uniformly by iontophoretic application of Ca ions. In this case, both the speed and the extent of shortening at the middle of the preparation were very limited as compared to the experiments shown in figure 1, because there were no non-activated sarcomeres which could be readily stretched by activated ones. As can be seen in figure 2, c, the A-band width was found to increase appreciably (by about 10%) during the course of slow sarcomere shortening, reaching a steady value when the sarcomere shortening at the middle part stopped due to the balance of force between the sarcomeres. This implied the A-band lenghtening during 'isometric' contraction, again indicating that the thick filament shortening does not take place during the generation of physiological force in each sarcomere. It was noticed that the extent of the A-band lengthening in the 'isometric' condition could well account for the elastic extension of the series elasticity at P_0 in intact muscle fibres[14], suggesting that the series elasticity in horseshoe crab striated muscle may mainly reside in the A-band. It is now being investigated whether the increase in A-band width shown in figure 2, c, is attributable to the elastic strain of the thick filaments[15] or to their misalignment as a result of force generation in each sarcomere. The present results, of course, do not exclude the possibility that the thick filament shortening, which has been studied extensively by Dewey and his coworkers[6-8], plays a role in some long-term muscle performance or during extreme sarcomere shortening below 4 μm.

1 We wish to thank T. Taniguchi, M. Kusama and M. Goto for their excellent technical assistance in taking cinematographic recordings.
2 A.F. Huxley and R. Niedergerke, Nature *173*, 971 (1954).
3 H.E. Huxley and J. Hanson, Nature *173*, 973 (1954).
4 G.W. de Villafranca, J. Ultrastruct. Res. *5*, 109 (1961).
5 G.W. de Villafranca, J. Ultrastruct. Res. *9*, 156 (1963).
6 M.M. Dewey, R.J.C. Levine and D.E. Colflesh, J. Cell Biol. *58*, 574 (1973).
7 M.M. Dewey, B. Walcott, D.E. Colflesh, H. Terry and R.J.C. Levine, J. Cell Biol. *75*, 366 (1977).
8 M.M. Dewey, R.J.C. Levine, D.E. Colflesh, B. Valcott, L. Brann, A. Baldwin and P. Brink, in: Cross-bridge Mechanism in Muscle Contraction, p.3. Ed. H. Sugi and G.H. Pollack. University of Tokyo Press, Tokyo 1979.
9 H. Tanaka, M. Tanaka and H. Sugi, J. Biochem., Tokyo *86*, 1587 (1979).
10 N.M. de Clark, V.A. Claes and D.L. Brutsaert, J. gen. Physiol. *69*, 221 (1977).
11 A.J. Hodge, J. biophys. biochem. Cytol. *2*, suppl. 131 (1956).
12 R.J. Podolsky, J. Physiol. *170*, 110 (1964).
13 H. Sugi, J. Physiol. *242*, 219 (1974).
14 S. Gomi and H. Sugi, Zool. Mag. *88*, 509 (1979).
15 H. Sugi, in: Cross-bridge Mechanism in Muscle Contraction, p.85. Ed. H. Sugi and G.H. Pollack. University of Tokyo Press, Tokyo 1979.

Catalase activity in skeletal muscle of varying fibre types

R.R. Jenkins and J. Tengi

Biology Department, Ithaca College, Ithaca (N.Y. 14850, USA), 21 March 1980

Summary. Slow oxidative skeletal muscles of rats and hamsters exhibit significantly greater catalase activity than fast oxidative muscles. Furthermore, regions of a single muscle may vary significantly.

Catalases are heme-containing porphyrin enzymes which exhibit ubiquity in cells containing cytochrome systems[1]. They exhibit tissue specificity, which appears to be epigenetic, and are found in highest concentrations in the liver, kidney and erythrocytes[2]. Recently, catalase was found in skeletal muscle[3]. Although catalase has been studied in great detail since it was first described by Thenard[4] in 1818, there is still no agreement as to its physiological role. Furthermore, there is a paucity of information related to the function of this enzyme in skeletal muscle. The data related to muscle that have been published have been faulted by the failure of investigators to report the muscle type which was sampled[5]. It is well known that skeletal muscles vary in the proportion of fibre types which they contain and that the enzyme profile is the principle criterion for the identification of the muscle fiber type. The purpose of our study was to determine the catalase activity in muscles of varying fibre types.

Methods. The data were obtained from 10 male and female Sprague-Dawley derived rats weighing between 250 and 300 g, and 10 male and female Syrian hamsters weighing between 150 and 175 g. The animals were decapitated and exsanguinated. The muscles were rapidly dissected out, rinsed and kept in ice cold Ringer's solution while awaiting homogenization. The muscles were blotted dry, weighed, and minced with scissors on a cold glass plate. A 10% (w/v) homogenate was formed with 15 strokes in a Thomas glass/teflon tissue grinder using deionized glass distilled water. Catalase in the crude tissue preparation was assayed by the oxygen cathode method according to Goldstein[6]. The unit of enzyme activity is the amount of enzyme which releases 1 μmole of O_2 per min at 30 °C, pH 7 and 0.033 M perborate. Protein was determined by the method of Lowry et al.[7].

Results and discussion. These data demonstrate that there is a significant difference between the catalase activity of the

Muscle catalase activity by muscle fibre type (μ/mg protein)

	Rats	Hamsters
Soleus (slow oxidative)	5.75±0.44*	3.60±0.26*
Extensor digitorum longus (EDL) (fast oxidative glycolitic)	2.60±0.41	1.84±0.30
Gastrocnemius		
red	3.63±0.34**	
white	1.42±0.30	

Data are means±SE. * Significantly different from EDL $p \leq 0.05$; ** significantly different from white gastrocnemius $p \leq 0.05$.

soleus (slow oxidative) and the extensor digitorum longus (fast oxidative glycolytic) skeletal muscles[8], both in rats and hamsters. The data further demonstrate that there may be a significant difference in catalase activity between the regions of a single muscle. Such is the case with the lateral and medial heads of the rat gastrocnemius muscle. Failure to take such differences into account might result in erroneous conclusions. These data demonstrate that samples for catalase assays must be taken not only from the same muscle, but from the same muscle region if comparisons are to be made in skeletal muscles.

1 C. Masters and R. Holmes, Physiol. Rev. *57*, 816 (1977).
2 E.T. Nishimura, S.N. Carson and G.W. Patton, Cancer Res. *26*, 92 (1966).
3 W.T. Stauber and J.C. Bird, Cytobios *9*, 83 (1974).
4 L.J. Thenard, in: Traite de Chemie, 6th edn, vol. 1, p. 529. Crochard, Paris 1834.
5 N.C. Kar and C.M. Pearson, Clinica chim. Acta *94*, 277 (1979).
6 D.B. Goldstein, Analyt. Biochem. *24*, 431 (1968).
7 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
8 M.A. Ariano, R.B. Armstrong and V.R. Edgerton, J. Histochem. Cytochem. *21*, 51 (1973).

Irreversible inhibition of sodium transport by the toad urinary bladder following photolysis of amiloride analogs

Melanie H. Cobb and W.N. Scott

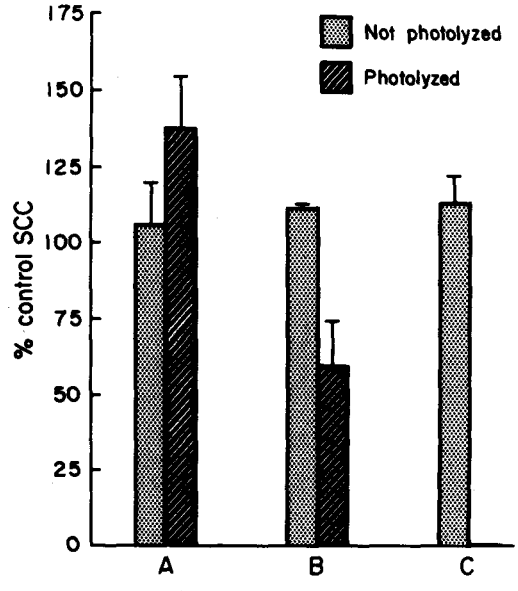
Departments of Physiology and Ophthalmology, Mount Sinai School of Medicine, New York (NY 10029, USA), 12 August 1980

Summary. Active sodium transport was completely and irreversibly inhibited in toad urinary bladders photolyzed in the presence of iodoamiloride.

Identification of the membrane components involved in the specific entry of sodium into mucosal cells is essential for the elucidation of the biochemical mechanisms associated with the active transcellular transport of the ion by epithelial tissues. Amiloride (N-amidino-3,5-diamino,6-chloropyrazine-carboxamide)[1], a rapidly reversible inhibitor of sodium transport, evidently acts by binding to the site in the apical membrane responsible for sodium permeation[2]. The apparent specificity of amiloride for the sodium entry site is such that the amount of amiloride bound by tissues has been used to determine the number of sodium channels[3]. An amiloride analog that could be used as an affinity label would be an elegant tool for the study of apical membrane sodium permeability; a radioactive amiloride affinity label might be used to identify, isolate and characterize the membrane components comprising the passive sodium entry mechanism.

The design of an effective amiloride-based affinity label has been hampered by the fact that even relatively simple derivatization of amiloride is associated with a loss of drug activity. For example, although results from in vivo studies of amiloride analogs as antagonists of the effects of deoxycorticosterone in the dog suggest that analogs with derivatized guanidine amino groups are potent diuretics[1], in isolated epithelial tissues such as the toad bladder these analogs retain no more than 1% of the sodium transport inhibitory activity of amiloride (Cobb, unpublished data). The discrepant results from the intact animal studies are probably due to the conversion of analogs to more active forms in vivo, since the parent compound, amiloride, can be recovered in the urine of these animals. Benos and Mandel[4] found that bromoamiloride (N-amidino-3,5-diamino,6-bromo-pyrazinecarboxamide), an amiloride analog in which the chlorine atom is replaced by bromine, will, after photolytic activation, irreversibly inhibit approximately 30% of the short circuit current (SCC) across the frog skin. In the following study we report the complete irreversible inhibition of the SCC across the toad urinary bladder by iodoamiloride, an amiloride analog which offers the potential for preparation with radioiodine to yield a photoaffinity label with very high specific activity.

Hemibladders excised from Dominican toads were mounted in a 2-section Ussing chamber in Ringer's solution of the following composition: 85 mM NaCl, 4 mM KCl, 17.5 mM $NaHCO_3$, 0.8 mM $MgSO_4$, 0.8 mM KH_2PO_4, 1.5 mM $CaCl_2$ and 10.5 mM glucose. The SCC was measured in both halves of the hemibladders, which were then incubated for 30 min in Ringer's solution alone or containing 500 µM bromoamiloride or 20–200 µM iodoamiloride. One half of each hemibladder was photolyzed, with a Hanovia mercury lamp through a skylight filter (> 320 nm), for 3 times for 30-sec intervals in a period of 10 min. The hemibladders



Irreversible inhibition of sodium transport by photolysis of amiloride analogs. After measurements of the SCC were made, hemibladders were treated for 30 min with Ringer's solution, *A* without amiloride analogs, *B* with 500 µM bromoamiloride, or *C* with 100 µM iodoamiloride. Following photolysis of one half of each tissue as described in the text, both halves of the tissues were rinsed with 3 changes of Ringer's solution for 30 min after which the SCC was measured again. Photolyzed tissue, right bar; unphotolyzed tissue, left bar.