It is not our intention here to speculate on the nature of the process or processes that are sensitive to protein synthesis inhibitors, but the possibility that there are common processes in the development of tolerance to several drugs does not seem unlikely.

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LETTERS TO THE EDITOR

A modified agar diffusion assay for amphotericin B

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The agar diffusion method of assay for amphotericin B described by Kramer & Kirshbaum (1960) and by Platt, Levin & others (1972) has been criticised as having a poor slope i.e. small difference in inhibition zone size between highest and lowest standards, and background activity due to the assay buffer alone. The poor slope leads to wide variations of assay results and is a consequence of the poor diffusion of this polyene antibiotic in agar.

Large-plate, agar-diffusion is the most generally useful method for antibiotic assay and it was felt that any improvements that could be made to the diffusion assay for amphotericin B would be welcome. We have found that the replacement of the recommended high pH phosphate buffer (Kramer & Kirshbaum, 1960; Platt & others, 1972; B.P. 1973; U.S.P. XIX, 1975) by a high pH carbonate/bicarbonate buffer greatly improves the dose-response for the assay and removes any background interference.

The test organism used is *Saccharomyces cerevisiae* SC 1600 (Squibb Culture Collection) which is stored in liquid nitrogen as in the method described by Beezer, Newell & Tyrrell (1976). The assay agar is as previously described by Kramer & Kirshbaum (1960) and by Platt & others (1972). Large (30 cm × 30 cm) glass bottomed plates are used with 250 ml of agar per plate. The inoculum is tested before assay to find the optimum concentration to be used. Sixty four wells are punched into each plate and these are filled in a latin square design with duplicate standards at two concentrations and two different samples at two concentrations per line.

Amphotericin B raw materials and pharmaceutical dosage forms are primarily dissolved in dimethylsulphoxide (DMSO) to a concentration of 500 μg amphotericin B ml^{-1}. Further dilutions are made in pH 10·6 carbonate/bicarbonate buffer (0·1 M) of the following composition, 4·505 g anhydrous sodium carbonate; 0·63 g sodium bicarbonate; distilled water to 1 litre; pH 10·6. The final concentrations of amphotericin B required are 4 and 1 μg ml^{-1}, with a final DMSO con-

* Correspondence.

centration of 10%. 75 μl of these solutions are filled into the wells as described above. After incubation at 37° for 16–18 h the resultant zones of inhibition are read and the results calculated.

As can be seen from Table 1, the dose response is 36% greater with the suggested carbonate/bicarbonate buffer than with the phosphate buffer recommended in the B.P. It was thought that this might have been a consequence of the ionic strength of the buffer, but, as also shown in Table 1, this is not so. As the ionic strength of both buffers is decreased so the dose-response is improved, but in each instance, apart from the highest ionic strength buffers, the carbonate/bicarbonate buffer gives the largest difference between the standard concentrations. In addition to slope improvement, zone definition with the use of the carbonate/bicarbonate buffer is greatly enhanced, thus reducing zone reading errors which previously could have constituted an inordinate proportion of the response. Finally, unlike the phosphate buffer, the carbonate/bicarbonate buffer itself (i.e. when used as a blank) does not produce a zone of inhibition and, therefore, any errors due to background interference are removed.

Table 1. *Dose response produced by phosphate buffers and carbonate/bicarbonate buffers of various ionic strengths.* Each result is shown with its 95% confidence interval ($P = 0.05\%$).

Buffer	Ionic Stgth (μ)	Average size inhib. zone (mm) 4 μg ml^{-1}	1 μg ml^{-1}	Diff. (mm)
Phosphate*	0·62	19·0 ± 2%	16·7 ± 3%	2·3
Carb/bicarb.	0·62	23·7 ± 3%	22·7 ± 3%	1·0
Phosphate	0·2	20·7 ± 2%	16·6 ± 4%	4·1
Carb/bicarb.	0·2	22·4 ± 2%	17·1 ± 4%	5·3
Phosphate	0·13	21·1 ± 2%	16·1 ± 3%	5·0
Carb./bicarb.**	0·13	22·0 ± 2%	15·7 ± 4%	6·3

* B.P. recommended buffer.
** Suggested buffer.

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The problems associated with the use of 2,2′-pyridylisatogen tosylate in evaluating the allegedly purinergic innervation of peripheral organs

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The ability of 2,2′-pyridylisatogen (PIT) to block the inhibitory effects of adenosine triphosphate (ATP) was originally measured on the isolated taenia of guinea-pig caecum so that a subsequent evaluation of the 'purinergic' nature of the non-adrenergic inhibitory innervation could be made (Spedding, Sweetman & Weetman, 1975). This course of action was adopted because Burnstock and his co-workers had accumulated the bulk of their evidence that ATP mediates the atropine-resistant non-adrenergic inhibitory innervation on this tissue (Burnstock, Campbell & others, 1970; Burnstock, 1972; Satchell, Lynch & others, 1972; Satchell, Burnstock & Dann, 1973). In our hands PIT failed to block the inhibitory response to field stimulation in the taenia. Indeed, PIT slightly increased the effect of stimulation at 2Hz (after PIT, 50 μM for 30 min, the response was 114 ± 7% of the control, n = 6), whereas the effects of exogenous ATP were less than half the control values (Spedding & others, 1975). In the same study, when a slightly different experimental design was adopted, PIT (50 μM for 30 min) did not modify the

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