the peroxidation of membrane lipids of erythrocytes from normal and anemic subjects are summarized in the table. In experiments in which intact red cells were exposed to very low concentrations of H_2O_2 introduced by vapor diffusion, MDA formation was significantly less in the case of anemic erythrocytes. This difference from normal erythrocytes was, however, nearly abolished on pretreatment of the cells with NEM. At higher concentrations of H_2O_2 added direct to the red cell suspension, MDA formation was higher in both normal and anemic erythrocytes but the difference between the 2 groups was not significant.

Again, lipids isolated from red cells or from red cell ghosts, from normal and anemic groups did not show any appreciable difference in the extent of peroxidation. In agreement with the observations of Stocks and Dormandy[15] isolated lipids yield much less MDA (per mg lipid P) compared to intact red cells.

From the foregoing results one finds that the susceptibility to peroxidation, of isolated lipids of red cell membranes, is not appreciably increased in IDA. In intact anemic erythrocytes, on the other hand, membrane lipids seem to be better protected at least at low concentrations of H_2O_2. It is possible that the protective agent is glutathione (GSH); two lines of evidence suggest this: a) pretreatment of red cells with NEM abolishes the observed difference in the peroxidation of lipids between the normal and anemia groups (table) and b) the red cell GSH levels are distinctly higher in IDA (2.47 ± 0.31 mmoles/l packed cells in IDA as against 1.73 ± 0.24 mmoles/l in normal erythrocytes in the subjects reported here). One would expect GSH to be even more effective in vivo in anemic erythrocytes on account of the higher activities of the enzymes glutathione reductase[21] and glutathione synthetase[22]. GSH presumably exercises its protective effect largely and directly through the GSH-peroxidase reaction. In addition, GSH has also been reported to be an essential requirement for a soluble, heat-labile inhibitor of lipid peroxidation distinct from GSH-peroxidase[23]. It would be interesting to know whether such an inhibitor is present in the erythrocytes and, if present, how its level is affected by iron deficiency.

In conclusion, the results presented here indicate that if the life-span of red cells is reduced at all in IDA, the reduction cannot be attributed to an increased susceptibility of membrane lipids per se to peroxidation.

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0014-4754/84/020173-02$1.50 + 0.20/0

Skinned smooth muscle: Time course of force and ATPase activity during contraction cycle[1]

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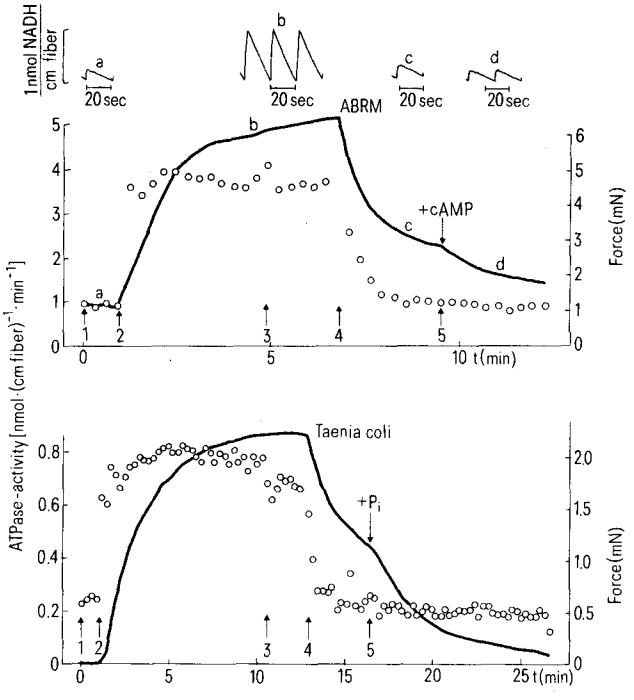
Summary. The time course of ATPase activity and force has been determined during contraction and relaxation in skinned (hyperpermeable) anterior byssus retractor muscle, ABRM, of *Mytilus edulis* and compared with corresponding measurements on skinned taenia coli of guinea-pigs. Following a calcium-induced contraction, lowering the $[Ca^{++}]$ to 10^{-8} M rapidly reduces ATPase activity within 2 min to resting levels while force declines only to about 30–50% of maximal tension within the same time. Thus slow relaxation is due to a 'catch-like-state' which is common to different kinds of smooth muscles and can be reduced with cAMP in ABRM and by P_i in taenia coli.

After cessation of active contraction, certain types of molluscan smooth muscle[2] and vertebrate smooth muscle[3] relax extremely slowly while the rate of energy expenditure is rapidly reduced to resting levels. During slow relaxation, tension is not actively maintained; the muscle is said to be in catch or latch. The catch phenomenon may be demonstrated using skinned or demembranated fibers of the anterior byssus retractor muscle of *Mytilus*[4] in which the



ATPase activity (open circles) during contraction relaxation cycle (solid curve) of skinned anterior byssus retractor muscle (ABRM, upper panel) and guinea-pig taenia coli (lower panel).

Upper panel: 1 Fiber bundle (thickness 200 µm) of ABRM skinned by freeze-drying[4] suspended in relaxing solution (pH 6.5, 20 °C) containing 150 mM KCl, 5 mM ATP, 5 mM $MgCl_2$, 20 mM imidazole, 10 mM NaN_3, 4 mM PEP (phosphoenol pyruvate), 5 mM EGTA, 100 U/ml PK (pyruvate kinase), 138 U/ml LDH (lactate dehydrogenase), 1.5 mM NADH, 0.2 mM A_2P_5 (P^1, P^5-(Di(adenosine-5') pentaphosphate, 1 mM DTE (1.4-dithio-L-threitol).

2 Contraction induced by increasing Ca^{++} to 2 µM with Ca-EGTA (cf. Portzehl et al.[14]).

3 Calcium-EGTA of high buffer capacity (5 mM EGTA) replaced by 0.5 mM Calcium-EGTA buffer (Ca^{++}=2 µM). Note that tension cost (ATP splitting rate per force)[4] is comparable to that in contracting living ABRM[2].

4 Ca^{++} to 10^{-8} M with 5 mM EGTA. After sudden removal of Ca^{++}, ATPase activity drops rapidly whereas the tension declines much more slowly: 'catch'.

5 Addition of c-AMP (0.1 mM) accelerates relaxation. The upper tracings show original records of ATP consumption (NADH consumption) at the times a, b, c, d marked on the force trace. The ATPase activity was measured using the NADH coupled enzymic assay method (cf. Güth and Junge[6] as modified from Griffiths et al.[7]). Changes in NADH fluorescence were detected with a microscope spectrophotometer (Zeiss) viewing the narrow perfusion chamber in which the fiber was mounted between an AME 801 force transducer and the shaft of a Ling Dynamics model 101 vibrator. The chamber was perfused with ATP salt solution containing the NADH coupled enzymic assay system: When perfusion stopped, NADH levels declined with a linear time course and they rose again during reperfusion.

Lower panel: Guinea-pig taenia coli fiber bundle skinned by extraction with triton-X-100[5] were *(1)* relaxed in solution (pH 6.7, T=20 °C) containing 30 mM imidazole, 7.5 mM ATP, 10 mM $MgCl_2$, 1 mM NaN_3, 1 mM PEP, 4 mM EGTA, 100 U/ml PK, 140 U/ml LDH; 0.6 mM NADH, 0.2 mM A_2P_5, 1 mM DTE, 0.1 µM calmodulin At *(2)* and *(3)* contraction was induced by raising the calcium ion concentration to 20 µM with Ca-EGTA (10) and terminated at *(4)* by lowering Ca^{++} to 10^{-8} M with EGTA. Note that ATPase activation preceded tension development during contraction, and ATPase inactivation was ahead of tension decline during relaxation, induced by lowering the Ca^{++} to 10^{-8} M (at *4*); relaxation is accelerated by addition of 6 mM P_i (at *5*).

time course of calcium dependent ATPase activity and force can be determined. We report here that during slow relaxation of these skinned fibers the ATP splitting rate is as low as in the relaxed state preceding contraction, suggesting that crossbridges are non-cycling. These results are compared to those previously obtained in 'hyperpermeable' guinea-pig taenia coli[5] where a similar dissociation of ATP-splitting rate and force development has also been observed[6].

Materials and methods. Fiber bundles of *Mytilus* anterior byssus retractor muscle (ABRM) were skinned by freeze-drying[4] while guinea-pig taenia coli was demembranated with 1% triton-X-100[5]. The time course of isometric force and the ATP-splitting rate were measured as described by Güth and Junge using a NADH coupled enzymic assay method[6] modified from Griffiths et al.[7].

Results and discussion. The upper panel in the figure shows a contraction-relaxation cycle of the ABRM skinned by freeze drying. Concomitantly, the ATP splitting rate was continuously monitored in the same fiber bundles. Note that force (solid line) and ATPase activity (open circles) increase when the calcium ion concentration is increased from 10^{-8} M to 2×10^{-6} M, but interestingly, ATPase activity increases much faster than force. When the calcium ion concentration was suddenly lowered again to 10^{-8} M Ca^{++} by replacing the 0.1 mM EGTA Ca-buffer (2×10^{-6} M Ca^{++}) with 5 mM EGTA, the ATPase activity drops rapidly whereas force decreases much more slowly and not completely. Thus, 2 min after removal of calcium ions by EGTA, ATPase activity has already declined to a resting value while about 50% of the initial tension is still maintained. As found previously[8,9], addition of cyclic AMP (0.1 mM) rapidly abolished the state of slow relaxation (catch-like state). This acceleration of relaxation occurred without significant change in ATPase activity. After increasing Ca^{++}-concentration again to 2×10^{-6} M, ATPase activity and force rose again to the level of a previous contraction. We observed a qualitatively very similar response in a skinned vertebrate smooth muscle (guinea-pig taenia coli): The lower panel of the figure shows that during contraction induced by increasing the Ca^{++}-concentration, force (solid line) increases more slowly than ATPase activity (open circles). After removal of Ca^{++}, the ATPase activity drops rapidly to the resting level whereas force declines much more slowly. Güth and Junge[6] suggested that crossbridge detachment may be impeded by the low calcium ion concentration under these conditions. During slow relaxation, tension is not actively maintained (no tension recovery after quick release) and it could be abolished by inorganic phosphate (6 mM, cf. Schneider et al.[10]) without any change in the ATP splitting rate.

It would appear, therefore, that in skinned, smooth muscle a catch-like tension can be maintained passively without calcium activation and ATP splitting suggesting that crossbridges are not cycling. A similar state has been described in living vertebrate smooth muscle[11,12] and it has been shown[3,13] that, under these conditions, tension can be maintained at low energy cost.

1 The support by the Deutsche Forschungsgemeinschaft and the excellent technical assistance of Mrs K. Winnikes are gratefully acknowledged.

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0014-4754/84/020174-03$1.50 + 0.20/0

Trans-epoxide hydrolase: A key indicator enzyme for herbivory in arthropods[1]

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Summary. An epoxide hydrolase selective for a trans-epoxide substrate is more commonly associated with arthropod herbivory than is a cis-selective epoxide hydrolase. The distinct selectivities in epoxide hydrolase activities between herbivorous pests and entomophagous arthropods used in their biological control may aid design of integrated pest management systems.

The diet of an arthropod is usually controlled by perception of key chemical and physical attributes of its food. Recognition and acceptance of food often occurs prior to ingestion[2], however an arthropod must contend with the physiological consequences of its choice. Overingestion of toxins can result in decreased fitness or death, and limit host range[3,4]. Generally, enzymatic detoxification[5] is the most direct and dependable way for an arthropod to survive a chemical insult.

Plants biosynthesize trans- and higher substituted olefins including fatty acids, phenolics, alkaloids and terpenoids that are rare or absent in animals. Many of these phytochemicals are defenses against herbivory[6-8]. By contrast, cis-olefins generally have constitutive and homeostatic functions in both plants and animals[9]. Epoxidation of olefins largely by cytochrome P-450 monooxygenases (MFOs) can produce epoxides harmful to an organism[5]. The enzyme epoxide hydrolase (EH) catalyzes hydration of the epoxide, thereby detoxifying it to a more excretable 1,2-dihydroxy metabolite[10,11]. This reaction is advantageous since epoxides have high reactivities, and are common in the arthropod's environment. Exposure to epoxides can occur through extranutritional dietary chemicals[6-9], environmental pollutants (e.g. dieldrin)[10], and endogenous hormones (e.g. juvenile hormone)[12]. We recently found that a herbivorous mite had both a much higher MFO and EH activity for a trans-epoxide than a carnivorous mite. Surprisingly, cis-EH activity had the reverse tendency, favoring the predaceous mite[13]. This observation caused us to examine in greater detail the association of trans and cis EH activities with feeding specialization in arthropods.

Epoxide hydrolase activities in 30 species of macro- and microarthropods were measured. Most species were field collected; some were obtained from private or commercial sources. Actively feeding adults or last instar larvae (Aa, Cr, Da, Hc, Ma, Md, Pr, Sf only; fig.) were surveyed. The trans- and the cis-EH activities for each species were plotted in the figure, and widely differing activities (up to 350-fold) were found. Chewing herbivores had much higher trans-EH activities (greater than 11-fold on the average, $p < 0.005$ by Student's t-test and used hereafter) than

Epoxide hydrolase in adult chrysomelidae beetles relative to their host range

Subfamily Tribe Species	Gut epoxide hydrolase* trans	 cis	 trans/cis	Plant families consumed**
Galerucinae				
Luperini				
Diabrotica longicornis	38.5 ± 7.3 ad	2.59 ± 0.35 a	14.9 ± 3.7 ab	17
D. undecimpunctata howardi	28.0 ± 6.6 ade	2.46 ± 0.58 ae	11.4 ± 0.6 b	14
D. virgifera	25.7 ± 5.7 bd	1.79 ± 0.33 ae	14.4 ± 1.2 a	13
Acalymma vittata	38.0 ± 6.1 ad	4.57 ± 0.52 b	8.32 ± 0.67 c	16
Galerucini				
Trirhabda virgata	44.8 ± 5.3 a	35.0 ± 3.2 c	1.28 ± 0.05 d	1
Chrysomelinae				
Doryphorini				
Leptinotarsa decemlineata	18.2 ± 0.9 be	8.04 ± 0.11 d	2.26 ± 0.12 e	5
Chrysomelini				
Plagiodera versicolora	4.00 ± 0.96 c	7.24 ± 0.96 bd	0.55 ± 0.06 f	1
Alticinae				
Altica woodsi	3.23 ± 0.16 c	1.89 ± 0.13 ae	1.71 ± 0.20 de	1
Criocerinae				
Crioceris asparagi	2.84 ± 0.84 c	1.46 ± 0.23 e	1.94 ± 0.36 de	1

* Activity in nmoles diol formed/min/mg protein; mean ±SE for 6 determinations on 2 population samples from consecutive seasons except for Pv and Aw, where only 1 population was sampled. Pools of midgut tissue from 20 to 40 field collected adults for each species were used for activity measurements. Species with same letter are not significantly different at $p < 0.05$. ** Host range was based primarily from work cited[3,22-26].