[11]. In accordance with this hypothesis, the changes in the activity of PLA-2 "trigger" the above-described imbalance in the lipid composition of the skin.

Thus, it was established that the lipid composition of the skin changes in PP, this probably altering the recognition of biologically active ligands on the level of PM.

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EXPERIMENTAL PHARMACOLOGY

Effects of Ultrasound, Polyene Antibiotics, and Dyes on Acid Phosphatase Activity of Yeastlike *Candida* Fungal Cells

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The importance of seeking or developing novel agents and methods, as well as their various combinations, that would enhance the fungicidal potential of therapy in patients with candidiasis, stems from the low efficacy of currently available treatments and the rising prevalence and incidence of this mycosis [1,5]. There are reports that purulent skin lesions and wound infections respond well to treatment with a combination of drugs and ultrasound [4,6,9,10], but the impact of such combinations on fungi, in particular those of the genus *Candida*, has not been investigated, and it is therefore not possible to provide any pathogenetically substantiated recommendations on the application of this method in the treatment of candidiasis.

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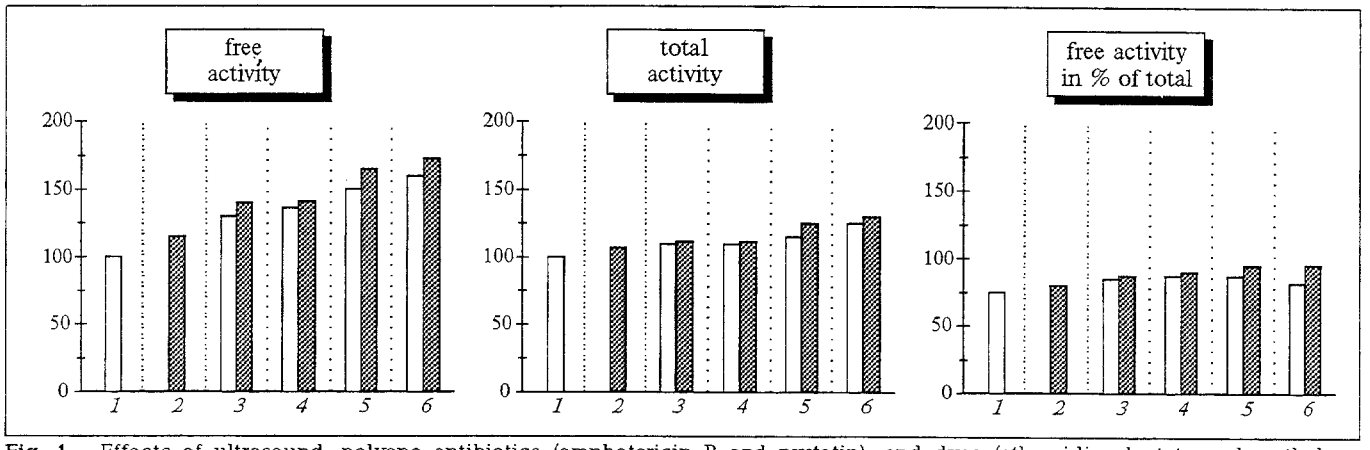

Fig. 1. Effects of ultrasound, polyene antibiotics (amphotericin B and nystatin), and dyes (ethacridine lactate and methylene blue) on acid phosphatase activity in *Candida* fungi. The values are percentages. *1*) Intact fungi (notexposed to US and chemicals); *2*) fungi exposed to US; *3*) fungi treated with methylene blue; *4*) fungi treated with ethacridine lactate; *5*) fungi treated with amphotericin B; *6*) fungi treated with nystatin.

One manifestation of the structural and functional impairments in microorganisms is an altered function of lysosomes, which play an important role in the life of any cell, and lysosomal activity may therefore be a criterion by which the severity of damage caused to fungi can be judged [3,8].

In view of the foregoing, the purpose of the present study was to measure the total and free activity of acid phosphatase, a marker enzyme for lysosomal membranes, in yeastlike *Candida* fungi after their treatment with a polyene antibiotic (amphotericin B, nystatin) or a dye (methylene blue, ethacridine lactate) with or without prior exposure of these cells to ultrasound.

MATERIALS AND METHODS

A total of 10 series of tests were carried out using 24-hour cultures of yeastlike *Candida* fungi grown on a dense Sabouraud medium. From the cultured cells suspensions were prepared whose density and dose was the same in all test samples (5×10^9 reproductive bodies). In each test series, both free and total alkaline phosphatase (AP) activities were measured and their percentage ratio was used as the criterion of the ease with which substrate-enzyme interaction had proceeded, considering that this reflected in large measure the stability of the lysosomal membranes in the fungal cell after their exposure to the agents used.

In the 1st (control) series, AP activities were measured in intact fungal cells. In the 2nd series, they were measured in fungal cells that had been continuously sonicated for 20 min using an ultrasound generator (frequency 2640±2.64 kHz; sonication power 1 W/cm^2). In the 3rd, 5th, 7th, and 9th series, unsonicated fungi were incubated with one of the drugs or dyes (amphotericin B, nystatin, methylene blue, or ethacridine lactate) added in a subbactericidal dose after titration with microbiological methods, tak-

TABLE 1. Acid Phosphatase Activity in *Candida* Fungi Treated with an Antifungal Antibiotic (Amphotericin B or Nystatin) or a Dye (Methylene Blue or Ethacridine Lactate) and Ultrasound (US). The Values are Means±SEM ($n=6-7$)

Treatment group	№ of experiment	Free activity (A_{free}), μg P/mg	Total activity (A_{total}), μg P/mg protein×min	A_{free}/A_{total}, %
None (control)	1	47.7±0.6	65.4±0.3	72.9±0.6
US alone (control)	2	54.6±0.8	69.8±0.4	78.6±0.6
Methylene blue + US	3	61.7±0.6	72.8±0.6	84.8±1.2
Methylene blue alone	4	66.2±1.0	74.4±0.1	89.1±1.4
Ethacridine lactate + US	5	64.2±0.3	72.6±0.5	88.3±0.6
Ethacridine lactate alone	6	68.0±0.4	75.3±0.3	90.7±0.8
Amphotericin B + US	7	71.8±0.2	78.0±0.5	92.2±0.5
Amphotericin B alone	8	80.7±0.1	83.4±0.2	96.9±0.09
Nystatin + US	9	75.8±0.1	83.7±0.2	90.6±0.09
Nystatin alone	10	84.0±0.1	86.4±0.6	97.2±0.5

Note. In all cases there were statistically significant differences ($p<0.01$) between group 1 and all other groups, between group 2 and groups 3 through 10, and between groups 3 & 4, 5 & 6, 7 & 8, 9 & 10, 3 & 7, 4 & 8, 5 & 7, 6 & 8, 3 & 9, 4 & 10, 5 & 9, and 6 & 10. For the A_{free}/A_{total} series the difference was insignificant between groups 3 & 4 ($p>0.1$) and 5 & 6 ($p>0.05$).

ing into account the volume and density of the fungal suspension. In the 4th, 6th, 8th, and 10th series, cultured fungi exposed both to a drug or dye (in the same dose as above) and to ultrasound were used.

AP activities were measured spectrophotometrically [7] in homogenates prepared by triturating the precipitate of a fungal culture in an ice bath for 2 min (120 rpm). The results were processed statistically using the parametric the Student *t* test [2].

RESULTS

Exposure to ultrasound alone (2nd series) was found to increase significantly both the total and free activities of the enzyme (by 6.7% and 14.4%, respectively) relative to their control values (Table 1, Fig. 1).

Unsonicated fungi incubated with methylene blue (3rd series) exhibited significantly higher AP activities than did the control fungi and those exposed to ultrasound alone. Still greater increases in enzyme activity occurred after treatment with both methylene blue and ultrasound.

Similar changes in the free and total AP activities were caused by ethacridine lactate (5th and 6th series), especially in sonicated fungi (Table 1 and Fig. 1)

AP activities were altered to a still greater extent by the polyene antibiotics, particularly in drug-treated and sonicated fungi (8th and 10th series).

Thus, as shown in the present study, the proportion of free AP activity, which reflects the possibility of substrate-enzyme interactions and the degree of lysosomal membrane stability, was lowest (apart from in the control series) in fungal cells exposed to ultrasound alone, was increased significantly in those treated with methylene blue or ethacridine lactate, and reached almost 100% in those exposed to both a polyene antibiotic and ultrasound. These results indicate that one mechanism by which ultrasound, dyes, and polyene antibiotics act on *Candida* cells is functional activation of their lysosomes with a consequent intensification of autolytic processes in the cells. The highest fungicidal effect was observed after combined treatment with ultrasound and antibiotics. The elevated functional activity of the lysosomal apparatus in *Candida* cells, leading to intensified catabolic and autolytic processes therein, may well be one of the key factors responsible for the therapeutic efficacy of dyes and polyene antibiotics in candidiasis, particularly when used in combination with ultrasound.

The results of this study lead to the conclusion that the treatment of candidiasis with polyene antibiotics should preferably be supplemented by exposure of *Candida* fungi to ultrasound, as this would enhance the fungicidal effect.

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