Nonspecific esterases are one of the most complex of isozymic systems. As such, the cellular functions of individual species have for the most part remained undefined[7]. A major step toward elucidating cellular functions necessarily involves the ability of the investigator to solubilize and resolve as many of the component enzyme species as possible. The behavior of specific isozymes can then be monitored under various experimental conditions to further determine their functional integration into cellular processes. Triton X-100 has afforded the opportunity to enhance solubilization efficiency and resolution of mosquito esterases.

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2 E.J. Houk, W.J. Cruz and J.L. Hardy, Comp. Biochem. Physiol. *61B*, 291 (1978).
3 E.J. Houk, W.J. Cruz and J.L. Hardy, Insect Biochem, in press.
4 A.P. Beranek, Ent. exp. appl. *17*, 129 (1974).
5 E.J. Houk, unpublished observations.
6 D. Angellis, N.R. Inglis and W.H. Fishman, Am. J. clin. Path. *66*, 929 (1976).
7 T.K. Shnitka, Electron Microscopy of Enzymes, Vol.3. Ed. M.A. Hayat. 1973.

Histochemical demonstration of differences in AMP deaminase activity in rat skeletal muscle-fibres[1]

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Summary. The histochemical method for AMP deaminase confirms that rat slow-twitch fibres contain less AMP deaminase activity than fast-twitch fibres. However, the enzyme appears similarly localized in all fibre types.

Skeletal muscle normally contains high levels of AMP deaminase (E.C.3.5.4.6), and it is well established that the ammonia produced by working muscle arises from AMP deamination[4,5]. Using a new histochemical method for the demonstration of AMP deaminase activity, Fishbein et al.[6,7] have discovered the occurrence of muscle AMP deaminase deficiency in some humans. In addition, Fishbein[6] reported that skeletal muscle fibres could be differentiated on the basis of their staining for AMP deaminase. However, Shumate et al.[8], while confirming the utility of the histochemical method for identifying muscle AMP deaminase deficient patients, have found no significant differences in staining between human fibre types. This seems surprising since it is probable that human slow-type fibres contain significantly less AMP deaminase activity than fast-type fibres.[9]

In contrast to human muscle, rat muscle fibre types have been well characterized with respect to AMP deaminase activity[10]. Rat slow-twitch oxidative (SO) fibres contain less than half the activity of either fast-twitch glycolytic (FG) or fast-twitch oxidative-glycolytic (FOG) fibres. Moreover, SO fibres, in contrast to fast fibres, produce little or no ammonia from AMP during work[5]. Therefore, the purpose of this study was to determine whether rat skeletal muscle fibre types show differences in histochemical staining for AMP deaminase.

Materials and methods. Plantaris and soleus muscles dissected from 250–350 g. Sprague-Dawley rats were frozen in isopentane cooled in liquid nitrogen, and 10-µm sections were cut at −25 °C. The histochemical method for AMP deaminase was slightly modified from the method of Fishbein[6,11]. Sections were incubated at 23 °C for 1 h in a medium containing 3.2 mM dithiothreitol, 1.2 mM nitroblue tetrazolium, 0.2 M KCl, 1.2 mM AMP, and 1 mM sodium azide at pH 6.1. Following incubation, sections were rinsed in 150 mM KCl - 1.5 mM sodium citrate, pH 6.0, cleared in acetone, and mounted in glycerin jelly. Individual fibres were characterized as light, intermediate, or dark, based on their relative staining intensity. Histochemical staining for AMP deaminase was dependent upon the presence of AMP, and was completely blocked by 50 µM coformycin, a specific inhibitor of AMP deaminase[12,13]. Inclusion of sodium azide in the medium slightly enhanced the contrast between fibres. Serial sections were stained for Ca^{++}-activated myosin ATPase (E.C. 3.6.1.3) by the method of Guth and Samaha[14] (preincubation at pH 10.4), and for succinate dehydrogenase[15] (E.C. 1.3.99.1.) or SDH. On the basis of these 2 reactions, fibres were classified as SO (low ATPase, high SDH), FOG (high ATPase, high SDH), or FG (high ATPase, low SDH)[16]. Reagents were obtained from Sigma Chemical Co., St. Louis, Mo., USA.

Results and discussion. As shown in figure 1a rat muscle fibres show differences in staining intensity for AMP deaminase. The FG fibres were always darkly stained (n = 180), while the SO fibres were never darkly stained (79% light, 21% intermediate, n = 73). These extremes are consistent with the differences in biochemically assayed AMP deaminase activity in these 2 fibres[10]. Similarly, in the soleus muscle (figure 2a), which contains predominantly SO fibres and no FG fibres, there were no darkly stained fibres. On the other hand, the differences in AMP deaminase staining were not sufficient to unambiguously define all 3 fibre types. For example, the FOG fibres of the plantaris muscle stained with variable intensity (7% light, 45% intermediate, 48% dark, n = 201). This could have been predicted, since this fibre type has an intermediate activity of AMP deaminase[10]. Thus, it is possible to identify the low and high AMP deaminase fibres histochemically and with the aid of myosin ATPase and SDH sections further separate rat skeletal muscle into its 3 fibre types.

We have recently shown that rat SO fibres, in contrast to fast fibres, produce little or no ammonia during intense contractions[5]. This difference could not be explained solely on the basis of the lower total AMP deaminase activity in SO fibres. One possible explanation might be that the enzyme is localized in different subcellular regions in slow as compared to fast fibres. However, oil-emersion microscopy revealed that the AMP deaminase histochemical reaction produced a diffuse pattern of formazan deposits, throughout the intracellular region (figure 3a), which was similar in all fibre types. This similar localization may be due to the binding of this enzyme by myosin[17], a result also suggested by the cross-striations illustrated in the longitudinal section (figure 3b). Thus, the lack of physiological AMP

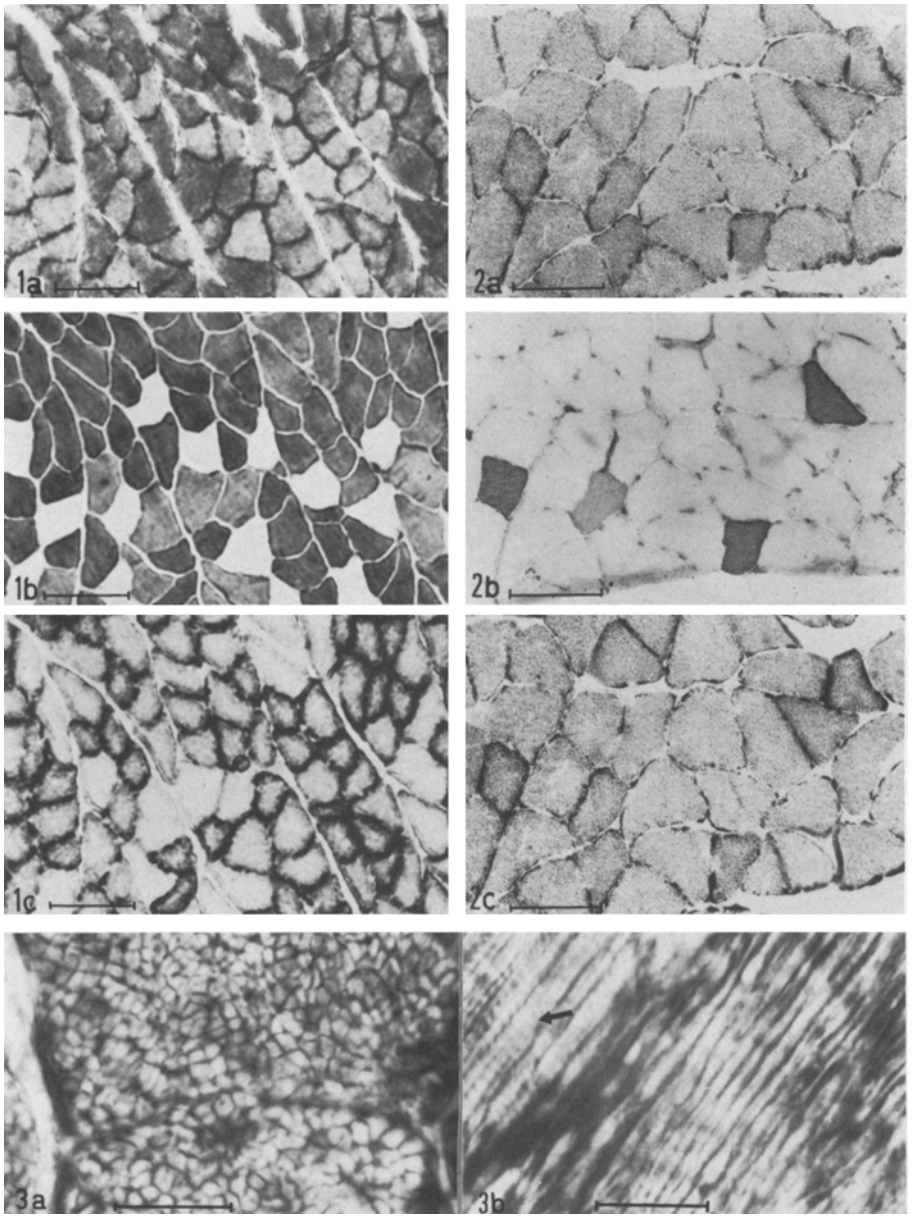

Fig. 1. Transverse serial sections of plantaris muscle stained for *a* AMP deaminase, *b* myosin ATPase (pH 10.4 preincubation), and *c* succinate dehydrogenase. Bars equal 100 μm.

Fig. 2. Transverse serial sections of soleus muscle stained for *a* AMP deaminase, *b* myosin ATPase (pH 10.4 preincubation), and *c* succinate dehydrogenase. Bars equal 100 μm.

Fig. 3. *a* Transverse and *b* longitudinal sections of 2 neighboring fibres of plantaris muscle. Note the intermyofibrillar and A-band AMP deaminase staining. Bars equal 10 μm.

deamination in SO fibres is probably due to inhibition of the enzyme or to unavailability of substrate in these fibres even during vigorous contractions.

In summary, rat skeletal muscle fibres stained for AMP deaminase show a similar pattern, but differences in staining intensity which are consistent with their known levels of AMP deaminase activity.

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4 J.M. Lowenstein, Physiol. Rev. *52,* 382 (1972).
5 R.A. Meyer and R.L. Terjung, Am. J. Physiol. Cell Physiol. *6,* 111 (1979).
6 W.N. Fishbein, Fed. Proc. *37,* 331 (1978).
7 W.N. Fishbein, V.W. Armbrustmacher and J.L. Griffin, Science *200,* 545 (1978).
8 J.B. Shumate, R. Katnick, M. Ruiz, K. Kaiser, C. Frieden, M.H. Brooke and J.E. Carroll, Muscle Nerve *2,* 213 (1979).
9 W.N. Fishbein, J.L. Griffin, K. Nagarajan, J.W. Winhert and V.W. Armbrustmacher, Clin. Res. *27,* 274A (1979).
10 W.W. Winder, R.L. Terjung, K.M. Baldwin and J.O. Holloszy, Am. J. Physiol. *227,* 1411 (1974).
11 Dr. W.N. Fishbein, Armed Forces Inst. Path. Washington, DC, personal communication.
12 R.P. Agarwal and R.E. Parks, Jr, Biochem. Pharmac. *26,* 663 (1977).
13 Coformycin was a gift of Dr H. Umezawa, Institute of Microbial Chemistry, Tokyo, Japan.
14 L. Guth and F.J. Samaha, Exp. Neurol. *25,* 138 (1969).
15 M.M. Nachlas, K. Tsou, E. DeSouza, C. Cheng and A.M. Seligman, J. Histochem. Cytochem. *5,* 420 (1957).
16 J.B. Peter, R.T. Barnard, V.R. Edgerton, C.A. Gillespie and K.E. Stempel, Biochemistry *11,* 2627 (1972).
17 B. Ashby, C. Frieden and R. Bischoff, J. Cell Biol. *81,* 361 (1979).