gle batches of ingredients low in chromium.

The characteristics of the postulated chromium pool are unknown at the present time. The effect of glucose loading on plasma chromium may be mediated through an increase in circulating insulin, since chromium elevation can be evoked in rats by injections of insulin as well as of glucose (*11*). However, a simple calculation excludes the hormone as a possible carrier for the appearing chromium; one molecule would have to bind close to 1000 atoms of chromium.

Possibly a physiological carrier substance for chromium appears in blood as a response to increased insulin, with a high enough affinity for the tissue-bound element to carry it into the circulation. One such compound may be reduced glutathione which has been reported to rise in blood after administration of insulin, and which also reacts with trivalent chromium (*12*). Until such carrier substances are defined and more is known about chromium metabolism, the absence of a chromium response to glucose, while perhaps suggestive of low chromium stores, cannot be considered as evidence of a deficiency state.

The significance of the described changes in plasma chromium with glucose loading for the regulation of glucose tolerance is unknown at this time. Transport of a potential co-factor for insulin to peripheral receptor sites would seem to be a reasonable hypothesis in view of previous studies in the rat. If relative chromium deficiencies exist in man, the relationship of plasma chromium to circulating insulin and the effect of chromium supplementation on the relative effectiveness of insulin may be worth study.

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Structure-Disrupting Ions: Detection of Qualitative Change in an Enzyme

Abstract. *Preparations of erythrocyte glucose-6-phosphate dehydrogenase from normal individuals show similar degrees of inhibition by neutral salts. Preparations from Caucasian subjects with different hereditary deficiency syndromes differ from each other and from normal subjects, indicating a qualitative difference in these specific "deficient" enzymes. Sensitivity to neutral salts may be a means of detecting an amino acid substitution.*

Neutral salts at high concentrations disrupt the structure of diverse macromolecules in an order of increasing effectiveness; for anions the order is, $CH_3COO^- < Cl^- < NO_3^- < Br^- < I^- < ClO_4^- < SCN^-$ and for cations, the order is $(CH_3)_4N^+ < NH_4^+ < K^+, Na^+ < Li^+$ (*1*). Because the orders in which these ions inhibit the activity of widely different enzymes are similar to the structure-disruption orders, it was concluded that inhibition results from disruption of organized enzyme structure (*2*). Given such a conclusion, it follows that amino acid modifications which result in the structural change of a given enzyme may be reflected by a change in salt sensitivity. This was the case when native myosin and myosin modified by *p*-chloromercuribenzoate were compared (*2*).

Mutational change in the amino acid sequence of an enzyme is in essence a modification, similar to that described above, except that it occurs by amino acid substitution during synthesis. We considered that a mutational change, if it causes the enzyme to exist in an altered structural form, should change its sensitivity to the ordered sequence of ions.

Total activity of a given enzyme in a given tissue is classically evaluated by assay of a tissue fraction under ideal conditions. Deviations from values normally observed can arise from both qualitative (altered amino acid sequence) and quantitative (altered number of otherwise normal enzyme molecules) variations in the enzyme. We now report that the salt sensitivity of erythrocyte glucose-6-phosphate dehydrogenase from healthy medical students clearly differs from the enzymes of individuals who have hereditary deficiencies in total activity.

To avoid complications from variation in the relative contribution of 6-phosphogluconate dehydrogenase under the differing conditions of assay, glucose-6-phosphate dehydrogenase was purified by a modification of the method of Kirkman (*3*) with all steps conducted at 0° to 4°C. After elution of the enzyme from diethylaminoethyl cellulose, a 70 percent ammonium sulfate precipitation was carried out, and this pellet was resuspended. The solution was dialyzed for 4 hours against 100 volumes of 0.1*M* sodium phosphate buffer, *p*H 6.3, containing 0.1 m*M* nicotinamide-adenine dinucleotide phosphate (NADP), 7 mM β-mercaptoethanol and 2.7 m*M* ethylenediaminetetraacetate. The dialyzate was checked with Nessler reagent and found to be free of ammonium ion. The dialyzed enzyme was centrifuged at 85,000*g* for 20 minutes and used for the subsequent assays. Kirkham and Hendrickson (*4*) have shown that glucose-6-phosphate dehydrogenase from human erythrocytes exists in monomer and dimer forms and that dissociation occurs when the molecule is stripped of NADP. Therefore, we prepared the enzyme in the presence of this cofactor and handled all samples in the same manner.

The purified enzymes were assayed spectrophotometrically in a 3.0 ml reaction mixture containing 0.6 μmole of

Table 1. Salt concentrations (molar) required to effect 50 percent inhibition of human erythrocyte glucose-6-phosphate dehydrogenase. N-1 and N-2 are normal individuals; C.L. and F.C. are patients with hereditary deficiencies.

Enzyme source	*p*H 7.0		*p*H 8.5		*p*H 9.5	
	$NaClO_4$	NaCl	$NaClO_4$	NaCl	$NaClO_4$	NaCl
N-1	0.069	0.68	0.074	0.72	0.10	0.46
N-2	.071	.71	.076	.74	.12	.47
C.L.	.046*	.27*			.080*	.31*
F.C.	.020*	.79	.016*	.51*	.020*	.50

* 20 percent difference from mean of normals.

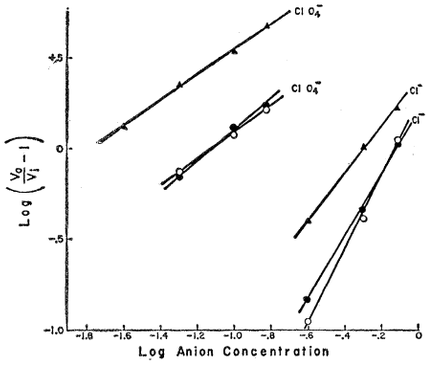

Fig. 1. Inhibition of glucose-6-phosphate dehydrogenase by sodium salts at *p*H 8.5. V_0 is the velocity in absence of added salts, and V_1 is the velocity in presence of salts; ●—● and ○—○, normals; ▲—▲, F.C. Conditions of assay are described in text.

NADP, 1.8 μmole of glucose-6-phosphate, 30 μmole of $MgCl_2$ and 300 μmole of buffer, with and without salts, at 25°C. Buffers were potassium phosphate at *p*H 7.0, tris-hydrochloride at *p*H 8.5, and glycylglycine at *p*H 9.5. Reactions were started by addition of enzyme, and initial linear velocities were determined by following the generation of NADPH at 340 mμ. Although total activity of glucose-6-phosphate dehydrogenase per gram of hemoglobin in the normal subjects markedly exceeded that in the individuals with hereditary deficiencies, equal amounts of activity were used in all assays. Assays were conducted in duplicate. On several occasions, reactions were repeated with a twofold quantity of substrate and cofactor, the results revealing that original assays were conducted at or near saturation. After purification, the activity of 6-phosphogluconate dehydrogenase in the preparation (assayed under similar conditions with 1.6 μmole of 6-phosphogluconate and 0.6 μmole of NADP) did not exceed 1 percent that of glucose-6-phosphate dehydrogenase.

Both NaCl and $NaClO_4$ were chosen for inhibition studies because these anions are near opposite ends of the ordered anion series (*1*). Usually, concentrations of NaCl in reaction mixtures were 0.25, 0.50, and 0.75*M* while those of $NaClO_4$ were 0.05, 0.10, and 0.15*M*.

Results, plotted by the method of Johnson, Eyring, and Williams (*5*), are shown in Fig. 1. A 19-year-old white male (F.C.) of Italian extraction responded to the ingestion of fava beans with hemolysis. Salt concentrations required for 50 percent inhibition (the point where log $[(V_0/V_1) - 1]$ is equal to zero) indicate that enzyme from F.C. is inhibited to a considerably greater degree by both salts than is enzyme from the normal individuals.

Similar experiments were carried out at *p*H 7.0 and 9.5. Salt concentrations required for 50 percent inhibition in the two normals and F.C. as well as C.L., a 12-year-old white boy with congenital nonspherocytic hemolytic anemia, are shown in Table 1. At all points, values for the two normal individuals are quite similar with variations of less than 5 percent except with $NaClO_4$ at *p*H 9.5. The enzyme from F.C. shows a sensitivity to $NaClO_4$ that is 3 to 5 times that of normal subjects at all *p*H's and a sensitivity to NaCl that is 1.4 times that of normals at *p*H 8.5. When compared with the normals, the enzyme from C.L. is about 1.5 times as sensitive to $NaClO_4$ at *p*H 7.0 and slightly more sensitive at *p*H 9.5. It is 2.6 times as sensitive to NaCl at *p*H 7.0 and 1.5 times as sensitive at *p*H 9.5.

To rule out the possibility that changes in salt sensitivity may occur during purification, the inhibitory effects of 1.0*M* sodium acetate; 0.5*M* NaCl, $NaNO_3$, and NaBr; 0.2*M* NaSCN and 0.1*M* $NaClO_4$ on the activity of the enzyme from F.C. were tested at *p*H 8.5 several times during and after purification. Degree of inhibition by a given salt (i) after resuspension, (ii) after dialysis, (iii) after a 16-hour period, and (iv) in the presence of 3.0 mg of serum albumin was found to be essentially identical (within 3 percent). Further, it was noted that the anions can be arranged in the usual order of increasing inhibitory effectiveness: $CH_3COO^- < Cl^- < NO_3^- < Br^- < ClO_4^- \cong SCN^-$ (*2*). Addition of 6-phosphogluconate dehydrogenase (previously removed during purification) to an activity approximating 5 percent of that of the glucose-6-phosphate dehydrogenase caused no change in observed velocity or degree of salt inhibition.

These observations suggest that glucose-6-phosphate dehydrogenase from the two individuals with hereditary deficiencies of this enzyme differ from normals and from each other as a result of an amino acid substitution. While the amino acid sequences of this enzyme from the erythrocytes of normal and subjects with hereditary deficiency have not been determined, there is a good deal of other information (*p*H optima, electrophoretic mobility, kinetic analysis, heat stability, and so forth) suggesting that, for the types of hereditary deficiency herein studied, this is the case (*6*).

Departure of the total activity of an enzyme from normal values in a given tissue as a result of heredity or neoplasia can be due to either a quantitative or a qualitative change in the enzyme. These findings provide evidence that differentiation between these possibilities may, in some instances, be made on the basis of activity-inhibiting effectiveness of neutral salts.

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7. It must be emphasized that mutational alteration of amino acid sequence or modification of an already existing residue, particularly if not drastic, might occur without significant or clearly detectable change in salt sensitivity.
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