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PHARMACOLOGICAL APPLICATION OF ANIMAL CALORIMETRY

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ABSTRACT

Heat dissipation is a sensitive indicator for the effects of pharmaceutical agents on living systems. Informations are gained not only by observing changing levels, but also by comparing dynamic patterns of metabolic activity. Modern microcalorimeters are sufficiently sensitive and provide a high time resolution to perform these measurements with small animals. The simultaneous record of heat dissipation and oxygen consumption in an open flow system permits a thermochemical interpretation of the calorimetric data and provides insight into the specific effects of pharmaceutical agents.

Experiments were performed with aquatic oligochaetes, zooplankton, and fish eggs and larvae. The combination of Streptomycin and Penicillin inhibited aerobic rates but did not exert any influence under anoxic conditions. Streptomycin and Neomycin, on the other hand, stimulated heat dissipation under aerobic as well as anoxic conditions. Oxygen consumption was not increased to the same extent as the calorimetric signal: This indicates the activation of anoxic mechanisms during periods of highly increased metabolic activity. Hence direct calorimetry is a more sensitive and general method than respirometry in pharmacological and toxicological investigations. The combination of the two methods greatly enhances the value of both analytical tests.

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INTRODUCTION

Antibiotics play an important role in the aquaculture of invertebrates and fish (Bardach et al, 1972; D'Agostino, 1975; Fisher and Nelson, 1978). The increased range of application of pharmaceutical substances, the increasingly large number of different antibiotics, and their use in different combinations produce a demand for improved methods to test the sublethal effects of various agents on aquatic organisms. Little is known about the influence of antibiotics on lower animals as compared to the detailed analyses of the antibiotic action on microorganisms (for a review see Dalla Via, in press).

MATERIAL AND METHODS

Direct calorimetric experiments were performed with a LKB 2107 flow sorption microcalorimeter as described previously (Gnaiger, 1979). The 0.5 cm^3 glass flow vessel served as the animal chamber. Prethermostated water was equilibrated with air or a gas mixture, and pumped at 5.7 $cm^3 \cdot h^{-1}$ through the experimental system via a gold capillary tube. After the establishment of reference levels of metabolic heat loss, antibiotics were added to the 400 cm^3 water reservoir which was constantly stirred by a magnetic impeller.

A twin-flow microrespirometer (Gnaiger, in press) was developed for simultaneous respirometric measurements. Two YSI polarographic oxygen sensors are interpolated in the flow path at the inlet and outlet of the calorimeter. Since the p_{O_2} of the water in the reservoir is known, the sensor at the inflow into the calorimeter is being calibrated, while the sensor at the outlet signals the drop in oxygen due to respiration. Calibration and measuring position are alternately changed by two 4-way microvalves. Switching the position of the oxygen sensors does not interrupt or reverse the flow through the animal chamber and hence does not disturb the calorimetric signal.

Animals were acclimated to experimental temperatures. Salmonid fish, Salvelinus alpinus from an experimental fish farm (Fisch-

zucht Thaur), were observed before hatching (eggs) and after hatching but before feeding was commenced (larvae). Experiments with the crustacean plankter, Cyclops abyssorum tatricus (Copepoda), were performed immediately after sampling the population of the lake Kalbelesee (Vorarlberg). Commercially available "Tubifex" were purchased, the species Lumbriculus variegatus (Oligochaeta) was sorted out and kept in the laboratory without food.

The effects of combinations of two antibiotics were tested: 1) Streptomycin- and Neomycin-sulfate. These are stable in aqueous solution and exhibit optimum antimicrobial activity in the pH range 7.4 to 8.0. Minimum inhibitory concentrations of Streptomycin range from 0.5 to 25 mg . dm^{-3} for gram-positive and gram-negative bacteria, and up to 150 mg . dm^{-3} for Neomycin and gram-positive bacteria. Rapid development of resistance is observed. 2) Streptomycin-sulfate and Penicillin G. Penicillin is fairly unstable in water, shows highest antimicrobial activity between pH 6.0 and 6.5, and is extremely active against gram-positive bacteria, but only at concentrations > 100 mg . dm^{-3} against the gram-negative. For a compilation of the literature on antibiotics see Dalla Via (in press).

RESULTS

Aerobic and anoxic environmental conditions

Streptomycin and Neomycin stimulate the metabolic activity of L.variegatus at concentrations of 20 to 200 mg . dm^{-3}. This effect is observed under aerobic conditions as well as under environmental anoxia, and elevated levels of heat dissipation are maintained over extended periods of time (Gnaiger, 1980a). On the contrary, addition of Streptomycin and Penicillin to the medium inhibits the metabolism after a transitory stimulation (Fig. 1). The stability of the lower level of heat loss even after replacement of the antibiotic medium by pure water points to an irreversible effect.

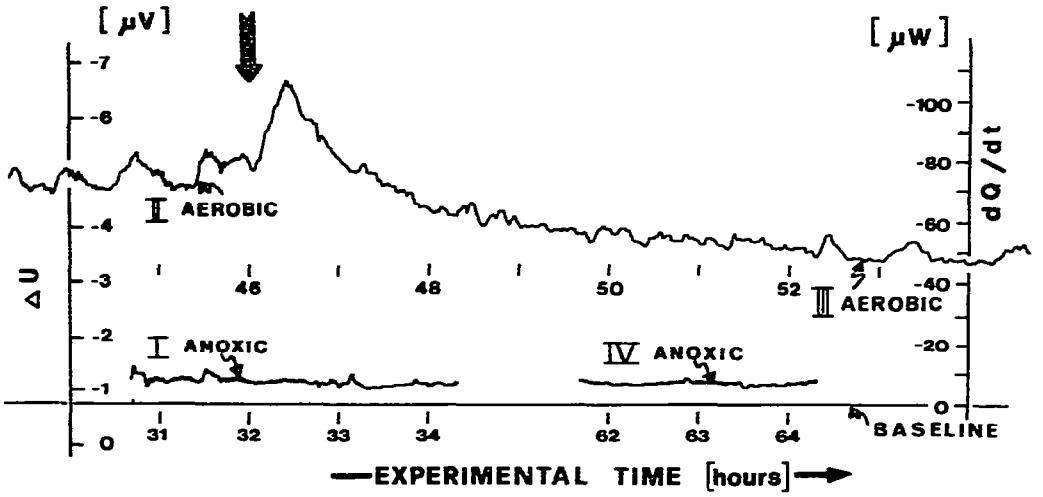

Fig. 1: Heat dissipation, $dQ.dt^{-1}$ [µW], of Lumbriculus variegatus under aerobic and anoxic conditions, before and after application of Streptomycin and Penicillin. Four sections of the recorder trace in an experiment with 10 animals (101.8 mg total wet weight; 20°C; flow rate of 5.7 $cm^3.h^{-1}$) are shown. I: anoxic metabolism without antibiotics; II: aerobic metabolism without antibiotics; Arrow: addition of 500 $mg.dm^{-3}$ Streptomycin-sulfate and 1000 $mg.dm^{-3}$ Penicillin G; III: constant level of aerobic heat dissipation after application of antibiotics; IV: anoxic metabolism with antibiotics. The numbers indicate the time of the experiment [hours] corresponding to each section. The antibiotics inhibited aerobic heat dissipation by 30%, while the anoxic rate was not affected.

Patterns of heat dissipation

Streptomycin and Penicillin not only reduced the metabolic level of the oligochaetes, but also tended to decrease the short-term fluctuations of the power-time curves, once the new steady state was reached (Fig. 1). However, a more disturbed pattern of heat dissipation appeared upon exposure of the copepods to these antibiotics (Fig. 2). The patterns may be related to locomotory activities of the animals which result in different structures of the power-time curves.

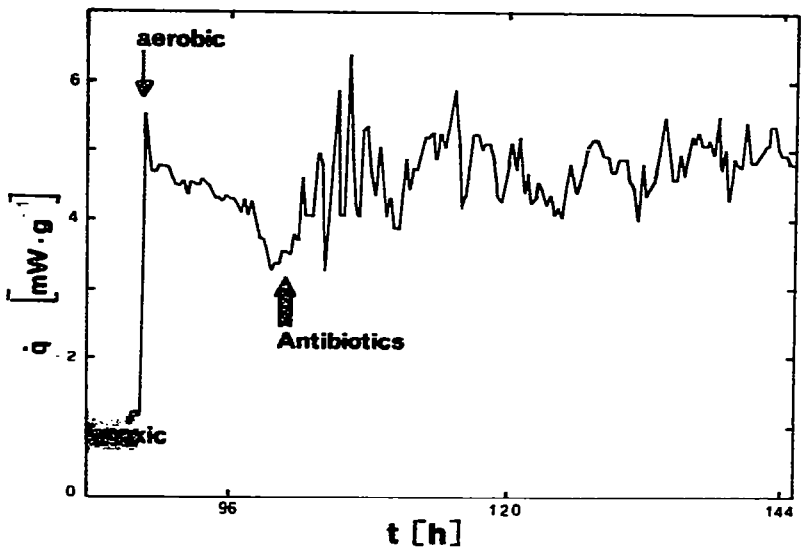

Fig. 2: Specific rate of heat dissipation, $\dot{q}$[mW.g^{-1} dry weight], during aerobic recovery in Cyclops abyssorum tatricus, and stimulating effect of Streptomycin-sulfate and Penicillin G (200 mg.dm^{-3} each) on metabolic rate and pattern of heat dissipation. 40 animals (1.418 mg dry weight); 6°C; flow rate of 5.65 cm^3.h^{-1}; interpolated points are 20 minute averages of the power-time curve (after Gnaiger, in press).

Fish eggs and larvae: calorimetry and respirometry

Eggs of S.alpinus were not affected by Streptomycin and Neomycin. The experimental sections shown in Fig. 3 are taken from transitory periods after anoxic exposure. The slightly changing levels of heat dissipation are due to the slow aerobic recovery of the salmonid fish eggs (Gnaiger, 1979). No baseline drift of the calorimeter occurred during these periods as evidenced by the stable thermostat temperature (Fig. 3B) (see Kaufmann and Gnaiger, 1981). The appearance of some structure in the power-time curve was frequently observed during aerobic recovery and can therefore not be attributed to the addition of antibiotics (compare Fig. 3A and B). After hatching, however, the protection by the egg membrane is lost, and the larvae are seriously disturbed by the exposure to the antibiotics (Fig. 4)

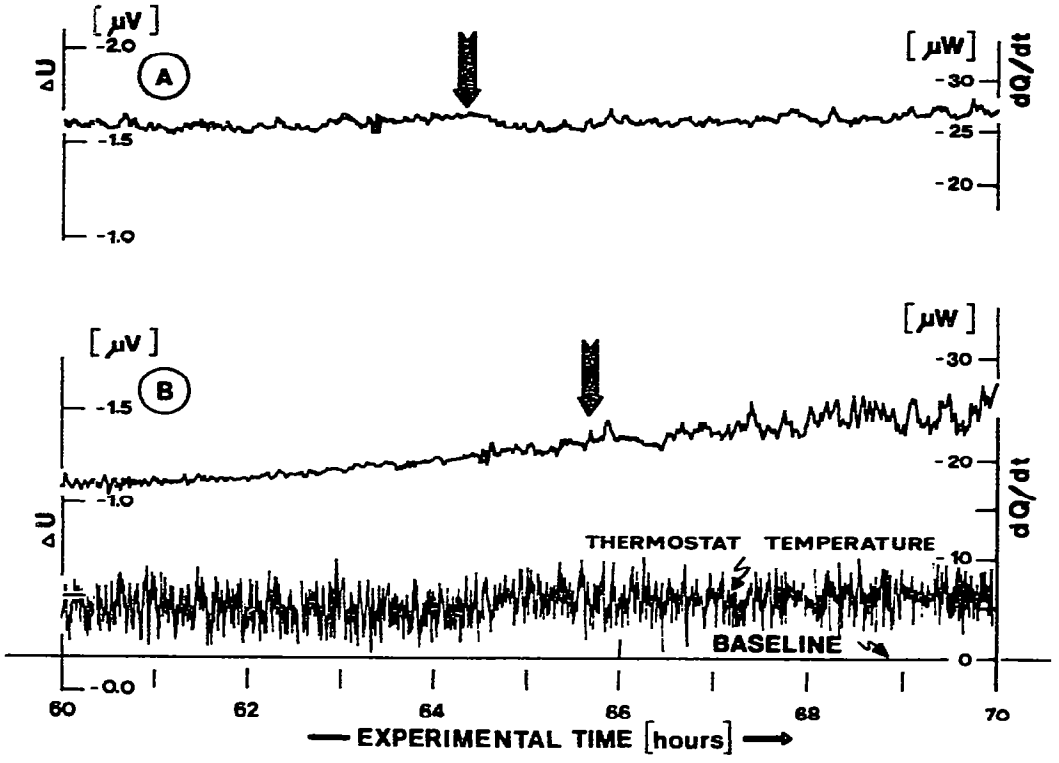

Fig. 3: Effect of Streptomycin- and Neomycin-sulfate (200 mg.dm^{-3}; arrows) on heat dissipation, dQ.dt^{-1}[µW], of eggs of Salvelinus alpinus in two experiments during aerobic recovery from anoxic exposure. 4 eggs (about 20 mg dry weight) in each experiment; 8.5°C; flow rate 3.3 cm^3.h^{-1}. Eggs are not affected by the antibiotics indicating that the egg membrane is impermeable to these substances. The absence of interfering microbial contamination is also documented. For a discussion of thermostat temperature (noise is about ± 0.002°C) in relation to baseline stability see Kaufmann and Gnaiger (1981).

Streptomycin and Neomycin induced a pronounced peak of heat dissipation and oxygen uptake of the larvae. During this period heat dissipation was increased almost threefold, whereas oxygen consumption was doubled only. The remaining gap between the direct calorimetric measurement and the respirometric estimate

of heat loss is due to anoxic processes sustaining high metabolic activity. After the initial heat burst, metabolism levelled off at high and fluctuating rates showing correspondence between direct and indirect calorimetry (Fig. 4).

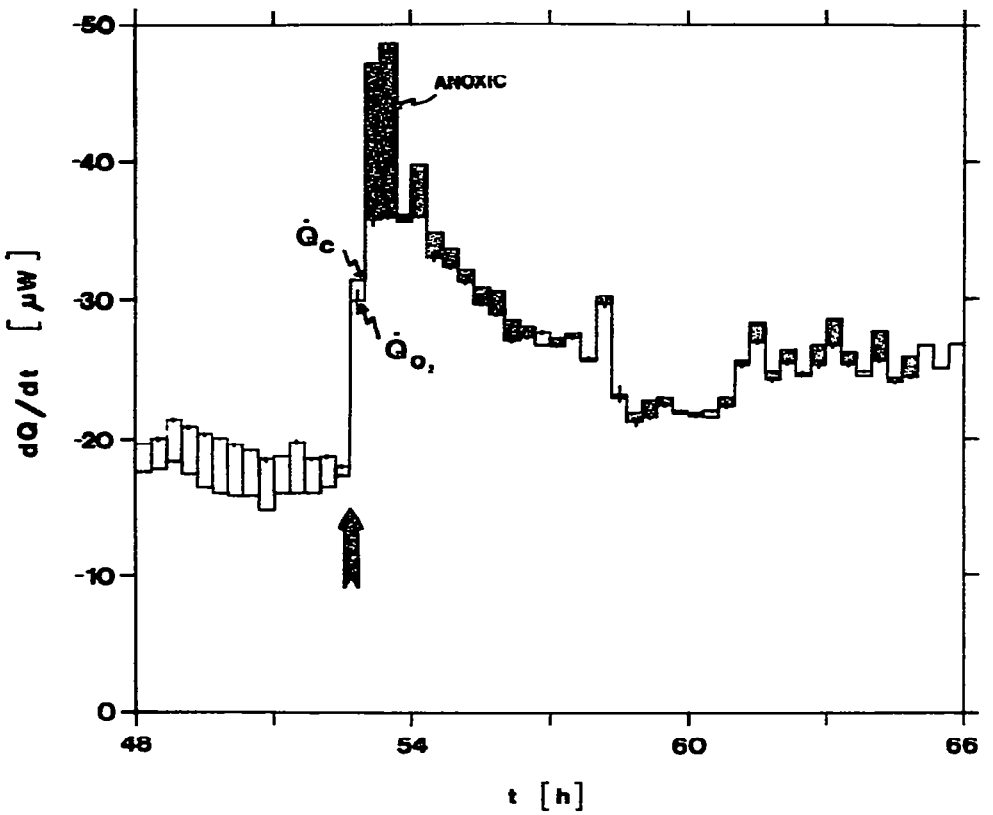

Fig. 4: Effect of Streptomycin- and Neomycin-sulfate (200 mg.dm^{-3}; arrow) on heat dissipation, dQ.dt^{-1}[µW], of a larval Salvelinus alpinus (5.1 mg dry weight; 8°C; flow rate 5.7 cm^3.h^{-1}; 48% air saturation). 20 minute averages of direct calorimetric measurements of heat loss, $\dot{Q}_c$, are shown by thick lines. The indirect estimations of heat loss, $\dot{Q}_{O2}$, were obtained from simultaneous oxygen consumption measurements. The oxycaloric equivalent of 451 kJ.mol^{-1} O_2 was obtained under normoxic conditions. The raw data of oxygen consumption were corrected for instrumental lag using a first order exponential approximation. The vertical bars indicate the most probable range of uncertainty due to the variable time constant of the respirometer (Gnaiger, in press). The simultaneous direct and indirect calorimetric measurements indicate the high anoxic contribution to the stress reaction (hatched area).

DISCUSSION

Numerous microcalorimetric analyses of the action of drugs on microorganisms have been reviewed by Wadsö (1977) and Beezer and Chowdnry (1980). There is also an increasing pharmacological application of microcalorimetry in the study of plant tissues (Anderson and Lovrien, 1979) and animal cells and tissues (Spink and Wadsö, 1976; Wadsö, 1977; Kemp, 1980; Pätel et al, 1980). The present report, however, represents the first microcalorimetric investigation of antibiotic effects on aquatic animals.

The scarce information on antibiotic influences of animal metabolism stands in contrast to the frequent application of these drugs. In biochemical, physiological and ecological experiments antibiotics are added to prevent interferences by microbial metabolism (Yetka and Wiebe, 1974). However, the above results demonstrate, that animals are also influenced to a variable extent by some commonly used bacteriostatic agents. Therefore any neglection of these effects leads to an erroneous interpretation of the experimental data. For instance, metabolic rates may be increased by 50% (Fig. 4) or decreased by 30% (Fig. 1) over extended periods of time depending on the antibiotic combination and on the organism. Hence uncritical application of antibiotics in bioenergetic studies cannot be recommended. Open flow calorimetry presents a quick method to quantify the antibiotic side effect on the animals; corrections are then possible in experiments where the use of these drugs cannot be avoided.

In addition, direct calorimetry provides the unique possibility to test the influence of chemical agents on metabolic rates under aerobic as well as anoxic conditions (Gnaiger, 1980a). The exclusive inhibition of aerobic respiration by Streptomycin and Penicillin (Fig. 1) may be taken as a suggestive indication, that the mechanism of action on the animals is specific to the respiratory chain. Streptomycin and Neomycin are known to be inactive against bacteria under anoxia (Dalla Via, in press).

However, these antibiotics exhibit a stimulating effect on heat dissipation of L.variegatus even in the absence of oxygen (Gnaiger, 1980a).

Simultaneous calorimetric and respirometric measurements provide experimental values of the caloric equivalent of oxygen consumption. The theoretical oxycaloric equivalent in dissipative metabolism, $\Delta H_k(O_2)$, ranges from -438 to -496 $kJ.mol^{-1}$ O_2 for different substrates (fat and carbohydrate; protein ranges intermediate) and different buffers (intra- and extracellular) (Gnaiger, 1980 b). Under the influence of drugs there may be a change not only in absolute levels of metabolism, but also in the ratio of heat dissipation to oxygen consumption. The increase of the apparent oxycaloric equivalent up to -600 $kJ.mol^{-1}$ O_2 can be explained only by the activation of anoxic mechanisms which amount to 25% of the total heat loss during the immediate stress response (Fig. 4).

A drastic increase of the oxycaloric equivalent is also known in yeast exposed to Nystatin (Schaarschmidt et al, 1979). Reis and Büger (1967) interprete the dependence of the caloric equivalent of oxygen consumption on pharmaceutics by uncoupling of the respiratory chain. Their thermodynamic discussion is erroneous, since the use of theoretical values of $\Delta H_k(O_2)$ already rests on the assumption of a purely dissipative catabolism of substrates without net gain of ATP or other energized products (Gnaiger, 1980 b). Uncoupling per se cannot increase the oxycaloric equivalent. A decreased $\sim P/O_2$ ratio, however, increases the caloric equivalent of molar ATP-turnover, $\Delta H_k(\sim P)$: for the same amount of substrate and oxygen metabolized, the same amount of heat is dissipated but less ATP is generated to be used in the ATP-cycle (Gnaiger, 1980 b, c). An increase in calorimetric and respirometric rates does not signal an equal increase of metabolism in terms of ATP-turnover if uncoupling occurs. Glycolytic substrate level phosphorylations in anoxic metabolism, as indicated by an increased apparent $\Delta H_k(O_2)$, could compensate for the lower ATP-coupling coefficients during exposure to uncoupling agents. This proposition adds to the attractiveness of simultaneous direct and indirect calorimetry in the analytical test of drugs.

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