Stretch Induced Formation of ATP-^{32}P in Glycerinated Fibres of Insect Flight Muscle

In a preceding communication MANNHERZ[1] showed that, in glycerinated fibre bundles of insect flight muscles, ^{32}P-labelled inorganic phosphate may exchange with the terminal phosphate of ATP. He implicated that this reaction may be dependent on the amount of negative work absorbed by the fibres. In this paper it will be shown that the rate of exchange can be greatly increased by applying repeated quick stretches to the fibres. Stretch also activates the actomyosin ATPase in glycerinated insect fibrillar muscles[2] and causes a large delayed increase in contractile tension (followed by isometric oscillations[3]), but no work.

Methods. Glycerinated bundles of 8–10 fibres (5 mm long, 70 µm thick) of flight muscles of *Lethocerus maximus* were attached to a driven oscillation apparatus described earlier[3] and suspended in 0.3 ml solution containing 15 m*M* ATP, 15 m*M* $MgCl_2$, 10 m*M* Na-azide, 4 m*M* Ca-EGTA, 20 m*M* imidazole-buffer (pH 6.8; 20 °C) and varying amounts of ^{32}P-labelled inorganic phosphate. In some experiments iodoacetate or oligomycin were added. At the end of the experiment, the ^{32}P incorporated into the ATP was quantitatively transferred into glucose-6-phosphate by adding glucose (0.08 *M*) and Hexokinase (Boehringer). The labelled glucose-6-phosphate was separated from $^{32}P_i$ by paper chromatography[4] and quantitatively. evaluated by liquid scintillation counting[1].

Results. The Figure shows the chromatographic separation of ^{32}P-labelled glucose-6-phosphate from $^{32}P_i$ in an experiment where the fibre bundle was held isometrically (dotted line) and, subsequently, repeatedly stretched (continuous line), each for period of 60 min. The diagram demonstrates that, in the sample from the stretch experiment, the area of the fast moving peak (glucose-6-^{32}P) is larger than in the case of the isometric experiment. It hence indicates a considerable increase of the rate of ATP-^{32}P formation by the mechanical stretches.

The Table shows that in bundles of 8 or 9 fibres held isometrically contracted (about 5 dynes per fibre) 0.3–1‰ of added P_i was found to be incorporated after 60 min. Repeated rectangular stretch and release (duration: 0.3 sec, rate: 1 or 1.5 per sec) of about 2–6% length change inducing 10–20 dynes tension development per fibre caused in all experiments an increase of the exchange to 160–550% of the 'isometric' exchange. Removal of calcium from the incubation solution with EGTA caused relaxation and reduction in the exchange rate.

Discussion. Assuming an average P_i concentration of about 0.3 m*M* in the incubation medium the calculated ^{32}P exchange between inorganic phosphate and ATP is of the order of 0.1–0.5 pmoles/cm fibre min. This exchange reaction is approximately 1‰ of the forward reaction – the ATP splitting – rate under the conditions of the experiment; see also[5].

The experiments show that the ATP splitting process by the contractile proteins may be partially reversible and that not only the rate of ATP splitting but also the rate of back reaction can be greatly increased mechanically by stretching the contractile structures. On the other hand, an exchange of ^{32}P in ADP with the terminal phosphate of ATP could not be found[6].

In conclusion, the tension or stretch induced exchange reaction suggests the formation of a high energy intermediate (ADP-actomyosin?) in tension development[7].

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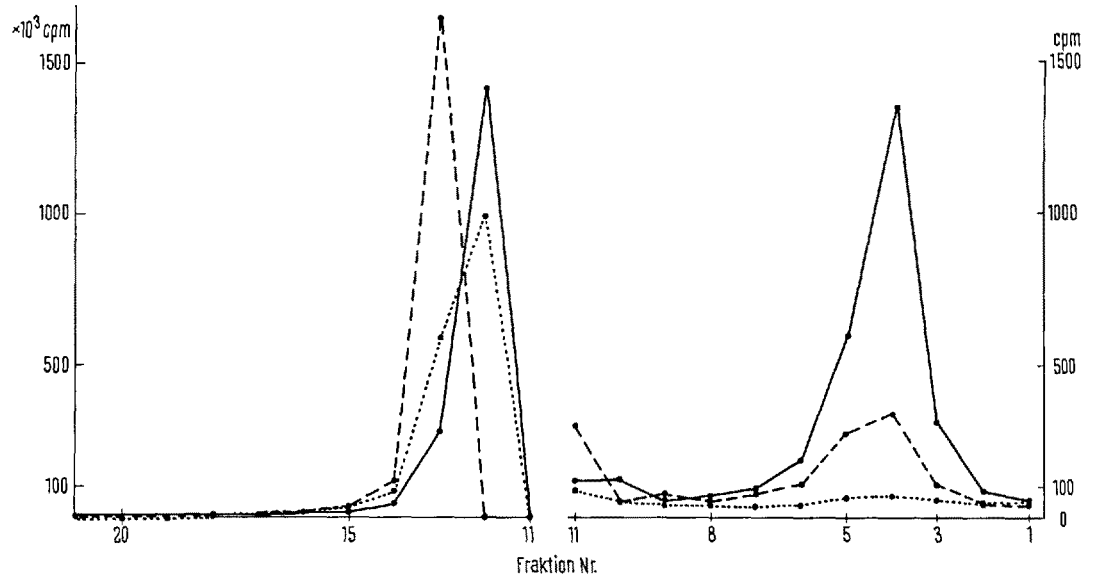

Stretch induced P_i-ATP exchange. Detection of exchanged ^{32}P (transferred from ATP into glucose-6-phosphate) by chromatographic separation. Fraction 3–5 = glucose-6-phosphate; fraction 12–15 = inorganic phosphate. · · ·, controle (without fibre); – – –, isometric contraction; ——, repeated quick stretches (1 per sec).

^{32}P exchange in fibre bundles during isometric contraction (A) and during application of rectangular quick stretches (B)

Experiment No.	A Isometric contraction	B Stretch	B/A
1	0.94	1.53	1.63
	0.80	1.47	1.84
2	1.08	1.94	1.79
	0.63	–	–
3	0.36	0.64	1.78
	–	0.38	–
4	0.31	0.52	1.71
	0.32	0.72	2.25
5	0.37	1.35	3.65
	0.32	1.70	5.32

ATP-^{32}P in $^0/_{00}$ of inorganic ^{32}P. For additional data see text.

Zusammenfassung. Wiederholte Dehnung von glycerinierten Fasern aus Insektenflugmuskeln erhöht die Rate des ^{32}P-Austausches zwischen P_i und ATP. Offenbar bildet sich eine energiereiche Zwischenverbindung im kontraktilen Protein (ADP-Myosin).

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Cocarcinogenic Croton Oil Factor A_1 Stimulates Lipid Synthesis in Cell Cultures

Croton oil factor A_1 (12-*O*-Tetradecanoyl-phorbol-13-acetate = TPA)[1,2] was found to influence protein and nucleic acid metabolism in vivo[3,4] and in vitro[5]. Effects on lysosomes and on mitochondria in cell cultures have been reported[6,7] and recently localization of TPA in plasma membranes by autoradiography was claimed[8]. These effects on cell organelles suggest that membranes may be involved in the biochemical mechanisms at action of TPA. Also certain physico-chemical properties of this compound indicate that direct interactions with cell membranes might be possible. Therefore we studied the incorporation of radioactive choline into lecithin, known to be incorporated into membranes.

HeLa- and L-cells were distributed in 1 ml portions into roller tubes and rolled overnight (12 rph) (400,000 cells/ml; HeLa-medium: 80% Gey's solution + 10% lactalbumine (2,5%) + 10% calf-serum; L-cell medium: 90% TCM-199 + 10% calf serum). The culture medium was replaced by TCM-199 containing 0.5% dimethyl-sulfoxide (DMSO) and TPA in the final concentrations indicated. After 5 h of incubation, ^3H-labeled choline (specific activity 250 mC/m*M*; Amersham/England) was added (2 μC/tube) and incubation continued for 1 additional h. The incorporation was stopped by removing the medium and cooling the roller tubes in an ice bath. A cell lysate was prepared by adding 1 ml 0.2% sodium dodecylsulfate solution per tube, shaking on a mechanical vibrator and incubation for 20 min at 37 °C. 0.1 ml aliquots were placed on filter paper disks. To process such disks a modified MANS-NOVELLI-procedure[9] was used: 3 extractions with cold 5% TCA removed non-incorporated acid soluble choline, residual TCA was removed by pressing the disks on filter paper. In later experiments, residual TCA was removed by an additional extraction with 5% cold acetic acid followed by drying at 60 °C.

As a control the same cell lysates were processed by extraction with chloroform/methanol (3/1)[10] to obtain the lipid material. From the latter, non-incorporated choline was extracted with 1 *N* H_2SO_4. The radioactivities remaining in the organic phases are practically identical with filter paper values obtained from corresponding lysates as described above. More than 95% of the radioactivity was extractable from the paper disks with chloroform/methanol. This comparison indicates that TCA-insoluble

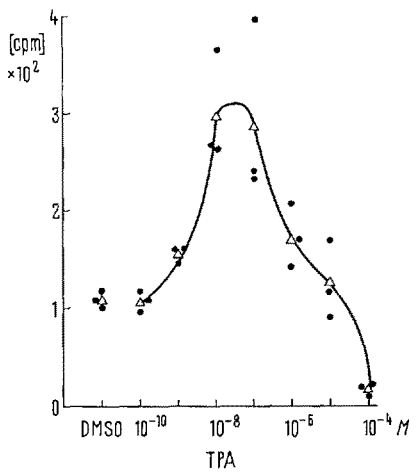

Fig. 1. Croton oil factor TPA dependent incorporation of ^3H-choline into TCA-insoluble material of HeLa-cells. For each concentration of TPA 3 tubes have been incubated for 6 h, worked up and measured (●). The average value from 3 tubes is indicated (▲).

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