AGE-RELATED CHANGES IN THE ACTIVITY OF ERYTHROCYTIC CARBONIC ANHYDRASE DURING EARLY NEONATAL PERIOD IN BUFFALO CALVES

S. P. S. GILL, RAJVIR SINGH, S. P. S. SINGHA and M. S. SETIA
Department of Veterinary Physiology, Punjab Agricultural University, Ludhiana 141 004, India.

TRANSITION from intrauterine to extrauterine life imposes a considerable acclimatization stress on the new born animal even under normal conditions of parturition. The process of parturition itself causes insufficiency of fetoplacental circulation, which gives rise to minor or major disturbances of oxygen supply and carbon dioxide elimination. Due to oxygen deficiency glycolysis takes place under anaerobic conditions, which leads to depression of energy production and increased lactic acid production, resulting in neonatal acidosis which affects vital cell functions and ultimately leads to death[1,2]. Light alkalosis is exhibited between 24 and 48 hr after birth with higher[3] pCO_2. Carbonic anhydrase, a metallo-enzyme present in the RBC, catalyses the reversible hydration of carbon dioxide and water to form carbonic acid and is chiefly involved in the transport of carbon dioxide and maintains acid base homeostasis.

Investigations were, therefore, conducted to study the changes in the erythrocytic carbonic anhydrase activity in buffalo calves from birth to 15 weeks of age.

Blood samples were collected in heparinized glass vials by juglar vein puncture from 15 new born calves starting from birth till 15 weeks of age at weekly intervals. All the calves were born through natural process of parturition without any external assistance and were reared under standard management conditions at the university dairy farm. The hematocrit values were determined immediately, the blood samples were cooled and the cells were separated from the plasma by centrifugation. The cell pellet was resuspended in normal saline (volume equal to the volume of plasma removed) and recentrifuged. The washing was repeated thrice. The cells were hemolysed by adding distilled water equal to the volume of plasma removed. The hemolysate was further diluted ten times and its carbonic anhydrase content was assayed by the method of Roughton and Booth[4]. The protein content was also analysed[5] The level of zinc in the

Table 1 *Levels of carbonic anhydrase, zinc, PCV and carbonic anhydrase: PCV ratio in the RBC hemolysate of buffalo calve's blood during different weeks of growth*

Age in weeks	Carbonic anhydrase mol.CO_2 released/1/ Sec/mg prot. $\times 10^{-7} \pm SE \times 10^{-8}$	Zinc ug/l ± SE	PCV ± SE	Carbonic anhydrase/PCV ratio
0	4.20 ± 8.40	367.75 ± 74.71	$47.91 \pm 1.14^{**a}$	$6.15\times10^{-9} \pm 2.27\times10^{-9}$
1	4.30 ± 1.20	337.00 ± 86.27	$44.14 \pm 1.40^{**ab}$	$9.37\times10^{-9} \pm 3.31\times10^{-9}$
2	4.50 ± 4.20	271.00 ± 74.71	$41.83 \pm 1.50^{**b}$	$7.94\times10^{-9} \pm 7.20\times10^{-10}$
3	4.50 ± 4.40	474.00 ± 66.82	$42.50 \pm 1.66^{**b}$	$9.29\times10^{-9} \pm 1.16\times10^{-9}$
4	4.03 ± 3.40	333.00 ± 52.83	$39.50 \pm 1.57g^{**bc}$	$1.12\times10^{-8} \pm 3.39\times10^{-9}$
5	4.80 ± 3.40	337.00 ± 49.81	$39.08 \pm 1.51^{**bc}$	$1.20\times10^{-8} \pm 1.24\times10^{-9}$
6	3.20 ± 3.40	389.13 ± 52.82	$39.09 \pm 1.58^{**bc}$	$8.89\times10^{-9} \pm 1.04\times10^{-9}$
7	3.60 ± 4.60	385.90 ± 47.25	$38.09 \pm 1.45^{**bc}$	$8.38\times10^{-9} \pm 1.59\times10^{-9}$
8	7.80 ± 3.60	399.10 ± 47.25	$38.69 \pm 1.45^{**bc}$	$9.94\times10^{-9} \pm 3.26\times10^{-9}$
9	8.30 ± 3.80	329.11 ± 49.81	$37.69 \pm 1.45^{**bc}$	$2.29\times10^{-8} \pm 1.17\times10^{-8}$
10	5.20 ± 7.30	487.78 ± 49.81	$36.54 \pm 1.75^{**bcd}$	$1.41\times10^{-8} \pm 2.05\times10^{-9}$
11	4.60 ± 5.30	394.50 ± 52.83	$35.46 \pm 1.45^{**bcd}$	$1.30\times10^{-8} \pm 1.41\times10^{-9}$
12	5.40 ± 1.08	404.00 ± 47.25	$34.14 \pm 1.45^{**bcd}$	$1.31\times10^{-8} \pm 2.44\times10^{-9}$
13	5 50 ± 8.30	399.55 ± 4.05	$35.21 \pm 1.40^{**bcd}$	$4.30\times10^{-7} \pm 2.44\times10^{-9}$
14	3.90 ± 2.80	400.90 ± 47.25	$34.00 \pm 1.40^{**bcd}$	$1.31\times10^{-8} \pm 1.63\times10^{-9}$
15	4.40 ± 2.10	373.00 ± 56.47	$35.21 \pm 1.40^{**bcd}$	$1.31\times10^{-8} \pm 7.54\times10^{-10}$

** $P<0.01$ Duncan's Multiple Range Test: Means with different superscripts differ significantly.

hemolysate was determined by atomic absorption spectrophotometry according to Ludmilla[6]. The data so obtained were statistically analysed according to Steel and Torrie[7].

The specific activity of the metalloenzyme carbonic anhydrase during different weeks of growth is shown in table 1. No significant differences were observed in the specific activity of the enzyme in relation to the age of the animal indicating that the quantity of the enzyme remains constant from birth onwards. No significant differences were observed in the zinc content of the hemolysate (table 1) which further substantiates our findings that the quantity of the enzyme carbonic anhydrase remained constant from birth onwards.

The PCV (packed ceil volume) levels, however, showed significant ($P<0.01$) differences between different weeks of growth (table 1). The levels showed a decline till 11 weeks of age. The highest values were found on the day of birth. There was a decline of 18.72% by the 5th week. Thereafter the decline was less steep (13.85%) till the 11th week. When the ratio of the enzyme levels to PCV was calculated it was seen that the ratio increased to ten-folds from birth till 15th weeks of age. The increase in the enzyme content per unit PCV was erratic from the day of birth till 10 weeks of age but from the 11th week onwards there was a uniform elevation by 10 folds as compared to the previous 10 weeks. PCV is directly related to erythrocyte count and haemoglobin content[8]. Hence from our data it is evident that the amount of carbonic anhydrase in the red blood corpuscles (RBC) increases as the animal matures.

These results clearly indicate that the level of the enzyme per RBC increases from birth onwards and to lower the pCO_2 during the early neonatal period[3], the body compensates by having higher blood volume[9] and PCV thereby increasing the total amount of the enzyme available for catalysing the transport of carbon dioxide and maintaining proper H^+ concentration. During early neonatal period there is increased thyroidal activity[10] and glucose utilization[11] which is necessary for functional and metabolic adaptation of the new born. This leads to excessive production of carbon dioxide. To maintain the body homeostasis the animal responds by increasing the availability of carbonic anhydrase for elimination of CO_2.

24 November 1986; Revised 21 February 1987

1. Lampe, L., *Intenziv Szuloszoba*, Medicina Budapest.
2. Walser, K. and Maurer-Schweizer H., *Tierarztl Praxis*, 1978, **6**, 451.
3. Maurer-Schweizer H. Wilhelmn, U. and Walser, K., *Tierarztl Wschr*, 1977, **90**, 192.
4. Roughton F. J. W. and Booth V. H., *Biochem. J.*, 1946, **40**, 309.
5. Meulemans, O., *Clin. Chem. Acta.* 1960, **5**, 757.
6. Ludmilla, D., *Clinical analysis by atomic absorption spectroscopy*, Varian Techtron Pvt. Ltd., Australia, 1976, p. 37.
7. Steel, R. G. D. and Torrie J. H., *Principles and procedures of statistical analysis a biometrical approach, 2nd edn.* McGraw Hill and Kogakusha, Tokyo, 1980, p. 137.
8. Swenson M. J., *Duke's physiology of domestic animals* , Comstock Publishing Associates, Cornell University Press, Ithaca, London 1970, p. 39, 8th edn.
9. Sodhi, S. P. S., Ph.D. thesis, Punjab Agricultural University, Ludhiana, 1979, p. 169.
10. Setia. M. S. and Verman P. N., *Indian J. Anim. Res.*, 1983, **17**, 17.
11. Setia, M. S. and Verman, P. N., *Z. Tierphysiol. Tierernachr. Futtermittelkd.*, 1980, **44**, 93.

RESPONSE OF PEROXIDASE ISOENZYMES TO CHEMICAL SEX MODIFICATION IN CUCUMBER (*CUCUMIS SATIVUS* L.)

E. A. EL-GHAMRINY, N. SINGH and V. K. VERMA
Division of Vegetable Crops, Indian Agricultural Research Institute, New Delhi 110 012, India.

It has been established that normal plant growth and development is governed by endogenous plant hormones. The exogenous applications of chemicals at 2-true leaf stage of the plants modifies the development and sex ratio in different genera of cucurbits.

The biochemical analyses were performed in monoecious cucumber var. 'Khira Poona' to find out the changes in peroxidase isoenzymes patterns in tips, leaves and flower buds to chemical treatment and to understand, why 2-true leaf stage is so critical for effective sex modification and also why ethrel behaves in different manner when compared to that of silver nitrate. It was also worthwhile to study the