AN AUTOMATED ANALYSIS OF CREATINE PHOSPHOKINASE NOT REQUIRING DIALYSIS

by

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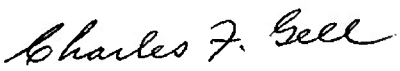

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SUMMARY PAGE

THE PROBLEM

To devise an automated procedure for measuring the activity of creatine phosphokinase which would avoid many of the complications and inaccuracies of previous analyses while retaining maximum economy and flexibility. This procedure would simplify the study of enzymatic changes in Naval personnel in stressful environments.

FINDINGS

A procedure was devised which makes it possible to analyze serum or tissue extracts for creatine phosphokinase activity by the colorimetric procedure described, without dialysis or extraction of protein, thus reducing both the number of manipulations and major wastes of reagents and sample.

APPLICATIONS

This improved procedure for the measurement of creatine phosphokinase adds significantly to the potentialities for use of this enzyme system for detection of stress or tissue damage under research conditions and to its ready availability for determination of myocardial infarction, debilitating myopathies, brain tumors or other pertinent clinical conditions. A coordinated system for simultaneous measurement of creatine phosphokinase and lactic dehydrogenase was also made available by the work described. This procedure is useful to research and clinical Naval personnel in submarine medicine.

ADMINISTRATIVE INFORMATION

This investigation was conducted as a part of Bureau of Medicine and Surgery Research Work Unit MR005.01.01-0053B -- Biochemical Mechanisms of Response to Environmental Stresses - Hyperbaric and Submarine. The present report is No. 7 on this Work Unit. The manuscript was approved for publication on 22 July 1970. It has been designated as Submarine Medical Research Laboratory Report Number 637.

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ABSTRACT

A method, designed for simplicity, has been described for the automated analysis of creatine phosphokinase. The procedure is not only applicable for measurement of serum enzyme activity, for which the analysis of this enzyme serves its most important clinical function, but also for the activity of a wide variety of other tissues. Since no protein precipitation or dialysis step is required by the method, minimal equipment is needed and significant economies are effected in sample size and reagent utilization.

A combined analytical procedure for creatine phosphokinase and lactic dehydrogenase activities is described and the clinical importance of these techniques when employed together is briefly discussed.

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INTRODUCTION

Analytical procedures for creatine phosphokinase (CPK) E.C. 2.7.3.2, have been devised which depend upon measurement of easily labile phosphate from creatine phosphate formed by reaction 1 proceeding in the forward direction[1], measurement

$$\text{Creatine} + \text{ATP} \underset{\text{Mg}}{\overset{\text{CPK}}{\rightleftharpoons}} \text{Creatine phosphate} + \text{ADP} \qquad (1)$$

of creatine produced by the reverse process[2] or measurement of ADP* formed during the reaction[3]. In the latter case, pyruvate kinase is employed to convert phosphoenolpyruvate (+ADP) to pyruvate (+ATP) which in turn oxidizes NADH to NAD^+ in the presence of lactic dehydrogenase, with the NADH-NAD^+ conversion being measured spectrophotometrically.

While the reaction coupled to NADH-NAD^+ provides for the possibility of analyses requiring continuous recording, methods depending upon measurement of creatine have generally been adopted for automated procedures.[4-7] The reaction mixtures are much simpler than those required by the enzyme-coupled system and no selective auxiliary reactions such as the hydrolysis of creatine phosphate in the presence of ADP and ATP are required. Since the colorimetric analysis for creatine is both widely used[2, 4-6] and can be employed with simple colorimeters as contrasted to spectrophotometers[3] or fluorometers[7], this method has been chosen for the studies described here. The additional advantage of the system chosen is that a dialysis operation or protein precipitation step is not required for satisfactory analytical results.

MATERIAL AND METHODS

A minimal Autoanalyzer** system consisting of sampler, proportioning pump, 37° water bath, colorimeter and recorder has been utilized. Although such a complete system provides considerable convenience, various substitutes in the apparatus may be made with quite satisfactory results. Any controlled temperature water bath, for example, into which a coil of tubing may be immersed to provide an appropriate delay period for the reaction to proceed may be substituted.

For analysis of sera of experimental animals, the following concentrations of reactants[4-8] have been chosen to yield optimum or nearly optimum reaction rates with an occasional trade-off between reactivity and expense of more costly reagents. Buffer-substrate -- 0.1 M Tris-acetate pH 7.0

Abbreviations employed -- ATP = Adenosine triphosphate; ADP = Adenosine diphosphate; NADH = Nicotine adenine dinucleotide, reduced form; NAD^+ = Nicotine adenine dinucleotide, oxidized form; EDTA = Ethylenediamine-tetraacetate; Tris = Tris (hydroxymethyl)-aminomethane; NEM = N - ethyl maleimide.

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pared to .025 M EDTA, the concentration chosen for employment in the rou-

Even more significant, however, than the savings relating directly to

containing .003 M magnesium acetate, .0022 M cysteine, .0027 M creatine phosphate and .0019 M ADP. Cysteine, creatine phosphate and ADP are weighed

of experimental conditions are provided in the flow diagram shown in Figure 1.

RESULTS

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A combined analytical procedure for creatine phosphokinase and lactic dehydrogenase activities is described and the clinical importance of these techniques when employed together is briefly discussed. This method was devised specifically to apply to the enzymatic evaluations of the effects of submarine and diving environments.

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