phosphatase activity of the actomyosin made with cardiac myosin was less than that made with skeletal myosin at all Ca^{++} levels, the sensitivities to changes in Ca^{++}-concentration were similar (Fig. 1). Activation of both cardiac and skeletal myosin adenosine triphosphatase activities by actin was almost complete at a Ca^{++} concentration of $10^{-5}M$, while the interaction between actin and myosin was abolished when the concentration of free Ca^{++} fell below $10^{-7}M$. The pCa^{++} at the midpoints of these sigmoid curves, corresponding to one-half of the maximal activation of myosin adenosine triphosphatase activity by the white skeletal actin, was 6.25 ± 0.15 in the case of reconstituted actomyosin made with white skeletal myosin and 6.20 ± 0.14 when cardiac myosin was used. Likewise, no significant differences were observed when the reconstituted actomyosins made with tropomyosin-containing actins from cardiac and white skeletal actins were compared.

The present findings do not support the view that differences in the Ca^{++}-sensitivities of the contractile proteins of cardiac and white skeletal muscle account for the lower velocity of contraction and relaxation in the myocardium. The possibility remains that the slower contraction of the heart is due to the weaker adenosine triphosphatase activity of cardiac myosin (*7*) or to the smaller amount of sarcoplasmic reticulum present in the myocardium (*10*). Furthermore, the marked ability of the heart to alter its contractility, the tension developed at a given fiber length, may reflect failure of the sparse sarcoplasmic reticulum to provide optimal amounts of Ca^{++} for the actomyosin filaments under normal conditions. If this is the case, increasing the availability of Ca^{++} to these systems could enhance contractility (*11*).

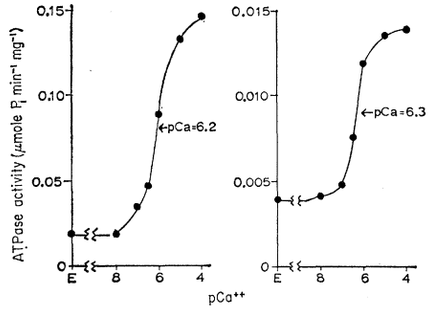

Fig. 1. Comparison of the Ca^{++}-dependence of the initial rates of adenosine triphosphate (ATP) hydrolysis by reconstituted actomyosins made with the same skeletal actin and either white skeletal (left) or cardiac (right) myosin. Reactions were carried out at 25°C with 0.5 mg/ml myosin and 0.167 mg/ml actin in 0.08*M* KCl, m*M* MgATP and 20 m*M* histidine at *p*H 6.80. Free Ca^{++} was calculated from the ratio EGTA/CaEGTA; EGTA + CaEGTA = 1.0 m*M*. The abcissa, pCa^{++}, = −log $[Ca^{++}]$. The activities labeled *E* were determined in 1 m*M* of EGTA and approximate the adenosine triphosphatase activities of the myosins alone.

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References and Notes

1. W. Hasselbach, *Federation Proc.* **23**, 909 (1964); R. Podolsky and L. L. Costantin, *ibid.*, p. 933.
2. S. Ebashi and F. Lipmann, *J. Cell Biol.* **14**, 389 (1962); W. Hasselbach and M. Makinose, *Biochem. Z.* **333**, 518 (1961).
3. A. Weber and S. Winicur, *J. Biol. Chem.* **236**, 3198 (1961); A. Weber and R. Herz, *ibid.* **238**, 599 (1963); ——— and I. Reiss, *J. Gen. Physiol.* **46**, 679 (1963); ———, *Proc. Roy. Soc. London Ser. B.* **160**, 489 (1964).
4. S. Ebashi and F. Ebashi, *J. Biochem.* **55**, 604 (1964).
5. A. Szent-Györgyi and B. Kaminer, *Proc. Natl. Acad. Sci. U.S.* **50**, 1033 (1963); N. Azuma and S. Watanabe, *J. Biol. Chem.* **230**, 3847 (1965).
6. A. M. Katz, *J. Biol. Chem.* **241**, 1522 (1966).
7. ———, D. I. Repke, B. Rubin, in preparation.
8. S. Chaberek, and A. E. Martell, *Organic Sequestering Agents* (Wiley, New York, 1959).
9. H. H. Taussky and E. Shorr, *J. Biol. Chem.* **202**, 675 (1953).
10. K. R. Porter and G. E. Palade, *J. Biophys. Biochem. Cytol.* **3**, 269 (1957); D. W. Fawcett, *Circulation* **24**, 336 (1961); F. O. Simpson and S. J. Oertelis, *J. Cell Biol.* **12**, 91 (1962); P. A. Nelson and E. S. Benson, *J. Cell Biol.* **16**, 297 (1963).
11. G. A. Langer, *Circulation Res.* **17**, 78 (1965).

12. Supported by research grants HE-08515 from the PHS and 65-G-61 from the American Heart Association. One of us (A.M.K.) is an Established Investigator of the American Heart Association.

26 January 1966

Plasma Chromium after Glucose Administration

Abstract. *Sharp increases in the concentration of chromium in plasma were found in five subjects with normal glucose utilization after administration of glucose by mouth. This rise was not observed in two diabetics when glucose tolerance was impaired; however, it appeared when glucose tolerance was improved and when trace amounts of trivalent chromium were given as a dietary supplement. The source of chromium which became elevated was most likely an internal pool. Possibly there is a relation between chromium and insulin function.*

There is increasing evidence that trivalent chromium may be a micronutrient required for optimum utilization of glucose. Restriction of chromium intake in rats results in progressive impairment of tolerance to intravenous glucose (*1*) and decreasing effectiveness of insulin on glucose utilization and galactose entry in the epididymal fat pad (*2*). The impairment can be rapidly reversed by administration in vivo or in vitro of trace amounts of the element. Chromium by itself is not a hypoglycemic agent and is active only when insulin is present. A hypothesis to explain its action postulates its participation in a ternary complex between insulin and sulfhydryl receptor groups on insulin-responsive membranes (*3*). A more complete chromium deficiency in rats results in a more severe impairment of glucose tolerance; the impairment is associated with a significant incidence of a diabetes-like state characterized by hyperglycemia and glycosuria during fasting (*4*). The deficiency is accompanied by impairment of growth, decreased longevity and an increase in the quantity of aortic lipids (*5*).

A survey of chromium concentration in tissues of a sample of the United States population (*6*) showed a decline from birth to old age which suggested the existence of a partial deficiency. This suggestion was further supported by the results of a clinical study in which oral supplements of microgram amounts of chromium for 15 to 120 days improved glucose tolerance in four of six diabetics (*7*). During some of these studies, concentrations of chromium in plasma were determined to ascertain the effect of supplementation. The amounts of chromium in the plasma during or after fasting were initially within our normal range of 17 to 45 nanograms per milliliter, as determined in 20 putatively normal subjects. Except for one patient, chromium concentration in the plasma rose during the course of chromium supplementation, the amount depending on time and dosage. When Cr concentrations were determined during tests for glucose tolerance, we made a series of

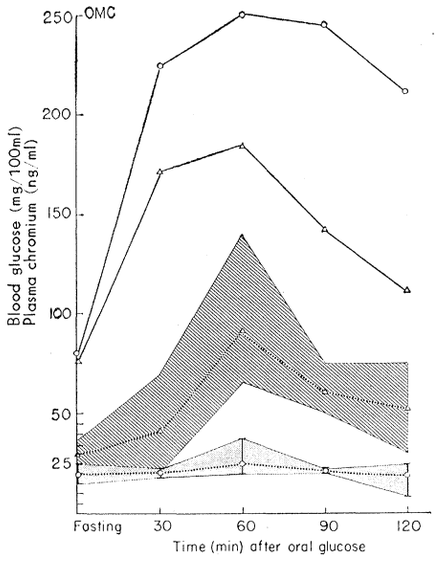

Fig. 1. Blood glucose (solid lines) and plasma chromium (dotted lines) during glucose tolerance tests (mean and range). Circles, means of four tests during impairment; triangles, means of three tests during relative improvement.

unexpected observations. We found sharp rises in plasma chromium concentrations after oral administration of glucose both in these subjects and in a group of healthy volunteers whose glucose tolerance was normal (*7*).

Chromium concentrations were measured by atomic absorption spectroscopy after ashing of 5 ml of plasma with nitric, perchloric, and sulfuric acids, oxidation with permanganate, and extraction of the oxidized, hexavalent chromium into methyl isobutyl ketone (*8*). Under these conditions, the limit of detection of chromium was 6 ng/ml; a linear concentration-absorption curve was obtained from 0 to 150 ng/ml, and the error of the method was 25 to 30 percent at 10 ng/ml and 8 to 10 percent at 150 ng/ml. Recovery of known amounts of chromium added to plasma samples exceeded 90 percent with a single extraction. Plastic syringes, test tubes, pipettes, aluminum hub needles, and sodium heparin used to handle venous blood samples yielded no detectable chromium by this assay. Blood glucose was determined in duplicate by a glucose oxidase method (*9*).

Five healthy male subjects between the ages of 19 and 30 years received 100 g of glucose by mouth, after an overnight fast. Samples of venous blood were drawn immediately before and at 30-minute intervals after administration of glucose. A rise in plasma chromium was found in all five (Table 1), but an initial decline preceded the rise in two subjects (J.B. and M.C.). While it is impossible to establish a response pattern from this small number of cases it is of interest that the maximum elevation of chromium appeared earlier when glucose disappearance was relatively rapid, and its magnitude appeared to be inversely related to the maximum rise in blood glucose. In subject D.E., blood glucose rose only slightly, and a relatively large rise in blood chromium occurred at 30 minutes; subject J.B. showed the largest rise in blood glucose, and plasma chromium declined before it rose to a maximum at 120 minutes. The elevations after the glucose administration did not correlate with the concentrations during fasting.

In contrast to this behavior of plasma chromium in healthy young subjects, the plasma of a 38-year-old diabetic (L.M.) with impaired glucose tolerance exhibited a flat curve after a glucose load. Chromium levels at fasting and at 30 minute intervals after administration of glucose were 20, 15, 19, 20, and 21 ng/ml. A second patient with maturity-onset diabetes (O.M.), on whom seven tests with simultaneous chromium determinations were performed, also failed to show a significant rise of plasma chromium in the four tests associated with impaired glucose tolerance (Fig. 1). In both patients, however, plasma chromium rose in response to glucose when glucose tolerance was relatively improved. Results for O.M. are shown in Fig. 1; those for L.M. were 15, 13, and 80 ng/ml, at fasting and at 1 and 2 hours after the glucose load. In a third diabetic (C.M.) only one test was performed during a period of chromium supplementation when his glucose tolerance was nearly normal, and a pronounced rise of plasma chromium from 38 ng/ml at fasting to 50, 137, 75, and 75 ng/ml was detected at 30-minute intervals after the load. A fourth patient (R.C.), whose glucose tolerance remained impaired with chromium supplementation, showed a similar rise in plasma chromium from a fasting concentration of 45 to 80 and 35 ng/ml at 1 and 2 hours after glucose.

The results can be summarized as follows. (i) Normal glucose tolerance tests in five healthy subjects, as well as improved tests in three diabetics, were associated with a rise in plasma chromium, after administration of glucose. (ii) Chromium supplementation was associated with a rise in plasma chromium after glucose administration, in four diabetics regardless of whether or not it improved glucose tolerance. (iii) Five tests on two diabetics without chromium supplementation, who exhibited impaired glucose tolerance, failed to show the chromium elevation after glucose.

The source of the elevation of plasma chromium concentration during and after ingestion of the glucose was most likely from an internal pool. Chromium in the glucose solution (15 ng/ml) could have contributed a maximum amount of 3 μg, in the unlikely case that it was completely absorbed (*10*). This is far less than the minimum estimate of 168 μg needed to raise the mean plasma concentration from 30 to 90 ng/ml (Fig. 1) in an estimated 2.8-liter volume. A significant contribution from the test meals taken by diabetics is equally unlikely since they were consistently prepared in sin-

Table 1. Concentration of blood glucose (mg/100 ml) and plasma chromium (ng/ml), at intervals after oral administration of D-glucose (100 g, 42 percent, wt/vol.).*

Subject	Concentration at intervals after administration									
	0 min		30 min		60 min		90 min		120 min	
	Glu (mg)	Cr (ng)	Glu (mg)	Cr (ng)	Glu (mg)	Cr (ng)	Glu (mg)	Cr (ng)	Glu (mg)	Cr (ng)
D.E.	69	27	98	83	65	65	74	56	59	25
M.C.	80	34	112	22	80	51	83	78	65	34
R.P.	75	23	135	25	99	49	72	61	64	45
H.C.	84	28	159	33	146	58	112	63	132	51
J.B.	80	28	162	12	123	30	108	39	86	49
Mean	78	28	133	35	103	51†	90	59‡	81	41
Same subjects on 240 ml of distilled water										
Mean	75	25	74	27	75	29	71	28	72	25

* Dexol Solution, Scientific Products, Evanston, Illinois. † Average of individual rises significant ($P < 0.05$). ‡ $P < 0.01$.

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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References and Notes

1. K. Schwarz and W. Mertz, *Arch. Biochem. Biophys.* **85**, 292 (1959); W. Mertz, E. E. Roginski, H. A. Schroeder, *J. Nutr.* **86**, 107 (1965).
2. W. Mertz, E. E. Roginski, K. Schwarz, *J. Biol. Chem.* **236**, 318 (1961); W. Mertz and E. E. Roginski, *ibid.* **238**, 868 (1963).
3. G. D. Christian, E. C. Knoblock, W. C. Purdy, W. Mertz, *Biochim. Biophys. Acta* **66**, 420 (1963).
4. H. A. Schroeder, *Life Sci.* **4**, 2057 (1965).
5. ———, W. H. Vinton, Jr., J. J. Balassa, *J. Nutr.* **80**, 48 (1963); H. A. Schroeder and J. J. Balassa, *Amer. J. Physiol.* **209**, 433 (1965).
6. H. A. Schroeder, J. J. Balassa, I. H. Tipton, *J. Chron. Dis.* **15**, 941 (1962).
7. W. H. Glinsmann and W. Mertz, in press.
8. F. J. Feldman and W. C. Purdy, *Anal. Chim. Acta* **33**, 273 (1965).
9. M. E. Washko and E. W. Rice, *Clin. Chem.* **7**, 542 (1961).
10. W. Mertz, E. E. Roginski, R. C. Reba, *Amer. J. Physiol.* **209**, 489 (1965).
11. W. H. Glinsmann, R. C. Reba, W. Mertz, manuscript in preparation.
12. F. Binkley, G. M. Christensen, Feng Chi Wu, *J. Biol. Chem.* **192**, 29 (1951); C. L. Rollinson, in U.S. Atomic Energy Commission Symposium on Radioactive Pharmaceuticals, Oak Ridge Institute of Nuclear Studies, 1–4 Nov. 1965, in press.
13. We thank Richard J. Doisy and Robert A. Levine for suggestions and criticism.
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23 February 1966

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