Chemotaxis in *Escherichia coli* analysed by Three-dimensional Tracking

HOWARD C. BERG & DOUGLAS A. BROWN

Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80302

Chemotaxis toward amino-acids results from the suppression of directional changes which occur spontaneously in isotropic solutions.

If a capillary tube containing an attractant is inserted into a suspension of motile bacteria, the bacteria accumulate near the mouth where the concentration of attractant is relatively high. Pfeffer[1] introduced this technique as an assay for chemotaxis in 1884. In 1901, Rothert[2] and Jennings and Crosby[3] noted that bacteria often swam past the capillary but returned after failing to enter regions of much lower concentration. It is now generally thought that chemotactic bacteria actively avoid regions of lower concentration by backing up or by choosing new directions at random[4,5]. This is not obvious in *Escherichia coli*, since these bacteria repeatedly change their directions even in the absence of an applied stimulus.

To study this motion in detail, we built a microscope which automatically follows individual cells[6]. We have used this on mutants of *E. coli* K12[7]: a wild type[8], a nonchemotactic mutant[8], an uncoordinated mutant[9], a mutant defective in taxis toward serine[10,11] and a mutant defective in taxis toward aspartate[11]. The results which we describe here demonstrate that the response to serine and aspartate at concentrations of order 10^{-5} M is not an avoidance response; when cells swim down gradients of these amino-acids their motion is indistinguishable from that in isotropic solutions; when they swim up the gradients they change direction less frequently.

In another communication[12] we discuss a solution to the diffusion equation which allows us to compute the concentration of attractant outside the mouth of a capillary, and, thus, to follow cells in defined gradients.

Motion in Isotropic Solutions

The motion appears as an alternating sequence of intervals during which changes in direction are gradual or abrupt—we call these "runs" and "twiddles", respectively. Genetic and environmental differences in behaviour are associated chiefly with the lengths of runs. Fig. 1 illustrates this for the wild type and a nonchemotactic mutant. A number of results of a quantitative run-twiddle analysis are given in Table 1.

Runs are long in *cheC* 497, short in *unc* 602, and of intermediate length in AW405 (Table 1). Mutants able to respond to a restricted set of attractants swim much like the wild type; the motion of the serine-blind mutant AW518 is essentially identical to that of AW405; the aspartate-blind mutant AW539 has somewhat shorter twiddles and somewhat longer runs (0.11 ± 0.18 s and 1.3 ± 2.1 s, respectively). The speed is nearly uniform during runs, but the bacteria slow down or stop on twiddling (Fig. 2). The mean change in direction from the end of one run to the beginning of the next is less than 90° (Table 1). If the bacteria chose a new direction at random, the probability of an angle change between θ and $\theta + d\theta$ would be $1/2 \sin\theta \, d\theta$, the mean value of θ would be 90°, and the standard deviation would be 39.2°. The distribution observed, however, is skewed toward small angles (Fig. 3). If changes in direction were random, the skew would be toward large angles because the digital

Table 1 Run-twiddle Analysis of Mutants Swimming in a Homogeneous, Isotropic Medium

Strain Type	AW405 Wild type	*Unc* 602 Uncoordinated	*CheC* 497 Nonchemotactic
Number of bacteria tracked	35*	10	14
Total tracking time (min)	20	3.0	2.7
Mean speed (μm/s)†	14.2 ± 3.4	14.4 ± 3.9	20.0 ± 4.9
Mean twiddle length (s)‡	0.14 ± 0.19	0.14 ± 0.24	0.10 ± 0.13
Mean run length (s)	0.86 ± 1.18	0.42 ± 0.27	6.3 ± 5.2
Mean change in direction from run to run (°)	68 ± 36	74 ± 33	33 ± 15
Mean change in direction during runs (°)	23 ± 23	18 ± 23	35 ± 22
Mean angular speed while twiddling (°/word)§	56 ± 29	54 ± 27	41 ± 32
Mean angular speed while running (°/word)§	14 ± 9	19 ± 9	9 ± 6

Data points (words) were generated at the rate of 12.6 per second. The beginning of a run was scored if the angular speed§ was less than 35°/word for three successive words. The end of a run was scored if the angular speed was greater than 35°/word for two successive words or if it was greater than 35°/word for one word, provided, in the latter case, that the change in the average direction between successive pairs of words was also greater than 35°. The angular speed is sensitive to short term fluctuations in the data. These depend on the ways in which the bacteria wobble and on the time constants (0.08 s) of the circuits which precede the analogue-to-digital converter. The time constants, the recording rate and the value 35°/word were chosen empirically by comparing results of digital analyses with plots of the kind shown in Fig. 1.

* Experiments done with three different cultures.

† The values are the means ± one standard deviation. In the calculation of the mean speed the mean for each bacterium is weighted equally, and the standard deviation is the standard deviation in the mean.

‡ In this and in subsequent entries in the table each twiddle or run is weighted equally; the standard deviations are of the same order of magnitude as those found with a single bacterium.

§ The angular speed is the change in the direction of motion from one word (data point) to the next.

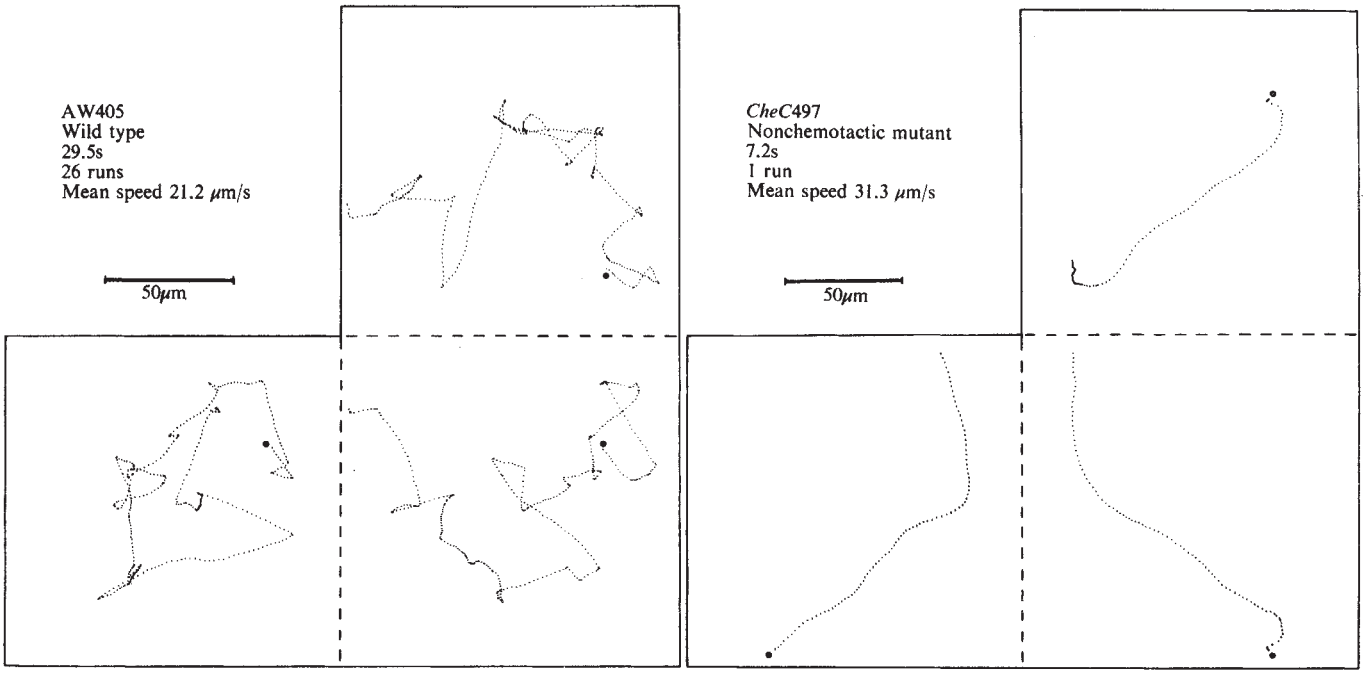

Fig. 1 Digital plots of the displacement of a wild type bacterium, AW405, and a generally nonchemotactic mutant, *cheC* 497, at the rate of 12.6 words (data points) per second. Tracking began at the points indicated by the large dots. The plots are planar projections of three-dimensional paths. If the left and upper panels of each figure are folded out of the page along the dashed lines, the projections appear in proper orientation on three adjacent faces of a cube. The cultures were grown in a minimal salts medium on glycerol, threonine, leucine, and histidine, as described by Hazelbauer *et al.*[10]. They were washed twice at 4° C with a solution containing 10^{-2} M sodium phosphate (*p*H 7.0), 10^{-4} M EDTA (ethylenediamine tetraacetate) and 10^{-3} M magnesium sulphate and diluted at room temperature to an optical density of 0.01 (590 nm) in a solution containing 10^{-2} M sodium phosphate (*p*H 7.0), 10^{-4} M EDTA, and 0.18% (w/v) hydroxypropyl methylcellulose (Dow Methocel 90 HG). They were tracked as such at 32.0° (viscosity 2.7 cp) in a tantalum and glass chamber 2 mm in diameter and 2 mm high.

analysis ignores the smallest changes (Table 1, legend). Changes in direction also occur during runs (Table 1). The drift is about what one would expect from rotational diffusion: the root-mean-square angular deviation of a 2 μm diameter sphere occurring in t sec in a medium of viscosity 2.7 cp at 32° C is 29 $\sqrt{t}$ degrees.

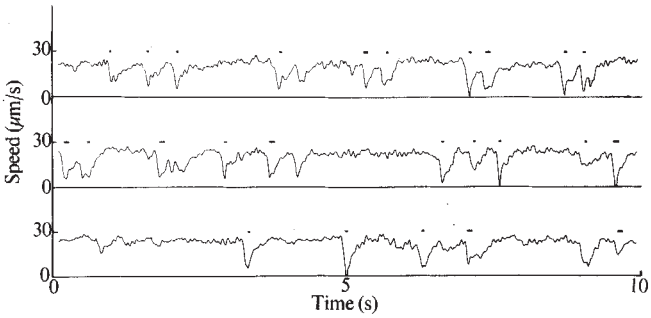

Fig. 2 The speed of the wild type bacterium of Fig. 1 displayed by an analogue monitor. The recording has been divided into three parts, each 9.8 s long; the figure should be read from left to right and top down. Twiddles occurred during the intervals indicated by the bars. Note the consequent changes in speed. The longest run can be seen at the left end of the bottom trace. It appears in the upper panel of Fig. 1 angling downwards and slightly to the left, five runs from the end of the track. It is 45 words or 3.57 s long.

The shortest twiddles and the shortest runs are the most probable (Fig. 4). The distribution of twiddle lengths is exponential (Fig. 4*a*). The distribution of run lengths is exponential for *unc* 602 (not shown) but only approximately so for AW405 (Fig. 4*b*). If for AW405 one allows for variations in mean run length for different bacteria, the curvature in the semi-log plot of the aggregate run-length data vanishes (Fig. 4*c*). From calculations of autocorrelation functions of sequences of twiddles and of sequences of runs we conclude that twiddles and runs of different length occur at random. The statistics are Poisson; for a given organism in a given isotropic environment the probability per unit time of the termination of a twiddle or the termination of a run is a constant.

The wild type is known to have chemoreceptors for serine, for aspartate and for a number of sugars[7]. If serine is added to suspensions of AW405 (no gradients), the run-length distributions remain exponential but shift dramatically toward longer runs (Fig. 5); the twiddles are suppressed. Calculations of the autocorrelation functions indicate that runs of

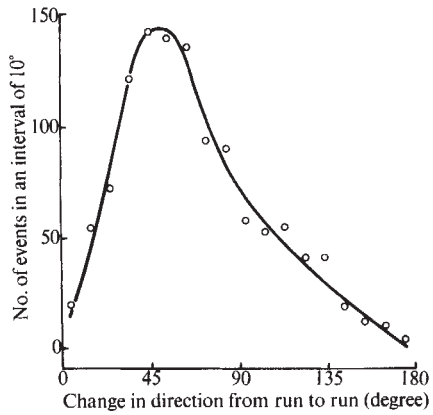

Fig. 3 Distribution of changes in direction from the end of one run to the beginning of the next for the wild type bacteria of Table 1. The distribution was constructed from 1,166 events by summing the numbers falling in successive 10° intervals. If the analysis is confined to the shortest twiddles, the distribution is skewed even farther toward small angles (mean and standard deviation 62±26°).

different length still occur at random. The shift does not occur with aspartate (Fig. 5), even though the chemotactic responses to aspartate and serine are nearly the same[10,11]. The shift due to serine involves the serine chemoreceptor; it does not occur in the serine-blind mutant AW518. The shift is not a metabolic effect, since it can be generated by a non-metabolite sensed by the serine chemoreceptor (experiment done with α-amino-isobutyric acid[11] and the aspartate-blind mutant AW539, which also shows the shift with serine). It does not occur in the uncoordinated mutant *unc* 602. Adler[7] notes that "serine slightly inhibits chemotaxis toward all other attractants, and this inhibition remains unexplained". If chemotaxis results from the suppression of twiddles (see below), serine should inhibit chemotaxis generally, provided that the mutants tested have a functional serine receptor.

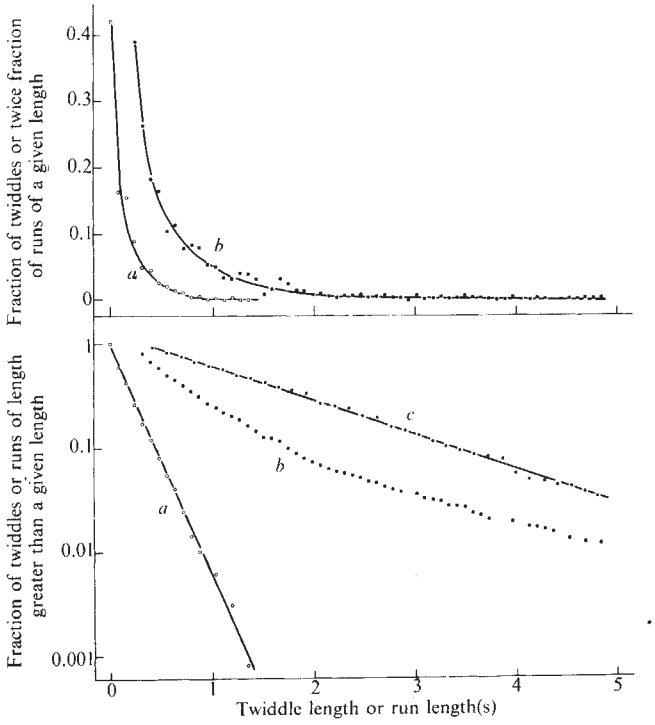

Fig. 4 Top: the fractional number of twiddles (*a*) or runs (*b*) of different lengths for the wild type bacteria of Table 1. There were 1,201 twiddles and runs. All the twiddles are plotted, but runs longer than 5 s are not (lengths 5.1, 5.4, 6.0, 6.4, 7.0, 7.1, 7.1, 7.2, 7.9, 7.9, 7.9, 12.9 and 24.2 s). Bottom: the same data plotted as the logarithm of the fractional number of twiddles (*a*) or runs (*b*) of length greater than a given length[13]. Curve *c* was obtained by scaling the run lengths of each bacterium so that its mean run length was equal to the ensemble mean.

Serine has other effects on the motion of the wild type which are less dramatic but which have a similar concentration dependence. The mean speed increases by about 40%; the mean twiddle length, the mean change in direction from run to run, and the mean angular speed while running all decrease by about 40%. With aspartate there is a slight increase in speed, but the other changes are not significant.

Motion in Gradients

Gradients were generated by diffusion of attractants from capillary tubes of the kind used by Adler[7], which we inserted through a flat side wall of the tracking chamber. In preliminary experiments we found the response (the number of bacteria entering a capillary in 1 h) to be negligible when bacteria were used at an optical density of 0.1 (590 nm; about 10^8 bacteria/ml.); the clouds of bacteria which accumulated near the mouths of the capillaries sank. At optical densities of order 0.01 the response to an attractant was proportional to the optical density, and the dependence on the concentration of the attractant was similar to that described by Adler[7,11] (experiments at 32° with serine, aspartate, α-amino-isobutyric acid and α-methyl-DL-aspartate, all in tracking medium).

Table 2 Run-twiddle Analysis of the Wild Type Swimming in Gradients

Attractant	Serine		Aspartate	
	Control	Gradient	Control	Gradient
Number of bacteria tracked	11	34	23	24
Total tracking time (min)	7.1	14.8	11.1	11.0
Mean run length (s)	0.83±0.88	1.67±2.56	0.83±0.90	0.90±1.56
Mean concentration of attractant (μM)	0	9.5±2.7	10	8.4±2.0
Mean distance from mouth of capillary (μM)	—	577±112	—	644±88
Mean value of $(\partial C/\partial r)/C$ (mm^{-1})	—	2.5±0.4	—	2.4±0.4

The cells were prepared as described in the legend of Fig. 1, except that the suspension used for the aspartate control also contained 10^{-5} M aspartate. Capillaries were used in the gradient experiments but not in the controls. For each data point we computed the distance from the mouth of the capillary to the bacterium being tracked (r), the angle between the direction of motion of the bacterium and the gradient (the "inclination", 0° for motion radially down the gradient), the concentration of the attractant at the bacterium (C, as defined by equation (4), ref. 12, with $r_c=0.01$ cm, $C_0=2.0\times10^{-3}$ M, $D_{serine}=1.0\times10^{-5}$ cm^2/s and $D_{aspartate}=0.89\times10^{-5}$ cm^2/s), the steepness of the gradient at the bacterium ($\partial C/\partial r$), the time rate of change of the concentration at the bacterium (dC/dt), and the logarithmic derivatives $(\partial C/\partial r)/C$ and $(dC/dt)/C$.

Mean speeds, twiddle lengths, changes in direction and angular speeds are not shown; the values are essentially identical to those for AW405 (Table 1).

Results of the run-twiddle analysis for the gradient experiments are given in Tables 2 and 3. Runs are longer in the gradients (Table 2) than we would expect from the concentration dependence (Fig. 5). This is true for runs which move the bacteria up the gradient but not for runs which move them down the gradient (Table 3). The differences in the up-gradient and down-gradient data are dramatic when the run-length distributions are examined (Fig. 6). For serine, the distri-

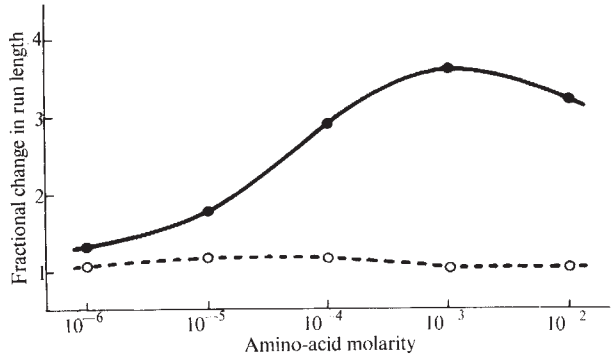

Fig. 5 Changes in mean run length caused by serine (●) or aspartate (○). The bacteria (AW405) were diluted in the tracking medium (Fig. 1, legend) to an absorbance of 0.02 (590 nm). Aliquots of this suspension were mixed with equal volumes of tracking medium containing L-serine or L-aspartate (Calbiochem A grade). Controls were made by omitting the amino-acids. The ratios of the mean run lengths observed in the presence and in the absence of the amino-acid are plotted as a function of concentration.

bution of runs down the gradient (Fig. 6*b*) is similar to the distribution in a 9 μM isotropic solution; for aspartate, it is indistinguishable from the control. From the data of Fig. 6 and from calculations of autocorrelation functions of sequences of runs (not separated into subsets), we conclude that the statistics are still Poisson. When a bacterium moves up the gradient the probability per unit time of the termination of a run decreases; when it moves down the gradient the probability reverts to the value appropriate to an isotropic solution of similar concentration. At the concentrations we have studied, the stimulus is sensed and acted on only when the bacterium swims up the gradient.

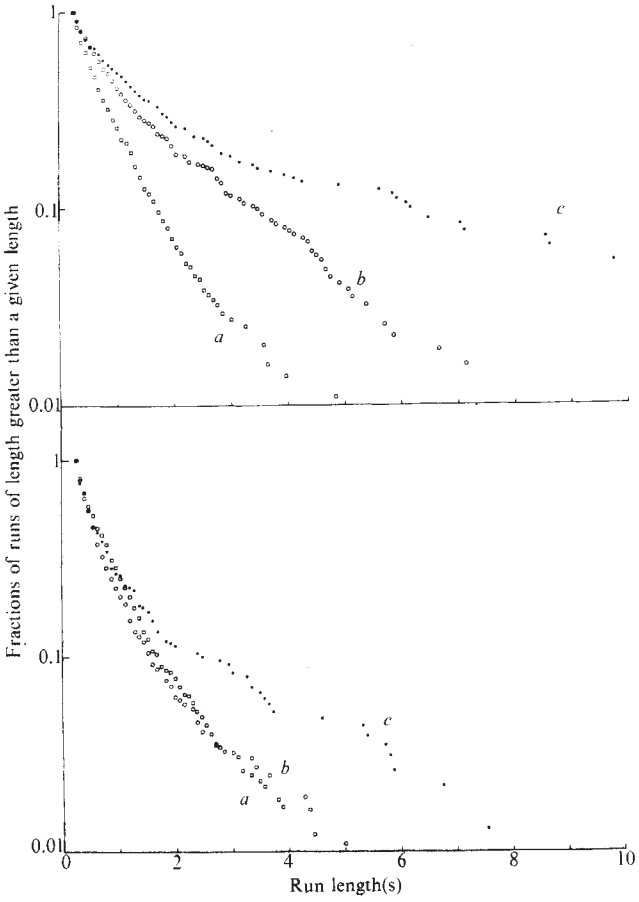

Fig. 6 The data from the serine (top) and the aspartate (bottom) experiments (Tables 2 and 3) plotted as the logarithm of the fractional number of runs of length greater than a given length. *a*, Runs in the control experiment; *b*, runs down the gradient; *c*, runs up the gradient.

Further proof of the assertion that motion away from the capillary is not sensed can be obtained from computation by linear regression[14] of the correlation between the length of a run and the mean value over the run of dC/dt, $(dC/dt)/C$, $-\partial C/\partial r$, or $-(\partial C/\partial r)/C$. These correlations are all positive (correlation coefficients of order 0.12 ± 0.02). If the analysis is confined to runs which move the bacteria up the gradient, the correlation coefficients are larger (of order 0.19 ± 0.03); if it is confined to runs which move the bacteria down the gradient, the coefficients are statistically insignificant (-0.02 ± 0.02). This implies that when the bacterium swims down the gradient there is no functional relationship between the length of a run and the derivatives of the concentration with respect to space or time. This is true both for serine and aspartate.

There is nothing in our data to suggest that the bacteria are able to steer in the direction of the gradient while running or that the motion is topotactic[4]. When they twiddle, the change in direction is still biased toward small angles, as in Fig. 3, but the angle chosen does not depend on the direction of the gradient; there is no correlation between the inclination at the end of a run (Table 2, legend) and the change in direction from run to run. Nor is there any correlation between the length of the run and the change in direction.

Table 3 Analysis of Runs which Move the Bacteria Up the Gradient or Down the Gradient

Attractant	Serine	Serine	Aspartate	Aspartate
Net displacement of runs	Up	Down	Up	Down
Mean concentration (μM)	10.0 ± 2.8	9.2 ± 2.6	8.8 ± 1.9	8.1 ± 1.9
Mean run length (s)	2.19 ± 3.43	1.40 ± 1.88	1.07 ± 1.80	0.80 ± 1.38
Mean run length expected from the control run length (Table 2) and the concentration dependence (Fig. 5) (s)	1.48	1.45	0.82	0.82

The runs of the gradient experiments (Table 2) divided into two subsets according to whether the net displacement of a run is toward or away from the mouth of the capillary (up-gradient or down-gradient). The mean speed was only slightly larger for runs up the gradient than for runs down the gradient (2% for serine, 7% for aspartate).

An accurate calculation of the mean rates at which the bacteria drift up the gradients could be made from the information in Tables 2 and 3 if we knew the functional dependence of the mean run lengths (for runs up the gradient) on inclination; the data at hand are inadequate. If we assume that the run-length bias is proportional to $\cos\theta$ ($90° < \theta < 180°$), the drift rate in serine is about 2.0 μm/s, and the drift rate in aspartate is about 0.9 μm/s. The value for serine is in rough agreement with that obtained by Dahlquist, Lovely and Koshland[15] for suspensions of *Salmonella* in exponential gradients of comparable steepness.

Mechanisms

When a bacterium runs, its flagellar filaments work together in a bundle of the kind photographed in *E. coli* by Ramsey and Adler (Ramsey, S. W., and Adler, J., private communication), or in *Salmonella* by Mitani and Iino[16]. When it twiddles the bundle probably loosens or comes apart. When the bundle re-forms, the cell goes off in a new direction. The direction chosen depends on the change in orientation of the bundle relative to the body of the cell. Smaller changes in direction require smaller changes in orientation and occur in shorter periods of time (Fig. 3). The stability of the bundle is improved by interaction with chemoreceptors. The association of an attractant with a receptor increases the stability even more. If the attractant is serine, the stability of the bundle is affected by both the average level of association (Fig. 5) and the rate at which it increases (Fig. 6). If it is aspartate, only the rate of increase is important (Fig. 6).

We do not know what the molecular structure of the twiddle generator is or how it is able to perturb the flagellar bundle. We do know it operates on Poisson statistics (Fig. 4) and that its firing rate can be suppressed by chemoreception. It is likely that the generator is built from elements which are missing or defective in generally nonchemotactic mutants. When the generator and the chemoreceptors are uncoupled, the generator runs free, and the mutants are uncoordinated.

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Random Packing of Equal and Unequal Spheres in Two and Three Dimensions

WILLIAM M. VISSCHER & M. BOLSTERLI

Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico 87544

A new computer simulation of random packing of spheres is applied to two- and three-dimensional problems with a uni-directional gravitational force.

THE ramifications of the sphere packing problem are multifarious. They are found, for example, in metallurgy, ceramics, soil science, biology, physics, chemistry, and many fields of engineering. The problem may be stated as follows: given spheres with radii distributed according to a prescribed probability density, and given that they are packed together randomly by some rule to be specified, what is the nature of the resultant heap? Density, average number of contacts, radial distribution function, and size distribution of interstices are a few of the quantities which have obvious importance in the fields mentioned above and which have been calculated or measured experimentally.

We approach the problem of random packing of spheres by means of a Monte Carlo computer simulation of the physical process of dropping spheres into a bin. Our packing rule differs from previous ones[1,2] in that our spheres position themselves under the influence of a unidirectional (vertical) gravitational force, rather than toward a centre of attraction.

The computer code in three dimensions is set up as follows: In order to avoid difficulties with the sides of an array, periodic horizontal boundaries are assumed, so that a ball at (x, y, z) reappears at $(x \pm L_x, y \pm L_y, z)$. Balls are dropped sequentially from a random point above the $L_x \times L_y$ bin. When a ball is dropped it hits ball m or the floor. If it has contacted ball m, it then rolls down in a vertical plane on m until it is in contact with m and n. Then it rolls downwards in contact with both m and n until it makes contact with p. If the contact with m, n, and p is stable, it stops. If not, it rolls on the double contact that goes down most steeply, and so on. Any time a ball contacts the floor, it stops.

In two dimensions the code is similar but simpler in that only the discs in the top layer need be checked for hits or contacts.

New features of our calculation include domain structure in 2d random packing, computer simulation of jiggling the container, the effect of machining errors on the buildup of a regular hexagonal close packed (h.c.p.) array of balls, densities of binary random mixtures of spheres in 2d and 3d, and simulation of shape irregularities, interparticle attractions or stickiness.

Two-dimensional Calculations: Buildup of Heaps of Hoops

The 2d simulation code has been used to build stacks of discs. An example of a binary mixture is shown in Fig. 1. The discs were dropped from random points above the periodic baseline.

Fig. 2 shows an array of discs, monosize except for the bottom layer, which is chosen from a uniform random distribution of radii between $R = 1.0 \pm 0.2$. This uneven base causes the structure to look quite random for a while, but soon order reappears in the form of domains of nearly square structure, tipped at about 45° from the vertical. These domains are of the order of 8 or 10 discs on a side, and fill most of the stack. The same kind of domain also appears if the array is started with a row of uniform discs with subsequent discs randomly chosen from a uniform distribution with a very small spread like $R = 1 \pm \frac{1}{2} \times 10^{-6}$; the smaller the spread the longer the regular hexagonal structure persists. (In the following we call this the fuzzy monosize distribution with spread 10^{-6}.)

These results contrast with those obtained by Kausch *et al.*[3] who found that ordered domains will form spontaneously, but that the ordered domains constituted only a small fraction of