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]).

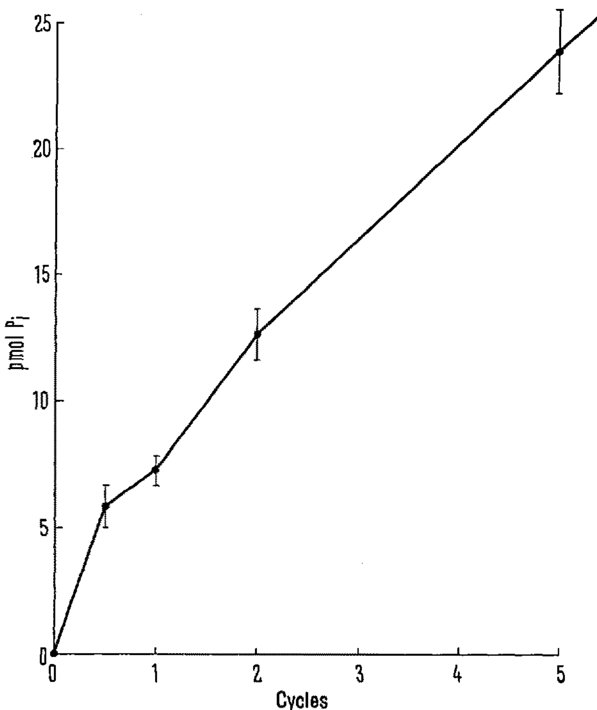


Fig. 3. Time course of stretch-induced ATP splitting after quick stretch. Ordinate: pmoles of P liberated per cm fibre (normalized for 40 dynes tension) after stretching as a function of the duration of stretch (number of isometric oscillation periods, see text. 1 cycle ≃ 100 msec). DLM fibres *L. maximus*,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

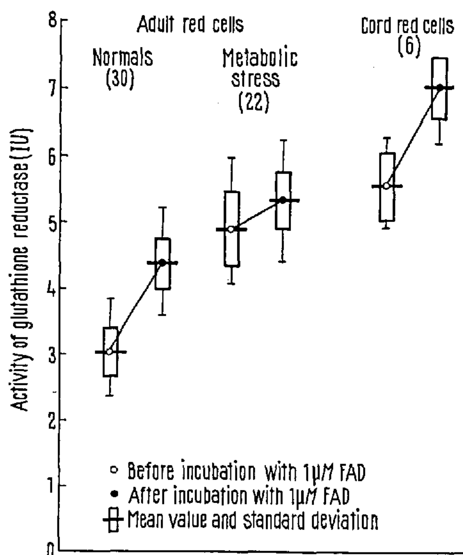
Hemolysates were prepared as previously described[5]. GR activity was assayed at 37°C according to the method of LONG and CARSON[6] in hemolysates with 130 m*M* *Tris* EDTA buffer (pH 7.6), 5.3 m*M* oxidized

glutathione (GSSG), and 0.29 mM NADPH using a Gilford Model 2000 Automatic Recording Photometer. Total GR activity in hemolysates was measured after 30 min of incubation at 37 °C with 1 μM FAD according to the method of BEUTLER[3]. The amount of the inactive form of GR represents the increase in GR activity after incubation with FAD. The saturation percentage was calculated by dividing the GR activity of the active form by the total activity obtained after incubation with FAD.

Results are shown in the Figure. Mean GR activity in red cells from 30 normal adults was 3.04 ± 0.36 IU (the active form). After the addition of FAD to the hemolysates, this increased to 4.38 ± 0.37 IU, an increase of 1.34 ± 0.37 IU (the inactive form). Thus, in normal adults 69.4% of GR is in the active form, and 30.6% of GR is in the inactive form. In the red cells of 22 adult patients with severe metabolic disorders, as described above, total GR activity was 5.35 ± 0.43 IU and 91.8% of GR was in the active form.

In contrast, mean GR activity in 6 samples of normal cord red cells was 5.57 ± 0.51 IU (active form). After addition of FAD to the hemolysates, this increased to 7.03 ± 0.45 IU, an increase of 1.46 ± 0.65 IU (the inactive form). Thus, in normal cord red cells, 79.2% of GR is in the active form, and 20.8% of GR is in the inactive form.

The percentage of GR in the active form in cord red cells is only moderately greater than that in the red cells of normal adults, and is not as elevated as in severe metabolic disorders. However, it is noteworthy that the total amount of GR in normal cord red cells is considerably higher than that in the red cells of normal adults or even of patients with severe metabolic disorders. This increased total GR activity cannot be ascribed to a younger red cell population in cord blood since GR activity is not related to the mean age of the red cell population[7]. Thus, the increase of total GR activity in cord red cells may be related to at least 2 possible causes; 1. increased synthesis of the enzyme, or 2. the presence of an isozyme of GR in cord red cells different from that of adult red cells. The latter is a consideration in light of the work of SCHROETER and TILLMANN[8] with hexokinase (HK). They have demonstrated that the higher HK activity of red cells of new-borns is due to the presence of HK isozyme type I, which is different from that of adult red cells (type III). Although, to our knowledge, studies on isozymes of GR in human cord red cells have not been reported, it is possible that normal cord red cells have an isozyme (a fetal type) of GR that is analogous to that for HK. This fetal type GR isozyme may then account for the increased activity, as in the case with HK[9].



Activation of glutathione reductase by FAD in normal adult red cells, adult red cells from patients with metabolic stress, and cord red cells.

Zusammenfassung. Nachweis, dass foetales Blut mehr Erythrocyten-Glutathionreduktase enthält als Erwachsenenblut und auch der prozentuale Anteil der aktiven Enzymform etwas grösser ist. Diese Befunde sind ein erster Hinweis auf das Vorkommen eines besonderen foetalen Isoenzyms.

Y. YAWATA and K. R. TANAKA

Department of Medicine, Harbor General Hospital, Torrance (California 90509, USA), and UCLA School of Medicine, Los Angeles (California 90024, USA),
25 January 1971.

[1] A. ICEN, Scand. J. clin. Lab. Invest. *20*, Suppl. 96, 1 (1967).
[2] G. E. J. STAAL, J. VISSER and C. VEEGER, Biochim. biophys. Acta *185*, 39 (1969).
[3] E. BEUTLER, J. clin. Invest. *48*, 1957 (1969).
[4] R. T. GROSS, R. BRACCI, N. RUDOLPH, E. SCHROEDER and J. A. KOCHEN, Blood *29*, 481 (1967).
[5] K. R. TANAKA, W. N. VALENTINE and S. MIWA, Blood *19*, 267 (1962).
[6] V. W. K. LONG and P. E. CARSON, Biochem. biophys. Res. Commun. *5*, 394 (1961).
[7] G. J. BREWER, in *Biochemical Methods in Red Cell Genetics* (Ed. J. J. YUNIS; Academic Press, New York 1969), p. 156.
[8] W. SCHROETER and W. TILLMANN, Biochem. Biophys. Res. Commun. *31*, 92 (1968).
[9] This study was supported by U.S. Public Health Service Research Grant No. HE-08757 from the National Hearth and Lung Institute and by General Research Support Grant No. RR 05551 from the National Institutes of Health. We thank Dr. H. E. KATTLOVE for reviewing the manuscript, Dr. V. A. MEDRANO for his assistance in obtaining blood specimens, and Mrs. P. CHAO and Mrs. H. G. CASTALDO for their technical assistance.

Neurogenic Responses of Resistance and Capacitance Vessels

Besides data providing the similar patterns of reflex responses in resistance and capacitance parts of skeletal muscle vascular bed[1,2] there are studies[3,4] revealing these responses to be opposite in direction under some pressor reflexes. Directional differences for resistance and capacitance responses were also shown to be possible in the abdominal aorta vascular bed under depressor reflex initiated from the right heart and the pressor synocarotid reflex[4]. Resistance vessels both in skeletal muscle and in splanchnic vascular beds are known to be constricted under pressor reflexes[5,6]. As to the responses of capacitance vessels recording simultaneously