically and proved useless in the case No. 1 recorded in the foregoing section on ultra-violet light before ultra-violet treatment was requested. The mercurials have been used in Veterinary Medicine but not with universal acclamation, though 'Penotrane' has been very useful. 'Acriflavine', Brilliant Green and Crystal Violet in the pure state all gave an 18 mm. zone, while 0.5% Methyl Violet produced a zone of 12 mm., 0.1% Proflavine hemisulphate did not produce any zone, as already noted. Clinically, any beneficial result from the use of these dyes has been very slow to appear.
The most interesting substances zoning chiefly in this region at the concentrations tested are the surface active detergents, both the quaternary ammonium compounds which are cationic in action and the anionic detergents of commerce which are generally either secondary alkyl sulphates, alkane sulphonates or alkyl aryl sulphonates, present at approximately 20% active agent in a vehicle. In Table 5 the activity of a number of these compounds is illustrated.

TABLE /

TABLE 5

THE ANTIMYCOTIC ACTIVITY OF SEVERAL SURFACE ACTIVE AGENTS AS SHOWN BY THE PAPER DISC METHOD.

Surface Active Agent	Radius of Zone of Inhibition after 14 Days at 37°C.
1% w/v Benzalkonium Chloride	18 mm.
1% w/v Cetrimide B.P.	15 mm.
1% w/v Dequadin[a] Chloride	15 mm.
1% w/v M. & B. 1732 [b]	11 mm.
1% w/v Teoquil [c] methosulphate	23 mm.
1% w/v Sodium Lauryl Sulphate	18 mm.
1% v/v 'By-Prox' [d]	8 mm.
'Daz' (Pulv.) [d]	11 mm.
'Omo' (Pulv.) [d]	14 mm.
'Surf' (Pulv.) [d]	12 mm.
'Tide' (Pulv.) [d]	14 mm.
'Teepol' [d]	15 mm.

(a) Decamethylene-bis-4-aminoquinaldinium.
(b) New Quarternary Ammonium Compound.
(c) Hexadecamethylene-bis-isoquinolinium.
(d) Commercial Detergents.

For Manufacturers' names see Appendix B - Tables 4 and 5.

A few preliminary tests showed that all these surface active agents retained their fungistatic activity in low dilutions, as was reported by Collier et alia (1955) and Babbs et alia (1956) to be true of Teoquil and Dequadin Salts when used against their test strains. Fungicidal and fungistatic activity of all these substances was therefore investigated and the results are reported below. In addition 'Captan', Boric Acid, Monosulfiram, Ammonia and Lysol were examined with Phenol for comparison.

Determination of Fungistatic Activity

Culture suspensions from the Strain C.516 and six freshly isolated strains, SP. 19, 20, 22, 46, 70 and 75 were prepared as described in the section on ultra-violet light. To serial dilutions in 10 ml. of Nutrient Broth (Oxo No_2) 0.04 ml. of culture suspension was added. After incubation at $37^{\circ}C$ for fourteen days the results were determined visually. All experiments were in at least triplicate and in Table 6 the mean minimal inhibitory concentration of the substances tested against seven strains of T. discoides are shown.

Results: These are recorded in the Table 6.

Phenol, at a concentration of 125 µg/ml. of medium inhibits the growth of T. discoides. Lysol was approximately /

TABLE 6

ANTIMYCOTIC ACTIVITY OF SEVERAL CHEMICALS AGAINST SEVEN STRAINS OF T. DISCOIDES 'IN VITRO'

Chemicals Tested	Minimal Fungicidal Concentration gm/100 ml. at 20°C.			Minimal Inhibitory Concentration µg/ml. after 14 days in Nutrient Broth at 37°C.
	10 min.	60 min.	12 hours	
Phenol	2.5	-	-	125
Lysol (f)	0.5	-	-	30
Ammonia	7.5	1.0	-	-
Boric Acid	>5.0	>1.0	<1.0	200
Captan (e)	0.5	0.25	<0.05	5
Monosulfiram	<0.25	-	0.05	1.6
Sod. Hypochlorite	0.025	-	-	1.0
Quaternary Ammonium Compounds				
Benzalkonium Chloride	1.0	<0.2	<0.05	0.5
Cetrimide B.P.	>2.0	>2.0	<0.05	1.0
Dequadin (a) Chloride	>0.5	<0.5	<0.05	1.0
M. & B. 1732 (b)	1.5	<0.5	<0.05	4.0
Teoquil (c) methosulphate	2.0	>1.0	<0.05	0.15
Surface Active Detergents				
Sod. Lauryl Sulphate	>1.0	>1.0	<0.25	2.0
'By-Prox' (d)	>10.0	<1.0	<0.25	1.5
'Daz' (d)	>10.0	<1.0	<0.25	20.0
'Omo' (d)	>10.0	<1.0	<0.25	10.0
'Surf' (d)	>10.0	<1.0	<0.25	14.0
'Teepol' (d)	>10.0	<1.0	<0.25	-
'Tide' (d)	>10.0	<1.0	<0.25	12.5

(a,b,c & d) See footnote to Table 5

(e) N-trichloromethyl-thiotetrahydrophthalamide

(f) Solution of Cresol with Soap B.P.

approximately four times as active while Boric Acid was not fungistatic below 200 µg/ml. 'Captan', Monosulfiram and Sodium hypochlorite were fungistatic for T. discoides at the low concentration of 5, 1.6 and 1 µgm/ml. respectively. The quaternary ammonium compounds were also fungistatic in low concentrations, 'Teoquil' being most efficient, proving inhibitory at 0.15 µgm/ml. and M. & B. 1732 least so, requiring 4 µgm/ml. The relative degree of activity shown for these compounds by this technique was similar to that demonstrated by the paper disc technique during screening.

The activity of the surface active anionic detergents was of a similar order to that of the quaternary ammonium compounds when the approximate concentration of 20% active agent in the commercial products was taken into account. By-Prox was outstandingly active, being inhibitory at a concentration as low as 1.5 µgm/ml., apparently ten times as active as the other compounds. Sodium Lauryl Sulphate was inhibitory at a minimal concentration of 2 µgm/ml.

DETERMINATION OF FUNGICIDAL ACTIVITY

Spore suspensions were prepared as for the fungistatic tests. Klarmann and Wright (1948), Klarmann (1948) and Collier (1955) found dried bovine ox bile in their media /

media neutralised the action of quaternary ammonium compounds. By experiment it was found that the same held good for the surface active anionic detergents tested. To 0.1 ml. of serially diluted test substance 0.04 ml. of spore suspension was added. After ten minutes, at 20°C, ten ml. of Nutrient Broth, containing 2% dried ox bile, was added, the containers shaken and incubated at 37°C for ten days when the results were read visually. In the case of the other products tested the exposure to the test solution was as described but at the end of the ten minutes two loopfuls (4 mm.) of the test mixture were transferred to 10 ml. of the ox bile broth. In the case of Lysol, Phenol and 'Captan' it was noted by comparative tests of the two methods that ox bile did neutralise these compounds, but Boric Acid retained the inhibitory properties in the ox bile broth. Incubation and reading was as described. On at least triplicate results against the seven strains described above, the Mean Minimal Fungicidal Concentration of each substance was determined and is illustrated in Table 6.

To test the effect of time on the minimal fungicidal concentration the tests were repeated with the exposure time extended to one hour and twenty-four hours. The results are also recorded in Table 6.

Results of Tests for Fungicidal Activity

From the Table it can be seen that Phenol at a concentration of 2.5 gm. per 100 ml. of water was fungicidal in ten minutes, a result which agrees with the findings of Collier (1955), and in the case of Lysol the result was approximately that expected.

Ammonia which was fungicidal at 7.5% concentration in ten minutes was efficient at 1% when the exposure was for one hour. Boric Acid was not fungicidal against T. discoides at 5% concentration after ten minutes or 1% after an hour, but no growth was obtained after twelve hours' exposure to this concentration. The figures for 'Captan' show that the efficiency of the drug as a fungicide increased with exposure time, from 0.5% after ten minutes to 0.25% after one hour, and to less than 0.05% after twelve hours. In ten minutes Monosulfiram was fungicidal at a concentration of less than 0.25%, which is below the concentration normally used therapeutically, and after twelve hours the effective concentration was 0.05%. Sodium hypochlorite was the most potent fungicide under the test conditions, being effective in 0.25% aqueous solution within ten minutes.

In /

In the case of the quaternary ammonium compounds after ten minutes' exposure to Benzalkonium Chloride, M. & B. 1732 and 'Teoquil', at a minimum concentration of 1.0%, 1.5% and 2% respectively, no spores remained alive, whilst after sixty minutes the effective concentrations were below 0.2% and 0.5% respectively in the case of the first two, but was above 1% for 'Teoquil'. 2% Cetrimide and 0.5% 'Dequadin' Chloride were not fungicidal even with an exposure time of sixty minutes, but after twelve hours' exposure time all five compounds were fungicidal. Sodium Lauryl Sulphate was not fungicidal at 1% in sixty minutes, though only a small growth was obtained as opposed to a heavy growth after ten minutes' exposure. After twelve hours in the 0.25% solution no spores survived.

The commercial surface active detergents were not fungicidal in ten minutes at 10% concentration, but after one hour in 1% solutions no growth was obtained. After twelve hours, 0.25% concentrations of all these products, which is the normal average concentration recommended for household washing purposes, proved fungicidal.

Discussion on 'invitro' antimycotic test results

The aim of these tests was to indicate the potentiality /

potentiality of the drugs as either therapeutic agents or fungicides for disinfecting purposes. The results in Table 6 indicate that Captan, Monosulfiram, Sodium hypochlorite, the quaternary ammonium compounds and the surface active detergents are potentially useful therapeutically, if Klarmann's (1948) statement that fungistatic activity is more important in clinical work than fungicidal activity be true. Of the compounds examined, Lysol and Sodium hypochlorite are apparently the most efficient and are cheap fungicidal agents for disinfecting purposes. Where there are objections to these compounds because of odour, poisoning or bleaching, a 1% solution of either ammonia or one of the surface active detergents listed may prove useful. At 0.25% which is the normal working concentration of the detergents, no growth resulted after exposure for twelve hours, suggesting the practicability of decontamination fabrics by soaking overnight in the detergents prior to washing the articles.

IV

STUDIES ON THE ANTIMYCOTIC ACTIVITY OF SOME CHEMICALS 'IN VIVO'

(i) On Experimental Infections

A. Infection of Guinea Pigs with T. discoides

The hair on the trunks of four female 350 gram. guinea pigs was clipped very short (1/20th mm.) over the thorax and abdomen. Guinea Pig No. 2 was inoculated, intradermally by dental syringe, at six sites on the left side with 0.05 ml. of a culture suspension of Strain C516 containing approximately 294,000,000 spores per ml. On the right side of the same animal a similar dose of arthrospores of the Strain 'F' was given. The animals numbered 1, 3 and 4 were inoculated by pasteur pipette with eighteen different strains of T. discoides recently isolated on Nutrient Agar slopes. Pipettes were drawn to a gauge of 2 mm. After cutting the ends of the capillary part was ground slightly to remove rough edges. Culture was scooped up into the end of the pipette and the latter gently but firmly pressed into the skin with a slight rotary movement until a circular impression remained in the epidermis. Effusion of blood was avoided but care taken to damage some hair follicles. Measurements were made on the eleventh, sixteenth, nineteenth /

nineteenth and twenty-fourth days after inoculation. Fig. 24 shows the left side of animal No. 2, and Fig. 25 the right side of animal No. 1 at the eleventh day from inoculation.

Reference to the measurements in Table 7 shows that at the eleventh day the duplicate lesions of each strain matched except in the cases of SP 1, SP 7, and SP 46, where the discrepancy probably reflects an error of judgment at the time of inoculation. At the sites on animal No. 2, where the inoculum was placed below the keratinised layers, poor lesions developed, though the 8 mm. by Strain 'F' was of a similar order to the lesion developed on another animal in a subsequent group when inoculated by pipette. Reference to the measurements of the control lesion in Table indicates that except in the case of Sp 13, C.516 and 'F' the lesions reached a maximum size of 10 to 18 mm. by the sixteenth to nineteenth day after inoculation. The lesions developed as described by Ormsby and Montgomery (1948). The early stages were not easily detected even on the white guinea pigs. Progress to a lesion of 8 mm. diameter took place within two or three days from the appearance of a papule. It can be seen that development to a climax was rapid. At about the sixteenth to nineteenth day ulceration took place on many, and thereafter /

thereafter healing took place very rapidly, leaving a hairless patch which persisted for a further period of approximately one month in most cases. The inflammatory reaction, scab formation, ulceration and rapid healing were characteristic and in contrast to the lesion on bovines.

Experiment No. 2

After completion of the healing stage in these animals eight guinea pigs similar in every way to the previous four, with the exception that they were now four weeks older at inoculation, were inoculated by pipette. Four strains which had produced marked lesions in the previous experiment were used. One strain was used on six sites on one side of each guinea pig. In addition to these strains six sites were inoculated with Strain 'F' and with a field strain 'Black' said to be very virulent for cattle.

At only twenty-six of the ninety-six sites inoculated did lesions develop. (Table 8). These were later in appearing, and generally speaking, did not reach a climax as early as on the previous occasion. The reason for this is obscure. During the two weeks prior to inoculation and over the experimental period, a liberal ration of grass was fed and the animals were in good condition. It had been noted that the persistence of /

of the small slough at the site of inoculation delayed the healing time of treated lesions in particular, and in an attempt to avoid this, less trauma had been caused at the sites than on the previous occasion. It is felt that this factor was of importance. Two older guinea pigs subsequently inoculated in the same fashion as the first four failed to develop lesions however.

CONCLUSIONS:

Guinea pigs, under certain circumstances, can be infected with T. discoides and though most strains produce well-marked lesions, there were exceptions which could not be anticipated by consideration of the virulence for cattle, as strains 'F' and 'Black' were very virulent for cattle in the field. The rapid development to a climax from the time one can be certain a good 'take' has been obtained, and the rapid healing, leaves little time for the assessment of antimycotic agents. The factors responsible for the unpredictable results in experiment No. 2 require further experimental study before the guinea pig could be recommended as a suitable experimental animal. The use of young animals may be an important factor.

B. /

TABLE 7

The Antimycotic Activity of Benzalkonium Chloride and Cetrimide B.P. 'in vivo'

Treatment	Strain of Trichophyton discoides	Diameter of lesions at 11 days after inoculation. mm. Cont.	mm. Treat.	Diameter of lesions at 16 days after inoculation. mm. Cont.	mm. Treat.	Diameter of lesions at 19 days after inoculation. mm. Cont.	mm. Treat.	Diameter of lesions at 24 days after inoculation. mm. Cont.	mm. Treat.	Guinea Pig No.
1% Benzalkonium Chloride on 11th, 16th and 19th days	SP 12	8	8	10	7	11	7	H	H	1
	SP 11	8	8	10	7	16	7	Small Scab	H	
	SP 15	12	12	16	10	16	10	Small Scab	H	
	SP 22	9	9	16	6	16	5	H	H	3
	SP 46	7	3	16	H	16	H	H	H	
	SP 50	6	6	16	6	16	5	H	H	
	SP 45	10	8	13	10	16	5	H	H	4
	SP 47	12	12	11	8	14	4	H	H	
	SP 70	12	12	18	8	16	4	H	H	
	C 516	3	3	3	H	H	H	H	H	2
	C 516	3	3	3	H	H	H	H	H	
	C 516	3	3	3	H	H	H	H	H	
1% Cetrimide on 11th, 16th and 19th days	SP 7	10	3	14	3	12	H	H	H	1
	SP 6	10	10	12	10	14	5	H	H	
	SP 3	10	10	12	10	14	8	H	H	
	SP 13	3	3	H	H	H	H	H	H	3
	SP 19	8	8	16	8	16	4	H	H	
	SP 20	10	10	16	10	16	10	H	H	
	SP 1	7	2	8	H	10	H	H	H	4
	SP 33	10	6	11	6	13	4	H	H	
	SP 34	12	9	12	8	16	5	H	H	
	F	3	3	8	3	8	H	H	H	2
	F	3	3	8	3	8	H	H	H	
	F	3	3	8	3	8	H	H	H	

TABLE 8

The Antimycotic Effect of Several Chemicals 'in vivo' (Guinea Pig)

Treatment given on 15th, 17th and 20th days after inoculation.	Strain of Trichophyton discoides	Diameter of lesion at 15 days after inoculation. Cont. mm.	Treat. mm.	Diameter of lesion at 17 days after inoculation. Cont. mm.	Treat. mm.	Diameter of lesion at 20 days after inoculation. Cont. mm.	Treat. mm.	Diameter of lesion at 24 days after inoculation. Cont.	Treat.	Diameter of lesion at 26 days after inoculation. Cont.	Treat.	Guinea Pig No.
0.1% Benzalkonium Chloride	F	8	8	8	12	8	12	H	Scab	H	H	12
0.2% Cetrimide	SP 46	8	8	12	8	14	8	Scab	H	H	H	5
	SP 46	8	11	12	11	14	10	Scab	H	H	H	5
	SP 70	8	8	11	8	12	7	Scab	Scab	H	H	6
	SP 22	-	9	-	12	-	14	-	14	-	Scab	9
1% Captan	SP 46	8	8	12	10	14	12	Scab	H	H	H	5
	SP 20	12	14	16	14	16	14	Scab	Scab	H	H	10
1% Suspension Dequadine Chloride	SP 70	10	8	11	8	12	6	Scab	Scab	H	H	5
	SP 70	10	14	11	12	12	10	Scab	Scab	H	H	7
1% M. & B. 1732	SP 46	8	8	12	10	14	14	Scab	H	H	H	5
	SP 46	8	8	12	10	14	10	Scab	H	H	H	5
	SP 22	12	14	16	16	16	14	Scab	Scab	H	H	10
	F	8	8	8	10	8	10	H	Scab	H	H	12
1% Teoquil Methosulphate	SP 70	10	8	11	8	12	6	Scab	Scab	H	H	5
	SP 70	10	14	11	17	12	18	Scab	18	H	H	7
0.1% Thiomersal	F	8	8	8	10	8	10	Scab	Scab	H	H	12
Aqua Distilled	SP 70	8	8	11	12	12	12	Scab	Scab	H	H	6
Untreated	SP 70	4	-	4	-	12	-	Scab	-	30 days H	-	11
Untreated	Black	8	-	8	-	8	-	H	-	H	-	12

55b.

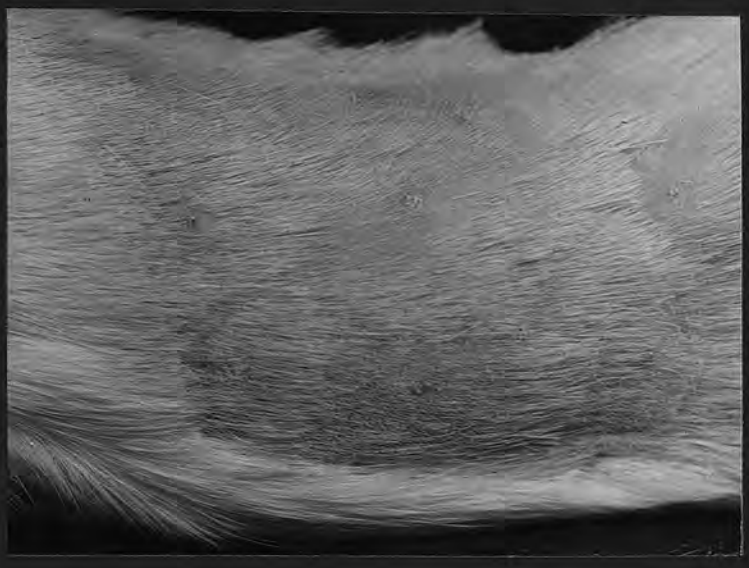

Fig. 24 Infection of Guinea Pig with T. discoides. Guinea Pig No. 2 by Strain C.516. Tenth day after inoculation.

Fig. 25 Infection of Guinea Pig with T. discoides. Guinea Pig No. 1 by Strains SP3, SP6, and SP7 (left to right) Tenth day after inoculation.

B. Assessment of Antimycotic agents using experimentally infected guinea pigs.

The lesions which developed on the guinea pig in the groups described above were used to test a few drugs. These agents, at the chosen concentration, were brushed on the lesion with a tooth brush. On individual guinea pigs one lesion due to each of the strains used on the animal was left untreated as a control. A lesion treated with Thiomersal served to demonstrate the comparative effect of a well-known therapeutic fungicide. On the first four guinea pigs treatment was given and measurements made on the eleventh, sixteenth and nineteenth days, while on the others these procedures were carried out on the fifteenth, seventeenth and twentieth days owing to the slower development of the lesions.

Results: Tables 7 and 8 show the results.

It is clear from the time by which healing had taken place in both control and treated lesions that a clear cut answer was not obtained. In the case of some solutions 'in vivo' fungistatic action was clearly demonstrated however.

1% Benzalkonium Chloride

This solution arrested the development of all lesions to which it was applied.

0.1% Benzalkonium Chloride

The treated lesion developed better than the control.

1% Cetrimide B.P.

This Solution arrested the development of all lesions to which it was applied.

0.2% Cetrimide B.P.

The growth of three lesions was stopped but on the lesion due to Strain SP.22 extension continued. There was unfortunately no other lesion on this animal to act as a control.

1% Dequadin Chloride Suspension

This suspension appeared to exert a better effect than the measurements indicate.

1% M. & B. 1732

No beneficial effect was exerted on two of the lesions and on the other two the antimycotic action was weak.

1% Teoquil methosulphate

The extension of one lesion was arrested, but the other, a rather active lesion, developed apparently unhindered by the drug.

1% Captan

No assessment can be made on the data, though that it did not arrest development of the lesion due to SP 46 is evident.

0.1% Thiomersal

The lesion continued to extend after the first treatment and healed at the same time as the untreated control lesion.

Distilled Water (Control)

Rubbing with a wet brush did not hinder the development of the lesion.

Discussion of the antimycotic action of drugs on experimentally infected guinea pigs.

The rapid development to a climax followed by rapid healing of control lesions did not allow clear conclusions to be drawn. It may be that by an alteration in technique or by the adaptation of a strain of T. discoides to the guinea pig by passage that better conditions for drug testing can be achieved.

On the few tests made 1% Benzalkonium Chloride and 1% Cetrimide B.P. Solutions were worthy of clinical trial on cattle. The weaker concentrations, known to be fungistatic 'in vitro' were not reliably efficient 'in vivo' though further evidence is necessary. Little can be said of the other drugs without further study, except perhaps in the case of 1% 'Dequadin' Chloride Suspension. One of the more soluble Salts of 'Dequadin' would probably be more efficient and better to handle.

Toxicity

No work designed to test the toxicity of the drugs was undertaken. As a class the quaternary ammonium compounds are non-toxic to tissue (McCulloch et al. 1948). Collier et alia (1955) and Babbs et alia (1956) have reported the toxicity of bisquaternary ammonium compounds 'Teoquil' and 'Dequadin' to be low. It was noted that at 1% concentration all the quaternary ammonium compounds produced some skin irritation. This took the form of a dry exfoliative dermatitis which would not therapeutically, in animals at least, be any disadvantage and might even be beneficial.

(ii) By clinical trial

Monosulfiram, Benzalkonium Chloride and Cetrimide B.P. were tested on cattle naturally infected with T. discoides. In all cases the agent was brushed on to the lesions with a stiff brush. The quaternary ammonium compounds w ere applied to a wide area around each lesion, resulting in some cases, in a complete soaking of the head and neck, and in the case of two calves treated with Cetrimide, over the whole body surface. The action of these two quaternary ammonium compounds made wetting of the coat and soaking of the scabs relatively easy.

Results: These are illustrated in a tabulated form in Tables 9, 10 and 11 and in the Figures in Appendix D.

Monosulfiram 25%

Though in the first three cases treated the result was satisfactory, the effect on subsequent groups was not outstanding, as can be seen by reference to the number of treatments necessary to effect a cure. In Groups B and C, eight treatments were required, and in the case of the latter it is probable that the drug was aided by spontaneous healing, as a similar group of animals on the same premises the following summer healed spontaneously by the end of September. As stated in the /

the British Veterinary Codex (1953) benefit from treatment was slow to take place. In Group D the drug, under normal farm conditions, failed to control the disease.

Cetrimide B.P.

Five cows with recent lesions were treated with a 1% Solution every third day for five treatments. The result was very satisfactory. (Group E).

Two Groups were then treated (F AND G), the concentration of Cetrimide being increased to 2% and the interval between treatment to seven and eight days respectively, between the first two dressings. Group F was treated again on the fourteenth day, but the animals in Group G were not treated until the thirtieth day when cultures from the few visible lesions were negative. It appeared that two dressings with an interval of seven days between was satisfactory. Group H was then treated with a 1% solution, with the same time intervals between the first two dressings. By the twenty-first day healing was indicated by the appearance of hair on the lesions. The remaining scab material was removed by scrubbing with the 1% solution, leaving a clean skin on which hair grew relatively quickly. Two calves (Group I) grossly affected were then washed with a 1% solution on only /

only two occasions at an interval of seven days with satisfactory results. The last case treated, the cow in Group J, had a very inflamed lesion, extending radially very rapidly, on which a 2% solution was used at three day intervals. It is clear that the results here were not so satisfactory as viable spores were present after twelve days' treatment.

On thirty-six of the thirty-seven cases, Cetrimide proved satisfactory. In all cases extension of the lesion ceased after the first application, indicating the fungistatic effect of the drug 'in vivo',

Benzalkonium Chloride

On a group of twelve cattle the action of 1% Benzalkonium chloride applied twice with a seven day interval between treatment was effective. The few scabs visible on the thirtieth day were removed by scrubbing with the drug. While cultures at the eighth day were positive no viable spores remained on the thirtieth day. As in the Cetrimide treatment the extension of lesions ceased after the first treatment.

Discussion on Clinical Trials.

It was evident that Monosulfiram was not a satisfactory /

satisfactory treatment for cattle though six dogs affected with T. mentagrophytes were cured in ten to fourteen days by daily application of the same concentration of the drug.

Both Benzalkonium Chloride and Cetrimide gave promising results, though the resistance of one case indicates the need for caution in interpretation, and the necessity of further trials.

TABLE 9

CLINICAL TRIALS

CATTLE

MONOSULFIRAN 25%

Group	Cattle Used			Lesions	Details of Treatment	Remarks
	Nos.	Age	Sex			
A	3	1½Yr	N	1st and 2nd had both well develpped lesions on head and neck, and small body lesions. 3rd had only small lesions on head.	Initially and on the 4th and 15th days.	No estension of lesion after first dressing. 4th Day: Clean skin where no haemorrhage initially. 15th Day: A little scab on 1st and 2nd, but good growth hair on 3rd case
B	2	1½Yr	N	On head, neck and body	Initially and on 4th, 10th, 41st, 44th, 47th, 50th and 53rd days.	Extension ceased after 1st treatment. 10th Day: Skin mostly clean under easily removed scabs. 41st Day: Still lesions. A few adjoining old lesions, others new.
C	20 21	1½Yr 1½"	F N	Numerous lesions on head, neck and body.	Initially and on 1st, 2nd, 6th, 10th, 18th, 20th and 27th days.	18th Day: 27 with lesions 20th Day: 24 do. 27th Day: 16 do. No further treatment.
D	12 Cows 6 Heifers 10 Calves			Head, neck, thorax, sacral and peroneal. Isolated and under 2" diameter.	Initially and on approx. 7th, 15th, 23rd, 35th days; and longer in some cases.	Extension ceased after 1st dressing, but if left 15 days between treatments lesions were active. Labour costs in handling and dressing too high.

TABLE 10

CLINICAL TRIALS

CATTLE

CENTRIMIDE B.P.

Group	Cattle Used			Lesions	Details of Treatment	Remarks
	Nos.	Age	Sex			
E	5	4Yr	F	Head, Neck. Several 1" to 1½" Sacral and Flank 1" to 4" dia.	1% Initially and on 3rd, 6th, 9th and 12th days.	Growth of lesions ceased after first treatment. 15th Day: Hair growing on clean skin on head and neck. 21st Day: Hair growing on all lesions.
F.	4 1	1Yr 3"	N F	Head and neck lesions up to 3" dia.	2% Initially and on 7th and 14th days.	Lesions ceased to grow after first treatment. 14th Day: Hair was growing through scabs or on clean lesions. Recovered without further treatment.
G.	12	1Yr	N	Head, neck, thorax, flank, sacrum and peritoneum. Lesion average 1". A few large areas 4" x 5".	2% Initially and on 8th and 30th days. (Only 5 treated on 30th day.)	All lesions ceased extending after 1st treatment. 8th Day: Five animals with small lesions cured. Only odd lesions, e.g. edge of ears were then active. These and three of large lesions with scab were positive on culture. 30th Day: All lesions healing. Only five had scabs which proved negative culturally.
H	4 11	2Yr 2"	N F	Numerous lesions up to 2" dia. Head, neck especially perioccular and on sacral and back regions.	1% Initially and on 7th and 21st days.	Lesions ceased to grow after first treatment. 21st Day: Hair wasgrowing through scabs or on clean skin. 40th Day: Hair normal on all lesions.

CENTRIMIDE B.P. (TABLE 10 Contd.)

Group	Cattle Used			Lesions	Details of Treatment	Remarks
	Nos.	Age	Sex			
I	2	3 mths	F	Confluent lesions over whole skin, except legs	1% Initially and on 7th day.	Animals lathered all over, then allowed to dry. No further treatment necessary.
J	1	4Yr	F	Flank lesions. Fig. 9 Very little crust. Skin thick and inflamed. No pus.	2% Initially and on 3rd, 6th, 9th, 12th, 15th, 18th and 24th days.	This lesion, painful to touch, was growing at 5mm radially daily. Growth ceased after 1st treatment. 12th Day: Scab still adherent and culturally positive. Smaller lesions improved. 18th Day: There was a dry dermatitis so no further treatment until 24th day, when last treatment was given. Fig. 10 shows case at this stage.

TABLE 11

BENZALKONIUM CHLORIDE

Group	Cattle Used			Lesions	Details of Treatment	Remarks
	Nos.	Age	Sex			
K	12	1Yr	N	Head, neck, thorax, flank, sacral and peronial. Lesions 1" A few large areas 4" x 5"	1% Initially and on 8th and 30th days. (Only 4 treated on 30th day.)	Extension ceased after one treatment. 8th Day: Two completely cured (small lesions). Most lesions looked inactive. Only lesions in places difficult to soak, e.g. edge of ear were active. These, and several badly crusted lesions, were positive in culture. 30th Day: All appeared cured except one with thick crust on frontal region. This was negative culturally, as were samples from the other three with possibly active lesions. Four animals with scabs were treated, removing the scabs without haemorrhage. 40th Day: Good growth of hair.

GENERAL DISCUSSION AND CONCLUSIONS

The Survey disclosed 888 (2.89%) affected cattle in a total of 30,766 animals in 518 herds, of which 133 herds (25.3%) were infected, and that in all the outbreaks of ringworm the causal dermatophyte was T. discoides. The main factor influencing the incidence of the disease was age; 7.43% of calves but only 0.43% of adults were affected. Contrary to commonly held views the survey revealed no seasonal variation in the incidence, though the type of herd did influence the incidence. Within the area of Northern Britain surveyed, no significant variation in the overall incidence of ringworm could be detected between the different counties. The difference in overall incidence in affected herds in this area compared with the area of East Anglia surveyed by Mortimer (1955) is associated with a higher percentage of young stock in his sample. Whether the low incidence in adult cattle is due to immunity or reduced opportunity for infection is undetermined. The proved survival of arthrospores in dry material for over four years in the case of two Trichophyta of bovine and equine origin, and for one and a half years in the case of T. mentagrophytes and M. canis from cats and dogs, and the ineffective action of ultra-violet light as a practical method of killing spores in debris, has demonstrated the importance of this factor in Public Health and Veterinary Preventive Medicine and the need for reliable fungicides.

Lysol, representing the Cresol type of disinfectant, and Sodium hypochlorite, have been shown to be fungicidal at concentrations below those normally used for disinfecting purposes. While the possibility of using the surface active anionic detergents for decontamination of infected material has been pointed out, further study of the basic components which are generally either the secondary alkyl sulphates or alkane sulphonates or alkyl aryl sulphonates may prove rewarding in the search for a therapeutic agent to meet the stated requirements. The difference in the length of hydrocarbon chains of the components is likely to influence the antimycotic efficiency of the individual product as is the case with the bisquaternary ammonium compounds (Collier et alia 1955) and the fatty acids. The apparent superiority of By-Prox as a fungistatic agent is in all probability ascribable to this factor. Though after twelve hours' exposure all five quaternary ammonium compounds were fungicidal at less than 0.05% concentrations there was wide variation between them at the end of ten or sixty minutes. None were efficient in low enough concentrations for use as contact fungicides. Benzalkonium chloride and Cetrimide in clinical trials gave encouraging results, though the resistance of one case to the latter compound indicates that extended trials are desirable. These are not always so easy to arrange as /

as Sellars (1956) claimed. It is often difficult to arrange controls, uniform intervals between treatments and sufficient affected animals on a farm within reach of the investigator. With dermatophytes, the age of the lesion is clearly important, for manifestly, lesions about to heal spontaneously give false values to any agents tested. To overcome this at least for the screening of drugs, 'in vivo' an experimental infection in a laboratory animal is desirable, and the need has been recognised by investigators (Smith 1956). They have been restrained, however, by the fear of infection of the attendants and spread of the disease to their stock animals. It has been the experience during the present study that T. discoides produced discrete lesions on guinea pigs without secondary lesions appearing or spread to contact guinea pigs occurring, which contrasts with the behaviour of T. mentagrophytes on the guinea pig (Menges and Georg 1956). By isolating the test unit, arranging for appropriate handling of the animals and equipment by attendants with rubber gloves no spread need occur. The results of the therapeutic tests on the experimental lesions confirmed the potentialities of such a method provided a suitable combination of experimental animal and dermatophyte can be arranged.

SUMMARY

1. In a Survey the results of which were examined statistically, of 30,766 cattle in 518 herds, 888 (2.89%) cattle were affected with ringworm caused by T. verrucosum var discoides. 133 of the herds were affected.

2. Young stock were affected more frequently than mature cattle, the overall incidence in calves being 7.34% and in adults 0.43%. In Rearing Herds the incidence was higher than in Dairy or Beef Herds.

3. The incidence did not appear to be influenced by season of the year, location of the herd, nutrition, the lighting of the cattle sheds or the presence of lice on the cattle.

4. Two outbreaks of T. discoides infection of the horse were recorded, and the occurrence of T. mentagrophytes infection in the pig was described for the first time in Britain.

5. The arthrospores of T. discoides and T. equinum were demonstrated to survive for over 4½ years and 4 years respectively, and those of T. mentagrophytes and M. canis for 1½ years when stored in dry skin scrapings.

6. Experimentally it was shown that while ultra-violet light was lethal to the spores of T. discoides, hair, scabs, on /

on lesions and skin itself all combined to protect the dermatophyte. Sunshine did not appear to influence the spread of cattle ringworm.

8. Using a Paper Disc Technique one hundred and fifty-six chemicals were screened for antimycotic activity against T. discoides. These have been listed according to their activity and discussed.

9. Eighteen of the screened substances were examined in liquid medium for fungistatic and fungicidal activity against T. discoides. 'Captan', Monosulfiram, Sodium hypochlorite, five quaternary ammonium compounds and seven surface active anionic detergents were fungistatic at less than .005 mg. of active agent per ml. of medium. As fungicides, Lysol, Captan, Monosulfiram, and Sodium hypochlorite were efficient in ten minutes. Three quaternary ammonium compounds, Benzalkonium chloride, M. & B. 1732 and Teoquil, but none of the surface active detergents were fungicidal in ten minutes. By increasing the exposure time to one hour and twelve hours, a progressive increase in fungicidal efficiency was demonstrated for both classes of compounds. The potentialities of these substances as therapeutic agents and disinfectants is discussed.

10. /

10. On guinea pigs experimentally infected with twenty-one strains of T. discoides the antimycotic activity of seven drugs was studied. 1% Solution of Benzalkonium chloride and of Cetrimide exhibited measurable antimycotic activity. The use of the guinea pig for this purpose has been discussed.

11. From the results of clinical trials of Monosulfiram, Benzalkonium chloride and Cetrimide on cattle, Benzalkonium chloride and Cetrimide appeared to be of value and to merit further trial

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A P P E N D I X A.

CATTLE RINGWORM SURVEY.

TABLES 1 and 2

CATTLE RINGWORM SURVEY

APPENDIX A

TABLE 1

The number of affected and non-affected cattle for the various age groups of animals and type of farm

For all cattle examined

		October and November 1955					December 1955 and January 1956				
		Adults	Heifers	Bullocks	Calves	Total	Adults	Heifers	Bullocks	Calves	Total
Dairy	A	13	24	15	91	143	2	25	14	13	54
	NA	2771	1819	322	1003	5915	1070	750	149	466	2435
Beef	A	1	4	5	44	54	0	12	15	18	45
	NA	392	344	875	358	1969	258	159	623	262	1302
Rearing	A	1	6	3	32	42	17	12	13	45	87
	NA	315	367	287	352	1321	791	485	352	710	2338
Total	A	15	34	23	167	239	19	49	42	76	186
	NA	3478	2530	1484	1713	9205	2119	1394	1124	1438	6075
Total Cattle		3493	2564	1507	1880	9444	2138	1443	1166	1514	6261

For cattle in affected herds only

Dairy	A	13	24	15	91	143	2	25	14	13	54
	NA	996	528	152	404	2080	199	155	83	116	553
Beef	A	1	4	5	44	54	0	12	15	18	45
	NA	208	147	219	203	777	89	41	378	61	569
Rearing	A	1	6	3	32	42	17	12	13	45	87
	NA	150	143	52	139	484	131	163	111	161	566
Total	A	15	34	23	167	239	19	49	42	76	186
	NA	1354	818	423	746	3341	419	359	572	338	1688
Total Cattle		1369	852	446	913	3580	438	408	614	414	1874

APPENDIX A

TABLE 1 (Contd.)

The number of affected and non-affected cattle for the various age groups of animals and type of farm

For all cattle examined

		February and March 1956					April and May 1956				
		Adults	Heifers	Bullocks	Calves	Total	Adults	Heifers	Bullocks	Calves	Total
Dairy	A	3	44	0	27	74	0	23	2	34	59
	NA	1167	747	85	452	2451	1468	1006	133	626	3233
Beef	A	6	25	5	0	36	0	6	0	25	31
	NA	147	122	244	108	621	231	176	773	227	1407
Rearing	A	1	1	4	10	16	0	17	1	75	93
	NA	300	201	88	90	679	378	549	304	387	1618
Total	A	10	70	9	37	126	0	46	3	134	183
	NA	1614	1070	417	650	3751	2077	1731	1210	1240	6258
Total Cattle		1624	1140	426	687	3877	2077	1777	1213	1374	6441

For cattle in affected herds only

Dairy	A	3	44	0	27	74	0	23	2	34	59
	NA	601	420	25	266	1312	287	245	46	104	682
Beef	A	6	25	5	0	36	0	6	0	25	31
	NA	136	105	56	56	353	48	51	87	78	264
Rearing	A	1	1	4	10	16	0	17	1	75	93
	NA	78	35	12	30	155	117	228	143	182	670
Total	A	10	70	9	37	126	0	46	3	134	183
	NA	815	560	93	352	1820	452	524	276	364	1616
Total Cattle		825	630	102	389	1946	452	570	279	498	1799

CATTLE RINGWORM SURVEY

APPENDIX A

TABLE 1 (Contd.)

The number of affected and non-affected cattle for the various age groups of animals and type of farm

For all cattle examined

		July and August 1956					Total for Year				
		Adults	Heifers	Bullocks	Calves	Total	Adults	Heifers	Bullocks	Calves	Total
Dairy	A	3	54	4	35	96	21	170	35	200	426
	NA	1089	843	205	556	2693	7565	5165	894	3103	16,727
Beef	A	0	8	21	2	31	7	55	46	89	197
	NA	228	354	456	217	1255	1256	1155	2971	1172	6554
Rearing	A	0	2	0	25	27	19	38	21	187	265
	NA	177	115	157	192	641	1961	1717	1188	1731	6597
Total	A	3	64	25	62	154	47	263	102	476	888
	NA	1494	1312	818	965	4589	10,782	8037	5053	6006	29,878
Total Cattle		1497	1376	843	1027	4743	10,829	8300	5155	6482	30,766

For cattle in affected herds only

Dairy	A	3	54	4	35	96	21	170	35	200	426
	NA	267	192	32	158	649	2350	1540	338	1048	5276
Beef	A	0	8	21	2	31	7	55	46	89	197
	NA	53	75	64	33	225	534	419	804	431	2188
Rearing	A	0	2	0	25	27	19	38	21	187	265
	NA	62	68	80	61	271	538	637	398	573	2146
Total	A	3	64	25	62	154	47	263	102	476	888
	NA	382	335	176	252	1145	3422	2596	1540	2052	9610
Total Cattle		385	399	201	314	1299	3469	2859	1642	2528	10,498

There were no data available for the months of June and September 1956.

CATTLE RINGWORM SURVEY

APPENDIX A TABLE 2

EFFECT OF TYPE OF HERD ON INCIDENCE IN VARYING CLASSES OF STOCK

ADULTS

	Affected		Not Affected		
	Obs.	Exp.	Obs.	Exp.	Total
Dairy	21	32.92	7565	7553.08	7586
Beef	7	5.48	1256	1257.52	1263
Rearing	19	8.60	1961	1971.40	1980
	47		10782		10829

$$X^2 = \begin{matrix} 4.32 & 0.02 \\ 0.42 & 0.00 \\ 12.23 & 0.05 \end{matrix} = 17.04$$

BULLOCKS

	Affected		Not Affected		
	Obs.	Exp.	Obs.	Exp.	Total
Dairy	35	18.38	894	910.62	929
Beef	46	59.70	2971	2957.30	3017
Rearing	21	23.92	1188	1185.08	1209
	102		5053		5155

$$X^2 = \begin{matrix} 15.03 & 0.30 \\ 3.14 & 0.06 \\ 0.36 & 0.00 \end{matrix} = 18.89$$

HEIFERS

	Affected		Not Affected		
	Obs.	Exp.	Obs.	Exp.	Total
Dairy	170	169.05	5165	5165.95	5335
Beef	55	38.34	1155	1171.66	1210
Rearing	38	55.61	1717	1699.39	1755
	263		8037		8300

$$X^2 = \begin{matrix} 0.01 & 0.00 \\ 7.24 & 0.24 \\ 5.58 & 0.18 \end{matrix} = 13.25$$

CALVES

	Affected		Not Affected		
	Obs.	Exp.	Obs.	Exp.	Total
Dairy	200	242.55	3103	3060.45	3303
Beef	89	92.60	1172	1168.40	1261
Rearing	187	140.85	1731	1777.15	1918
	476		6006		6482

$$X^2 = \begin{matrix} 7.46 & 0.59 \\ 0.14 & 0.01 \\ 15.12 & 1.20 \end{matrix} = 24.52$$

A P P E N D I X B.

THE ANTIMYCOTIC ACTIVITY OF CHEMICALS

PAPER DISC TECHNIQUE

TABLES I to VII

APPENDIX B.

TABLE 1.

Chemicals failing to produce a zone of inhibition

Ammonia 10%
Atropine Sulphate (Pulv.)
Aureomycin (Pulv.)
Barium Chloride (Crystals)
Benzadrine (2%)
Benzole (100%)
Brom-cresol purple (Pulv.)
n-Butyl Alcohol (100%)
Carbon Tetrachloride (100%)
Chloramphenicol (10% Topical)
Chrysoidon Y (1%)
Chlorozol Fast Pink (Pulv.)
Chlorparacide 20% (Pulv.)
Dapsone (25% Paste)
Dettol 2%
Dihydroxyanthraquinone B. Vet. C. (Pulv.)
Diethyl Carbamazine 2% Sol.
Diethylamine Acetarsol 9.4%
Ethyl Alcohol 75%
Glycerol 100%

Harrington's Solution	Hydrog. Perchlor.	gm. 1.6
	Ac. Hydrochlor. Dil.	ml. 112
	Sp. Vini Meth.	ml. 1078
	Chrysoidin Y	mg. 16
	Aqua. Dest.	ml. 596

Hexamethonium Bromide (Pulv.)
Liq. Plumbi. Subacet. (Pulv.)
Lisapol (100%)
Methyl Benzoate
Oestroform (B.D.H.) (ester of oestrogenic hormones)
Orange G (Pulv.)
Oxalic Acid
Oil (Wate Engine)
Phenol 5%
Phenolphthalein (Pulv.)
Phlorizin (Pulv.)
Piperazine Adipate (Pulv.)
Piperazine Citrate (Pulv.)
Piperazine B.P.C. (Crystal)
Pot. Permanganate (Crystal)
Prostigmin (Roche) 0.05% Sol.
Proflavine Hemisulphate 0.1% Sol.
Pyridine 100%

APPENDIX B. (Contd.)

TABLE 1 (Contd.)

Salicylic Acid (Pulv.)
Salicylic Aldehyde (100%)
Salicyl Sulphonic Acid 20%
Santonin (Pulv.)
Saponin (Pulv.)
Sodium Carbonate 4%
Sodium Glycarsamate 30%
Sodium β - Naphthaquinone - 4 - Sulphonate (Crystals)
Sodium Propionate 10%
Sodium Sulphacetamide 6%
Stilboestrol D (in oil 10 mgm./ml.)
Sulphamilamide 1%
Sulphadimidine (Pulv.)
Sulphathiazole Sod. (45%)
Sulphaquinoxaline (36% Sol.)
Terramycin 2.5% Sol.
Tetrachlorethylene 100%
Urea (Crystals)
Urethane (Crystals)
Uranium Nitrate (Crystals)
Vit E. (∝ -tocopheral acetate) 10 mgm. solid
Zinc Sulphate (Crystals)
Zinc Sulphanilate (Pulv.)

TABLE II

Chemicals producing a zone of 1-5 mm. Radius

Acetone (100%)	2 mm.
'Agroxone' (n-chlorophenoxyacetic acid)	5 mm.
Benzaldehyde (100%)	5 mm.
Captan (0.5%) (N-trichloromethyl-thiotetrahydrophthalamide)	5 mm.
Copper Sulphate (2% Sol.)	2 mm.
Dettol 20% (one-fifth Dil. of Chloroxylenol B.P. 4.8% Terpineol B.P. 9%)	1 mm.
Dicotox (2:4 dichlorophenoxyacetic acetate)	5 mm.
Dibrogan Cream (Dibromopropamidine isothionate 0.15% Promezathine 2%)	5 mm.
Diphehan B.P. (Pulv.)	5 mm.
Evans Blue (Pulv.)	3 mm.
Gamma Benzine Hexachloride (5% in oil)	3 mm.
Hibitane (I.C.I.) (Pulv.)	4 mm.
Hydrarg. Iod. rub. 1/500 in Meth. Sp.	2 mm.
Liq. Iodi. Mitis B.P.C.	5 mm.
Lithium Antimony Theomalate 6%	2 mm.
Phenothiazine (Pulv.)	2 mm.
Sod. Hypochlorite 1%	3 mm.
Sulphur (Flowers of)	2.5 mm.
Tannic Acid (Pulv.)	3 mm.
Tinct. Benzoini Co. B.P.C.	

APPENDIX B. (Contd.)

TABLE III

Chemicals producing a zone of 6-10 mm. Radius

Acid acetic (Glacial) 100%	6 mm.
Benzidine (Solid)	6 mm.
Benzyl Benzoate 25% Suspension	10 mm.
Cocaine (Crystals)	9 mm.
'Cuprinol' (Copper Fungi Dressing for Wood)	10 mm.
Fuchsin, Basic 3%	9 mm.
Hydrarg. Perchloridum (Pulv.)	10 mm.
Iodoform (Pulv.)	6 mm.
Liq. Cresolis Sap. (50% Cresol) 5% Sol.	8 mm.
Naptharesorcinol (Pulv.)	9 mm.
Pentachlorphenol 2%	10 mm.
Phenol Liq. B.P.	10 mm.
Sec, Octyl Alcohol 100%	6 mm.
Soap ('Lux Flake')	7 mm.
Sodium Ricinoleate (Solid)	6 mm.
Sodium Taurocholate (Pulv.)	10 mm.
Tar Dermanent (P. D. & Co.)	7 mm.
Veratrine (Pulv.)	8 mm.

APPENDIX B. (Contd.)

TABLE IV

Chemicals producing an inhibitory zone of 11-15 mm. Radius

Benzalkonium Chloride 1%	18 mm.
Castillani Paint (Basic Fuchsin 10 gm. Acetone 5 ml. Boric Acid 1 gm. 5% Phenol 100 ml. Resorcin 10 gm.)	15 mm.
Cetrimide 1%	15 mm.
Copper Sulphate (Crystals)	12 mm.
Copper (Colloidal) ('Buisol' Horticultural Fungicide)	15 mm.
Daz (Pulv.) (Surface active anionic detergent, T. Hedley & Co. Ltd.)	11 mm.
Dequadin Chloride 1% (Decamethylene-bis-4-aminoquinaldinium Chloride, Allen & Hanburys Ltd.)	15 mm.
Di-iodohydroxyquinoline (Pulv.)	15 mm.
Di-phenyliodoniumphthalimide 1%	15 mm.
Formalin 4%	12 mm.
Lactophenol (Mounting Solution) (Aqua dest. 20 ml. Phenol 20 gm. Lactic Acid 20 ml. Glycerol 40 ml.)	15 mm.
M. & B. 1732 (New Quaternary Ammonium Compound, May & Baker)	11 mm.
Methyl Violet B. 0.5%	12 mm.
Monosulfiram 1%	15 mm.
Omo (Surface active detergent, Hudson & Knight Ltd.)	14 mm.
Quinodine (Pulv.)	12 mm.
Silenium disulphide 1% Suspension	15 mm.
Sodium Borate (Pulv.)	15 mm.
Sodium Fluoride (Pulv.)	13 mm.
Sodium Lauryl Sulphate 1%	18 mm.
Surf (Pulv.) (Surface active detergent, Lever Bros. Ltd.)	12 mm.
Tide (Pulv.) (Surface active detergent, T. Hedley & Co. Ltd.)	14 mm.
Teepol (Surface active agent, Shell Products Ltd.)	15 mm.

TABLE V

Chemicals producing an inhibitory zone of 16-20 mm. Radius

'Asterol' Ointment (Roche Lab. Ltd.) (6-(2-dimethylamino-thoxyl)-2-dimethylamino-benzothiazole) (Incomplete Inhibition)	20 mm.
Acriflavine (Pulv.)	18 mm.
Brilliant Green (Pulv.)	18 mm.
Cobalt Chloride (Crystals)	16 mm.
Crystal Violet (Pulv.)	18 mm.
'Histantin' (dl-1-P-chlorobenzhydryl-4-methyl piperazine 2% monohydrochloride + 0.1% chlorocresol, Burroughs Wellcome & Co.)	18 mm.
Naphtholethylene diamine (Pulv.) (Incomplete Inhibition)	18 mm.
Phenylmercuric Chloride 0.2%	17 mm.
Penotrane Tincture (0.04% organic mercurial content, Ward, Blenkinsopp & Co.)	17 mm.
Penotrane Solution (0.04% organic mercurial content, Ward, Blenkinsopp & Co.)	17 mm.
Undecylenic Acid 5% + Zinc Undecylenate 20% Ointment	20 mm.

APPENDIX B. (CONTD.)

TABLE VI

Chemicals producing an inhibitory zone of 21-25 mm. Radius

Boric Acid (Pulv.)	22 mm.
Benzalkonium Chloride (10% Sol.)	25 mm.
Cetrimide B.P. (20% Sol.)	26 mm.
Dichlorophen (2%)	22 mm.
Lysol (Solution of Cresol with soap) (50% Cresol content)	25 mm.
Potassium Iodide (Crystals)	24 mm.
Promethazine Hydrochloride 5% (Incomplete)	25 mm.
Teoquil Chloride 1% (Hexadecamethylene-bisisoquinolinium Chloride, Allen & Hanburys Ltd.)	23 mm.

TABLE VII

Chemicals producing an inhibitory zone greater than 25 mm. in Radius

Captan (Pulv.) (50% N-trichloromethyl-thiotetrahydrophthalamide)		40 mm.
Creosote (Beechwood)	over	75 mm.
Dettol 100% (9% Terpineol; 4.8% Chloroxylenol)		40 mm.
Hexachlorethane (Pulv.)	over	75 mm.
8-Hydroxyquinolinum (Pulv.)		26 mm.
Liq. Picis Carbonis B.P.		30 mm.
Mercurial Compounds:		
'Aerosan' (Organic mercurial fungicide 1.56 Mercury content)		26 mm.
'Aretan' (Organic mercurial fungicide for bulbs)		26 mm.
'Harvesan' (Organic mercurial fungicide for seed drying 1.5% Hg.)		26 mm.
'Tulisan' (Organic mercurial horticultural fungicide)		35 mm.
Hydrarg. Iod. Rub. (Pulv.)	over	75 mm.
Thiomersal 0.1%		45 mm.
Monosulfiram (25% Alcoholic Sol.)		29 mm.
Sod. Azide (Pulv.) (Incomplete inhibition)		35 mm.
Terpineol (25% emulsion)		62 mm.
Thymol (solid)		75 mm.
Trinitrophenol (1 in 1000 of Sat. Aq. Sol.)		75 mm.

APPENDIX C.

TRICHOPHYTON MENTAGROPHYTES

NATURAL INFECTION IN PIGS

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INTRODUCTION

While examining material submitted to them during the Survey of Animal Mycosis sponsored by the Agricultural Research Council in 1951 Ainsworth and Austwick were unable to find a dermatophyte in any of the samples of porcine origin. (Ainsworth and Austwick 1954).

Clinically, Pityriasis rosea in the pig (Glässer et alia 1950) resembles ringworm in man and is often erroneously so described. The natural infection in pigs due to Trichophyton mentagrophytes is described for the first time in Britain.

CLINICAL FINDINGS

During December 1955 a large white fattening pig of five months of age suffered for several weeks from pruritus and dermatitis. After six weeks the only evidence of the disease consisted of rounder patches of golden tinted hairs on the thorax and flanks. A six months old gilt had shown a similar lesion for a month. There were a number of vertical elongated fresh red traumatic lesions on both sides of the thorax as a result of the pruritus. Examination

of /

of the skin revealed a number of small red roughly circular areas on the neck and sides of the trunk while two larger lesions were noted. One of these, measuring 5" x 3½", occurred below and behind the right ear (Fig. 1), and the other, measuring 3" x 2", was present on the medial aspect of the left thigh above the level of the patella (Fig. 2). These lesions were red in colour, covered by a thin, brownish dried crust, under which, after clipping the hair and washing off the debris, numerous red upstanding hair follicles were visible. The circular lesions with a raised margin found in Pityriasis rosea are shown (Fig. 3) for comparison. With epilation forceps a number of hairs were removed and those with a root sheath were chosen for laboratory examination. After treatment for a few seconds with hot 10% Potassium hydroxide such a hair was flattened under a cover glass. Numerous spores of 3 μ were evident forming a sheath round the root of the hair (Fig. 4). Branching hyphae of 2 μ diameter were noted within the hair running chiefly lengthwise. The mycelium in many cases extended from the root bulb (Adamson's fringe) outwards for one-third the length of the hairs; branching suggesting outward /

outward growth was evident at the outer limit of mycelial development (Fig. 5). Longitudinal sectioning of the hair showed that the hyphae had invaded the cortex and medulla of the hair (Fig. 6). Spore formation within the hair occurred chiefly between the cortex and medulla.

Identification of Dermatophyte

After teasing a hair in sterile distilled water on a slide, innoculations on nutrient agar (Austwick 1954) and Sabourqud's glucose agar produced typical growths of Trichophyton mentagrophytes. This strain produced a purple colour on the latter medium and the surface growth was very granular in appearance.

Histological Examination

Two small pieces of skin were removed for histological study. Sections were stained by haematoxylin and eosin, or by the Periodic Acid Schiff Technique after fixation in Bouin's Fluid. Only in a few places where hyphae occurred in the early cornified layers or hair follicles was cornification or crusting of the epidermis notable. Occasional infiltrating cells, chiefly eosinophils, were present in the epidermis. In the dermis masses of infiltrating lymphocytes /

lymphocytes, plasma cells, fibroblasts and eosinophils were associated with numerous proliferating capillaries. The predominant cells in any one place were either lymphocytes or fibroblasts. At the base of an infected follicle a similar cellular infiltration was present. There was no evidence of secondary infection.

Sequelae

No treatment was carried out. Six weeks after the photographs were taken the lesions had healed without loss of hair. Nine weeks after clipping, the hair over the neck lesion was approximately 5 mm. long, and of the same golden tint noted in the first affected pig. Microscopically these hairs contained numerous brown coloured hyphae, which failed to grow on culture media. Fifteen weeks from the date of photographing, the hairs, only a few of which were of a golden colour, were of normal length. Microscopically only some granular material could be seen distributed along the substance of the hairs. These hairs also proved negative on cultural examination.

Two of three contact gilts developed a few small scattered areas of infection, the most obvious being 1" diameter /

diameter on the volar aspect of the right carpus. The pruritus in these cases lasted about two weeks and no extension of the areas occurred.

DISCUSSION

The origin of the infection has not been determined. This disease did not cause any loss of condition nor serious skin blemish. No hairs were shed and it is probable that the disease is more prevalent than the absence of published data suggests. In pigs kept in dirty surroundings or pigs with good coats of hair the lesions would not be outstanding and the scratching might well be erroneously related to the activities of mange mites or lice if the pigs are not carefully examined. The golden colour observed in the hairs of the recovered pigs can be related to the brown pigment seen in the dead hyphae within these hairs.

No fungi could be demonstrated culturally or histologically in material from a number of cases of Pityriasis rosea which agrees with the findings of the German workers referred to by Glässer, and contrasts with the ease with which Trichophyton mentagrophytes was recovered from the case here described.

SUMMARY /

SUMMARY

1. The occurrence of Trichophyton mentagrophytes infection in pigs in Britain is recorded.

2. The disease was self-limiting, healing in less than ten weeks from the time of lesion development under the conditions prevalent in the outbreak.

ACKNOWLEDGMENT

I wish to thank Professor G.C. Ainsworth for examining scrapings from Pityriasis rosea cases. For the same reason and for confirming the identity of the dermatophytes here reported I am indebted to Mr. P.K.C. Austwick. I should also like to acknowledge help from a number of members of the technical staff of this School - especially are thanks given to R.S. Hood, F.I.B.P., F.I.S.T., for the preparation of the photographs.

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Austwick, P.K.C. (1954). Vet. Record 66.224.

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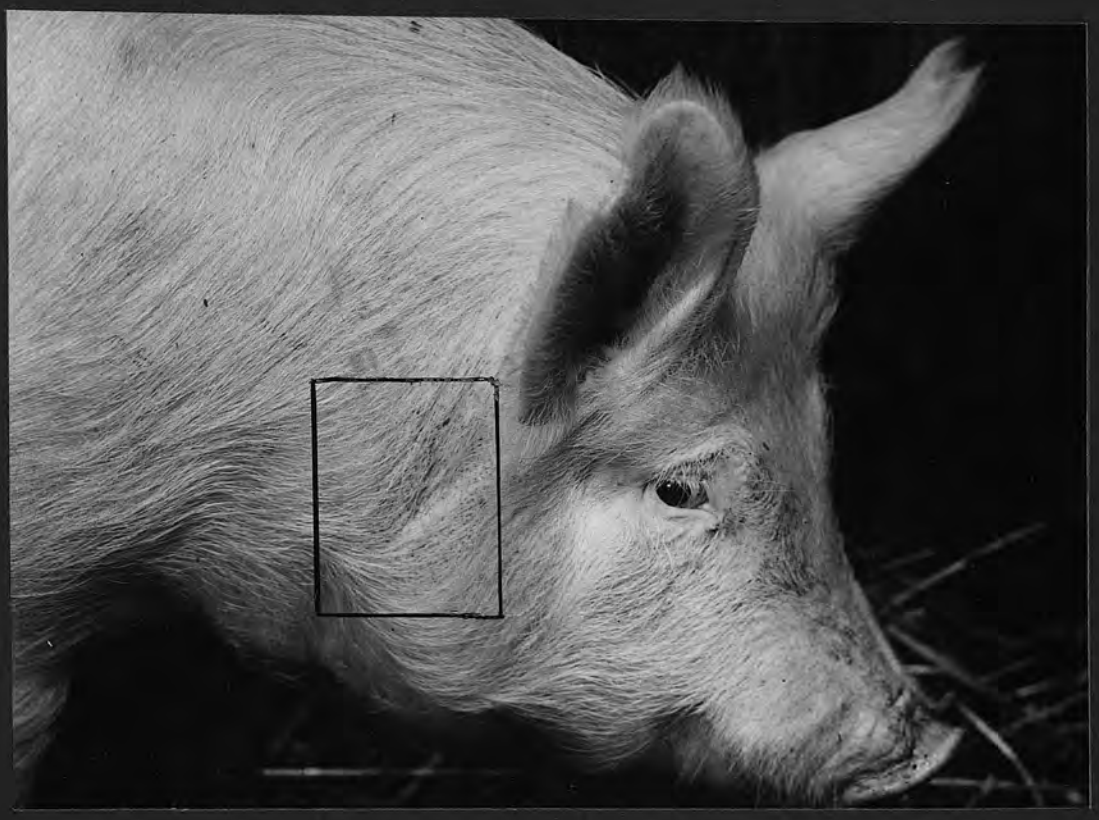

Fig. 1. T. mentagrophytes. 6 months old gilt. Skin lesion 5" x 3½".

Fig. 2. T. mengagrophytes. 6 months old gilt. Skin lesion 3" x 2".

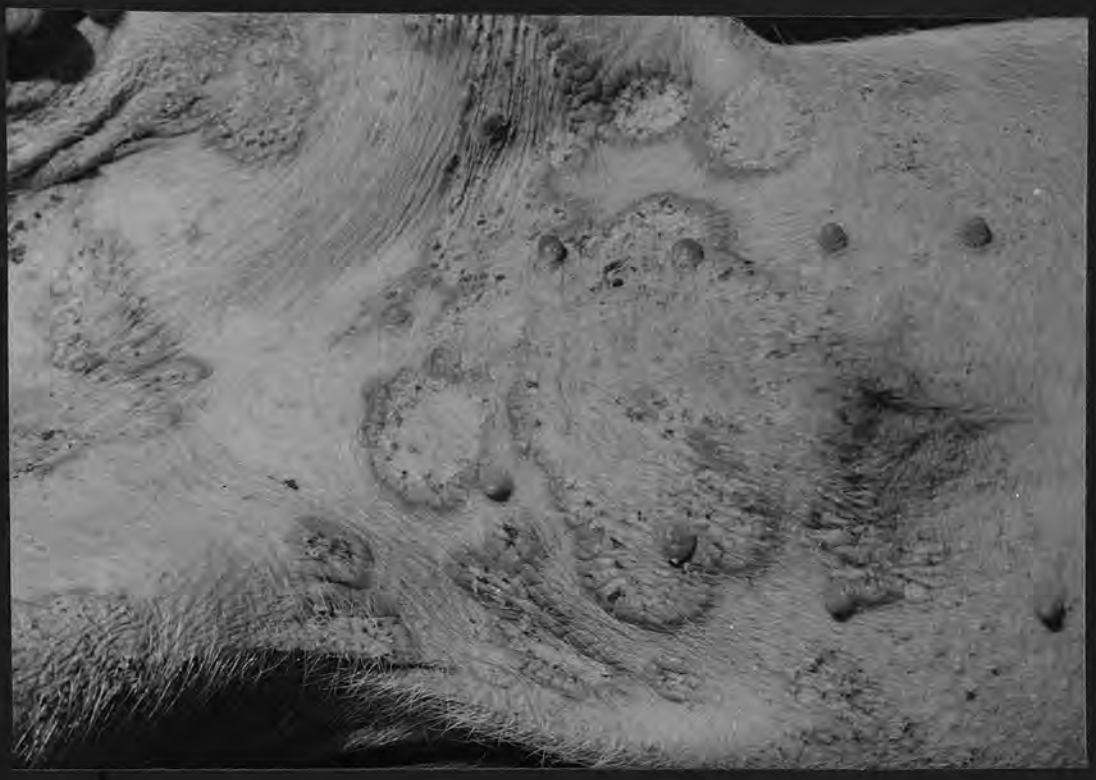

Fig. 3. Pityriasis rosea. Pig 3 months old.

Fig. 4. T. mentagrophytes. 6 months old gilt. Sheath of spores 3 u diameter around hair and endothrix hyphae of 2 u. 10% KOH unstained. X 130 approx.

Fig. 5. T. mentagrophytes. 6 months old gilt. Outer limit of mycelium in a hair. X 210 Modified Gram Stain (Claudius).

Fig. 6. T. mentagrophytes. 6 months old gilt. Hyphae within the medulla and cortex; spores between cortex and medulla. Periodic Acid Schiff Stain. X 480.

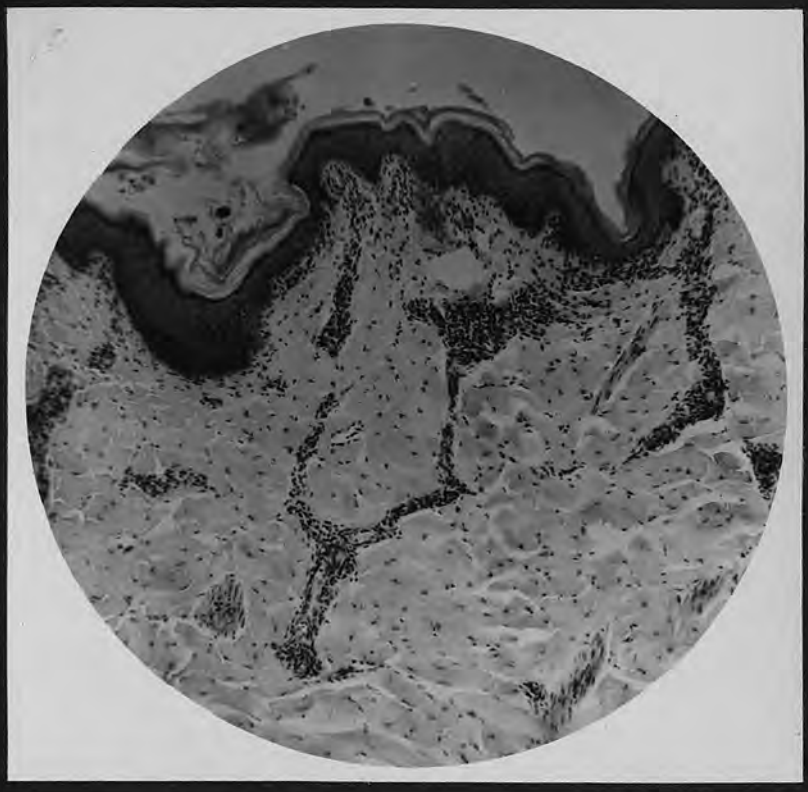

Fig. 7. T. mentagrophytes. 6 months old gilt. Cellular reaction. Haematoxylin and eosin. X150.

Fig. 8. T. mentagrophytes. 6 months old gilt. Infected hair follicle, cellular infiltration with lymphocytes, fibroblasts and eosinophils. H.& E. X150.

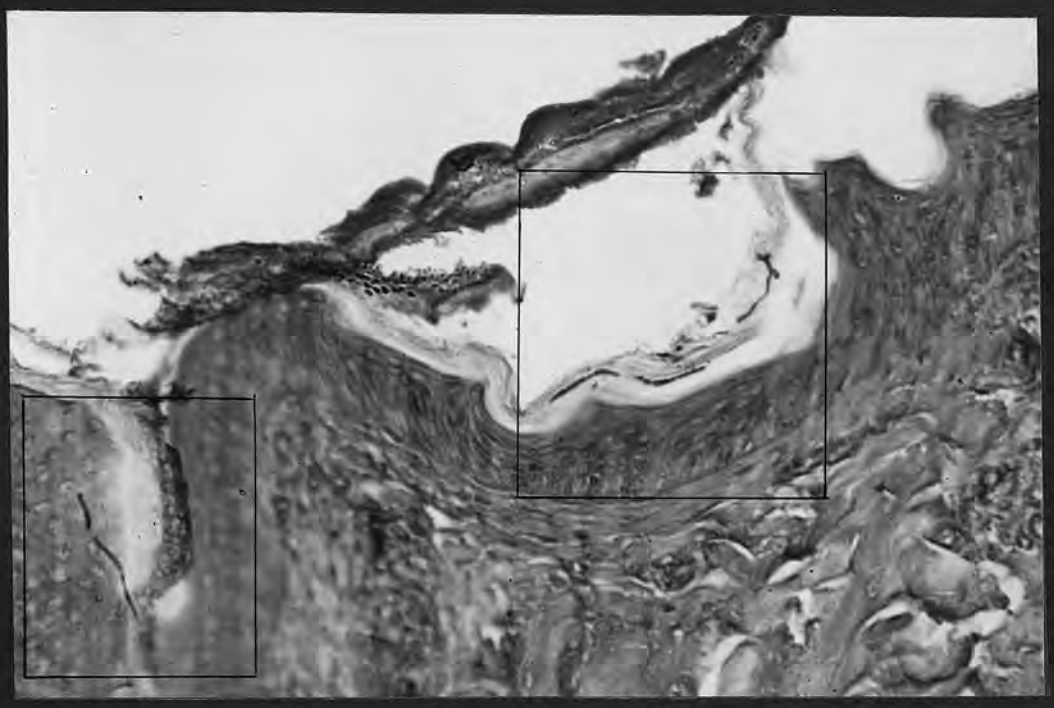

Fig. 9. T. mentagrophytes. 6 months old gilt. Mycelium in early cornified layers of epidermis and in a hair follicle. Periodic Acid Schiff Stain. X420.

Fig. 10. T. mentagrophytes. Mycelium in hair follicle. X800.

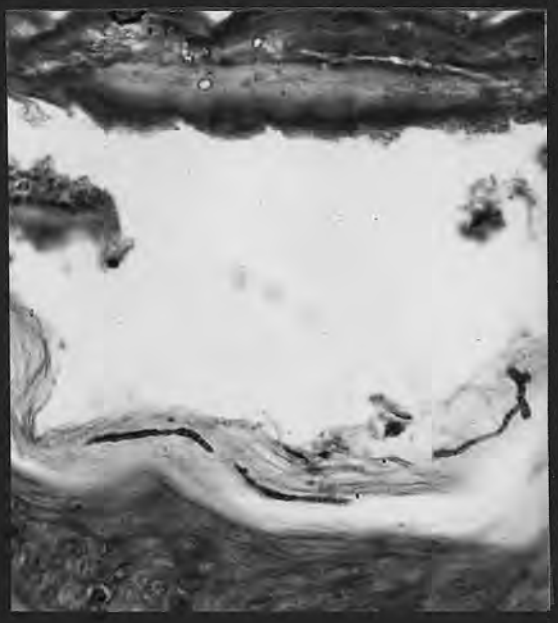

Fig. 11. T. mentagrophytes. Mycelium in early cornified layer. X800.

A P P E N D I X D.

CLINICAL TRIALS ON CATTLE.

FIGURES 1 - 12

Fig. 1. Initial lesions. Treated 25% Monosulfiram. Group A in Table 9.

Fig. 2. As in Fig. 1 but four days later.

Fig. 3. As in Fig. 1 but fifteen days later. Scabs came off easily during treatment. No further treatment was required.

Fig. 4. Initial lesions. Treated 25% Monosulfiram. Group A in Table 9.

Fig. 5. As in Fig. 4 but fifteen days later. Scabs came off easily during treatment. No further treatment necessary.

Fig. 6. Initial lesions. Treated 1% Cetrimide. Group H in Table 10.

Fig. 7. As in Fig. 6 but fourteen days later.

Fig. 8. As in Fig. 6 but forty days later.

Fig. 9. Initial lesions. Treated 2% Cetrimide. Group J in Table 10.

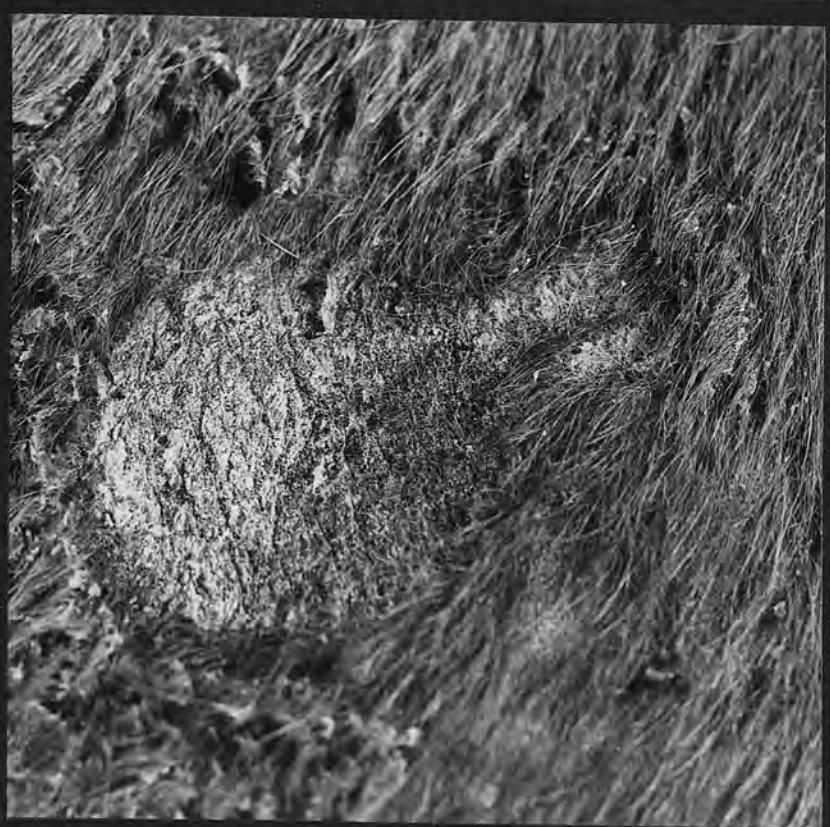

Fig. 10. As in Fig. 9 but twentyfour days later. Hair growing on lesions.

Fig. 11. Initial lesions. Treated 1% Benzalkonium Chloride. Group K in Table 11.

Fig. 12. As in Fig. 11 but forty days later. Hair on lesions now normal.