The fluorescence spectra of pig heart mitochondrial F_1-ATPase indicate the presence of at least 2 aromatic species. The fluorescence intensity of tyrosine in solution is much lower than that of tryptophan when the excitation wavelength is higher than 290 nm, because above this wavelength only tryptophan absorbs[14]. The increase in the ratio of the fluorescence emission of F_1-ATPase at 334 nm to that at 310 nm, with an increase in the excitation wavelength, therefore indicates the presence of tryptophan. In most proteins, when tryptophan is present, its fluorescence masks that of tyrosine. The latter is normally detected with a low yield and with a maximum emission around 304 nm only in the absence of tryptophan. The tyrosine fluorescence in proteins is normally diminished by the quenching effect of hydrogen bonds formed between tyrosine and amino acid side chains[9] or peptide carbonyl groups[15]. Thus, the high fluorescence of tyrosine in F_1-ATPase may indicate the presence of tyrosine which is exposed and not quenched; the quenching normally induced by the vicinal peptide carbonyl groups would be abolished either by helical conformation or by location of these peptide linkages in a nonpolar environment[15]. Similar spectra showing an important contribution of the tyrosine in the total fluorescence spectra have been described for myelin and for the basic protein of myelin[16]. Myelin basic protein and F_1-ATPase are both proteins which can strongly interact with lipids in membranes, and are considered to sit on 1 face of the membrane[17,18]. It would be interesting to know if this high tyrosine fluorescence is a general characteristic of such membrane proteins, and if F_1-ATPase reassociated with liposomes keeps this property as does basic protein in myelin.

The presence of tryptophan has not been detected in beef heart F_1-ATPase by amino acid analysis[7] or in chloroplast F_1-ATPase. However, it is possible that a low content of tryptophan may have been overlooked in a protein such as F_1-ATPase, which has a high mol.wt (360,000 for beef heart ATPase according to Senior[4]), especially because tryptophan is susceptible to degradation during the hydrolysis of the protein that must precede the amino acid analysis. It is unlikely that the tryptophan fluorescence observed in F_1-ATPase is due to a contaminant protein, for, when the spectra were done with an F_1-ATPase preparation that contained contaminants (detectable in sodium dodecyl sulfate gel electrophoresis as 2 small protein bands of mol.wt around 80,000), the contribution of tryptophan in the spectra was not increased as compared to spectra obtained with a preparation containing no visible contaminant.

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Imbalance in the activities of alkaline phosphatase and Na^+-K^+-ATPase in the brain of experimentally induced phenylketonuric squirrels *(Funambulus palmarum)*[1]

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Summary. Phenylketonuric squirrels have shown marked inhibition of alkaline phosphatase in the olfactory lobes and cerebral hemispheres, whereas the Na^+-K^+-ATPase remained less altered. In the pathogenesis of phenylketonuria inhibition of alkaline phosphatase at the level of 'Blood-Brain Barrier' (BBB), leads transport system to impaired functioning.

In phenylketonuria, the massive increase and accumulation of phenylalanine and its metabolites has been believed to cause permanent brain damage in men. The intricacies of altered brain functions in phenylketonuria are sill unsolved. Previous reports have, however, shown that the uptake of other amino acids into the brain is inhibited if the concentration of a single amino acid increases in the plasma[2,3]. It is far from clear how the entry of amino acids into the brain is repressed. An inhibition of enzymes mediating transport of amino acids, has, however, been presumed to be one of the causative factors. In order to evaluate this supposition, the author has investigated the effects on alkaline phosphatase and Na^+-K^+-ATPase in the brain of experimentally produced phenylketonuria in squirrels.

Materials and methods. 1. The 1st series of experiments were performed on experimentally induced phenylketonuric squirrels, produced by the method of Antonas et al.[4] i.e. 4 days treatment of 300 mg/kg p-chlorophenylalanine + 200 mg/kg L-phenylalanine. In all cases, control groups received injections of equal volumes of 0.9% saline.
2. The 2nd series of experiments was carried out by directly injecting intracerebrally and bilaterally, under ether anaesthesia 8.0, 32.0 and 64.0 µg of L-phenylalanine in a total volume of 4 µl. Control groups received 4 µl of 0.9% saline. After 1 h the animals were killed by fracturing the neck and

Imbalance in the activities of alkaline phosphatase and Na^+-K^+-ATPase in the olfactory lobes and cerebral hemispheres of intracerebrally L-phenylalanine injected and phenylketonuric squirrels (enzyme activities are expressed as μmoles of inorganic phosphate liberated/g wet wt of tissue/h at 37 °C)

Enzyme	Treatment	Olfactory lobes Mean ± SEM	% change	Cerebral hemispheres Mean ± SEM	% change
Alkaline phosphatase	Control	60.2 ± 1.871	-	75.2 ± 1.871	-
	8 μg	44.0 ± 2.236	(−26.9)*	66.8 ± 2.916	(−11)*
	32 μg	22.8 ± 1.871	(−62)*	36.8 ± 2.450	(−51)*
	64 μg	15.8 ± 2.916	(−73)*	24.0 ± 2.739	(−67)*
	Pheylketonuric	38.6 ± 1.621	(−58)*	50.6 ± 3.184	(−48)*
Na^+-K^+-ATPase	Control	110.8 ± 6.782	-	133.8 ± 8.720	-
	8 μg	112.0 ± 3.162	NS	130.2 ± 2.916	NS
	32 μg	105.0 ± 4.472	NS	112.0 ± 2.739	(−16)*
	64 μg	87.8 ± 5.568	(−20)*	103.0 ± 3.674	(−23)*
	Phenylketonuric	114.4 ± 66.214	NS	120.2 ± 2.142	(−14)*

* $p < 0.001$; NS, not significant; (−) % inhibition. Results are expressed as mean value of 5 experiments ± SEM; p-value represents significance of difference between control and experimental (based on Student's t-test).

the brain regions were rapidly removed, weighed, homogenized and centrifuged. The clear supernatant was removed and stored at −10 °C until the enzyme analysis was carried out. Alkaline phosphatase assay mixture was designed according to the method of Chakrabarti and Daginawala[5]. The Na^+-K^+-ATPase activity was assayed by determining the difference between the amount of ATP hydrolyzed in the presence and absence of ouabain[6]. The hydrolyzed inorganic phosphate was measured according to the method of Fiske and Subbarow[7]. Substrate and tissue blanks were incubated under identical conditions with each set of experiment.

The values obtained were calculated as percent change of enzymatic activity as follows:

$$\frac{C-P}{C} \times 100 = \% \text{ change}$$

C = values obtained for control group of animals for each set of experiments; P = values obtained in the brain of phenylketonuric squirrels, or intracerebrally phenylalanine injected group of animals. The enzyme activity is expressed as μmoles of inorganic phosphate liberated/g wet wt of tissue/h at 37 °C.

Results. Injections of L-phenylalanine at different concentrations show marked and statistically significant inhibition of alkaline phosphatase from olfactory lobes as well as from the cerebral hemispheres (table). Increasing concentrations of phenylalanine from 8.0 μg to 64.0 μg produced 26.9–73% inhibition of enzyme from olfactory lobes, whereas these concentrations are comparatively less inhibitory in the case of cerebral hemispheres (11–67%). Experimentally induced phenylketonuric squirrels showed 58 and 48% change in the alkaline phosphatase activity of the olfactory lobes and cerebral hemispheres (table).

Na^+-K^+-ATPase from both the regions of the brain, at 8.0 and 32.0 μg showed statistically insignificant alterations in activity (table). However, the 64-μg concentration of phenylalanine produced small but statistically significant changes in the Na^+-K^+-ATPase activity for olfactory lobes (20%) and cerebral hemispheres (23%). The experimentally produced phenylketonuric squirrels showed insignificant changes in the Na^+-K^+-ATPase activity in both regions of the brain.

Discussion. In phenylketonuria, mental retardation has been considered as one of the factors which results from the impairment of transport mechanisms in the brain. The anatomical sites of the BBB, brain capillaries and glial cells[8,9] show intense alkaline phosphatase activity[11,12]. Bhatt[12] has also reported the role of alkaline phosphatase in transport mechanisms at the level of the BBB. Therefore, the inhibition of alkaline phosphatase in the brain regions of phenylketonuric squirrels may be interpreted in relation to the repressed transport of metabolites at the level of BBB. Other studies have also reported that an elevated level of phenylalanine inhibits the uptake of other amino acids into brain both in vivo[3,10,13] and in vitro[14,15]. In agreement with these investigations author suggests that impaired uptake and incorporation of amino acids into brain proteins, owing to repressed alkaline phosphatase activity, may be one of the causative factors in the mental retardation in phenylketonuria. Antonas and Coulson[16] have also shown 50% reduction both in uptake and incorporation of leucine and a parallel reduction in the acid-soluble leucine pool in the proteins of the brains of experimentally phenylketonuric rats.

The results of present investigation do not support the idea that the Na^+-K^+-ATPase system is altered in phenylketonuria. Thus the theory that in phenylketonuria impaired transportation is due to the inhibition of Na^+-K^+-ATPase receives little support.

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