Effect of light of different wavelengths on the perfused isolated heart of *Periplaneta americana* (L.)[1]

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Summary. The isolated perfused heart of *Periplaneta americana* (L.) was exposed to light of different wavelengths. Heart rate was reduced by blue and green lights, not affected by red light, and was increased by infrared light.

Various attempts have been made to show extraocular light sensitivity among arthropods in general and insects in particular. Tucolesco[3] has shown that the larvae of *Tenebrio molitor* avoid light after decapitation, indicating dermal light sensitivity. According to Booth[4] the antennae of *Aphis fabae* carry receptors which are responsible for the animal's photokinetic activity. Although insects are known to exhibit pronounced alterations in their physiology and behavior in response to changes in the photoperiod, very little is known about the relation of extraocular sensory centres to photoperiodism. In *Papilio xuthus* Arikawa[5] has demonstrated 2 distinct photoreceptive areas on either side of the genital region, one sending afferents to the 6th pair of nerve roots and the other connecting the CNS through the 5th pair of nerve roots of the last abdominal ganglion. However, whether these receptors are functional in copulation and other reproductive behavior is not clear. Saunders[6] found the presence of photoreceptors in the brain and the last abdominal ganglion of *Periplaneta americana* and in the brain of *Hyalophora cecropia.* Campan[7] reported some electric light induced disturbances in the cardiac rhythm of *Calliphora vomitoria* (L.) and *Nemobius sylvestris* (Bosc.). However, very little is known about the cardiac responses of insects to lights of different wavelengths. Experimental results that demonstrate a distinct effect of light of different wavelengths on the isolated heart of *Periplaneta americana* (L.) are presented here.

Cockroaches were reared in the laboratory. The heartbeats of cockroaches and the effect of lights of different wavelengths were determined by Naidu's[8] method with some modifications. Since in the technique employed the animals were freed from the brain and ventral nerve cord, any indirect effects of the experimental stimuli on the cardiac rhythm were eliminated. Since continuous exposure to light is known to decrease the cardiac sensitivity to temperature variations[9], in the present work the heart was exposed to white light (40 W) to diminish the temperature effect. Further, the physiological solution[10] bathing the heart was kept circulating at the rate of 115–120 drops/min and actual measurements of the bathing fluid during the course of the experiment did not show any temperature variations. The steady state of normal heartbeat frequency was obtained after about 1 h. In different individual roaches it varied from 90 to 130 beats/min at 22 ± 1 °C. Effects of different photic stimuli were studied on different preparations after the white light had been switched off. Light of a given color was shone from above the preparation for 30 min continuously. The heartbeats were recorded every alternate minute and then, immediately, the preparation was exposed to normal light again to follow the recovery. The preparation was exposed to infrared light only for 6 min.

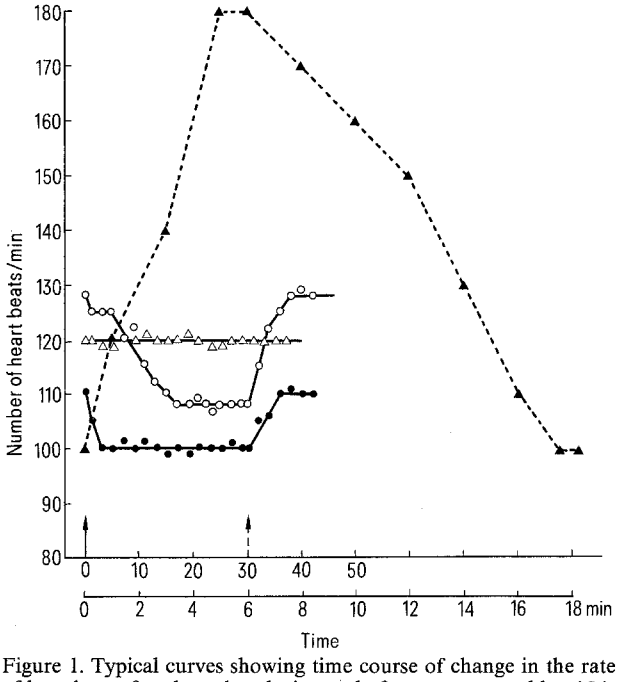

Figure 1. Typical curves showing time course of change in the rate of heartbeat of cockroaches during and after exposure to blue (○), green (●) red (△), and infrared (▲) lights. The solid and broken arrows respectively indicate the onset and cessation of the photic stimulus in each case. The time scale on the abscissa is different for infrared light (0–18) from that of blue, green and red lights (0–50).

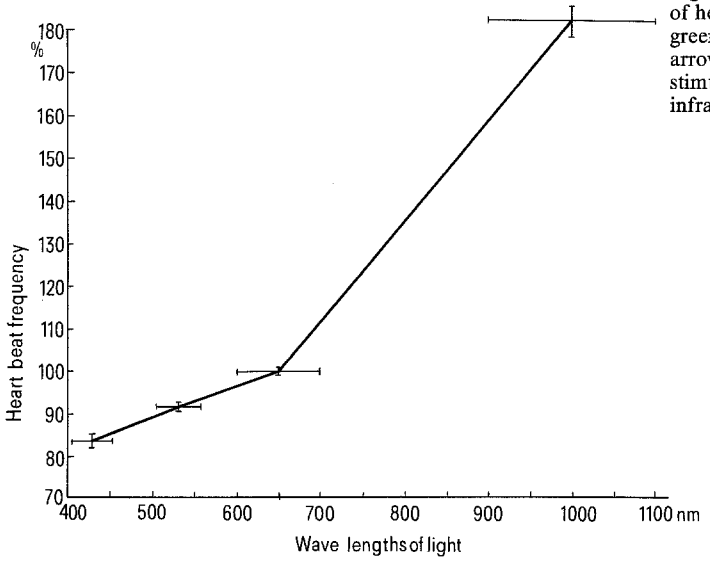

Figure 2. Relationship between heartbeat and stimulating wavelengths. Since the heart was stimulated by lights of different colors (and not by monochromatic lights) the range of stimulating wavelengths is shown in each case by the horizontal bar. The heartbeat frequencies plotted against blue (410–453 nm), green (510–565 nm), red (600–700 nm) and infrared (900–1100 nm) lights are measured at times indicated by the broken arrow in figure 1 and expressed as percentages of the controls (measured at zero time, solid arrow in fig. 1). The heartbeat frequencies plotted for blue and green lights are the means of those 100 cockroaches, while those in the case of red and infrared lights are the means of 50 values. The SD in each case is shown by a vertical bar.

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