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.*

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## 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.

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Table I. Effect of piperazine on the phospholipids of *Ascaris lumbricoides*

| Estimation | Normal level (mg P/100 g ± SE) | Level after incubation in a medium containing 2 mg/ml piperazine (mg P/100 g ± SE) | *P*-value | Effect (%) |
|---|---|---|---|---|
| Total phospholipid | ♂ 38.55 ± 3.24 | 29.68 ± 2.09 | 0.01 | 23 |
| | ♀ 53.36 ± 1.58 | 41.78 ± 0.71 | 0.01 | 22 |
| Lecithin | ♂ 14.16 ± 2.39 | 10.85 ± 0.05 | 0.02 | 24 |
| | ♀ 21.63 ± 1.61 | 17.20 ± 4.27 | 0.02 | 21 |
| Cephalin | ♂ 10.88 ± 0.38 | 7.16 ± 1.41 | 0.01 | 34 |
| | ♀ 14.16 ± 2.81 | 9.43 ± 0.89 | 0.01 | 33 |

*P*-value $< 0.02$ and $< 0.01$ are very significant.

Table II. Effect of piperazine on the activities of phospholipase C and choline kinase in *Ascaris lumbricoides*

| Enzyme | Normal activity[a] ± SE | Activity in presence of piperazine (1 mg/ml ± SE) | *P*-value | Effect (%) |
|---|---|---|---|---|
| Phospholipase C | $6.22 \pm 0.09 \times 10^{-6}$ | $8.22 \pm 0.09 \times 10^{-6}$ | $< 0.01$ | 28 (activation) |
| Choline kinase | $5.56 \pm 0.05 \times 10^{-5}$ | $5.05 \pm 0.05 \times 10^{-5}$ | $< 0.01$ | 10 (inhibition) |

*P*-value $< 0.01$ is highly significant. [a] m*M*/min/mg protein.

Phospholipid was estimated by the method of ZILVERSMIT and DAVIS[4]. Lecithin and cephalin were separated by TLC and estimated by the above method. Phospholipase C (EC. 3.1.4.3) was estimated by the method of KLEIMAN and LANDS[5] and choline kinase (EC. 2.7.1.32) by the methods of WITTENBERG and KORNBERG[6] and that of APPLETON et al.[7]. Enzyme protein was determined by the method of LOWRY et al.[8].

*Results.* Total phospholipid level together with the values of lecithin and cephalin and also the percentage effect on incubation in the medium containing piperazine at a concentration of 0.2% on these, are given in Table I. The enzyme activities and the percentage effect on these due to piperazine at a concentration of 1 mg/ml reaction medium are given in Table II.

*Discussion.* The results clearly indicate a decrease in the phospholipid content of the worms exposed to piperazine. Though the content is different, the percentage inhibition of phospholipids in both the male and female worms appears to be the same. Lecithin and cephalin constitute about 80% of the phospholipids of this parasite. The results presented clearly indicate that the observed decrease in phospholipid levels is mainly due to the decrease in lecithin and cephalin fractions. A concentration of 0.2% (2 mg/ml) in the external medium was used to produce a similar effect as observed in worms expelled after piperazine therapy. Under the experimental conditions, it brings about 30% stimulation of phospholipase C activity and about 10% inhibition of choline kinase activity. This shows that the observed decrease in phospholipid level may be due to increased degradation and decreased synthesis. This effect of piperazine on the phospholipid level of the worms looks interesting from the point of view of the mode of action of this drug. This aspect is currently under detailed investigation.

*Summary.* The phospholipid level in the human parasitic nematode *Ascaris lumbricoides* is decreased by piperazine, by partially stimulating catabolic enzymes such as phospholipase C and partially inhibiting anabolic enzymes such as choline kinase.

P. K. SASI and R. KALEYSA RAJ[9]

*Department of Biochemistry, University of Kerala, Trivandrum 695001 (India), 26 February 1975.*

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