ation, but was found to have many disadvantages:

(i) Fritted glass discs became clogged with dicyclohexylurea or triethylamine hydrochloride salt during the continued recycling of the reactants.

(ii) The reaction chamber was not very flexible to different quantities of polymer. This was especially noticeable because of the swelling of the polymer in such solvents as dimethylformamide (DMF) or methylene chloride. Similarly, one could not use large volumes of solvents to dissolve intermediates with low solubility in DMF, for example, *tert*-butyloxycarbonyl-nitroarginine or *tert*-butyloxycarbonyl-N^{im}-benzyl-L-histidine.

(iii) To avoid channeling flow, reversible flow was required, and a special circulating pump (peristaltic pump, Sigma-Motors, model TL) was essential. Further, suitable tubing for circulating both acids and solvents, such as DMF, is not available. "Tygon" was found to be suitable for acid conditions and "silastic" for DMF and methylene chloride, but it is inconvenient to change the tubing. Polyethylene tubing could not be used on this pump because it lacked proper flexibility.

(iv) Mixing was slower than in conventional shaking, and it was necessary to allow more time for mixing.

In view of these difficulties, we decided to construct an apparatus as illustrated in Fig. 3. It consisted of a 250- or 500-ml round-bottom flask, flattened at the bottom and fitted with two side arms. To side arm A was fitted, through a standard 24/40 joint, a bulb carrying a drying tube as an air-leak and a dropping funnel; the two octapeptides, L-alanine^3-L-isoleucine^5-angiotensin II (*6, 7*) and L-isoleucine^1-L-isoleucine^5-angiotensin II (*7, 8*), were synthesized with this apparatus in overall yields of 55 to 60 percent. However, low yields or impure products are not due to a faulty coupling or a faulty apparatus but to the final step in which the peptide is removed from the polymer. The best available procedure for the cleavage of peptide from the polymer requires the passage of hydrogen bromide through a suspension of the peptide-polymer in trifluoroacetic acid. Exposure for 15 to 20 minutes results in 50 to 60 percent yields of the angiotensin peptides, while exposure for 1 hour or more to obtain quantitative cleavage yields a complex mixture of products not hydrolyzable by leucine aminopeptidase (*9*) to the component amino acids. This final step warrants further investigation to improve the procedure of angiotensin synthesis.

Our apparatus appears to be very convenient. During the synthesis of the octapeptides it was found that the solvents or reagents could be transferred very conveniently, the polymer adhering to the sides of the side arm A could be washed in easily, mixing of the polymer and removal of solvents was very efficient, the flask could be immersed in ice water with simultaneous shaking for carrying out the coupling at low temperature, and the entire synthesis was carried out without opening or removing the flask from the shaker. For cleavage of peptide from the polymer, the peptide-polymer was washed twice with glacial acetic acid followed by trifluoroacetic acid. The polymer was then suspended in trifluoroacetic acid and a slow stream of hydrogen bromide bubbled through the fritted disc into the suspension. The polymer was filtered and washed with trifluoroacetic acid, and the peptide was obtained from the filtrate in the usual manner.

MAHESH C. KHOSLA*
ROBERT R. SMEBY
F. MERLIN BUMPUS
Research Division, Cleveland Clinic Foundation, Cleveland, Ohio 44106

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* Present address: Central Drug Research Institute, Lucknow, India.

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Creatine Phosphokinase in Thyroid: Isoenzyme Composition Compared with Other Tissues

Abstract. *Considerable creatine phosphokinase activity was demonstrated in thyroid glands of several species. After starch gel electrophoresis, thyroid exhibited a creatine phosphokinase isoenzyme pattern different from that of muscle, heart, and brain. The isoenzyme repertory of various tissues and a hybrid enzyme produced from muscle and brain enzyme are consistent with the postulated dimeric structure of creatine phosphokinase.*

In addition to those tissues (brain, and skeletal and cardiac muscle) previously reported to be rich in creatine phosphokinase (*1*), we have found significant concentrations of this enzyme in the thyroid gland of several species including man (*2*), confirming the finding of Hess *et al.* on a small sample of human thyroids (*3*). The creatine phosphokinase activity of thyroid tissue of various species is listed in Table 1.

Separation of isoenzymes was accomplished by starch gel electrophoresis. Aqueous homogenates (1:5 for muscle, heart, and brain; 1:3 for thyroid) of freshly obtained rabbit tissues were centrifuged at 100,000*g* for 1 hour and the supernatants were electrophoresed in borate buffer, *p*H 8.0, ionic strength 0.12, with a current of 5 volt/cm for 16 hours. The gel was repeatedly washed with 0.2*M* glycine buffer, at *p*H 6.75, because the subsequent staining reaction proceeds poorly at the higher *p*H. Creatine phosphokinase activity on the gel was demonstrated with the following reaction mixture: creatine phosphate $7.6 \times 10^{-3}M$, adenosine diphosphate $1.1 \times 10^{-3}M$, Mg acetate $1.0 \times 10^{-1}M$, glucose $3.3 \times 10^{-2}M$, nicotinamide adenine dinucleotide phosphate $9.2 \times 10^{-4}M$, nitroblue tetrazolium $5.9 \times 10^{-4}M$, phenazine methosulfate $1.4 \times 10^{-4}M$, hexokinase 490 international units per 100 ml, glucose-6-phosphate dehydrogenase 420 international units per 100 ml, prepared in 1.0*M* glycine buffer.

This linked reaction is a modification of Oliver's method (*4*) and results in deposition of a blue formazan at the sites of creatine phosphokinase activity in the gel. Adenylic kinase also yields formazan. Omission of creatine phosphate from the reaction mixture often revealed a faintly stained slow band of adenylic kinase.

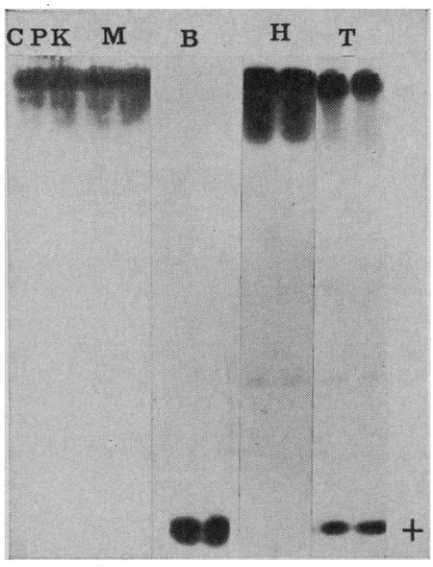

Fig. 1. Starch gel electrophoretic pattern of commercial crystalline enzyme (*CPK*, creatine phosphokinase) and of high-speed supernatants of muscle (*M*), brain (*B*), heart (*H*), and thyroid (*T*). Enzymatic activity was demonstrated by a method described in the text.

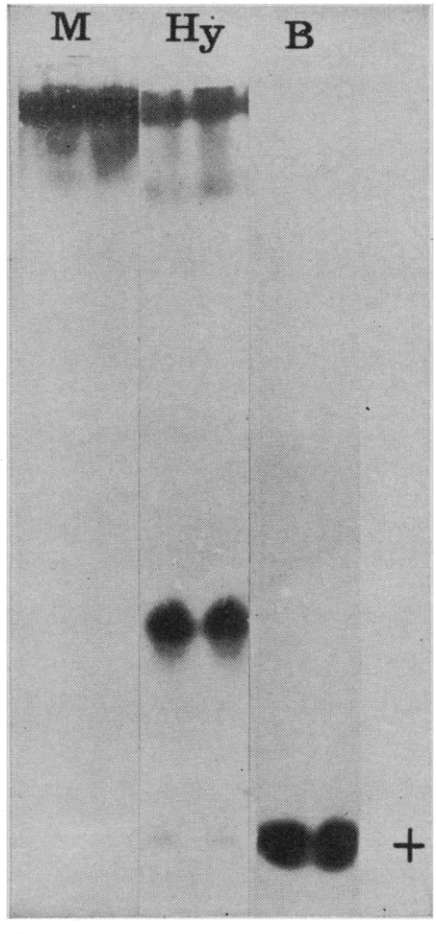

Fig. 2. Starch gel electrophoretic pattern of high-speed supernatants of muscle (*M*), brain (*B*), and hybrid enzyme (*Hy*). Enzyme stain as in Fig. 1.

Figure 1 is a combined photograph, demonstrating the migration of creatine phosphokinase isoenzymes on starch gel. Brain exhibits a single fast-moving anodal band; muscle, only a slow anodal component. Commercially available crystalline enzyme (prepared from rabbit muscle) gives a pattern identical with that of muscle supernatant. Myocardial enzyme exhibits a slow-moving and an intermediate band; thyroidal enzyme consists of slow, intermediate, and fast-migrating components.

A triple sub-banding can be discerned within the slow component of creatine phosphokinase. Supporting media other than starch, such as agar gel (*5*) or polyacrylamide gel (unpublished observations) invariably show the slow component as a homogeneous band. Therefore it is our belief that the sub-banding observed on starch gel does not represent true molecular heterogeneity. Microheterogeneity of enzyme molecules has been demonstrated, for example with lactic dehydrogenase when this protein was subjected to high-voltage electrophoresis (*6*) or treated with β-mercaptoethanol (*7*).

Prolonged storage of tissues or supernatants at −20°C resulted in reduction in the activity of the fast and intermediate bands of thryroidal and heart enzyme, respectively. The slow-moving component appeared to lose less of its activity with storage. Loss of enzymatic activity of creatine phosphokinase may occur owing to oxidation of thiol groups. The initial activity can be restored with the addition of cysteine (*8*). It is possible that those isoenzymes which lose activity more readily with storage have more exposed thiol groups than those more resistant to storage. However, addition of cysteine to the staining mixture is not feasible, since it results in a dark precipitate.

If a mixture of muscle and brain supernatants is frozen and thawed in the presence of 1*M* NaCl and 0.1*M* Na_2PO_4 a hybrid enzyme is formed. This is similar to the method of Chilson *et al.* for lactic dehydrogenase hybridization (*9*). On starch gel electrophoresis the isoenzymes of the parent molecules and a newly formed intermediate band are demonstrable (Fig. 2). Both the isoenzyme distribution of the tissues examined and the electrophoretic migration of the hybrid are consistent with the concept that creatine phosphokinase is a dimer, as suggested by other evidence (*10*). Similar conclusions have been reached by agar gel electrophoresis (*5*), although thyroid tissue was not tested.

Table 1. Creatine phosphokinase activity of thyroid tissue in various mammals. Results are expressed as micromoles of substrate converted per gram of wet tissue, per hour, at 37°C. Numbers in parentheses indicate number of specimens assayed.

Mammal	Range	Mean
Man (7)	690 to 2110	1027
Rat (7)	1312 to 5578	2074
Lamb (3)	563 to 1030	852
Calf (8)	510 to 2130	1309
Dog (2)	650 to 758	704
Rabbit (3)	1049 to 3551	2530

On the basis of available evidence, and in analogy with the well-known tetrameric nomenclature of lactic dehydrogenase, the following terminology is suggested for creatine phosphokinase isoenzymes:

Muscle: MM; *Brain*: BB; *Heart*: $\frac{MM}{MB}$

Thyroid: $\frac{MM}{(MB)}$; *Hybrid*: $\frac{MM}{MB}$

$\frac{BB}{}$ $\frac{BB}{}$

This nomenclature corresponds to the one used by Dawson *et al.* (*5*).

The invariable presence of significant amounts of creatine phosphokinase in thyroid tissue suggests that this enzyme may have a role in thyroid tissue metabolism or hormone biosynthesis, thus far not appreciated.

FRANK A. GRAIG*
J. CRISPIN SMITH
Montefiore Hospital and Medical Center, Bronx, New York 10467

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* Present address: Grasslands Hospital, Valhalla, N.Y. 10595.

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