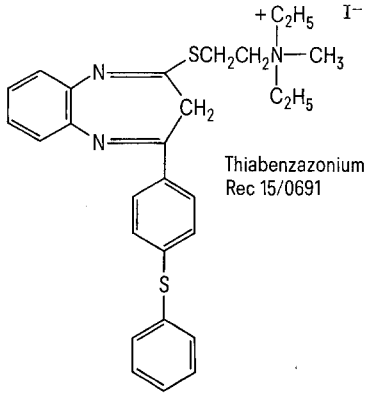
Table II. LD_{50} and p 0.05 fiducial limits of thiabenzazonium and of its cleavage products

Substance	Animal species	Route of administration	LD_{50} (mg/kg)
Thiabenzazonium	Mouse	os	9000 (6570–12300)
Thiabenzazonium	Moose	i.p.	42 (34–52)
Thiabenzazonium	Rat	os	> 10.000
Thiabenzazonium	Rat	i.p.	35 (28–43)
CA	Mouse	i.p.	> 3000
CB	Mouse	os	880 (704–1100)
CB	Mouse	i.p.	37 (34–40)

CA, 4,p-phenylthiophenyl-1,3-dihydro-2H-1,5-benzodiazepin-2-one. CB, 2-methyldiethylammoniumethylthiole iodide.



Thiabenzazonium
Rec 15/0691

In acid medium thiabenzazonium cleaves to 4,*p*-phenylthiophenyl-1,3-dihydro-2H-1,5-benzodiazepin-2-one and 2-methyldiethylammoniumethylthiole salt, which have no antimicrobial activity.

Antimicrobial activity. The in vitro minimal inhibitory concentrations (MIC) are given in Table I, in comparison with chloramphenicol and bacitracin.

The bactericidal activity against a number of Gram positive strains (2 *Stp. pyogenes humanus* gr. A, 2 *Stp. equisimilis* gr. C, 2 Stp. species gr. G, 3 *Stp. mitis viridans*, 2 *Diplococcus pneumoniae*, 1 *St. aureus* and 2 *Corynebacterium diphtheriae*) after 5, 15, and 30 min of contact at 2 concentrations of the substance (5 µg/ml and 10 µg/ml) was studied and thiabenzazonium was found active against *Streptococcus*, *Diplococcus* and *Corynebacterium*.

The phenol coefficient, determined according to the Rideal-Walker method, showed a bactericidal activity of thiabenzazonium about 250 times higher than that of phenol. In vitro *St. aureus* and *Stp. pyogenes* did not develop resistance against thiabenzazonium during 10 successive transfers.

Virucidal activity was tested against Influenza APR-8 virus and Influenza A2 Ann. (Arbor 60) virus. The infectivity was then evaluated on embryonated Leghorn eggs as previously described[3]. After 1 h of contact at 37 °C, thiabenzazonium inactivated APR-8 virus at a concentration of 0.125 µ*M*/ml and the A2 virus at a concentration of 0.0156 µ*M*/ml.

Toxicity. The toxicity of thiabenzazonium after a single administration is uneventful and the animals die 10–20 h after oral or i.p. treatment. The LD_{50}, given in Table II, show that there is a striking difference between the oral and the i.p. toxicity of thiabenzazonium, probably due either to a poor intestinal absorption of the drug, or to a splitting into less toxic substances. A 6 months chronic toxicity study in rats with 4, 40 and 400 mg/kg/die orally and in dogs with 4, 30 and 200 mg/kg/die orally was also practically uneventful. Also fetal toxicity studies in rats and rabbits yielded similar uneventful results.

Conclusion. Thiabenzazonium is a substance with potent antimicrobial properties, especially on some Gram positive bacteria which are agents of oropharyngeal infections. The drug shows also virucidal activities on influenza-virus strains. These antibacterial and antiviral properties, combined with a very low oral toxicity, allow one to classify thiabenzazonium as an antimicrobial drug, potentially indicated for local treatment and for prophylaxis of oropharingeal infections.

Riassunto. Si descrivono la sintesi, le caratteristiche fisico-chimiche e le proprietà antimicrobiche del thiabenzazonio, un nuovo derivato ammonico quaternario della 1,5-benzodiazepina. Il thiabenzazonio è dotato di una spiccata attività batteriostatica e battericida su alcune specie microbiche Gram-positive, che spesso sono responsabili di infenzioni del cavo orofaringeo.

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Paracrystallization of Actomyosin

The interaction of actin and myosin lies at the basis of muscular contraction. For this reason actomyosin, as gels or threads, has been used as a useful contractile model[1,2]. There would be merit in refining such a model by assembling actomyosin into a thick and thin filament order similar to that of the muscle sarcomere. This paper reports the formation of such aggregates having a degree of order not previously achieved[3–5].

Natural actomyosin of high purity and retaining calcium sensitivity was prepared from leg muscle of the hen[6]. It was dissolved to 0.4–0.6 mg/ml in a relaxing medium of high ionic strength (6 m*M* ATP, 6 m*M* $MgCl_2$, 2 m*M* EGTA, 0.01 *M* imidazole, 0.5 *M* KCl, pH 7.0), and then dialyzed for 24 h at 2 °C against a similar medium (ionic strength 0.15) in which KCl was reduced to 0.05 *M*, allowing thick myosin filaments to

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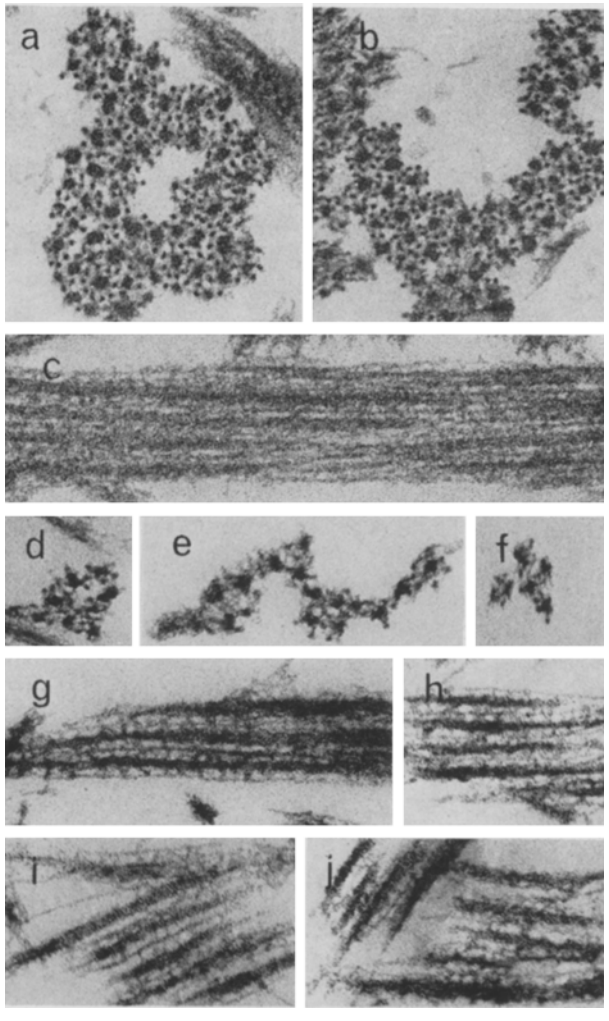

Fig. 1. Electron micrographs of paracrystalline hen actomyosin. ×100,000. a–b) Transverse sections through paracrystals, showing the ordering of thick and thin filaments. c) A longitudinal section through the same preparation. Thick and thin filament alignment can be seen. d–f) Transverse sections through smaller paracrystals. g–j) Longitudinal sections through small paracrystals, showing increased detail, with thick and thin filament alignement and cross-bridging.

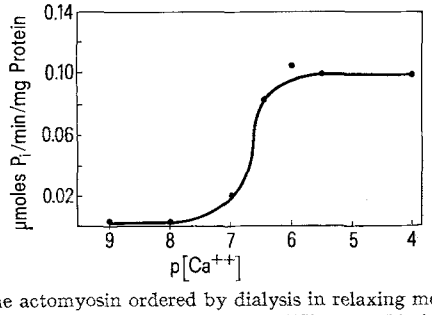

Fig. 2. The actomyosin ordered by dialysis in relaxing medium was further dialyzed for 24 h against 0.15 *M* KCl, 0.01 *M* imidazole, pH 7.0. It was centrifuged at 2,000 × *g* for 10 min and resuspended in the enzymic reaction medium, 6 m*M* ATP, 6 m*M* $MgCl_2$, 2 m*M* EGTA, 0.01 *M* imidazole, 0.05 *M* KCl, pH 7.0. The EGTA was titrated with Ca^{++} to give the required pCa values[1]. Protein concentration in the reaction was 0.4–0.6 mg/ml; the incubation temperature was 25 °C. The reaction was stopped at 20 min with 6% perchloric acid. In some experiments the further dialysis step to remove relaxing medium was not carried out. In these, the relaxing medium in which the paracrystals were formed was retained as the basic substrate adjusted to the appropriate pCa levels. Despite this, the relationship shown was still obtained.

form. The low temperature and absence of calcium ions prevented any significant hydrolysis of ATP in the dialysate over the 24 h period.

Gross aggregation occurred, since, on centrifugation (2,000 × *g* for 10 min) 90% of the protein precipitated. The precipitate was fixed in glutaraldehyde and osmium tetroxide and embedded in epoxy resin. Sections were stained with uranyl acetate and lead citrate.

Electron micrographs of transverse sections of the aggregates showed a high degree of order of thick and thin filaments (Figure 1, a and b). The ratio of thick to thin filaments was close to 1:4, reminiscent of certain insect muscles[7] or the double-overlap zones of F-actin in shortened vertebrate muscle[8].

The thick filaments were usually circular, but varied considerably in diameter (mean, 15 nm). The thin filament diameter was ~6 nm. The centre-to-centre distance of adjacent thick filaments was ~35 nm. Between adjacent thick and thin filaments it was ~20 nm. The lattice dimensions were therefore within the range found in striated muscle[9,10]. Figure 1c is a longitudinal section of the aggregates, showing thick and thin filaments in parallel.

Regions of lesser aggregation, giving a greater degree of resolution, are shown in transverse sections (Figure 1, d, e and f). Bridging between the 2 sets of filaments can be seen. Longitudinal sections (Figure 1, g, h, i and j) show definite cross-bridges, at intervals of ~30 nm. Only those cross-bridges oriented close to the plane of the section will be clearly distinguished. Because of their abundance in this plane, it is assumed that most or all of the cross-bridges from each thick filament and at closer than 30 nm exist between the 2 sets of filaments. It cannot be assumed, however, that the cross-bridge interval would be the 14.3 nm of whole muscle[11].

Paracrystalline aggregates form at ATP and magnesium concentrations between 2 and 10 m*M*. Judged from a survey of many sections from 14 preparations, this order is retained in preparations raised to 25 °C.

Figure 2 shows the ATP-ase activity of paracrystalline actomyosin over a range of Ca ion concentrations. The characteristic sygmoidal relationship was obtained[1,12]. At pCa < 6, maximum ATP-ase activity was observed. At pCa 8–9 activity was extremely low, varying between 0 and 5% of that at pCa 4. In 5 of the 12 experiments it was zero. This means that the regulating system (troponin-tropomyosin complex) in the paracrystals was fully functional.

The conventional view is that cross-bridging between thick and thin filaments does not occur in relaxed, living muscle[11]. It is surprizing that natural actomyosin can be assembled from a relaxing medium into aggregates with what appears to be close to a full complement of cross-bridges. This is despite the fact that the preparations have an active regulating system, the destruction of which would have caused filament linking (although not necessarily with filament ordering) even at the low calcium level of a relaxing medium.

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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 (~7 nm)[13] 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 ~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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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[1,2], and the latter illustrated and mentioned in the normal papillary muscle of the cat[3]. 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[1].

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[4]. From our indirect inferences, therefore, we support the view of Yarom and Meiri[1], 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[2,5]. Pepe[2] mentions that, in longitudinal sections through the

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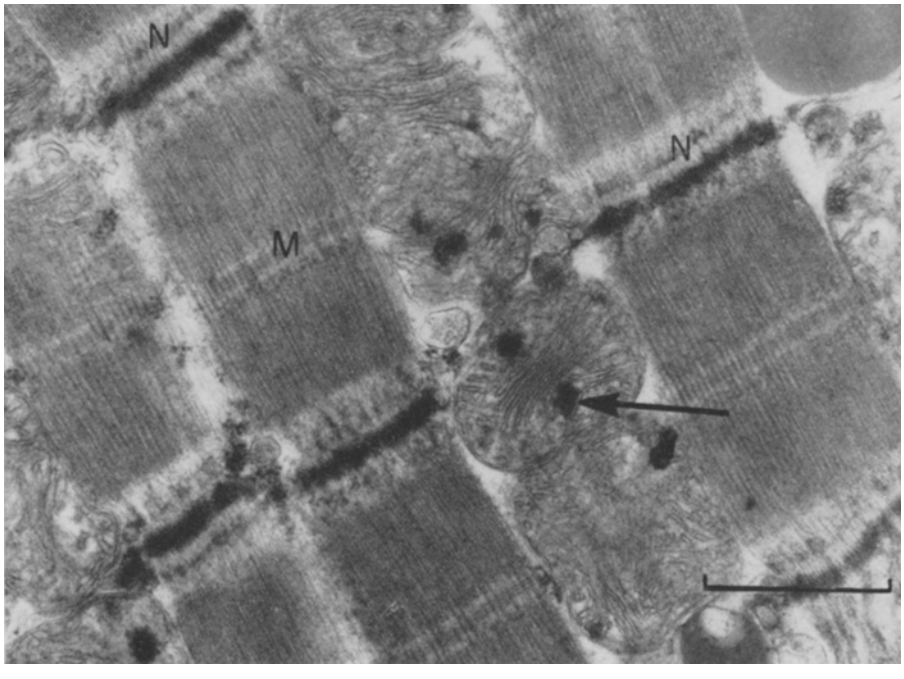

Electron micrograph of ischemic monkey myocardium. The N-line (N), observed within the I-bands of many sarcomeres, appears prominent as do the intramitochondrial condensations (arrow). The M-band (M) can be seen in one sarcomere as having 5 lines. The bottom line indicates 0.5 μm. ×48,800.