

percent) of the dry weight of arils. Mammals common at nearby *Tetragastris* trees (9), which produce sugary fruits sweet to the taste, avoided *Virola* day and night. Spider monkeys smelled and rejected as many arils as they consumed; this fruit was not an important part of their diet; see A. Hladik and C. M. Hladik, *Terre Vie* 23, 25 (1969).

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22. An alternative but less intuitive procedure shows that dispersal success is negatively correlated with the weight of the arillate seed ($r = -0.66$, $P = .002$). Partial correlation controlling for aril weight shows little influence ($r_{\text{part}} = -0.78$, $P < .001$); partial correlation controlling for seed weight shows decisive influence ($r_{\text{part}} = 0.32$, $P > .1$). There is no correlation between aril and seed weight ($r = -0.10$, $P > .3$). The percentage of seeds dispersed simi-

larly shows a strong correlation with the difference between aril and seed weights.

23. Neither aril weight, seed weight, nor their ratio correlate with fecundity ($P > .1$).

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26. We thank H. Dingle, W. Glanz, E. G. Leigh, A. S. Rand, V. L. Sork, and anonymous reviewers for comments on the manuscript. Research was supported by the University of Iowa, Smithsonian Tropical Research Institute, and BSRG grant PS07035-13 of the National Institute of Health. B. Oskroba and M. Trlica were responsible for nutritional analyses.

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Nerve Terminals Are as Metabolically Different as the Muscle Fibers They Innervate

Abstract. The rate at which glucose enters nerve terminals in muscle was estimated indirectly by measuring changes in miniature end-plate potential frequency. D-Glucose entered nerve terminals in muscles with a fast twitch more rapidly than it entered those with a slow twitch. This suggests that nerve terminals in fast- and slow-twitch muscles differ in their rate of metabolism.

A motor unit is composed of a motor neuron and the muscle fibers that it innervates. Motor units can be divided into those that contract rapidly, type F, and those that contract slowly, type S. These units differ in their physiological properties, such as rate of firing, and in the histochemical properties of their muscle fibers (1, 2). Differences in neuronal metabolism would also be expected. This report presents evidence that glucose enters type F nerve terminals more rapidly than it enters type S terminals.

Experiments were performed on nerve-muscle preparations from Sprague-Dawley rats (100 to 200 g). Conventional methods for intracellular recording were used. The bathing solution contained 160 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM Na-Hepes, and 11 mM D-glucose (pH 7.0 to 7.4; temperature, 32° ± 0.5 C°). Hyperosmotic neurosecretion (3, 4) was used to estimate glucose entry into single nerve terminals (5). Miniature end-plate potential (MEPP) frequency (f) was recorded by inserting a microelectrode into a muscle fiber near the nerve ending. Measurements were made 5 to 8 minutes (f_5) and 17 to 20 minutes (f_{20}) after sucrose was added to the solution to make it hyperosmotic. The recorded frequencies were then compared to the resting MEPP frequency (f_0). Figure 1 shows that there is a linear relation between $\log(f_5/f_0)$ or $\log(f_{20}/f_0)$ and the osmotic gradient across the nerve terminal membrane. These results are similar to those reported by Hubbard *et al.* (6).

A decrease in MEPP amplitude would cause small MEPP's to be lost in the am-

plifier noise and would therefore simulate a decrease in MEPP frequency. To avoid this complication I used small rats, for which the mean MEPP amplitude was 0.95 mV; data for cells in which the resting potential dropped by more than

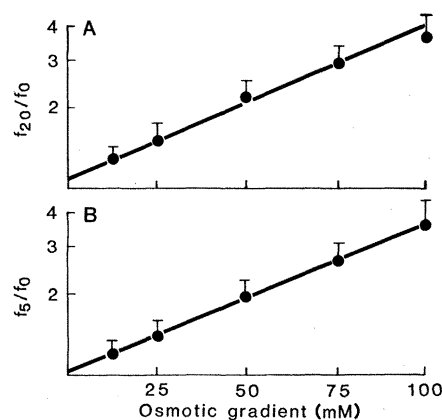


Fig. 1. (A and B) Increase in MEPP frequency as a function of osmotic gradient across the nerve terminal membrane. Each mean and standard deviation is based on ten cells. Ordinate: the ratio of mean MEPP frequency 5 to 8 minutes (f_5) and 17 to 20 minutes (f_{20}) after the osmotic gradient was increased with sucrose to the resting MEPP frequency (f_0), which was based on a 10-minute recording. Note logarithmic scale. Abscissa: the difference between test and control osmolarities. The lines represent least-squares linear regressions. The results shown are from recordings made in the diaphragm; similar results were found in the extensor digitorum longus and soleus when osmotic gradients up to 30 mM were used.

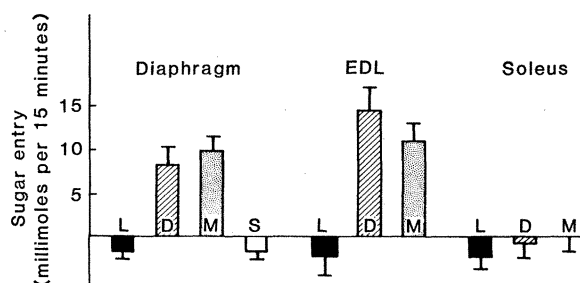


Fig. 2. Rates of entry of various sugars into presynaptic nerve terminals in the diaphragm, EDL, and soleus. Abbreviations: L, L-glucose; D, D-glucose; M, 3-O-methyl-D-glucose; and S, sucrose. Each mean and standard error is based on 15 cells. L-Glucose did not penetrate into nerve terminals in any of the muscles tested; D-glucose and 3-O-methyl-D-glucose entered nerve terminals in the diaphragm and EDL but not in the soleus.

The statistical significance of these differences is evident even in the smallest difference [for transport of D-glucose in the diaphragm versus transport of 3-O-methyl-D-glucose in the soleus, $P < .01$ (Student's *t*-test)]. Whereas the rates at which D-glucose and 3-O-methyl-D-glucose enter nerve terminals in the diaphragm and EDL varied, no significant difference was detected by analysis of variance. Analysis of variance also failed to detect a significant difference in rate of entry among the other sugars. These results suggest that D-glucose and 3-O-methyl-D-glucose enter nerve terminals in the diaphragm and EDL more rapidly than they enter nerve terminals in the soleus.

bathing solution caused the normal initial increase in MEPP frequency (f_s/f_0) but a subsequent decrease (as measured by f_{20}/f_0), indicating that these sugars enter the nerve terminal in a stereospecific manner. Both D-glucose and 3-O-methyl-D-glucose entered nerve terminals in the diaphragm and extensor digitorum longus (EDL), but did not enter nerve terminals in the soleus. The lack of a difference in the rate of entry of D-glucose and 3-O-methyl-D-glucose shows that nerve terminal metabolism of glucose does not distort the estimate of glucose entry, since 3-O-methyl-D-glucose is not metabolized (7).

The proportion of type F nerve terminals is about 20 percent in the soleus muscle, 60 percent in the diaphragm, and 95 percent in the EDL (8, 9). The present experiments show that there is a positive correlation ($r = .63$, $P < .001$) between the proportion of type F nerve terminals in the three muscles and the rate of D-glucose entry, suggesting that glucose enters type F nerve terminals more rapidly than it enters type S terminals. Type F nerve terminals can be divided into those associated with fast oxidative-glycolytic muscle fibers and those associated with fast glycolytic ones (10). No fine distinction between the two type F nerve terminals can be made.

The functional significance of these findings is not known, but it is tempting to speculate about the relation between glucose transport and the rate of cell metabolism. Glucose transport is usually closely linked to the rate of cell metabolism (11). Cells with rapid glucose transport often do not regulate glucose entry into the cell, while cells with slow glucose transport do. I assume that the slower entry of glucose into type S nerve terminals means that glucose transport into these terminals is regulated. Since type S motor units are capable of sustained activity, slow entry of glucose suggests that the type S nerve terminal may have a large energy store, such as glycogen or lipid, or may be able to rapidly increase glucose transport as needed during activity. Type F motor units show brief bursts of activity and may be able to survive on the glucose provided by their more active transport systems. Synaptosomal preparations show evidence of two carriers that differ in their affinity for glucose (12). Hence it is conceivable that, depending on its metabolic requirements, a given neuron may only have carriers of high or low glucose affinity.

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The Role of Zinc and Follicle Cells in Insulin-Initiated Meiotic Maturation of *Xenopus laevis* Oocytes

El-Etr *et al.* (1) showed that high concentrations of insulin (up to 400 $\mu\text{g/ml}$) initiated meiotic maturation in most full-grown *Xenopus laevis* oocytes and suggested that amphibian oocytes may thus serve "as a model system for the study of the poorly understood mechanism of insulin action." We have cultured growing *X. laevis* oocytes in the presence of 1 μg of insulin per milliliter and have found maturation never occurs unless progesterone is added to the medium (2).

We therefore tested the effect on manually dissected, full-grown (> 1.2 mm in diameter) oocytes of two insulin preparations (3) we have previously used for oocyte culture (4), but used the highest concentration (40 $\mu\text{g/ml}$; 1 U/ml; 7 μM) for which data were reported by El-Etr

et al. (1). The results (Table 1, experiment 1) indicated that the insulin preparation (lot 615-D63-10) we have routinely used for oocyte culture (2, 4) did indeed initiate germinal vesicle breakdown (GVBD) in some of the oocytes, thus confirming the results of El-Etr *et al.* (1). Spontaneous maturation did not occur in the absence of hormone, and progesterone initiated a 100 percent response, thus establishing appropriate controls for our experimental system. Our second insulin preparation (lot 615-1082B-108-1) appeared to be inactive (Table 1, experiment 1). The active insulin was a crystalline, Zn^{2+} -precipitated preparation [Zn^{2+} content = 0.7 percent by weight as determined by atomic absorption spectrometry (3)]; the inactive

Table 1. Initiation of meiotic maturation in full-grown *X. laevis* oocytes by insulin and Zn^{2+} . Results are expressed as the percentage incidence of germinal vesicle breakdown. For each test in experiments 1 and 3 we used 37 to 46 oocytes derived from three females; for each test in experiment 2 we used 26 to 30 oocytes derived from two females. Donor females were previously untreated by any hormone. In experiment 1, insulin was used at a concentration of 40 $\mu\text{g/ml}$ and the medium was solution O-R2 (13). In experiments 2 and 3, insulin was used at a concentration of 100 $\mu\text{g/ml}$; solution O-R2 was also modified by eliminating 1 mM Na_2HPO_4 and replacing 5 mM Hepes-NaOH buffer with 5 mM tris-Cl buffer. This modification avoids precipitation of Zn^{2+} salts. Oocytes incubated at 21°C were observed for up to 24 hours for the appearance of a large white spot indicating germinal vesicle breakdown (9); scoring was routinely confirmed by piercing the animal pole with a fine needle and gently squeezing the equator with a forceps so as to extrude the germinal vesicle, if present (7, 14).

Addition	Experiment			
	1	2	3	
			Without Pronase	With Pronase
None	0	0	0	0
Progesterone (1 $\mu\text{g/ml}$)	100	100	100	94
hCG (50 U/ml)			93	0
Zn^{2+} -insulin (lot 615-D63-10)	21	69	83	26
Zn^{2+} -insulin (lot 615-D63-10), dialyzed*		12	36	0
Zn^{2+} -insulin (lot 615-07J-256)			68	17
Insulin (lot 615-1082B-108-I)	0	38		
Insulin (lot 050YB7)			32	2
ZnCl_2 (10^{-3}M)		100	100	93
ZnCl_2 (10^{-4}M)		41	38	46
ZnCl_2 (10^{-5}M)		7	10	16
ZnCl_2 (10^{-5}M) + insulin (lot 615-1082B-108-I or 050YB7)		50	63	11

*Dialysis was for 24 hours at 4°C against 0.001N HCl, with Spectrapor 6 membranes (2000 molecular weight cutoff).