Monoamine oxidase activities in different strains of mice

Strain	Age	Specific activity, SE				Relative activity, SE			
		Brain		Liver		Brain		Liver	
C57B1/6J (4)	18–20 days	11.82	0.44[a]	166.67	5.75[b]	0.336	0.012[b]	5.74	0.20[b]
CBA/J (8)	18–20 days	9.26	0.25[a]	97.80	3.40[a]	0.249	0.015[b]	2.58	0.20[a]
$B6CBAF_1$ (8)	20 days	9.37	0.52	104.65	7.77	0.256	0.02	3.23	0.30
C57B1/6J (7)	6 weeks	13.40	1.48	104.03	16.03	0.387	0.043	4.26	0.75
CBA/J (6)	6 weeks	11.37	0.66	93.65	5.93	0.317	0.023	3.37	0.36
DBA/2J (6)	6 weeks	12.80	0.56	133.52	8.02	0.349	0.011	4.84	0.17

Specific activity = nmole Kynuramine deaminated/mg protein/30 min. Relative activity = μmole Kynuramine deaminated/g tissue/30 min. SE, standard error; ( ) number of mice; [a] Student t-test, $p < 0.05$; [b] $p < 0.01$.

The mice were killed by decapitation. The brains and livers were then excised and frozen immediately at –70 °C. On the day of assay the organs were weighed and homogenized in 9 volumes of 0.25 M sucrose. The homogenates were then centrifuged at $1500 \times g$ for 10 min to remove cellular debris and the supernatant again centrifuged at $18{,}000 \times g$ for 20 min to pellet the mitochondria. The mitochondria were then resuspended in a volume equal to 3× the wet weight of the organ. Protein was determined by the LOWRY method[18] and MAO according to WEISSBACH et al[19]. Since interference occurred with mitochondrial suspensions from brain materials at the adsorption peak of the substrate, kynuramine dihydrobromide (360 nm) we read the adsorptions at 328.5 nm which is the peak for the reaction product, 4-hydroxyquinoline. As the reaction is equimolar we express all the results as the amount of substrate utilized. Enzyme activity was halted either by boiling the homogenate for 10 min or the addition of 0.6 ml of 10 mM iproniazid phosphate to the reaction mixture, but was unaffected by the addition of 10 μmoles of potassium cyanide.

Results and discussion. None of the mutations tested affected MAO as we found no differences in MAO between any of the mutants and their respective littermate controls (data not shown). However, there were significant differences ($p < 0.05$) between some of the mutants i.e. reeler (*rl*), jimpy (*jp*^{msd}) and wobbler lethal (*wl*). Since these differences did not result from an effect of the mutant genes themselves they must have resulted from differences in the genetic background or inbred strains. Our results are presented in the Table. For example strain C57BL/6J has significantly higher levels of MAO in both brain and liver than strain CBA/J. The hybrid between them ($B6CBAF_1$) is like CBA/J suggesting dominance for low over high MAO activity. The fact that differences in MAO between strains occurred only up to 20 days of age but not at 6 weeks may reflect genotype-dependent differences in rates of organ development and maturation. Thus, our data confirm the age differences in levels of MAO observed by others[13–15].

[18] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).

[19] H. WEISSBACH, T. E. SMITH, J. W. DALY, B. WITKOP and S. UDENFRIEND, J. biol. Chem. *235*, 1160 (1960).

Serum Enzyme Activities in the African Elephant (*Loxodonta africana*)

I. R. F. BROWN[1] and P. T. WHITE[2]

Department of Chemical Pathology, St.George's Hospital Medical School, University of London, Blackshaw Road, London SW17 0QT (England); and Department of Zoology, Makerere University, P.O. Box 7062, Kampala (Uganda), 6 February 1976.

Summary. The serum activities of aspartate aminotransferase, alanine aminotransferase, α-hydroxybutyrate dehydrogenase and creatine phosphokinase have been measured in the African elephant. In general, the values were broadly comparable with those of man except that alanine aminotransferase was much lower and creatine phosphokinase higher. No variation due to age, sex, season or location was observed.

Measurements of the levels of the enzymes found in blood serum are widely used in clinical laboratories as diagnostic aids in the treatment and assessment of human disease. Increasing use of such assays is also being made in veterinary laboratories in the care and management of domestic animals. The African elephant, *Loxodonta africana*, is an animal of some commercial importance and, since we are currently involved in a wide ranging study of the elephant in the game parks of Uganda, we decided to measure the serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), α-hydroxybutyrate dehydrogenase (α-HBD, lactate dehydrogenase-1-isoenzyme) and creatine phosphokinase (CPK) and to find out what factors, if any, caused variations in these levels.

Material and methods. Blood was obtained immediately after death from elephants shot in the Rwenzori and Kabalega National Parks in Uganda. Serum samples were frozen as soon as practicable and then flown to the United Kingdom for analysis.

AST, ALT, α-HBD and CPK were all estimated using optimized standard methods[3] obtained in kit form

Table I. Serum enzyme levels in the African elephant

Enzyme	No. of animals (♂ = male, ♀ = female)	Age (range in years)	Mean enzyme activity (U/l)	Range (U/l, 95% probability)	Human reference values (U/l)
AST	64 (17♂, 47♀)	0.5–60	19.7[a]	3–117	10– 42
ALT	58 (18♂, 40♀)	2 –60	3.5	0– 10	10– 55
α-HBD	65 (17♂, 48♀)	0.5–60	131.5[b]	31–232	70–230
CPK	27 (8♂, 19♀)	2 –60	152.9[c]	0–324	0–100

[a] Omitting 3 very high values of 371, 480 and 925 U/l. [b] Omitting 2 very high values of 437 and 464 U/l. [c] Omitting 1 very high value of 720 U/l.

Table II. Correlations between elephant serum enzymes

Correlation	No. of animals	Correlation coefficient (r)	p
α-HBD and AST	67	0.794	< 0.001
CPK and α-HBD	24	0.633	< 0.001
CPK and AST	24	0.559	< 0.01
α-amylase and AST	23	0.254	> 0.1
α-amylase and α-HBD	23	0.169	> 0.1

(Boehringer Mannheim GMBH) and modified for use on an LKB reaction rate analyzer. Only non-haemolyzed specimens were analyzed and strict quality control procedures ensured analytical reliability throughout. All activities are expressed in Units per litre (U/l).

Results and discussion. Table I shows the results obtained for the 4 enzymes. 4 animals were found to have exceptionally high values for one or more of the enzymes and these high results were omitted from the calculations of the means. The AST showed a log normal distribution and statistical analysis was therefore carried out on log transformed values. The figures were then converted to the absolute values shown in Table I. The mean elephant AST and α-HBD values are very similar to those we obtain in humans. However, the elephant AST range is increased almost 4-fold compared with that of humans. In contrast, all 58 ALT values were low and, indeed, many of them were indistinguishable from zero. The CPK results were very variable with a wide range and were on average 3 times the normal human values.

The present results can perhaps be criticized on two grounds and care should, therefore, be taken in attaching too much importance to the absolute values obtained. Firstly, the substrate concentrations used in the assays were those which give optimum activities for the human serum enzymes. It is quite possible that these assay conditions are not the most suitable for measuring enzyme activities in elephant sera. This would apply particularly to the α-HBD assays in which one is trying to selectively measure the activity of the lactate dehydrogenase-1-isoenzyme (LDH-1-isoenzyme). However, the conditions for the enzyme assays are being defined and a valid comparison both with the results from humans and between results from individual elephants can, therefore, still be made.

Secondly, there was inevitably a time lag between the time of collection and initial freezing of the samples in Uganda and the time of eventual analysis in the United Kingdom. It is possible that the activities of the enzymes altered during the period prior to analysis. In an attempt to assess this, 10 samples were analyzed a second time for the enzymes 10 weeks after the initial assay. Statistical analysis showed that the mean difference between the results was not significantly different from zero. We can say, therefore, that the elephant serum enzyme activities did not change during the period of storage and assay.

In a comparative study on the Indian elephant, *Elephas maximus*, 12 animals were found to have a mean AST value similar to that found in the dog whereas the ALT value was considerably lower[4]. Moreover, the serum aminotransferase levels in the dog are very similar to those found in man[5]. The present results are, therefore in agreement with these relative activities and would seem to indicate that, compared with other mammals including the pig, cow, goat, sheep and horse[5], the elephant, both African and Indian, has low levels of the serum aminotransferases.

Since there are marked differences between species both in the distribution of LDH isoenzymes and in their properties[6], it is difficult to say with certainty that our α-HBD results are in fact measuring the LDH-1-isoenzyme. In cattle, sheep, and pigs, it has been found that α-HBD activity is far better correlated to total LDH activity than to the thermostable LDH isoenzyme (LDH-1-isoenzyme)[6]. This is in contrast with the situation in man where there is a very good correlation between the α-HBD and heat stable LDH.

AST, CPK and LDH-1-isoenzyme are all found in high concentrations in heart muscle. In addition, AST occurs in liver and kidney in high concentration. One might, therefore, expect a relationship between the levels of these 3 enzymes in serum and this is what we find with our elephant results (Table II). There is a highly significant positive correlation between the 3 enzymes. Furthermore, there is no similar relationship between another

[1] Department of Biochemistry, Faculty of Medicine, University of Khartoum, P.O. Box 102, Khartoum, Sudan.
[2] Department of Biological Sciences, Njala University College, P.M.B. Freetown, Sierra Leone.
[3] Recommendations of the Deutsche Gesellschaft für Klinische Chemie in Z. klin. Chem. klin. Biochem. *8*, 658 (1970).
[4] G. Nirmalan and S. G. Nair, Res. vet. Sci. *10*, 176 (1969).
[5] H. J. Zimmerman, M. A. Schwartz, L. E. Boley and M. West, J. Lab. clin. Med. *66*, 961 (1965).
[6] S. Tollersrud, Acta vet. scand. *11*, 510 (1970).

elephant serum enzyme we have studied in more detail[7], α-amylase, and the present group of enzymes (Table II). This is not unexpected since serum amylase is primarily of pancreatic and salivary origin.

Analysis of all the enzyme results showed that there was no variation due to the age or sex of the animals nor any due to the season or location. The serum aminotransferase levels have been reported to alter due to a variety of environmental and other factors in some breeds of healthy cattle[8–12] but not in others[13]. These include age, weight, sex, stage of lactation, season and environmental temperature. However, in our present results we found no evidence of such factors having an effect in the African elephant, although 4 individual animals did have one or more of the enzyme levels well outside the 95% probability limits. All liver function tests (including ALT levels) in these animals were normal and none of them had any other chemical evidence of disease. In the absence of further information, therefore, the causes of these high levels must remain unknown[14].

[7] R. L. SKINNER, I. R. F. BROWN and P. T. WHITE, Comp. Biochem. Physiol., in press (1976).
[8] L. R. BOOTS, W. L. CRIST, D. R. DAVIS, E. W. BRUM and T. M. LUDWICK, J. Dairy Sci. *52*, 211 (1969).
[9] D. R. DAVIS, W. L. CRIST and T. M. LUDWICK, J. Dairy Sci. *49*, 731 (1966).
[10] J. M. RAKES, O. T. STALLCUP and J. D. ROUSSEL, J. Dairy Sci. *50*, 999 (1967).
[11] W. L. CRIST, T. M. LUDWICK, E. W. BRUM and D. R. DAVIS, J. Dairy Sci. *50*, 998 (1967).
[12] L. R. BOOTS, T. M. LUDWICK and E. R. RADER, J. Dairy Sci. *53*, 1587 (1970).
[13] P. J. MYLREA and P. J. HEALEY, Austr. vet. J. *44*, 570 (1968).
[14] We wish to thank Mr. A. OMAR, Director, Uganda National Parks, and the National Research Council of Uganda for permission to carry out this study. We are also grateful to Dr. S. K. ELTRINGHAM and Dr. E. EDROMA, former and present directors of the Uganda Institute of Ecology for their cooperation, to Mr. R. C. MALPAS for help in the collection of serum samples and to Professor J. A. OWEN in whose Department the analyses were carried out. Financial support was provided by the Makerere Research Grants Committee.

Labile Protein-Methyl Ester: Comparison Between Chemically and Enzymatically Synthesized[1]

S. KIM and W. K. PAIK

The Fels Research Institute and Department of Biochemistry, Temple University School of Medicine, Philadelphia (Pennsylvania 19140, USA), 1 December 1975.

Summary. The rate of hydrolysis of protein-methyl ester, the enzymatic product of S-adenosylmethionine: protein-carboxyl methyltransferase (EC.2.1.1.24) acting on oxidized ribonuclease, was measured at pH 7.1 and 8.6 at 37 °C. The half-life of the hydrolysis of the ester is 25 min at pH 7.1, and 4 min at 8.6. The rate of hydrolysis of the enzymatically formed esters at pH 7.0, in 0.1 *M* phosphate buffer, was about 25 times faster than that of esters formed chemically by reaction with methanol in HCl. The lability of the enzymatically synthesized protein-methyl ester suggests that the esterification is specific to sites such that ionization of neighboring amino acid side chains enhances the rate of the hydrolysis.

Among the various methylated amino acid residues in proteins (polypeptides) formed post-translationally by enzymatic methylation[2], the one that is formed by the methylation of carboxyl group is unique in that the product is labile in aqueous alkaline pH[3]. It has been indicated that the methyl group is linked as an ester bond at the free carboxyl groups of protein[3,4]. To support the contention further, it has been reported that the methyl-accepting capacity of substrate protein is lost after the blockage of free carboxyl groups by chemical modification[5]. Protein methylase II (S-adenosylmethionine: protein methylase II (S-adenosylmethionine: protein-carboxyl methyltransferase, EC.2.1.1.24) that methylates (esterifies) free carboxyl groups of protein substrate has been purified from various mammalian tissues[6,7]. The present communication reports the comparative rate of hydrolysis of enzymatically and chemically formed protein-methyl ester.

Materials and methods. S-Adenosyl-L-(methyl-^{14}C) methionine, 60 mCi/mmole was obtained from New England Nuclear Corporation, Boston, Mass., and ^{14}C-methanol, 58 mCi/mmole from Amersham/Searle Corporation, Arlington Heights, Illinois. γ-Aspartic and δ-glutamic acid methyl esters were obtained from Schwartz/Mann. Bovine pancreatic ribonuclease A (5 × crystallized) was obtained from Sigma, and other reagents were from local sources and of the best commercial grade available. Protein methylase II was purified from calf thymus[6] and the preparation transferred 6440 picomoles of methyl groups/min/mg protein using denatured calf thymus cytosol protein (F-P-100, ref.[3]) as substrated.

Enzymatic methylation (esterification) of oxidized ribonuclease. The general conditions for the methylation were essentially the same as those reported previously[3]. The incubation mixture contained 20 mg oxidized pancreatic ribonuclease, 0.3 ml of citrate-phosphate buffer, pH 6.0, 0.3 ml (30 μ g) of protein methylase II and 47.8 μ moles of S-adenosyl-L-(methyl-^{14}C) methionine in a final volume of 0.6 ml. The mixture was incubated at 37 °C for 1 h, at which time 23.9 μ moles of S-adenosylmethionine and 0.1 ml of protein methylase II was added again, and the incubation was continued for an additional period of 2 h. The reaction was terminated by the addition of 10 ml of cold ethanol-1 *N* HCl (39:1, v/v) and the resulting protein precipitate was removed by centrifugation. The precipitate was washed 5 times with the

[1] Acknowledgments. This work was supported by Research Grants AM 09603 from the National Institute of Arthritis and Metabolic Diseases, CA 10439 and CA 12226 from the National Cancer Institute, 1-P01-HD-05874 from the National Institute of Child Health and Human Development, National Institutes of Health, GM 20594-03 from National Institute of General Medical Sciences, USA.
[2] W. K. PAIK and S. KIM, Science *174*, 114 (1971).
[3] S. KIM and W. K. PAIK, J. biol. Chem. *245*, 1806 (1970).
[4] M. LISS, M. MAXAM and L. J. CUPRAK, J. biol. Chem. *244*, 1617 (1969).
[5] S. KIM and W. K. PAIK, Biochemistry *10*, 341 (1971).
[6] S. KIM, Arch. Biochem. Biophys. *157*, 476 (1973).
[7] S. KIM, Arch. Biochem. Biophys. *161*, 652 (1974).