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Ringworm carriage and its control in mice

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INTRODUCTION

The carriage of *Trichophyton mentagrophytes* (Robin) Blanchard by symptom-free laboratory mice has been described by La Touche (1957), Dolan, Kligman, Kobylinski & Motsavage (1958) and Mackenzie (1961). It may often be only the development of infections in the animal handlers which draws attention to the carriage of the fungus by the mice. These ringworm infections in man may take the form of a rapidly developing eczematous and vesicular lesion and the mouse carrier constitutes a significant hazard to the laboratory animal worker.

In radiobiological and oncogenic experiments where the animal may be studied throughout its life span, killing infected mice as suggested by Parish & Craddock (1931) is impracticable and the adoption of control measures is essential.

The study to be described was initiated when six out of thirteen workers in contact with a colony of about 1500 mice belonging to a radiobiological laboratory developed ringworm (Fig. 1). In each case *T. mentagrophytes* of the mouse variety was isolated from skin scrapings taken from the patient's lesions. Less than 1% of the mice showed any signs of fungal infection.

MATERIALS AND METHODS

Animals

The mouse colony studied was of two strains, an inbred C_3H/Bi colony and a smaller breeding nucleus of BALB/c mice, randomly bred within the closed colony. The mice were kept at 65° F. in a small, air-conditioned room, and no mice were introduced from outside.

Ten other strains (A, AH, AKR, CBA, C 57 Bl, C 57 Br, C 57 L, DBA, ICI and Swiss mice) were examined in other laboratories.

Caging of mice

Breeding mice were housed in galvanized metal carriers at one end of the room, together with weaned litters. After 3 weeks, the litters were ear-marked and transferred to mesh drawers hanging in a battery rack, where they remained until placed in metal carriers again for experimental use. Breeding and experimental mice were kept in the same room since space did not allow any other arrangement and in general the breeding mice did well. The total number of mice in the room averaged 1500, with 50–60 breeding C_3H pairs, and variable numbers in stock and

experiments during the year. The numbers of the BALB/c mice in stock were always smaller, and work in clearing up the outbreak of ringworm was concentrated on the more valuable inbred stock.

Culture technique

The number of mice carrying the fungus was determined by rubbing a Petri dish of malt-extract agar (containing 40 μg./ml. chloramphenicol and 0·5 mg./ml. cycloheximide) over the backs of the mice. The plates were incubated at 25° C. and the numbers of colonies of *T. mentagrophytes* which developed were counted from 5 to 7 days onwards.

Incidence of ringworm carriage in the colony

Random samples of approximately 10% of the mice were examined at 6-monthly intervals from January 1962 to December 1964. In addition, groups of mice representing the different categories of animal within the colony were investigated to determine the effect of age, length of time spent in the stock rack and irradiation upon the level of carriage. Evaluation of the weight of infection in experimentally treated groups was made by examining each mouse individually at weekly intervals, and counting the number of colonies of *T. mentagrophytes* isolated per mouse sweep plate. This gave both the percentage of mice in the group carrying the fungus, and the weight of carriage among the carriers.

Dipping technique

The dip used was the acaricidal dip described by Bateman (1961): this is a mixture of 2 g. DMC (di-*p*-chlorophenyl methylcarbinol, Sherwin Williams Inc., Cleveland, Ohio) dissolved in 3 g. ethanol, and 67 g. Tetmosol (25% tetraethylthiuram monosulphide in industrial alcohol, I.C.I. Ltd., Wilmslow, Cheshire) made up to 1 l. with warm tap water. The dip is kept at 37° C., and the mice are pulled through the dipping bath for 5–10 sec., so that the whole animal is immersed except for the tip of the muzzle. The animals are then momentarily completely immersed. After 10 min. the procedure is repeated; the first dip wets the fur and the second penetrates it. The double treatment is repeated after an interval of 3 weeks. This procedure is quite harmless to young mice provided that they are kept warm whilst drying, but is not without risk to mice over 1 year old, since some have died, apparently of cold, afterwards.

Antifungal activity of the dip constituents

Tetmosol and DMC were separately suspended in distilled water in the concentrations used in the complete dip, i.e. 1·7% tetraethylthiuram monosulphide and 0·2% DMC, and inoculated with 0·02 ml. of a suspension containing 10^5 spores of *T. mentagrophytes* per ml. After shaking for ½, 1 and 2 hr. drops were removed and streaked out on malt-extract agar.

Tetmosol and DMC in peptone glucose broth in the above concentrations and in tenfold dilutions were inoculated with 0·02 ml. of the same suspension of *T. mentagrophytes* and the minimum inhibitory concentrations determined.

Human hair samples were soaked in Tetmosol and DMC in the dip concentrations for 1 hr., the solutions were filtered off and the hair was air-dried. Samples of hair were placed in 25 ml. universal containers containing sterile distilled water and inoculated with a dense suspension of *T. mentagrophytes*. Untreated hair in distilled water was similarly inoculated as a control. The samples were incubated at 25° C. and examined at different time intervals after inoculation.

Level of mite infestation

The older mice in the closed colony were infected with mange mites, both *Myobia musculi* (Shrank) and *Myocoptes musculinus* (Koch). The degree of infestation was determined by the method of Tuffery and Broach (personal communication). With flamed forceps equal-sized tufts of hair were plucked from the head, back and belly, and examined in liquid paraffin under low magnification (× 100). The degree of infestation was judged on an arbitrary scoring method:

0	No trace	5–6	Dead mites
1–2	Empty eggs only seen	7–8	Live mites
3–4	Live eggs		

The scores were added at each site, to give a maximum of 20, with a total maximum per mouse of 60.

Experimental transfer of mites and ringworm

An attempt was made to transfer both mites and ringworm to non-infected animals by boxing them with mice known to carry both. Old, heavily infected experimental mice were placed with young C_3H weanlings free from both infestations. Mice were boxed in groups of five, either two donors to three recipients or one to four. Individual mite and colony counts were made on each mouse at weekly intervals.

RESULTS

In the initial survey of the colony, the fungus was isolated from over 90 % of a 10 % random sample of C_3H/Bi stock and a slightly lower percentage of the BALB/c. Whilst all except the very youngest mice were carriers, less than 1 % showed any signs of infection.

Clinical symptoms in the stock mice differed in the two strains. The agouti C_3H mice developed bald patches, usually on the back or belly, but culture of tail skin scrapings in this strain was negative. The BALB/c mice, unless experimentally irradiated, never showed hair loss, but some developed raised brown circular scabs on the tail, from which *T. mentagrophytes* was isolated. La Touche (1957) found that in two unnamed strains of mice fungal carriage on the tail was an important factor in maintaining infection in a colony.

Effect of dipping

As both strains of mice in this colony were also infested with mites, it was thought that the fungus might find a more favourable environment on mouse skin that had been abraded by mite bites. As a first step in cleaning up the colony

an acaricidal dip (Bateman, 1961) was used. This not only cleared the mice of mites but reduced fungal carriage (Davies & Shewell, 1964).

Since April 1963 breeding C_3H pairs have been routinely dipped before pairing, and from December 1963 experimental mice for long-term experiments have been dipped before being used. The success of these measures in reducing carriage of fungus and decreasing the risk to those handling the mice is shown in Fig. 1.

Only one worker became infected after the breeding stock were dipped, and there have been no infections since mice for long-term experiments have been dipped before being used. The overall incidence of mice carrying the fungus has

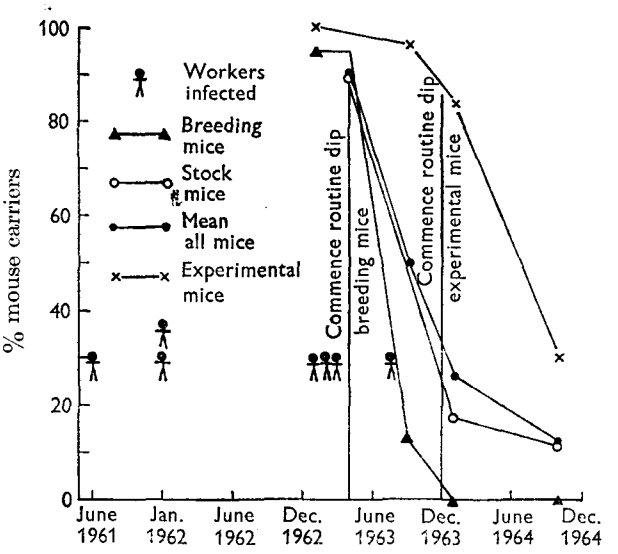

Fig. 1. Effect of routine dipping of all breeding mice from April 1963 and mice for experiment from December 1963 onwards.

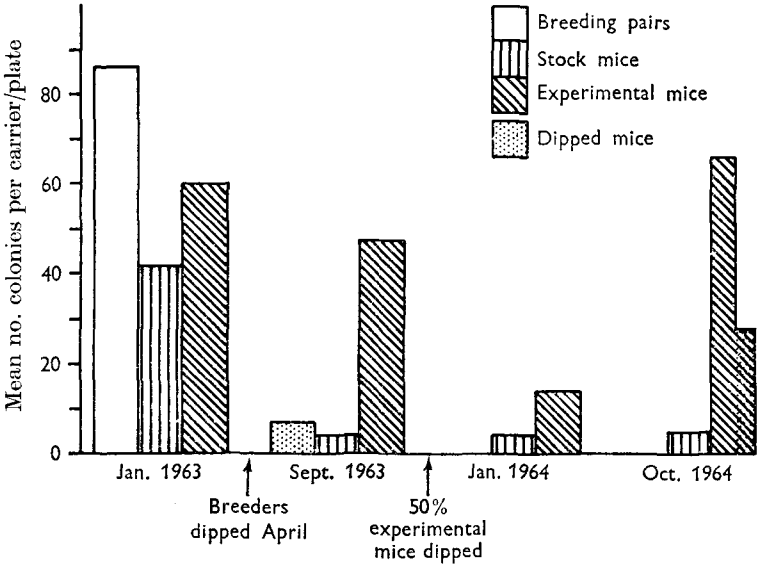

Fig. 2. Effect of dipping on the weight of fungal carriage.

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ultimately been reduced to only 12 % despite the presence in the room of experimental mice from long-term irradiation experiments which are heavily infected, but were considered too valuable to risk dipping.

Figure 2 shows the effect of dipping on reducing the weight of fungal carriage in the different groups of mice in the colony. It may be seen that the degree of carriage varied among the different groups of mice. Thus in October 1964, although 11 % of the stock mice (made up of mice born to dipped parents but not themselves dipped) were carriers, the mean number of colonies isolated from individual mice carrying fungus was only 5/plate (Fig. 2) while the undipped experimental mice had a mean count of 66 colonies/plate.

Variations in carriage of undipped mice

(*a*) *With age*

Table 1 shows the incidence of carriage in stock mice of different ages before any control measures were introduced. The percentage of carriage among the stock mice increased with increasing age (or increasing time spent in the mesh drawers). There was no sex difference in carriage rate.

Table 1. *Incidence of fungal carriage in C_3H stock mice*

Age	No. examined	Percentage mice carrying fungus
3 weeks	8	25*
8 weeks	12	66
12 weeks	12	75
3–6 months	12	92
6 months–1 year	12	100
Over 1 year	10	100

* Figure not based on a random sample, but that found in the weanlings of two litters where both parents were carriers.

(*b*) *Effect of whole-body irradiation*

Some of the experimental mice are the long-term survivors of whole-body irradiation experiments: these animals have always been found to yield higher colony counts than non-irradiated carriers of comparable age. For example, the mean colony count $\pm$ S.E. of irradiated undipped 13-month-old mice in December 1963 was $34 \pm 7{\cdot}5$ while that of undipped 13-month-old stock mice was $16 \pm 3{\cdot}2$ ($P < 0{\cdot}001$).

Antifungal activity of the dip constituents

The action of the dip in reducing the frequency and weight of fungal carriage might have been due to the removal of the mites, to antifungal activity, or to both. The compounds DMC and Tetmosol were tested separately on infected mice: both were found to be antifungal and DMC slightly the more effective (Davies & Shewell, 1964). In a more critical evaluation of antifungal activity, it was found that no colonies of *T. mentagrophytes* grew on malt-extract agar after 7 days

incubation, when the inoculating suspension had been shaken with either DMC or Tetmosol in the concentrations in which they were used in the complete dip. The shortest period of shaking tested, 30 min., was enough to prevent growth. Suitable dilutions of samples from the higher concentrations of both compounds grew *T. mentagrophytes* when transferred to malt-extract agar, showing these compounds to be fungistatic in their activity. The minimal inhibiting concentrations were found to be 0·01 % for the tetraethylthiuram monosulphide and 0·1 % for DMC.

Fungal growth on human hair samples after 10 days incubation was only visible to the unaided eye on the untreated hair. Both the DMC and Tetmosol-soaked samples appeared to be clear. After 6 weeks' incubation, however, when examined microscopically, the characteristic pitting due to the development of *T. mentagrophytes in vitro* was evident in the treated as well as the untreated hair. The frequency of the pits was greatest in the untreated hair.

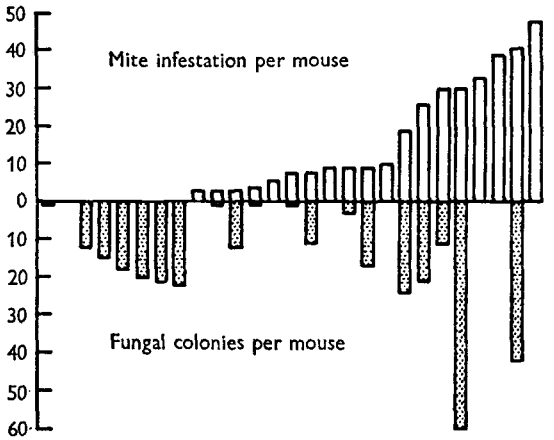

Fig. 3. Sample of twenty-seven stock mice showing level of mite infestation and number of colonies of *T. mentagrophytes* developing per mouse sweep plate.

Relationship with mite infestation

There was no correlation between the carriage of fungus and the presence of mites in the untreated stock ($\chi^2 = 0{\cdot}22$, $0{\cdot}7 > P > 0{\cdot}6$). Some mice carried the fungus and were free of mites on repeated examination; others had high mite scores and no fungus (Fig. 3). However, the highest concentrations of fungus were found on mice with mites, thus providing some support for the hypothesis that mite-abraded skin provides good lodgement for fungal multiplication.

Though it has been shown that irradiated mice gave significantly higher fungal colony counts than non-irradiated animals of the same age, there was no significant difference in mite score between the two groups (mean $\pm$ S.E. for irradiated mice = $13 \pm 2{\cdot}7$, non-irradiated mice = $13 \pm 2{\cdot}9$).

Experimental transfer of mites and fungus

No difference in transfer was found when there were two donors to three recipients, or one to four (Fig. 4 showing the pooled results).

A week after boxing the mice together, mites were found on, and a few fungal

colonies isolated from, the recipient mice. Although the mite count on the recipient mice increased until, by 4 weeks, it equalled that of the older mice, there was no comparable increase in fungal carriage which remained low throughout. When the experiments were repeated similar results were obtained showing the transmission of ectoparasites from one mouse to another does not parallel the carriage of ringworm spores.

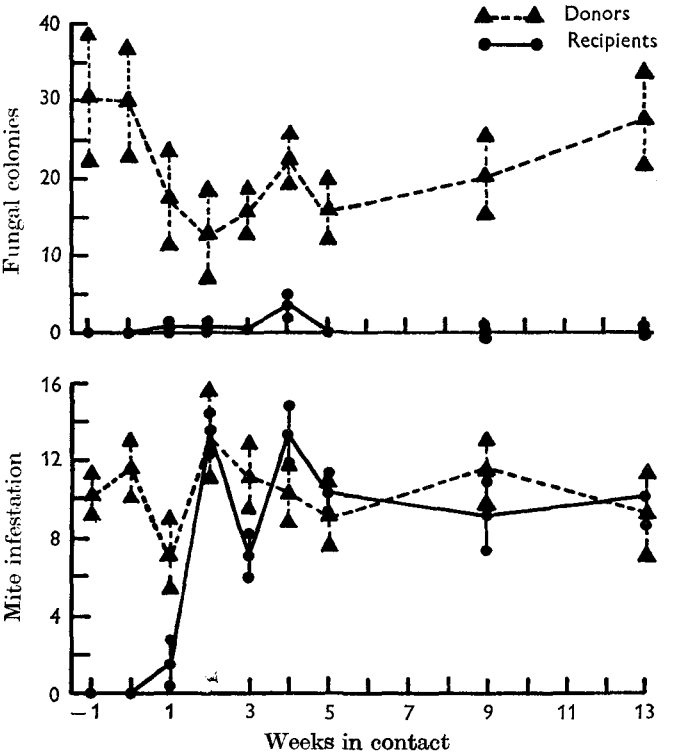

Fig. 4. Effect of contact o ı the transfer of mites and *T. mentagrophytes* from carriers to non-carriers during a 3-week period in boxes. (Points given represent mean values ± standard error.)

Incidence in other laboratories

During the past 2 years as opportunity arose, we examined mice for fungal carriage in other laboratories and from commercial sources; the strain of mouse, incidence and weight of carriage and degree of human infection, when known, are given in Table 2. Mice were purchased on three occasions from source 1 which had a low incidence of light carriage, 2–3 % of the mice being carriers and an average of three or four colonies being isolated per mouse sweep plate. When the incidence of carriage is high, however, the weight of carriage may also be high, and colonies of these mice constitute a hazard for the handler. In the C_3H mouse colony described, when 88 % of the breeding stock were fungal carriers, and the mean colony count ± S.E. per mouse sweep plate was 68 ± 8, three workers developed ringworm within 3 months. In the Leeds colony with a 100 % carrier rate and a mean carriage weight of 103 ± 21 colonies per mouse sweep plate, both the regular animal handlers were infected within a 4-month period.

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Table 2. *Incidence and weight of fungus carriage by mice in relation to proved human infections*

Source	Mouse strains	Pre-valence of mouse carriers (%)	Wt. of carriage, mean no. cols./ mouse	Human cases
L.A.C.	A, AKR, CBA, C57Br, A2G, C57Bl, C57L, DBA	0	0	None for at least past 6 years
W.F.I.	ICI	0	0	None
Source 1	Swiss	2	3	Not known
2	Swiss	8	2	Not known
3	C57Bl	90	4	Not known
S.M.H.M.S.	C_3H, BALB/c	90	61	Three cases in 4 months
Leeds (Fox: Leeds)	CBA, AH, C57Bl	100	103	Two cases in 4 months

DISCUSSION

The acaricidal efficacy of the combined DMC-Tetmosol dip has been clearly demonstrated by Bateman (1961) and Whiteley & Horton (1962). The present study shows that both the active acaricidal constituents are also fungistatic. When tested *in vitro* the antifungal activity of Tetmosol was greater than that of DMC by a factor of 10, but *in vivo* DMC appeared to be the more effective. This may be explained by the physical effect of DMC on the animals' fur. Mice dipped in DMC or a mixture of Tetmosol and DMC feel greasy to the touch for from 5 to 7 days after dipping and their fur stands up in peaks; the fur of mice dipped in Tetmosol alone, however, is groomed to normal appearance in 3–4 days.

DMC, a tertiary alcohol, is commercially available as a yellow wax, insoluble in water, soluble in alcohol but precipitating out of alcoholic solutions of less than 50%. Although the alcoholic concentration of the complete dip is approximately 20% the DMC appears to remain in suspension and this may be due to adsorption on the sulphide precipitated when the Tetmosol is mixed with warm water.

The antifungal effect of the dip would appear to be independent of its acaricidal action, since no relationship has been demonstrated between ectoparasite and fungal infection.

It has been shown that in mouse colonies where the incidence of carriage is high, the weight of carriage may also be high, and it is colonies of this type that are associated with infection in those handling the mice. Sweeping the back of a mouse with a Petri dish of malt-extract agar is a simple technique and we suggest that when the mean carriage per mouse rises to ten or more colonies there is an unnecessary hazard for the animal handler which may be eradicated with the dip described.

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