Consequently one might expect differences in primary structure of the creatine kinases. Determinations of amino acid compositions of purified brain enzymes from sparrows however do not indicate significant differences.

It is evident that only experiments on purified enzymes can definitely decide which hypothesis is correct. Such experiments are in progress in our laboratories. If it can be confirmed that the new isoenzymes are different conformations of the brain enzyme or its subunits, it is very likely that this is not a random phenomenon but has physiological implications, since first of all different organs show typical differences in relative amounts of these isoenzymes. Furthermore it is not very probable that a mutation, which only led to a random phenomenon, should persist over a long period of immense morphological and physiological divergence in bird's evolution[10,11].

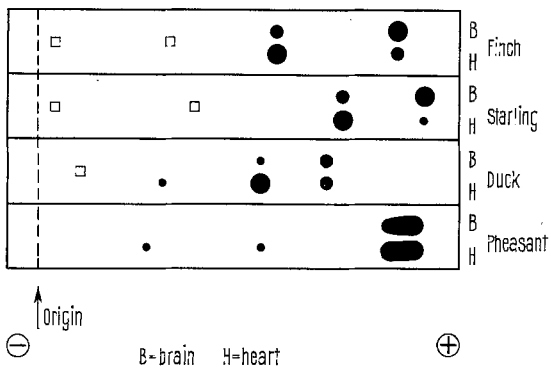

Fig. 2. Tracings of creatine kinase isoenzymes from brain- and heart-extracts of various birds (finch, *Fringilla coelebs*; starling, *Sturnus vulgaris*; duck, *Aythya fuligula*; pheasant, *Chrysolophus pictus*). Conditions of electrophoresis see Figure 1. The relative intensities of isoenzyme bands (compare Figure 1) are indicated by the different size of the spots. The positions of isoenzymes which have been observed in muscle tissue of adult birds are indicated by open squares.

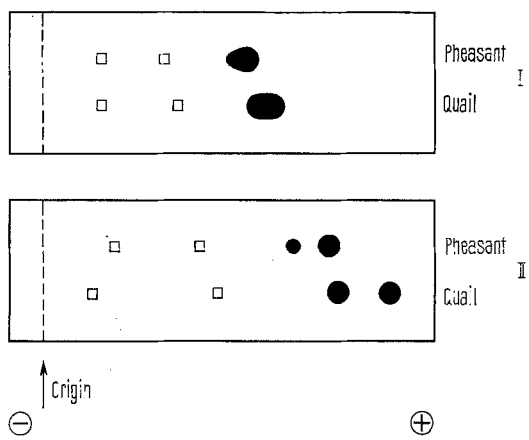

Fig. 3. Electrophoretic separation of creatine kinases in supernatant fractions from brain homogenates in (I) veronalbuffer 0.02 *M*, pH 8.6, 40 mA, 75 min and (II) citrate-NaOH-buffer 0.01 *M*, pH 6.5, 30 mA, 90 min. Pheasant, *Chrysolophus pictus*, quail, *Francolinus francolinus* (Galliformes). The positions of the other isoenzymes (MM/MB) are indicated by squares.

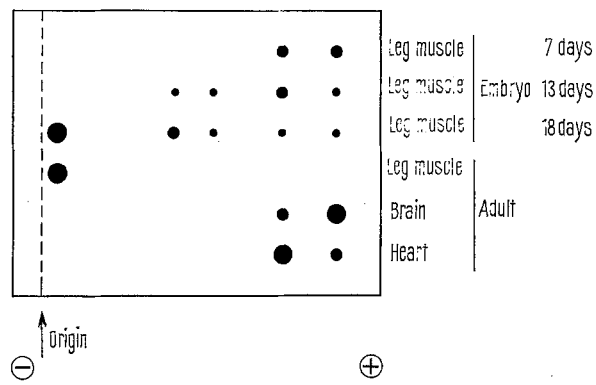

Fig. 4. Ontogenetic changes of isoenzyme patterns in developing leg muscle (domestic duck). The patterns in various organs from the adult duck are added for comparison (lower 3 separations). Conditions as given in Figure 1.

Zusammenfassung. Durch Abwandlung der elektrophoretischen Auftrennungsmethode wird das Vorkommen einer neuen Isoenzymbande der Kreatinkinase, das ursprünglich auf die Vogelordnung Passeriformes beschränkt zu sein schien, allgemein in verschiedenen Geweben von Vögeln nachgewiesen. Eine weitere neue Isoenzymbande wird in bestimmten Entwicklungsstadien der Skelettmuskulatur beobachtet. Es wird diskutiert, dass diese neuen Isoenzyme alternative thermodynamisch mögliche Konformere der Gehirn-Kreatinkinase (BB-Enzym) oder ihrer Monomere (B) sein könnten. Anhand der Untersuchungsergebnisse wird aufgezeigt, dass diesen vermutlich durch Konformationsunterschiede bedingten Isoenzymen physiologische Bedeutung zukommen könnte.

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[10] Supported by 'Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung' and by a grant from 'Muscular Dystrophy Associations of America, Inc.'.

[11] The technical assistance of Miss Evelyne Mathys is gratefully acknowledged.

Creatine of Mouse Brain: Evidence of Active Uptake from Blood

The existence of a distinct concentration gradient of creatine between plasma and brain in man and animals is known and well documented for some time[1]. Although it was shown more recently that cerebral tissue is capable of synthetizing creatine from suitable precursors[2,3], it is yet to be determined whether intracerebral formation of creatine constitutes the sole source for the brain to replenish its comparatively high content of this compound. To examine the possible contribution of peripheral creatine to the cerebral pool by way of its transfer from blood to brain, we have studied the distribution

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of creatine-1-^{14}C between plasma and brain in mice. Preliminary data of these experiments will be presented.

White male mice weighing 25–30 g received into the tail vein 0.1 ml physiological saline containing 0.80 μc of creatine-1-^{14}C (The Radiochemical Centre, Amersham, England; specific activity 12.8 mc/m*M*). The animals were killed by decapitation at various time intervals thereafter and the radioactivity of plasma and brain (dpm/ml and dpm/g, respectively) was determined (Table I). The total creatine content of plasma and brain (free and phosphate-bound creatine) was assayed by the colorimetric diacetyl method[4,5]. The specific activity of plasma creatine was determined by thin-layer chromatography of trichloroacetic acid (TCA) extracts of plasma after the removal of TCA with diethyl ether (Table II).

The data of Table I on the distribution of radioactivity between plasma and brain at several time intervals after the injection of the label reveal that creatine enters the brain. While the activity in plasma keeps falling, at first rapidly then more slowly, radioactivity of the brain is steadily increasing to reach maximum levels between 36 and 48 h of the experiment. During this period fairly stable levels of both plasma and brain radioactivity were found indicating that a steady state between plasma and brain, and other organs and body tissues as well, was attained regarding the exchange of creatine-1-^{14}C and the release of newly formed labelled creatinine.

To determine, therefore, more specifically the distribution of creatine-1-^{14}C between brain and plasma, the percentage of plasma radioactivity attributable to creatine-1-^{14}C was determined (Table II). The radioactivity of brain tissue was not further corrected on the assumption that all the radioactivity present in brain was originally taken up as creatine. Values of 19.4 and 22.9, respectively, were then obtained for the distribution ratio of labelled creatine between brain and plasma at 2 time intervals. These figures are in close agreement with the ratio of 19.4 found for the normal distribution of creatine between brain and plasma in mice.

The results of these experiments demonstrate that in mice creatine is being taken up by brain against a concentration gradient even if the fact is taken into consideration that up to 60% of the total creatine of brain may be present as phosphocreatine[6]. This observation is in line with results of in vitro studies on the uptake of creatine by cerebral tissue slices[7]. In vivo uptake of creatine by brain has been studied in the past when rabbits were given large oral doses of creatine twice daily for 7 days[8]. Even so, there was no demonstrable increase in brain creatine levels and it was concluded that creatine does not enter the brain. This study has shown that uptake of creatine by brain is rather slow and it seems possible that the rate of uptake is quantitatively related to the conversion of creatine to creatinine and the release of the latter from brain. In this case there would be no net uptake of creatine precluding the demonstration of an influx of creatine into the brain when inert material was used.

Table I. Total radioactivity in plasma and brain tissue of mice receiving creatine-1-^{14}C intravenously

	Time elapsed since injection of creatine-1-^{14}C				
	5 min	8 h	24 h	36 h	48 h
Plasma (dpm/ml)	88,777 ± 7,941	4,767 ± 2,039	2,622 ± 534	1,373 ± 221	1,386 ± 423
Brain (dpm/g)	3,885 ± 837	10,050 ± 2,516	15,153 ± 3,317	18,363 ± 2,281	18,748 ± 1,640

Values represent the mean of 5 animals or more. For the counting procedure aliquots of plasma or brain tissue (hemispheres) were dissolved in a solubilizing base (NCS, Nuclear Chicago) and the total radioactivity of the samples was determined by liquid scintillation counting. Counts were converted to disintegrations on the basis of a standard quench correction curve.

Table II. Brain-to-plasma ratios of creatine and creatine-1-^{14}C in mice

	Total creatine[a] Plasma mg/100 ml Brain mg/100 g	Creatine-1-^{14}C dpm/ml (g) after 36 h	48 h
Plasma	6.84 ± 2.54 (24)	945[b]	819
Brain	132.70 ± 23.35 (40)	18,363[c]	18,748[c]
Brain / Plasma	19.4	19.4	22.9

[a] Total creatine after hydrolysis of the perchloric acid extract at 65 °C for 10 min. Figures in parentheses indicate number of animals. [b] Specific activity is expressed as dpm of creatine-1-^{14}C per milliliter of plasma. For the determination of the specific activity plasma of 5 animals was pooled, treated with trichloroacetic acid and extracted with diethyl ether. Creatine and creatinine were added as carrier and suitable aliquots were separated on cellulose layers in *n*-butanol - glacial acetic acid - water (60:15:15)[9]. The individual spots were scraped off and eluted with 0.1 *N* hydrochloric acid for the determination of radioactivity by liquid scintillation counting. [c] Dpm in brain represent total radioactivity per gram of tissue.

Zusammenfassung. Bei Mäusen wurde die Verteilung von Kreatin-1-^{14}C zwischen Plasma und Gehirn nach einer einmaligen i.v. Gabe von Kreatin-1-^{14}C untersucht. Ein Fliessgleichgewicht stellte sich 36–48 h nach Verabreichung der Substanz ein, wobei Kreatin gegen ein Konzentrationsgefälle aus dem Plasma in das Gehirn übertritt. Während dieser Zeit war die Radioaktivität im Gehirngewebe um das 20fache höher als im Plasma.

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