Consumption of molecular oxygen of growing cultures of *F. lini* in the presence of various substrates and of cyanide ions

Substrate	Period of O_2-determination	Difference of O_2-uptake of normal respiration (%)
$10^{-3}M$ KCN	1–8 h after addition	−25
$10^{-4}M$ KCN		−24
$10^{-5}M$ KCN		−11
Deoxycorticosterone	Before hydroxylation	0
Digitoxigenin	Before hydroxylation	0
Deoxycorticosterone	During hydroxylation	−32
Digitoxigenin	During hydroxylation	+12
Deoxycorticosterone + digitoxigenin	During hydroxylation of deoxycorticosterone	+ 9[a] to + 23[b]
Deoxycorticosterone + digitoxigenin	During hydroxylation of digitoxigenin	+41

[a] Beginning of the hydroxylation. [b] End of the hydroxylation.

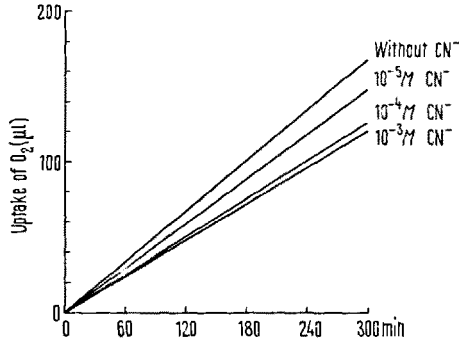

Fig. 1. Respiration of growing cultures of *Fusarium lini* in the presence of KCN^+ (Conditions of culture cf.[11]). The curves are based on the mean values of a great number of independent incubation experiments.

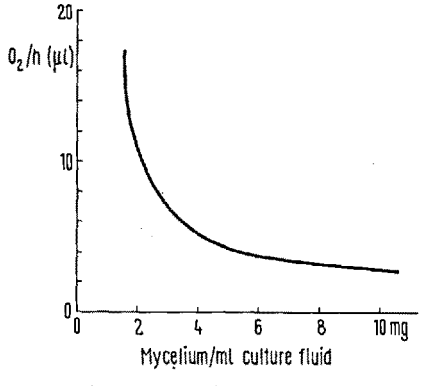

Fig. 2. O_2-consumption of *F. lini* and concentration of mycelium in the culture fluid after 6–8 days of incubation.

This enhancement is also observed when deoxycorticosterone or digitoxigenin are added to the cultures of *F. lini*. It takes place during the period of the hydroxylation the rate of which is also enhanced at this moment. The maximum of O_2-consumption is encountered if cyanide, deoxycorticosterone and digitoxigenin are simultaneously present.

In the course of these investigations it became apparent that the respiration of the micro-organism depends upon the quantity of molecular oxygen dissolved in the culture fluid and the amount mycelium formed. A semiquantitative estimation of this relationship is demonstrated by the curve of Figure 2 in which the O_2-uptake is plotted against the weight of dried mycelium per volume culture fluid. It shows that the amount of O_2 consumed decreases with the increasing quantity of mycelium present.

The experiments described demonstrate that the respiration of *F. lini* is influenced by both deoxycorticosterone and digitoxigenin during their hydroxylation. Addition of KCN effects inhibition of the O_2-consumption of the micro-organism, the extent being dependent of the concentration of the cyanide ions. However, with $10^{-3}M$ KCN an enhancement of respiration is observed which is also maintained in the presence of the steroidal substrates. The results are in agreement with the view that the extraneous steroids added to the culture inhibit the respiration of the micro-organism. By the hydroxylation the extraneous substance is converted into a more water soluble form and can thus be removed more rapidly from the closer environment of the organism. By this process of detoxification normal respiration is achieved[17]. The enhancing effect of cyanide can be explained by their blocking action on the cytochromoxidase of the mitochondria[18,19].

Zusammenfassung. Die Atmung von wachsenden Kulturen von *Fusarium lini* (BOLLEY) wird durch Cortexon und Digitoxigenin während ihrer Hydroxylierung erhöht. 0,001-molares Kaliumcyanid, allein und in Gegenwart von Cortexon und Digitoxigenin, bewirkt ebenfalls eine Erhöhung des Sauerstoffverbrauchs. In kleineren und grösseren Konzentrationen hingegen hemmt Kaliumcyanid die Atmung. Die vom Mikroorganismus aufgenommene Sauerstoffmenge nimmt mit dessen Wachstum ab.

E. WEISS-BERG and CH. TAMM

Institut für Organische Chemie der Universität Basel, CH-4056 Basel (Switzerland), 6 April 1971.

[17] G. WIX and K. ALBRECHT, Nature, Lond. *183*, 1279 (1959).

[18] R. KATO, E. CHIESARA and P. VASSANELLI, Experientia *18*, 453 (1962) in the case the oxidation of drugs by liver microsomal enzyme systems.

[19] Acknowledgment. This work was supported by the 'Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung' (Projects No. 2535 and 3976).

Myofibrillar ATP-Splitting in the Elementary Contractile Cycle of an Insect Flight Muscle

The elementary contractile cycle in force generation is probably a cyclic attachment and detachment of a cross-bridge between thick and thin myofilaments, which may be associated with the splitting of ATP[1]. The possibility of cross-bridge synchronization was recently considered by SCHÄDLER et al.[2], when they obtained oscillations of the isometric tension in glycerinated flight muscles subjected to quick stretch, for oscillation frequency roughly corresponded to the molecular turnover-number of the actomyosin ATPase. On the assumption that one such isometric oscillation period does correspond to the period of a cross-bridge cycle, it may be possible

to find out: 1. whether fast and slow flight muscles differ in the rate of cross-bridge cycling, 2. the number of molecules of ATP split in one bridge cycle, and 3. the course of ATP-splitting from moment to moment in an oscillatory cycle, after suddenly turning on the ATPase by quick stretch.

Methods. Glycerinated fibre bundles of *Lethocerus maximus* and *Lethocerus annulipes* dorsolongitudinal flight muscles (DLM) were mounted to a driven oscillation apparatus[2] and suspended in ATP salt solution (15 m*M* K–ATP, 15 m*M* $MgCl_2$, 10 m*M* imidazole, 4 m*M* Ca-EGTA, 2 m*M* Na-Azid, pH 6.9), 20 °C. Repetitive rectangular stretches of 15 to 1000 msec duration could be produced by applying squarewave pulses (Grass-Stimulator Type Sy) to the Godoman VP 47 Vibrator. Length, tension and ATP-splitting were measured as described earlier[3].

Results. 1. Comparison of fast and slow muscle: When glycerinated fibre bundles were suspended in ATP salt-solution and optimally activated with calcium ions (about 10^{-5} *M*), contractile tension and ATPase activity were in the range 5–10 dynes per fibre and 5–10 pmoles P per cm fibre per sec. A quick stretch by 5–10% L_o applied within 5 msec caused a delayed rise of tension (up to 40 dynes) and an abrupt activation of the ATPase up to 50 pmoles per sec (*Lethocerus maximus* fibres) or 80 pmoles (*L. annulipes* fibres) when maximally activated (40 dynes/fibre). As in previous experiments[2] the stretch-induced tension oscillated though the recording conditions remained isometric (Figure 2). The mechanical effect on contractile tension and ATPase activity could be graded by varying the extent of quick stretch; in this case stretch-induced tension and stretch-induced ATPase activity were always proportional to each other (Figure 1), suggesting that stretch recruits more cross-bridges[5]. The slopes of the straight lines (corresponding to the maintenance heat in live muscles) were related to the respective speeds of contraction. Thus, the fibres from *L. maximus* with 50 pmoles per sec maximal ATPase activity developped maximal tension within 65 msec, they oscillated at a frequency of 7 per sec (Figure 2) and they are known to operate in vivo at 25 oscillations per sec (the wing beat frequency at 35 °C). In the fibres from the fast flight muscles of *L. annulipes* the ATPase activity was 60% higher at maximal tension which was developed within 40 msec, and followed by oscillations of 12 Hz (at 20 °C); the operation frequency of flight muscles in live *Lethocerus annulipes* (30 °C) is 40 Hz[4].

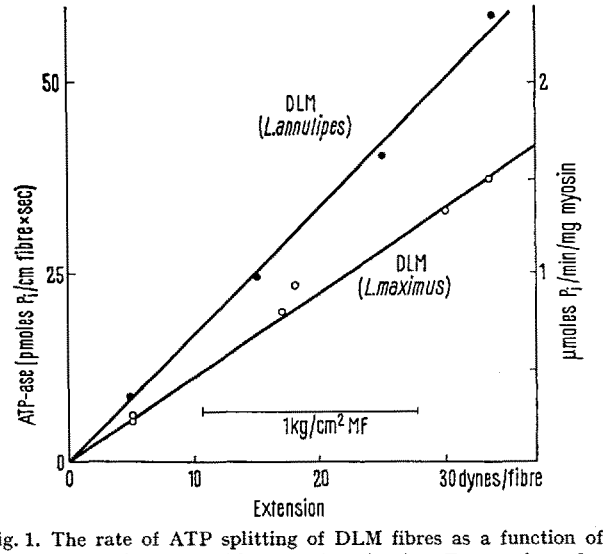

Fig. 1. The rate of ATP splitting of DLM fibres as a function of contractile tension induced by stretch-activation. Preparation: glycerinated dorso-longitudinal muscle fibres of *Lethocerus maximus* or *Lethocerus annulipes*. Conditions, see methods.

2. ATP split per cross-bridge cycle: Let us assume, that the frequency of isometric oscillations corresponds in fact to the turnover frequency of partially synchronized cross-bridges (cf.[2]). Under conditions of maximal activation and cross-bridge recruitment, 1 cm DLM fibre of *L. maximus* split 7 pmoles ATP per cycle (= 50:7) and 1 cm DLM fibre of *L. annulipes* split the same amount (80:12). Electronmicroscope – and biochemical investigations[7,8] revealed the existence of about 3 pmoles cross-bridges per cm length of fibre or 3–4 pmoles of myosin, corresponding to 6–8 pmoles ATP splitting sites (cf.[6]). It may then be concluded that in maximally activated glycerinated flight muscle fibres of the fast and slow bug, about 2 molecules of ATP were split in each cyclic operation of the cross-bridge. The frequency of isometric oscillations (7 or 12) obviously did correspond to the respective enzymic turnover numbers of the ATP splitting sites. This finding is another strong argument for the above assumption, that the isometric oscillation frequency corresponds to the frequency of synchronized cross-bridge movement. It further suggests, that this frequency determines the speed of contraction (Figure 2) as well as the ATPase activity per tension, but not the contractile tension.

3. Burst of ATP-splitting after quick stretch: The above results apparently contradict an earlier finding of only one molecule ATP split per cross-bridge cycle[2]. In those experiments the fibres were continuously stretched while in the present experiments the fibres were subjected to (repetitive) squarewaves of stretch and release, whereby each extension period lasted for only one oscillation period (e.g. 100 msec). From the ATP splitting of the

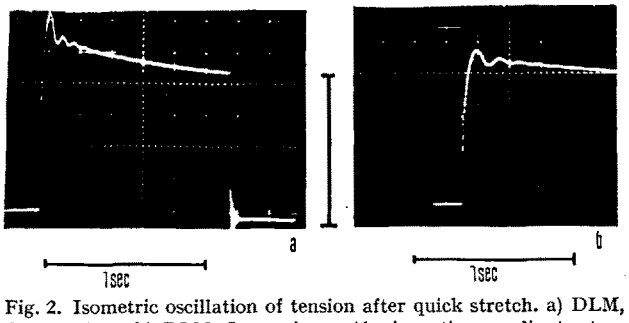

Fig. 2. Isometric oscillation of tension after quick stretch. a) DLM, *L. annulipes*; b) DLM, *L. maximus*. Abscissa, time; ordinate, tension. Vertical bar: 30 dynes/fibre. Composition of ATP saltsolution, see methods.

[1] H. E. HUXLEY, Science *164*, 1356 (1969).
[2] M. SCHÄDLER, G. STEIGER and J. C. RÜEGG, Experientia *25*, 942 (1969).
[3] G. J. STEIGER and J. C. RÜEGG, Pflügers Arch. ges. Physiol. *307*, 1 (1969).
[4] S. B. BARBER and J. W. S. PRINGLE, Proc. R. Soc. B *164*, 21 (1966).
[5] J. C. RÜEGG and H. STUMPF, Pflügers Arch. ges. Physiol. *305*, 34 (1969).
[6] S. LOWEY, H. S. SLAYTER, A. G. WEEDS and H. BAKER, J. molec. Biol. *42*, 1 (1969).
[7] M. K. REEDY, Am. Zoologist *7*, 465 (1967).
[8] R. A. CHAPLAIN and R. T. TREGEAR, J. molec. Biol. *21*, 275 (1966).

summed extension periods the splitting per period (or per oscillation cycle) could then be easily calculated on the assumption, that stretch activated ATPase was turned on and off rather abruptly by the process of stretch and release. The discrepancy suggests that stretch increased the ATPase activity which, however, declined by about 50% shortly after stretching. Consequently we attempted to determine the amount of ATP splitting from moment to moment after repetitive stretch activation. The stretch ATPase was stopped by quick releases applied after the desired time following the quick stretch, e.g. after 17, 33, 50, 100, 200, 300 or 500 msec corresponding to a fraction of the cycle or to e.g. 1, 2, 3, 4 or 5 cycles of oscillation. Naturally, the amount of ATP split in each extension was found to be a function of the extension time, which was conveniently expressed in terms of oscillation periods rather than seconds (Figure 3), in order to normalize the results of about 70 fast and slow DLM fibre bundles. It may be seen from the time progress curve, that about 6 pmoles of ATP per cm fibre were quickly hydrolyzed, long before the first oscillation cycle was completed (e.g. 50 msec after stretching). After this initial burst reaction the rate of hydrolysis approached the steady rate of only 3 pmoles per cycle in 1 cm fibre (cf.[2]).

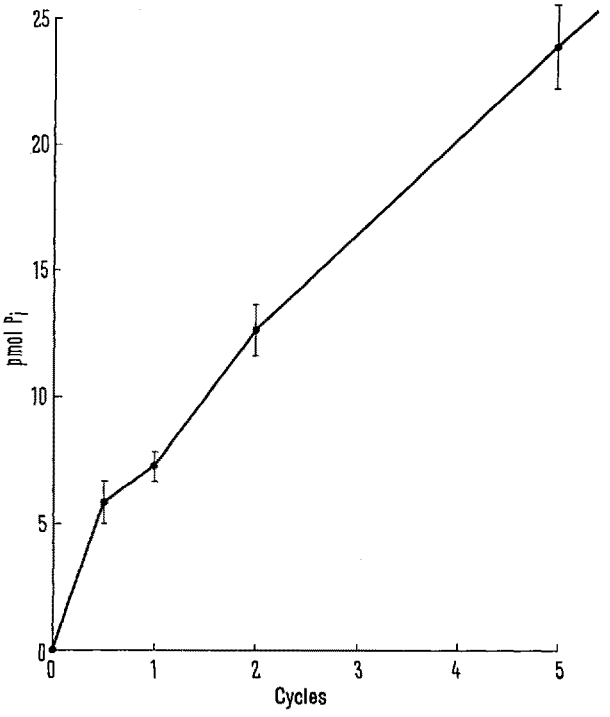

Fig. 3. Time course of stretch-induced ATP splitting after quick stretch. Ordinate: pmoles of P liberated per cm fibre (normalized for 40 dynes tension) after stretching as a function of the duration of stretch (number of isometric oscillation periods, see text. 1 cycle ≅ 100 msec). DLM fibres *L. maximus*, 20 °C.

Discussion and conclusion. It has been shown that in extracted insect flight muscle of *Lethocerus* about 80% of the maximal tension output and 80% of the ATPase activity may be controlled mechanically, by stretch and release. The time progress curve of ATP splitting within the first 200 msec after quick stretch suggests that muscle extension activates the contractile ATPase with very little delay (if any) and this finding seems to rule out a number of suggested more indirect mechanisms of stretch activation implicating e.g. diffusional delays. The time course of ATP splitting also suggests the splitting of 2 molecules of ATP per cross-bridge cycle during the early tension development (the cross-bridge stroke?) and the splitting of only 1 molecule per cycle during tension maintenance; for a number of reasons it is quite unlikely that the low steady state activities are rate-limited by diffusion. The early burst is reminiscent of the early phosphate release (2 moles P per mole of myosin) observed about 50 msec after combination of myosin with its substrate (Lymn and Taylor[9]). Both findings could be taken to mean that after ATP-splitting, ADP is still bound to myosin when phosphate is released[10].

Zusammenfassung. In glyzerinierten fibrillären Muskelfasern von *Lethocerus* werden schon 20 msec nach maximaler «Dehnungsaktivierung» der ATP-ase (um 5000%) 2 Mol ATP pro Mol Myosin gespalten, worauf die Kontraktionsspannung mit der Wechselzahl von Myosin-ATPase und Querbrücken (etwa 10 Hz) oszilliert.

W. Breull

Institut für Zellphysiologie der Ruhr-Universität, D-463 Bochum-Querenburg (Germany),
21 December 1970.

[9] R. W. Lymn and E. W. Taylor, Biochemistry *9*, 2975 (1970).

[10] Supported by Grant No. RU 154/3 of the Deutsche Forschungsgemeinschaft. The excellent technical assistance of Geraldine Fischer and the helpful discussions of Prof. J. C. Rüegg are gratefully acknowledged.

Activation of Glutathione Reductase by Flavine Adenine Dinucleotide in Human Adult and Cord Red Cells

The enzyme, glutathione reductase (GR), which regenerates GSH in the presence of reduced nicotine adenine dinucleotide phosphate (NADPH), appears to be important in maintaining the integrity of red cells. Recently, it has been reported that GR is present in at least 2 forms: the active form associated with flavine adenine dinucleotide (FAD), and the inactive form without FAD[1–3]. Gross et al.[4], reported that GR activity in cord red cells is significantly greater than that of normal adult red cells. However, the mechanism for this increased GR activity and the role of FAD in activation of GR in cord red cells is not known. In this study, we compared the total level of GR and the amount of the active form associated with FAD in normal cord red cells with that in red cells from normal adults and from patients with severe metabolic disorders, e.g., severe uremia and cirrhosis of liver, as well as G-6-PD deficiency and various hemolytic anemias.

Hemolysates were prepared as previously described[5]. GR activity was assayed at 37 °C according to the method of Long and Carson[6] in hemolysates with 130 m*M* *Tris* EDTA buffer (pH 7.6), 5.3 m*M* oxidized