# HIGH ACTIVITY OF CREATINE KINASE IN MITOCHONDRIA FROM MUSCLE AND BRAIN AND EVIDENCE FOR A SEPARATE MITOCHONDRIAL ISOENZYME OF CREATINE KINASE

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Creatine kinase (CK) was shown to be present in numerous kinds of animals (Ennor,1961;Kuby et al.,1962).The enzyme was found mainly in muscular,electric and nervous organs.But until now no data were available on the intracellular distribution of CK in these organs.

During studies concerning $P^{32}$incorporation into isolated pigeon breast mitochondria we observed $P^{32}$ labelling of creatine phosphate.By filtering centrifugation it was demonstrated that the observed creatine phosphate was actually formed inside the mitochondria (Heldt,1962).

In studies on fractionated enzyme extraction of rat heart and pigeon breast muscle homogenates Pette and Luh found the occurrence of CK in the fraction of mitochondrial enzymes(Pette and Luh,unpublished,1962).But it was not established that CK was really part of the mitochondria.

In this paper it is shown that high levels of CK are contained in isolated mitochondria controlled for the absence of cytoplasmic contamination.

## Experimental procedure

Mitochondria were prepared and extracted according to procedures described in detail elsewhere (Jacobs,Heldt and Klingenberg,in prep.).As a measure of purity of the mitochondrial preparations a maximum freedom from lactate dehydrogenase was taken.The extraction was done in a 0.1 M phosphate buffer of pH 7.2 which contained 0.01 M reduced

glutathione to stabilize the enzyme.The activity of CK was measured under standard conditions(Bücher et al.,1964).For agar gel electrophoresis total and mitochondrial extracts were prepared as 10% homogenates of tissue or a tissue equivalent of mitochondria in the mentioned phosphate-glutathione medium.-Cytoplasmic extracts were prepared by careful extraction of a 10% tissue dilution in 0.3 M sucrose,0.01 M triethanolamine-HCl,pH 7.2(Delbrück et al.,1959).-1 µl samples of high speed supernatants were employed for electrophoresis. Technique of agar gel electrophoresis was based on methods developed in this institute(Wieme,1959;Küchemann,1962).Preparation of agar gel and test reaction for the enzyme in the agar layer were performed according to Burger et al.(1964).

## Results

1.)The activity of CK which was shown to be present in various types of isolated mitochondria(Jacobs and Heldt,1963) is given in table 1. While mitochondria from muscle and brain contain considerable activity of CK,virtually no activity of CK was detected in mitochondria from liver and kidney.

| mitochondria | CK $\left[\frac{\mu\text{moles DPNH}}{\text{min}\cdot\text{gr prot}^*}\right]$ | | n |
|---|---|---|---|
| | $\bar{x}$ | $\sqrt{s^2}$ | |
| Pigeon,skelet.muscle | 1730 | 352 | 10 |
| heart | 700 | 164 | 7 |
| Rat, skelet.muscle | 1010 | 160 | 11 |
| heart | 960 | 180 | 11 |
| brain | 895 | 242 | 6 |
| liver | <10 | | 4 |
| kidney | <10 | | 5 |

Table 1. Activities of creatine kinase (CK) in isolated mitochondria(*per gram mitochondrial biuret protein)
n = number of mitochondrial preparations
$\bar{x}$ = arithmetic mean value, $\sqrt{s^2}$=standard deviation

2.)A comparison between the CK of the total organ and the activity of the mitochondria is given in table 2.The mitochondrial activities as referred to the mitochondrial pro-

tein are compared with the total activities of the tissues as referred to fresh weight by means of the "cytochrome a factor" (Schollmeyer and Klingenberg,1962;Klingenberg,1964).

| | activity of total organs | activity of mitochondria | activity of mitochondria as part of total |
|---|---|---|---|
| | $\left[\frac{\mu\text{moles DPNH}}{\text{min}\cdot\text{gr fr}}\right]$ | $\left[\frac{\mu\text{moles DPNH}}{\text{min}\cdot\text{gr fr}}\right]$ | |
| Rat,skelet.muscle M.quadriceps | 396 | 26 | 7% |
| Pigeon,skel.muscle M.pectoralis | 406 | 100 | 25% |
| Rat,heart | 111 | 53 | 48% |

Table 2. Comparison between total and mitochondrial CK activities of various muscular tissues

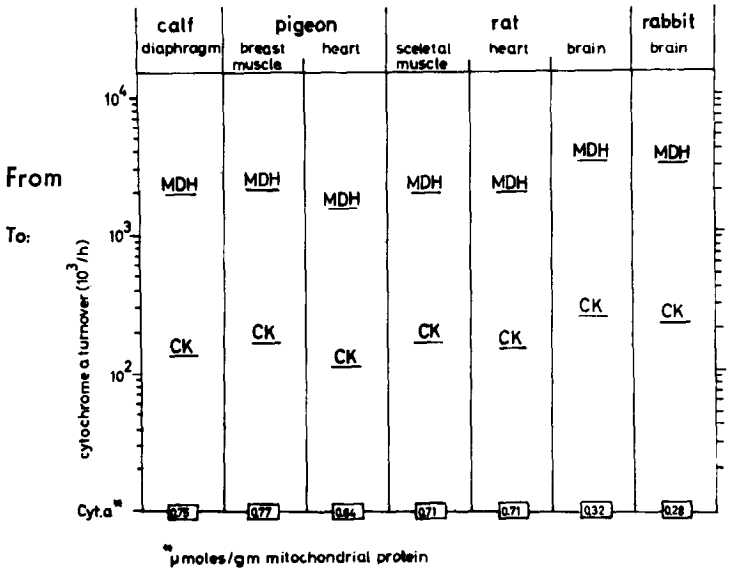


Figure 1

3.)In figure 1,mitochondrial CK and malate dehydrogenase (MDH) activities have been plotted versus the content of cytochrome a on a logarithmic scale:This enzyme pattern exhibits a constant relation between CK and the two other enzymes.

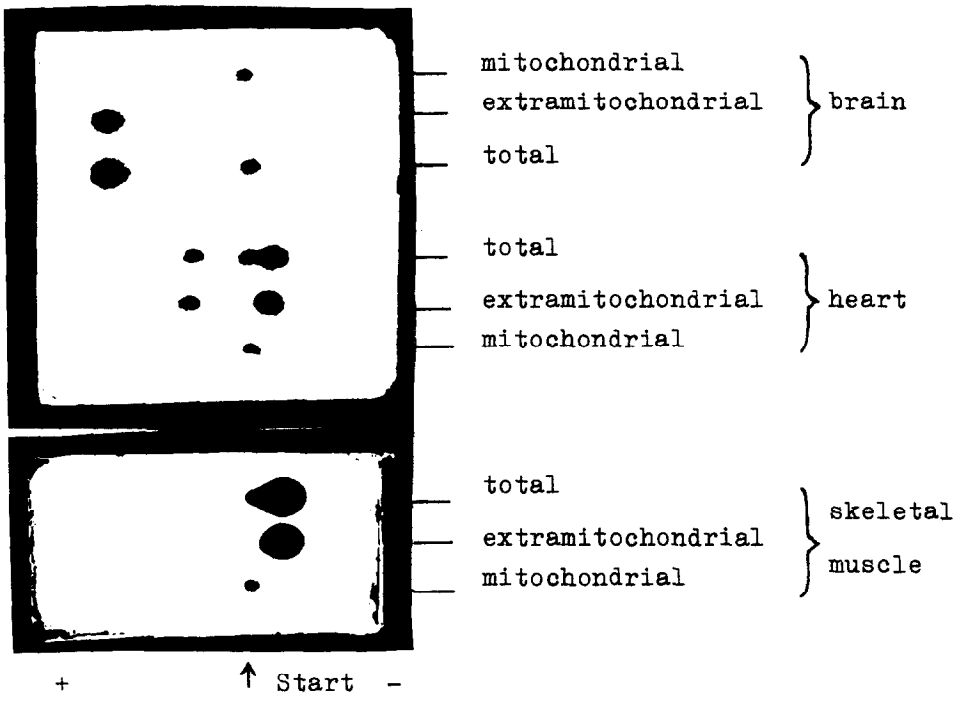


Figure 2. Agar gel electrophoresis of total,extramitochondrial and mitochondrial extracts from various rat organs. Conducted at 0°C,150 V,30-50 mA for 45 minutes on 0.8% purified agar buffered with Tris-NaCl pH 9.0

4.)By means of agar gel electrophoresis intra- and extramitochondrial creatine kinases can be separated as isoenzymes. Figure 2 shows the distribution of CK in total,extramitochondrial and mitochondrial extracts from brain,heart and skeletal muscle of the rat.Spots A,B and D represent different extramitochondrial isoenzymes of CK,while spot C is demonstrated to be of mitochondrial origin.Mitochondrial CK from various organs shows identical behaviour in contrast to the differences between extramitochondrial enzymes.

## Discussion

The results show that a considerable amount of creatine kinase (CK) activity is actually located in mitochondria from muscle and brain.The data also show a constant relation between the CK activity in these mitochondria and the "constant proportion group" of the enzymes of the respiratory chain and the citric acid cycle (Pette et al.,1962). Constant proportion groups of enzymes of the main pathways have been shown to exist in widely varying types of tissues. (Bücher and Pette,1961).In the case of mitochondrial CK the constant proportion appears to be limited only to those tissues which utilize creatine.Whenever CK occurs in a tissue,the activity of the mitochondrial part is determined by the constant proportion to the respiratory chain.Thus it is found in all cases investigated at the same relatively high level.

By the electrophoretic investigations a recent report by Burger et al.(1964) is confirmed as far as the extramitochondrial CK is concerned.These authors showed the heterogeneity of CK from different organs by means of agar gel electrophoresis.They failed to detect mitochondrial activities as they investigated tissue extracts,which were prepared with isotonic sucrose.

The agar gel electrophoresis shows a uniformity of the intramitochondrial enzymes of various organs in contrast to the heterogeneity of the extramitochondrial enzymes.One may draw a parallel to the finding that the mitochondrial CK forms a constant proportion group with the enzymes of the respiratory chain.Extramitochondrial CK on the other hand does not seem to be a member of a constant proportion group (Pette,unpubl.).

Hitherto in two other cases extra- and intramitochondrial localization of enzymes was shown to be combined with the occurrence of separate extra- and intramitochondrial isoenzymes: Malate dehydrogenase (Siegel et al.,1961;Englard et al.,1962) and glutamate - oxalacetate transaminase (Morino et al.,1963;Morino et al.,1964).The isoenzymes have been isolated and shown to be different in their physical and chemical properties.Creatine kinase is another example of this type of intra- and extramitochondrial isoenzymes.

The considerable activity of mitochondrial CK makes it probable that the enzyme is part of a still unknown pathway of mitochondrial phosphate metabolism. Observations on reactions of isolated mitochondria with external creatine and creatine phosphate indicate that high-energy phosphate is transferred by this way between intra- and extramitochondrial compartments (Jacobs and Klingenberg, unpublished). Research on the phosphorylation and dephosphorylation of high-energy phosphate during rest and work of muscle and brain has to account for this new way of phosphate transfer.

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