

myofibrils, the M-band is observed to display 5 lines, which are perpendicular to the long axis of the thick filaments. As seen in our sections, some of the 5 lines vary in density or are missing, depending upon the plane of section through the myofibrils. PEPE² assumes that the M-band material composing these 5 lines is attached to the tail-to-tail abutments of myosin molecules. It has also been demonstrated that the M-band material is composed of 2 polypeptide chains whose significance is correlated with changes in sarcomere length⁶. It appears in both normal³ and hypoxic myocardium of mammals.

Our findings should not be considered unusual since the sliding filament model for muscular contraction applies to both cardiac and skeletal muscle, and the arrangement of the fibrils is consistent in both cardiac and skeletal muscles.

⁶ B. L. EATON and F. A. PEPE, J. Cell Biol. 55, 681 (1972).

⁷ H. S. BENNETT and J. H. LUFT, J. Biophys. biochem. Cytol. 6, 113 (1969).

⁸ M. L. WATSON, J. Biophys. biochem. Cytol. 4, 475; 727 (1958).

⁹ E. S. REYNOLDS, J. Cell Biol. 17, 208 (1963).

¹⁰ The authors wish to thank the Northwestern Ohio Chapter of the American Heart Association for support of this work.

This study was made on ischemic monkey myocardium 4 h after occlusion of the coronary vasculature of the area from which this specimen was removed. The tissue was immediately immersed in cold 3% glutaraldehyde buffered to pH 7.4 and fixed for 2 h. The specimens were post-fixed for 2 h in cold 1% OsO₄ solution buffered to pH 7.5 with s-collidine⁷, dehydrated in ethanol, then immersed in propylene oxide and embedded in Araldite-Epon. The sections obtained with ultramicrotomy were stained with uranyl acetate⁸ and lead citrate⁹.

Zusammenfassung. Es gelingt der elektronenoptische Nachweis, dass auch beim Herzmuskel des Säugers (Affen) die M-Bande aus 5 distinkten Linien besteht und bei Hypoxämie im Bereich der I-Linie eine dunkle Zwischenbande (N-Linie), die auf Ablagerungen von Ca-Salzen zurückzuführen ist, erscheint.

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The Antibacterial Action and R-Factor-Inhibiting Activity by Chlorpromazine

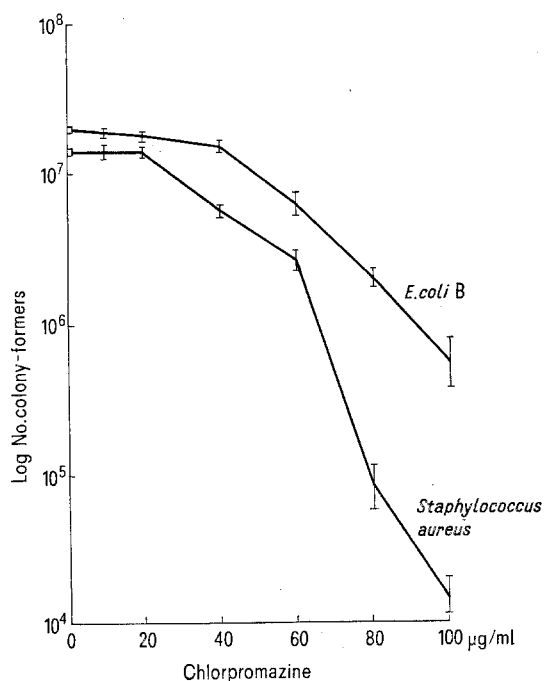
In a previous paper we reported that chlorpromazine (CPZ) a fenothiazine derivate at 1.0 mM concentration completely inhibited the growth of *B. anthracis* strain VR¹. Using the agar diffusion method we have now compared the antibacterial effect of CPZ and 2 related compounds, levomepromazine (Tisercin) and promethazine (Pipolphen) produced by E.Gy.T., Budapest, on a number of Gram-positive and Gram-negative bacteria. CPZ at a concentration of 12–25 µg was effective against *Staphylococcus aureus*, *Diplococcus pneumoniae*, *Coryne-*

bacterium Hofmanni, *B. anthracis* VR and at a concentration of 100–125 µg on *E. coli* B., *Proteus vulgaris* and *Klebsiella pneumoniae* respectively. Levomepromazine was found to be as effective as CPZ, whilst promethazine was the less active since its minimum inhibitory concentration on Gram-positive bacteria was as high as 125 µg and 125–250 µg on Gram-negative bacteria. It is of interest that fenothiazine compounds tested in this study had no antibacterial effect on *Pseudomonas aeruginosae*, even at a concentration of 1250 µg.

We failed to obtain any CPZ resistant colonies from the VR strain of *B. anthracis*. When CPZ (final concentration 31 µg/ml) was added to a nutrient broth culture of exponentially growing (0.3 O.D. at 620 nm) cells of *B. anthracis*, the culture partially lysed and the majority of cells became Gram-negative. Levomepromazine and promethazine at the same concentration did not significantly influence the growth rate of *B. anthracis*.

In further experiments, when CPZ was added at 37°C to washed suspensions of exponential phase cells in saline of *E. coli* and *Staphylococcus aureus*, we observed a bactericidal effect. As shown in the Figure the bactericidal effect of CPZ is more marked against *Staphylococcus aureus* than *E. coli*.

The R-factor-inhibiting activity of CPZ was tested on the polyresistant strain of *E. coli* K₁₂². The bacteria were cultivated in the presence of 50 µg/ml CPZ for 72 h, then the antibiotics sensitivity of the cells were tested. It was found that 81 of the 547 colonies were not able to grow at 37°C for 24 h on the nutrient agar-plates containing 50–50 µg/ml streptomycin, tetracycline and chloramphenicol and sulphadimidine 400 µg/ml. The ethidium-bromide treated cells served as a control. In this case 25 of the 240 colonies proved to be sensitive to the antibiotics. On the basis of these observations, we can say that at a



Bactericidal Effect of Chlorpromazine.

¹ J. MOLNÁR and B. PRÁGAI, Acta microbiol. hung. 20, 171 (1973).

² N. DATTA, A. M. LAWN and E. MEYNELL, J. gen. Microbiol. 45, 365 (1966).

concentration of 50 $\mu\text{g/ml}$ CPZ eliminated the R-factor from 15% of the cells of a polyresistant 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 dehydrogenase³, succinoxidase, succinodehydrogenase⁴, alters the permeability of bio-membranes and inhibits oxydative-phosphorylation⁵ and phagocytosis of human leucocytes in vitro⁶.

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.

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³ O. A. SHEMISA and L. A. FAHIEN, *Molec. Pharmac.* 7, 8 (1971).

⁴ E. W. HELPER, M. J. CARVER, H. P. JACOB and J. A. SMITH, *Arch. Biochem. Biophys.* 76, 354 (1958).

⁵ F. LETERIER, J. CANVA and J. F. MARIAUD, *C.r. hebdom. Séanc. Acad. Sci. Paris.* 273, 2668 (1971).

⁶ B. KVARSTEIN and H. STORMORKEN, *Biochem. Pharmac.* 20, 119 (1971).

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) present¹. In fluorescence studies on amphibian skeletal muscle JOBSIS and DUFFIELD² have suggested that the area enclosed by a fluorescence transient waveform $\int \Delta F \cdot dt$, 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.

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 (mM) NaCl, 118.0; KCl, 4.75; MgSO₄·7H₂O, 1.18; KH₂PO₄, 1.18; CaCl₂·2H₂O, 2.54; NaHCO₃, 24.8 and aerated with 95% O₂ - 5% CO₂. This solution was also used for bathing the isolated muscle with the addition of 10 mM sodium pyruvate (pH 7.4).

The myothermic apparatus has been described previously³. 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 above³. 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.

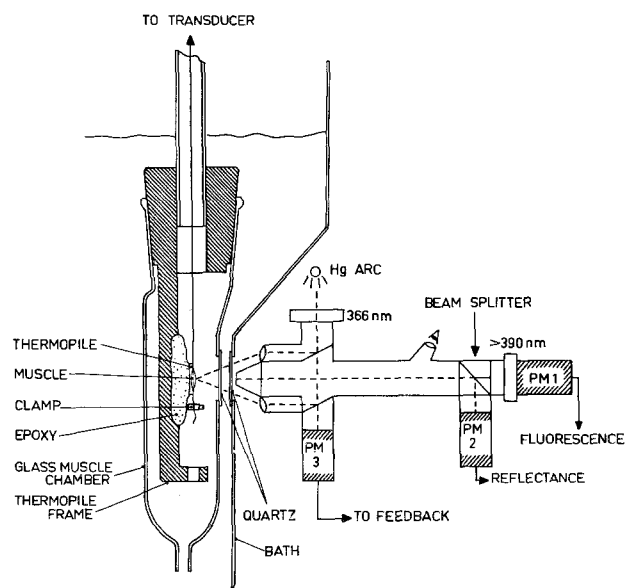


Fig. 1. Schematic diagram of apparatus for simultaneous recording of heat and fluorescence. Filters are labelled according to the wavelengths transmitted. The physiological solution was drained from the muscle-thermopile chamber for myothermic and optical recording.

¹ B. CHANCE, J. R. WILLIAMSON, D. JAMIESON and B. SCHOENER, *Biochem. Z.* 341, 357 (1965).

² F. F. JOBSIS and J. C. DUFFIELD, *J. gen. Physiol.* 50, 1009 (1967).

³ C. L. GIBBS, W. F. H. M. MOMMAERTS and N. V. RICCHIUTI, *J. Physiol., Lond.* 191, 25 (1967).