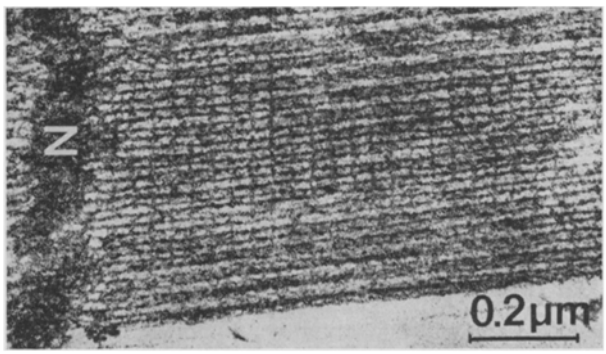

Fig. 4. 'Thin' longitudinal section through a fibre which had developed tension in a rigor solution, was relaxed by Mg-pyrophosphate, transferred back into the rigor solution and fixed there. The center of the micrograph shows a single layer of actin filaments with lateral projections consisting of the actin ends of cross bridges. If the micrograph is sighted at a glancing angle perpendicular to the filaments, the projections form lines with regular distances. (Z = Z-line).

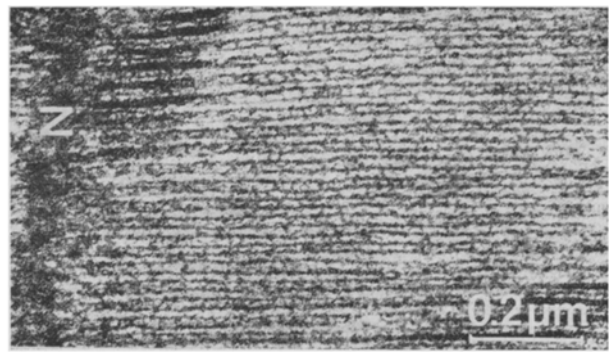

Fig. 5. 'Thin' longitudinal section through a fibre fixed after relaxation by Mg-pyrophosphate, showing a single layer of actin filaments. The lateral projections of the filaments are attached in irregular distances (Z = Z-line).

Until now the results have been discussed as if all cross bridges behaved in the same manner during PP relaxation. However, it is not yet clear if this simplification is permitted. Regarding single actin-myosin layers of PP-relaxed fibres, most of the micrographs indeed show similar orientation of the myosin heads to that of fibres relaxed with ATP[7,8]. On some micrographs, however, the cross bridges appear to prefer an angled position. Further experiments, together with X-ray diffraction controls (in conjunction with Mrs. PHYLLIS ARMITAGE and Dr. R. T. TREGEAR, Oxford) will be necessary to exclude the possibility that artifactual cross bridge movements are happening during the fixation procedures. But, in any case, the present experiments suggest that PP is capable of inducing a reversible change of cross bridge configuration[10].

Zusammenfassung. Elektronenmikroskopische Aufnahmen von einfachen Filament-Lagen Mg-Pyrophosphat-erschlaffter Fasern der dorsolongitudinalen Flugmuskulatur von *Lethocerus spec.* gleichen weitgehend dem Bild ATP-erschlaffter Muskeln. Optische Transformationen scheinen dagegen die Charakteristika von ATP-erschlafften Fasern und solchen, die sich im Rigor mortis befinden, zu kombinieren. Die Überführung der Fasern aus einer Mg-Pyrophosphat- in eine Rigor-Lösung stellte das Rigor-Muster wieder her.

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3′-5′- cAMP and Lysosome Membrane Labilization

In our preliminary experiments[1] it was suggested that 3′–5′-cAMP produces an endogenous activation of phospholipase A enzyme in tissue homogenate. The aim of the following experiments would be to investigate the effect of exogenous 3′–5′-cAMP on the ultrastructure of lysosome membrane prepared from rat liver tissue, studying the release of lysosomal enzymes: acid phosphatases and β-glucuronidases from lysosomes.

The labilization of the lysosome membrane and an increased level of lysosomal enzymes in extralysosomal space indirectely indicates a substantial transformation of lysosomal membrane structure, presumably the predominance of micellar configuration[2].

Material and methods. Male rats of the Wistar strain were used; 7 g of the chilled liver were weighed out and lysosomes were prepared according to WEISSMANN[3].

The following procedure was a modification of KARLSSON's techniques[4]. The lysosome-rich sediment obtained after washing with sucrose was resuspended in 10.0 ml of ice-cold salt phosphate buffer solution (pH 7.4)[5] containing 0.25*M* sucrose and 5 g human serum albumin (resuspended sediment). In order to determine the total activity of lysosomal enzymes per ml of suspension, 0.7 ml of the suspension was mixed with 2.3 ml of the above mentioned sucrose – HSA buffer with added Triton X-100 (final concentration 0.15%).

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Table I. Activity of acid phosphatases

No. of experiments	Total activity	Free activity			
		Control	cAMP ($10^{-3}M$)	Theophyllin ($10^{-4}M$)	cAMP+Theophyllin
	(phosphatase/ml of resuspended sediment μmol P/ml/10 min)				
12	181.3 ± 6.3	29.4 ± 3.1	28.4 ± 4.3	33.6 ± 3.1	62.8 ± 5.9[a]

Table II. Activity of β-glucuronidases

No. of experiments	Total activity	Free activity			
		Control	cAMP ($10^{-3}M$)	Theophyllin ($10^{-4}M$)	cAMP+Theophyllin
	(β-glucuronidase/ml of resuspended sediment umol phenolphtalein/ml/10 min)				
11	33.0 ± 2.5	18.1 ± 2.0	18.0 ± 2.3	18.5 ± 1.7	22.6 ± 0.5[a]

[a] $p < 0.01$.

The second sample made up for the determination of control free activity of lysosomal enzymes was composed of 2.0 ml of the suspension and 1.0 ml of the buffer. The third sample was made up for the determination of the changed free activity of lysosomal enzymes in consequence of in vitro administered agents and was composed as the second sample. The agents were resolved in 1.0 ml of buffer solution.

All samples were incubated at 37°C for 45 min. After incubation they were cooled in ice-cold water and then centrifuged at 4°C for 20 min at 15.000 *g*. 0.1 ml of the supernatants was then incubated for 10 min at 37°C in 1.9 ml of acetate buffer (0.05 *M*, pH 5.4) with β-glycerophosphate and phenolphtalein glucuronide as substrates to determine the acid phosphatase[4] and β-glucuronidase activity[6].

Results and discussion. The 3′–5′ cAMP ($10^{-3}M$) and theophyllin ($10^{-4}M$) increased the permeability of lysosome membrane prepared from rat liver tissue with respect to acid phosphatase (Table I) and to a certain extent β-glucuronidase (Table II) enzymes and this change in permeability indirectly suggests that the micellar organisation, as a more labile structural arrangement, prevails. The 3′–5′-cAMP and theophyllin, alone, could not produce this effect, their remarkable common effect, we think depends on the inhibition of cAMP phosphodiesterase enzyme by theophyllin.

There are, however, two questions to be discussed concerning the results of experiments: At the administration of cAMP + theophyllin is it a disruption or increased permeability of lysosome membrane that took place? b) Why does the percent-value of the increased free activity of acid phosphatase and β-glucuronidase enzymes differ under the same effect? (40%, respectively only 30% increase).

a) As to the first question the change of permeability seems more probable: This is supported by the presence of intact lysosomes in lysosome fraction at electronmicroscopical control and by the insignificant increase of supernatant protein concentration (the increase is less than 10%). b) The difference in the release of the two enzymes also rather suggests specific change of membrane permeability than lysosome membrane disruption.

It was observed that exogenous lysolecithin[7] and Na-desoxycholate (this latter is a known activator of phospholipase A enzyme)[8] increase the proportion of the micellar state within membranes and therefore increase the permeability and facilitate the fusion between adjacent membranes. However, these materials are exogenous agents and serve only as a model to investigate a physiological mechanism. The above results indirectly suggest the possibility that the cAMP would be an endogenous, physiological material, which may start an ultrastructural rearrangement of the lysosomal membrane.

Zusammenfassung. Es wird gezeigt, dass 3′–5′-cAMP ($10^{-3}M$) und Theophyllin ($10^{-4}M$) die Permeabilität derjenigen Lysosmen-Membranen steigern, die aus Rattenleberzellen in bezug auf saure Phosphatase and in bezug auf β-Glucuronidase-Enzyme präpariert wurden.

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Lack of Major Cytoplasmic Protein Contamination of Rat Liver Nuclear Chromatin

It was suggested by JOHNS and FORRESTER[1] that non-specific contaminating proteins, possibly of cytoplasmic origin, could be removed from calf thymus chromatin by washing in 0.3–0.35 *M* NaCl. In recent studies, we have observed that many different nonhistone proteins are solubilized from both rat liver and rat kidney chromatin by treatment with 0.3 *M* NaCl[2,3]. In order to determine if these proteins represent cytoplasmic contamination of nuclear chromatin, the following experiment was performed.