concentration of 50 μg/ml CPZ eliminated the R-factor from 15% of the cells of a polyresistent strain of *E. coli*, whilst 100 μg ethidium-bromide, under the same experimental conditions, eliminated the plasmid from 10.5% of the cells. The auxotrophic properties of the $R^-$ cells were the same as the $R^+$ cells.

It is possible that CPZ has a multifocal action on bacteria, since it inhibits different enzymes in mammalian tissues e.g. glutamate dehydrogenase[3], succinoxydase, succinodehydrogenase[4], alters the permeability of biomembranes and inhibits oxydative-phosphorylation[5] and phagocytosis of human leucocytes in vitro[6].

*Zusammenfassung.* Nachweis der antimikrobiellen Wirkung der bekannten Neuroleptika Chlorpromazin, Levomepromazin und Promethazin, wobei Chlorpromazin die beste bakterizide Wirkung hatte. Die grampositiven Bakterienstämme waren empfindlicher gegen Chlorpromazin als die gramnegativen, während Chlorpromazin keinerlei Wirkung auf die Vermehrung von *Pseudomonas aeruginosa* hatte.

J. Molnár, J. Király and Yvette Mándi

*Institute of Microbiology, University Medical School, Dom tér 10, H–6720 Szeged (Hungary), 18 November 1974.*

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## Simultaneous Recording of Heat and Fluorescence Following Contraction of Isolated Cardiac Muscle

It has been established that when cardiac muscle is irradiated with UV-light the intensity of fluorescent emission is linearly related to the amount of reduced nicotinamide adenine dinucleotide (NADH) present[1]. In fluorescence studies on amphibian skeletal muscle Jobsis and Duffield[2] have suggested that the area enclosed by a fluorescence transient waveform $\int \Delta Fl.dt$, should be linearly proportional to the flux of adenosine diphosphate (ADP) through the respiratory chain during mitochondrial oxidative phosphorylation. This means that the time integral of a fluorescence change following a muscle contraction should be proportional to the total energy cost of the contraction. In order to test this postulate we have made simultaneous recordings of heat production and fluorescence changes in isolated cardiac muscle performing isometric and isotonic contractions.

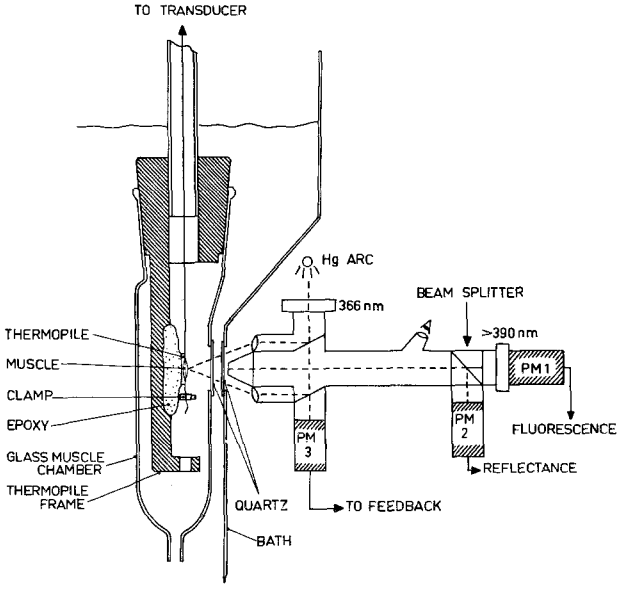


Fig. 1. Schematic diagram of apparatus for simultaneous recording of heat and fluorescence. Filters are labelled according to the wavelengths transmitted. The physiological solution was drained from the muscle-thermophile chamber for myothermic and optical recording.

Papillary muscles from the right ventricles of stunned rabbits were isolated after perfusing the coronary circulation with 20 to 30 ml of physiological solution containing (m*M*) NaCl, 118.0; KCl, 4.75; $MgSO_4 \cdot 7H_2O$, 1.18; $KH_2PO_4$, 1.18; $CaCl_2 \cdot 2H_2O$, 2.54; $NaHCO_3$, 24.8 and aerated with 95% $O_2$ – 5% $CO_2$. This solution was also used for bathing the isolated muscle with the addition of 10 m*M* sodium pyruvate (pH 7.4).

The myothermic apparatus has been described previously[3]. It consists of a thermopile made up of over 100 active silver-constantan junctions. These junctions are contoured into a groove in which the papillary muscle sits. Certain modifications were made to the thermopile to facilitate fluorescence measurements: in order to minimize background fluorescence the thermopile and frame were coated with black non-reflective, non-fluorescent paint everywhere except for a strip about 0.5 mm wide extending down the middle of the groove occupied by the papillary muscle. This groove also housed a flattened piece of platinum about 4 mm in length which was used as a stimulating electrode at the tendinous end of the muscle. The other stimulating electrode was mounted on a screw which could be rotated so as to position the tip of the electrode against the ventricular end of the muscle as it lay in the groove of the thermopile. This arrangement was used in order to minimize optical artifacts during fluorometric recording but it also allowed lower stimulus strengths to be used than with the previous methods where both electrodes were cantilevered from above[3]. Heat loss from the muscle-thermopile system was practically exponential so that heat records could be electrically corrected with very little error, but to ensure accuracy the uncorrected heat records were compared gravimetrically with a reference standard obtained by liberating a known amount of energy into the muscle-thermopile system.

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The fluorometric apparatus was similar to that described previously[4] with additional feedback compensation for instability of the arc lamp[5]. As can be seen in Figure 1 the preparation was illuminated with 366 nm radiation from the epi-illumination attachment of a microscope. The intensity of the emitted light which peaks at 480 nm was recorded with a photomultiplier tube. The a.c. output from the photomultiplier tube was demodulated and displayed as a d.c. oscillographic signal. The level of 366 nm reflected light was also monitored to serve as a check on the optical alignment of the preparation in the light field. If, following a contraction, this reflectance did not return to baseline then the record was rejected.

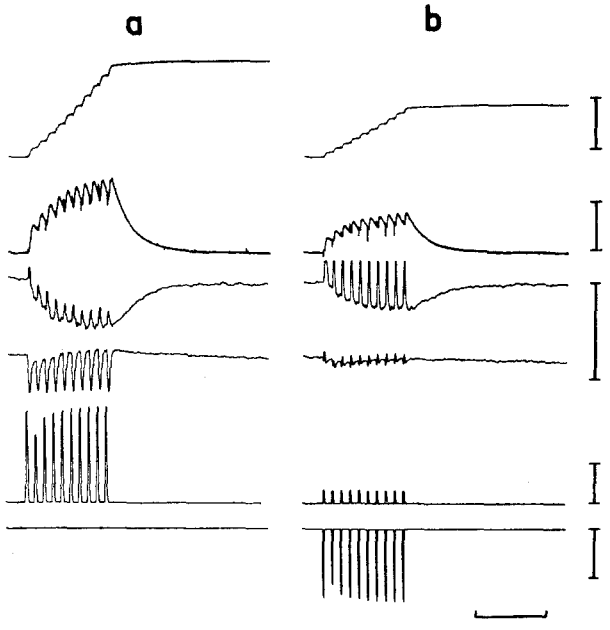


Fig. 2. Original records from isometric (a) and isotonic (b) contractions of a papillary muscle. From above down the traces show heat production, thermoelectric output, fluorescence, reflectance, tension and muscle length. The calibrations from above down are 60 mJ/g of muscle, 5 μV, 20% of the resting optical baseline, 25 m$N$/mm$^{-2}$, 0.5 mm· The horizontal line calibration is 30 sec.

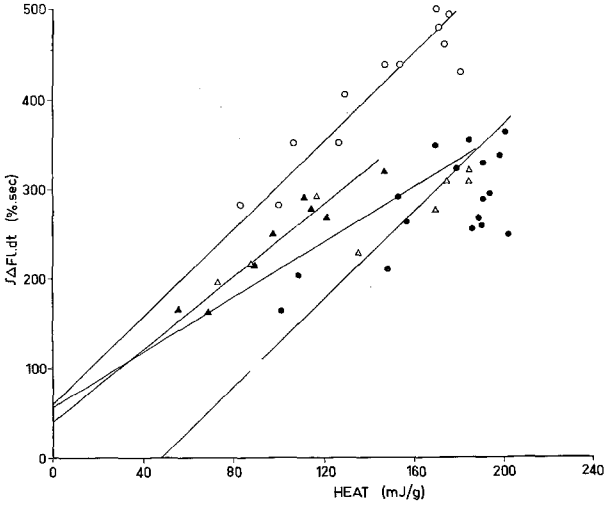


Fig. 3. Relationships between fluorescence-time integral ($\int \Delta F1/dt$) and heat production obtained from 4 papillary muscles and corrected for stimulus heat artifact. Least square regression lines have been fitted with correlation coefficients of ○, 0.93; ▲, 0.96; △, 0.85; ●, 0.77.

The critical problem in combining the two recording techniques is to allow for the limited working distance from the front of the microscope objective (16 mm) and still have adequate thermal isolation of the thermopile from the laboratory surroundings. This problem was overcome by using a cylindrical stainless steel container as the reference water bath containing well stirred water circulated to it from an external thermostat[6]. The container was surrounded by a wall of plastic foam which was held in place by an outer housing moulded from fibreglass. A hole large enough to accomodate the microscope objective was made in the insulating layers in line with a quartz window fixed to the inside of the stainless steel cylinder. Another quartz window was also fitted into the front of the glass organ bath containing the preparation and bathing medium. The clearance between the 2 quartz windows was only 2 mm but this space, occupied by temperature-stabilized flowing water, was sufficient to allow adequate thermal isolation (Figure 1). When the physiological solution was drained from the organ bath the heating effect of the arc lamp was found to be equivalent to about half of that due to the resting metabolism of the muscle. The steady temperature resulting from these two heat sources formed the resting baseline against which the active heat production of contracting muscles was measured.

Figure 2 shows myothermic, optical, and mechanical signals recorded oscillographically during isometric and lightly loaded isotonic contractions of a papillary muscle at 22 °C. In each of the two sets of records the muscle was stimulated electrically to contract ten times at a rate of 0.25 Hz. The recording conditions were a) isometric contraction, b) isotonic contraction against a load of 9.8 m$N$. The individual traces shown in Figure 2 are, from above down, heat production (corrected for heat loss), thermoelectric output (uncorrected), fluorescence (> 390 nm), reflectance (366 nm), tension and muscle length. The corrected heat records were obtained by recording the thermoelectric output on magnetic tape and replaying the signals through a heat loss corrector. The newly-gained advantage of simultaneous recording is that it affords a calibration of the fluorometric technique hitherto unavailable except for amphibian skeletal muscle[7]. The tissue fluorescence recorded under the present conditions arizes largely from the fluorescence of reduced nicotinamide adenine dinucleotide, NADH[2,4,7]. If, as has been argued for cardiac muscle[4], this fluorescence is mitochondrial in origin, then it is possible to use the fluorometric measurements to estimate the energy consumption of cardiac muscle contraction.

Four papillary muscles were stimulated to contract isometrically or isotonically against various loads after the pattern illustrated in Figure 2. The time-integrals of the resulting fluorescence changes were measured by tracing the fluorescence waveforms and using linear interpolation during the optical artifacts occurring during each mechanical event. The tracings were cut out and weighed, and the weights were plotted against the weights of similar tracings of the uncorrected thermoelectric signals. These latter weights were verified to be proportional within 2% error to the amplitudes of the corrected records of heat production.

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In Figure 3 we show data from the 4 experiments with pyruvate as substrate where fluorescence-time integral has been plotted against the total energy production. For each muscle the energy production was varied by allowing the muscle to contract isotonically against a range of afterloads. Use of a *t*-test revealed that none of the intercepts was significantly different from zero. The results are clearly compatible with the assumption of JOBSIS and DUFFIELD[2]. Preliminary experiments also show that this linear proportionality between fluorescence-time integral and energy production is found in other substrates, but that the proportionality factor for a particular muscle depends on the substrate present.

In twitch contractions of skeletal muscle a similar relationship between energy production and time-integral of fluorescence probably exists since in toad sartorius muscle the variation in fluorescence-time integral with load[8] is very similar to the energy variation with load[9]. The relationship apparently breaks down for tetani as shown by the simultaneous heat and fluorescence measurements of GODFRAIND-DE BECKER[7] on amphibian skeletal muscle and from measurements we have made on mammalian skeletal muscle[10]. In metabolically inhibited preparations several authors have demonstrated that a linear relationship exists between phosphocreatine (PC) break-down and energy production (heat + work) in many types of contractions[11–13]. However recent tetanic energy balance studies[14,15] have shown a greater initial energy production in metabolically inhibited amphibian skeletal muscle than would have been predicted on the basis of measured PC and ATP breakdown. The complicated fluorescence data obtained at high stimulus rates or under tetanic conditions may reflect complexities produced by high glycolytic activity as suggested by JOBSIS and DUFFIELD[2] and GODFRAIND-DE BECKER[7], or may reflect the possibility that unknown reactions contribute to total energy production. However, in aerobic cardiac muscle, where total energy production (initial and oxidative recovery heat) is being measured[16–18] the postulate of JOBSIS and DUFFIELD[2] does seem to be confirmed.

The techniques described in this report are now available to study the relationship between pyridine nucleotide fluorescence and total energy production in isolated papillary muscle. This should allow the fluorescence measurements to be calibrated according to the prevailing metabolic conditions and hence provide much needed information as to the nature and extent of the conditions under which the fluorescence-time integral is proportional to total energy turnover.

*Zusammenfassung.* Nachweis, dass bei isometrischer und bei isotoner Arbeit des Papillarmuskels des Kaninchens die Veränderung der Fluoreszenz direkte Rückschlüsse auf die durch die Arbeit verbrauchte Energie erlaubt.

J. B. CHAPMAN, C. L. GIBBS and H. VOGELSANGER[19]

*Department of Physiology, Monash University, Clayton 3168 (Victoria, Australia), 30 December 1974.*

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## Temperature Sensitivity of Bromosulphophthalein Clearance by the Liver

Clearance of bromosulphophthalein (BSP) is used as an index of hepatic function[1] and for the indirect measurement of hepatic blood flow in experimental animals[2,3] and man[4]. In a perfused rat liver, the transport maximum for BSP in the formation of bile is reduced by decrease in temperature[5,6]. Therefore the evaluation of BSP clearance in hypothermic animals may be affected by the temperature dependence of this procedure. This paper reports an investigation of the clearance of BSP by an isolated perfused rat liver at 37 °C and 27 °C in relation to the estimation of hepatic blood flow.

*Materials and methods.* Liver donors were fed male Wistar albino rats weighing between 250 and 300 g. The liver perfusion system was based on the method used by HEMS et al.[7] and incorporated the modifications suggested by HEMS and WHITTON[8]. Bovine serum albumin used in the perfusate was dialyzed against 3 changes of Kreb's bicarbonate buffer (pH 7.4) over a 48 h period to remove small vasoactive peptides. A water-jacket on the bulb-oxygenator ensured that the temperature of the perfusate was maintained constant. The perfusion pressure (14 cm water) was determined by the height of the perfusate reservoir in the oxygenator above the liver. Perfusate flow rate was measured directly by collecting the outflow in a graduated cylinder. Cannulation of the bile duct allowed direct collection and measurement of bile flow. Perfusate samples were centrifuged at 1000 *g* and the supernatant used. Lactate was assayed using lactate dehydrogenase (Worthington)[9] after precipitation of protein with perchloric acid. Samples for BSP estimation (0.2 ml) were added to 0.5 ml of 0.05 *N* sodium hydroxide and the optical density measured at 565 nm. Results are expressed as mean ± standard error of the mean and are compared by Student's *t*-test.

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