Cartesian Diver Studies on Respiration of Rat Bone Marrow Cells in the Presence of Erythropoietin and Anti-Erythropoietin

It is known that erythropoietin (ESF), elaborated by the kidney, stimulates heme synthesis in bone marrow cells in vitro[1]. Since respiration of bone marrow cells has been investigated by various methods[2], the purpose of the present investigation was to measure respiratory activity of bone marrow cells in the presence of ESF and anti-ESF. In this respect, the Cartesian diver technique was of major interest because of the sensitivity of the system, the ability to use small quantities of cells, and the fact that CO_2 may influence cellular metabolism[3,4].

Methods. Bone marrow suspensions from male rats, Long-Evans strain, were used for all experiments. Sterile suspensions were obtained by flushing the bone marrow with Ca^{++}–Mg^{++} free salt solution, and then resuspending in Pucks or Hanks balanced salt solution. In experiments where ESF or anti-ESF was added, bone marrow from transfusion-induced plethoric rats was used[5]. The ESF was prepared from anemic human urine and had a specific activity of 4 U/0.25 µl. Anti-ESF was prepared according to the method of SCHOOLEY and GARCIA[6]. The Cartesian diver technique, which allows one to use micro samples of cells, has been described elsewhere[3,7,8].

Results. Preliminary experiments using the micro-ampulla diver technique of ZEUTHEN[7], demonstrated that respiration of normal bone marrow cells is slightly lower in the presence of glucose as compared to without glucose (Crabtree effect). In Table I, all determinations were made with 200–300 cells/diver and 0.1 *N* NaOH as an absorbant at 37°C. These results are not unusual in view of the numerous reports of reduced respiration of bone marrow and other elements in the presence of glucose[9–12].

Table I. Ampulla diver determinations of bone marrow cells in Pucks salts with or without 0.018 *M* glucose

ΔVO_2/Cell/h $\times 10^{-6}$µl	
Cells without glucose	Cells with glucose
0.97 ± 0.17 (4)	0.73 ± 0.15 (4)

Controls (no cells) = 0.52, 1.10×10^{-5}

Number of determinations in parenthesis. Average ± S.D.

Table II. Respiration of bone marrow cells in Hanks balanced salt solution with or without ESF, anti-ESF and 10^{-3} *M* azide

ΔVO_2/Cell/Hr $\times 10^{-6}$µl			
Cells	Cells + 4 units ESF	Cells + ESF + anti-ESF	Cells + 10^{-3} *M* Azide
1.25	0.46	0.73	0.28
1.05	0.53	0.87	0.24
1.04	0.61	1.15	0.41
0.79	0.56	0.92	
0.93	0.67	1.35	
0.91	0.34	0.99	
0.92	0.53	0.89	
	0.63	0.97	
0.99 ± 0.14	0.54 ± 0.10	0.98 ± 0.18	0.34 ± 0.06

Controls (no cells) = 8.95, 7.50, 6.65×10^{-4}

Micro stoppered standard divers were used with a gas phase of 5% CO_2 + 95% air, and 400–2000 cells/diver. Each figure represents an average. 4 h determination for 1 diver. Final total average calculated ± S.D.

Studies on plethoric bone marrow cells (ESF responsive) demonstrated that cell respiration was lower in the presence of ESF, and that inhibition was overcome in the presence of anti-ESF (0.54 vs 0.99×10^{-6}) (0.98 vs 0.99×10^{-6}) (Table II). All determinations were made with micro-standard stoppered divers, with a gas phase of 5% CO_2 + 95% air and absorbant seals of $NaHCO_3$[8]. In divers without hormone, an equal amount of protein (albumin) was added to compensate for the added hormone in experimental divers.

Discussion. It is of interest to note that respiration in the presence of ESF was lower than normal control cells, and that this inhibition was overcome in the presence of anti-ESF. Inhibition by azide suggests that a functional cytochrome system exists. Apparently some of the bone marrow elements are responding to the hormone in such a way that the overall aerobic respiration of the cell population is decreased. Since a Crabtree effect may exist, it is possible that a increased anaerobic metabolism could account for the reduced respiration[13–14].

Résumé. L'étude de la respiration des cellules de la moelle des os du rat fut faite avec un plongeur Cartésien. Ces cellules, avec ou sans glucose, repirent à raison de $0{,}73 \pm 0{,}15$ et $0{,}97 \pm 0{,}17 \times 10^{-6}$ µl O_2/cellule/h. Comparée aux mesures de contrôle, la respiration des cellules médullaires de rats pléthoriques est plus fabile en présence de ESF ($0{,}45$ contre $0{,}99 \times 10^{-6}$ µl O_2/cellule/h). Cette inhibition fut supprimée par la présence d'anti-ESF ($0{,}98$ contre $0{,}99 \times 10^{-6}$ µl O_2/cellule/h).

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