Serum Creatine Activity : A kinetic study in patient of hyper and hypothyroidism

H.G. Hassan ,L.M.Hassan
Department of Chemistry,College of Science , University of Sulaimani
Department of Chemistry,College of Education, University of Salahaddin

Abstract

Serum Creatine Kinase (SCK) activity was investigated in hyper and hypothyroidism objects. Estimated Levels were compared with healty controls .

Optimization of SCK activity has been achieved using 0.05 ml of the Serum and 25 m mol/L of the substratet creatine phosphate . Reaction mixture was incubated at 37C for 10 minutes . Data obtained were reflects an elevation in the enzme activity in hyperthyroidism objects. Determination of the physical parameters (Vmax and Km) were obtained applying lineweaver- Burk Plot analysis. All detemined parameters were established using buffer phosphate at optimum P^{H} Value of 6.4.

Introduction

It was realized that throid hormones (T3,T4,and ,TSH) were appear to be synthesized with in thyroglobulin (1) . The building up of the hormones occurred using iodinated tyrosine residues in thyroglobulin .

A coupled reaction of mono- and di-iodinated derivation lead to the formation of T3 and T4 (2). TSH is a glycoprotein synthesized by the the pituitary gland . Thyroid dysfunction (hypo and hyper)were diagnosed by serum T3 and T4 levels variation (3) it was aeen that higher levels of serum T3 and T4 (hyperthyroidism) can block TSH synthesis and release , while their low levels can stimulated TSH synthesis (hypothyroidism) (4). A health cell condition is a measure of the conversion of T4 in to T3 , which is catalysed by 5- diiodinase

(5), this is modified by the pituitary gland (6). Creatine kinase as a key enzyme play a significant role in assessment of the thyroid function as a clinical score and measurements of these thyroid peripheral thyroid hormones (7).

Thyroid hormones increase the activites of many enzymes including CK(8) .This process was prohilbited by protein synthesis inhibitors (9). Most disorders are associated with protein biosyntesis for example severe hypothyroidism resulted in an elevation in CK activity (10) while the disorder hyperthyroidism was seen to cause a decreases in the CK activity (8).

It was understood that mass concentrations of SCK isoenzymes and the increased catalytic activity concentrations of serum total CK gradually decreased in inverse proportion to the increased concentrations of T3 and T4 (11). Studying the energetic behavior of SCK and its effects in cases studied was not comprensive by literatures there fore, the propose of this paper is to highlights the kinetic Parameters behaviors of SCK and to investigate energy contents. Data obtained were concentrated the central Hawler City .

Materials and Methods

Chemical: All chemicals used in this project were of high Analar grade.

A CK –K it from RANDOX Lab was used for determination of CK activity.

Sampling: Hypothyroidism patients (20 samples) of adult ages , thein were 10 male and 10 females hyperthyroidism patients (20 samples) of adult ages they were 8 males and 12 females . Both samples were diagnosed by consultants and proved by thyroid function tests. They are no accompanied disease. Healty normal (20 samples) were used as controls , they were of adult age (4 males and 16 females).

Blood Collection and Serum Isolation:

Blood (5ml) was collected from patients and healthy control objects by vienopuncture, The blood left at room temperature for 30 -06 min . After clot formation the Serum isolated by centrifugation at 3000 rpm for 10min . Serum obtained wasa use on the same day of experiment or within days after storing at (-20C)

Determination of SCK Activity

Creatine kinase **(CK)** activity was done utilizing creatine phosphate as substrate to act as the initial catalyst for a series of reactions resulting in the formation of NADPH as outlined the coupled enzyme assay shown below using a colorimetric method (12):

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatine} + \text{ATP}$$

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{Glucose-6-p} + \text{ADP}$$

$$\text{Glucose-6-P} + \text{NADP}^{+} \xrightarrow{\text{G6p-DH}} \text{Gluconate-6-p} + \text{NADPH}$$

$$2\text{NADPH} + \text{NBT} \xrightarrow{\text{Diaforase}} \text{diformazan} + 2\text{NADP}$$

The NADPH produced is proportional to CK activity and is used to reduce nitro blue tetrazolium (NBT) in the presence of diaforase to give the blue / violet color of diaformazan which has an absorption maximum around 560 nm. The reaction is stopped by the addition of hydrochloric acid

Determination of V max km values

Using different concentrations of the substrate creatine creatine phosphate (25,17,12,5,8,5 Mm) , Vmax and Km values determined from Michaelis –Menten analysis . These values were confirmed using lineweaver- Burk plot analysis . The determined data includes both hypo and hyperthyroidism sera and compared with that of controls. The protocol applied was as that used in determining the SCK activity.

Biostatistical analysis

A T-test analysis was applied for investigating data obtained

Results

Investigating SCK activity , data obtained shows an elevation of the activity in hypothyroidism and decrease in case of hyperthyroididm

(550.79+25.81 and 27.03+2.687 U/L respectively) when compared with normal activity (111.58 5.63 U/L) fig (1).

Determination of Vmax and values . data obtained from hypothyroidism reflect higher value of Vmax compared with control , while Km reflect lower value than that of control table (1). An inverse values were obtained for Vmax and Km in case of hyperthyroidism.

These values of Vmax and Km were achieved using Michaelis – Menten analysis fig(2) .

The data analysed were confirmed by plotting lineweaver – burk plot fig (3) . Studying the effect of the enzyme concentration on the reat reaction , a procedure using increases volume of the serum has applied which give an increased linearity in enzyme activity fig (4).

Discussion

Creatine kinase (CK,EC2.7.3.2)is an enzyme(a type of protein)found in muscle and brain . Normally , very little CK is found circulating in the blood . Elevated levels `indicates damage to either muscle or brain possibly from myocardial infaction muscle disease , or stroke.

It was also found that there were response relations between smoking and serm CK concentrations, and ankle-reflex time in the women with overt hypothyroidism (7). Different causes has been noticed leading to elevation in CK levels (13).

As hypothyroidism resulted in a deficiency of thyroid activity , characterized by decrease in basal metabolic rate (BMR) fatigue , and lethargy , these parameters were covering energy content , thus , the study was focusing on investigating of CK activity and its activity in hypothyroidism when compared with control .

The mechanism of the changes in these analyses in hypothyroidism may be related to increased leakage from skeletal – muscle cells or diminished clearance from the circulation or both (8) .

The above discussion can be predicts due to the thyroxin replacement therapy study that seen to be due to the normalization occurs in SCK levels . Thyroid hormone replacement therapy resulted in resolution . Clinical Symptoms and a marked reduction in the SCK level such SCK level in patient with hypothyroidism under scores the important assessing thyroid function in patient with weakness even when systemic symptoms and singns of hy pothyroidism are moderali or absent (14).

Studying physical parameters table(1), Vmax and Km, it was seen that using 37C as incubated temperature, is a convenient temperature for increasing the affinity of the enzyme to its substrate.

Decreased value in Km (hypothyroidism) can be explained to be due to the increase in enzyme affinity , while increased value of V max compared with control could be due to a change occurring in the catalytic site conformation or /number in the total of the enzyme which leading to increase in the enzyme activity and affinity (15).

References

1. Harrisons,T.R. (1998) principles of medicine volume 2-4th ed .
2. Jyne,F. and Michael , Sh .(1999) Evaluation of thyroid function in health and disease (www.thyroidmanager or h /chapter6.6-textHtm1-9).
3. Fabio ,M,N. ;Nadia,C.; Gabriele,S.;Laura,M.; Luigi ,M. and Eleuterio,F. (1997), J.Clin Endo ,82(10): 3315-3318.
4. Alper,G.; Vali,CI. And Miyase,Bl. (1997).J.Clin , 14(3):180-183.
5. Davidsons . (2000). 18 thed .U.K .
6. Green Span,S.F. and Grardener ,G. (2001).Clin .Enod 6thed Grow Hill.USA.
7. Beat Muller,MID; Henry Zuikuski,M.D.; Peter Huber , PHD; John , G.; R atc lif, MD, and Jean.Jacques Staub, M.D. (1995). J.MEDICINE. 333:964-969.
8. Docherty,J.;Harrop,J.S.; Hine,K.; Hopton ,R. and Jaylor ,C.(1984).J.Chem .30(10:42-45.
9. Waelboeck ,M.; Vanobberghen,E. and Myts ,P. (1979).J.Biol . 254: 7736-7740.
10. Finsterer ,J.; Stollberger ,C.; Croossegger , C. and Kcroiss,A. (1999)J.M ed :52(4):205-208.
11. Miyamato, T.; Nagasaka, A;, Kato,K.;Masunaga,R.; Kotake,M.; Kawabe, C.; Naki ,A.;Mokun,T. and Oda,N. (1994). J.Clin. Chem.32: 589-593.
12. Oliver,IT. (1995).J.Biochem. 61:116.
13. Joseph,F. (1987), Medi library Corbett Jane Vincent Laboratory Tests and Dignostic proced ure with Nursing Diagnoses 2nd Norwalk.
14. Soott, KR.; Simmons, Z. and Boyer ,PJ. (2002). J.Medi 26(1):141-144 .

15. Diler ,R.A.(1004) An MSc thesis . College of Medicine Salahaddin UNIV.

Table (1): Km and Vmax values for serum enzymes in (Normal , Hypo and Hyper) thyroidism

Parameter	Normal	Hypo	Hyper
Km(mM)	19.77	13.29	250.34
Vmax(U/L)	322.58	1428.57	312.50

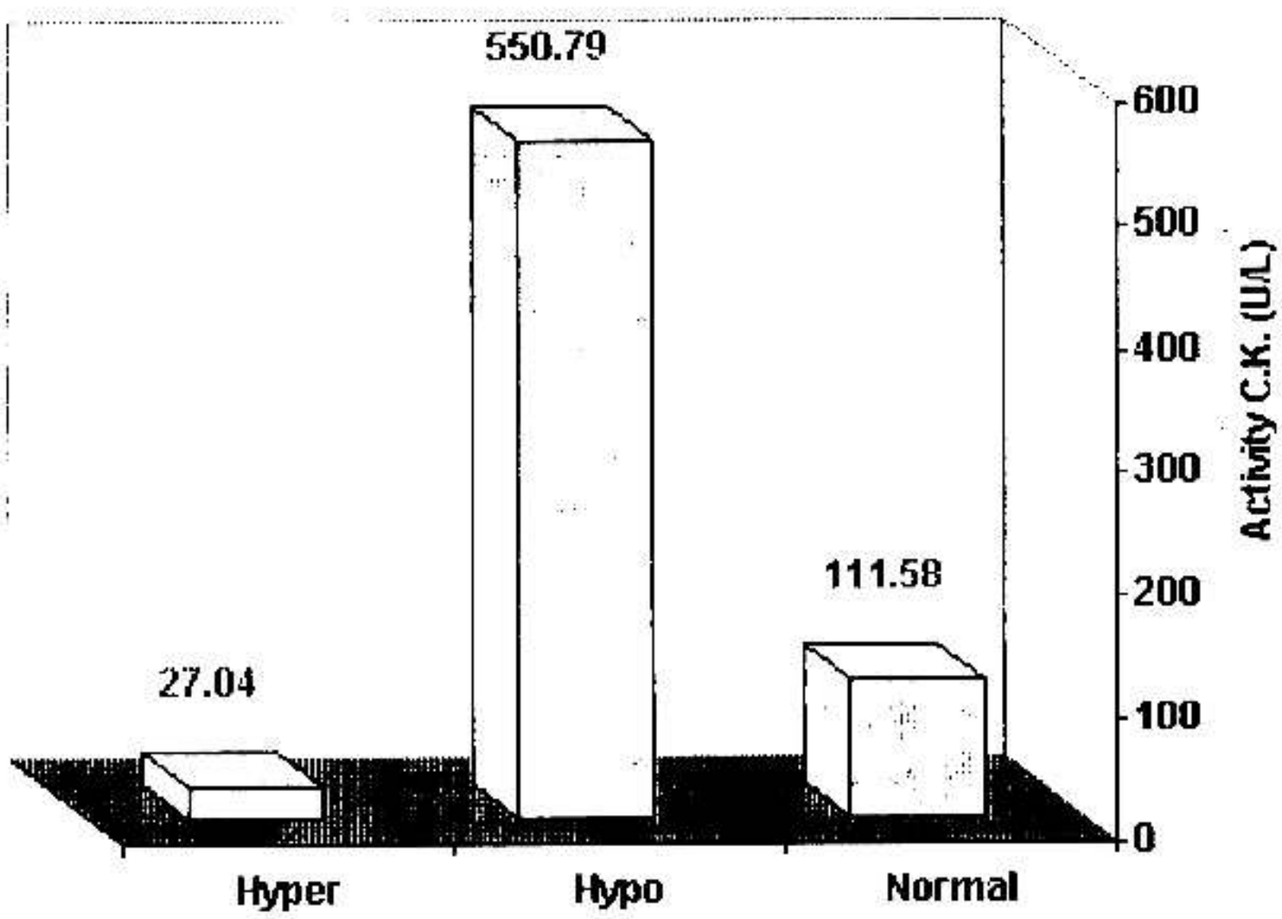

Fig (1) Serum CK Activity in Normal , Hypo and Hyper groups

Activity C.K.

SE	±	Mean	
5.632	±	111.58435	Normal
52.817	±	550.7914	Hypo
2.682	±	27.035	Hyper

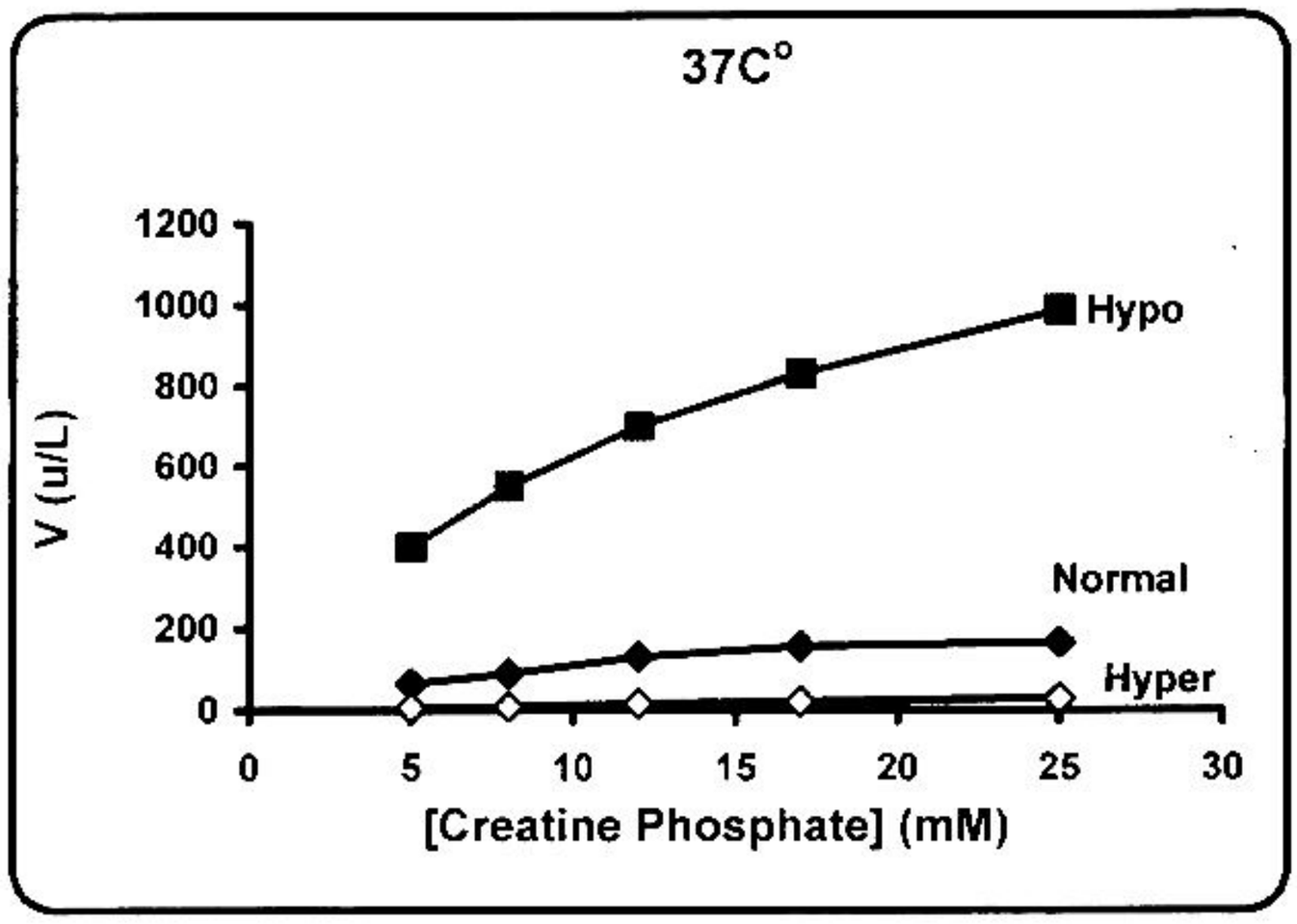

Fig (2) Michael's menten plots for Serum CK activity in Hypo , Normal and Hyper thyrodisim cases

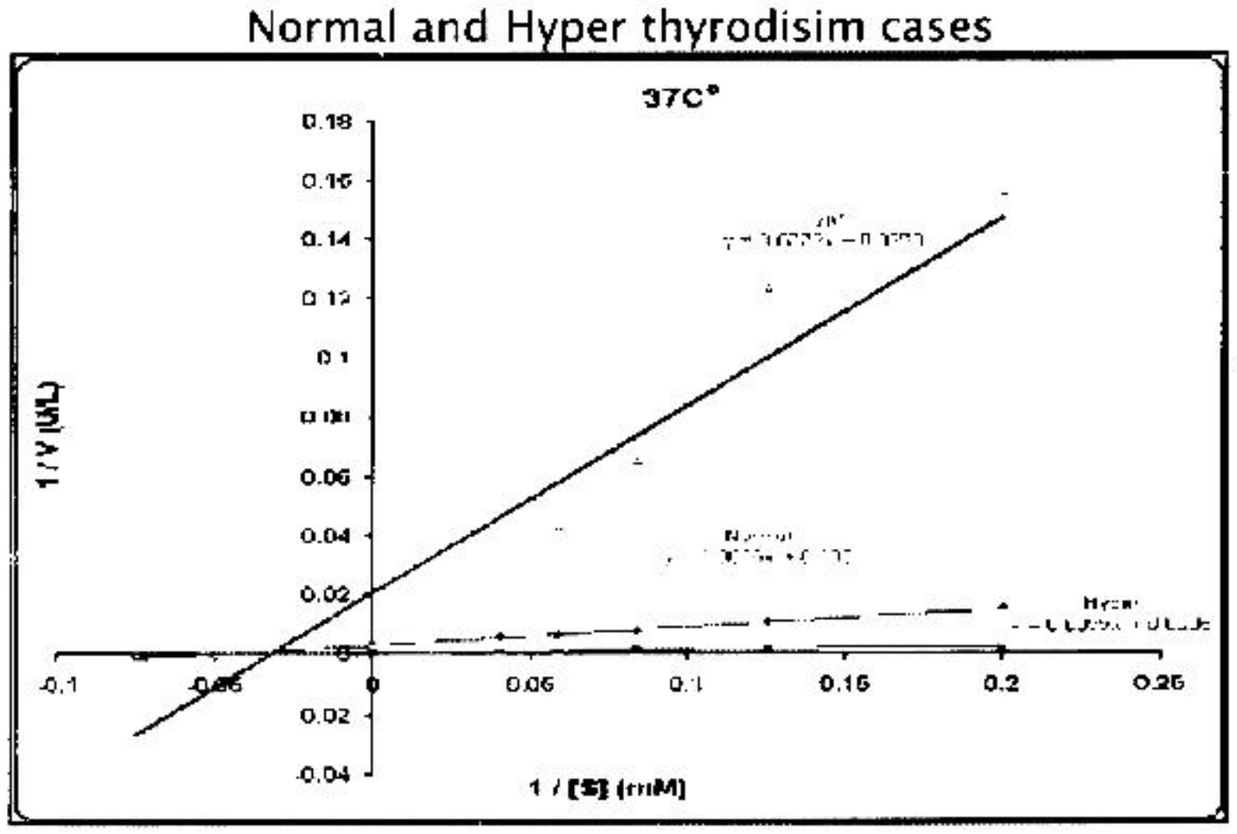

Fig (3) Lineweaver - Burk Plot for Normal, Hypo and Hyper CK activity

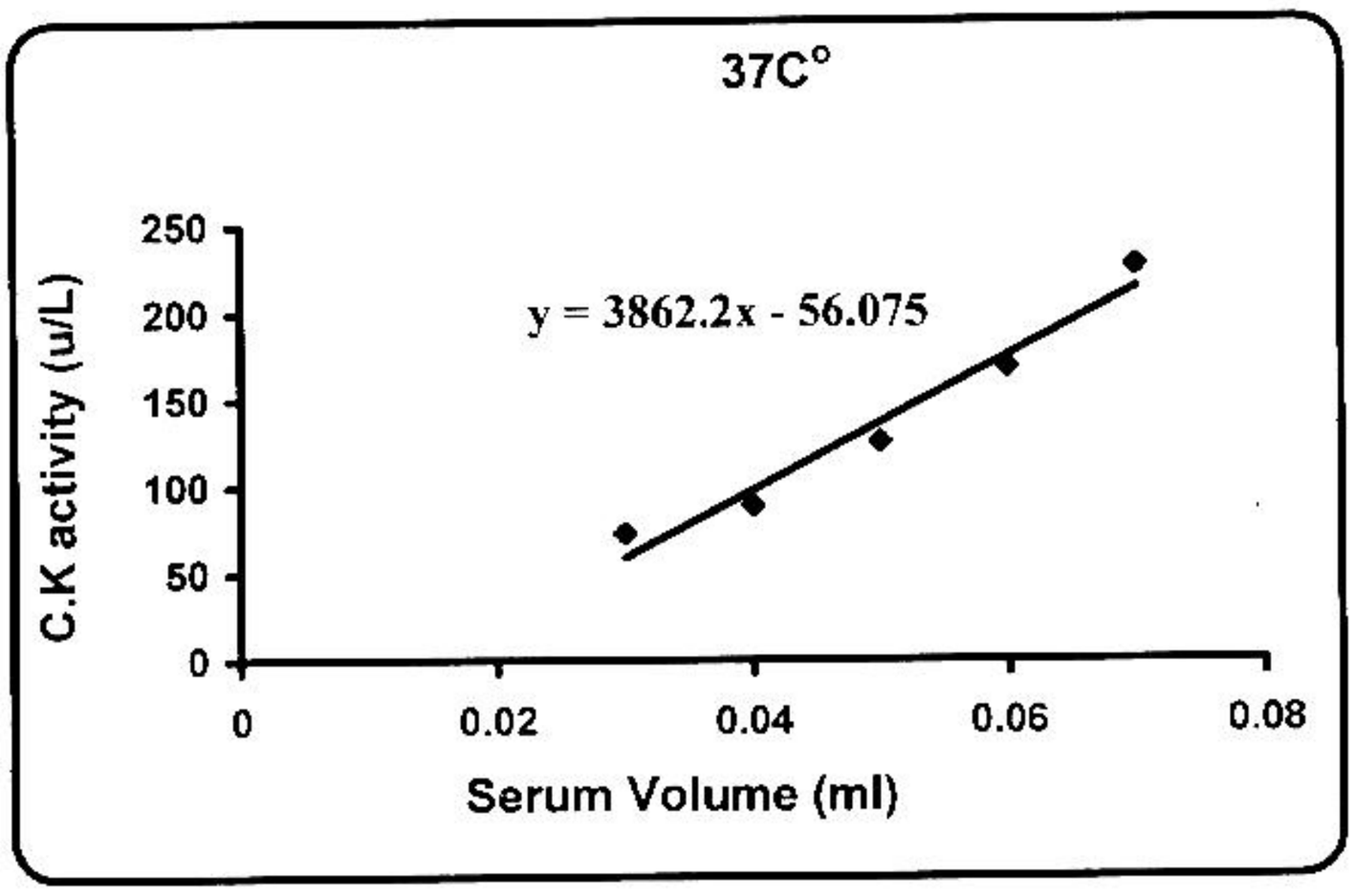

Fig: (4) Increasing CK activity using increased serum.

Volume of serum (ml)	Absorbance (nm)	Activity (u/L)
0.03	0.091	73.528
0.04	0.111	89.688
0.05	0.156	126.048
0.06	0.209	168.872
0.07	0.281	227.048

نشاط انزيم الكرياتين كاينيز :دراسة حركية في مصول مرض الفرط والضمور الدرقي

حامد غفوري حسن و لطفية محمد حسن
قسم الكيمياء، كلية العلوم ، جامعة السليمانية
قسم الكيمياء، كلية التربية ، جامعة صلاح الدين

الخلاصة

تم بحث انزيم الكرياتين كاينيز في مصول دم مرضى الفرط الدرقي وقد قورنت النتائج بمثيلاتها من المصل الطبيعي .

قيست النتائج تحت شروط مثلى ،اذ استخدم تركيز ركيزة فوسفات الكرياتين 0,05 مليمول / لتر وفي درجة حضن لمزيج التفاعل 37 م ومدة 10 دقائق . اوجدت النتائج التي حصل عليها ارتفاعا في نشاط الانزيم في حالة الضمور الدرقي .

ان نتائج دراسة الدوال الفيزيائية الحركية (السرعة القصوى وثابت ميكيلس –منتن) قد أثبتت باستخدام علاقة لينيويفير – بيرك الخطية .لقد أجريت التجارب باستعمال المحلول المنظم الفوسفات وبدرجة اس هايدروجيني 6,4.