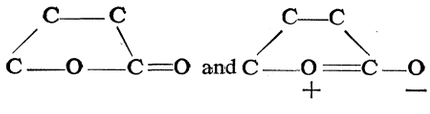
deviation. The values for Δ C-O (Table 1) in the ring oxygen bonds show a positive trend; this is a marginal observation, but it appears to be more definite for the free sugar than the pyranoside.

In the axial series, there is a distinction between the C(1)-O(1) bonds for the free sugars and the pyranosides. In the former, the shortening is in agreement with the results for the equatorial series, but in the glycosides it is much smaller or not observed. Rather it is the disproportionation of the two ring oxygen bond lengths that is more striking, with the C(1)-O(5) shorter and the C(5)-O(5) longer than average. This observation requires more confirmation with greater experimental precision, since a shift in the ring oxygen position will simultaneously lengthen one bond and shorten the other. However, it appears from these results, that there is a correlation between the C(1)-O(1) bond shortening and the lengths of the adjacent ring oxygen bonds that merits serious consideration. When the C(1)-O(1) bond is short, both the ring oxygen bonds are long; whereas a "normal" C(1)-O(1) bond is accompanied by differences between the two ring oxygen bonds.

The spread in the C-C and C-O bond lengths, given in parentheses in columns 1 and 2 of Table 1, generally exceeds 3σ. While this might be ascribed to a consistent underestimate of the standard deviations obtained from least-squares refinement procedures, it is important to realize that the steric environment formed by the adjacent bonds is different, to a more or less degree, for every C-C or C-O link in these molecules. By excluding the possibility of variations in single-bond length of the order of 0.005 Å, we might be missing a clue to further research.

The feature that does emerge quite definitely from this survey is the shortening of the anomeric C(1)-O(1) bonds. Comparable bond shortenings are also shown in Table 1 for two accurate determinations on γ-lactones, which are interpretable in terms of the valence-bond resonance between



This anomeric effect appears therefore to be an electronic perturbation of about half of the magnitude of the γ-lactone resonance. The term "anomeric effect" is familiar in carbohydrate chemistry (*10*). It has been used to describe the phenomenon that the axially substituted α-glycosides are more stable than the equatorially oriented β-isomers; even when the latter corresponds to the smaller conformational instability factors. The effect has been interpreted in terms of dipole-dipole interaction between the electrons in the anomeric bonds and the lone-pair electrons on the ring oxygens (*11*). The bond-shortening anomeric effect may well involve an enhancement of electron density in the anomeric bond (hence a shorter and stronger bond) at the expense of the ring oxygen electron density, but we do not yet have a theoretical model which permits us to calculate quantities, such as bond orders, that can be directly related to the interatomic distances.

Sundaralingam (*12*) has discussed the evidence for C-O bond-shortening in the furanose moiety of nucleotides and obtains a striking relationship between C-O bond length and C-C-O bond angles, which suggests that changes in valence angles and bond distances can be correlated through hybridization theory. No simple furanose molecules have been studied, and the precision of the observations which Sundaralingam seeks to correlate is less than in this study, by reason of the added complexity of the molecules. In general, however, a similar pattern of bond-shortening in the formally saturated glycosidic part of the molecules is observed.

HELEN M. BERMAN
SHIRLEY S. C. CHU
G. A. JEFFREY

Crystallography Laboratory,
University of Pittsburgh,
Pittsburgh, Pennsylvania 15213

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Erythrocyte Abnormality in Human Myopathy

Abstract. *Erythrocyte ghosts isolated from myopathic patients responded to 10^{-4} molar ouabain with a dramatic increase in adenosine triphosphatase activity, while identical preparations from normal donors were inhibited by the same drug. These results have been interpreted in terms of a disease-related change in membrane integrity bearing upon function of the transport enzyme.*

Myopathies have been attributed to a deficiency in ion transport, and this in turn has been associated with the cellular membranes. It has also been suggested (*1*) that the defect may be generalized to the extent that membranes of the erythrocytes share the fault. In the white Pekin duck (*2*) there occurs, as a function of myopathy, reversal of the characteristic inhibitory response of membrane adenosine triphosphatase to the cardiac glycoside, ouabain. The transport enzyme ($Na^+ + K^+$ adenosine triphosphatase) from normal muscle sarcoplasmic reticulum and from hemolyzed erythrocytes was inhibited by $10^{-4}M$ ouabain; identical preparations from myopathic animals were stimulated by the glycoside. We related this to changes in membrane integrity—changes correlated with demonstrable myopathy.

In our study, we compare the responses of adenosine triphosphatase of erythrocyte ghosts (obtained from myopathic and normal human donors) to cardiac glycoside.

Blood (5 to 10 ml) was collected from each donor in a Vacutainer tube containing 6 mg of ethylenediaminetetraacetate (EDTA) as an anticoagulant. Within 4 hours, blood cells were hemolyzed in 2 m*M* tris buffer, *p*H 7.4, with 0.02 m*M* EDTA. After centrifugation at 10,000*g* for 30 minutes, the hemolyzed cells were washed three to five times with the same buffer containing 15.5 m*M* NaCl. Between each centrifugation, the ghosts were homogenized with a Teflon pestle in a Potter-Elvehjem tissue mill at moderate speed. The white ghosts obtained were either used immediately after suspending them again in an equal volume of 0.1*M* tris buffer with 0.25*M* sucrose, *p*H 7.2, or were stored at −10°C. A temperature of 2° to 4°C was maintained throughout the procedure.

Adenosine triphosphatase activity was assayed from a reaction mixture containing 0.1 ml of erythrocyte-ghost suspension, 0.8 ml of substrate (0.7 mg of sodium adenosine triphosphate per milliliter of reaction mixture with 1m*M* NaCl, 2m*M* KCl, and 1m*M* $MgCl_2$ in 0.1*M* tris-HCl buffer with 0.25*M* sucrose), and 0.1 ml of water or inhibitor. Because of the retention of sodium during the preparation, the concentration in the incubation mixture is higher than indicated. Nonetheless, the concentrations here have been chosen as optimum for inhibition by ouabain, though total adenosine triphosphatase activity is somewhat less than maximum. After incubation for 20 minutes at 42°C, protein was precipitated by adding 0.1 ml of 50 percent trichloroacetic acid. Inorganic phosphate in the supernatant was measured by a modified Fiske procedure (*3*). Protein in ghost suspension was determined by the phenol method (*4*).

The clinical diagnoses of the myopathic donors are indicated in Table 1. Donor 8 was originally thought to be normal. Our experimental results indicated variation from the normal. We then consulted his attending physician who advised us of a family history of cerebral atrophy; he exhibits symptoms of this as well as of limb abnormality. Normal donors were staff members or psychiatric patients. Identical results to those obtained with the blood of normal donors were consistently obtained with the use of blood from the blood bank.

The characteristic response of membrane systems to ouabain (and similar cardiac drugs) is lowering of the rate of catalytic hydrolysis of adenosine triphosphate (*5*). In our study, the adenosine triphosphatase activity of red blood cell ghosts from normal human donors was consistently inhibited by $10^{-4}M$ ouabain. Conversely, ghosts prepared identically but obtained from myopathic donors predominantly showed a stimulation of activity in the presence of ouabain. Thus, patients described as exhibiting spontaneous muscular dystrophy and myotonia provided consistent examples of membrane adenosine triphosphatase activity which could be stimulated by ouabain. Of the patients diagnosed as Duchenne (*6*), one (donor 14) appeared normal by our criteria, while two of the children (donors 19 and 20) were abnormal, though to a lesser degree, in that ouabain neither stimulated nor inhibited their red blood cell adenosine triphosphatase. Two limb-girdle muscular dystrophy patients were normal in the adenosine triphosphatase response to ouabain.

A mode of action for the variation of response to ouabain has been suggested (*7*), though the mechanism remains unknown. Additional study of the possible distinction between categories of muscular dystrophy is required.

These findings are in accord with the common supposition that defects of certain muscle diseases involve aberrant phenomena of ion transport. We interpret the results to indicate also that the abnormality of which the myopathy is a result includes red blood cells. Thus, what we have observed is an alteration of the activity pattern of the transport enzyme, possibly in consequence of a relationship to membrane structure. We suggest that the analysis may have diagnostic value and is relatable to genetic interpretation and functional explanation basic to myopathic defect.

Table 1. Effect of ouabain ($10^{-4}M$) on adenosine triphosphatase activity in erythrocyte ghosts obtained from normal and myopathic donors. Donors 1, 4, 10, 11, 23, and 24 were females. Adenosine triphosphatase activity of individual preparations, without ouabain, in micromoles of inorganic phosphate per milligram of protein per minute (average protein 0.9 mg/ml) for donors 1 through 24, respectively, was: 0.20, 0.34, 0.32, 0.27, 0.28, 0.84, 0.68, 0.62, 0.10, 0.34, 0.43, 0.19, 0.22, 0.41, 0.13, 0.20, 0.36, 0.42, 0.18, 0.31, 0.41, 0.58, 0.68, and 0.22. Group I (donors 1 through 7), mean percentage of change with $10^{-4}M$ ouabain, −49.285 (S.D., 20.870; S.E., 7.888); group II (donors 9 through 24), mean percentage of change, 24.625 (S.D., 43.346; S.E., 10.836). Values indicate significance at the 1 percent level ($P < .01$). For information on donor 8 (an adult showing no stimulation), see text.

Donors		Stimulation or inhibition (%)
No.	Age	
	Normal	
1	45	−82
2	26	−55
3	34	−71
4	40	−33
5	27	−44
6	Adult	−29
7	50	−31
	Spontaneous muscular dystrophy	
9	40	+50
10	18	+15
11	36	+68
	Myotonia	
12	Adult	+47
13	34	+60
	Pseudohypertrophic muscular dystrophy (Duchenne type)	
14	17	−77
15	8	+77
16	9	+35
17	Child	+25
18	2	+50
19	8	0
20	11	0
21	Child	+60
	Limb-girdle muscular dystrophy	
22	44	−44
23	45	−22
24	40	+50

HARRY DARROW BROWN
SWARAJ K. CHATTOPADHYAY
ANIL B. PATEL
Department of Biochemistry, University of Texas Medical Branch, Galveston 77550

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