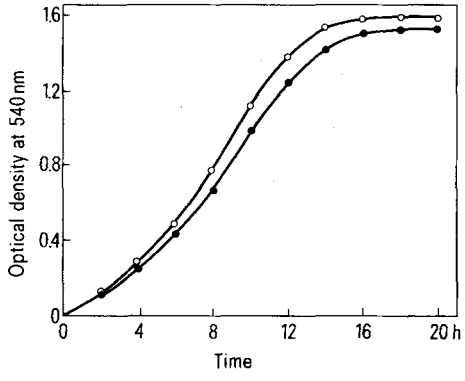
Since GS has a low K_m for ammonia, it can, together with GOGAT provide an efficient ammonia assimilatory pathway when the ammonia concentration in the environment is low.

The effect of sucrose and succinate on the activities of the enzymes was studied, as these carbon sources were found to be essential for the asymbiotic fixation of nitrogen[7,17,18]. GDH activity was found to be considerably higher and that of GS/GOGAT lower when succinate was used as carbon source instead of sucrose (table 3). Sucrose-grown cultures also showed higher levels of α-ketoglutarate and lower levels of ammonia than succinate-grown cultures (table 3). Growth rates were nearly identical on both carbon sources (doubling time was about 2 h). α-Ketoglutarate and ammonia are known to regulate GS activity in *Escherichia coli*[19–22]. Thus a higher activity of GS in sucrose grown cultures as compared with succinate grown cells may be due to the increased concentration of α-ketoglutarate. The comparatively low activity for GDH in sucrose-grown cultures could be due to the reduced levels of ammonia.



The growth curve of *Rhizobium meliloti* grown in the presence of succinate (○) and sucrose (●).

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A comment on the design of experiments to estimate the Michaelis-Menten parameters of enzyme-catalysed reactions

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Summary. Michaelis-Menten parameters can be estimated by measuring initial velocities (v) in replicate at 2 concentrations of substrate, one much lower than K_m and the other much higher than it. Analysis of simulated experimental data suggests that this design will probably give more precise estimates of K_m and V than the conventional design in which v is measured at several different concentrations of substrate.

The biochemical literature is full of papers in which the Michaelis-Menten equation is fitted to initial velocity data. In some of them the object of the experiment was solely to estimate K_m and V, and not to find out if the equation fitted the data: for example, in the search for kinetic variants of an enzyme. In these instances, one might intuitively expect that the most efficient experimental design would be to measure the initial velocity (v) in replicate at only 2 concentrations of substrate (s), the 1st much lower than a provisional estimate of K_m and the 2nd much higher than it. (In terms of a Hanes or Lineweaver-Burk plot, this is equivalent to drawing a straight line between 2 points spaced as far apart as possible.) In this note we examine the validity of the expectation, by analysing simulated experimental data.

Methods. Two experimental designs were compared. In the 2-substrate concentration design A v was measured 6 times at each s and in the conventional design B it was measured once at each of 12 concentrations. Thus in both cases an experiment comprised 12 points.

Simulations were carried out as previously described[1]. A set of perfect (i.e. error-free) data was formed by setting $K_m = V = 1$ and calculating v at chosen values of s: 0.2 and 3.0 for design A, and between 0.2 and 4.0 inclusive for

design B. Sets of 'experimental' data (500) were generated from the perfect data by using series of normally-distributed pseudo-random numbers. 2 sorts of random error were incorporated:
AN: normally-distributed eror of constant absolute magnitude, v having an SD of 0.10 (= 10% of V).
RN: normally distributed error of constant relative magnitude, v having a coefficient of variation of 10%.
The Michaelis-Menten equation was fitted to the data by 2 numerical methods:
WLS: weighted non-linear least-squares[2], the weighting factor being unity for data sets AN, and the reciprocal of the square of the predicted velocity for RN.
RLP: the revised version of the direct linear plot, in which the axes are scaled in K_m/V and $1/V^3$.
Results and discussion. The results (table) are for K_m rather than V because the former is the more difficult to estimate reliably; those for V were similar. It is evident that the 2-concentration design (A) was a little less precise than the conventional one (B) when the error was of constant absolute magnitude (AN), presumably because of the high coefficient of variation of the lower velocity (60%). On the other hand it was considerably the more precise when the error was of constant relative magnitude (RN). Since in general errors in v increase with the magnitude of v[4], one may conclude that design A will usually give the more precise estimates of K_m and V. Certainly this is our experience when assaying erythrocyte acetylcholinesterase[5]. We have also found the 2-concentrations design helpful when deciding whether an inhibitor acts in a linear competitive, uncompetitive or mixed fashion (unpublished work). Finally, the table shows that, as one would expect, the method of least-squares (WLS) is more efficient than the distribution-free alternative (RLP).
The major limitations of the 2-concentration design are that it gives no information as to whether or not the Michaelis-Menten equation fits the data, and that it requires a provisional estimate of K_m. However, these are relatively unimportant when, for example, kinetic variants of an enzyme are being sought, because one is looking for differences in kinetic behaviour and will have a standard value of K_m. The design also has a number of peripheral advantages; such as: 1. There is no need to weight the data when least-squares is used, unless standard errors of K_m and V are required. In contrast, incorrect weighting of the data from the conventional design is likely to give biased estimates of K_m and V as well as of their standard errors. 2. Running replicate assays at only 2 concentrations of substrate is convenient in practice, and gives a day-by-day check on the reproducibility of one's methods.

Values of K_m from the 2 experimental designs

Experimental design	Error type	
	AN	RN
Weighted least-squares (WLS)		
A	1.056 ± 0.451 (0.950)	1.004 ± 0.098 (1.000)
B	1.106 ± 0.442 (1.010)	1.025 ± 0.159 (1.021)
Revised linear plot (RLP)		
A	1.105 ± 0.620 (1.036)	1.007 ± 0.105 (1.006)
B	1.093 ± 0.523 (0.991)	1.036 ± 0.178 (1.024)

Values of K_m are mean ± SD (median in parentheses) of 500 simulated experiments. Design A: 6 replicate initial velocities at each of 2 concentrations of substrate; design B: 1 velocity at each of 12 concentrations of substrate.

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Evidence against cyclic GMP acting as a direct modulator of active sodium absorption in rat cecum[1,2]

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Summary. Cyclic GMP concentrations were measured in rat cecum mucosa in vivo when the net absorption of sodium, chloride and fluid was stimulated by methylprednisolone (MP). Whereas Na-K-ATPase specific activity was increased by MP, suggesting enhanced active sodium transport, cyclic GMP levels remained unaffected.

Cyclic guanosine 3′,5′-monophosphate (cGMP) has been considered to be related to sodium absorption in the mammalian intestine[4-6] and toad urinary bladder[7], although the postulated connection is poorly understood. Under some conditions, elevated cGMP concentrations in the epithelial cell are associated with increased active sodium absorption[4] whereas in others, the correlation is inverse[5,7]. Recently, it has been reported in a preliminary communication that the activity of guanylate cyclase (GC) in rat ileum was increased by methylprednisolone[8] (MP) treatment which enhances sodium absorption and the specific activity of the Na^+-K^+-activated adenosine triphosphatase (Na-K-ATPase) in the small and large intestine[9]. It may, therefore, be anticipated that cGMP levels also increase under MP. The present experiments were designed to test this assumption in the cecum mucosa of the rat. Although we confirm that the glucocorticoid stimulated sodium transport and Na-K-ATPase specific activity, cGMP concentrations were not significantly affected.
Materials and methods. Male Wistar rats (180–220 g) were kept on a standard diet with free access to water. MP (Urbason®, crystalline suspension, Hoechst), 30 mg/kg b.wt