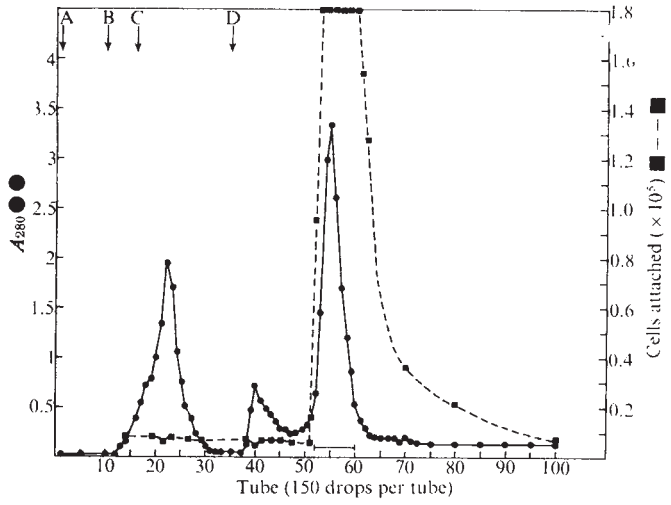


**Fig. 7** DEAE-11 chromatography of 2nd $AmSO_4$ fraction. At each arrow, the following solutions were added to the 4.5 × 20 cm column: (A) 100 ml of diluted factor; (B) 60 ml of 0.25 strength standard buffer; (C) 200 ml of 0.65 strength standard buffer; (D) elution with 1l of 0.25 M NaCl + 0.1 M imidazole (*p*H 6.8). Tubes 52 to 60 were pooled.

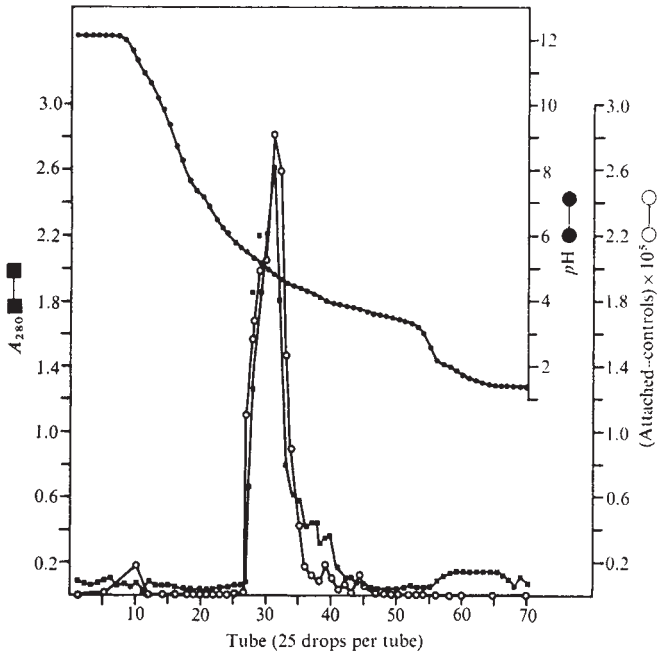


**Fig. 8** Isoelectric focused in a LKB 110 ml column, in a sucrose gradient containing 1.4 ml *p*H 3–6 LKB ampholytes and 0.14 ml *p*H 3–10 LKB ampholytes.

produced by most tissue culture cell lines[5]. Finally, cell attachment to collagen has been shown here to depend on physiological parameters; namely, a requirement for divalent cations as well as a specific high molecular weight protein.

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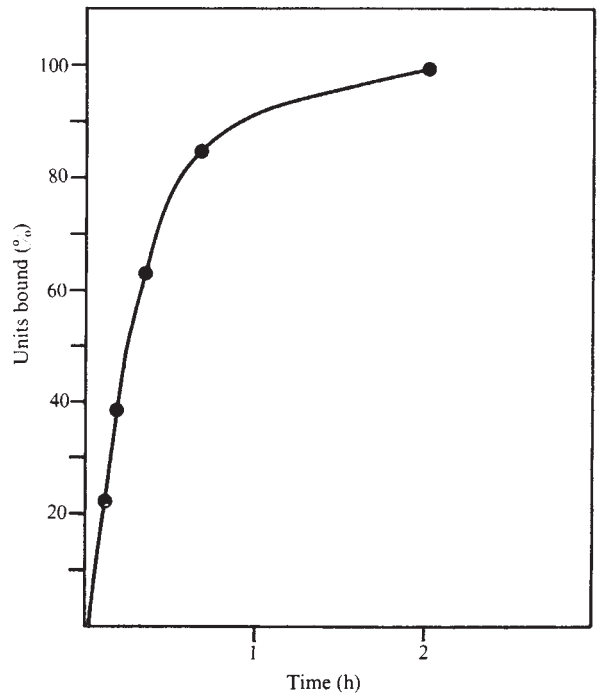


**Fig. 9** Binding of purified attachment factor to collagen in the absence of divalent cations or cells. Collagenised plates were treated with a series of dialysed factor concentrations in 0.9% NaCl and treated as described in the text. The washed plates were assayed for attachment activity in Eagle's medium (with $Ca^{2+}$ and $Mg^{2+}$ + 200 μg $ml^{-1}$ bovine serum albumin).

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1 Stoker, M. G. P., O'Neill, C., Berryman, S., and Waxman, V., *Int. J. Cancer*, **3**, 683 (1968).
2 Flaxman, B. A., Lutzer, M. A., and Scott, E. J. V., *J. Cell Biol.*, **36**, 406 (1968).
3 Harness, R. D., *Biol. Rev. Cambridge*, **36**, 399 (1961).
4 Bailey, A. J., *Comprehensive Biochem.*, **26B**, 297 (1962).
5 Goldberg, B., and Green, H., *Proc. natn. Acad. Sci., U.S.A.*, **59**, 1110 (1968).
6 Jackson, D. S., and Cleary, E. C., *Methods of biochemical analysis*, **15**, 25 (1967).

## Effect of zinc on haemoglobin binding by red blood cell membranes

RESULTS of recent studies in our laboratory have suggested that zinc plays an important role in sickle cell anaemia. A significant proportion of sickle cell patients are zinc deficient[1]. Zinc binds to haemoglobin and increases oxygen affinity[2,3]. Sickle cells treated *in vitro* with zinc show markedly improved filterability at concentrations too low to be explained on an oxygen affinity basis[4], and zinc may interact with the membranes to affect filterability. A recent report suggests that sickling involves the accumulation of calcium[5] which is known to reduce red cell membrane deformability[6]. We have now found that zinc decreases the amount of haemoglobin associated with red cell membranes and inhibits the effect of calcium in causing haemoglobin retention by membranes.

Single stage red cell membranes were prepared by the technique of Hoffman[7] with minor modifications. Agents to be incorporated into these membranes were added during

Table 1 Haemoglobin retention by ghost cells

| Sample type | Hb (g% in initial packed cells) | Hb (g% in final ghost pellet) | Mean cell volume ($\mu m^3$) | Hb (g% corrected for change in MCV) | % Retention of Hb | t test |
|---|---|---|---|---|---|---|
| No addition (control) | 25.21±1.8 | 1.86±0.6 | 73.6±1.9 | 1.86±0.6 | 7.4±2.5 | |
| | | | | | | $t = 6.3$ $P < 0.001$ |
| $ZnSO_4$ ghosts | 24.94±0.8 | 0.79±0.2 | 22.9±2.5 | 0.25±0.1 | 1.0±0.3 | |
| $CaCl_2$ ghosts | 25.81±1.0 | 21.79±2.4 | 21.5±1.6 | 6.39±0.9 | 24.6±4.1 | $t = 4.5$ $P < 0.01$ |
| $CaCl_2 + ZnSO_4$ ghosts | 24.48±2.1 | 10.41±4.0 | 22.1±2.3 | 2.50±0.5 | 10.7±2.6 | |

All values are the mean ±1 s.d. of four observations. $ZnCl_2$ had approximately the same effects when substituted for $ZnSO_4$.

haemolysis. The principle of this technique is based on the knowledge that erythrocyte membranes lose their selective permeability at haemolysis and immediately thereafter, facilitating the introduction of various normally nonpenetrating compounds into the cells[8,9]. In our experiments normal red cells to be haemolysed were divided into four aliquots and treated as follows: no addition (control), zinc sulphate (1.5 mM), calcium chloride (1 mM), and calcium chloride (1 mM) plus zinc sulphate (1.5 mM). Hypotonic exposure lasted 20 min. In all but the control aliquot enough sodium chloride was added to achieve the same ionic strength as the calcium chloride plus zinc sulphate solution. After hypotonic exposure with 10 volumes of water the red cell membranes were resealed with isotonic saline–0.01 M Tris buffer, *p*H 7.4, and washed until the supernatant was clear of haemoglobin. The haemoglobin in these ghost cell preparations was measured by the cyanmethaemoglobin method. The size and the mean cell volume (MCV) of the ghost cells were calculated using a Coulter counter with multichannel particle size analyser and recorder. Haemoglobin concentrations in the membrane preparations were corrected for the decreased size of the red cell membranes compared with original red cells, since shrinkage of these ghost cells results in some increase in concentration of entrapped haemoglobin.

Table 1 shows that the amount of haemoglobin retained in the membrane preparations after final washing was much less in the presence of zinc compared with control membranes. In the presence of calcium a large amount of haemoglobin was retained, as reported before[10,11]. When zinc was present together with calcium, however, much less haemoglobin was retained compared with calcium alone, although in both cases the membranes were similar in size (both were small). Equimolar concentrations of lanthanum chloride, but not magnesium, also decreased the haemoglobin-retaining effect of calcium in these preparations (data not shown).

Calcium is known to interact with the interior of the red cell membrane, altering its configuration, decreasing passive permeability[10], decreasing deformability[6,11] and increasing haemoglobin retention[10,11]. Zinc seems to counteract the retention of haemoglobin by red cell membrane both in the presence and absence of added calcium. One effect of calcium may be to crosslink haemoglobin and membrane sites. If so, calcium may be involved in the pathogenesis of irreversibly sickled cells by promoting such crosslinking. It is tempting to speculate that zinc improves the filterability of sickle cells, and promotes the elution of haemoglobin from red cell membranes, by blocking our hypothesised calcium-induced crosslinking of haemoglobin to membranes.

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1 Schoomaker, E. B., Prasad, A., Oelshlegel, jun., F. J., Ortega, J., and Brewer, G. J., *Clin. Res.*, **21**, 834 (1973).
2 Oelshlegel, jun., F. J., Brewer, G. J., Prasad, A., Knutsen, C., and Schoomaker, E. B., *Biochem. biophys. Res. Commun.*, **53**, 560 (1973).
3 Oelshlegel, jun., F. J., Brewer, G. J., Knutsen, C., Schoomaker, E. B., and Prasad, A., *Clin. Res.*, **21**, 833 (1973).
4 Brewer, G. J., Dash, S., and Oelshlegel, jun., F. J., *J. clin. Invest.*, Abstr. (in the press).
5 Eaton, J. W., Skelton, T. D., Swofford, H. S., Kolpin, C. E., and Jacob, H. S., *Nature*, **246**, 105 (1973).
6 Weed, R. I., Lacelle, P. L., in *Red cell membrane structure and function* (edit. by Jamieson, G. A., and Greenwalt, T. J.), 318–338 (Lippincott, Philadelphia and Toronto, 1969).
7 Hoffman, J. F., *J. gen. Physiol.*, **45**, 837 (1962).
8 Hoffman, J. F., *J. gen. Physiol.*, **42**, 9 (1958).
9 Seeman, P., *J. Cell Biol.*, **32**, 55 (1967).
10 Weed, R. I., Lacelle, P. L., and Merrill, E. W., *J. clin. Invest.*, **48**, 795 (1969).
11 Palek, J., Curby, W. A., and Lionetti, F. J., *Am. J. Physiol.*, **220**, 19 (1971).

## Nuclear segregation in *Bacillus subtilis*

JACOB, BRENNER AND CUZIN[1] suggested that prokaryote nuclear segregation was achieved by cell surface extension between surface sites to which the chromosome is permanently attached (Fig. 1*a*). Their model (model A) showed nuclear segregation occurring in newly divided cells, with all surface extension in one cycle occurring between the nuclei. Thus at cell division, nuclei would be arranged symmetrically in the half cell but asymmetrically during the interdivisional period. If nuclear segregation occurs during mid-cycle and cell extension is continuous throughout the cycle, the nucleus cannot reach a symmetrical position (that is at 25% of the cell length) by the end of the cycle relying solely on length extension. To overcome these difficulties, Clark[2] proposed that the nucleus is always located at the junction of old and new membranes with growth occurring on one side of the nucleus (model B, Fig. 1*b*). At nuclear segregation, the growing point divides allowing growth of the cell envelope between the nuclei as shown in Fig. 1*b*. Nuclei remain attached at the site of envelope extension, giving a newborn cell with an asymmetrically arranged nucleus. Donachie and Begg[3] have presented evidence for terminal growth regions in slow growing cells as predicted by Clark's model. A third possibility is that the nucleus is located centrally in new born cells and moves to a position at the centre of a half cell at the time of segregation (model C, Fig. 1*c*). These, and other possibilities, can be explored by determining the position of nuclei in relation to cell length in exponential phase cells.

*Bacillus subtilis* (168/S) (an asporogenic derivative of 168 *tryp*$^-$ *thy*$^-$ able to grow on succinate as sole carbon