fruits is negatively associated with the incidence of intestinal metaplasia and atrophic gastritis in a high risk area for gastric cancer[14]. A conclusive risk estimate will require further studies on the in vivo formation of N-nitroso-5-vinyl-2-oxazolidone after consumption of goitrin-containing vegetables and on the genotoxic potential of this compound.

1 Mirvish, S.S., Toxicol. appl. Pharmac. *31* (1975) 325.
2 Sugimura, T., Fujimura, S., and Baha, T., Cancer Res. *30* (1970) 455.
3 Sugimura, T., and Kawachi, T., in: Gastrointestinal Tract Cancer. Eds Lipkin and R.A. Good. Plenum, New York 1978.
4 Weisburger, J.H., in: N-Nitroso compounds. Eds R.A. Scanlan and S.R. Tannenbaum. ACS Symposium Series 174 (1981).
5 Chisholm, M.D., and Wetter, L.R., Plant Physiol. *42* (1967) 1726.
6 Elfving, S., Ann. clin. Res. Suppl. 28, *12* (1980) 7.
7 Preussmann, R., Neurath, G., Wulf-Lorentzen, G., Daiber, D., and Hengy, H., Z. analyt. Chem. *202* (1964) 187.
8 Jorgensen, K.A., Ghattas, A.-B.A.G., and Lawesson, S.-O., in: N-Nitroso compounds, occurrence and biological effects. p. 159. IARC Scientific Publication No.41 (1982).
9 Ames, B.N., McCann, J., and Yamasaki, E., Mutation Res. *31* (1975) 347.
10 Miyahara, M., Miyahara, M., Kamiya, S., and Maekawa, A., Chem. pharm. Bull., Tokyo *29* (1981) 2366.
11 Hassner, A., and Reuss, H., J. org. Chem. *39* (1974) 553.
12 Fenwick, G.R., Heaney, R.K., and Mullin, W.J., CRC in Food Science & Nutrition *18* (1983) 123.
13 Fine, D.H., Challis, B.C., Hartman, P., and Van Ryzin, J., in: N-Nitroso compounds, occurrence and biological effects. IARC Scientific Publ. No.41 (1982).
14 Correa, P., Cuella, C., Fajardo, L.F., Haenszel, W., Bolanos, O., and de Ramirez, B., J. Natn. Cancer Inst. *70* (1983) 673.

0014-4754/84/050452-02$1.50 + 0.20/0

Simple integrated rate equations for reversible bimolecular reactions

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Summary. If the complete rate equations for reversible, one-step, bimolecular reactions are written with $P_e - P$ as the concentration variable (where P_e is the equilibrium, and P is the instantaneous, product concentration), the 3 possible stoichiometries can be reduced to a single straightforward differential equation. This can be solved very economically. For each stoichiometry,

$$\mathrm{A} \rightleftharpoons \mathrm{P} + \mathrm{Q}: \qquad k_1(-1/K_e)Dt = -\ln\left(1 - \frac{\Delta P}{P_e - P_o}\right) + \ln\left(1 - \frac{\Delta P}{D + P_e - P_o}\right)$$

$$\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{P}: \qquad k_1 Dt = -\ln\left(1 - \frac{\Delta P}{P_e - P_o}\right) + \ln\left(1 - \frac{\Delta P}{D + P_e - P_o}\right)$$

$$\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{P} + \mathrm{Q}: \qquad k_1(1 - 1/K_e)Dt = -\ln\left(1 - \frac{\Delta P}{P_e - P_o}\right) + \ln\left(1 - \frac{\Delta P}{D + P_e - P_o}\right)$$

where t is time, k_1 is the forward rate constant, K_e is the equilibrium constant, and ΔP is $P - P_o$. The terms $P_e - P_o$ and $D + P_e - P_o$ are the physically possible and physically impossible roots of the quadratic equation for $P_e - P_o$ in terms of the initial concentrations and K_e. D is the discriminant in this equation. All 3 quantities can be calculated if the equilibrium constant is known. A plot of t against $\ln\{[1 - \Delta P/(D + P_e - P_o)]/[1 - \Delta P/(P_e - P_o)]\}$ should be a straight line for any second order reaction. For each stoichiometry, $P_e - P_o$ approaches A_o, the initial concentration of the first reactant, as the equilibrium constant increases. When a second reactant is present, $D + P_e - P_o$ approaches B_o. The limiting equation is then that of an irreversible bimolecular reaction. For $\mathrm{A} \rightleftharpoons \mathrm{P} + \mathrm{Q}$, D approaches $-K_e$ as the equilibrium constant becomes large, and the value of the second logarithmic term in the integrated equation approaches zero. The limiting equation is that of an irreversible, unimolecular reaction.

Rates of reaction are ordinarily calculated from measurements of product (or reactant) versus time. The usefulness of integrated rate equations, which express the quantity actually measured, has therefore long been recognized. However, even for reactions as common and simple as 1-step bimolecular processes, the standard textbook derivations become painfully cumbersome as soon as anything more complex than an irreversible reaction is considered.

To circumvent this, bimolecular reactions, and reactions that display second-order kinetics, have usually been treated as a series of separate special cases: $\mathrm{A} + \mathrm{B} \rightarrow \mathrm{P}$, where the reaction is irreversible and $A_o = B_o$, or $\mathrm{A} + \mathrm{B} \rightarrow \mathrm{P} + \mathrm{Q}$, where $A_o \gg B_o$, or etc.[2]. The result is a series of equations that are difficult to reconcile and have little intuitive appeal, and plotting methods that apply only in special cases. An additional very serious consequence is that integrated equations for enzyme-catalyzed reactions, whose potential utility is widely recognized, have either been impossible to obtain or too complex to be of practical value.

It is possible, by choosing the right concentration variable, to solve the differential equations for $\mathrm{A} \rightleftharpoons \mathrm{P} + \mathrm{Q}$, $\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{P}$, and $\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{P} + \mathrm{Q}$ in a simple and uniform way. With a single exception, where the mathematical limit is not trivial, the resulting equation covers all possible initial conditions. The equation is straightforward, has a simple mathematical meaning, can easily be analyzed in terms of the shape of the product versus time curve, and can be used to make a simple logarithmic plot of any second-order reaction. This approach leads to an intuitive appreciation of uncatalyzed second-order processes, and is directly applicable to enzyme-catalyzed reactions.

Derivation. Examples of more-or-less standard textbook derivations can be found in Moore[3], Frost and Pearson[4], Hammes[5], etc. Szabo[6] obtains an involved result that is applicable to all initial conditions. In all these derivations, the reaction

variable used depends on initial concentrations; i.e. $A = A_o - x$, $P = P_o + x$, etc. In the derivation given here, equilibrium concentrations are used instead, and the concentration variable is $P_e - P$ (or $A - A_e$, etc.) instead of $P - P_o$ or $A_o - A$.

Consider the reaction $A + B \rightleftharpoons P$ as an example. Substitute $A = A_e + P_e - P$, $B = B_e + P_e - P$, and $A_eB_e = P_e/K_e$ in the rate equation:

$$\begin{aligned} dP/dt = k_1(AB - P/K_e) &= k_1[(A_e + P_e - P)(B_e + P_e - P) \\ &\quad - P/K_e] \\ &= k_1[A_eB_e - P/K_e + (A_e + B_e)(P_e - P) + (P_e - P)^2] \\ &= k_1[(P_e - P)/K_e + (A_e + B_e)(P_e - P) + (P_e - P)^2] \\ &= k_1(P_e - P)(A_e + B_e + 1/K_e + P_e - P) \end{aligned}$$

Now define a constant D, which is independent of the concentration variable $P_e - P$, such that $D = A_e + B_e + 1/K_e$. This gives

$$A + B \rightleftharpoons P: \qquad dP/dt = k_1(P_e - P)(D + P_e - P)$$
$$D \equiv A_e + B_e + 1/K_e$$

Carrying through the same process for the other 2 stoichiometries, defining D as appropriate, gives

$$A \rightleftharpoons P + Q: \qquad dP/dt = -k_1(P_e - P)(D + P_e - P)/K_e$$
$$D \equiv -(P_e + Q_e + K_e)$$
$$A + B \rightleftharpoons P + Q: \qquad dP/dt = k_1(1 - 1/K_e)(P_e - P)(D + P_e - P)$$
$$D \equiv [K_e(A_e + B_e) + P_e + Q_e]/(K_e - 1)$$

Notice that each of the 3 equations has the same form, namely $dP/dt = (\text{constant})(P_e - P)(D + P_e - P)$. When applied to a unimolecular reaction, this process gives $dP/dt = k_1(1 + 1/K_e)(P_e - P)$. The term $D + P_e - P$ is characteristic of a second order reaction.

By defining a constant C containing k_1, etc., for each of the stoichiometries[2], a single differential equation can be obtained. It can easily be solved by the method of partial fractions, as follows:

$$\frac{dP}{dP} = C(P_e - P)(D + P_e - P) = C \Big/ \left[\frac{1}{(P_e - P)(D + P_e - P)}\right]$$

$$\frac{dP}{dt} = CD \Big/ \left[\frac{1}{P_e - P} - \frac{1}{D + P_e - P}\right]$$

$$CD \int_0^t dt = \int_{P_o}^{P} \frac{dP}{P_e - P} - \int_{P_o}^{P} \frac{dP}{D + P_e - P}$$

$$CDt = -\ln\left(1 - \frac{\Delta P}{P_e - P_o}\right) + \ln\left(1 - \frac{\Delta P}{D + P_e - P_o}\right)$$

It is of course possible to write this equation as 1 term, $\ln\{[1 - \Delta P/(D + P_e - P_o)]/[1 - \Delta P/(P_e - P_o)]\}$, and plots of this term versus t will give straight lines with slopes CD. However, for reasons that will become clear, it is conceptually easier to think of the equation as the sum of 2 terms.

Analysis of the second-order term. The term $D + P_e - P_o$ occurs characteristically in second-order equations. $D + P_e - P_o$ is the physically impossible root of the quadratic equation for $P_e - P_o$ in terms of K_e and the initial concentrations, and D is the discriminant in this equation. This can be shown in the following way, again using $A + B \rightleftharpoons P$ as an example. In the definition of D, $D \equiv A_e + B_e + 1/K_e$, substitute the stoichiometric identities $A_e = A_o + P_o - P_e$ and $B_e = B_o + P_o - P_e$. Then $D = A_o + B_o + 1/K_e - 2(P_e - P_o)$. Rearranging,

$$2(P_e - P_o) = A_o + B_o + 1/K_e - D$$

Solving the quadratic equation for $P_e - P_o$ gives

$$2(P_e - P_o) = A_o + B_o + 1/K_e \mp \sqrt{(A_o + B_o + 1/K_e)^2 - 4(A_oB_o - P_o/K_e)}$$

where the physically possible concentration, $P_e - P_o$, requires a minus sign. Thus D is the positive value of the square root and $P_e - P_o + D$ is the impossible root. Algebraic expressions for all 3 stoichiometries are shown in table 1.

Integrated equations for bimolecular reactions can therefore be thought of as the sum of 2 logarithmic terms, each containing 1 root of the quadratic for $P_e - P_o$. For a unimolecular reaction, there is but 1 root and 1 logarithmic term.

Limiting expressions for the integrated equations. The 2 roots, $P_e - P_o$ and $D + P_e - P_o$, approach limits in certain circumstances. These limiting cases are the special ones given in many textbooks. It is useful to examine the conditions under which the limiting equations apply.

As K_e increases, $P_e - P_o$ of course approaches A_o. When a second reactant is present, $D + P_e - P_o$ approaches B_o, and D approaches $B_o - A_o$ rather more slowly[7]. The integrated equation is that ordinarily given for an irreversible second order reaction:

$$A + B \rightarrow P \pm Q:$$
$$k_1(B_o - A_o)t = -\ln(1 - \Delta P/A_o) + \ln(1 - \Delta P/B_o) \qquad \text{or}$$

$$k_1(B_o - A_o)t = -\ln\frac{A_o(B_o - \Delta P)}{B_o(A_o - \Delta P)}$$

If products are absent initially, A_o and B_o are within 1% of $P_e - P_o$ and $D + P_e - P_o$ if K_e (for $A + B \rightleftharpoons P + Q$) or K_eA_o (for $A + B \rightleftharpoons P$) is ≥ 110 and $B_o \geq 10\ A_o$. The equilibrium constant needed rises quickly as B_o approaches A_o, reaching 10^3 at $B_o = 1.1\ A_o$ and 10^4 at $B_o = A_o$. Adding product at time zero has relatively little effect; the K_e needed for an error less than 1% increases only 4 fold when P_o or $Q_o = A_o$.

Neither side of the equation above approaches a limit as quickly as do the roots; this results from the fact that A_o has a value greater than the number it approximates and B_o has a value less than the number it approximates. When $B_o = 1.1\ A_o$,

Table 1. Roots and discriminants of the quadratic equations

Stoichiometry	Discriminant, D	Possible root, $2(P_e - P_o)$*
$A + B \rightleftharpoons P$	$\sqrt{(A_o + B_o + 1/K_e)^2 - 4(A_oB_o - P_o/K_e)}$	$A_o + B_o + 1/K_e - D$
$A \rightleftharpoons P + Q$	$-\sqrt{(P_o + Q_o + K_e)^2 + 4(K_eA_o - P_oQ_o)}$	$-(P_o + Q_o + K_e) - D$
$A + B \rightleftharpoons P + Q$	$\sqrt{\left[\frac{K_e(A_o + B_o) + P_o + Q_o}{K_e - 1}\right]^2 - 4\left(\frac{K_eA_oB_o - P_oQ_o}{K_e - 1}\right)}$	$\frac{K_e(A_o + B_o) + P_o + Q_o}{K_e - 1} - D$

* In each case, the impossible root is $2(P_e - P_o + D)$.

a 1% error in $B_o - A_o$ requires an error of 0.05% or less in approximating each of the roots; the equilibrium constant needed is now 2.2×10^4. The error in the logarithmic side of the equation depends slightly on the position in the progress curve, but in fact it resembles the error in $B_o - A_o$ quite closely.

It is apparent that both sides of the integrated equation approach zero when K_e is large and B_o approaches A_o. The limiting equation in this case is the same as that for an irreversible second-order reaction with but 1 substrate. Integrating $-dA/dt = k_1 A^2$ gives $k_1 A_o t = (\Delta P/A_o)/(1 - \Delta P/A_o)$, which is another standard textbook example. A reaction must have an equilibrium constant greater than 4×10^6 for this equation to be accurate within 1%.

Experimentally, reactions with 2 reactants are often treated as pseudo-first-order reactions when 1 reactant is present in excess. In the second-order equation, $\Delta P/(D + P_e - P_o)$ can be rewritten as $[\Delta P/(P_e - P_o)]/[P_e - P_o)/(D + P_e - P_o)]$. $(P_e - P_o)/(D + P_e - P_o)$ must be zero for the reaction to be pseudo-first-order. If $B_o = 10\ A_o$, $(P_e - P_o)/(D + P_e - P)$ is 0.1 when K_e is very large, and decreases to 0.077 when K_e is 5. For any equilibrium constant greater than 1, $(P_e - P_o)/(D + P_e - P_o)$ is reduced to 0.01 or less only if $B_o \geq 100\ A_o$.

Another pseudo-first-order situation occurs if the reaction $A \rightleftharpoons P + Q$ is taken to be irreversible. Again, the second logarithmic term must approach zero. It can be written as $\ln \{1 - [\Delta P/A_o]/[A_o/(D + P_e - P_o)]\}$; $A_o/(D + P_e - P_o)$ approaches zero as K_e/A_o increases. An equilibrium constant of $K_e/A_o \geq 10^4$ is needed for $A_o/(D + P_e - P_o) < 0.01$ when P_o and Q_o are zero.

Limiting shapes for second-order progress curves. It is helpful to have a picture of the shape of the expected progress curves for second-order reactions, and to compare them with the shape for a first-order reaction. The second logarithmic term in the integrated equation for these reactions can be thought of as being compounded from the progress curve, $\Delta P/(P_e - P_o)$, and a fraction, $(P_e - P_o)/(D + P_e - P_o)$. This fraction has readily definable limiting values, which can be obtained either mathematically or with a programmable desk calculator. The limiting values and the conditions needed to obtain them are shown in table 2. Using these limiting values, both logarithmic terms can be calculated for the duration of the progress curve.

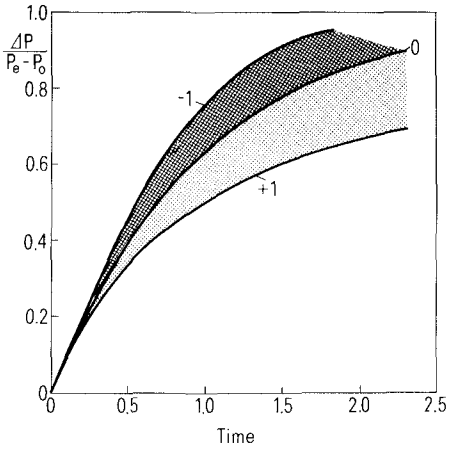
In order to display only the shape of the progress curve, it is necessary to eliminate the effect of the absolute magnitude of the rate constant. A simple way to do this is to set the initial slope of the plot to 1. This is in effect what one does in choosing the axes of an experimental plot so that the progress curve subtends a 45° angle at the origin. For a plot of $\Delta P/(P_e - P_o)$ versus t, the initial slope is $(dP/dt)_o/(P_e - P_o)$. $(dP/dt)_o$ can be obtained for each stoichiometry by setting $P = P_o$ in the appropriate differential equation. Substituting $(dP/dt)_o$ into the integrated equation then eliminates C, which contains the rate constant, and gives the same expression for all 3 second-order stoichiometries:

$$t\,[(dP/dt)_o/(P_e - P_o)] = \frac{-\ln\left(1 - \frac{\Delta P}{P_e - P_o}\right) + \ln\left(1 - \frac{\Delta P}{D + P_e - P_o}\right)}{1 - \frac{P_e - P_o}{D + P_e - P_o}}$$

The corresponding equation for a first-order-reaction is:

$$t\,[(dP/dt)_o/(P_e - P_o)] = \ln\left(1 - \frac{\Delta P}{P_e - P_o}\right)$$

In the figure, t has been plotted against the fractional reaction for the extreme values of $(P_e - P_o)/(D + P_e - P_o)$, after setting



Progress curves for second-order-reactions. The equation used to calculate the curves is described in the text. The values of $(P_e - P_o)/(D + P_e - P_o)$ used are indicated on the figure. The dark area represents the stoichiometry $A \rightleftharpoons P + Q$, and $A + B \rightleftharpoons P + Q$ when $K_e \leq 1$. The light area represents $A + B \rightleftharpoons P$ and $A + B \rightleftharpoons P + Q$ when $K_e \geq 1$. The central curve on the figure, where the ratio equals O, is also the progress curve for a first-order-reaction.

Table 2. Conditions under which $(P_e - P_o)/(D + P_e - P_o)$ approaches its limits

Stoichiometry	$(P_e - P_o)/(D + P_e - P_o)$ equal to		
	-1	0	$+1$
$A \rightleftharpoons P + Q$	$K_e/A_o \to 0$	$K_e/A_o \to \infty$	
$A + B \rightleftharpoons P$		$K_e A_o \to 0$	$K_e A_o \to \infty$ $B_o = A_o$
$A + B \rightleftharpoons P + Q$	$K_e \to 0$ $P_o = Q_o$	$K_e = 1$	$K_e \to \infty$ $B_o = A_o$

the initial slope, $(dP/dt)_o/(P_e - P_o)$, equal to 1. The curve for a first order reaction is the same as that for a second-order-reaction when $(P_e - P_o)/(D + P_e - P_o)$ is equal to zero.

Discussion. The integrated equations presented here could be used to characterize bimolecular reactions under conditions that have not previously been accessible experimentally; i.e., where the equilibrium constant is close to 1 and/or where the reactant concentrations are similar. In addition to the initial concentrations, which will be known, the equations presented depend only on the forward rate constant, k_1, and the equilibrium constant. If K_e is known, $P_e - P_o$ and $D + P_e - P_o$ can be calculated, and progress curves plotted as t versus $\ln [1 - \Delta P/(D + P_e - P_o)]/[1 - \Delta P/(P_e - P_o)]$ will give straight lines with slopes of CD, where C is $-k_1/K_e$ or k_1 or $k_1(1 - 1/K_e)$, depending on the stoichiometry. Alternately, if K_e is not known accurately, the progress curves could be fit by computer, using non-linear regression techniques[8,9] in order to obtain best-fit values of both K_e and k_1.

Perhaps a more important application of this work lies in the area of enzyme-catalyzed reactions. The mathematical principles described here are directly applicable to nearly all second-order, steady-state reactions that are hyperbolic (as opposed to co-operative). A preliminary account of this work has appeared[10] where, however, the notation is ponderous because the significance of the impossible root was not fully understood. Less complex equations have since been obtained[11]. These equations depend only on the stoichiometry of the reaction, and not on previous knowledge of the mechanism. Within this theoretical framework, then, it should be possible to carry out a kinetic analysis of an enzymic reaction much more efficiently than is now possible by initial rate methods; com-

plete time courses can be used rather than just the extrapolated initial rates. We are presently testing the practicality of this experimental technique, using an irreversible reaction with the stoichiometry A→P + Q.

1 Acknowledgments. I thank Dr. Athel Cornish-Bowden for many helpful discussions. This work was partially supported by a grant from Utah State University.

2 *A, B, P,* and *Q* are instantaneous concentrations of reactants and products; the subscripts o and e indicate initial and equilibrium concentrations respectively. K_e is the equilibrium constant; k_1 is the forward rate constant. ΔP is $P - P_o$. C is $-k_1/K_e$ for $A \rightleftharpoons P + Q$, k_1 for $A + B \rightleftharpoons P$, and $k_1(1 - 1/K_e)$ for $A + B \rightleftharpoons P + Q$. D is defined, and discussed at length, in the text.

3 Moore, W.J., in: Physical Chemistry, 4th edn, p. 333. Prentice-Hall, New York 1972.
4 Frost, A.A., and Pearson, R.G., in: Kinetics and Mechanism, 2nd edn, p. 12, 185. Wiley, New York 1961.
5 Hammes, G.G., in: Principles of Chemical Kinetics, p. 5. Academic Press, New York 1978.
6 Szabo, Z.G., in: Comprehensive Chemical Kinetics, vol. 2, p. 43. Eds C.H. Bamford and C.F.H. Tipper. Elsevier, Amsterdam 1969.

7 Mathematically, a sufficient condition for these limits is, for $A + B \rightleftharpoons P$, $K_e A_o \gg 1 + P_o/A_o$. For $A + B \rightleftharpoons P + Q$, it is $K_e \gg 1 + (P_o + Q_o)/A_o + P_o Q_o/A_o^2$.

8 Wilkinson, G.N., Biochem. J. *80* (1961) 324.
9 Johansen, G., and Lumry, R., C.r. Trav. Lab. Carlsberg *32* (1961) 185.
10 Boeker, E.A., Trans. biochem. Soc. *10* (1982) 214.
11 Boeker, E.A., Biochem. J., in press.

0014-4754/84/050453-04$1.50 + 0.20/0

The effects of diltiazem on *Periplaneta americana*[1]

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Summary. The effects of the calcium antagonist diltiazem on nerve and muscle in the cockroach *Periplaneta americana* were examined. Diltiazem was observed to inhibit myogenic and glutamate-induced contractions of the visceral muscle while having either a potentiating, an inhibiting or a biphasic effect against proctolin-induced contractions. Against the isolated nervous system, diltiazem induced an increase in spontaneous discharge activity, followed by nerve block. Injection of diltiazem into cockroaches produced behavioral and toxic effects.

Diltiazem is a representative of a new class of pharmacological agents, the calcium antagonists. Recently, Ishida and Shinozaki[4] reported that diltiazem had differential effects on glutamate potentials and excitatory junctional potentials in an invertebrate preparation, the crayfish neuromuscular junction. In view of these interesting results, we examined the effects of diltiazem on neuromuscular transmission in visceral muscle of *Periplaneta americana* where there is evidence for both glutamate and proctolin as neurotransmitters and very few pharmacological tools to differentiate between them[5].

Materials and methods. Adult male *Periplaneta americana*[5] were used in all experiments. Bioassays using innervated and deganglionated hindguts were as previously described[6]. Hindgut neural stimulation was performed at 6–15 Hz applied in 2–10 sec trains at 30 sec intervals and the response quantified as reported earlier[5]. Diltiazem hydrochloride (active agent, 99.7% pure, m.p. 214 °C), from Nordic Laboratories Inc. Laval, Quebec, was added to the supply of saline perfusing the gut muscle to give a final concentration of 0.15, 0.3 or 0.6 mM. Bioresmethrin (92% pure) was from Wellcome Research Laboratories, Berkhamsted, U.K. Isolated nervous systems were obtained for recording by excising the ventral nerve cord with associated tracheae and pinning the nerve cord to the parawax floor of a recording chamber under saline that had been oxygenated for 30 min to 1 h. The trachea associated with the nerve cord were allowed to reach the surface of the saline as conduits for oxygen diffusion. Nerve cords consisted of the metathoracic and abdominal ganglia or in some experiments (where recordings were made from the proctodeal nerve) the IV, V and VI abdominal ganglia alone. Using a suction electrode, recordings were obtained from the proctodeal nerve where it joins the cercal nerve and from nerve 2A of the second abdominal ganglion. The signals were amplified through a W.P.I. DAM-6 differential amplifier, viewed on a Nicolet 2090-II digital storage oscilloscope, and recorded on a Phillips PM 8120 X-Y plotter. Toxicological studies were carried out by intrahemocoelic injections of 10 µl of diltiazem dissolved in cockroach saline, with the syringe introduced between the abdominal sclerites on the dorsal surface lateral to the heart. Treated cockroaches and controls were then placed on their backs in groups of 3–6 in glass beakers covered with muslin at 22–25 °C for observation of behavior and mortality effects.

Results. The cockroach hindgut preparation routinely displays spontaneous, apparently myogenic, contractions and responds with a sustained contracture to both glutamate and proctolin application[7]. The presence of diltiazem in the perfusion buffer at a concentration of 0.15–0.6 mM resulted in a marked reduction of spontaneous myogenic contractures within 1–2 min and changed the muscle response characteristics to glutamate and proctolin application (fig. 1 A, B). The response of the muscle lost its phasic character resulting in a tonic response resembling that produced by high potassium challenges (data not shown). The amplitude of these responses changed gradually before reaching stability after 16–30 min exposure of the preparation to diltiazem. Glutamate responses were inhibited in a dose-dependent manner (fig. 1C). The effect on proctolin responses was considerably more variable, the response observed (N = 18) being potentiation, inhibition, or potentiation followed by inhibition. These effects were reversible; continuous saline perfusion resulted in the recovery of the normal gut properties within a period of 4–6 min. Neurally evoked contractions were diminished in amplitude within 1 min and abolished within a period of 3–5 min by perfusion with 0.3 mM diltiazem. As this may be a direct effect of diltiazem on proctodeal nerve function, the action of diltiazem on spontaneous nerve activity in the isolated cockroach nervous system was investigated. When recordings were made from the severed distal end of the proctodeal nerve, spontaneous spike activity resembling that reported by Brown and Nagai[8] was observed. This firing pattern was stable for a period of over 1 h. Applications of diltiazem at final concentrations of 0.3–15 mM resulted in a transient increase in spontaneous firing activity followed by a decrease in firing frequency leading to nerve block (fig. 2A). This nerve block condition was seen in all proctodeal nerve recordings (N = 5) with onset occurring more rapidly at higher diltiazem concentrations. To test the generality of this effect,