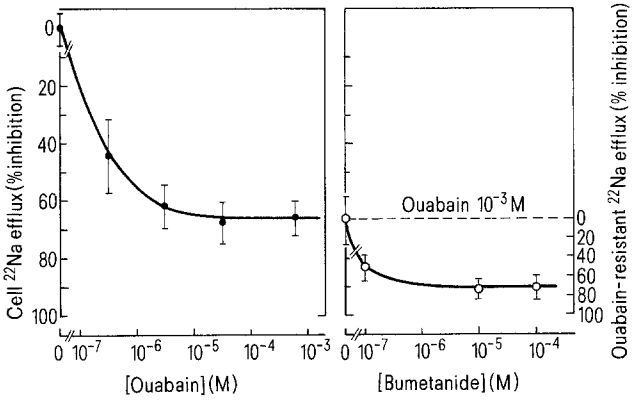

Figure 3. The effect of different concentrations of ouabain and bumetanide on ^{22}Na efflux rate from peripheral mononuclear cells. Cells were incubated at 37 °C for 2 h in PBS medium containing ^{22}Na (10^5 Bq/ml) after which the cells were rapidly washed with unlabeled medium. Cells were further incubated in PBS medium and duplicate samples were taken at two different time intervals and assayed for cell radioactivity. Ouabain was added at different concentrations and the effect of increasing bumetanide concentrations was assayed in the presence of 10^{-3} M ouabain. Rate constants ± SD were calculated from least square fits of single exponentials, the data are represented as % inhibition of ^{22}Na efflux rates in the absence of ouabain and bumetanide.

20% of total Na efflux and had a value of 0.05 ± 0.02 nmoles/10^6 cells × min.

Bumetanide- or furosemide-sensitive Na fluxes have been described in various cell types[15-17]. Extensive studies done in red cells[15,18] and cultured fibroblasts[16] showed it to be mediated by a Na, K, Cl-cotransport system. It is tempting to postulate that bumetanide-sensitive Na efflux in human lymphocytes is mediated by such a system; however, the thorough investigations and kinetic analysis required are hampered in lymphocytes by the limited amount of cells which could be obtained from human blood, and will have to await the development of new techniques.

The role of PGE in lymphocytes is not fully understood. They are known to be involved in immunological and blastogenic responses[19,20]. In these cases variations of Na transport properties have also been reported to occur. We have therefore tested the effect of PGE on Na transport pathways in PMC. PGE_1 and PGE_2 at concentrations $> 10^{-6}$ M markedly reduce (80%) the bumetanide-sensitive Na efflux rate without altering ouabain-sensitive or ouabain- and bumetanide-resistant Na efflux (table 2). The action of PGEs is known to be mediated by variations in cAMP level[21]. In our hands, the addition of dibutyryl cAMP (2 mM), similarly to PGE_1 and PGE_2, reduced the bumetanide-sensitive Na efflux without affecting the ouabain-sensitive or the ouabain- and bumetanide-resistant fluxes. It is interesting to note that isobutyl-methylxanthine (0.5 mM), a phosphodiesterase inhibitor, has no effect on Na transport pathways (table 2). These results are similar to those observed in mouse lymphocytes and human fibroblasts[16,22]. It is clear that regulatory mechanisms controlling cotransport fluxes are tissue and cell specific, as the cotransport system in avian erythrocytes is stimulated by agents which elevate cAMP[1]. On the other hand, the effect of PGE on Na transport might not be mediated solely by cyclic nucleotide variations; a role for calcium and phospholipid metabolism should be considered, and further analysis is required to determine their implication.

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1 Palfrey, H.C., Feit, P.W., and Greengard, R., Am. J. Physiol. *238* (1980) C139.
2 Ellory, J.C., and Stewart, G.W., Br. J. Pharmac. *75* (1982) 183.
3 Canessa, M., Brugnara, C., Cusi, D., and Tosteson, D., J. Gen. Physiol. *87* (1985) 113.
4 Karlish, S.J., Ellory, J.C., and Lew, V.L., Biochem. biophys. Acta *646* (1981) 353.
5 Canessa, M., Bize, I., Adragna, N., and Tosteson, D.C., J. gen. Physiol. *80* (1982) 149.
6 Dagher, G., Brugnara, C., and Canessa, M., J. Membr. Biol. *86* (1985) 145.
7 Palfrey, H.C., and Greengard, P., Ann. N.Y. Acad. Sci. *372* (1981) 291.
8 Palfrey, H.C., and Rao, M.C., J. expl Biol. *106* (1983) 43.
9 Averdunk, R., and Lauf, P.K., Expl Cell. Res. *93* (1975) 331.
10 Segel, G.B., and Lichtman, M.A., J. Cell. Physiol. *93* (1977) 277.
11 Grinstein, S., Rothstein, A., Sarkadi, B., and Felfand, E.W., Am. J. Physiol. *246* (1984) C204.
12 Kurnick, J.T., Ostberg, L., Stegagno, M., Kimura, A.K., Orm, A., and Sjoberg, O., Scand. J. Immun. *10* (1979) 563.
13 Segel, G.B., Lichtman, M.A., Gordon, B.R., MacPherson, J.L., and Nusbacher, J., Transfusion *16* (1976) 455.
14 Quastel, M.R., Segel, G.B., and Lichtman, M.A., J. cell. Physiol. *107* (1981) 165.
15 Wiley, J.S., and Cooper, R.A., J. clin. Invest. *53* (1974) 745.
16 Owen, N.E., and Prastein, M.L., J. biol. Chem. *260* (1985) 1445.
17 Russell, J.M., J. gen. Physiol. *81* (1983) 909.
18 Schmidt, W.F., and McManus, T.J., J. gen. Physiol. *70* (1977) 59.
19 Gemsa, D., in: Lymphokines, vol. 4, p. 335. Academic Press, New York 1981.
20 Novogrodsky, A., Rubin, A.L., and Stenzel, K.H., J. Immun. *122* (1979) 122.
21 Goodwin, J.S., and Ceuppens, J., J. clin. Immun. *3* (1983) 295.
22 Diez, J., Braquet, P., Verna, R., and Garay, R.P., in: Adv. Cycl. Nucl. Prot. Phosph. Res., vol. 17, p. 621. Eds P. Greengard et al. Raven Press, New York 1984.

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NADH-methemoglobin reductase activity in the erythrocytes of newborn and adult mammals

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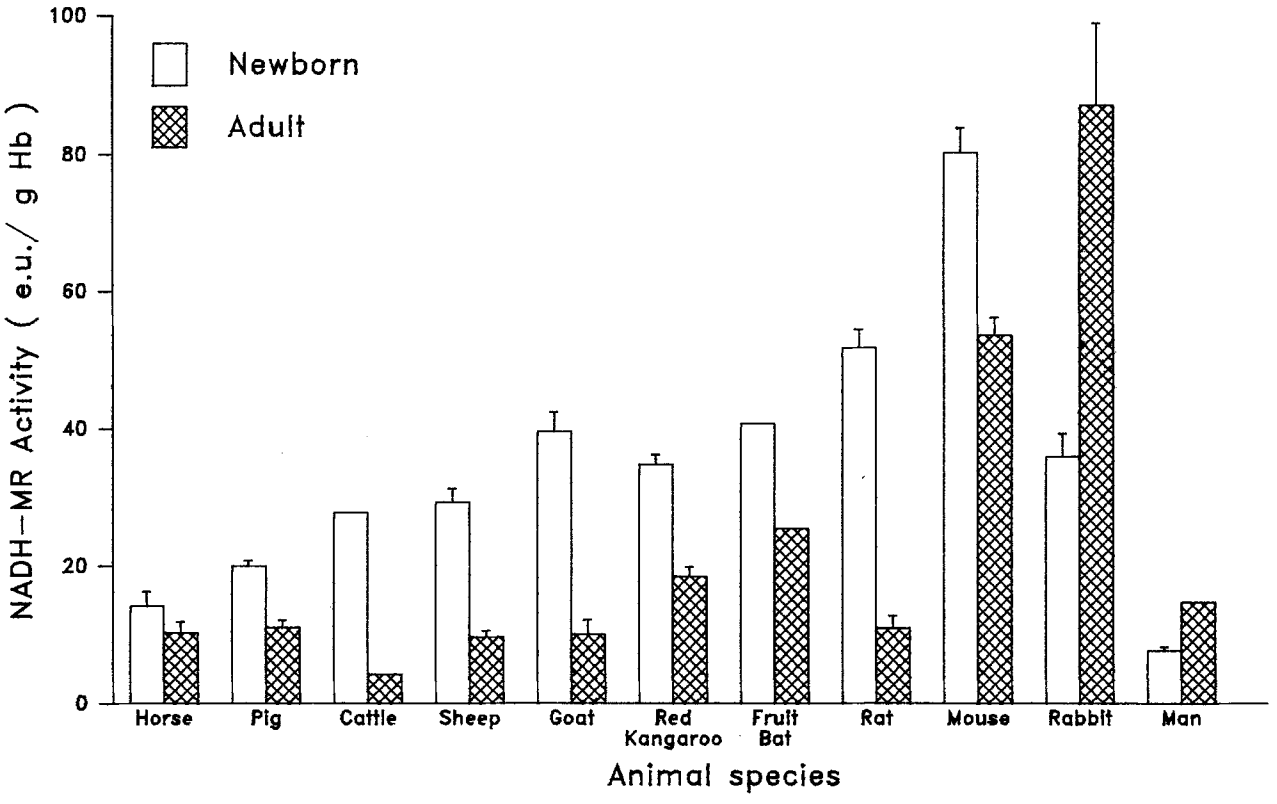
Summary. NADH-MR activity was measured in the erythrocytes of newborn and adult horses, pigs, cattle, sheep, goats, red kangaroos, fruit bats, rats, mice, rabbits and humans. Our results fail to support an earlier hypothesis that higher NADH-MR activity may be an adaptation to increased ruminal nitrite production leading to accelerated oxidation of fetal hemoglobin.
Key words. Erythrocytes; NADH-methemoglobin reductase; mammals; newborn; adults.

The enzyme primarily responsible for reducing methemoglobin in mammalian erythrocytes is NADH-methemoglobin reductase (NADH-MR)[2], (also called cytochrome b_5 reductase[3] and NADH-ferricyanide reductase[4,5]). Several cases of methemo-



Activity of NADH-MR in the erythrocytes of newborn and adult mammals ($\bar{x} \pm$ SEM).

globinemia due to deficiency of NADH-MR have been reported in man and animals[2,6-8].

The activity of this enzyme in the erythrocytes of infants is significantly lower than that of adult humans[2]. However, the erythrocytes of newborn cattle have an enzyme activity several times higher than that found in adult cattle[9]. Based on these observations, Smith and Beutler[9] suggested that a higher activity of NADH-MR in the newborn cattle may be a physiological adaptation to protect fetal oxyhemoglobin against oxidation by nitrites that are normal intermediates of rumen fermentation. The present investigations were undertaken to test this hypothesis in some other mammalian species.

Blood samples were obtained from the following animal species:

Species	Newborn	Adult
Horse	5	5
Pig	14	14
Cattle	1	1
Sheep	6	9
Goat	6	6
Red kangaroo	15	8
Fruit bat	1	2
Rat	16	3
Mouse	8	4
Rabbit	12	3
Human	3	2

The blood was centrifuged for 10 min at 200 × g and the erythrocytes washed with 0.145 M NaCl. A 1:20 dilution of erythrocytes was made in a stabilizing solution[10]. The activity of NADH-MR in the hemolysate was measured by the method of Board[5] and was expressed as enzyme units (e.u.)/g Hb.

As shown in the figure, all 11 species of mammals studied in the present investigation may be classified into one of two groups; one in which the activity of NADH-MR was greater in the newborn than in the adult and second in which the situation was reversed. The majority of species studied are in the first group, with only man and rabbit in the second category. Thus our results fail to support the hypothesis that higher NADH-MR activity may be an adaptation to increased ruminal nitrite production leading to accelerated oxidation of foetal hemoglobin.

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2 Kiese, M., Methemoglobinemia. A Comprehensive Treatise. C.R.C. Press, Cleveland 1974.
3 Leroux, A., Torlinski, L., and Kaplan, J. C., Biochim. biophys. Acta *481* (1977) 50.
4 Passon, P. G., and Hultquist, D. E., Biochim. biophys. Acta *275* (1972) 62.
5 Board, P. G., Clinica chim. Acta *109* (1981) 233.
6 Harvey, J. W., Ling, G. V., and Kaneko, J. J., J. Am. vet. med. Ass. *164* (1974) 1030.
7 Letchworth, G. J., Bentrick-Smith, J., Bolton, G. R., Wootton, J. F., and Family, L., J. Am. Anim. Hosp. Ass. *13* (1977) 75.
8 Scott, E. M., and Hoskins, D. D., Blood *13* (1958) 795.
9 Smith, J. E., and Beutler, E., Nature *211* (1966) 757.
10 Beutler, E., in: Red Cell Metabolism: A Manual of Biochemical Methods, p. 11. Grune and Stratton, New York 1975.

0014-4754/86/11/121264-02$1.50 + 0.20/0