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# COMPARATIVE EFFECTS OF CHLORTETRACYCLINE AND CORTISONE ON A LOCAL MONILIAL LESION IN THE MOUSE

BY

F. O'GRADY AND R. E. M. THOMPSON

*From the Bland-Sutton Institute of Pathology, the Middlesex Hospital, London*

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The course of a local, closed monilial lesion in the thigh of the mouse is described. Treatment of animals suffering from this lesion with subcutaneous chlortetracycline in near-toxic doses leads to a persistent suppression of the lesion indistinguishable from a curative effect. Treatment with subcutaneous cortisone produces suppression of the lesion during treatment with subsequent " rebound."

Super-injection of the lesions with chlortetracycline produces a very large and persistent swelling. Treatment of these lesions with subcutaneous chlortetracycline in near-toxic doses produces suppression and " rebound " resembling that seen in lesions super-injected and treated with cortisone. Aspirin, chosen for its insolubility and acidity, and turpentine, chosen for its irritant effect, did not reproduce the effects of chlortetracycline or cortisone.

While it is possible tnat the suppressive effect of chlortetracycline and cortisone is in certain circumstances related, the local irritant effect of chlortetracycline plays an important part in the enhancement of these lesions.

Shortly after the introduction of penicillin, Geiger, Wenner, Axilrod and Durlacher (1946) described a case of generalized moniliasis following the administration of this antibiotic. Two years later, Weinstein (1947) drew attention to the spontaneous occurrence of new infections during the course of treatment with streptomycin or penicillin, and Zimmerman (1950) described further cases of generalized moniliasis following the use of these drugs. The possible importance of these findings was recognized and led some workers to explore the means by which these changes were brought about. Campbell and Saslaw (1949) claimed that the addition of streptomycin to the culture medium improved the growth of certain fungi, and Foley and Winter (1949) observed that the mortality rate of chick embryos and rabbits infected with *Candida albicans* was increased by treatment with penicillin.

Interest in these problems has been increased by reports of the frequent occurrence of moniliasis following treatment with chloramphenicol and chlortetracycline. The majority of those reporting these cases have supported the idea that overgrowth and increased invasiveness of monilia are dependent upon the suppression of normal bowel flora which accompanies the oral administration of these drugs (Tomaszewski, 1951; Woods, Manning and Patterson, 1951), and some *in vitro* results supporting this have been reported by Paine (1952). Some, however, believed that certain concentrations of the drug exert a direct stimulant effect on the growth of monilia (Moore, 1951) and it has been suggested that this effect is independent of antibiotic activity (Pappenfort and Schnall, 1951). Other workers have suggested that, either as a result of vitamin deficiency following the suppression of intestinal bacteria (Harris, 1950), or as a more direct effect of the drug (Seligmann, 1952, 1953), the defence mechanisms of the host are suppressed by chlortetracycline.

The possibility than an agent used for the treatment of infection may exert a depressant effect upon the defences of the host clearly deserves study. The work of Seligmann (1952, 1953) on the mortality of monilia-infected mice treated with chlortetracycline led him to postulate that chlortetracycline exerts an effect upon the response of the host analogous with that of cortisone. As a first step in the further study of this view we have compared the effect of cortisone and chlortetracycline on a measurable monilial lesion established in the mouse thigh. The object of this has been to see whether enhancement of the lesion

results from chlortetracycline treatment, and whether any changes which occur are comparable with those produced by cortisone.

## Materials and Methods

*Inoculation.*—Two strains of *C. albicans* were used which had been recently isolated, one from a patient suffering from a urinary infection and the other from a patient suffering from vaginitis.

An overnight broth culture of the organism was flooded on plain agar plates which were then incubated at 37° for 48 hr. The growth was harvested with a spatula and suspended in normal saline. Direct counts of this suspension were made and a final inoculum containing 150 million organisms/ml. was prepared and used immediately. Purity of the inoculum was confirmed by culture.

Mice, in groups of 10, were inoculated intramuscularly in the left thigh as previously described (Selbie and O'Grady, 1954) with 0.1 ml. of this suspension using a 1 ml. syringe fitted with a 26 s.w.g. intradermal needle. Control groups of animals were similarly inoculated in the thigh with chlortetracycline 2 mg., 5 mg., or 15 mg.; cortisone acetate 1 mg.; acetylsalicylic acid 1 to 50 mg.; or turpentine 10 to 100 mg. Each drug was dissolved or suspended in 0.1 ml. normal saline. The medio-lateral diameters of the thighs were measured daily with sliding callipers and the average increase, compared with the normal thigh, calculated.

*Subcutaneous Treatment.*—The mice in different groups infected in this way received subcutaneous injections, in the flank at the time of infection and daily for 4 subsequent doses, of chlortetracycline 2 mg., 5 mg., or 15 mg.; cortisone acetate 1 mg.; acetylsalicylic acid 1 to 50 mg.; or turpentine 10 to 100 mg. Each dose of the drug was dissolved or suspended in 0.5 ml. of saline.

*Intramuscular and Combined Treatment.*—Further groups of animals were also infected in the thigh, but using 0.05 ml. of double strength inoculum ($300 \times 10^6$ orgs./ml.), and an injection was immediately made into the infected thighs of chlortetracycline 2 mg. or 15 mg., cortisone 1 mg., aspirin 1 mg. or 50 mg., or turpentine 10 mg. Each drug was dissolved or suspended in 0.05 ml. saline and injected, where necessary, through a larger needle. These "super-injected" groups, therefore, received the same total volume of inoculum as those singly inoculated. Some groups of animals "super-injected" in this way were further treated subcutaneously with the same drugs at the time of inoculation and for 4 subsequent doses.

The various combinations of infection, intramuscular injection, super-injection, and treatment are summarized in Table I.

TABLE I

SCHEME OF TREATMENTS OF THE 17 GROUPS OF ANIMALS

With each substance, a number of dose levels were employed.

| *A. Intramuscular Injection* | *B. Subcutaneous Treatment* |
|---|---|
| 1. Monilia | S.C. treatment with |
| 2. Chlortetracycline | Monilia (given i.m.) 6. Chlortetracycline |
| 3. Cortisone | 7. Cortisone |
| 4. Aspirin | 8. Aspirin |
| 5. Turpentine | 9. Turpentine |
| *C. Super-injection* | *D. Combined Treatment* |
| Thigh "super-injected" with | Thigh "super-injected" with / Subcutaneous treatment with |
| Monilia (given i.m.) 10. Chlortetracycline | Same dose of |
| 11. Cortisone | Monilia (given i.m.) 14. Chlortetracycline |
| 12. Aspirin | 15. Cortisone |
| 13. Turpentine | 16. Aspirin |
| | 17. Turpentine |

## Results

### *Course of the Untreated Lesion*

Infection of the thigh with *C. albicans* as described gave rise to a swelling which showed a peak size usually on the second day after inoculation followed by a small decline which was in turn succeeded by a second peak size usually about the 5th day. The size of the swelling then subsided to a plateau which might be maintained for a

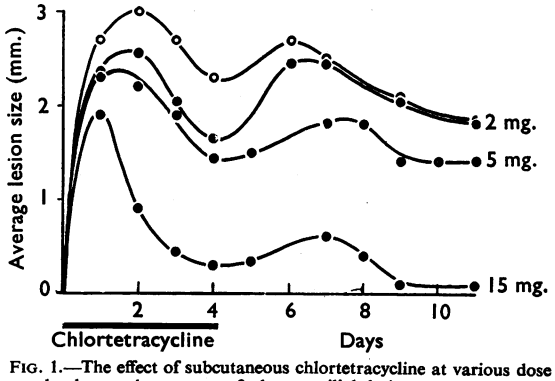


FIG. 1.—The effect of subcutaneous chlortetracycline at various dose levels on the course of the monilial lesion. O, Controls; ● Treated.

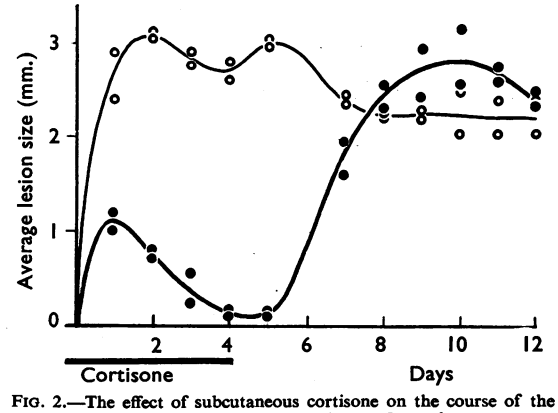


FIG. 2.—The effect of subcutaneous cortisone on the course of the monilial lesion. O, Controls; ●, Treated.

further week before resolution began (Figs. 1 and 2). The second peak might be greater than the first.

Microscopically, the lesion consisted of a granuloma within the connective tissue of the thigh. At the edge of the granuloma, proliferating monilial hyphae with some yeasts could be seen. The initial infiltration with polymorphs was soon succeeded by chronic inflammatory cells, histiocytes, macrophages and capillary loops. Pockets of pus containing numerous polymorphs and scanty monilia persisted, however, throughout the development of the lesions.

### *Effect of Treatment*

#### *Chlortetracycline*

*Subcutaneous Treatment.*—The effect of subcutaneous chlortetracycline on the course of the lesion is shown in Fig. 1. Treatment at the 2 mg. dose level reduced the size of the lesion, but on cessation of treatment there was a return to the control size. Increasing the dose to 5 mg. reduced the size of the lesion still further and delayed its subsequent return to the control size. With toxic doses of chlortetracycline (15 mg.) swelling of the infected thigh disappeared by the third day and remained absent thereafter.

The largest dose of chlortetracycline given, 15 mg. for five doses, was very close to the cumulative LD50 for these mice and a number of deaths occurred amongst the treated animals. Subcutaneous chlortetracycline, particularly in these large doses, gave rise to marked inflammatory changes in the skin and subcutaneous tissue; infarcted areas were common and sloughing of the overlying skin sometimes occurred.

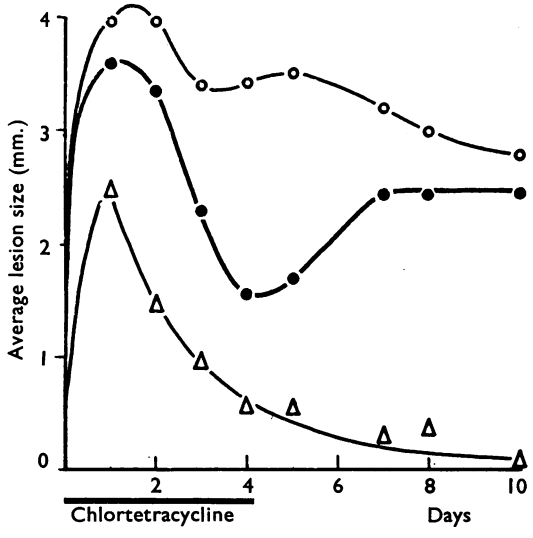


FIG. 3.—Comparison of the course of lesions produced by Δ, chlortetracycline (1 mg.); O, monilia plus chlortetracycline (1 mg.); and ●, monilia plus chlortetracycline (1 mg.) treated with subcutaneous chlortetracycline (1 mg.).

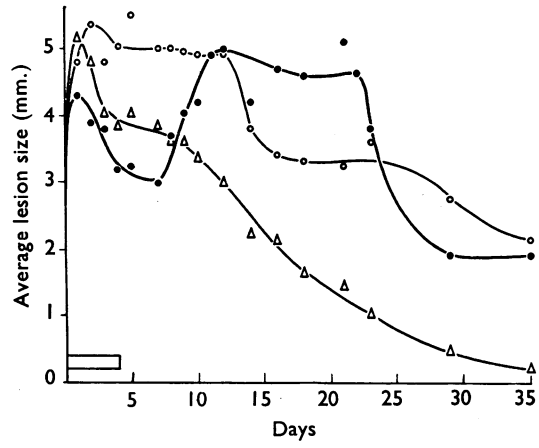


FIG. 4.—Comparison of the course of lesions produced by Δ, chlortetracycline (15 mg.); O, monilia plus chlortetracycline (15 mg.); and ●, monilia plus chlortetracycline (15 mg.) treated with subcutaneous chlortetracycline (15 mg.). The duration of treatment is shown by the hollow rectangle.

*Intramuscular and Combined Treatment.*—The intramuscular injection of chlortetracycline alone gave rise to a swelling maximal on the 1st day but which often showed a small secondary peak at 5 to 8 days. Super-injection of thighs already infected with monilia gave rise to very large and sustained swellings (Figs. 3 and 4). Subcutaneous chlortetracycline produced changes resembling those obtained in the animals only infected with monilia. When very large doses (15 mg.) of chlortetracycline were given (Fig. 4) the reduction in the lesion size persisted for 2 to 3 days after withdrawal of the drug, and was then followed first by a sharp rise to the control size and then by an increase above the control size, subsequently called " rebound."

#### *Cortisone*

*Subcutaneous Treatment.*—Five daily doses of 1 mg. cortisone acetate reduced the size of the lesions to zero, but following withdrawal of the drug there was a rapid " rebound " rise above the control size (Fig. 2). The difference between the means of the treated and untreated groups, calculated on day 10, is highly significant ($P<0.001$).

*Intramuscular and Combined Treatment.*—Intramuscular cortisone alone produced no recognizable swelling at 1 day; on the contrary, a

reduction in the size of the limb often occurred (Fig. 5). Injection of cortisone into limbs infected with monilia decreased the swelling for 2 days, but subsequently the swelling became greater than in the control animals. Subcutaneous treatment of doubly inoculated animals produced a reduction in size followed by "rebound" (Fig. 5). The difference between the means of the control and

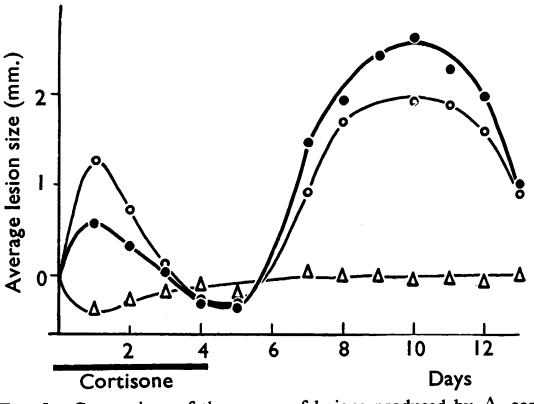


FIG. 5.—Comparison of the course of lesions produced by Δ, cortisone; O, monilia, plus cortisone; ●, monilia plus cortisone treated with subcutaneous cortisone.

treated groups calculated on day 10 is highly significant ($P<0.01$). This effect closely resembled that seen in animals treated with subcutaneous cortisone (Fig. 2).

*Aspirin and Turpentine*

*Subcutaneous Treatment.*—In doses up to and including the LD50 for these animals, these agents produced only a small sustained reduction in the size of the lesions.

*Aspirin*

*Intramuscular and Combined Treatment.*—Very large doses of aspirin alone given into the thigh produced a small swelling only (Fig. 6). Super-injection of monilia-infected thighs gave rise to an insignificant increase in swelling, and in doubly inoculated animals the addition of subcutaneous treatment produced only minor changes (Fig. 6).

*Turpentine*

*Intramuscular and Combined Treatment.*—In contrast, the intramuscular injection of turpentine produced a very large swelling comparable with that resulting from the largest tolerated dose of chlortetracycline (Figs. 4 and 7). This swelling had its peak at the first day and subsequently declined logarithmically. Super-injection of infected thighs gave rise to a similar large swelling, but after the first 3 days, when the greater part of the swelling due to turpentine had subsided, the lesions followed the control course closely (Fig. 7). Treatment of such doubly inoculated animals with repeated subcutaneous turpentine was without effect on the course of the lesions apart from a small overall reduction in size (Fig. 7).

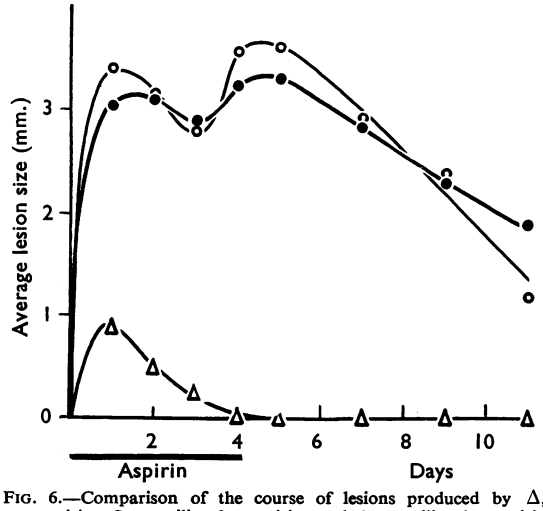


FIG. 6.—Comparison of the course of lesions produced by Δ, aspirin; O, monilia plus aspirin; and ●, monilia plus aspirin treated with subcutaneous aspirin.

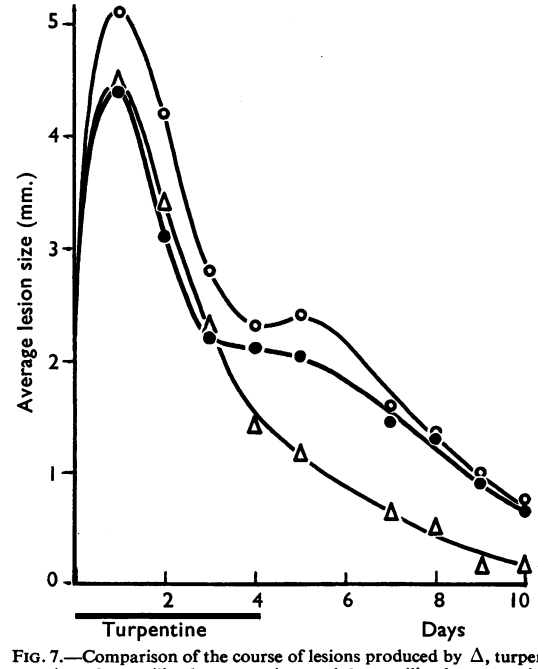


FIG. 7.—Comparison of the course of lesions produced by Δ, turpentine; O, monilia plus turpentine; and ●, monilia plus turpentine treated with subcutaneous turpentine.

## Discussion

The possibility that chlortetracycline exerts a cortisone-like effect on monilial lesions has been examined using a monilial infection established at a site which is normally sterile. In this way, any changes which might be mediated by effects upon local normal flora have been excluded. The objects have been to determine whether the effects on this lesion of cortisone and of chlortetracycline are in any way similar, and to investigate to what extent the effects of chlortetracycline are the result of toxic or inflammatory processes.

Comparison of the effects of cortisone and chlortetracycline on the monilial infection reveals two similarities and two differences. The similarities are best seen in the animals receiving both intramuscular and subcutaneous injections (Figs. 3, 4, and 5). Both drugs produce a decrease in size which is followed by a rapid increase when treatment is stopped. In addition, if the chlortetracycline dose is high enough, this increase continues above the control value, as in those treated with cortisone (Figs. 4 and 5). This resembles closely the " rebound " increase or reappearance of activity which has been described in a number of conditions following suppression with cortisone (Ragan, 1950; Boland, 1950; Le Maistre, Tompsett, Muschenheim, Moore, and McDermott, 1951; Vollmer, 1951).

The first of the two differences is also shown by the groups receiving both intramuscular and subcutaneous injections. Intramuscular injection of cortisone alone produces a small reduction in the size of the thigh and in combination with monilia gives rise to swellings smaller than the controls (Fig. 5). Chlortetracycline, on the other hand, injected alone into the thigh produces a large swelling and in combination with monilia gives rise to a very large and persistent mass. The other difference occurs in the groups receiving the drugs subcutaneously only. The suppression and " rebound " resulting from cortisone typified in Fig. 2 cannot be reproduced by subcutaneous chlortetracycline in any dosage (Fig. 1). The reduction in lesion size which results from subcutaneous chlortetracycline becomes increasingly prolonged with increased dosage, but " rebound " does not occur even if chlortetracycline is given for two days only.

Both the important features of the response to cortisone can, therefore, be reproduced by chlortetracycline only in certain circumstances. There is, in addition, a local irritant response to chlortetracycline which is precisely the reverse of that to cortisone.

The possibility that this local irritant or toxic effect of chlortetracycline might be responsible for all the phenomena described led to a study of the effects of a number of unrelated substances chosen for their physical or biological properties. It is sufficient at present to say that the effects of cortisone and chlortetracycline have not been reproduced by any unrelated substance studied so far. Two examples of such substances have been chosen to illustrate some of the properties examined. Aspirin is a substance which corresponds fairly closely with chlortetracycline in acidity and solubility—physical properties of potential importance in the present connexion. Moreover, it has a therapeutic effect which resembles that of cortisone (Hailman, 1952). In the present experiments, it is clear that the action of aspirin does not resemble either that of chlortetracycline or that of cortisone.

Turpentine was chosen for its irritant properties, and sufficient was given to produce an irritant response in the thigh comparable with that of chlortetracycline. The failure of turpentine to reproduce the remainder of the effects of chlortetracycline makes it appear that chlortetracycline is not acting as a simple irritant. It must be observed, however, that neither aspirin nor turpentine gave rise to such persistent swellings as chlortetracycline, nor did either produce such severe tissue damage, though both were given to some groups in toxic doses.

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