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

* Correspondence.

Table 1. *The effects of 2-2'-pyridylisatogen tosylate (PIT) on isolated tissues.*

Preparation	Conc (μM)	Time (min)	Effect	Ref
Taenia	25	30	Blocks ATP-induced relaxations	1
Taenia	2·5	10	Relaxation	1, 2
Liver mitochondria	10	2	Inhibition of ADP-stimulated respiration	2
Diaphragm	20	30	Contracture	3
Stomach	50	30	Non-specific block of drug-induced relaxations	2
Bladder	50	30	Non-specific block of nerves	4
Ileum	12·5	15	Inhibition of arachidonic acid-induced contractions	2
Ileum	25	30	Non-specific antagonism of drug-induced contractions	2

The diaphragm was prepared from rat, the other preparations being of guinea-pig tissues. The references are: 1. Spedding & others, 1975; 2. Spedding, 1977; 3. Unpublished results; 4. Weetman & Turner, 1977.

response to field stimulation but did block ATP. This, we feel, is critical evidence against ATP being the transmitter mediating the inhibitory effects on the taenia.

Thus we were surprised to read that Kažić & Milosavljević (1977) found PIT provided evidence in favour of the purinergic mediation of the excitatory effects of atropinized coaxially stimulated terminal ileum of the guinea-pig. The properties of PIT are such that, in our view, the increase in responses of the ileum to stimulation at 3 Hz may well be explained by one of the actions of the drug that are unrelated to ATP-receptors (Table 1). For example, the concentration of PIT employed by Kažić & Milosavljević, although very low, is sufficient to produce some degree of inhibition of oxidative functions in mitochondrial preparations. Should this also occur in smooth muscle preparations, a leakage of Ca^{2+} from the mitochondria into the cell fluid would be envisaged, which could lead to increases in the amplitudes of evoked contractions. Release of Ca^{2+} from mitochondria could also explain the contractures of skeltal muscle induced by PIT (Table 1).

The many actions of PIT limit the usefulness of this drug in evaluating the proposed purinergic nature of peripheral innervations. Nevertheless, PIT is the best ATP-receptor antagonist currently available. There is a clear need for the synthesis and evaluation of more isatogen derivatives in order to develop a specific ATP-receptor blocking drug. Meanwhile, findings such as those reported by Kažić & Milosavljević (1977) provide only circumstantial support for Burnstock's hypothesis.

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