activities of phosphorylase *a* and phosphorylase *a* + *b* in the endocardial layers were higher ($p < 0.01$) than those in the epicardial layers. The PCr level of the endocardial layers, however, was lower ($p < 0.05$) than that of the epicardial layers. No significant differences were detected in the ATP level between the endo- and epicardial layers. The ligation of the small branch resulted in a marked decrease in glycogen level in each of the two layers, and caused a conversion of phosphorylase from *b* form to *a* form. The rate of decrease in glycogen level after the ligation in the endocardial layers was faster than that in the epicardial layers. The level of G6P and of lactate in each of the two layers increased rapidly after the ligation. The level of ATP in each of the two layers was not appreciably altered by the ligation. The PCr level in each of the two layers decreased markedly until at least 7 min after the ligation. The rate of decrease in the endocardial PCr level was more rapid than that in the epicardial PCr.

2. *Nitroglycerin-treated dogs.* Nitroglycerin (20 µg/kg) was injected instead of saline in this series of experiments. In non-ischemic hearts, the levels of glycogen, G6P, lactate and ATP, and the activities of phosphorylase *a* and phosphorylase *a* + *b* in each of the two layers did not differ from those obtained in control dogs. The level of PCr in each of the two layers, however, was slightly lower than that obtained in control dogs. Nevertheless, the level of PCr in the endocardial layers was always lower than that in the epicardial layers. The ligation of small branch of coronary artery produced neither marked decrease in glycogen level nor rapid conversion of phosphorylase from *b* form to *a* form in both the layers. The level of ATP in each of the two layers was not altered by the ligation. After the ligation, the level of PCr in the epicardial layers decreased slightly, but that in the endocardial layers did not.

Zusammenfassung. Nachweis, dass die Unterbindung schon eines kleinen Astes der Koronararterie des Hundes im Endokard eine stärkere Steigerung der anaeroben Glykolyse als im Epikard bewirkt. Vorbehandlung mit Nitroglycerin hemmt die Steigerung der durch die Ligatur bedingten anaeroben Glykolyse.

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Interaction of Drugs: A Mathematical Model and its Application in Bacteriology

In a previous paper[1] it was reported that novobiocin combined with tetracycline had a synergistic bactericidal effect on *Pseudomonas pseudomallei*. This communication centres on the analysis of the mathematical model that has been put forward to represent the relation between response and dose of drugs, alone and in combination, and on the graphic presentation of the values indicated in the model. Two combinations only are presented here, the novobiocin-tetracycline and the kanamycin-chloramphenicol; the former is bactericidal on *P. pseudomallei*, strain 6[1], the latter on *Escherichia coli*, strain 80[1].

Mathematical model. The concept of additivity of drugs is predicated by the following model. Let N denote the number of bacteria present at time t (in h), and N_0 the number of bacteria present originally ($t = 0$). In the absence of drugs, it is assumed that bacteria grow at a rate proportional to the amount present $\frac{dN}{dt} = kN$ where k denotes the growth rate. The rate of bactericidal activity of drugs, single or in combination, is proportional to the number of bacteria present and the amount of drug(s) used:

$$\frac{dN}{dt} = kN - czN = -(cz - k)N \qquad (1)$$

where z denotes the amount of drug(s), single or mixed in fixed proportion, and c is a constant which denotes the specific bactericidal activity of drugs, single or in combination. By solving equation (1), we have

$$N = N_0 e^{-(cz-k)t}$$

and, after a fixed time interval t_1 (24 h, e.g.), the corresponding bacterial count N_1 satisfies the equation

$$\frac{N_0}{N_1} = \frac{1}{e^{-(cz-k)t_1}} = e^{(cz-k)t_1}. \qquad (2)$$

We define the log response q to a drug dose as

$$\log_{10}\frac{N_0}{N_1}.$$

This quantity represents conveniently, on a decreaings scale, the number of viable bacteria in function of the dose tested at time $t_1 = 24$ h:

$$q = \log_{10}\frac{N_0}{N_1} = \log_{10}[e^{(cz-k)t_1}] = (cz-k)\,t_1 \log_{10} e = 0.434\,(cz-k)\,t_1. \qquad (3)$$

According to this mathematical model, the log response will vary linearly with the total amount z of drug(s) tested, single or mixed in fixed proportion. Since

$$q = [(0.434\,c)\,z - 0.434\,k]\,t = (c'z - k')\,t \qquad (4)$$

the specific rate of bactericidal activity c' depends only on the amount and proportion of drugs tested z while the rate of growth k' depends only on the strain of bacteria used.

Let us suppose that several drugs D_1, D_2, D_3 ... that have bactericidal rates c'_1, c'_2, c'_3 ... are tested in combination in the following amounts, respectively, z_1, z_2, z_3 ... We shall say that the action is additive if eq. (1) becomes

$$\frac{dN}{dt} = kN - (c_1 z_1 + c_2 z_2 + c_3 z_3 \ldots)\,N$$

so that

$$q = \log_{10}\frac{N_0}{N_1} = 0.434\,(-k + \sum_n c'_n z_n)\,t_1 = (\sum_n c'_n z_n - k')\,t. \qquad (5)$$

When two drugs are tested in combination, then

$$q = (c'_1 z_1 + c'_2 z_2)\,t_1 - k'\,t_1. \qquad (6)$$

A given pair of drugs exhibits isobole additivity at a particular level of response q, if the corresponding isobole is rectilinear. If the isobole is either markedly convex or concave toward the origin, the combined effect is said to be synergistic in the first case, and antagonistic in the

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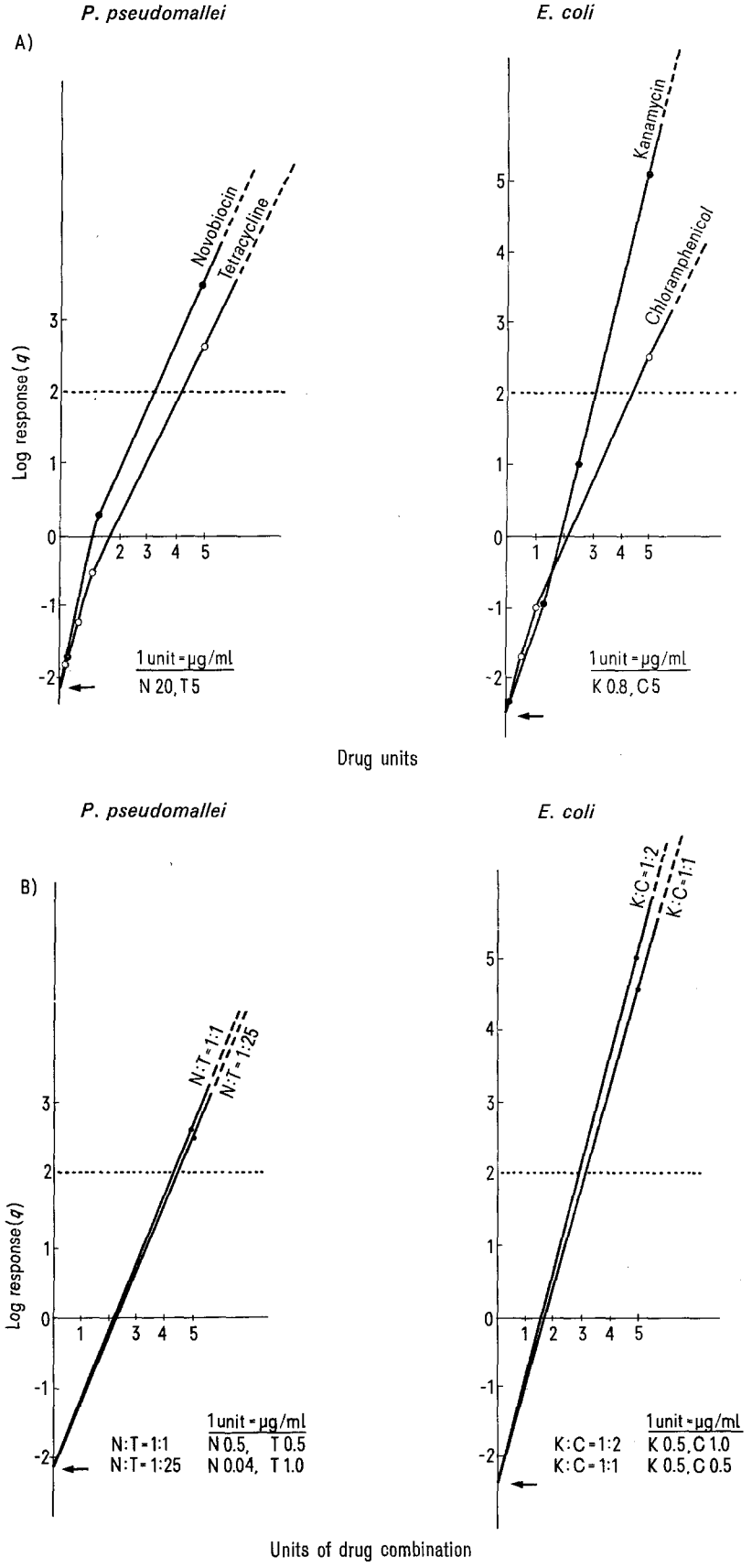

Figs. A and B. The log response of *Pseudomonas pseudomallei* (left) and of *Escherichia coli* (right) to drugs after 24 h incubation. A) Single drugs. B) Drugs mixed in fixed proportion. N, novobiocin; T, tetracycline; K, kanamycin; C, chloramphenicol. Points indicate experimental values. The dotted line at $q = 2$ represents 99% kill and arrow indicates the q value of the drug-free control. A) and B) are the graphic expression of eq. (4).

Log response (q) of *P. pseudomallei* and *E. coli* to drugs, single and in combination. Estimated and relative doses of each antibiotic for 99% bactericidal activity of the combination

Strain	Drug	Dose tested (μg/ml)	q values [a]	Estimated dose [b] for $q = 2$ (μg/ml)	Relative dose [c]	Drug effect [d]
P. pseudomallei	Novobiocin	0.2	−2.05			
		2.5	−1.75			
		25.0	0.29			
		.....	2.00	64.4		
		100.0	3.54			
	Tetracycline	1.0	−1.80			
		2.5	−1.21			
		5.0	−0.51			
		.....	2.00	21.0		
		25.0	2.66			
	Novobiocin/tetracycline	*N* 2.5, T 2.5	2.65	*N* 2.15, T 2.15	*N* 0.03, T 0.10	S
		N 0.2, T 5.0	2.55	*N* 0.18, T 4.40	*N* 0.003, T 0.20	S
	No drug	...	−2.11			
E. coli	Kanamycin	0.2	−2.20			
		1.0	−0.90			
		2.0	1.05			
		.....	2.00	2.5		
		4.0	5.04			
	Chloramphenicol	2.5	−1.68			
		5.0	−0.98			
		.....	2.00	22.1		
		25.0	2.49			
	Kanamycin/chloramphenicol	*K* 2.5, C 2.5	4.62	*K* 1.55, C 1.55	*K* 0.62, C 0.07	(A)
		K 2.5, C 5.0	5.04	*K* 1.47, C 2.94	*K* 0.59, C 0.13	(A)
	No drug	...	−2.31			

[a] $q = \log_{10}$ viable bacteria after 24 h incubation expressed as % of inoculum size. [b] Estimated dose for 99% bactericidal activity, i.e. 1% viability ($q = 2$). [c] Dose of each drug expressed as a fraction of 1 unit of drug acting alone. [d] S, marked synergism; (A), additive effect suggested.

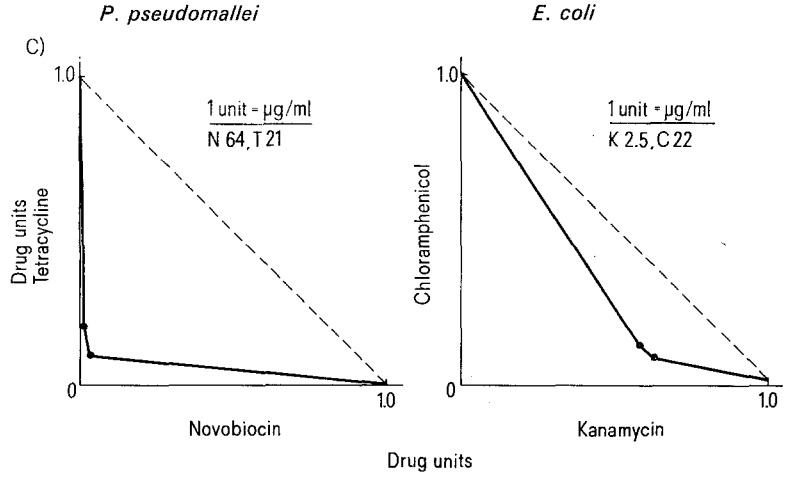

Fig. C. Isobolograms of estimated 99% kill of the novobiocin-tetracycline combination against *P. pseudomallei* (left) and of the kanamycin-tetracycline combination against *E. coli* (right). Isobolograms show drugs mixed in variable proportions and are constructed from the data estimated graphically from Figures A and B. Each point represents the relative dose of each drug in the pair; this is expressed as a fraction of 1.0 unit, i.e. of the amount needed by the same drug for the same effect (99% kill) when the drug is acting alone. Isobolograms are the graphic expression of eq. (6). The brocken line represents a theoretical additive effect. The sharply convex isobole of the novobiocin-tetracycline combination (left) indicates a marked synergistic effect.

latter[2]. The extent to which the experimental data fit the mathematical model of strict additivity implied in eq. (6) has been discussed[1]. The growth rate k' and the bactericidal rates c'_1 and c'_2 are determined experimentally by testing in parallel assays, respectively, growth in absence of drugs and in presence of each drug of the pair (see Table). Results are recorded as the number of viable bacteria expressed as % of the inoculum size after 24 h incubation. The log response is then plotted.

Graphic presentation. This entails the construction of 3 graphs for each combination tested. The data that are estimated graphically from the first and second graph are used to construct the third, which is the isobologram. Figures A–C show the 3 graphs for the novobiocin-tetracycline combination at left and those for the kanamycin-chloramphenicol combination at right. Experimental data and estimated values are presented in the Table. In the first 2 graphs (Figures A and B), the log response of individual strains is plotted on an arithmetic scale as a function of varying amounts of drugs, single (Figure A) and combined in fixed proportion (Figure B). In both graphs, the dose that gives the highest kill is measured as 5 units along the abscissa and the weight equivalent of 1 unit is indicated. The dose that produces 99% kill (i.e. 1% viability, $q = 2$) is obtained graphically and is the standard level of effectiveness of drugs, both single and mixed in fixed proportion. In the case of single drugs, this dose is obtained from the number of drug units and the weight equivalent of 1 unit (μg/ml); whereas in the case of drug combinations, the dose of each drug in the pair is obtained from the number of units of combination and the weight equivalent of each drug in 1 unit (μg/ml). The dose that produces 99% kill ($q = 2$) can also be obtained by inter- or extrapolation, using the basic, simple algebra of the interpolation formula, and is calculated as

$$d_1 - \frac{(d_1 - d_2)(q_1 - 2)}{q_1 - q_2} \quad \text{or} \quad d_1 + \frac{(d_2 - d_1)(2 - q_1)}{q_2 - q_1}$$

where d_1 and d_2 are the doses tested (μg/ml), and q_1 and q_2 are their log responses, respectively.

In constructing isobolograms for 2 drugs (Figure C), the dose of each drug needed for its independent effect, i.e. 99% kill, is measured respectively as 1 unit along each of the 2 axes on an arithmetic scale, and the weight equivalent of 1 unit is indicated. The relative dose of each drug in the pair is plotted; this is obtained by dividing the dose of each drug that in combination gives 99% kill by that of the same drug acting alone. In the present study, a synergistic effect is obtained and is assumed to warrant clinical consideration when the concentration of each drug in the pair is 0.2 unit or less. Accordingly, the corresponding isobole is markedly convex.

Results and discussion. During the last 3 decades several mathematical models have been put forward for representing the relation between the dose of drug combination and the level of response[2,3]. The model presented here has computational convenience; its graphic presentation allows to compare the bactericidal activity of drugs, single or in combination, between bacteria and bacterial strains, and their synergistic effect, if present. The use of a biological unit scale is essential in comparing the relative effect of different pairs and indicates more clearly the upper limit of synergism for a pair of drugs (see Table). It can be seen that the kanamycin-chloramphenicol combination is 100- to 1000-folds more bactericidal than that of novobiocin with tetracycline (see Table and Figure B). However, only the latter is synergistic, and highly so, since as little as 0.003 unit of novobiocin is needed for the bactericidal effect of the combination (see Table and Figure C, left); accordingly, this isobole is sharply convex. Whereas concentrations of kanamycin as high as 0.59 unit, or over one half of the concentration for its independent effect, are needed for the bactericidal action of the kamanycin-chloramphenicol combination this isobole is somewhat convex and suggests an additive effect only, according to the criteria used in this study.

Résumé. On présente un modèle mathématique sur la relation entre le dosage des antibiotiques en combination et le niveau de réponse des bactéries. On décrit la présentation graphique du modèle et une méthode établie pour l'interprétation du synergisme.

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Temperature Dependent Aqueous Solubility of Actinomycin D

Actinomycin D (act-D) or dactinomycin, an antineoplastic drug, is known to be soluble in 95% ethanol, propylene glycol, and water/glycol mixtures[1]. An effective tool, this compound is employed quite frequently in the elucidation of various biological processes and the mechanism of action of a whole host of drugs[2,3]. We currently use act-D with great success to induce experimental ascities and pleurisy[4,5] in animal models. A problem associated with the use of act-D has been the impression that it is insoluble in an aqueous medium. In the course of our study a reciprocal relationship between the solubility of act-D in an aqueous medium and temperature was serendipitously observed. This prompted the investigation of the temperature dependent solubility of act-D at 3 concentrations.

Actinomycin D was obtained in 5 mg vials from Sigma Chemical Company, St. Louis, Missouri. A small amount (0.5 ml) of PO_4 buffer (0.155 M, pH 7.4) was added to the vial so as to suspend the drug. The suspension was then frozen and, when latter thawed, the drug passed into solution. While still cold this solution was transferred to another vessel. The vial was then washed several times with cold buffer until all the drug was removed. Final concentrations of the drug were 0.25, 0.5, and 1.0 mg/ml.

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