

hypotonic lysis in EDTA. In addition, in experiments 8 and 17, we also followed the method of membrane preparation and incubation of Brown *et al.*, except that we used 3 mg of EDTA per milliliter compared to 0.6 to 1.2 mg/ml. Incubation was carried out for 40 to 60 minutes at 37°C with air as the gas phase, compared to 20 minutes at 42°C used by Brown *et al.* The reaction was stopped by the addition of 0.5 ml of 10 percent trichloroacetic acid. After centrifugation, the amount of inorganic ^{32}P liberated in the protein-free supernatant was determined by the radioactivity method of Siegal and Albers (3). We also used this method for the determination of the release of inorganic orthophosphate in the two experiments 8 and 17 in which we reproduced Brown's method; Brown *et al.* used a chemical method. All incubations were carried out in triplicate. Mean values of the adenosine triphosphatase activity in control experiments as well as in experiments with ouabain added are shown in Table 1. In each case, the Mg^{++} -dependent, Na^+ , K^+ -activated adenosine triphosphatase activity was inhibited in the presence of ouabain.

Freezing and thawing of the erythrocyte ghosts (experiments 5, 7, 12, and 16) increased the enzymic activity, but ouabain inhibition was still observed. In this last-named group of studies, the lesser degree of inhibition is consistent with the further release of only Mg^{++} -dependent adenosine triphosphatase activity if the membranes are disrupted further (freezing). When we followed Brown *et al.*'s method of membrane preparation and incubation (experiments 8 and 17), we did not observe either the high adenosine triphosphatase activity reported (1) or the absence of ouabain inhibition of activity. No significant differences were detected in either the degree of enzyme activity or in the ouabain inhibition when the cells of patients were compared to those of normal control subjects.

The high activities of adenosine triphosphatase reported by Brown *et al.* (1) (200 nmole of inorganic phosphorus liberated per minute per milligram of protein) are difficult to explain; that is, stoichiometrically, there was more inorganic phosphate released than there was sodium adenosine triphosphate added, calculated on the basis of the amount of membrane protein in their medium and the total incubation time of 20 minutes.

Stimulation of Na^+ , K^+ -activated

adenosine triphosphatase activity has been reported (4), but only with the ouabain concentrations of approximately 10^{-10} mole/liter. Although altered (decreased) adenosine triphosphatase activity in erythrocyte membranes in uremic patients with hemolytic anemia has been reported (5), this condition has not been documented in patients with muscular dystrophy. It has not been possible to correlate the data of Brown *et al.* (1) with either the above observations (4, 5) or with our data.

Our experience suggests that the Na^+ , K^+ -activated adenosine triphosphatase activity of erythrocyte ghosts from normal subjects and from patients with muscular dystrophy are inhibited by ouabain in a concentration of 10^{-4} mole/liter.

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References and Notes

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7. Values of adenosine triphosphatase activity obtained with the method of membrane preparation and incubation described in (1).
8. We thank Dr. F. Anderman (Montreal Neurological Institute) for referring patients with Duchenne dystrophy; Mrs. C. Stanford, Mrs. E. Summers, and E. Whittington for technical assistance. Supported by grants from the Muscular Dystrophy Association of Canada, and the Medical Research Council of Canada.

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The specifics of our method were chosen to optimize the ouabain-response phenomenon. Klassen and Blostein did not follow the procedure we described. They used different metal concentrations, different buffer concentration, different incubation time, and temperature, and they omitted sucrose from medium.

Our legend (Table 1, p. 1578) should read 10^{-8} mole. Conditions chosen, while different from some used by other workers, are nonetheless well within the bounds of enzymology convention.

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Aquaculture: Amplification and Correction

Inquiries from colleagues received since my article on aquaculture appeared (1) suggest the following corrections and amplifications: (i) to page 1100, column 3, paragraph 2; and (ii) to page 1102, column 3, paragraph 3, and to Table 1.

With regard to (i), the enormous numbers of food organisms mentioned as necessary to sustain tank-reared pelagic fish larvae were based on the assumption that every cubic centimeter in the entire water volume must contain sufficient numbers of plankters at all times even though only a limited quantity of them is removed by grazing. The Fishery-Oceanography Center of the Bureau of Commercial Fisheries at La Jolla where the experiments I mentioned were undertaken informs me that it is not necessary to make such massive collections of plankton for rearing the relatively small numbers of larvae which they require for the purpose of gaining insight into problems of larval survival at sea. The culture of pelagic fish larvae by the bureau was and is not conducted with aquaculture in mind but to shed light on mechanisms which control year-class strength of pelagic fish stocks.

With regard to (ii), the per hectare production of oyster flesh in both the above mentioned places is at variance with other, supporting figures in my text that would indicate it to be 20 and not 58 metric tons. I regret to have made a mistake in the conversion of figures from British and Japanese measuring systems into the metric one. On checking again with the Hiroshima Provincial Fisheries Laboratory, I find that the per hectare yields of oyster flesh from rearing sites in the Japan Inland Sea in fact range from 12 to 30 metric tons with an average of 18 to 20 metric tons: the per hectare dollar value of these oysters would then be \$23,100 and not \$67,000, as stated in the table. It should be noted once more that this harvest comes from an open aquatic ecosystem with tidal interchange, and the primary production under a much larger area of water than that of the rearing sites proper is responsible for it.

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