INTERACTIONS OF ORAL STRAINS OF *CANDIDA ALBICANS* AND LACTOBACILLI

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The increased incidence of candida infections since the advent of antibiotic therapy is well known (Wolfson, 1949; Finland, 1951; Keefer, 1951; Woods *et al.*, 1951; McVay and Sprunt, 1951; Editorial, J.A.M.A., 1951). We have found a rise also, over the past five years, in the incidence of *Candida albicans* in the mouths of healthy young adults. Using the same methods of sampling as in a previous study, (Young *et al.*, 1951) and a comparable college-student group as subjects, we have seen the percentage of candida-positive individuals in the test population rise from 48.6 to 62.1, while the quantity of yeasts in the saliva increased even more drastically (table 1). Since about 86 per cent of these persons are also carriers of oral lactobacilli, the two organisms commonly coexist in the healthy mouth. The present study was undertaken to determine what effects they might exert on each other, and whether a change in their relationship might be involved in the increase in oral candidas.

MATERIALS AND METHODS

The strains of *C. albicans* used were isolated from healthy human mouths and identified by the method of Martin and Jones (1940). The lactobacilli, also obtained from healthy oral cavities, were classified according to *Bergey's Manual* and other sources; the strains used corresponded closely to descriptions of *Lactobacillus acidophilus*. A minimum of 3 and a maximum of 9 strains of each organism were used in the various tests. The inocula were standardized by nephelometry.

After some experimentation, peptonized milk broth, with 1 per cent glucose added, was chosen as a medium complete for both organisms. For enumeration of candidas and lactobacilli from mixed cultures, however, media were used which would support the growth of only one of the organisms: Sabouraud's agar (4 per cent maltose, not acidified) for the yeasts, and Rogosa's medium for the lactobacilli. The latter do not grow at all on the Sabouraud's medium. An occasional candida will grow on Rogosa's medium, but none of the strains used in these studies did so. Vitamin assay media (Difco) were used in the nutritional studies.

To facilitate identification of all colonies produced on the mixed plates, surface inoculations only were used, 0.1 ml of appropriate dilutions of the cultures being transferred to the agar surface, then spread evenly with a sterile rod. Five plates were made of each dilution, and results from countable plates averaged. To ensure optimal growth of the lactobacilli, all cultures were incubated in an atmosphere of carbon dioxide.

RESULTS

Using standard inocula, broth cultures were made of *C. albicans* alone, oral lactobacilli alone, and the 2 organisms together. Plate counts for each type of organism were made at 6, 12, 24, 48, 72, 96, and 120 hr, and typical growth curves are shown in figures 1 and 2. There was a consistent, marked decrease in the candidas when grown with lactobacilli, and the amount of decrease was the same regardless of the strains used. The lactobacilli, on the other hand, showed an increase when grown in mixed culture. All strains tested showed this increase at 24 hr, but the degree of stimulation was greater with some strains of lactobacilli than with others, ranging from 24 per cent to 72 per cent, with most strains showing a 65–72 per cent increase.

It was noted that after 24 hr incubation, cultures containing lactobacilli were more strongly acid (pH 3.7–4.2) than pure cultures of the yeasts (pH 5.3–5.8). This suggested the possibility that the decrease in *C. albicans* in mixed cultures might be due in part to lactic acid accumulation; accordingly, cultures of the yeasts were made, adding lactic acid in lieu of the lactobacilli. Varying quantities of acid were used, bringing the pH to the range reached in the mixed cultures. To determine the specificity of the effect, tubes of

TABLE 1

Incidence of oral Candida albicans in healthy young adults

Year	No. Tested	Positive for Candida	Positives with Candida Counts Over 1000/ml Saliva
		per cent	*per cent*
1949–50	584	48.6	9.7
1954–55	111	62.1	47.8

identical acidity were prepared, substituting tartaric acid for lactic. The results are shown in figure 3. Lactic acid in the quantities used produced a marked decrease in *C. albicans*; tartaric acid at the same pH had a much smaller effect.

In view of the fastidious nutritional requirements of the lactobacilli, it seemed possible that the stimulation of these organisms by *C. albicans* might be attributed to vitamins supplied by the latter. Both organisms were streaked on a variety of vitamin-deficient agars, all of which supported the growth of the yeasts. Of 6 such media used, 2 also gave good growth of lactobacilli: the thiamin-deficient and the folic-acid-deficient. The lactobacilli were, however, unable to grow in pure culture in the absence of riboflavin, niacin, pantothenate, or pyridoxine.

Mixed inocula were diluted so as to yield discrete colonies distributed evenly over the plates, and when these were placed on media adequate for both organisms, 3 types of colonies could be distinguished: the small, gray colonies of the lactobacilli; larger, white colonies resembling those of the candidas, and differing from them only in that the edges were a little less sharply

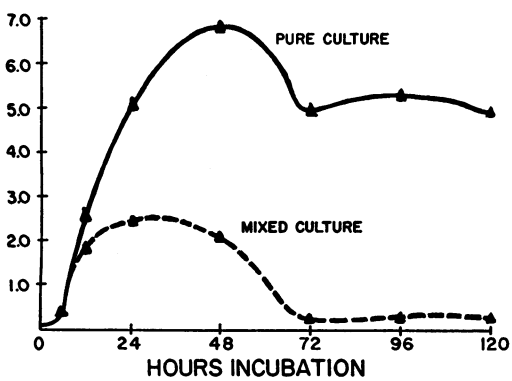


Figure 1. Growth of *Candida albicans* in pure culture and in mixed culture with lactobacilli. Figures on ordinate are lactobacilli per ml × 10^8.

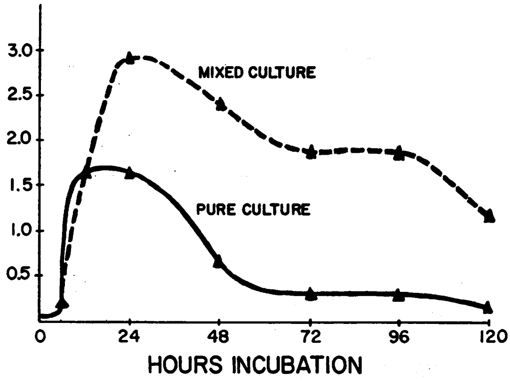


Figure 2. Growth of *Lactobacillus acidophilus* in pure culture and in mixed culture with yeast. Figures on ordinate are yeast per ml × 10^7.

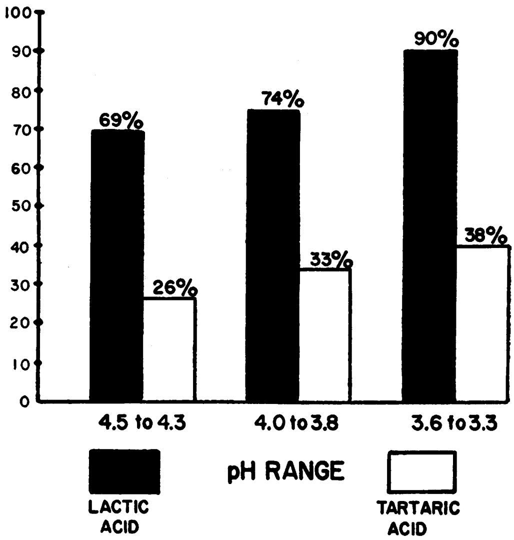
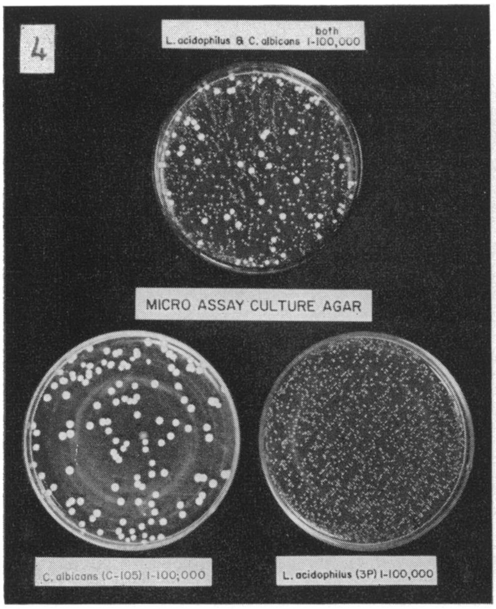
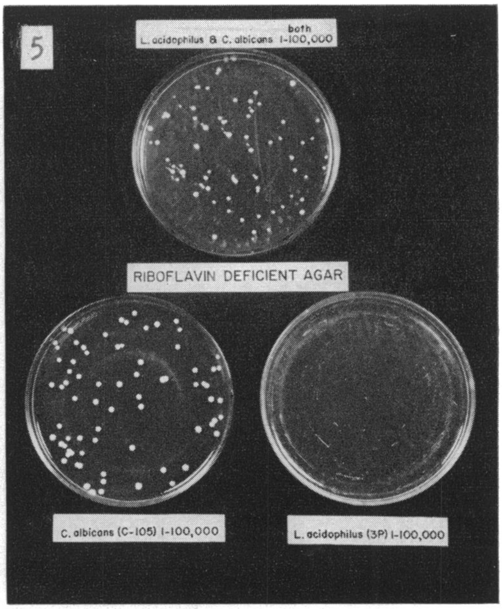
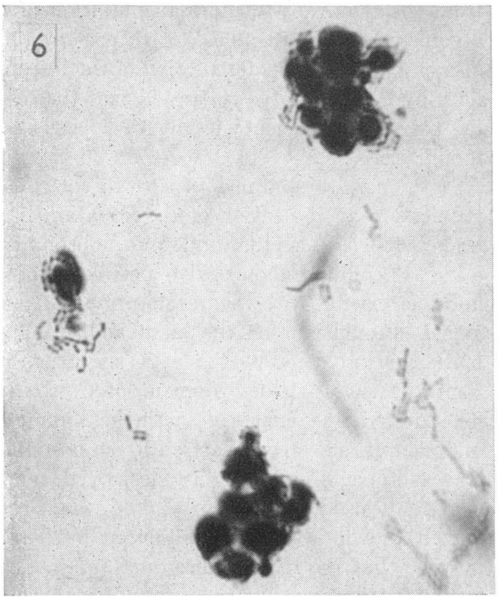


Figure 3. The effect of lactic and tartaric acids on the growth of *Candida albicans*. Figures on ordinate are per cent decrease in *C. albicans*.

defined; and a few typical candida colonies. On media deficient for the lactobacilli, only the last 2 were seen (figures 4 and 5). Microscopic examination of the atypical white colonies showed a mixture of yeasts and lactobacilli, evenly distributed, with the bacteria apparently adhering to the surface of the larger yeasts (figure 6). Smears made from all portions of these colonies on both adequate and deficient media showed a homogeneous mixture, and the large numbers of lactobacilli made it clear that these organisms had not merely survived, but had grown luxuriantly on the deficient media in the presence of







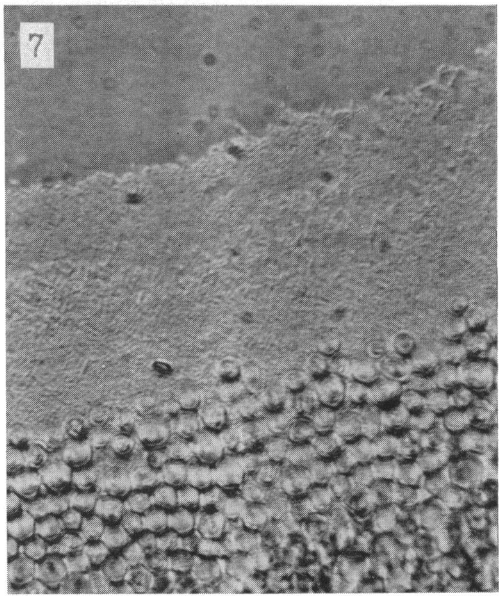


Figure 4. Growth of lactobacilli, candidas, and mixed inoculum on a medium adequate for both organisms. Small, gray lactobacillus colonies are numerous on the mixed plate. A few of the larger, opaque colonies contain only yeasts; most of these are mixed colonies.

Figure 5. Growth of lactobacilli, candidas, and mixed inoculum on a medium inadequate for lactobacilli. Most of the colonies on the mixed plate contain both types of organism.

Figure 6. Smear from mixed colony, showing lactobacilli adhering to candida cells. × 1500.

Figure 7. Edge of mixed colony on riboflavin-deficient agar, showing border of lactobacilli. These organisms are also mixed with the yeasts throughout the interior of the colony. × 900.

TABLE 2

Growth of Candida albicans and Lactobacillus acidophilus on various media

Agar	Inoculum				
	C. albicans growth	*L. acidophilus* growth	*C. albicans* and *L. acidophilus* colony type		
			C. albicans	*L. acidophilus*	Mixed
Rogosa	−	+	−	+	−
Sabouraud	+	−	+	−	−
Tomato juice	+	+	+	+	+
Thiamin-deficient	+	+	+ (few)	+	+
Folic acid-deficient	+	+	+ (few)	+	+
Riboflavin-deficient	+	−	+ (few)	−	+
Niacin-deficient	+	−	+ (few)	−	+
Pantothenate-deficient	+	−	+ (few)	−	+
Pyridoxin-deficient	+	−	+ (few)	−	+

the yeasts. Direct microscopic examination of the colonies showed that the semi-translucent edges were composed of masses of lactobacilli (figure 7). A summary of the results obtained with the various media is shown in table 2.

The tendency of these organisms to produce mixed colonies was investigated further. Since the cultures in question were made by spreading the inoculum evenly, instead of streaking to isolate individual cells, streaked plates were made from the mixed inocula; these also produced mixed colonies, except in the most isolated portions, where pure colonies of each type appeared. Yeasts mixed with a variety of other bacteria failed to form the heterogeneous masses; even when contiguous, typical pure colonies of each organism developed. The lactobacilli, on the other hand, also produced mixed colonies with staphylococci.

Finally, as a check on the interactions suggested by these results, plates of the 2 organisms were inoculated by the drop method of Rosebury *et al.* (1954). As might be expected, the inhibition of the candidas by the lactobacilli was not as striking as in the liquid medium, but could be noted, especially when the ratio of lactobacilli to yeasts was 1000 to 1. The stimulation of the bacteria by the candidas was likewise most clearly seen when the latter predominated by the ratio of 1000 to 1.

DISCUSSION

A number of theories have been advanced to explain the relationship between antibiotic therapy and moniliasis, among them that of Paine (1952), who introduced the idea that intestinal infections with *C. albicans* might result from the removal of a normal inhibition of this organism by *Escherichia coli*. Lighterman (1951), also emphasizing the destruction of the intestinal flora by the antibiotic, suggested that the resulting B-vitamin deficiency might predispose to oral moniliasis.

If the results obtained *in vitro* in the present studies are an indication of the situation in the oral cavity, it seems that under normal conditions a kind of counterbalance exists between candidas and lactobacilli, with the yeasts providing nutritional stimulation for the lactobacilli, and the lactic acid produced by the latter serving to prevent the excessive development of *C. albicans*. The destructive effect of lactic acid on other organisms has been emphasized by Dubos (1953). The lactobacilli are readily destroyed by the commonly used antibiotics, while *C. albicans*, as is well known, is markedly resistant to them. It may be that the increase in oral *C. albicans* following antibiotic therapy results from the destruction of the normally inhibitory lactobacilli, the role of the latter being comparable to that suggested by Paine for *E. coli*.

If, on the other hand, the destruction of the normal intestinal flora by the drug produces a vitamin deficiency, this, as well as the direct action of the antibiotic, would depress the lactobacilli, while leaving the yeasts relatively unaffected. Thus a disturbance of the counterbalance between these organisms may also be a factor in the relation between B-vitamin deficiency and moniliasis.

SUMMARY

Oral strains of lactobacilli and *Candida albicans* grown in mixed culture showed a decrease in candidas and an increase in lactobacilli when compared with pure cultures.

Inhibition of candidas comparable to that produced by the lactobacilli was obtained by the addition of lactic acid in amounts sufficient to reduce the pH to the level reached in mixed cultures. Tartaric acid at the same pH had a much smaller effect.

In 4 vitamin-deficient media which would not support the growth of lactobacilli alone, these organisms grew well in the presence of *C. albicans*. The growth usually took the form of mixed colonies, containing large numbers of both organisms.

It is suggested that in the normal mouth a counterbalance exists between these 2 organisms, with the candidas providing nutritional stimulation for the lactobacilli, and the latter producing lactic acid which prevents the excessive development of the yeasts. Alteration of the balance by antibiotics may be a factor in the increased incidence of candidas in healthy mouths, as well as the increase in oral moniliasis.

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