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Serum enzyme studies in muscle disease

Part I Variations in serum creatine kinase activity in normal individuals

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It has been recognized for many years that in patients with muscular dystrophy there are substantial increases in the activity of certain enzymes, *e.g.*, aldolase and the transaminases, in the serum. Extensive surveys by several workers have shown that the changes are particularly striking in cases of the Duchenne type muscular dystrophy (decreasing as the disease progresses), smaller in the limb-girdle and facio-scapulo-humeral types of dystrophy, and slight or absent in cases of neurogenic muscular weakness and wasting (Dreyfus and Schapira, 1955; Dreyfus, Schapira, and Schapira, 1954, 1958; Schapira, Dreyfus, Schapira, and Demos, 1960; Thomson, Leyburn, and Walton, 1960; Hughes, 1962). Hence they are of value in helping to distinguish conditions of the latter group from the muscular dystrophies. Of the enzymes studied, creatine kinase (A.T.P.: creatine-phospho-transferase) appears to display the largest increase.

Recent studies (Chung, Morton, and Peters, 1960; Schapira *et al.*, 1960; Aebi, Richterich, Colombo, and Rossi, 1962; Hughes, 1962; Walton, Pearce, Pennington, and Barwick, 1962) have shown that there is an elevation of serum creatine kinase in some of the female relatives of patients with the Duchenne type muscular dystrophy. The results indicate that many, but not all, the known carriers of this sex-linked recessive gene display a level of serum enzyme activity above the normal range. The importance of being able to establish whether or not a woman is a carrier is evident. In order that the maximum use may be made of the serum creatine kinase level as a criterion for detecting carriers it is important to possess adequate data concerning variations occurring in normal individuals under a wide variety of physiological conditions. The main reason why this is necessary is that wide variations in levels of serum enzyme activity, *e.g.*, of aldolase, have been shown by Thomson (1962) to occur in relation to physical exercise and parturition. Minor elevations of enzyme activity occurring under varying physiological circumstances could conceivably make it difficult to detect carriers in whom the increase is usually small. We have therefore undertaken a study of the influence of various factors upon the activity of the enzyme in healthy individuals. The use of estimations of the serum creatine kinase in the diagnosis of the carrier state, of preclinical muscular dystrophy, and of other hereditary and non-hereditary myopathies will be the subject of subsequent publications (Pearce, Pennington, and Walton, 1964).

MATERIAL

The volunteer subjects used in this investigation were healthy young adult members of the nursing, medical, and technical staff of the General Hospital and Royal Victoria Infirmary and healthy women attending an ante-natal clinic. Samples were also taken from children and young adult patients not suffering from myopathic disorders. Studies were carried out on 19 normal adult females in the fasting state after 12 hours' bed rest, and under similar circumstances in nine adult males. In four females the blood samples were taken before and after food, in three before, during, and after strenuous physical exercise, and in two the samples were taken from forearm veins before and after ischaemic work. In 21 subjects estimations were carried out at various stages of pregnancy and in 16 at various stages of the menstrual cycle. Samples were also taken from four children under the age of 1 year, from four between the ages of 1 and 5 years, from three aged 5 to 10 years, and from three between 10 and 15 years of age.

METHOD

Blood samples (5 ml.) were withdrawn by venepuncture, allowed to clot, and centrifuged. The sera were frozen

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and kept in a cabinet deep-freeze until assayed. Measurements on two specimens showed that a single freezing did not significantly alter the creatine kinase activity, although repeated freezing and thawing caused the activity to decrease. Storage in the frozen state for at least two weeks does not appear to have any effect upon the activity of the enzyme.

Creatine kinase activity was estimated by measuring the formation of creatine from creatine phosphate and adenosine diphosphate (A.D.P.) by the method of Ennor and Rosenberg (1954). The detailed procedure was similar to that used by Hughes (1962) with the following modifications: 1 A lower concentration (4·2 mM) of cysteine was used in the medium, since this was found to be adequate for maximum activity: 2 To avoid the appearance of turbidity which was occasionally encountered during the creatine determination, the p-chloromercuribenzoate was added at a later stage. The enzyme reaction was stopped by adding zinc sulphate and barium hydroxide (0·2 ml. of each), after which 0·7 ml. of the supernatant and 0·3 ml. of p-chloromercuribenzoate were used for the creatine estimation. 3 The blank tubes (to which were added water instead of A.D.P.) were also incubated for 30 minutes.

The enzyme activity is expressed as micro-moles of creatine formed per hour per millilitre of serum at 37°C.

RESULTS

The results are given in Tables I to VII. The samples for estimation of basal activity were taken in the

TABLE I

LEVEL OF ACTIVITY OF CREATINE KINASE IN NORMAL ADULT SUBJECTS

	Creatine Kinase Activity (units)	*Range*	*Mean*
Females			
O.H.	1·0		
J.H.	1·3		
J.H.	1·0		
R.R.	1·6		
J.H.	1·5		
I.R.	3·5		
V.C.	1·7		
S.H.	1·9		
H.M.	2·1	1·0-4·0	1·7
J.B.	2·0		
S.T.	1·1		
B.M.	1·3		
R.T.	2·7		
A.M.	2·5		
M.E.	1·0		
L.D.	1·2		
M.M.	1·0		
J.W.	2·4		
S.T.	4·0		
Males			
P.R.	3·6		
H.M.	1·6		
R.B.	3·0		
G.R.	0·4		
T.B.	1·3	0·4-3·6	1·5
S.B.	1·4		
C.S.	0·8		
H.D.	1·0		
L.M.	0·8		

S.D. = 0·72 5% limit = 3·1 1% limit = 3·5

TABLE II

EFFECT OF FOOD ON BASAL ACTIVITY OF CREATINE KINASE

Subject	*Creatine Kinase Activity (units)*		
	Before Food	*Thirty Minutes after Food*	*Change*
V.C.	1·7	1·6	−0·1
S.H.	2·1	1·8	−0·3
S.T.	1·1	1·7	+0·6
A.M.	2·5	2·6	+0·1

TABLE III

EFFECT OF STRENUOUS EXERCISE ON BASAL ACTIVITY OF CREATINE KINASE

Subject	*Creatine Kinase Activity (units)*					
	Rest	*1 Min.*	*15 Min.*	*30 Min.*	*45 Min.*	*60 Min.*
J.B.	2·0	1·8	1·6	1·9	1·4	—
S.T.	1·1	1·1	1·1	—	1·1	—
B.M.	1·3	1·0	1·2	—	1·0	0·8

TABLE IV

EFFECT OF ISCHAEMIC EXERCISE ON BASAL ACTIVITY OF CREATINE KINASE

Subject	*Creatine Kinase Activity (units)*		
	Before	*After*	*Change*
V.S.	1·6	1·3	−0·3
M.A.	1·0	1·0	+0

TABLE V

VARIATIONS IN CREATINE KINASE ACTIVITY DURING PREGNANCY

Subject	*Creatine Kinase Activity (units)*	
First trimester (0 to 13 weeks)		
P.McG.	1·3	
F.P.	0·7	
C.C.	1·4	Mean 1·55
S.B.	2·6	
S.M.	2·0	
J.A.	1·3	
Second trimester (14 to 26 weeks)		
W.M.	1·1	
C.L.	3·6	
J.O.	1·6	
M.K.	4·4	Mean 1·8
J.L.	0·6	
M.A.	0·4	
T.A.	0·7	
A.D.	1·7	
A.J.	2·0	
Third trimester (27 to 40 weeks)		
M.S.	1·7	
H.S.	0·4	
M.W.	2·5	Mean 1·3
M.W.I.	0·6	
R.B.	1·4	
M.A.	1·0	

TABLE VI

VARIATIONS IN CREATINE KINASE DURING THE MENSTRUAL CYCLE

Day of Cycle	*Subject*	*Creatine Kinase Activity (units)*	*Mean*
0 to 6	I.S.	1·7	
	S.H.	1·9	2·1
	I.S.	2·8	
	I.S.	2·1	
7 to 14	V.C.	1·7	
	S.T.	4·0	
	B.M.	1·3	2·0
	A.M.	2·5	
	J.R.	1·3	
	M.M.	1·0	
15 to 22	S.T.	1·1	
	J.B.	2·0	1·5
	J.S.	1·6	
	J.R.	1·5	
23 to 30	I.S.	1·5	1·35
	L.O.	1·2	

TABLE VII

ACTIVITY OF CREATINE KINASE IN CHILDREN

Age (yr.)	*Patient*	*Creatine Kinase Activity (units)*	*Mean Creatine Kinase Activity*
0 to 1	L.McB.	3·5	
	S.H.	3·3	3·6
	P.M.	4·5	
	R.A.	3·0	
1 to 5	B.Y.	2·6	
	C.B.	6·2	2·8
	J.M.	1·4	
	G.N.	0·9	
5 to 10	B.B.	2·1	
	R.E.	2·6	1·6
	G.O.	1·0	
10 to 15	M.S.	1·1	
	S.W.	1·4	1·3
	M.T.	1·3	

fasting state after 12 hours of bed rest (Table I). In four subjects (Table II) further blood samples were taken after a large breakfast and a further three subjects were exercised strenuously, being asked to run three times up and down two flights of stairs. Observations were made at intervals after the exercise (Table III). Ischaemic work was performed in the arm in two subjects (Table IV) while the brachial artery was occluded with a sphygmomanometer and blood was taken from the antecubital vein.

It will be seen that in 19 normal females the basal activity varied from 1·0 to 4·0 units with a mean of 1·7 units. In nine males without evidence of muscle disease the range was 0·4 to 3·6 with a mean of 1·5 units. No significant change in the serum activity of creatine kinase occurred after a heavy meal, after exercise, or after ischaemic work. Apart from two patients in whom the activity was unusually high in the second trimester, no significant deviations from the normal range were observed during pregnancy or at various stages of the menstrual cycle. We also found that the results in different age groups in healthy adult subjects showed no significant variations. The level of serum creatine kinase is significantly higher in infants and in children under the age of 5 years. This elevation is most marked in infancy, and gradually decreases with increasing age of the child.

DISCUSSION

The mean activity of serum creatine kinase in the normal females in our series under basal conditions was close to the figure (1·62) given by Hughes (1962) for normally active females. Hughes obtained a consistently higher value in males than in females, but our results do not show any such distinctive trend.

It is evident that none of the factors studied had any significant effect on the activity of creatine kinase, though there is a very slight suggestion of a difference between the two halves of the menstrual cycle. It is of particular interest that exercise produced no observable effect. Several workers have studied the effect of exercise on serum enzyme levels in men and animals, and varying results have been reported. Thus Tessari and Parrini (1961) found that lactate dehydrogenase increased during light exercise in young men, but, on the other hand, Critz and Merrick (1962) reported a fall in aspartate aminotransferase (S.G.O.T.) after exercise in untrained subjects but no difference in well-trained athletes. Casula, Cherchi, and Spinazzola (1961) found occasional moderate increases in aldolase, lactate dehydrogenase, and aminotransferases. Our own results would suggest that physical exertion is not likely to be an important variable influencing normal serum creatine kinase levels, although it is clear that it can affect other enzymes. These findings are similar to those of Hughes (1963). We have shown that when samples of blood are taken and the creatine kinase level is estimated the result is not significantly affected by a previous meal, by previous exertion, by pregnancy, by the stage of the menstrual cycle, or by the age of the adult patient. This is obviously relevant to the interpretation of the results obtained in the investigation of patients suffering from muscular dystrophy and their relatives.

We have data (to be published) which indicate that serum creatine kinase levels are raised in patients with the Duchenne type of dystrophy, and that a less striking increase is found frequently in patients suffering from the limb-girdle and facioscapulohumeral types of muscular dystrophy (Pearce *et al.*,

1964). Of particular importance is the frequent finding of high levels of creatine kinase in carriers and probable carriers of the Duchenne dystrophy, and a possible elevation in some sibs of such patients. The increase in the carriers is definite but is not of the same order as in affected patients. The interpretation of these smaller elevations of serum creatine kinase levels is only possible in the light of data demonstrating the range of variation which may occur in the normal individual.

SUMMARY

To facilitate the interpretation of the significance of alterations in serum creatine kinase activity in the blood of patients with muscular dystrophy and their close relatives, estimations of the activity of this enzyme have been carried out on serum samples obtained from normal subjects under a wide variety of physiological conditions. Physical exercise and the ingestion of a large meal did not significantly affect the results. During pregnancy, and at the different stages of the menstrual cycle, the results did not change significantly. The activity in males and females did not differ significantly, the means being 1·7 and 1·5 units. The upper limit of normal activity was 3·5 units, the results being expressed in micromoles of creatine/hr./ml. serum. Slightly higher values were seen in infancy and early childhood.

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