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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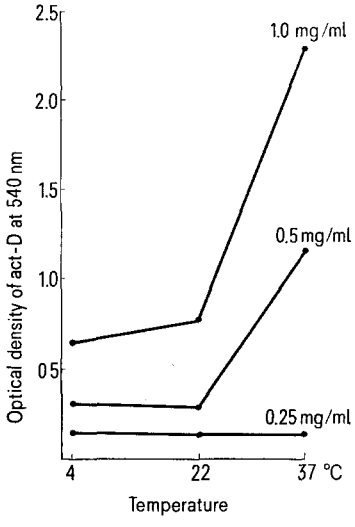
With an increase in temperature the drug came out of solution and the resulting turbidity was read at 540 nm on a Beckman Spectrophotometer with an attached Haake Constant Temperature Circulator. Readings were taken while the solutions were maintained at 4°, 21–22° (room temperature), and 37 °C.

The results of this study are summarized in the Figure. Optical density was partially due to the yellow-orange color of the drug. At 4 °C each concentration was clear and free from turbidity. It was obvious the drug was completely in solution and optical density was wholly concentration dependent at this temperature. At room temperature only the highest concentration of act-D (1.0 mg/ml) began to come out of solution and thus registered an increased turbidity. This insolubility was heightened 3-fold at 37 °C. Actinomycin D at the lower concentrations of 0.25 and 0.5 mg/ml stayed in solution at room temperature. At 37 °C, 0.5 mg/ml became insoluble with a 4-fold increase in turbidity; 0.25 mg/ml remained in solution.



The turbidity of actinomycin D at different temperatures. 3 concentrations of the drug in phosphate buffer (0.25, 0.5, 1.0 mg/ml) were maintained at 4°, 21–22° (room temperature), and 37 °C. Turbidity was measured, (as a change in optical denstiy), spectrophotometrically at 540 nm. Optical density was partially due to the color of the drug. The variation in optical density at 4 °C was wholly concentration dependent; the drug was completely in solution at this temperature.

The physico-chemical property of increased solubility with decreasing temperature is not unique to act-D but has been observed with other compounds[6]. By a freeze-thaw technique concentrations of act-D in aqueous medium can be realized as high as 0.5 mg/ml at room temperature. For rapid bioavailability, however, we suggest the administration of act-D at 0.25 mg/ml. Solutions containing 0.5 mg/ml are liable to precipitate, at body temperature, at the site of injection. This, then, eliminates the necessity of using foreign vehicles to dissolve act-D and thus the possibility of their effects[7].

Résumé. L'actinomycine D est solubilisée dans un milieu aqueux par une technique de congélation suivie de dégel, ce qui permet d'atteindre des concentrations de 1 mg/ml à 4 °C et de 0.5 mg/ml à la température du laboratoire. Pour obtenir une absorption rapide de l'actinomycine D les concentrations ne doivent pas dépasser 0.25 mg/ml.

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Vesiculation of the Nuclear Envelope of the Liver and Kidney of the Mouse

The development of vesicle structures from the nuclear envelope has been described in various species of oocytes[1–4] and in the gas gland cells from *Perca fluviatilis*[5], the development of such structures from the nuclear envelope of a mammalian somatic cell has not been observed. We here report the development of small, single membrane-bounded vesicles from the outer element of the nuclear envelope of both the liver and kidney cells of the mouse and large nuclear envelope protrusions in proximal convoluted tubule cells of the kidney.

In cells of both liver and kidney proximal tubules the contour of the outer nuclear membrane was frequently irregular and puckered. Fingerlike projections and pockets were pinched-off to form small, electron transparent vesicles, 50–100 nm in diameter. A complete developmental series has been traced in both liver and kidney, as is shown in Figures 1 and 2. There was no evidence that the vesicle profiles represented sections of microtubules or lamellae derived from the nuclear envelope.

Although there was no apparent association between the position of the nucleolus within the nucleus or with mitochondria or Golgi apparatus with sites of small vesicle production, there was an association with the endoplasmic reticulum. Especially in the liver, rows of small vesicles occurred between the parent nuclear envelope and adjacent lamellae of endoplasmic reticulum. Such vesicles were often flattened, suggesting their conversion into elements of endoplasmic reticulum but no evidence was found of their fusion with lamellae.

Nuclear envelopes of some 15% of kidney cells were also observed with large local protrusions which were apparently associated with the production of double membrane bound vesicles containing material of apparently nuclear origin. These protrusions were almost always

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