nism of vitamin A action on brain maturation is not yet clear.

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1 Winick, M., and Noble, A., Devl Biol. *12* (1965) 451.
2 Winick, M., Brasel, J. A., and Rosso, P., Nutrition and Development, p. 49. Wiley Interscience, New York 1972.
3 Enesco, M., and Leblond, C. P., J. Embryol. exp. Morphol. *10* (1962) 530.
4 O'Toole, B. A., Frackin, A. R., Warkany, J., Wilson, J. G., and Mann, G. V., J. Nutr. *104* (1975) 1513.
5 Takahashi, Y. I., Smith, J. E., Winick, M., and Goodman, D. S., J. Nutr. *105* (1975) 1299.
6 Sharma, H. S., and Misra, U. K., Acta vitaminol. enzymol. *7* (1985) 85.
7 Dobbing, J., and Sands, J., Biol. Neonate *19* (1971) 363.
8 Shukla, R. R., Joshi, H. C., and Misra, U. K., Biol. Neonate *44* (1983) 243.
9 Sharma, H. S., and Misra, U. K., Z. Ernährungswiss. *26* (1987) 116.
10 Sharma, H. S., and Misra, U. K., Z. Ernährungswiss. *27* (1988) 119.
11 Malathi, P., Subarao, K., Shastry, P. S., and Ganguly, J., Biochem. J. *87* (1963) 305.
12 Munro, H. N., and Fleck, A., Methods biochem. Anal. *15* (1966) 113.
13 Burton, K., Biochem. J. *62* (1966) 315.
14 Dische, Z., The Nucleic Acids, vol. 1. Academic Press, New York 1955.
15 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. *193* (1951) 265.
16 Sharma, H. S., Sriram, K., and Misra, U. K., Nutr. Rep. Internat. *34* (1986) 221.
17 Sharma, H. S., and Misra, U. K., Biol. Neonate *50* (1986) 345.

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Localisation of acetylcholinesterase in rat myotubes in the presence of β-endorphin and β-endorphin-(1-27)

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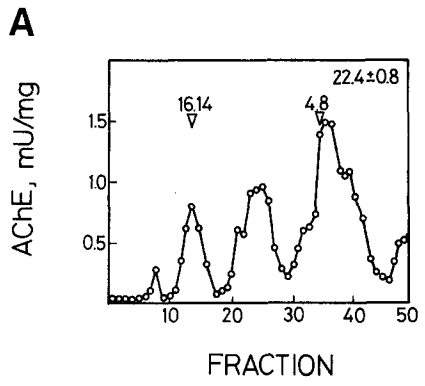
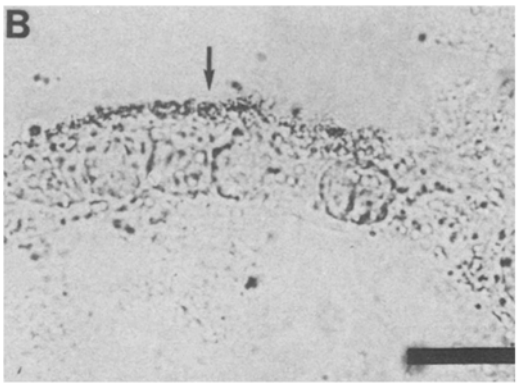
Summary. In rat embryo skeletal myotubes, acetylcholinesterase is present, as multiple forms, and can be detected in deposits at the cell surface. Myotubes cultured in the presence of β-endorphin, exhibit an increased predominance of the globular (precursor) forms of the enzyme, which are largely restricted to intracellular sites associated with nuclei. In the presence of β-endorphin-(1-27), the relative proportions of the different forms of acetylcholinesterase is similar to that seen in the controls, but the enzyme is intracellular and has a characteristic focal localisation in the myotube.
Key words. Acetylcholinesterase; myotubes; β-endorphin.

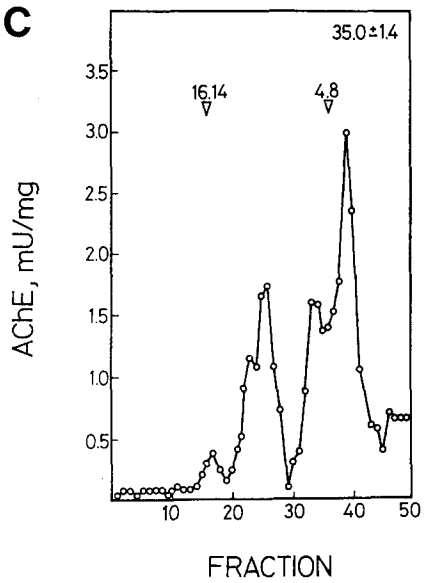
Acetylcholinesterase (AChE, EC 3.1.1.7) in skeletal muscle is under the trophic control of the motor nerve. Studies on developing myotubes in vitro have shown that both electromechanical activity [1, 2] and soluble factors [3, 4] can regulate the activity of the different molecular forms of the enzyme. β-Endorphin, a neuropeptide which coexists with acetylcholine in motoneurones of developing rodents [5, 6] can exert a regulatory influence over AChE, reducing the proportion of the asymmetric A_{12} form, and increasing that of the globular (G_1, G_2, G_4) forms of the enzyme [7]. β-Endorphin and its C-terminally-truncated derivative, β-endorphin-(1-27), are released by embryonic spinal cord in vitro [7]. In this report, we show that a striking redistribution of AChE accompanies the changes in enzyme activities in rat myotubes cultured in the presence of the peptides.

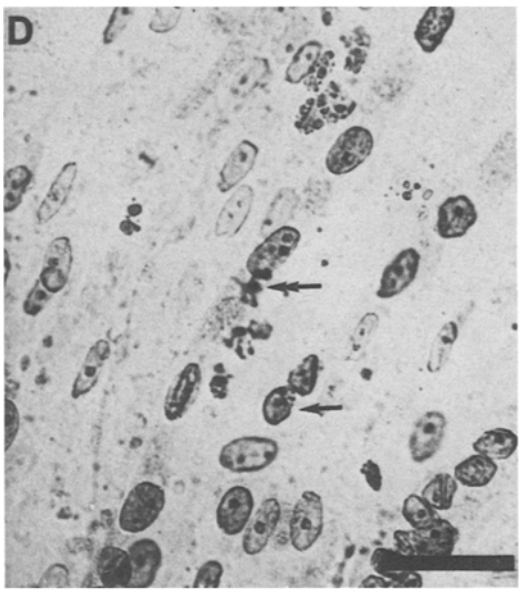
Myotubes were cultured from rat embryos of 20–21 days gestation, as previously described [7]. After fusion had occurred, the cells were cultured for a further 3 days and then transferred to a maintenance medium containing 0.25% chick embryo extract, 0.2 U/ml insulin, 50 μg/ml transferrin and 32 ng/ml progesterone. In some experiments the cells were exposed to β-endorphin or β-endorphin-(1-27) for up to 7 days. Cells were either harvested and the AChE molecular forms extracted and analysed as previously described [7], or they were stained histochemically for AChE using a modified direct colouring reaction [8, 9] in phosphate buffer (pH 7.5, reaction time 120 min) after the method of Koelle [10]. AChE was detected using acetylthiocholine iodide as substrate in the presence of 0.1 mM tetraisopropylpyrophosphoramide, and butyrylcholinesterase (BuChE) was detected using S-butyrylthiocholine chloride as substrate in the presence of 10 μM 5,5′-dithiobis(4-alkyldimethylammoniumphenyl)pentan-3-one dibromide (reaction time 5 h).

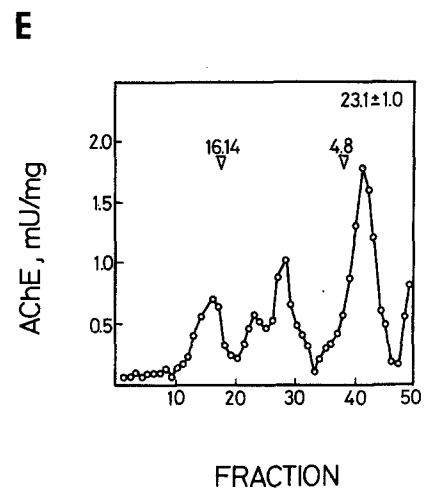
In control cultures, three major peaks of AChE were detected, of which a significant proportion of the enzyme was the asymmetric, A_{12} form (fig., A). In rat myotubes, this form is restricted in its localisation to the cell surface [9]. Most of the AChE was at or near the cell-surface (fig., B). In β-endorphin-exposed cultures the AChE activity was recovered principally as low and medium-sized AChE forms (fig., C) which are precursors to the A_{12} form. In these cells the AChE was seen to be present exclusively in the nuclear envelope (fig., D, arrowed) and Golgi apparatus (double-arrowed), and no cell mem-

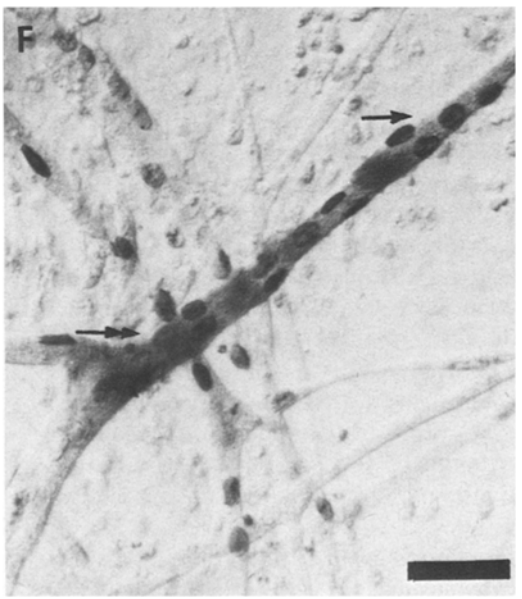












A Activity of molecular forms of AChE in control myotubes. AChE forms are separated on 5–20% sucrose density gradients with gradient meniscus to the right. Gradient markers are indicated in S values. The A_{12} form of AChE coincides with the 16.14S marker with globular forms appearing in later fractions. Specific activity of enzyme (mU/mg) is shown in the upper right-hand corner (mean ± SEM, n = 6); *B* reaction product was seen in patches near the cell membrane (arrowed) in the same cultures; *C* activity of molecular forms and specific activity of AChE in cells exposed to β-endorphin as in *A*; *D* reaction product seen in perinuclear sites (arrowed) and Golgi system (double-arrowed) during exposure to β-endorphin, *E* activity of molecular forms and specific activity of AChE in cells exposed to β-endorphin-(1-27) as in *A*; *F* reaction product is seen in association with AChE-positive nuclei during exposure to β-endorphin-(1-27) as for β-endorphin (cf. *D*). Note also 'hotspots' of AChE associated with one or more nuclei (double-arrowed). Bars in *B, D, F* indicate 50 μm.

brane-associated activity was evident. In cultures exposed to β-endorphin-(1-27) (30 nM) the relative predominance of the different forms of the enzyme was essentially the same as in controls (fig., E, cf A). The histological localisation of AChE was similar to that in the presence of β-endorphin but fewer nuclei were AChE-positive. However, β-endorphin-(1-27)-exposed cultures were unique in that dense reaction product was focally distributed in the cytoplasm as well as being associated with AChE-positive nuclei in these areas (fig., F). BuChE was barely detectable in control cultures and was seen mainly in association with myotube nuclei. Its distribution was unaffected by the peptides. These findings indicate that multiple effects of naturally-occurring β-endorphin derivatives can regulate the distribution of AChE, and the nature of the effect depends on the length of the C-terminus. β-endorphin alters the interconversion of low-molecular weight, globular (G_1, G_2, G_4) asymmetric and (A_{12}) AChE forms, stimulating the accumulation of the globular AChEs within the cell. β-endorphin-(1-27) stimulates the lateral localisation of the enzyme into active regions of the cell, reminiscent of those seen at sites of nerve contact. At the time of innervation, AChE loses its diffuse localisation in the rat muscle cell and becomes associated with specialised nuclei which become associated with the future motor endplate [10]. Our observations are consistent with the possibility that β-endorphin and related peptides, which may be released from motor nerve terminals in immature rats [11], exert a trophic control over muscle AChE.

1 Rieger, F., Koenig, J., and Vigney, M., Devl Biol. *76* (1980) 358.
2 Brockman, S. K., Younkin, L. H., and Younkin, S. G., J. Neurosci. *4* (1984) 131.
3 Bulger, J. E., Randall, W. R., Nieberg, P. S., Patterson, G. T., McNamee, M. G., and Wilson, B. W., Dev. Neurosci. *5* (1982) 474.
4 Fernandez, H. L., Patterson, M. R., and Duell, M. J., J. Neurobiol. *11* (1980) 557.
5 Haynes, L. W., Smyth, D. G., and Zakarian, S., Brain Res. *232* (1982) 115.
6 Haynes, L. W., and Smith, M. E., Neurosci Lett. *55* (1985) 125.
7 Haynes, L. W., Smith, M. E., and Smyth, D. G., J. Neurochem. *42* (1984) 1542.
8 El Badawi, A., and Schenk, E. A., J. Histochem. Cytochem. *15* (1976) 580.
9 Inestrosa, N. C., Silberstein, L., and Hall, Z. W., Cell *29* (1982) 71.
10 Kupfer, C., and Koelle, G. B., J. exp. Zool. *116* (1951) 397.
11 Haynes, L. W., and Smith, M. E., J. Physiol. (Lond.) *351* (1984) 22P.

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The effect of moderate hemodilution with Fluosol-DA or normal saline on acetaminophen disposition in the rat

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Summary. Hemodilution with 40 ml/kg of Fluosol or saline reduced the acetaminophen V_d and the acetaminophen sulfate Cl_M at 48 or 72 h, respectively. Fluosol hemodilution increased the acetaminophen renal excretion at 24 and 72 h. But at 48 h, Fluosol hemodilution either inhibited the renal secretion of acetaminophen or enhanced its reabsorption.
Key words. Acetaminophen; Fluosol; sulfation; glucuronidation; hemodilution.

Perfluorochemical (PFC) emulsions are being evaluated as potential blood substitutes or acellular oxygen carrying substances because of their ability to dissolve oxygen. Numerous animal studies have demonstrated that severe hemodilution with PFC emulsions can sustain life. PFC emulsions have been used clinically in man for blood loss replacement, oxygen delivery to ischemic tissues, myocardial infarction, coronary angioplasty, spinal cord injury, sickle cell crisis, gangrene, chemotherapy of solid tumors and severe anemia [1].

PFC particles are captured by the reticuloendothelial system and reach a maximum hepatic content in 2 days [2]. The PFC particles have been found in Kupffer cells, hepatocytes, mononuclear phagocytes, and 'foamy' macrophages [3]. Not unexpectedly, the disposition of several drugs is altered immediately after PFC administration [4]. Recent studies have indicated that Fluosol®-DA (Fluosol) hemodilution alters cytochrome P-450 mediated drug metabolism in a time-dependent and dose-dependent manner [5]. The current investigation determines if the conjugation pathways of sulfation and glucuronidation involved in acetaminophen disposition are altered by Fluosol or normal saline hemodilution in a time-dependent manner.

Materials

Fluosol was donated by Alpha Therapeutic Corporation (Los Angeles, California), and was prepared as directed within 0.5 h of use. Fluosol is a 20% (w/v) perfluorode-