1967

Creatine phosphokinase in the newborn

Judith Harris
University of Nebraska Medical Center

CREATINE PHOSPHOKINASE IN THE NEWBORN

Judith Harris

Submitted in Partial Fulfillment for the Degree of

Doctor of Medicine

College of Medicine

February 1, 1967

Omaha, Nebraska

TABLE OF CONTENTS

In the list of enzyme determinations used for diagnosis, creatine phosphokinase (CPK) is one of the more recent determinations available. In 1934, Lohmann(18) first described the enzyme CPK and showed with extracts of skeletal muscle that the two labile phosphate groups of adenosine triphosphate (ATP) were transferred to creatine in the reaction:

ATP + Creatine ⟶ ADP + Phosphocreatine.

In 1935, Lehmann(17) showed the reversibility of the reaction. Both men claimed that the dephosphorlation of ATP proceeded to adenylic acid and that two molecules of phosphocreatine were formed. In 1943, Banga, as quoted by Ennor(6), was able to partially purify the enzyme. She stated that another enzyme was responsible for the transfer of phosphate from ADP to creatine forming the second molecule of phosphocreatine. For many years chemists studied the enzyme, attempting to purify it, ascertain its specific properties, and localize it in the various body organs and tissues. Narayaswami in 1952 described the properties of CPK present in cerebral cortical tissue(19), and Wood, in 1963, (31) continued this investiga-tion using ox brain to further purify the enzyme.

PROPERTIES AND FUNCTION

Creatine phosphokinase catalyzes the reversible reaction

ATP + Creatine ⇌ ADP + Phosphocreatine.

The forward reaction proceeds optimally at a pH of 10.5 or greater(6); the reverse reaction at a pH of 7.2(6). The enzyme

has been found to be stable at a pH range of 5.5 to 11.8 when stored two hours at 0°C (32).

Temperature has a marked effect on enzyme activity over long hours of storage. The enzyme can be stored twelve hours at -10°C with no loss of activity. By ninty-six hours, eighty percent of the activity remains. When stored at 4°C only eighty percent activity remains after twelve hours; after twenty-four hours of storage forty-six percent of the activity remains. When the enzyme is stored for twelve hours at 25°C, sixty-six percent activity remains; after twenty-four hours of storage there is only twenty-seven percent of the activity remaining(24).

Cysteine or sulfhydryl groups are without effect on the forward reaction above, but produce acceleration of the reverse reaction with increasing concentration. Cysteine does not affect the equilibrium of the reaction(6).

Calcium and magnesium ions have been shown to rapidly increase the activity of the enzyme with increasing ion concentration(6,32). There is no enzyme activity in the absence of either ion(6). Manganese ions can replace magnesium ions as the activating metal ion (30). Preincubation of the CPK with manganese ions causes inactivation of the CPK, which can be stopped but not reversed, by cysteine(30). Inhibition by manganese ions causes loss of the reactive sulfhydryl groups which are in close proximity to the substrate

binding sites of the CPK (30).

The enzyme CPK has been shown to be inhibited in its activity by a variety of chemical substances, including p-chloromercuribenzoate, N-ethylmaleimide, iodoacetate, dinitro-o-cresol, phosphate, citrate, fluoride, aged sodium thioglycollate (6,30,32). The enzyme is activated by thiols, including thioglycollate, cysteine, and glutathione (6, 30).

CPK cannot catalyze a reaction with adenosine monophosphate (AMP) as a phosphorus acceptor (31), substantiating Banga's statement that the second molecule of phosphocreatine formed in the overall reaction:

ATP + Creatine ---> Phosphocreatine + Adenylic acid

requires two separate enzymes.

IDENTIFICATION OF ENZYME ACTIVITY

After the discovery of the enzyme CPK and its purification, researchers developed several techniques to identify enzyme activity in the serum and organs of animals, including man. The enzyme was found to be in the cytoplasm of cells in brain, heart, and skeletal muscle, or loosely bound to protein particles in serum or tissue (31). Since it was isolated in greatest quantities in these three tissues, it was earlier theorized, and later shown, to be composed of three isoenzymes, one for heart, skeletal muscle, and brain.

Eppenberger, et al(7), undertook extensive research tracing the embryonic development of the enzyme CPK in rats and chicks. Using fetal and newborn animals, they took samples of brain,

heart, and skeletal muscle and ran CPKphoresis on these samples. They found that the isoenzyme of brain was the first to appear, and it could be identified early in all three tissues, remaining in brain as the only isoenzyme of CPK. The heart isoenzyme develops next, and finally that of skeletal muscle.

Veen and Willebrands (28), using the following technique, determined the presence of three fractions of the enzyme CPK, and tested many tissues for the presence or absence of each of the three fractions, and the relative quantity of each fraction. Using agar gel electrophoresis, they based the final quantity of identifiable enzyme fraction on the following reactions.

1. ADP + Phosphocreatine $\overset{\text{CPK}}{\rightleftharpoons}$ ATP + Creatine

2. ATP + Glucose $\overset{\text{Hexokinase}}{\rightleftharpoons}$ ADP + Glucose-6-PO_4

3. Glucose-6-PO_4 + NADP $\overset{\text{Glucose-6-PO}_4\text{ dehydogenase}}{\rightleftharpoons}$ 6-phospho gluconic acid + $NADPH_2$

4. $NADPH_2$ + Phenazine Methosulfate (PMS) $\longrightarrow$ NADP + reduced PMS

5. Reduced PMS + Nitroblue Tetrazolium (NBT) $\longrightarrow$ PMS + reduced NBT (violet color)

Based on the amount of color formed on the gel strip, they quantitated their results on normal tissues, + small amount, ++ moderate amount, +++ large amount, of the fraction of CPK. Table 1 is a tabulation of their results.

Table 1

Tissue	Fraction 1	Fraction 2	Fraction3
Heart Muscle	±	+++	+++
Skeletal Muscle	-	-	+++
Brain	+++	-	±
Liver	+	-	-
Kidney	+	-	-
Lung	+	-	-
Spleen	+	-	-
Red Blood Cells	±	-	-

CPK Fraction in Various Tissues

Duel and Van Breenen (3) selected agar gel electrophoresis for identifying creatine phosphokinase isoenzymes. They compared preparations of CPK from brain, heart, and skeletal muscle, assaying homogenates of gel strips for enzyme activity by dipping the gel into substrate and subsequently into phosphate reagent solutions providing a color reaction for the determination of the isoenzymes. Using these same gel homogenates, they also covered them with a substrate containing gel followed by detection of loss of fluorescence due to oxidation of $NADH_2$. Using both techniques, comparable results were obtained. They found that the enzyme migrated both toward the anode and cathode. The isoenzyme band from brain migrated with greater electrophoretic motility than did the single band obtained from skeletal muscle. Heart preparations contained five bands, two of which migrated in a pattern similar to the bands from skeletal muscle and brain.

In clinical medicine, most work with pathologic sera has been based on the total CPK determination, using serum as the source of enzyme. Okinaka, at al (23), were among the first to describe a method for determination of the total CPK. They begin each test with two solutions, A and B, which differ in that solution B contains creatine as the substrate. Solution A contains ATP, $MgSO_4$, tromethamine, and serum. The reaction is begun by the further addition of ATP and incubated thirty minutes at 38°C. The reaction is stopped by adding cold

trichloracetic acid. Then the inorganic phosphorus generated is determined by the method of Fiske and Subbarow(8) using acid molybdate solution and Enol's solution. A blue color develops which can be determined colorimetrically using a known standard of phosphorus. The difference in the quantity of phosphorus in Solutions A and B is equal to the transphorylated phosphorus generated from the phosphocreatine obtained in the following reaction:

$$\text{ATP} + \text{Creatine} \xrightleftharpoons{\text{CPK}} \text{Phosphocreatine} + \text{ADP}.$$

This method is used at the University of Nebraska Hospital Laboratory, available from the Sigma Company in St. Louis, Missouri, in a kit containing all the reagents. Using this method, the results are expressed in Sigma units per milliliter of CPK. One Sigma unit of CPK will phosphorylate one millimicromole of creatine per minute at 37°C.

Preston(24) compared the colorimetric method of Okinaka to a spectrophotometric method of Tanzer, which includes an auxillary reaction incorporating phosphoenol pyruvate and phosphate kinase(27). His indicator system consists of the conversion of generated pyruvate to lactate in the presence of reduced diphosphopyridine nucleotide (DPNH). He then measures the quantity of DPNH consumed per unit time using the following chemical reactions:

1. $\text{ATP} + \text{CREATINE} \xrightleftharpoons{\text{CPK}} \text{ADP} + \text{Creatine phosphate}$

2. ADP + Phosphoenol Pyruvate $\rightleftharpoons$ Pyruvate + ATP

3. Pyruvate + DPNH + H $\overset{\text{LDH}}{\rightleftharpoons}$ Lactate + DPN

with the summation of the reactions being:

Creatine + PEP + DPNH + H $\overset{\text{CPK}}{\rightleftharpoons}$ Creatine Phosphate + Lactate + DPN

The spectrophotometric determination is also available in kit form, in the Boehringer CPK-Test Kit, from Calbiochem, Los Angelos. One unit of CPK is the amount of enzyme per milliliter of serum which at 340 mu and 25°C causes a change in DPNH absorbance of 0.001 per minute in a 3.0 ml assay volume. Preston found poor correlation between these two methods, preferring the spectrophotometric method.

Because of the variation in techniques available for the total CPK determination, clinical studies comparing actual laboratory values are difficult, but the trends shown in these studies do compare well. The newer technique of isoenzyme determination is not currently available in most clinical laboratories.

CLINICAL APPLICATION

As the laboratory investigation into the characteristics of the enzyme CPK proceeded, clinicians began studying the relationship between CPK activity and disease.

The diagnosis of acute myocardial infarction can be aided with the total CPK determination. Much investigation has been conducted, concluding that serum levels of CPK are elevated

as much as fifty percent within three to six hours post infarction(22,29). The peak of activity is within twelve to twenty-four hours after the infarction(22). This information is especially useful in early cases when the lactic dehydrogenase (LDH) and serum glutamic-oxalacetic transaminase (SGOT) levels have not yet risen above normal. Also, the test can be performed in less than two hours after obtaining the blood speciman.

Since CPK is found in skeletal muscle, much work has been done to determine its application in the human myopathies. In progressive muscular dystrophy, a genetic disorder, the activity of CPK has been found to be markedly elevated in the patient(23). Also, studies have been conducted on the parents and normal siblings of these patients. In the Duchenne type muscular dystrophy, an X-linked recessive disease, the total CPK activity of affected individuals is markedly elevated. There is also elevated CPK activity in some of the female siblings and in the mothers of the affected patient(20), thus affording a method of identifying the carrier state. Studies in patients with motor neuron disease, anterior poliomyelitis, polyneuritis, and myasthenia gravis, have revealed these patients to have normal serum CPK activity(23). In the muscle of the patient with muscular dystrophy, total CPK activity is decreased when compared to normal muscle, and the isoenzymes of this muscle manifest no characteristic abnormality

in their pattern(13).

Exercise requiring prolonged muscular activity increases serum CPK activity(11), but the values in men, women, and children are different for the same activity. Children have greater levels of CPK activity than adults, and men have greater levels than women(11).

In altered thyroid states, the serum CPK activity is also affected. In hypothyroid patients the value may be more than five to six times normal levels(9). Hyperthyroid patients have normal to low normal levels of CPK activity(10). Several hypotheses have been proposed to explain the elevation of CPK in hypothyroid patients, but no real explanation has been proven.

Acheson and her colleagues studied twenty-four patients for fourteen days following cerebral vascular accidents. Fifteen of her patients had elevated serum CPK activity(1). The maximun elevation occurred in two to six days, with an average of three days. The levels returned to normal by fourteen days. Seven patients had a second small rise in the CPK activity at day eight or nine, which was significant. Using CPK levels alone, Acheson,et al, were not able to differentiate cerebral infarction from localized intracerebral hematoma. They found no correlation between the level of CPK activity and the severity of the cerebral lesion. However, they did feel that a high level of CPK activity correlated

with a poor prognosis and death.

In obstetrics, the concentration of CPK has been studied during pregnancy. Pregnant women were found to have normal levels when compared to nonpregnant women(5), except late in pregnancy when the CPK activity is elevated(15). No correlation could be found between the serum level of CPK and the duration of the pregnancy, age, or parity of the pregnant woman(5). During delivery, about eighty percent of the women had elevated levels of CPK(15). The first day post partum the serum CPK activity increases, but by post partum day six, all women studied had normal levels(5). Cord blood levels of CPK are elevated, being about two times normal(15).

In patients with acute and chronic hepatitis or cirrhosis of the liver, serum levels of CPK have been found to be normal(23). In hepatic coma, however, the serum CPK activity is increased(23). In advanced metastatic carcinoma, some patients may have elevated serum CPK activity(22).

THE EXPERIMENT

Since serum CPK has been found to be elevated following a cerebral vascular accident, it was postulated that newborn infants who have suffered from cerebral hypoxia might also have increased CPK activity. Since no studies were available in the literature investigating normal newborn serum CPK activity, this study was conducted to determine the normal serum CPK activity in infants less than twenty-four hours

of age.

During June, 1966, twenty newborn infants at University Hospital were selected as subjects of this study. An infant was included in the study if his one minute Apgar was five or greater, if no rescusitation was necessary for spontaneous respiration, if his birth weight was 2500 grams or greater, if his newborn physical examination was within normal limits, and if there was no family history of congenital anomalies, mental retardation, neurologic disorders, or muscular dystrophy. One child born by Caesarean section, and one child who was a frank breech presentation, were included in the study.

All laboratory measurements were conducted by the same technician. Blood specimens were collected in capillary tubes, using heel blood as the source of the blood sample. The samples were taken to the laboratory and centrifuged immediately. All determinations were done on serum within three hours after collection. Any sample having CPK activity greater than twenty-five Sigma units was diluted and the CPK determination repeated. Serum CPK was determined using the Sigma Company Kit based on Okinaka's colorimetric determination, as previously described in this paper.

The Sigma Company established normal values of less than twelve Sigma units, and elevation as greater than twenty-five Sigma units. Three infants had CPK activity levels less than twelve Sigma units. Five others had values between twelve and

twenty-five units. The highest value obtained was 114 Sigma units. The mean value for twenty tests was 41.6 Sigma units per ml., with a standard deviation of 32.4 Sigma units. For thirteen female infants the mean was 32.3 Sigma units; for seven male infants the mean CPK was 59 Sigma units. (Table 2)

DICUSSION

Eighteen normal infants less than twenty-four hours of age and two infants forty-six and fifty-two hours of age were studied to determine serum activity of creatine phosphokinase in the newborn infant. It was found that three infants had activity levels less than twelve Sigma units, the level considered normal in adults. Several factors were then considered as possibly contributing to the high CPK values.

It is known that children normally have greater than normal CPK levels when exercising than when at rest. This exercise must be strenuous and prolonged. Each child, when blood collection was initiated, cried vigorously. Also, the trauma to the foot when the sample was obtained causes a small amount of trauma to the foot musculature. However, the amount of activity when the child cried was not great, as they were all quiet during most of the collection. Also, the amount of trauma to each child was about the same, and this should be reflected equally among all children, with no child having a small CPK value.

Table 2

Sex	Age in Hours	Duration of Labor-Hours	Apgar-1 minute	Weight Grams	CPK Sigma units
F	24	---	10	2900	3
F	19	17 51/60	6	3700	11.4
F	5	3 50/60	7	2799	18
F	21	9 27/60	9	2962	18
F	25	11 40/60	9	3595	19.5
F	12	9 30/60	10	3019	24
F	27	1 46/60	8	4272	24
F	13	1	5	3427	26 -Breech
F	45	9 35/60	10	4586	30
F	22	none	8	3700	32-C-section
F	22	6	8	3150	40
F	52	9 3/60	10	2836	60
F	5	11 14/60	9	3005	114
Mean	23	7.5	8.4	3406	32.3
M	11	7 33/60	8	3350	6
M	7	4 20/60	8	3705	36
M	28	10 29/60	9	3444	36
M	23	25 30/60	9	3312	73
M	22	4 45/60	6	4139	80
M	24	5 26/60	9	3444	90
M	16	16	9	3487	92
Mean	19	10.4	8.3	3554	59
Mean-M & F		8.7	8.4	3460	41

Hemolysis of the red blood cells could not be considered a factor contributing to the high CPK activity, as studies (28) have shown that the red blood cell does not contain CPK.

Parity of the mother was considered a possible factor contributing to the elevated CPK levels, believing that the trauma of birth would be greater in the primagravida than in the multipara. However, there was no correlation between parity of the mother and the CPK activity, so this factor was eliminated as a cause of elevated CPK activity. (Table 3).

Birth weight was another factor considered, but no correlation could be found between birth weight and CPK activity. (Table 4).

Duration of labor was considered as a possible factor, but no correlation could be found between the duration of labor and the CPK activity. (Table 5).

Age, in hours, was also considered a factor contributing to the elevation of the CPK activity. The younger the infant, the more the trauma of birth was continuing to affect the child, with its muscular compression and activity. However, there was no correlation between the age of the child and the CPK values. (Table 6).

Since no correlation could be established for each of these factors, they were considered as having some effect in the elevation of the serum CPK levels, but not the complete explanation for the high levels obtained. Perhaps the best

explanation is that newborn infants normally have CPK activity levels which are greater than the adult, due to 1) the trauma to muscle and brain tissue at birth secondary to the molding of the child in the pelvis, 2) the propertion of muscle mass and brain tissue to the total weight of the infant, and 3) the muscular activity of the infant when awake and crying.

At the present time, only the crudest means are available to the physician to assess birth trauma in the newborn infant. This is particularly true for brain damage. If an infant has severe, gross, brain damage, it is obvious, but it would seem that minor degrees of brain damage are far more common. This is substantiated by the fact that the cause of cerebral palsy and mental retardation cannot be determined by reviewing obstetrical and neonatal records.

The work of Acheson, et al, certainly suggests that brain damage results in release of creatine phosphokinase into the peripheral circulation. This study was conceived in an effort to determine how widely normal newborn levels vary, and if they correlate with various obstetric parameters. No correlation was found, but this does not negate the value of the study. What it does show, is that if this determination is to be of value for this purpose, it is necessary to identify that enzyme which arises from brain tissue and distinquish it from that arising from muscle in the newborn. At the present time a practical laboratory technique for doing this , clinically is

not available. However, it can be seen from previously cited material that the isoenzymes of creatine phosphokinase can be separated, and the isoenzyme which arises from brain is distinct. Hopefully, a practical laboratory test to identify it will soon be available.

The value for clinical application of such a test is obvious, since at the present time, there is no way to accurately identify the baby with minimal or moderate brain damage at birth. If this were possible, early corrective steps, special education, physiotherapy, might well minimize some of the sequelae of this brain damage.

SUMMARY

Measurement of serum creatine phosphokinase activity in clinical disease is a relatively new laboratory diagnostic tool. Twenty newborn infants, considered normal, were studied in an effort to determine the normal serum CPK activity in infants.

A review of the investigation into the properties of the enzyme and the methods for measuring enzyme activity has been presented. As the reagents become available, the isoenzyme determination of CPK activity will become an new diagnostic tool for the clinician in determining the source of enzyme elevation, either heart, brain, or skeletal muscle.

Table 3

Parity of Mother	Number	CPK Values
One	Eight	3, 6, 24, 40, 60, 73, 92, 114
Two	Four	26, 32, 36, 80
Three	One	90
Four	None	
Five	Four	11.4, 18, 19.5, 36
Greater than Five	Three	18, 24, 30

Parity and CPK

Table 4

Birth Weight	Number	CPK Values
2500-3000	Four	3, 18, 18, 60
3000-3250	Three	24, 40, 114
3250-3500	Six	6, 26, 36, 73, 90, 92
3500-3750	Four	11.4, 19.5, 32, 36
3750-4000	None	
4000-4500	Two	24, 80
4500-4750	One	30

Birth Weight in Grams and CPK

Table 5

Duration of Labor	Number	CPK Values
0-2	Three	24, 36, 32
2-5	Three	18, 36, 80
5-10	Seven	6, 18, 24, 30 40, 60, 90
10-12	Three	19.5, 36, 114
12-15	None	
15-20	Two	11.4, 92
Greater than 20	One	73

Duration of Labor in Hours and CPK

Table 6

Age of Infant	Number	CPK Values
0-10	Three	18, 36, 114
10-15	Three	6, 24, 26
15-20	Two	11.4, 92
20-25	Seven	3, 19.5, 32, 40 73, 80, 90
Greater than 25	Five	18, 24, 30, 36, 60

Age in Hours of Infant and CPK

BIBLIOGRAPHY

1. Acheson, J., et al: Serum Creatine Kinase Levels in Cerebral Vascular Disease, Lancet 1:1306-1307 (June 19) 1965.

2. Colombo, J.P.; Richterich, R.; Rossi, E.:Serum Kreatin-Phosphokinase:Bestimming und Diagnostische Bedeutung, Klinische Wochenschrift 40:37-44 (Jan) 1962.

3. Deul, D.H., and Van Breenen, J.F.L.: Electrophoresis of Creatine Phosphokinase from Various Organs, Clin Chim Acta 10:276-283, 1964.

4. Dreyfus, J.C., and Schapira, G.: Technique de Dosage de la Creatine Phosphokinase du Serum, Rev Franc d'Etudes Clin et Biol 6:700, 1961.

5. Emery, A., and Pascasio, F.: The Effects of Pregnancy on The Concentration of Creatine Kinase in Serum, Skeletal Muscle, and Myometrium, Am J Obst Gyn 91:18-22 (Jan) 1965.

6. Ennor, A.H., and Rosenberg, H.: Some Properties of Creatine Phosphokinase, Biochem J 57:203-212, 1954.

7. Eppenberger, H.M., et al: Ontogeny of Creatine Kinase Isoenzymes, Develop Biol 10:1-16, 1964.

8. Fiske, C.H.; and Subbarow, Y.: The Colorimetric Determination of Phosphorus, J Biol Chem 26:375-400, 1925.

9. Graig, F.A., and Ross, G.: Serum Creatine-Phosphokinase in Thyroid Disease, Metabolism 12:57-59, 1963.

10. Graig, F.A., and Smith, J.C.: Serum Creatine Phosphokinase Activity in Altered Thyroid States, J Clin Endocrin 25:723-731, 1965.

11. Griffiths, P.D.: Serum Levels of ATP:Creatine Phosphotransferase (Creatine Kinase). The Normal Range and Effect of Muscular Activity, Clin Chim Acta 13:413-420 (April) 1966.

12. Griffiths, P.D.: Serum Levels of Creatine Phosphokinase, J Clin Path 17:56-57 (Jan) 1964.

13. Kar, N., and Pearson, G.: Creatine Phosphokinase Isoenzymes in Muscle in Human Myopathies, Am J of Clin Path 43:207-209, 1965.

14. Kelly, S.; Kelly, W.; Swift, H.: Serum Creatine Phosphokinase in Four Generations of a Muscular Dystrophy Family, Am J Clin Path 45:377-380 (April) 1966.

15. Konttinen, A., and Pyorala, T.:Serum Enzyme Activity in Late Pregnancy, at Delivery, and during Puerperium, Scand J Clin Lab Invest 15:429-435, 1963.

16. Kumar, D., and Barnes, A.C.: Studies on the Mechanism of Action of Progesterone on the Human Myometrium, Bull John Hopkins Hosp 113:330-334, 1963.

17. Lehmann, H.: Uber die Enzymatische Synthese der Kreatin Phosphorsaure durch Umesterung der Phosphobrenztraubensaure, Biochem Z 281:271-291, 1935.

18. Lohmann, K.: Uber die Enzymatische Synthese der Kreatin Phosphorsaure; Zugleich ein Beitrag zum Chemismus der Muskelkontraktion, Biochem Z 271:264-277, 1934.

19. Narayanswami, N.: Creatine Phosphokinase in Mammalian Brain, Biochem J 52:295-301, 1952.

20. Nichol, C.J.: Serum Creatine Phosphokinase Measurements in Muscular Dystrophy Studies, Clin Chim Acta 11:404-407, 1965.

21. Nielson, L., and Luduigsen, B.: Improved Method for Determination of Creatine Kinase, J Lab Clin Med 62:159-168 (July) 1963.

22. Nissen, N.I., et al: Evaluation of Four Different Enzymes in the Diagnosis of Acute Myocardial Infarction, Brit Heart J 27:520-527, 1965.

23. Okinaka, S., et al: Serum Creatine Phosphokinase Activity in Progressive Muscular Dyatrophy and Neuromuscular Diseases, AMA Arch Neurol 4:520-525, 1961.

24. Preston, J., et al: Serum Creatine Phosphokinase:A Clinical and Laboratory Evaluation, AmJ Clin Path 44:71-79 (July) 1965.

25. Sax, S.M., and Moore,J.J.: Fluorometric Measurement of Creatine Kinase Activity, Clin Chem 11:951-958 (Oct) 1965.

26. Scopes, R.K.: Acid Denaturation of Creatine Kinase, Arch Biochem 110:320-324 (May) 1965.

27. Tanzer, M., and Gilvarg, C.: Creatine and Creatine Kinase Measurement, J Biolog Chem 234:3201-3204 (Dec) 1959.

28. Veen, K.J., and Willebrands, A.F.: Isoenzymes of Creatine Phosphokinase in Tissue Extracts and in Normal and Pathological Sera, Clin Chim Acta 13:312-316 (Mar) 1966.

29. Warburton, F.G.; Bernstein, A.; Wright, A.C.: Serum Creatine Phosphokinase Determinations in Myocardial Infarction, Brit Heart J 27:740-747, 1965.

30. Watts, D.C.: Studies on the Mechanism of Action of Adenosine 5-Triphosphate-Creatine Phosphotransferase: Inhibition by Manganese Ions and by p-Nitrophenyl Acetate, Biochem J 89:220-229 (Nov) 1963.

31. Wood, T.: Adenosine Triphosphate-Creatine Phosphotransferase from Ox Brain:Purification and Isolation, Biochem J 87:453-462, 1963.

32. Wood, T.: Adenosine Triphosphate-Creatine Phosphotransferase from Ox Brain:Properties and Function, Biochem J 89:210-220, 1963.