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ABSENCE OF CARBONIC ANHYDRASE IN RED CELL MEMBRANES

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SUMMARY

Carbonic anhydrase (carbonate hydro-lyase, EC 4.2.1.1) activity was assayed on freshly prepared human red cell ghosts. The amount of enzyme found was $< 0.008\%$ of the total protein present in the membrane. A comparison with the haemoglobin content led to the conclusion that carbonic anhydrase is not bound to the red cell membrane.

The question of whether red cell membranes contain bound carbonic anhydrase (carbonate hydro-lyase, EC 4.2.1.1) has been discussed for some time. Maren[1] stated that red cell ghosts contain none, but Enns[3] claimed that a fraction of the enzyme was either part of the cell ghosts or closely adhered to them. Recently, Enns[4] has said that red cell ghosts can be washed until a stable amount of carbonic anhydrase is obtained. Tappan[5], however, concluded that carbonic anhydrase was no more a part of the membrane than haemoglobin and Rosenberg and Guidotti[6] found $<0.01\%$ of the total protein in red cell membranes was carbonic anhydrase.

We investigated the matter further on red cell ghosts prepared as follows: Human red cells, less than 7 days old, were lysed and rewashed in a 7.5 mM Tris–HCl buffer (pH 7.5) at 4 °C (Rosenberg and Guidotti[6]) until creamy-white ghosts were obtained. The ghosts were then water-washed 3 times. Ghosts prepared by this method are assumed to retain almost all of their original proteins. Ghosts extensively washed in this manner have also been shown to maintain fully functional transport properties[7] and retain detectable activities of known membrane-bound enzymes, such as Na^+/K^+-dependent ATPase (EC 3.6.1.3)[8] and acetylcholinesterase (EC 3.1.1.7)[9]. The haemoglobin content of these ghosts was determined by the pyridine haemochromogen method (Dodge *et al.*[10]) and was found to be $1.1 \pm 0.2\%$ of the total protein present.

Carbonic anhydrase was assayed by the micromethod of Maren[2]. The detectability of membrane-bound carbonic anhydrase has been thoroughly investigated in

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both the liver[11] and the kidney[12]. The molar activities and inhibition kinetics of the membrane-bound enzyme are akin to those of the human red cell Fraction C, the prototype for most vertebrate tissue and red cell enzymes. One enzyme unit is defined as the amount that doubles the uncatalysed rate in this system which is saturated with CO_2 at 0 °C in a volume of 1.0 ml and using 0.05 M bartibal buffer, pH 7.9. Human red cells in this system were found to contain about 28 000 units/ml.

The uncatalysed time was 24 s. Ghosts containing 90 μg total protein gave readings consistently within <1 s of the uncatalysed time. Ghosts in a solution of up to 5% (v/v) Tween 80 gave readings identical to those of membranes in water only.

Since the method can detect a change of 1 s, the sensitivity may be reckoned as

$$\frac{24 - 23}{23} = 0.043 \text{ unit}$$

One enzyme unit corresponds to 0.16 μg of carbonic anhydrase by the following calculation. Human red cells contain 150 μmoles carbonic anhydrase per l (Maren[1]) or 4.5 g/l. One l contains $2.8 \cdot 10^7$ units and therefore 1 unit = 0.16 μg of carbonic anhydrase. In the calculation the difference in the activity between the two isoenzymes, B and C, in human red cells has been neglected, as we are concerned only with the overall or mean activity of the preparation. The activity in human red cells is essentially all due to C, even though there is about 5 times as much B as C (Maren[1]). One unit corresponds to 0.027 μg carbonic anhydrase C and 0.133 μg carbonic anhydrase B.

These ghosts, therefore, contain < 0.043 unit or < 0.007 μg of carbonic anhydrase (both isoenzymes). This represents < 0.008% of the total protein present. Table I compares the relative concentrations of haemoglobin and carbonic anhydrase in the red cell to that found in the membrane ghosts.

TABLE I

RELATIVE CONCENTRATIONS OF HAEMOGLOBIN AND CARBONIC ANHYDRASE IN RED CELLS AND MEMBRANE GHOSTS

	Red cells (g/l)	*Ratio*	*Ghosts (% protein)*
Haemoglobin	300	67	1.1
Carbonic anhydrase	4.5	1	<0.008

The data indicate that carbonic anhydrase is not concentrated in the membrane as would be expected for a bound component. This confirms the earlier findings[1,5,6] that red cell ghosts contain no bound carbonic anhydrase.

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