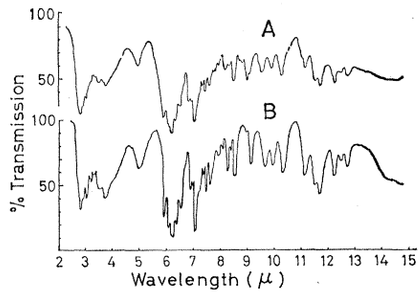

Fig. 1. Infrared spectra in KBr of (*A*) crystallized substance from frog heart extract and (*B*) a sample of synthetic *N*-acetyl-L-histidine monohydrate.

was carried out on wide sheets of Toyoroshi No. 50 paper with a mixture of pyridine, *n*-butanol, and water (1:1:1). The water eluate of corresponding sections of paper was concentrated and dried over calcium chloride in a desiccator, about 30 mg of crude crystals of the unknown component being obtained. This substance was ninhydrin-negative and its R_F value coincided exactly with that of synthetic acetylhistidine in all the aforementioned solvent systems.

Dinitrophenyl (DNP) derivatives of this substance and synthetic acetylhistidine gave colorless, diazo- and ninhydrin-negative spots of the same R_F value; the spots were detectable by ultraviolet absorption. The properties of the derivative were similar to those of imidazole-DNP-*N*-acetylhistidine. After hydrolysis in HCl these DNP- derivatives shifted to a lower R_F corresponding to that of imidazole-mono-DNP-histidine, which revealed a colorless, diazo-positive (yellow), ninhydrin-positive, and ultraviolet-absorbing spot.

The purified substance was identical with synthetic *N*-acetyl-L-histidine monohydrate (*4*) by infrared spectroscopy (Fig. 1), elementary analysis, and measurements of melting point and specific rotation.

The isolation of a simple acetylated amino acid from animal tissues has been reported heretofore only in two instances, acetylaspartic acid (*5*) from brain and acetylglutamic acid (*6*) from liver. They are both characterized by acetyl derivatives of acidic amino acid. The presence of acetylhistidine in frog heart is unique and is the first isolation of an acetylated basic amino acid.

Newt hearts also contain acetylhistidine. Thus acetylhistidine may be a common component of amphibian hearts.

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28 February 1966

Control of Myocardial Contraction: The Sensitivity of Cardiac Actomyosin to Calcium Ion

Abstract. *The control by calcium ion of the adenosine triphosphatase activity of cardiac actomyosin is similar to that of white skeletal actomyosin. This finding indicates that the slower contraction and relaxation of heart muscle do not reflect different levels to which free calcium ion concentration around the myofibrils must be adjusted during contraction and relaxation and suggests a mechanism whereby myocardial contractility may be regulated.*

It is possible that the slower contraction cycle in cardiac muscle, when compared with that of white skeletal muscle, is due to different concentrations of free Ca^{++} needed for activation of the myofilaments. Release of Ca^{++} by the sarcoplasmic reticulum of both cardiac and skeletal muscle now appears to constitute an essential step during excitation-contraction coupling, in which an action potential at the muscle cell surface initiates shortening and tension development by the myofilaments. Evidence that the sarcoplasmic reticulum is able to take up and store Ca^{++} has been obtained in studies of the intact muscle (*1*) and subcellular fractions isolated from muscle homogenates (*2*). Furthermore, both the adenosine triphosphatase activity and syneresis of isolated skeletal myofibrils and actomyosin are markedly inhibited by reduction of the Ca^{++} concentration to levels that can be achieved by the sarcoplasmic reticulum in the intact muscle (see *3*).

More recently it has been recognized that a tropomyosin-containing protein complex is necessary to mediate the inhibitory action of Ca^{++}-binding agents such as 1,2-bis-(-2-dicarboxymethylaminoethoxy) ethane (EGTA) (*4–6*) because reconstituted actomyosin made from highly purified actin and myosin is not responsive to these physiological changes in Ca^{++} concentration (*4*). In the present study, Ca^{++}-sensitive reconstituted actomyosins made with actin containing this tropomyosin complex and either skeletal or cardiac myosin have been compared.

Dog cardiac and rabbit white skeletal myosins were prepared by a method that minimizes contamination with actomyosin (*7*). Tropomyosin-containing "Straub" actins were prepared by extracting the acetone-dried muscle powders at room temperature (*6*) and reconstituted actomyosins were made by adding three parts, by weight, of myosin to one part of tropomyosin-containing F-actin (*6*). We prepared Ca^{++}-buffers by combining EGTA and CaEGTA (*3*), using the dissociation constants for the CaEGTA complex provided by Chaberek and Martell (*8*). Adenosine triphosphatase activities were calculated from the initial rates of liberation of inorganic phosphate, the latter determined by the method of Taussky and Shore (*9*). Traces of calcium were removed from adenosine triphosphate by treatment with Dowex 50.

Comparison of the Mg^{++}-activated adenosine triphosphatase activities of the reconstituted actomyosins prepared from the same tropomyosin-containing white skeletal actin and either dog cardiac myosin or rabbit white skeletal myosin revealed differences similar to those observed when these myosins were combined with tropomyosin-free actin (*7*).

At the KCl concentration of 0.08*M* used in these experiments, the actomyosin made with white skeletal myosin was eight to ten times as active as that made with cardiac myosin when the Ca^{++} concentration was greater than $10^{-4}M$. At this level of Ca^{++}, maximal activation of myosin adenosine triphosphatase by actin was seen. Although the adenosine tri-

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*).

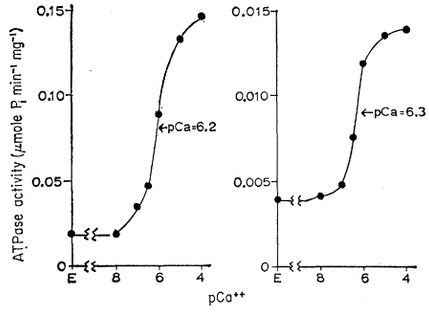

Fig. 1. Comparison of the Ca^{++}-dependence of the initial rates of adenosine triphosphate (ATP) hydrolysis by reconstituted actomyosins made with the same skeletal actin and either white skeletal (left) or cardiac (right) myosin. Reactions were carried out at 25°C with 0.5 mg/ml myosin and 0.167 mg/ml 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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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