

An apparent growth stimulant for *Candida albicans* released from tetracycline-treated bacterial flora

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(Received 15 October 1959)

Various explanations have been proposed to account for the increased incidence of moniliasis as a result of 'broad spectrum' antibiotic therapy, but it is generally believed that the suppression of the bacterial flora enables fungi to proliferate without competition. The pathogenicity of *Candida albicans* is, in any event, associated with the numbers of cells present and we have undertaken tests *in vitro* in an attempt to contribute more evidence on the mechanism of their proliferation in the presence of tetracycline-treated bacterial flora. The results are presented here as a preliminary communication.

DIRECT EFFECTS ON GROWTH

In agar, streptomycin sulphate, benzylpenicillin and tetracycline hydrochloride had little effect on the growth of five strains of *C. albicans*. Occasionally there was slight enhancement but this could not be considered significant. Yeast extract, riboflavine and 'vitamin B complex' accelerated multiplication, although not to a degree comparable with that of dextrose. Aerobic cultivation on serum agar previously inoculated with an emulsion of mouse faeces did not appear to affect the growth rate of *C. albicans*, thus suggesting that the established bacterial colonies did not check significantly the development of the yeast colonies. When similar preparations were treated with 1% tetracycline hydrochloride before inoculation with *C. albicans*, fungal growth appeared to be stimulated. The effect of tetracycline-treated faecal cultures was then studied further and the influence of a tetracycline-treated throat culture was also investigated.

EFFECT OF TETRACYCLINE-TREATED CULTURES

Materials and methods

A strain of *C. albicans* isolated from a patient was used in one series of tests conducted with a tetracycline-treated faecal culture, and an N.C.T.C. strain was employed in all the other tests.

The faecal cultures were prepared by emulsifying in serum broth fresh faeces obtained from healthy mice. These primary cultures were incubated at 37° C. for 24 hr. in two series and for 60 hr. in the third.

The throat culture was prepared by thoroughly swabbing the area of the tonsils and soft palate of a healthy human subject who had not had any form of chemo-

therapy for more than 9 months. The swab was put into 20 ml. of serum broth which was then incubated for 60 hr.

Smears and serum-agar subcultures of the faecal and throat cultures showed the presence of staphylococci, Gram-negative bacilli, diplococci, streptococci and Gram-positive bacilli.

Tetracycline hydrochloride was added to each primary culture to produce a concentration of 1% and the preparations were set aside for 24 hr. They were then centrifuged and the supernates were carefully decanted. In the case of the throat preparation and one faecal preparation, the clear liquids were, in addition, sterilized by filtration, the filtrates being tested for sterility.

The two series of tetracycline-treated faecal cultures which had not been sterilized by filtration were then transferred to groups of four sterile tubes, inoculated with a culture of *C. albicans* and their turbidities were examined after 24, 48 and 72 hr. incubation. Since it was thought possible that tetracycline-resistant bacteria might continue to grow after inoculation with *C. albicans* and so increase the turbidity, control tubes were employed in addition to the controls described below. Two were not inoculated with *C. albicans* and two were treated with nystatin (100 u./ml.) immediately before inoculation with the yeast. After incubation for 72 hr., none of these tubes showed increased turbidity.

The other faecal preparation and the throat preparation, which had been passed through sintered glass filters, were transferred to two series of sterile tubes and were made up in doubling dilutions in serum broth so that the whole series represented 1/1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and 1/128 dilutions of the appropriate filtrate. All the tubes were inoculated with 0.1 ml. of a 16 hr. culture of *C. albicans* and incubated. Turbidities were examined after 24, 48 and 72 hr. Subsequently the preparations were subcultured on serum-agar and serum-agar containing nystatin (100 u./ml.). Only *C. albicans* grew on the serum-agar and there was no growth of organisms on the medium containing nystatin.

Controls were prepared as follows. Portions of the faecal emulsions were autoclaved before incubation and treatment with tetracycline. Other portions were incubated in the usual way but were not treated with tetracycline before being centrifuged and inoculated with *C. albicans*. Tubes containing serum broth alone, serum broth plus tetracycline or serum broth plus 10% formaldehyde solution were inoculated with *C. albicans*.

Turbidities were measured by means of a Gallenkamp colorimeter utilizing an Ilford Green Filter no. 626 and special matched Gallenkamp tubes. The instrument was zeroed on the formalinized preparation and readings were taken after thorough agitation.

Results

In the two series of tetracycline-treated faecal preparations which were not sterilized by filtration after centrifugation it was found that multiplication of *C. albicans* was consistently and significantly greater in the preparations which originally contained non-sterile faecal emulsion than in the corresponding controls.

Stimulation of growth of *C. albicans* was also evident in the presence of the tetracycline-treated faecal and throat preparations which were sterilized by filtra-

tion after centrifugation. This stimulation increased with increasing concentration of filtrate, but it tended to fall off at a certain point probably because the culture filtrate had originally supported heavy growth over 60 hr. with depletion of nutrient materials and accumulation of waste products. There was no fresh serum broth in the 1/1 preparation, but increasing amounts in the dilutions from 1/2 to 1/128. In these series it was also noted that the amounts of mycelial elements showed variations which seemed to parallel the differences in turbidity. Subcultures on serum agar continued to show these filamentous differences. Counts, however, showed that the increased turbidities should be ascribed to multiplication of the yeasts as well as to the development of mycelium.

COMMENT

The results reported in this preliminary communication suggest that tetracycline acts on the bacterial flora of the human throat and the mouse intestine in such a way as to make available an unidentified 'growth factor' for *C. albicans*. Multiplication of yeasts as a result of tetracycline therapy would therefore seem to be due to such a factor rather than ecological changes.

Increase in the development of mycelial elements in the presence of filtrates from tetracycline-treated throat and faecal cultures was an incidental observation, but it is interesting to speculate on the possibility that this induced increase in the development of pseudomycelium might reflect a change from relatively saprophytic growth towards a form with greater pathogenic potential.