EXPERIMENTAL ORAL CANDIDAL INFECTION AND CARRIAGE OF ORAL BACTERIA IN RATS SUBJECTED TO A CARBOHYDRATE-RICH DIET AND TETRACYCLINE TREATMENT

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SUMMARY. Oral candidal infection and the carriage of oral bacteria in rats has been studied in animals on a high carbohydrate diet and treated with tetracycline. Candidal infection was not significantly enhanced by carbohydrate alone but was promoted by tetracycline; carbohydrate plus tetracycline was no more effective than tetracycline alone. Carriage of lactobacilli was enhanced by carbohydrate but streptococcal carriage was depressed; there was no effect on the number of rats carrying enterobacteria. Administration of tetracycline reduced the carriage of all three groups of bacteria but the isolation rate for enterobacteria increased towards the end of the experiment, becoming nearly the same as at the start. The prevalence of *C. albicans* did not vary with these changes in bacterial populations.

INTRODUCTION

Diet may influence the carriage of *Candida albicans* in the mouth. Bowen and Cornick (1970) found that the yeast disappeared from the mouths of tube-fed monkeys but reappeared when the animals were fed a high carbohydrate diet by mouth. Excess sugar may promote the growth and multiplication of yeasts (Knight and Fletcher, 1971) and when *C. albicans* was inoculated into the mouths of rats receiving a high carbohydrate diet, it persisted longer than in rats on a normal diet (Russell and Jones, 1973a). It is also noteworthy that stomatitis can be initiated in denture wearers with healthy palatal mucosa by repeated rinsing with sucrose (Olsen and Birkeland, 1976).

Tetracycline administration commonly predisposes to candidosis (Seelig, 1966a and b). In rats treated with tetracycline, candidal mycelial elements seem to penetrate the stratum corneum earlier than in untreated animals (Jones and Russell, 1973). Jones *et al.* (1976) found that rats treated with antibiotic and given oral inocula of *C. albicans* remained colonised longer than rats not given tetracycline. However, repeated inoculation is necessary to maintain prolonged colonisation and infection (Russell and Jones, 1975).

Tetracycline may act by interfering with the indigenous microflora which would otherwise suppress oral colonisation with *C. albicans* (Seelig, 1966b). In-vitro studies have shown that lactobacilli enhance (Isenberg *et al.*, 1960) and inhibit (Young *et al.*,

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1956) the growth of *C. albicans*. *C. albicans* was administered to germ-free and conventional chickens by Balish and Phillips (1966), who demonstrated that *Escherichia coli* provided protection against crop infection after oral challenge with *C. albicans*, whereas *Streptococcus faecalis* provided no such protection. Cormane and Goslings (1963) suggested that in-vivo interactions between *C. albicans* and bacteria arose primarily from competition for available nutrients, and that the presence of high concentrations of glucose favoured the growth of *C. albicans*. We now report a study of the effects of a carbohydrate-rich diet and tetracycline treatment on the carriage of oral bacteria in rats and its possible relationship to candidosis.

MATERIALS AND METHODS

Rats. A total of 120 Sprague-Dawley rats (males and females, average weight 100–110 g, 35 days old) were separated into 12 groups, each of 10 rats. Groups I–IV (40 rats) were uninoculated controls and groups V–XII (80 rats) were experimental groups. The groups received the following diets: groups I, V and IX, normal diet; groups II, VI and X, carbohydrate-rich diet; groups III, VII and XI, normal diet plus tetracycline; groups IV, VIII and XII, carbohydrate-rich diet plus tetracycline.

Diets. The normal diet was “standard- $\frac{3}{8}$ ” mouse and rat pellets (Messrs Oakes, Congleton, Cheshire). The carbohydrate-rich diet comprised powdered icing sugar 65.5%, dried skimmed-milk powder 32%, desiccated liver powder 2%, choline chloride 0.2% and dried multivitamins (“Vitament”, Beecham Animal Health, Brentford, Middlesex) 0.3%. Both diets were supplied to the rats *ad libitum*.

Antibiotic treatment. Rats in groups III, IV, VII, VIII, XI and XII were treated with tetracycline as described by Russell and Jones (1973*b*). For 5 days before the experiment (week 0), each rat was given 40 ml/day of a 0.1% aqueous solution of tetracycline to drink; subsequently each was given 40 ml/day of a 0.01% solution.

Candida challenge. Rats were given oral inocula of *C. albicans* in the yeast phase as described by Russell and Jones (1973*b*). Groups V, VI, VII and VIII received *C. albicans* in weeks 1, 2 and 3 and groups IX, X, XI and XII in weeks 1, 2, 3, 5, 7, 9, 11 and 13 of the experiment.

Microbiological examination. In week 1 (before receiving *C. albicans*) and weekly to the end of the experiment, the mouth of each rat was sampled by swabbing. The swabs were streaked immediately on to three plates: (1) Rogosa SL agar (Difco), incubated in candle jars at 37°C for 2–3 days for the isolation of lactobacilli—representative colonies were examined by gram-stained smears and tested for catalase (Cowan, 1974); (2) Mitis-Salivarius Agar (Oxoid), incubated in candle jars at 37°C for 48 h—colonies were examined by gram-stained smears and tested for catalase; (3) MacConkey Agar without salt (Oxoid), incubated aerobically at 37°C for 24–48 h—colonies were examined by gram-stained smears and tested for catalase and oxidase (Cowan, 1974). The swabs were then placed in 7-ml screw-capped bottles containing 3 ml of Sabouraud’s Liquid Medium (Oxoid) with penicillin 40 IU/ml and streptomycin 20 µg/ml and incubated at 37°C until visible growth appeared, or for 2 weeks before being discarded as negative. All bottles showing growth were sub-cultured on to plates of BiGGY Medium (Oxoid) for isolation of candida. The plates were incubated for 72 h at 37°C, and colonies were identified as *C. albicans* by the filamentation test of Taschdjian *et al.* (1960).

Post-mortem examination. Five rats from each group were killed 7 weeks after the beginning of the experiment; the remaining rats were killed after a further 7 weeks. The heads and tongues of all animals were examined histologically as described by Russell and Jones (1973*b*). Statistical comparisons between the different groups were made by the χ^2 test (Siegel, 1956).

Statistical analysis. For each treatment group 115 swabs were examined: i.e., from 10 animals sampled eight times and from five animals sampled a further seven times. The total numbers of swabs from which a specific organism was isolated were compared group by group by the χ^2 test (Siegal, 1956).

RESULTS

Animals receiving the carbohydrate-rich diet remained healthy and gained weight, as did the animals on the normal diet.

Carriage of C. albicans

Results of culture are presented in table I. For the sake of brevity, only the results for weeks 0, 1, 4, 7, 9, 11 and 14 are shown. *C. albicans* was not recovered from the mouth of any rat in the control group at any time throughout the experiment, nor from any rat in the experimental groups before inoculation with the challenge strain. After inoculation, *C. albicans* was recovered from all groups for the duration of the experiment, with the exception of group V. In this group, given *C. albicans* on three occasions only and not treated with tetracycline nor provided with the carbohydrate-rich diet, the yeast disappeared in the seventh week. In the early stages, there was no difference in the stability of oral colonisation with *C. albicans* between groups VI, VII and VIII. At week 8, six out of 10 rats in group VI, six out of 10 in group VII and eight out of 10 in group VIII were still colonised, but, thereafter, there was a fall in the number colonised which seems less rapid in group VIII. Similarly, there was a gradual reduction in the number of rats in group IX harbouring *C. albicans* in their mouths; these rats were given *C. albicans* repeatedly but were fed on a normal diet and not treated with tetracycline. This reduction seemed to be more rapid than in the other groups repeatedly given *C. albicans*.

There were significant differences in the recovery of *C. albicans* between group V and groups VI, VII and VIII and between group IX and groups X, XI and XII ($p < 0.001$). Thus, both tetracycline treatment and the carbohydrate-rich diet favoured

TABLE I
Isolation of C. albicans from rats

Group*	Percentage of rats from which <i>C. albicans</i> was isolated in week						
	0	1	4	7	9	11	14
I	0	0	0	0	0	0	0
II	0	0	0	0	0	0	0
III	0	0	0	0	0	0	0
IV	0	0	0	0	0	0	0
V	0	0	40	0	0	0	0
VI	0	0	70	60	40	20	20
VII	0	0	90	60	60	40	10
VIII	0	0	100	80	80	40	60
IX	0	0	50	30	20	20	20
X	0	0	70	60	80	60	60
XI	0	0	80	80	100	100	100
XII	0	0	100	100	100	100	100

* Groups I-IV, uninoculated controls; groups V-VIII, given *C. albicans* in weeks 1, 2 and 3; groups IX-XII, given *C. albicans* in weeks 1, 2, 3, 5, 7, 9, 11 and 13; groups I, V and IX, normal diet; groups II, VI and X, carbohydrate-rich diet; groups III, VII and XI, normal diet plus tetracycline; groups IV, VIII and XII, carbohydrate-rich diet plus tetracycline.

the oral carriage of *C. albicans* in the rats regardless of whether the challenge strain was administered once or several times. Repeated administration to rats on a normal diet, without tetracycline, also favoured persistence of the *C. albicans*.

Carriage of bacteria

Lactobacilli were found in the mouths of most rats at the beginning of the experiment but thereafter there was a gradual reduction ($p < 0.001$) in the number of tetracycline-treated rats that harboured lactobacilli, i.e., groups III, IV, VII, VIII, XI and XII, whether on the normal or the carbohydrate-rich diet, and whether given *C. albicans* or not (table II). There was an increase ($p < 0.001$) in the number of rats in

TABLE II
Isolation of lactobacilli from rats

Group*	Percentage of rats from which lactobacilli were isolated in week						
	0	1	4	7	9	11	14
I	60	60	70	60	60	60	80
II	70	80	80	90	100	100	100
III	60	50	50	20	0	0	0
IV	50	40	40	50	20	0	0
V	70	60	70	70	80	60	60
VI	80	80	90	100	80	100	100
VII	80	60	50	40	20	0	0
VIII	80	70	40	50	20	20	20
IX	60	60	60	60	60	80	100
X	70	80	80	100	100	100	100
XI	70	60	40	40	40	0	0
XII	70	60	30	40	60	20	20

* See footnote to Table I.

groups II, VI and X harbouring lactobacilli, i.e., in the rats fed the carbohydrate-rich diet but not treated with tetracycline, whether inoculated with *C. albicans* or not. The stability of colonisation by lactobacilli of rats in groups I, V and IX, i.e., those fed a normal diet and not given tetracycline, remained constant throughout the experiment and was not affected by the presence of *C. albicans*.

Streptococci were found in the mouths of all the rats at the beginning of the experiment but thereafter there was a gradual reduction in the number of tetracycline-treated rats that harboured streptococci in their mouths, and at the end of the experiment streptococci were not detectable (table III). The stability of colonisation in groups I, V and IX remained constant throughout the experiment; the presence of *C. albicans* had no effect on carriage of streptococci. The high-carbohydrate diet seemed to depress the carriage of streptococci ($p < 0.01$) but the reduction was by no means as evident as when tetracycline was used.

Enterobacteria were found in the mouths of all rats at the beginning of the experiment (table IV). Subsequently, in the tetracycline-treated rats there was a gradual reduction in the numbers colonised by enterobacteria until about week 10,

TABLE III
Isolation of streptococci from rats

Group*	Percentage of rats from which streptococci were isolated in week						
	0	1	4	7	9	11	14
I	100	100	100	100	100	100	100
II	100	100	80	70	100	100	100
III	100	70	60	40	20	20	0
IV	100	70	60	50	0	20	0
V	100	100	100	90	100	80	100
VI	100	100	80	80	60	80	100
VII	100	80	60	40	20	0	0
VIII	100	70	60	40	0	0	0
IX	100	100	100	100	80	100	100
X	100	100	70	70	80	100	100
XI	100	80	50	30	0	0	0
XII	100	70	60	30	0	0	0

* See footnote to table I.

TABLE IV
Isolation of enterobacteria from rats

Group*	Percentage of rats from which enterobacteria were isolated in week						
	0	1	4	7	9	11	14
I	100	100	100	90	80	100	100
II	100	100	90	70	80	100	100
III	100	70	70	50	20	60	100
IV	100	80	60	50	0	40	100
V	100	100	100	90	100	100	100
VI	100	100	100	70	100	100	80
VII	100	60	70	50	20	80	100
VIII	100	80	70	40	0	60	80
IX	100	100	100	100	80	100	100
X	100	100	90	70	80	100	100
XI	100	70	60	40	20	60	80
XII	100	80	60	40	0	20	80

* See footnote to table I.

when the organisms reappeared. Over the 14-week period, the total number of positive swabs for each group given tetracycline was lower ($p < 0.001$) than for the corresponding control group. At the end of the experiment a similar proportion of rats in all groups harboured them, and a difference apparent in the middle of the experiment was no longer seen. *E. coli* was isolated and was found to be resistant to a disk containing tetracycline 25 μg , to which the original strain was sensitive. The stability of oral colonisation by enterobacteria in groups I, II, V, VI, IX and X remained constant throughout the experiment; neither the high-carbohydrate diet nor the presence of *C. albicans* had any effect.

Histological findings

The treatment received by rats with histological evidence of oral candidosis is given in table V. No rat in the control groups was found to have oral candidal infection. Infection was found in all other groups except group V. This last group was given *C. albicans* only at the beginning, received a normal diet and was not given tetracycline; its members were not colonised by *C. albicans* at week 7 (table I). The numbers of sites of infection in each group are given in table II. At week 7, infection was sometimes superficial without alteration of the lingual papillae. In contrast, in four animals (three from group XII and one from group XI, i.e., animals repeatedly given *C. albicans* and tetracycline), infection on the dorsal surface of the tongue was associated with loss of the lingual papillae and with a flat-surfaced hyper- or parakeratotic epithelium. At week 14, infection occurred most frequently on the dorsal surface of the tongue. In all

TABLE V
Histological evidence of candidosis in the mouth and tongue of treated rats

Group	Treatment	Number of rats infected		Number of sites of infection	
		wk. 7	wk. 14	wk. 7	wk. 14
V	Initial <i>C. albicans</i> and normal diet	0	0	0	0
VI	Initial <i>C. albicans</i> and carbohydrate-rich diet	1	1	1	1
VII	Initial <i>C. albicans</i> , tetracycline and normal diet	1	1	1	2
VIII	Initial <i>C. albicans</i> , tetracycline and carbohydrate-rich diet	2	2	3	2
IX	Repeated <i>C. albicans</i> and normal diet	1	1	2	1
X	Repeated <i>C. albicans</i> and carbohydrate-rich diet	2	2	2	2
XI	Repeated <i>C. albicans</i> , tetracycline and normal diet	5	3	9	3
XII	Repeated <i>C. albicans</i> , tetracycline and carbohydrate-rich diet	4	4	7	5

5 rats were killed at week 7 and 5 at week 14. There were no sites of infection in control rats in groups I to IV.

groups at all sites of infection, the lingual papillae were replaced by a flat-surfaced layer of parakeratotic epithelium. Mycelial elements penetrated the parakeratotic layer of the epithelium but never the underlying stratum spinosum. Beneath the infected epithelium the corium was infiltrated by mononuclear cells. Sites of infection other than the tongue exhibited parakeratosis and no change in the sub-epithelial tissues was identified. These changes are similar to those described by Russell and Jones (1975) and Fisker *et al.* (1982). Statistical comparison between the groups showed a significant effect ($p < 0.05$) of tetracycline on the incidence of candidosis.

DISCUSSION

The present study confirmed that oral candidal infection is not easily induced in rats. Infection occurred in the mouths of only two of 20 rats given *C. albicans* but not given a carbohydrate-rich diet or tetracycline, in six of 20 rats on the carbohydrate-rich diet, in 10 of 20 rats treated with tetracycline and in 12 of 20 rats given a carbohydrate-rich diet and tetracycline. Thus, as in previous experiments (Russell and Jones, 1973), a

positive effect of a carbohydrate-rich diet alone on infection, as distinct from carriage, was not proven ($p > 0.2$), but tetracycline enhanced carriage and infection ($p < 0.02$). A carbohydrate-rich diet plus tetracycline did not have a greater effect than tetracycline alone on either colonisation or infection.

The promotion of candidal carriage by excess carbohydrate (table I, groups VI and X) is similar to the results of previous work that showed a positive effect of glucose supplementation on growth and multiplication of *C. albicans* in saliva (Knight and Fletcher, 1971) and enhancement of the adherence of *C. albicans* to epithelial cells by sucrose (Samaranayake and MacFarlane, 1980 and 1982).

Tetracycline treatment of rats on a high-carbohydrate diet did not significantly enhance candidal carriage, presumably because of the strongly positive effect of carbohydrate alone. However histological examination indicated that infection occurred in more sites in rats treated with tetracycline and receiving the high-carbohydrate diet than in animals receiving the drug or the diet alone.

Lactobacilli were found to be equally prevalent in the mouth in all groups at the beginning of the experiments. A carbohydrate-rich diet significantly favoured the presence of lactobacilli in the rats' mouths; tetracycline treatment always reduced the carriage of these organisms. The presence of *C. albicans* did not have any effect on the carriage of lactobacilli whatever the treatment regime, nor was the occurrence of *C. albicans* affected by the presence of lactobacilli. These results suggest that lactobacilli in the mouths of rats are not significant factors in promoting or preventing candidal colonisation. It would clearly be misleading to extrapolate to the human mouth the findings *in vitro* of Isenberg *et al.* (1960) and Young *et al.* (1956) that suggested direct interaction between *C. albicans* and lactobacilli.

Streptococci and enterobacteria were found in the mouths of all rats at the beginning of the experiment. The carbohydrate-rich diet alone had no effect on the prevalence of enterobacteria but depressed the carriage of streptococci. Tetracycline reduced the prevalence of streptococci and of the enterobacteria, even in rats receiving the carbohydrate-rich diet. The presence of *C. albicans* did not significantly alter the prevalence of either group of bacteria. A noteworthy finding was the reappearance of enterobacteria towards the end of the experiment in rats treated with tetracycline. This reappearance was not accompanied by the disappearance of *C. albicans*. This contradicts what one might expect from the finding by Balish and Phillips (1966) that *E. coli* protected chickens against infection by orally-administered *C. albicans*.

The growth of resistant micro-organisms that were not identified in the present study might account for the gradual fall in the prevalence of *C. albicans* in tetracycline-treated rats given *C. albicans* only at the start of the experiment. The findings in the present experiments relating to lactobacilli, streptococci and enterobacteria do not demonstrate any relationship between the presence or absence of these organisms and the carriage of *C. albicans*. Nevertheless, they cannot be taken as evidence to refute the widely accepted hypothesis that tetracycline promotes oral colonisation by *C. albicans* by eliminating competing bacteria. The complex interactions which may be involved have not been elucidated in this *in-vivo* work.

There is an apparent discrepancy between the clearly significant increase in candidal colonisation caused by the high-carbohydrate diet and the lack of a statistically significant effect on candidal infection. This may relate to a quite separate effect of carbohydrate on the influence exerted by oral bacteria on the pathogenic effect

of *C. albicans*. For example, Hassan and Russell (1983) showed that streptococci may prevent germination of *C. albicans* but this effect is much reduced if the streptococci have been grown with sucrose.

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