The light intensity (0.005095 $W \cdot cm^{-2} \cdot sec^{-1}$) and the distance (25 cm) between the filters (Hindustan-Pilkington Glass works, Asansol, Calcutta) and the preparation were the same in all cases. All experiments were performed in a dark room to exclude the influence of other environmental light conditions.

From the results (figs 1 and 2) it is clear that the heartbeat frequency decreased by $16.40 \pm 2.56\%$ when exposed to blue light (410–453 nm) and by $8.43 \pm 1.42\%$ on exposure to green light (510–565 nm). Surprisingly, the red light had no effect, but infrared increased it by $82.52 \pm 3.48\%$. However, these effects were reversible and the heartbeat frequency returned to normal within a few minutes after the cessation of the photic stimulation (fig. 1). In general, wavelengths shorter than 600 nm appear to inhibit the heart rate while those above 700 nm appear to stimulate it (fig. 2).

No obvious and perceptible change in the amplitude of the heartbeat was noticed in *Periplaneta americana* (L.) in contrast to the phasic effects of flickering light, noticed by Campan, on the heart rate of *Nemobius sylvestris* (Bosc.). Since all the sensory input was cut off in the present experiment, one is tempted to conclude that light influences the activity of heart cells or the nerve cells in the heart. Since similar experiments have not hitherto been performed on the isolated perfused insect heart further comparisons are not possible. It is apparent from the results that even isolated physiological systems without an environmental sensory input may be sensitive to photic stimuli.

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Potentiation of the biological activities of daunomycin and adriamycin by ascorbic acid and dimethylsulfoxide

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Summary. L-Ascorbic acid (0.57 mM) and dimethylsulfoxide (14.1 mM) were found to potentiate four times the antibacterial activities of daunomycin and adriamycin in the *Staphylococcus aureus* test. This effect, however, could not be demonstrated against eukaryotic cells and leukemia P 388 in mice.

The antitumor and antibacterial agents include some compounds displaying redox properties in a cellular environment, this playing an important role in their biological activity. Daunomycin and adriamycin, anthracycline antibiotics possessing a quinone moiety, are 2 such compounds and are effective anticancer agents in clinical practice. The redox cycle of the compounds leads to the formation of active free radicals, and as a specific consequence of this, severe cardiotoxic side-effects arise[2]. Various efforts have recently been made to prevent this cardiotoxicity. We report here the effects of L-ascorbic acid (AA) and dimethylsulfoxide on the activities of daunomycin and adriamycin in some biological systems.

Materials and methods. The materials were as follows: daunomycin · HCl (D · HCl, rubomycin · HCl, Medexport, USSR); adriamycin · HCl (A · HCl, Farmitalia); dimethylsulfoxide (DMSO, spectroscopic grade, Merck); 1,3-diphenylisobenzofuran (Aldrich-Europe); superoxide dismutase (SOD, EC 1.15.1.1, 2700 units/mg, Sigma); catalase (EC 1.11.1.6, circa 65,000 units/mg, Serva). Other chemicals were of analytical grade (Reanal).

For characterization of the antimicrobial activities of the compounds and combinations, the minimal inhibitory concentrations (MIC) were determined by the serial dilution technique. The strains and conditions of the experiments were as follows; for Staphylococcus aureus Duncan: medium type B243 (Difco), incubation at 37 °C; for *Tetrahymena pyriformis* strain GL: peptone-yeast medium[3], 25 °C; for *Saccharomyces cerevisiae S 288 cg*$^+$ (grande) and *S. cerevisiae S 288 cg°/6* (petite): medium containing 0.5% yeast extract (Difco), and 1.0% glucose, 30 °C. 10,000 cells of stock cultures were used to inoculate 3 ml of media. Evaluation was performed by measuring the turbidity at 520 nm in the culture of bacterium, and by microscopic cell counting using a Bürker chamber in the cultures of protozoan and yeast.

For examination of the antitumor activities, female BDF_1 mice weighing 20 g were inoculated i.p. with 10^6 P 388 leukemia cells on day 0. Treatment with the compounds was made with the specified dose i.p. on days 1, 2 and 3. The mean values of the survival time in groups of 6 mice were determined and the %T/C value, the percentage survival time of treated mice/control mice, was calculated.

Results. The antimicrobial activities of the combinations are shown in the table. The antibacterial activities of D · HCl and A · HCl were increased 2-fold by AA (0.57 mM), and a further 2-fold potentiation was achieved with DMSO (14.1 mM). At concentrations showing the maximum activity, the molar ratio of D · HCl:AA:DMSO was 1-4:257:6351.

$CuSO_4$ (10 nM) did not influence these effects. Radical scavenger compounds such as sodium benzoate (0.689 mM), ethanol (17 mM) and 1,3-diphenylisobenzofuran (0.0185 mM in 0.1% v/v DMSO) had no effect when added to mixtures 1 and 3. In mixtures 3 and 6, SOD, catalase and bovine serum albumin (10 µg/ml each) did not exert any decreasing effect. $NaBH_4$ (2.65 mM) decreased the activity of D · HCl 4-fold. At this molar excess of $NaBH_4$ the reduced form of D · HCl can be found, as was verified by TLC.

Tetrahymena pyriformis and *Saccharomyces cerevisiae* are less sensitive to D · HCl, and no potentiating effect of AA and DMSO was found against these cells. The petite mutant of *S. cerevisiae*, in which the mitochondrial aerobic respiration is missing, is less sensitive than the grande to D · HCl, in accordance with the data published for adriamycin[4]. As a peculiar effect of D · HCl and also its combinations with AA and DMSO, it was found that at half the

MIC value, that is at the highest concentration of D · HCl, where cell propagation still occurred, significantly enlarged cells were present; these formed chains and agglomerations, in contrast to the small and single form of the normal cells.

Antitumor test. The median survival time (± SD) of the control (given physiological saline) P 388 leukemia mice group was 11.0 ± 0.89 days. When D · HCl in a 4 mg/kg dose was given once on day 1, the survival time was 27.4 ± 2.8 days (% T/C - 249%), while with repeated treatment with this dose on days 1, 2 and 3 it was 21.6 ± 2.1 days (196%); a repeated dose of 0.5 mg/kg led to a survival time of 16.4 ± 0.55 days (145%). When the D · HCl in the above 3 treatment schedules was supplemented with AA (100 mg/kg) and DMSO (1000 mg/kg), the survival times were 28.6 ± 2.9 days (260%), 20.8 ± 1.8 days (189%) and 17.1 ± 1.0 days (155%) respectively, showing that there were no significant changes (defined as more than ± 25% difference) in survival times and in tumor development. This means that these combinations did not alter the effectiveness of D · HCl.

Discussion. The quinone structure of anthracycline may be reduced chemically and enzymatically[5,6]. This leads to the formation of the semiquinone, and reaction with O_2 results in the formation of the superoxide radical (O_2^-), H_2O_2 and the OH· radical, which damage DNA. Cells are defended enzymatically against these radicals[7], but in the cardiac muscle this mechanism is reduced[8].

We found that the activities of daunomycin and adriamycin against *Staphylococcus aureus* cells can be potentiated by L-ascorbic acid and DMSO. This effect was not reduced by radical scavenger compounds or enzymes. The *Staphylococcus* cell itself is characterized by a high catalase production. Cu^{++}, which in vitro stimulates the autoxidation of AA[9], dit not influence the effectiveness of the combinations.

Antimicrobial activities of the compositions

No. Composition	Minimal inhibitory concentrations (μM) *S. aureus** 2 days	5 days	*T. pyr.* 7 days	*S. cer.* g^+ 7 days	*S. cer.* $g^-/6$ 7 days
1 Daunomycin · HCl**	8.88	17.7	355	44.4	178
2 1 + AA (0.057 mM)	8.88	8.88			
3 1 + AA (0.57 mM)	4.44	8.88	355	44.4	178
4 1 + AA (2.34 mM)	8.88	17.7			
5 1 + AA (5.68 mM)	8.88	17.7			
6 3 + DMSO (14.1 mM)	2.22	4.44	355	44.4	178
7 3 + DMSO (1.41 mM)	4.44	8.88			
8 3 + DMSO (0.14 mM)	4.44	8.88			
9 3 + DMSO (0.014 mM)	8.88	8.88			
10 5 + DMSO (14.1 mM)	2.22	4.44			
11 1 + DMSO (14.1 mM)	8.88	17.7			
12 1 + $CuSO_4$ (10 nM)	8.88	17.7			
13 3 + $CuSO_4$ (10 nM)	4.44	8.88			
14 6 + $CuSO_4$ (10 nM)	2.22	4.44			
15 1 + $NaBH_4$ (2.65 mM)	35.4	70.8			
16 3 + $NaBH_4$ (2.65 mM)	35.4	70.8			

*Full namens of the strains: *Staphylococcus aureus* Duncan, *Tetrahymena pyriformis* GL, *Saccharomyces cerevisiae S 288* g^+ (grande) and $g^-/6$ (petit). **Double-scale serial dilutions for daunomycin HCl were made in 10 parallels. MIC indicates the concentration of D · HCl where no cell multiplication could be detected. SD = ± 0.0 in the table means that the differences in activities for doubled concentration series exceeded the value of the deviation of the experiments. When adriamycin · HCl was used instead of D · HCl, the MIC values for *S. aureus* Duncan were halved. In mixtures 1, 3 and 6 these values were 4.44, 2.22 and 1.11 μM respectively, after an incubation of 2 days. There was no inhibition of cell propagation at the given concentrations of materials. Values of MIC: $NaBH_4$ > 20 mM, AA > 10 mM, DMSO > 1000 mM.

L-Ascorbic acid is readily oxidized, so that interaction of AA with the process of enzymatic reduction of these anthracyclines, followed by autoxidation resulting in oxygen radicals, could be expected. There are numerous experimental data on the role and possible therapeutic application of AA[10,11]. Radical formation and DNA cleavage have been reported in an in vitro system[12], while in vivo its antioxidant, radical scavenging role has been demonstrated[9,13]. It is known that in vitro some nitro compounds mediate the reaction of ascorbate with oxygen. The consumption of oxygen in these experiments could be enhanced by DMSO and could be inhibited by superoxide dismutase and catalase or by Ehrlich cells containing these enzymes[14,15]. Sodium ascorbate was found to potentiate the growth inhibitory effects of certain agents of different structures on neuroblastoma cells in vitro, this being partly explained by the inhibition of catalase activity by ascorbate, which results in accumulation of toxic H_2O_2[16].

As regards the role of DMSO, its OH· radical scavenging effect is known[17], and accordingly DMSO is indirectly able to increase the electron transport processes[14]. The usefulness of DMSO in therapy has been discussed[18].

The role of the cellular reduction-oxidation cycle in effecting antibacterial activity has been shown for some antibiotics, e.g. streptonigrin[19] and toxoflavin[20].

The potentiating effect of AA and DMSO could be revealed only in the *Staphylococcus* bacterial test, and was demonstrated neither in 2 eukaryotic cell tests nor in the in vivo P 388 leukemia test, showing that this effect appears only in a particular system. The efficacies of combinations such as redox chemotherapeuticum, L-ascorbic acid and DMSO will also be examined in other tests.

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