

It is possible that the 'cross-bridges' of the paracrystals are not true links at all. The paracrystals may be formed in relaxing medium through weak electrostatic interaction of the filaments. In this situation the thin filaments would be held away from the thick filaments by a distance equal to the diameter of the globules ( $\sim 7$  nm)<sup>13</sup> that make up the myosin heads. These would appear as 'cross-bridges', and taking 15 nm as the diameter of the thick filament, would establish a minimum distance between thick filaments centres of  $\sim 30$  nm.

**Zusammenfassung.** Beschreibung der In-vitro-Bildung grosser, parakristalliner Aggregate des Hennen-Aktomyosins. Dicke und dünne Filamente der Aggregate

zeigen parable Anordnung, die für quergestreifte Muskeln typisch ist. Ebenfalls treten Querbänder zwischen dicken und dünnen Filamenten des entspannten Muskels auf, und das aktiv entspannte Proteinsystem des gereinigten Aktomyosins bleibt voll funktionell.

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<sup>13</sup> S. LOWEY, H. S. SLAYTER, A. G. WEEDS and H. BARKER, *J. molec. Biol.* 42, 1 (1969).

### N-Lines and M-Bands in Cardiac Muscle

During our studies on the effects of hypoxia on the monkey heart, a prominent N-line and an M-band composed of 5 separate lines were observed (Figure).

According to our bibliographic search, both the N-line and 5-lined M-band have been investigated only in skeletal muscle<sup>1,2</sup>, and the latter illustrated and mentioned in the normal papillary muscle of the cat<sup>3</sup>. Additionally, the N-line is often poorly resolved with routine electron microscopic preparation procedures, which we have used in the present investigation. Special techniques are usually needed to make the N-line visible, and it has been suggested that this line is the site for the storage of intracellular calcium<sup>1</sup>.

The N-line can be seen as a dense striation within the I-band (Figure). Its morphologic characteristics are similar to those of the substance constituting the Z-band and the dense particles within mitochondria. Since the N-line becomes very pronounced at the same time as the amorphous intramitochondrial condensations appear, it can be hypothesized that they may have a similar composition. The intramitochondrial densities are believed

to be calcium phosphate accumulations, and appear when muscle becomes ischemic<sup>4</sup>. From our indirect inferences, therefore, we support the view of YAROM and MEIRI<sup>1</sup>, who correlated the N-lines with calcium storage.

The 5-lined M-band in the middle of the H-zone is made up of 'M-material', which bridges the thick filaments<sup>2,5</sup>. PEPE<sup>2</sup> mentions that, in longitudinal sections through the

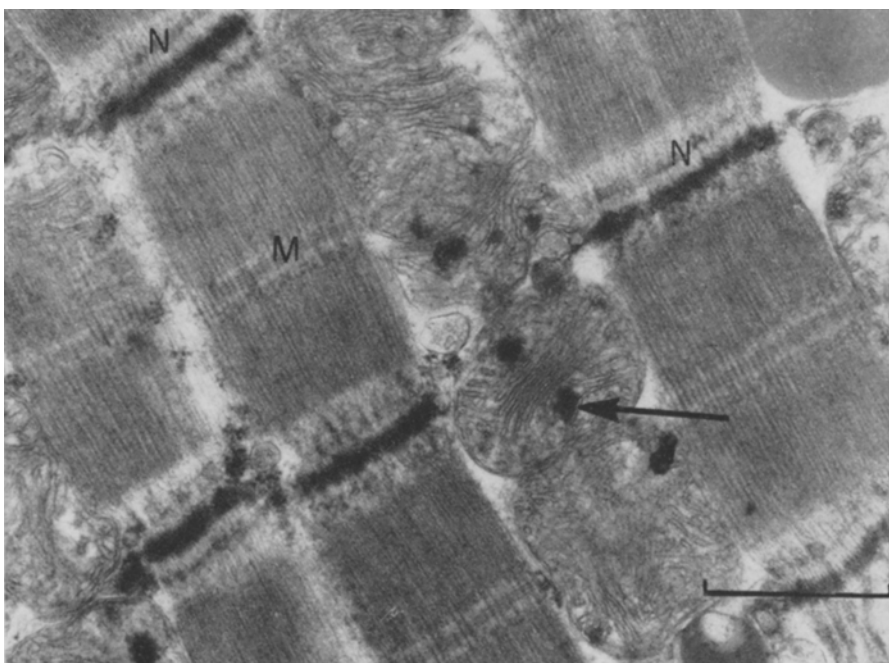
<sup>1</sup> R. YAROM and U. MEIRI, *Nature (New Biology)* 234, 254 (1971).

<sup>2</sup> F. A. PEPE, in *Progress in Biophysics and Molecular Biology* (Ed. J. A. V. BUTLER and D. NOBLE; Pergamon Press, Inc., New York, 1971), p. 77.

<sup>3</sup> N. S. McNUTT and D. W. FAWCETT, in *The Mammalian Myocardium* (Ed. G. A. LANGER and A. J. BRADY; John Wiley and Sons, New York, 1974), pp. 11, 12 and 14.

<sup>4</sup> R. B. JENNINGS and C. E. GANOTE, in *Effect of Acute Ischemia on Myocardial Function* (Ed. M. F. OLIVER, D. G. JULIAN and K. W. DONALD; Churchill Livingstone, Edinburgh 1972), p. 50.

<sup>5</sup> G. FANO, G. ASCANI NUVOLO, M. P. BECCHETTI, P. CHINEA, B. M. DOLCINI and C. DOLCINI, *Boll. Soc. ital. Biol. sper.* 49, 388 (1973).



Electron micrograph of ischemic monkey myocardium. The N-line (N), observed within the I-bands of many sarcomeres, appears prominent as do the intramitochondrial condensations (arrow). The M-band (M) can be seen in one sarcomere as having 5 lines. The bottom line indicates 0.5  $\mu$ m.  $\times 48,800$ .

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. PEPE<sup>2</sup> 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 length<sup>6</sup>. It appears in both normal<sup>3</sup> 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.

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

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

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

<sup>9</sup> E. S. REYNOLDS, J. Cell Biol. 17, 208 (1963).

<sup>10</sup> 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% OsO<sub>4</sub> solution buffered to pH 7.5 with s-collidine<sup>7</sup>, dehydrated in ethanol, then immersed in propylene oxide and embedded in Araldite-Epon. The sections obtained with ultramicrotomy were stained with uranyl acetate<sup>8</sup> and lead citrate<sup>9</sup>.

**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

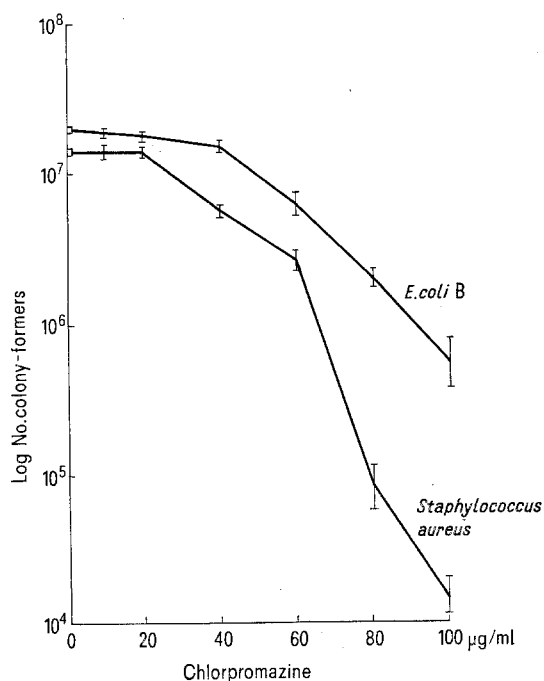
In a previous paper we reported that chlorpromazine (CPZ) a fenothiazine derivate at 1.0 mM concentration completely inhibited the growth of *B. anthracis* strain VR<sup>1</sup>. Using the agar diffusion method we have now compared the antibacterial effect of CPZ and 2 related compounds, levomepromazine (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* K<sub>12</sub><sup>2</sup>. 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.

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

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