Table 1 Haemoglobin retention by ghost cells

Sample type	Hb (g% in initial packed cells)	Hb (g% in final ghost pellet)	Mean cell volume (μm^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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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

source at a generation time of 115 min) was grown in either succinate or glucose based minimal medium to minimise the cell separation time[5] and to give substantial numbers of mononucleate cells[4]. Using Giemsa, nuclei appeared as small spherical dark blue bodies in a pale pink-to-colourless cytoplasm. The number and appearance of nuclei was highly reproducible under constant cultural conditions. Cells contain an average of 1.3 and 1.8 nuclei per unit on succinate and glucose media respectively. Each bacterial particle has been regarded as a unit even while containing a septum. The time interval between nuclear segregation and cell separation, calculated from the age distribution theorem[5] is 48.5 and 45 min for succinate and glucose (generation times 115 and 57 min). Large numbers of cells were photographed and, from the negatives projected on to a screen, positions and diameters of nuclei and lengths of cells were determined. Less than 5% of any population could not be scored because of poor staining and a slightly larger proportion was discarded that were not in focus. All measurements of the position of nuclei were taken from the distal edge of the nucleus to the centre of the cell. These data are shown in Fig. 2.

The critical difference between model C and the other two is the abrupt transition of the nucleus from the centre of the cell to the centre of the half cell. If nuclei move apart gradually at early stages of segregation the nuclear attachment sites should be sufficiently close that nuclei would overlap and be scored as one larger than average nucleus. When plotted against cell length, the distance between cell centre and the distal edge of the nucleus should vary continuously as the two nuclei become visable. Figure 2 indicates that this is not so, and in fact the distal edges of nuclei of 85% of binucleate cells are at least 2.5 radii from the cell centre. Nuclei vary only slightly in size between mononucleate and binucleate cells. The best line connecting binucleate and mononucleate cells has a slope of about 1.5 in both media indicating that the nucleus moves along the cell apparently three times faster than the rate of length extension. The frequency distribution of distances from the cell centre to centre of nuclei are shown for two size classes of binucleate cells in Fig. 3. The peak for all cells is between 20 and 24% from the centre, but there is a skew towards the centre in smaller cells. The data are shown relative to the average nuclear radius of mononucleate cells and there is only minimal overlap between mono and binucleate cells. The peak of the distribution of distance from the cell centre is slightly less than 25%, possibly because

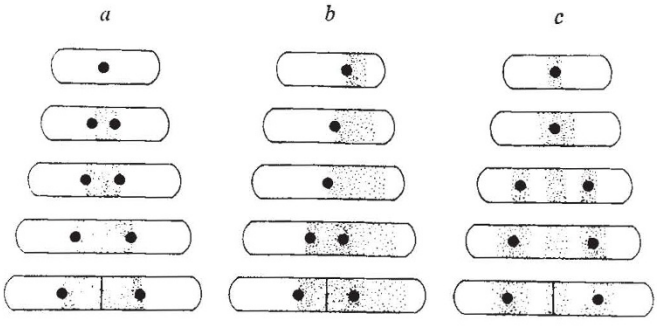

Fig. 1 Models of nuclear segregation in bacteria. *a*, The model of Jacob, Brenner and Cuzins[1] (model A). All surface growth occurs between the nuclei. *b*, The model of Clark[2] (model B) in which nuclei are rigidly attached to a particular site and growth occurs only to one side of the nucleus. *c*, An alternative model (model C) in which nuclei move to a site 25% of the cell length from the poles. Surface growth occurs to both sides of the nucleus. Most intense stippling indicates newest cell envelope.

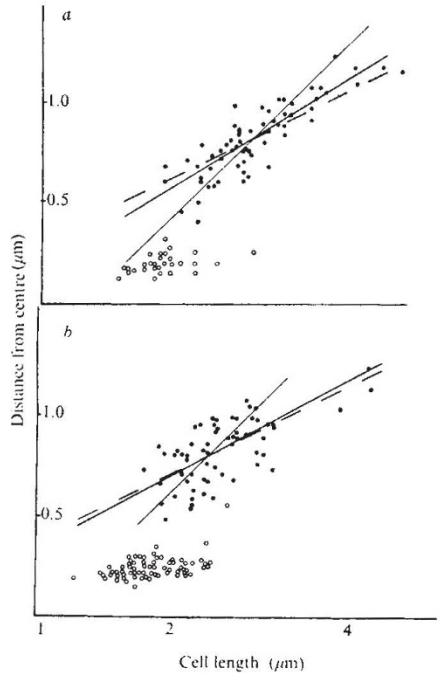

Fig. 2 Position of nuclei relative to cell length. *a*, Glucose grown cells (96 units in sample); *b*, Succinate grown cells (247 units in sample). To stain the nuclei, bacteria were heat fixed onto glass slides, treated with 1 N hydrochloric acid at 60° C for 7 min, washed in phosphate buffer (*p*H 7.4), and stained with Giemsa (diluted 1:10 with distilled water) for 5 min. ●, Binucleate cells; ○, mononucleate cells; — —, graph of 25% of cell length; —, graph of 50% of cell length; —, line of best fit (obtained by method of least squares) for distal edge of nucleus. Regression coefficients for the glucose grown cells (*a*) for distal edge and nuclear centre respectively were 0.31 (significantly different to 0.25 at 1% probability using Students' *t* test) and 0.27 (not significantly different at 5% probability) respectively. The correlation coefficients were 0.87 and 0.76 respectively. Regression coefficients for the succinate grown cells (*b*) for distal edge and nuclear centre respectively were 0.25 and 0.23 and were not significantly different to 0.25 at 5% probability. The correlation coefficients were 0.67 and 0.65 respectively.

cell length has been systematically slightly overestimated. As less than 5% of mononucleate cells show any deviation from the centre of the cell, the nuclei are probably located at 25% from the cell centre immediately before cell division.

Model B proposes that nuclei should be arranged asymmetrically in most cells of an exponential population. Less than 5% of mononucleate cells show any asymmetry on either medium, and the data in Fig. 3 indicate that nuclei of most binucleate cells are arranged symmetrically in each half cell.

Model C differs from A and B in the slope of the line relating nuclear position with cell length. Models A and B predict a slope of 0.5 whereas model C predicts a slope of 0.25. Although for both sets of data the scatter is considerable, the data favour model C rather than A or B (Fig. 1). Using autoradiography, Mendelson[6] has shown that nuclei in binucleate cells of *Bacillus subtilis* 168 are predominantly 25% from either pole but mononucleate cells were not included in his analysis.

The rapid transition (less than 6 min) indicated by these data may be illusory, as in growing cells the nucleus may be arranged more diffusely than stained preparations suggest. If so, the location of the fixed nucleus probably represents the focus of the chromosomes' strongest interactions with the cell surface. The nucleus, however, may form a loose link with a new site earlier in the cell cycle. In *Escherichia coli*, segregation occurs close to the time of

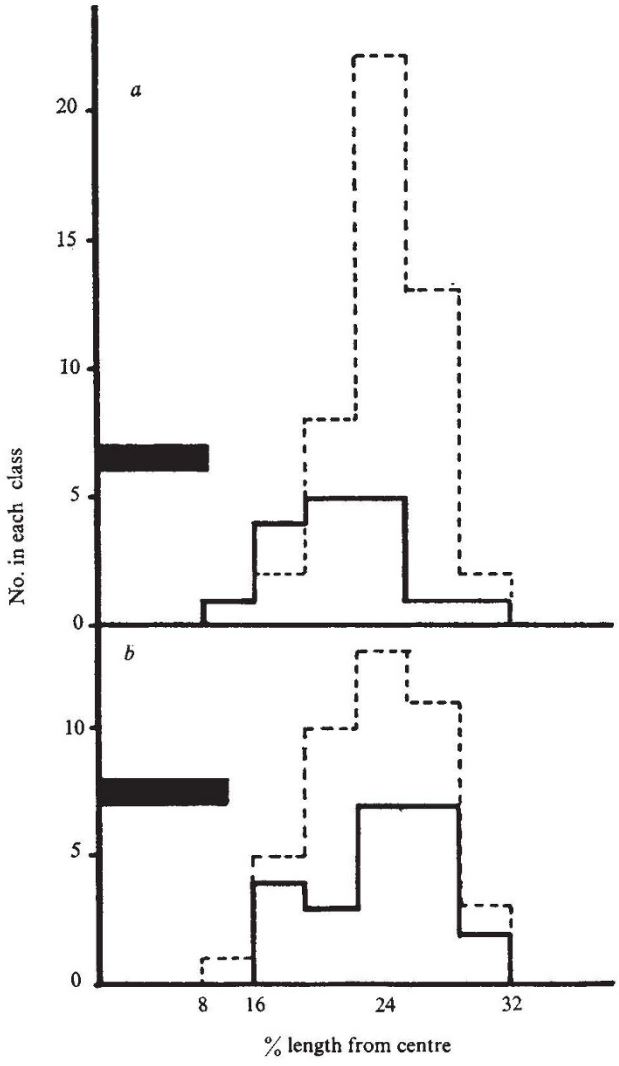

Fig. 3 Frequency distribution of distances from cell centre to centre of nuclei. *a*, Data from Fig. 2*a*. ——, Data from binucleate cells less than 2.8 μm long. - - -, Cells of greater length. *b*, Data from Fig. 2*b*. ——, Binucleate cells less than 2.6 μm long. - - -, Cells of greater length.

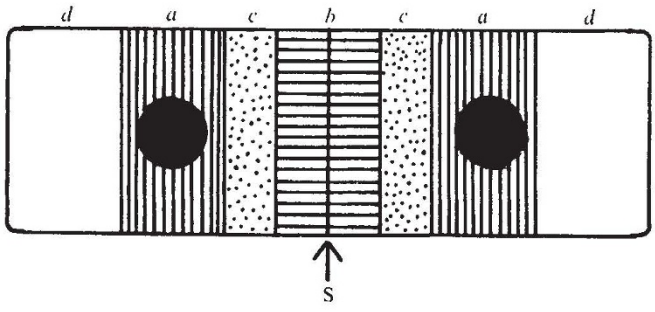

Fig. 4 Postulated origin of cell surface for a cell at division. Length of zone calculated assuming linear rate of surface extension from sites occupied by nuclei and that segregation occurs at mid-cycle (S, septum). *a*, Synthesised in interval between segregation and cell division; *b*, synthesised before segregation in current cycle; *c*, synthesised in interval between segregation and cell division in previous cycle; *d*, synthesised before segregation in previous cycle.

chromosome termination[7] and this may represent the signal for pre-existing weak interactions with new sites to become predominant.

If the site of nuclear attachment is a major site of cell envelope extension and the rate of length extension is linear at each growth zone, the pattern of surface growth shown in Fig. 4 follows from the pattern of nuclear segregation in model C. The diagram shows the postulated distribution of cell surface synthesised during four periods for a cell which is about to divide. Lengthwise growth between segregations (Fig. 4*b* and *c*) must equal half the length of a cell at segregation. Therefore, if nuclei move 25% of the cell length, they would move to the junction of old and new surface formed one generation previously. As this was the site occupied by the nucleus at the time of the previous segregation, 'the pre-existing weak interactions', discussed here, may have been established at this time.

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Chronic response of dogs to parathyroid hormone infusion

WHEN parathyroid hormone is infused chronically at a near-physiological rate, it causes hypercalcaemia by a mechanism fundamentally different from that which follows acute injections. Hormones, like drugs, have characteristic rates of metabolism and excretion and their distribution in the body is affected by binding to plasma proteins and membranes. Thus similar dependence of the pattern of response on entry rate is to be expected in the case of other hormones with short half lives, acting on multiple receptors of varying sensitivity.

As discussed in a recent review[1], parathyroid hormone (PTH) is an 84-residue single-chain polypeptide, which is probably secreted continuously at a rate regulated by the calcium concentration of the plasma. Its principal function seems to be to raise the calcium concentration in the plasma and extracellular fluid. Most of its multiple actions contribute to this effect and the rate of secretion of the parathyroid glands is inversely related to the circulating calcium concentration, providing close negative feedback control of the plasma calcium level. PTH accelerates bone breakdown and increases renal calcium retention and intestinal calcium absorption, this being the order of relative contribution of these actions to the hypercalcaemia elicited by injecting a large dose. Consideration of their individual dose-response curves, however, suggested a very different relative importance in normal physiology and led us to make a direct test of the effects of infusing minute doses continuously for periods of several weeks.