Serum Creatine Phosphokinase Activity in Altered Thyroid States

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ABSTRACT. Serum creatine phosphokinase activity was determined in euthyroid, hyperthyroid and hypothyroid subjects. Mean serum enzyme values (μmol substrate converted/ml/hr) were: 2.03 ($\pm$0.216) for healthy subjects, 1.71 ($\pm$0.257) for euthyroid patients, 0.72 ($\pm$0.08) for hyperthyroid patients and 13.22($\pm$3.22) for hypothyroid patients. Serial studies in hyper- and hypothyroidism demonstrated good correlation of serum enzyme activity with thyroid function. The assay of this enzyme was found to be useful in the assessment of thyroid status in patients who received exogenous iodine or diphenylhydantoin. Concurrent injury or inflammatory disease of skeletal or cardiac muscle invalidates the diagnostic value of CPK in hypothyroidism but increases its usefulness in hyperthyroidism. (*J Clin Endocr* **25:** 723, 1965)

AS PART of a study of the relationship between thyroid hormones and muscle tissue, variations in serum creatine phosphokinase (adenosine 5′-triphosphate-creatine phosphotransferase; EC 2.7.3.2) activity in hyper- and hypothyroidism were examined. Previously, elevated serum CPK[1] levels in hypothyroid patients were reported (1) and confirmed by two other investigators (2, 3). The present paper is concerned with serum CPK activity of euthyroid, hyperthyroid and hypothyroid subjects determined with the modified method of Hughes (4). Serial determinations in a number of patients before, during and after correction of their abnormal thyroid states have also been carried out. Some of the data have been presented previously (5).

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[1] Abbreviations used are: C: creatine; CP: phosphocreatine; CPK: creatine phosphokinase; HK: hexokinase; G-6-PDH: glucose-6-phosphate dehydrogenase; PK: pyruvate kinase; LDH: lactic dehydrogenase; ATP and ADP: adenosine tri- and diphosphate; PEP: phosphoenolpyruvate; pyr.: pyruvate; lac.: lactate; G: glucose; G-6-P: glucose-6-phosphate; 6-PG: 6-phosphogluconate; NADP and NADPH: nicotinamide adenosine dinucleotide phosphate, oxidized and reduced form; NAD and NADH: nicotinamide adenosine dinucleotide, oxidized and reduced form; T_3: triiodothyronine; T: desiccated thyroid; T_4: thyroxine; TSH: thyroid-stimulating hormone.

Materials and Methods

The reaction catalyzed by CPK is shown in the following equation:

$$ATP^{4-} + C \underset{\text{reverse}}{\overset{\text{forward}}{\rightleftharpoons}} ADP^{3-} + CP^{2-} + H^+$$

(CPK)

According to the direction in which the reaction proceeds various components of the reaction mixture can be used as indicators of enzyme activity. In general, the methods may be divided into 2 categories: those which directly measure a product formed or a group transferred (C, phosphate, H^+) and indirect methods in which auxiliary reactions are coupled with the CPK reaction. C is measured colorimetrically, in the presence of α-naphthol and diacetyl; inorganic phosphate is determined by the ammonium molybdate method; H^+ is determined titrimetrically. The indirect methods utilize the change in optical density at 340 mμ in the final step. The rate of change in optical density is pro-

portional to CPK activity. A schematic representation of the principal methods employed is shown below.[2]

We have had experience with most of the methods mentioned but prefer for serum assays Hughes' (4) modification of the Ennor and Rosenberg (7) procedure. This is a sensitive assay, suitable for processing a large number of samples. All determinations were done in duplicate. The color was measured in a Beckman DU spectrophotometer at 520 mμ. Normal or low activity sera were incubated for 30 min. Because of an apparent increase in serum enzyme activity with dilution (4), the incubation time was shortened for highly active sera. CPK activities are expressed as μmols C formed/ml of serum/hr at 37 C. CP, C, ADP, *p*-hydroxy mercuribenzoate, crystalline CPK and cysteine HCl were obtained from Sigma Chemical Company. α-Naphthol was obtained from Distillation Products Industries and freshly prepared solutions were kept in the dark until used because of their tendency to deteriorate upon exposure to light. Attempts were made to reduce the value of the blank by recrystallization of α-naphthol and redistillation of diacetyl. However, no significant reduction of the blank was effected. CP and ADP solutions were prepared weekly and kept frozen at −20 C; the cysteine was freshly dissolved every day in Mg^{++}-Tris buffer. Standard C solution was freshly prepared at frequent intervals. All other chemicals used were reagent grade.

Blood was allowed to clot at room temperature for 1 to 2 hr and the serum removed after centrifugation was kept frozen until assayed. Enzyme assays were performed within 1 week after the blood was obtained. Storage of sera up to 4 weeks did not affect CPK activity significantly.

The hyper- and hypothyroid subjects were in- or outpatients at Montefiore Hospital. All patients described in this paper were clear-cut cases of hyper- or hypothyroidism and could be easily diagnosed by clinical examination alone. In most cases the clinical diagnosis was supported by laboratory data.

The euthyroid subjects consisted of 2 groups. Sixteen individuals were members of hospital and laboratory staff, in good health, and had no evidence of endocrine, renal, cardiac or muscular abnormality. Since strenuous exercise may increase serum CPK level (16), all subjects were questioned with regard to their physical activity during the 24 hr preceding the taking of the blood sample. Male and female subjects are considered together, although some authors (9, 14) report lower enzyme levels in females. Twenty-eight hospitalized patients with no endocrine or muscle disorder, and no acute myocardial necrosis, constituted the other group of euthyroid subjects studied.

TSH-stimulation was performed over a 3-day period with daily subcutaneous injections of 10 U of thyrotropic hormone (Thytropar, Armour); 24-hr ^{131}I uptake in the neck was measured before the first dose and the day after the last injection of thyrotropin.

Results

Serum CPK. The individual serum CPK activities for the two groups of euthyroid subjects are listed in Tables 1 and 2. The mean value for the ambulatory subjects was 2.03 ± 0.216[3] and for the hospitalized patients 1.71 ± 0.257.[3] The serum enzyme levels for 25 hyperthyroid patients and for 30 hypothyroid patients are listed in Tables 3 and 4; laboratory confirmation of the thyroid status, wherever

[3] Standard error of means.

[2]

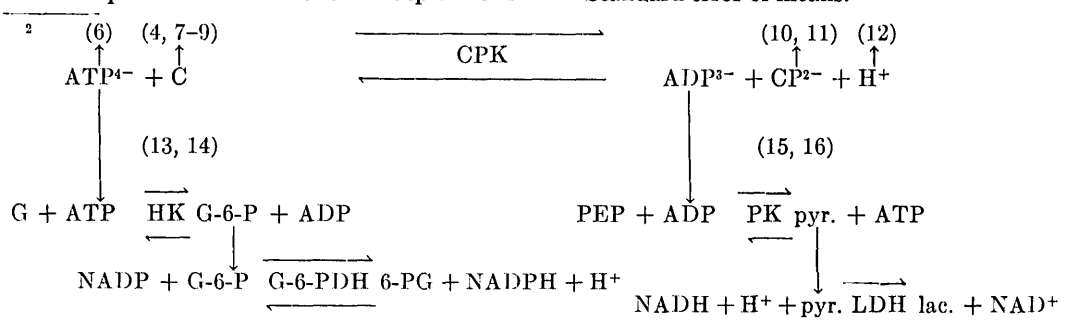

TABLE 1. Distribution of serum CPK values in healthy subjects

Subject	Age	Sex	CPK (μmol/ml/hr)
C.P.	25	F	1.40
J.S.	32	M	3.70
E.E.	24	F	1.60
F.G.	49	M	2.70
F.F.	54	M	1.35
L.B.	30	M	3.40
S.L.	27	M	2.56
S.K.	27	M	1.64
S.C.	28	M	2.94
M.S.	30	M	2.28
M.W.	41	F	1.0
F.B.	28	M	0.90
G.S.	18	M	2.85
R.B.	32	M	1.72
E.S.	45	F	1.35
U.B.	22	F	1.15

Mean: 2.03.
SE: ±0.216.

available, is also included in the tables. The mean serum CPK level for the hyperthyroid patients was 0.72 ± 0.08[3] and for the hypothyroid subjects 13.22 ± 3.22.[3] The distribution of values for each of the groups is presented graphically in Fig. 1. Employing Student's *t* test, significant differences ($p < 0.01$) were found between euthyroid and hypothyroid subjects and between euthyroid and hyperthyroid subjects. The differences between the two groups of euthyroid individuals are not significant.

Correlation between CPK levels and PBI. Fig. 2 demonstrates that generally there is good inverse correlation between PBI

TABLE 2. Distribution of serum CPK values in euthyroid patients

Patient	Age	Sex	Diagnosis	PBI (μg/100 ml)	^{131}I uptake (%)	CPK (μmol/ml/hr)
W.D.	41	M	Pituitary hypogonadism	4.0		3.18
A.G.	60	F	Euthyroid	7.9		1.62
D.A.	40	F	Euthyroid	4.9		1.21
M.S.	36	F	Euthyroid	4.5		2.08
L.A.	41	F	Euthyroid		34	1.26
M.M.	58	F	Rheumatic heart disease, congestive heart failure			0.82
S.F.	65	M	Coronary artery disease			1.39
L.L.	62	M	Cancer of stomach			0.79
I.C.	58	F	Cancer of rectum			3.63
E.L.	81	F	Coronary artery disease, nephrolithiasis			1.11
M.A.	23	M	Infectious mononucleosis			1.80
A.C.	72	M	Coronary artery disease			0.78
M.B.	58	F	Cancer of breast			2.38
S.M.	57	F	Lymphosarcoma			2.01
R.E.		F	Cancer of pancreas			0.71
Z.B.	55	F	Euthyroid		23	1.20
J.M.	41	F	Euthyroid	5.0	30	1.20
R.H.	74	F	Euthyroid on replacement therapy	5.6		3.02
S.S.	70	F	Euthyroid	4.8		2.25
J.P.	44	F	Euthyroid		30	1.36
A.L.	42	F	Euthyroid, post-^{131}I therapy	4.9	26	0.81
H.H.	35	F	Euthyroid on Tapazole	4.8		1.72
C.C.	18	F	Euthyroid, post-thyroidectomy	6.4	20	1.48
F.B.	53	F	Euthyroid on replacement, post-^{131}I therapy	4.0	21	1.54
R.P.	73	F	Euthyroid, post-^{131}I therapy		23	2.93
R.S.	54	F	Euthyroid	5.7		1.43
E.B.	65	F	Euthyroid (exogenous iodine)		4	1.37
C.W.	36	F	Euthyroid, post-^{131}I therapy	4.0	37	2.91

Mean: 1.71.
SE: ±0.257.

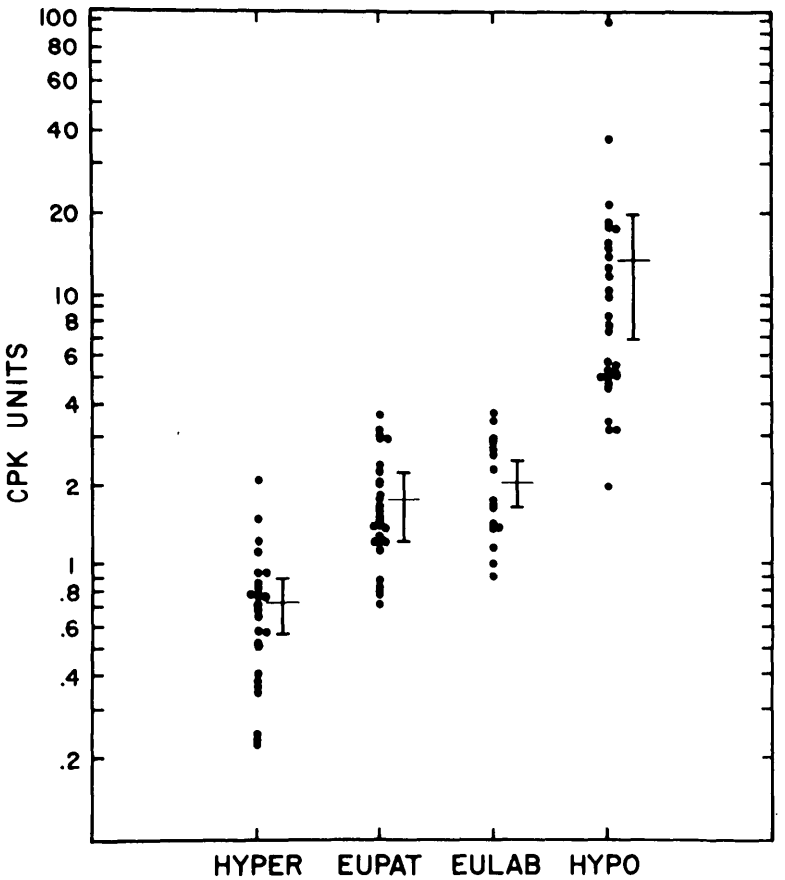

FIG. 1. Serum CPK activity in hyperthyroid (HYPER), euthyroid hospitalized patients (EUPAT), euthyroid laboratory staff (EULAB) and hypothyroid patients (HYPO). Horizontal lines represent the mean for each group with 2× standard error indicated by vertical bars.

values and serum enzyme levels. Pearson's coefficient of correlation (r) between the logarithms of CPK and PBI values is 0.363. The correlation ratio for the regression of CPK on PBI is 0.481 $\pm$0.11 (SE) and for the regression of PBI on CPK 0.675 $\pm$0.079 (SE). The correlation between 24-hr uptake of ^{131}I and serum CPK is less close. This is understandable as the accumulation of iodide by the thyroid is also influenced by extrathyroidal factors and by iodide stores in the gland (17).

Serial determinations of CPK. In individual patients serum CPK levels reflect changes in thyroid status, as shown in Fig. 3. Patient B.S. (*A.*) had severe myxedema and the highest CPK value we have ever encountered in hypothyroidism. After replacement therapy was instituted, a gradual decline in enzyme level occurred. Patient B.M. (*B.*) was hyperthyroid and was treated with radioiodine. The serum enzyme values rose to the normal range as she became euthyroid, and continued to rise when she became hypothyroid with a PBI of 0.8. Subsequent replacement therapy decreased the serum CPK to normal value. Patient M.M. (*C.*) was also treated with ^{131}I for hyperthyroidism and developed transient hypothyroidism. Prompt replacement therapy was given when she showed signs of mild hypothyroidism, and her serum CPK did not increase to above normal levels. Patient D.S. (*D.*) received methimazole therapy

TABLE 3. Serum CPK values in 25 hyperthyroid patients*

Patient	Age	Sex	^{131}I uptake (%)	Conversion ratio (%)	PBI (μg/100 ml)	CPK (μmol/ml/hr)
S.S.	58	F	79	84		1.2
A.G.	44	F	94	63		0.23
J.O.	61	F	77	92		0.40
K.E.	14	F	48	86		0.93
I.R.	38	F	58	82		0.52
L.J.	34	F	87	82		0.77
M.J.	23	F	76	81		0.86
T.M.	30	F	100	90		0.50
S.S.	28	F				0.34
K.M.	7½	F			8.0	0.38
M.G.	61	F	53	90		0.76
M.P.	30	M	65	90		1.44
E.S.	38	F	51	65	9.2	0.92
P.S.	26	M	81	90		2.07
D.G.	39	F	54	53		1.10
D.S.	47	F	77	90		0.68
M.S.	52	F	70	64	8.6	0.77
B.M.	55	F	75	88		0.82
T.S.	19	F				0.57
J.H.	22	F				0.64
M.M.	49	F	76	95	11.0	0.36
L.S.	29	F	51			0.57
A.R.	37	F	100	92	9.0	0.24
J.T.	32	F	100	72	19.5	0.22
M.H.	38	F	55	92	22.0	0.70

Mean: 0.72.
SE: ±0.08.
* Laboratory substantiation of diagnosis could not be obtained in 3 patients.

for hyperthyroidism with increase in serum CPK values as euthyroidism was achieved. After discontinuation of the drug she again became hyperthyroid. The changes in the thyroid status of this patient were also reflected in her serum CPK values. In each case a further parameter of thyroid function was determined and is also shown in Fig. 3.

Effect of TSH. Increased discharge of hormone from the thyroid gland lowers the serum CPK level transiently. This fails to occur when the thyroid is unresponsive, indicating that the effect is not due to TSH itself (Fig. 4). Patients *E.* and *F.* are euthyroid individuals who responded vigorously to the administration of TSH as indicated by the increase in ^{131}I uptake and PBI; a transient decrease in enzyme levels also occurred. The decrease in serum CPK exceeded the fluctuations which occur when daily determinations are done in patients. Patient *G.* had idiopathic myxedema and showed no response to thyrotropin stimulation; his highly elevated serum CPK showed no significant decrease.

Discussion

The CPK values found in euthyroid subjects are in good agreement with the normal values found by Hughes (4). The somewhat lower mean for the hospitalized patients than for the 16 healthy individuals either may be due to the fact that there was a predominance of females in the hospitalized group, or may mean that "sick" people have slightly decreased enzyme levels.

The values of serum CPK in the hypo- and hyperthyroid groups studied demonstrate an inverse relationship between serum enzyme levels and thyroid activity. Such a relationship has not been reported for any other serum enzyme.

TABLE 4. Serum CPK values in 30 hypothyroid patients*

Patient	Age	Sex	Diagnosis	^{131}I uptake (%)	PBI (μg/100 ml)	Cholesterol (mg/100 ml)	CPK (μmol/ml/hr)
R.W.	46	F	Post-^{131}I hypothyroidism	4			4.71
E.B.	40	F	Post-^{131}I hypothyroidism	1	2.6		7.70
S.S.	15	M	Primary hypothyroidism	1	0.9		37.04
A.M.	50	M	Hashimoto's thyroiditis with hypothyroidism				4.97
B.W.	41	M	Primary hypothyroidism, hyperparathyroidism				7.31
A.F.	50	F	Pituitary myxedema		2.5		5.26
U.S.	24	F	Primary hypothyroidism	1	0.9		3.16
F.R.	70	F	Primary hypothyroidism	5.8	2.0	407	5.68
E.G.	66	F	Post-thyroidectomy hypothyroidism	2.5	0.9		8.30
J.T.	32	F	Post-^{131}I hypothyroidism		2.1	377	18.30
C.G.	60	F	Primary hypothyroidism	1	0.9	600	13.83
J.A.	38	F	Post-^{131}I hypothyroidism	3.7			14.85
L.M.	57	F	Primary hypothyroidism		1.2		5.16
N.P.	46	F	Primary hypothyroidism		0.7	260	15.54
B.S.	59	F	Post-^{131}I hypothyroidism	1	2.1	488	99.85
M.C.	24	F	Post-^{131}I hypothyroidism	18	3.0		4.97
M.G.	62	F	Primary hypothyroidism		1.2		17.74
R.R.	30	F	Primary hypothyroidism	2.2	1.0		1.95
E.A.	43	F	Primary hypothyroidism	2	2.2	344	17.07
M.M.	63	F	Post-^{131}I hypothyroidism	18	3.1		3.40
T.R.	53	F	Primary hypothyroidism		2.1	500	9.70
I.M.	46	M	Primary hypothyroidism		2.4	404	11.66
L.B.	52	F	Primary hypothyroidism	1.5	1.1	318	10.20
F.G.	63	M	Post-^{131}I hypothyroidism	11	3.2		12.45
E.S.	49	F	Post-^{131}I hypothyroidism	25	2.0	226	5.57
S.K.	69	M	Pituitary myxedema	2.7		170	4.96
S.M.	60	M	Post-^{131}I hypothyroidism		1.7	331	3.16
V.M.	54	F	Post-^{131}I hypothyroidism	13	3.9	442	4.28
A.O.	23	F	Post-thyroidectomy hypothyroidism	2.6	1.2	442	20.13
E.Mc.	58	F	Primary hypothyroidism		2.6		17.67

Mean: 13.22.
SE: ±3.22.
* Laboratory data concerning thyroid status were unobtainable in 2 patients.

Elevation of serum glutamic oxalic transaminase has been observed by us in some cases of severe myxedema. Similar findings were obtained by B. Zumoff (personal communication). Elevation of serum malic dehydrogenase was described in both hyper- and hypothyroidism (18), and an increase in serum ribonuclease activity was reported in hyperthyroidism (19).

The reciprocal variation of serum CPK with thyroid status could be explained in various ways. The serum enzyme has identical electrophoretic mobility on starch gel with the muscle enzyme (20), and it is likely that in normal individuals it represents enzyme which leaked out of muscle. In altered thyroid states several other factors may be operative. Muscle tissue itself is severely affected both in hyper- and hypothyroidism (21–24), and it has been claimed that skeletal muscle plays an important role in the degradation of thyroxine (25). It is not known whether thyroid hormones alter the permeability of the sarcolemmal membrane, although such alterations could explain the changes in serum CPK. The permeability of cell membranes to large molecules in various thyroid states has not been studied.

An alternate explanation for the ob-

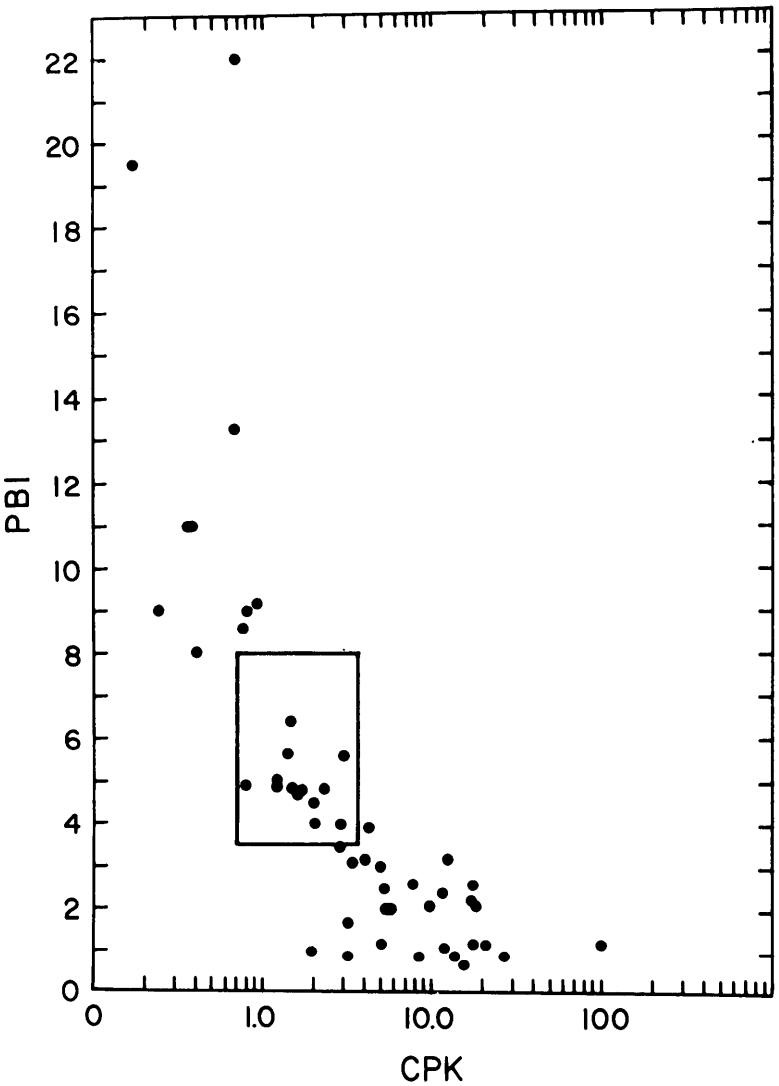

FIG. 2. Correlation between serum CPK activity and PBI values (μg/100 ml). Enclosed area represents the range of normal values for CPK and PBI. Patients represented in this area were all clinically euthyroid. The high and low PBI values were obtained from proven cases of hyper- and hypothyroidism, respectively. These cases are taken from Tables 1–4. The coefficient of correlation (r) between the logarithms of PBI and CPK values is 0.363. The correlation ratio for the regression of CPK on PBI is 0.48 ± 0.11 (SE) and for the regression of PBI on CPK 0.675 ± 0.079 (SE).

served variations in serum CPK could be that thyroid hormones influence the activity of this enzyme. No such evidence is available and, in fact, Tata (26) has shown that the soluble enzymes, among them CPK, are not affected by physiologic amounts of T_3.

Although it has been demonstrated (11, 27) that crystalline CPK is inhibited *in vitro* by thyroxin, this occurred with very high concentrations (*e.g.*, 24% inhibition at 10^{-4}M). We found no inhibition of the purified rabbit muscle enzyme or of the serum enzyme when L- or D-T_4 was added in a final assay concentration of 10^{-5}M. As the concentration of free T_4 in serum is 3.0×10^{-11}M (28), a direct inhibitory effect on CPK activity *in vivo* is improbable.

The observed changes in serum CPK could be related to the alteration of creatine metabolism in hyper- and hypothyroidism (29). Although there are no studies in man of serum CPK levels after creatine feeding, normal levels were found in a few patients with advanced

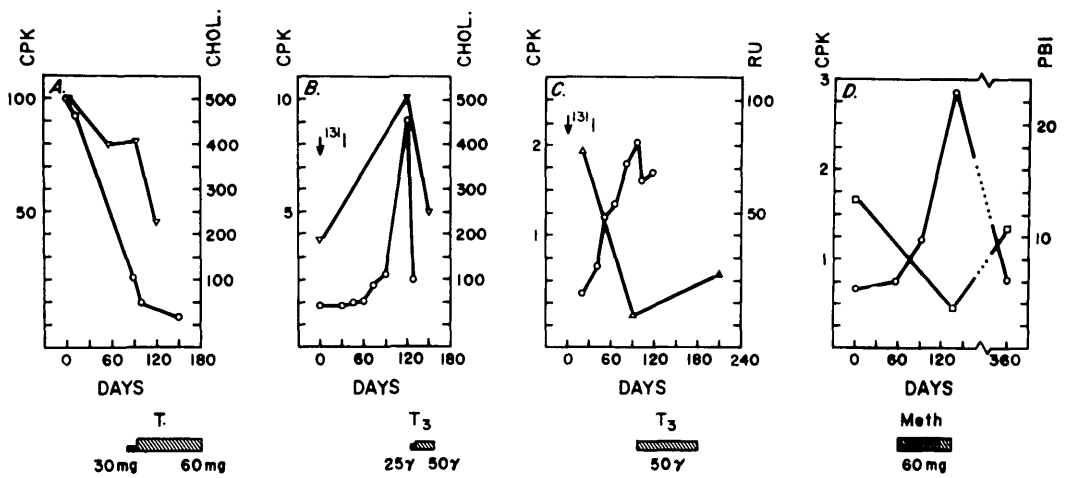

FIG. 3. Time course of serum CPK○ activity in relation to some indices of thyroid function. Chol. ▽ = cholesterol, mg/100 ml; RU△ = 24-hr thyroidal uptake of ^{131}I; PBI □ = protein-bound iodine, μg/100 ml. ^{131}I therapy indicated by arrow. Period of medication indicated by horizontal bars (T = desiccated thyroid; T_3 = triiodothyronine; Meth = methimazole). For discussion of cases see text.

renal failure (Graig, unpublished data). Creatine injections suppress creatine synthesis in rats (30) but fail to alter the serum CPK level. It is likely that the changes in serum CPK in altered thyroid states either reflect the general effect of thyroid hormones on anabolic and catabolic processes or result from changes in the release of this enzyme from muscle.

The measurement of serum CPK activity is not proposed as a routine test of thyroid function; however, it may have practical value in selected cases. Serum CPK is unaltered by circulating

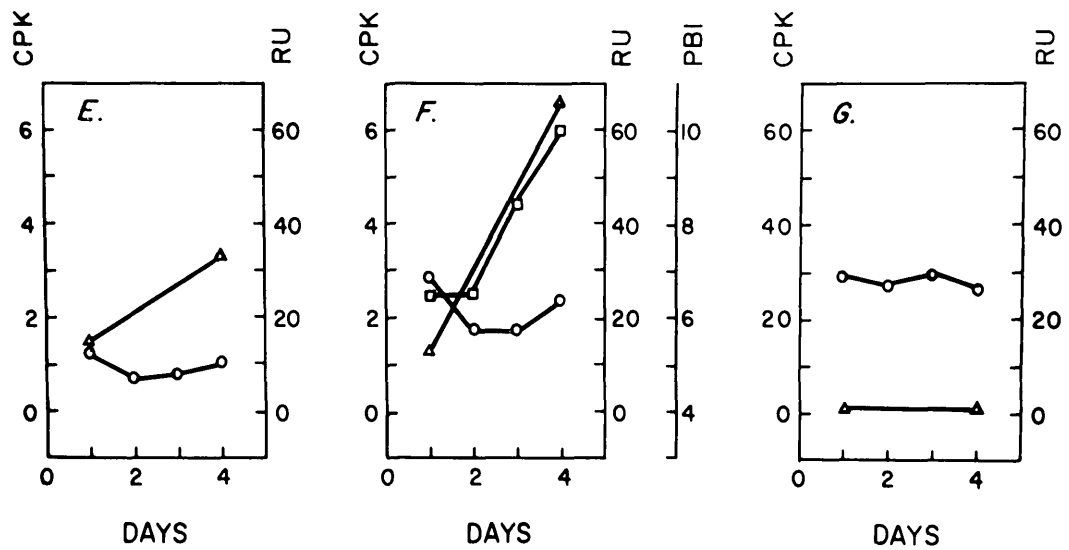

FIG. 4. Time course of serum CPK activity following the administration of TSH. Abbreviations, units and symbols as in Fig. 3. For description see text.

iodinated compounds and therefore it is a useful adjunct to the clinical examination in patients whose routine thyroid tests could not be evaluated. Unlike the PBI (31), the serum CPK level is uninfluenced by diphenylhydantoin and may be helpful in evaluating thyroid status in patients who, because of previous pituitary surgery, are continuously taking this medication. Serial serum CPK determinations show good correlation with thyroid activity and can be used for the early diagnosis of hypothyroidism that may follow ^{131}I therapy. Since the source of serum CPK is muscle, acute trauma (accidental or surgical) and widespread inflammatory process affecting skeletal or heart muscle may cause a rise in the serum activity of this enzyme. Certain degenerative diseases of muscle, especially Duchenne-type dystrophy, also result in highly elevated serum CPK levels (1, 3, 4, 9, 16). The differentiation of any of these diseases from hypothyroidism usually presents no difficulty. Their concomitant occurrence with hypothyroidism would invalidate the diagnostic significance of serum CPK determination. On the other hand, a significantly elevated serum CPK in the presence of hyperthyroidism has been helpful to us in calling attention to the presence of concurrent muscle disease.

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