elimination. Hyperoxygenation, by altering the balance between production and elimination rates, may permit the accumulation of hypertrophic histiocytes in some of the alveoli, enabling their detection. The fact that in our experiments most of the hypertrophic histiocytes are found in peripherally located alveoli, which probably evacuate their contents with more difficulty, adds substance to this interpretation. It must be admitted, however, that alternative interpretations are possible. Thus the facilitating action of hyperoxygenation could be related to completely different mechanisms, such as an interference with liver metabolism[11] resulting in diminished capacity to catabolize the drug. Although the mechanism responsible for the observed pulmonary histiocytosis is still unknown, the fact that it does exist is of great interest to investigators of neonatal physiology, since successive or concurrent hyperoxygenation and treatment with amphiphilic drugs are possible occurrences in pediatric practice.

1 This work was supported by grants from the Medical Research Council of Canada.
2 To whom reprint requests should be sent.
3 Acknowledgments. Skilled technical assistance was provided by Mr V. Kapal and Miss G.A. Calderwood, Jr. The authors wish to thank Dr I.M. Nielsen, H. Lundbeck and Company, Copenhagen, Denmark, for the generous supply of chlorphentermine.

4 D. Karabelnik, G. Zbinden and E. Baumgartner, Toxic. appl. Pharmac. *27*, 395 (1974).
5 S. Kacew, R. Narbaitz and T.C. Dubas, Toxic. appl. Pharmac. *47*, 185 (1979).
6 D.S. Bonikos, K.G. Bensch, S.K. Ludwin and W.H. Northway, Jr, Lab. Invest. *32*, 619 (1975).
7 H. Witschi and M.G. Côté, Chem.-Biol. Interact. *19*, 279 (1977).
8 S. Kacew and R. Narbaitz, Expl molec. Pathol. *27*, 106 (1977).
9 D.H. Bowden and I.Y.R. Adamson, Lab. Invest. *30*, 305 (1974).
10 B. Meyrick and L.M. Reid, in: Development of the Lung, p. 135. Ed. W.A. Hodson. Marcel Dekker, Basel 1977.
11 D.A. Baeyens and M.J. Meier, Aviat. Space Environ. Med. *49*, 980 (1978).
12 J. Yam, L. Frank and R.J. Roberts, Pediat. Res. *12*, 115 (1978).

Isoelectric focusing of mosquito esterases in the presence of Triton X-100[1]

E.J. Houk and J.L. Hardy

Naval Biosciences Laboratory, University of California, School of Public Health, Oakland (CA 94625, USA), 20 August 1979

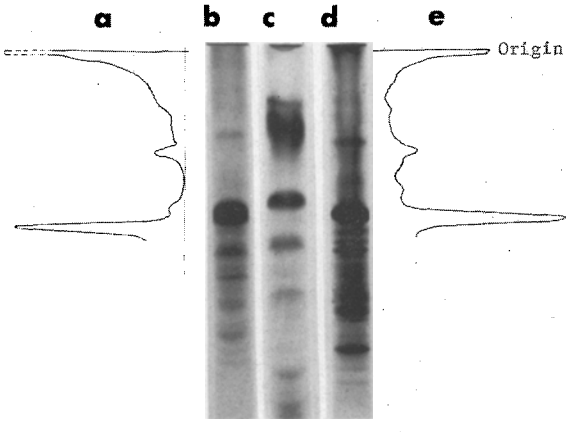
Summary. Triton X-100 improves nonspecific esterase solubilization from mosquito samples and also leads to increased resolution in an isoelectric focusing electrophoresis.

Houk et al.[2,3] observed that most electrophoretic studies of insect nonspecific esterases had dealt with H_2O and buffer soluble species. A single investigator had ventured to add a mild detergent, sodium deoxycholate, to aid in releasing membrane bound esterolytic material[4]. Deoxycholate does solubilize a greater amount of esterolytic activity into the 10,000 × g - 20 min supernatant as measured spectrophotometrically[5]. Subsequent electrophoresis revealed that new isozymic species were detected and the relative activities of some previously detected species were enhanced. However, substantial esterolytic activity remained at the origin and did not enter into the isoelectric focusing system[3]. In an attempt to release more nonspecific esterolytic material into the supernatant fraction, Triton X-100 was adopted as the solubilizing agent[6]. This report substantiates the increased solubilization and improved resolution of mosquito nonspecific esterase isozymes in a Triton X-100-isoelectric focused system.

Female mosquitoes, *Culex tarsalis,* origin and maintenance described elsewhere[2,3], were ground in Tris buffer (0.2 M; pH 6.7) containing either 0.05% (w/v) sodium deoxycholate or 0.5% (v/v) Triton X-100. The suspension was centrifuged at 10,000 × g - 20 min and the supernatant subjected to isoelectric focusing electrophoresis for either 24 h at a constant 200 V[2] or at constant power, 1/3 watt gel^{-1}, for 16 h. The Triton X-100 solubilization system was examined with and without additional Triton X-100 (0.1%; v/v) incorporated into the acrylamide gel matrix. The preparation of the acrylamide gels and subsequent histochemical detection of nonspecific esterases has been described[2,3].

A comparison of deoxycholate and Triton C-100 nonspecific esterase zymograms reveals an increase in the resolution and number of detectable isozymes in the mosquito, *C. tarsalis* (figure, b and d). In the deoxycholate system, substantial unsolubilized esterolytic activity at the origin is represented by a truncated peak (figure, a) whose actual height is considerably off scale. The Triton X-100 system (figure, e) is a complete representation of the apparent esterolytic activity at the origin, with a major extraneous optical contribution from the air-acrylamide gel interface.

Triton X-100 is requisite both in the sample and within the gel matrix for optimal results[6] (figure, c and d). The absence of Triton X-100 from the gel matrix leads to large scale aggregation of esterolytic material in the more alkaline regions (pH = 6.5) of the gel (figure, c).



Influence of solubilization medium and acrylamide gel composition on densitometric tracings and electropherograms of mosquito nonspecific esterases: *a* Deoxycholate solubilized samples with substantial residual activity at the origin, *b* deoxycholate solubilized electropherogram, *c* Triton X-100 electropherogram without Triton X-100 incorporated into the gel matrix, *d* electropherogram of Triton X-100 solubilized samples with Triton X-100 included in the gel and *e* Triton X-100 solubilized samples with a complete representation of residual activity at the origin.

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.

1 Acknowledgments. This research was supported by a US Army contract/grant DAMD 17-77-C-7018, US Army Medical Research and Development Command, Washington, DC and by the Office of Naval Research.
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]

R.A. Meyer[2], J. Gilloteaux[3] and R.L. Terjung

Department of Physiology, Upstate Medical Center, State University of New York, Syracuse (New York 13210, USA), 21 September 1979

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