EFFECTS OF ORAL INOCULATION OF *CANDIDA ALBICANS* IN TETRACYCLINE-TREATED RATS

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PLATES VIII AND IX

HOST factors, including treatment with anti-bacterial agents, are important in permitting the transition of *Candida albicans* from commensalism to parasitism (Winner, 1969). Oral candidosis has been induced experimentally in the adult rat, and appears not to be influenced by a reduction in salivary flow (Jones and Adams, 1970). It is no easier to induce in weanling rats than in adults (Jones and Russell, 1973), but a diet rich in carbohydrates favours carriage of *C. albicans* in the rat's mouth and perhaps affects its infectivity (Russell and Jones, 1973). The present study describes the effects of the oral inoculation of *C. albicans* in rats that were given tetracycline in their drinking water.

MATERIALS AND METHODS

Rats. Sixty Sprague-Dawley rats of either sex, with an average weight of 200 g, were divided into two groups of equal size. Group 1 received normal dietary cubes ("Standard, $\frac{3}{8}$ -in. mouse and rat pellets", Messrs Oakes, Congleton) and Group 2 a carbohydrate-rich diet which was originally designed to be cariogenic and contained 42 per cent. powdered icing sugar and 30 per cent. corn starch. Half of each group received *C. albicans* in the yeast phase and the other half *C. albicans* as a mycelial suspension. The rats were housed five to a cage.

For 5 days before inoculation with *C. albicans* the rats were given a 0.1 per cent. aqueous solution of tetracycline hydrochloride to drink at the rate of 40 ml per day for each rat. Subsequently, and for the remainder of the experiment, the concentration of antibiotic was reduced to 0.01 per cent., each rat again being allowed 40 ml per day.

Suspensions of *C. albicans*. A laboratory stock culture of *C. albicans*, originally isolated from a human carrier was used. To prepare a suspension of the yeast form, plates of Sabouraud's agar were flooded with a broth culture of the organism. After incubation at 35°C for 2 days the resultant growth was harvested in sterile saline, and washed twice by centrifugation before being re-suspended in normal saline at a concentration of 6×10^8 cells per ml.

To prepare the mycelial form, 200 ml of broth containing 0.1 per cent. peptone (Evans) and 1 per cent. sucrose, pH 5.5, was inoculated with 0.1 ml of a broth culture of *C. albicans*. The medium was incubated at 35°C for 5 days, during which sterile air was bubbled through the culture to prevent mat formation. The resultant growth was harvested by centrifugation, washed twice in saline and finally re-suspended in 20 ml saline. Some yeast cells were present but the preparation was predominantly mycelial.

Rats were inoculated orally with 0.1 ml of the appropriate suspension on days 6, 8 and 10 of the experiment. At the beginning of the experiment, before inoculation on the 6th day, and on days 13, 20, 27 and 34, the mouth of each rat was sampled by rubbing a swab on the tongue and mucosal surfaces. The swabs were placed in bottles of Sabouraud broth

Received 18 Aug. 1972; accepted 22 Aug. 1972.

containing penicillin (40 units per ml) and streptomycin (20 μ g per ml). The bottles were incubated at 35°C for 5 days and subcultured onto plates of Candida Medium (Oxoid). The characteristic colonies were confirmed as those of *C. albicans* by the germ-tube test (Taschdjian, Burchall and Kozinn, 1960).

Five rats from each sub-group were killed on day 13, five on day 20 and five on day 34, i.e., 3, 10 and 24 days after the final inoculation. The heads were decalcified *in toto* and sections from serial blocks were stained with haematoxylin and eosin and by the periodic acid-Schiff technique.

RESULTS

The results are summarised in the table. The fungus was not recovered from the mouth of any rat before the start of the experiment or after the

TABLE

Effects of oral inoculation with Candida albicans on rats given tetracycline† in the drinking water*

Diet	Phase of <i>Candida</i> inoculated	Number of rats in group	Type of examination made	Proportion of positive results‡ obtained (number positive/number examined) on day					
				1	6	13	20	27	34
Normal	Yeast	15	{ Cultural Histological	0/15	0/15	15/15	10/10	5/5	5/5
Normal	Mycelial	15		...	...	5/5	5/5	...	4/5
			{ Cultural Histological	0/15	0/15	15/15	10/10	4/5	4/5
				...	...	5/5	5/5	...	2/5
Carbohydrate-rich	Yeast	15	{ Cultural Histological	0/15	0/15	15/15	10/10	5/5	5/5
Carbohydrate-rich	Mycelial	15		...	...	5/5	5/5	...	2/5
			{ Cultural Histological	0/15	0/15	14/15	10/10	5/5	4/5
				...	...	2/5	5/5	...	0/5

* On days 6, 8 and 10.

† For dosage see text.

‡ Positive cultural result = *C. albicans* isolated from mouth swab; positive histological result = mycelial penetration of the oral epithelium.

administration of tetracycline for 5 days. *C. albicans* was however recovered consistently throughout the experiment from the mouths of all rats after inoculation with the yeast phase. Negative results were obtained on four occasions in rats inoculated with the mycelial phase, but in two of these rats a subsequent swab was positive. There was little difference between the results obtained in rats on normal diet and carbohydrate-rich diet.

Histological evidence of candidosis, i.e., mycelial penetration of the superficial layers of the oral epithelium, was found in 45 of the 60 inoculated rats; in 17 of 20 animals killed on day 13, in all 20 killed on day 20, and in 8 of 20 killed on day 34. Candidosis was found in 19 of the 30 animals receiving the diet rich in carbohydrates and in 26 of the 30 animals provided with a normal diet. Candidosis was present in 26 of 30 animals given the yeast form of the micro-organism and in 19 of 30 given the mycelial form. These differences are not significant at the 0.05 level.

In animals killed on day 13, infection occurred on the tongue, gum and buccal mucous membrane; the lower half of the mouth was more often

infected than the upper half. In areas showing infection, the yeast form was often present in large numbers on the surface and sometimes a pseudomembrane was formed. One week later (day 20) mycelia were found penetrating the orthokeratotic layer of the epithelium and only occasionally was parakeratosis seen. In some areas of infection the basal layers of the epithelium showed considerable inter- and intracellular oedema; inflammatory cells infiltrated the epithelium and were present in the underlying corium (fig. 1). On the tongue, mycelial penetration occurred at the base of the filiform papillae, and where the cellular layer was reached the mycelia either ran parallel to it or produced an indentation of it. The filiform papillae were usually preserved in areas of infection in animals killed on day 13 (fig. 2).

A very different picture was apparent in rats killed on the 34th day, i.e., 24 days after final inoculation. In these the lesions occurred much more frequently on the tongue than elsewhere and were notable for the loss of filiform papillae (fig. 3). There was a thick flat superficial orthokeratotic or parakeratotic layer and the epithelium was acanthotic and showed focal atypia (fig. 4). In some places the epithelium was penetrated by polymorphonuclear leucocytes which accumulated in the superficial layers of it.

The lesions in rats killed on the 20th day resembled those found in 13-day animals more than those found in rats killed on the 34th day. The histological findings were not influenced by the phase of the organism at inoculation or by the diet provided.

DISCUSSION

It has been suggested by some observers that treatment of patients with broad-spectrum antibiotics may potentiate their susceptibility to candidal infection (Pappenfort and Schnall, 1951; Solomon, 1961; Seelig, 1966), but others (McKendrick, Wilson and Main, 1967) have failed to demonstrate any change in the prevalence or salivary concentration of *C. albicans* in the mouths of patients receiving tetracycline. Our work indicates that tetracycline administration results in the persistence of inoculated *C. albicans* in the mouths of almost 100 per cent. of rats over 24 days. None of the uninoculated rats harboured *C. albicans* in their mouths after 5 days' medication with tetracycline and we have failed to identify the fungus as a commensal in the mouths of normal rats in many other studies. More recently we have shown that tetracycline administration *per se* for 20 wk does not cause *C. albicans* to appear in the oral flora of the rats in our colony. Previously (Russell and Jones, 1973) we demonstrated a rapid fall in the carriage rate after oral inoculation in rats not treated with tetracycline; after 2 wk the organism was present in the mouths of very few animals. The prolongation of carriage produced by feeding a carbohydrate-rich diet was slight in comparison with that achieved in the present work by tetracycline administration.

In the present experiments we again found that giving tetracycline is associated with a high rate of candidal infection. This is most striking in comparison with our previous experience (Jones and Adams, 1970; Adams and Jones, 1971; and Russell and Jones) that less than half of the rats could be infected by

similar techniques in the absence of tetracycline medication. Even when successful, the infection was less exuberant. The present work also demonstrates that candidal infection may produce very marked alteration in the histomorphology of the rat's lingual epithelium. Twenty-four days after inoculation, the normal papillary structure of the dorsal surface of the tongue had been lost at the sites of infection. The organism in its mycelial form apparently first penetrated the keratin layer of normal orthokeratotic epithelium inducing hyperplastic and inflammatory changes. The epithelium became parakeratotic and the papillary structure of the tongue epithelium was lost. The final appearance resembled that seen in candidal leukoplakia in man. The present study provides confirmatory evidence for the sequence of events proposed for human candidal leukoplakia by Cawson (1966), and for the view expressed by Cawson and Lehner (1968) that *C. albicans* causes some cases of human leukoplakia.

As regards the nature of the potentiating effect of tetracycline on *C. albicans*, Cormane and Goslings (1963) suggested that bacteria in the gastro-intestinal tract compete with the fungus for available nutrients or produce antagonists to it. By eradicating such bacteria the antibiotic permits candidal superinfection. Chlortetracycline has also been shown to increase growth of *C. albicans* in broth cultures, but no correlation between antibiotic stimulation of growth *in vitro* and antibiotic-based superinfection has been demonstrated (Huppert and Cazin, 1955).

Although the results of the present work did show some differences between the two dietary sub-groups and the two sub-groups given the organism in different phases, these differences were not significant at the 0.05 level and no conclusions can be drawn from them.

In the present experiments, tetracycline was administered before, during and after oral inoculation with *C. albicans*. It remains to be seen whether the fungus, once established with the aid of an antibiotic, can maintain itself in the absence of tetracycline. We are investigating this possibility.

SUMMARY

After being given tetracycline in drinking water (0.1 per cent.) for 5 days, rats were inoculated orally with *Candida albicans* on three occasions at 2-day intervals. Immediately after the first inoculation, the concentration of tetracycline in the water was reduced to 0.01 per cent. and this was maintained for the next 28 days. The rats' mouths were regularly colonised by *C. albicans*, the organism being recoverable throughout the experiment. Histological examination showed the organism in the tissues in 75 per cent. of the animals. Infection of the dorsal surface of the tongue produced a striking change in the histological appearance of the lingual epithelium, reminiscent of that found in candidal leukoplakia in man.

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FIG. 1.—Buccal sulcus of rat. Candidal mycelial elements penetrate the keratinised layer of the epithelium. There is intercellular oedema and inflammatory-cell infiltration of the deeper layers of the epithelium. PAS. $\times 40$.

FIG. 2.—Rat tongue. Candidal penetration of the filiform papillae is apparent. The dorsal lingual papillae are preserved 13 days after infection. PAS. $\times 40$.

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FIG. 3.—Rat tongue. Thirty-four days after infection the filiform papillae have been lost and the dorsal surface of the tongue is flat. PAS. $\times 40$.

FIG. 4.—Cellular atypia seen in areas of candidal infection. PAS. $\times 82$.

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