tighest pyruvate regulation may be expected in tissues with high levels of LDH_1 activities, because this isoenzyme is strongly inhibited with higher pyruvate concentrations. Our findings (Table) suggest that brain has the capability for such a control of LDH activity.

Summary. The highest lactate dehydrogenase (LDH) activity was found in thalamus, statistically significantly less in cerebral and cerebellar cortex and the lowest in pons. LDH_1 and LDH_{4+5} represented 58% and 23% of the total activity in cerebral cortex, 54% and 20% in thalamus, 42% and 4% in cerebellar cortex and 55% and 7% in pons, respectively.

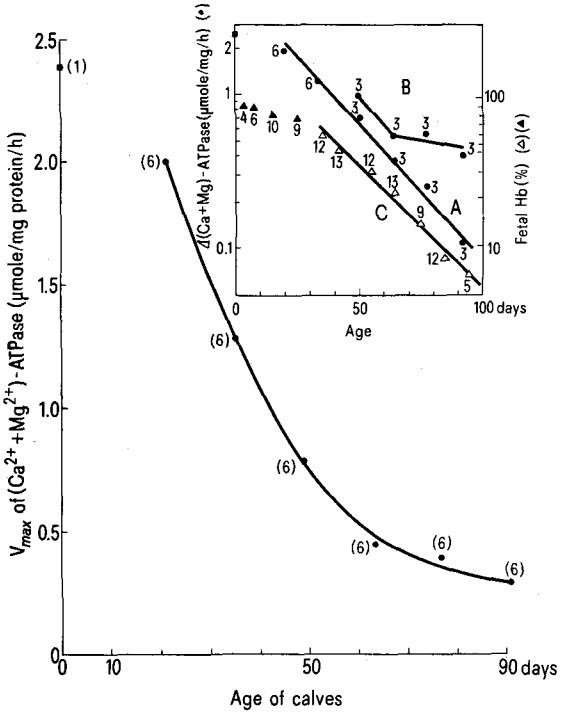
R. V. ŽIVKOVIĆ and B. M. DJURIČIĆ[18,19]

Institute of Biochemistry, Faculty of Medicine, Višegradska 26, Y-11001 Beograd (Yugoslavia),
15 July 1975.

[18] Authors wish to express their gratitude to T. C. DETWILER (Downstate Medical Center, New York) for his helpful criticism during preparation of manuscript.
[19] Requests for reprints should be sent to Dr. B. M. DJURIČIĆ.

Postnatal Decline of (Ca^{2+} + Mg^{2+})-Activated Membrane ATPase in Cattle Red Cells

(Ca^{2+} + Mg^{2+})-stimulated, membrane bound ATPase ((Ca^{2+} + Mg^{2+})-ATPase) in human red cells is believed to reflect the presence of an active outward Ca transport system[1–5]. In adult cattle erythrocytes, the (Ca^{2+} + Mg^{2+})-ATPase activity is only about 1/50 of that found in human cells[6,7]. However, in young calves the enzyme activity exceeds that of human cells and starts falling after the third week[6]. In the present study we examined the time course of this decline in a group of 6 calves of the Simmenthal breed, fed on artificial milk, supplemented with an oral dose of iron shortly after birth. This is compared with the time course of disappearance of fetal hemoglobin (fHb), measured in a group of 13 calves of the same breed, fed in the same way.



v_{max} of red cell membrane (Ca^{2+} + Mg^{2+})-ATPase, taken from 1/v vs. 1/Ca^{2+}-conc. plots, as function of age of calves. ■, red cells of one case obtained from umbilical vein at birth. Number of animals near points. Inset: Same observations, together with data for fetal hemoglobin from another group of calves. •, ordinate: v_{max} of (Ca^{2+} + Mg^{2+})-ATPase minus 0.015 µmole/mg/h (value for cows). A) fast growing calves; B) slowly growing calves. For the first two points the 6 animals were pooled. Later on the group was divided into fast and slowly growing animals, because a difference in weight gain became apparent after 50 days. ▲△, fetal Hb in percent of total Hb (value at infinity assumed to be zero). Note that disappearance of fetal Hb becomes rapide and exponential (linear in log plot) after 20 days. Therefore only the part of the curve marked by open triangles was used for calculation of regression line. Rate constant for decline of ATP-ase (line A) = 0.041 ± 0.002 d^{-1}, for fetal Hb (line C) = 0.037 ± 0.002 d^{-1}. Number of animals near points.

Equal amounts of fresh, washed red cells from 3 or 6 animals were pooled, membranes were prepared as described before[5] and (Ca + Mg)-ATPase assayed by measuring liberation of inorganic phosphate[5] in the following medium: (m*M*) Choline-Cl 110, imidazole-Cl 30, $MgCl_2$ 4, Na-ATP 2, ouabain 0.17, Ca-EGTA buffer or *tris*-EGTA 1, pH 7.0. Sample volume was 2.5 ml, mean protein concentration 0.64 mg/ml, temperature 37 °C and incubation time 90 min. ATPase requiring Ca alone showed negligible activity. The fraction of fHb was determined in calves with type A adult hemoglobin[8] by electrophoretic separation on cellulose acetate strips at pH 8.6 and scanning the stained strips (Ponceau S) photometrically[9]. Ca influx into intact fresh cells was measured after ATP depletion in the following way: 1 vol of washed cells was preincubated for 1 h at 37 °C in 30 vol of medium ((m*M*) NaCl 120, KCl 5, *tris*-Cl 30, iodoacetamide 5, inosine 5, pH 7.4) or starved for 17 h at 37 °C and preincubated with the metabolic poisons for 20 min. Then 1 m*M* $^{45}CaCl_2$ was added, samples taken at 1 h intervals and cells washed 4 times in 50 vol ice-cold medium without labelled Ca and inhibitors. An aliquot of cells was dried on a planchet and counted in a windowless Geiger-Müller tube. Results were corrected for the difference in self-absorption of medium and cells.

The Figure shows the time course of decay of the (Ca + Mg)-ATPase with age. Maximal rate (v_{max}) was found by plotting activation curves obtained with Ca^{2+}-concentrations between 10^{-6} and 10^{-5} *M* according to Lineweaver-Burk. K_{Ca} values varied between 0.83 and 2.6×10^{-6} *M* and showed no dependence on age. The

[1] H. J. SCHATZMANN, Experientia *22*, 364 (1966).
[2] H. J. SCHATZMANN and F. F. VINCENZI, J. Physiol., Lond. *201*, 365 (1969).
[3] K. S. LEE and B. C. SHIN, J. gen. Physiol. *54*, 713 (1969).
[4] E. J. OLSON and R. J. CAZORT, J. gen. Physiol. *53*, 311 (1969).
[5] H. J. SCHATZMANN, J. Physiol., Lond. *235*, 551 (1973).
[6] J. EKHOLM, unpublished, see D. J. HANAHAN, Biochim. biophys. Acta *300*, 319 (1973).
[7] H. J. SCHATZMANN, Nature, Lond. *248*, 58 (1974).
[8] R. M. GRIMES, C. W. DUNCAN and C. A. LASSITER, J. Dairy Sci. *41*, 1527 (1958).
[9] H. R. SCHEIDEGGER, Diss. Bern (1973).

inset of the Figure demonstrates that for 3 animals the decay is exponential during the whole time of observation and that the rate constant is similar to that for disappearance of fHb. For the other 3 animals, which gained weight at a lesser rate, the decay of (Ca + Mg)-ATPase slowed down at the end of the experiment. The difference between curve A and B is statistically significant ($p < 0.01$). Disregarding this final deviation in unthrifty animals, the half-life is 16.8 days for (Ca + Mg)-ATPase, which is similar to that of 18.8 days for fHb.

Ca influx into metabolically poisoned cells of 2 calves (2 and 3 weeks old) and 2 cows was measured in six 1 h periods to obtain an estimate of passive permeability of the membrane for Ca^{2+}. As extremely little Ca enters the cells in 1 h, the experimental error is large and the difference between calves (2.33 ± 0.96 µmole/l h ± SEM) and cows (0.47 ± 1.0 µmole/l h) not statistically significant. The average of cows and calves taken together of 1.4 ± 0.73 µmole/l h ± SEM) is only about 1/10 of the value found under similar conditions in human red cells[10].

Ekholm[6] first showed that not only red cell (Na + K)-ATPase but also red cell (Ca + Mg)-ATPase declines in maturing calves after the 3rd week. At 21 days we found a value of 2.0 µmoles/mg protein/h, whereas the value for adult cows is 0.015 µmoles/mg protein/h[7]. The present observation demonstrates that from the 3rd week onwards the activity disappears in an exponential fashion. The alteration bears on v_{max} and not on K_{Ca} and is thus due to a reduction of turnover rate or, more likely, of the number of sites per mg protein or per unit surface area. The very similar time course of decay of (Ca + Mg)-ATPase and replacement of fHb strongly suggests that when synthesis of adult Hb sets in, the cells are simultaneously equipped with the adult type membrane of low (Ca + Mg)-ATPase. The fact that the disappearance of (Ca + Mg)-ATPase slowed down significantly after the 60th day in unthrifty animals might be explained by a general impairment of protein synthesis in these.

The influx measurements indicate that cattle red cells are less permeable for Ca^{2+} than human red cells, taking into account that, in spite of a large difference in cell volume in the two species, the surface/volume ratio is comparable[11]. If activity of the (Ca + Mg)-ATPase is indicative of the rate of active outward Ca transport also in cattle, it may be predicted that in fetal cattle red cells the intracellular Ca^{2+} concentration must be exceedingly low and that even adult cattle red cells may be able to keep Ca^{2+} effectively out of the cytosol in view of the low passive permeability. The latter point is shown by direct measurements of overall Ca content of red cells in this species[6,7].

Summary. Activity of membrane bound (Ca^{2+} + Mg^{2+})-stimulated ATPase, associated with Ca^{2+} outward transport, in calf red cells is high at birth and declines with a rate constant of 0.041 d^{-1} after the 3rd week. The decline parallels the disappearance of fetal hemoglobin.

H. J. Schatzmann and H. R. Scheidegger

Veterinär-Pharmakologisches Institut der Universität, Länggass-Strasse 124, CH–3012 Bern (Switzerland), and Nutztier- und Pferdeklinik der Universität, CH–3012 Bern (Switzerland), 23 July 1975.

[10] V. L. Lew, Biochim. biophys. Acta *233*, 827 (1971).
[11] E. Ponder, *Hemolysis and Related Phenomena* (Grune & Stratton, New York 1948).

Effect of Piperazine on the Level of Phospholipids and on the Activities of Certain Enzymes of Phospholipid Metabolism in Human *Ascaris lumbricoides*

Piperazine is one of the widely used anthelmintic drugs in the treatment of ascariasis infection, caused by the parasitic nematode, human *Ascaris lumbricoides*. This drug is shown to paralyze the above nematode[1]. It is also shown that it causes inhibition of certain key enzymes in the glycolytic pathway in *Ascaris* species isolated from the pig[2] as well as from humans[3]. So far no information is available regarding the effect of this drug on the lipid constituents or the enzymes involved in its metabolism. The results presented in this communication indicate a significant decrease in the level of phospholipids, following incubation of the parasite in a medium containing sub-lethal concentrations of piperazine. It is further indicated that the decrease may be due to enhanced degradation and decreased synthesis of the phospholipids in presence of piperazine.

Materials and methods. Live round worms collected from the local hospitals were used for the study. The worms brought to the laboratory were incubated at 37 °C for a period of 24 h in modified Tyrode solution so as to contain about 50 ml per worm. Active worms were then separated into male and female ones and separately pooled into groups of 3 worms, each, so as to weigh about 5 g. These again suspended and incubated at 37 °C for a further period of 6 h in modified Tyrode solution containing a neutralized solution of piperazine hexahydrate to give a concentration of 0.2% free base. An identical set of experiments were conducted without piperazine for use as control. All the worms subjected to the piperazine treatment were found paralyzed at the end of the experimental period, while the control worms were as active as before. These worms then removed, wiped dry, frozen to death, homogenized and subjected to lipid extraction using ethanol ether 3:1 and chloroform methanol 1:1 one after the other. The pooled lipid extracts were then made up to a known volume and used for the estimation as well as for the fractionation of phospholipids. Whole worm homogenate was used for the enzyme studies.

[1] O. D. Standen, Br. med. J. *2*, 20 (1955).
[2] H. J. Saz and E. Bueding, Pharmac. Rev. *18*, 871 (1966).
[3] R. Kaleysa Raj and P. A. Kurup, Indian J. Biochem. *4*, 178 (1967).