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SHORT COMMUNICATION

Some effects of bacterial pyrogens and activated charcoal on polarographic maxima

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The adsorption of pyrogens from injection solutions is well known (Lees & Levvy, 1940; Brindle & Rigby, 1946), and to monitor the removal of pyrogens (Micka & Kalvoda, 1956) a polarographic method has been proposed; this consists of observing the form of the polarographic reduction wave of cadmium. In the presence of pyrogen no maximum is seen at the crest of the wave whereas after treatment of the solution with activated charcoal an acute maximum (Fig. 1B) can be observed. It is claimed that this corresponds to adsorption of the pyrogen onto the charcoal, the minimum amount of charcoal required for full maximum formation being used as a measure of the amount of pyrogen present. Farkas & Bridicksa (1964) have employed this polarographic technique for the routine quality control of pyrogen-free water. The same authors have similarly used the polarographic oxygen maximum. The suppression of the oxygen maximum by some pyrogenic solutions has been reported previously by Suzuki (1955).

It is known (Heyrovsky & Vascautzanu, 1931) that because the half-wave potential of cadmium lies close to the electrocapillary zero potential of mercury, the cadmium wave does not normally exhibit a maximum. It therefore seemed desirable to investigate more closely the effect of charcoal on the cadmium wave and also to determine the sensitivity of polarographic maxima to pyrogens.

Experimental

A Tinsley type 14/3 polarograph was used with a dropping electrode system made entirely of glass. The drop-time was 2·5 s on closed circuit and zero applied potential. The cell anode was a mercury pool. One of the two electrolysis cells used permitted the introduction of solutions from a burette. Cell solutions were deoxygenated with solvent-saturated oxygen-free nitrogen, and polarograms recorded with minimal damping. The water used was from a still previously shown to produce apyrogenic water. Potassium chloride (0·1 M) was used as a supporting electrolyte. Analar chemicals were used where possible: the gelatin was of B.P. quality. Activated charcoal was Norit N.K. freed from water-soluble impurities. The pyrogens used were: the dried purified lipopolysaccharide from *Salmonella abortus-equi*, the O-somatic antigen from *Shigella dysenteriae* (the International Pyrogen Reference Preparation) and the purified "3922 lipopolysaccharide *Escherichia coli* 0111: B4", the latter supplied by Difco Laboratories Ltd.

Current-time recordings were obtained with a current-calibrated double-beam oscilloscope.

Results and discussion

We were able to record the acute cadmium maximum only when charcoal was present in the cell (Fig. 1B). Despite rigorous attempts at pyrogen exclusion we have

been unable to record this maximum in the absence of charcoal. In all cases when charcoal was not present in the cell during recording, the polarogram was of the form shown in Fig. 1A.

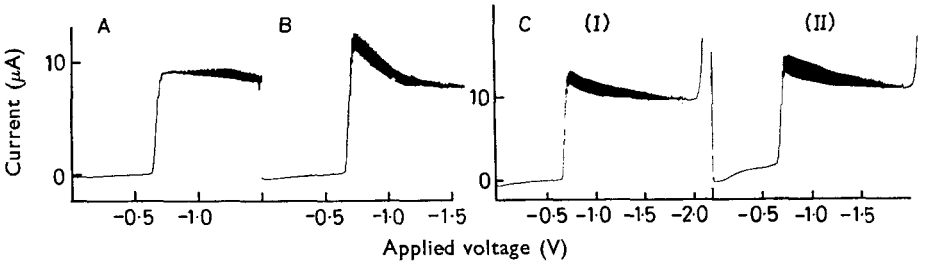

FIG. 1. Polarographic waves for (A) 1·0 mM cadmium chloride in 0·1M potassium chloride. (B) 1·0 mM cadmium chloride and 0·01% w/v activated charcoal in 0·1M potassium chloride. (C) 1·0 mM cadmium chloride and 0·01% w/v activated charcoal in 0·1M potassium chloride: (I) immediately after deoxygenation, (II) 18 h after deoxygenation without disturbing cell contents.

These results are at variance with those of Micka (1956), who stated that polarograms with maxima of identical height were recorded when the charcoal had been removed by sedimentation or by centrifugation. After centrifugation at 10,000 rev/min for 2 h we could not record the acute maximum from the supernatant liquid, whereas before centrifugation this had been possible, even with as little as 0·01% w/v charcoal present. After 18 h of undisturbed settling in the cell, the maximum was still in evidence (Fig. 1C II). Light scattering measurements showed that at this time there was still a detectable amount of charcoal in the capillary tip region (Fig. 2).

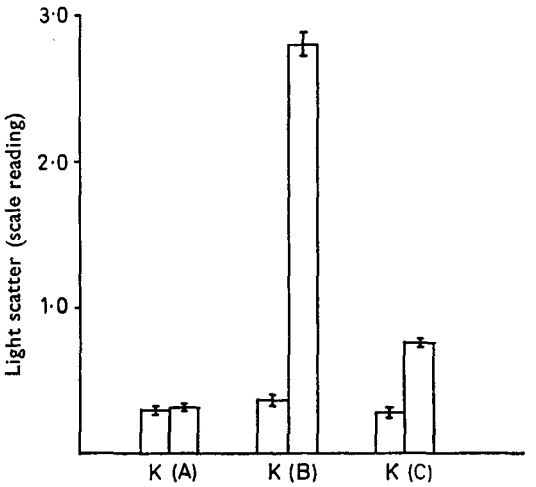

FIG. 2. Block diagrams to show the light scatter with standard errors produced by the cell contents: (A) before addition of charcoal, (B) immediately after deoxygenation of cell suspension containing charcoal, (C) 18 h after deoxygenation without disturbing cell contents. K is the cell solution (without added charcoal) as control.

The current-time curves obtained for charcoal suspensions both before and after sedimentation confirmed the presence of charcoal at the mercury drop surface (Fig. 3). All these observations led us to conclude that the role of charcoal in the recording

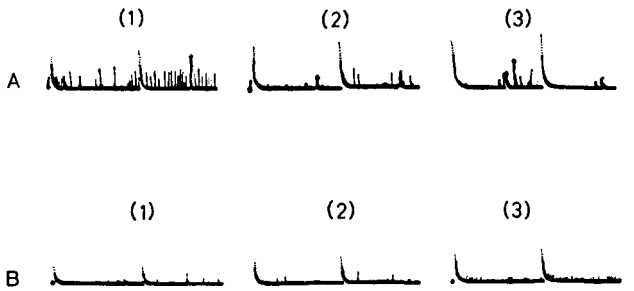

FIG. 3. Oscillographic current-time curves recorded from 0·1M potassium chloride containing 0·01% w/v activated charcoal: A, immediately after deoxygenation, B, 18 h after deoxygenation without disturbing cell contents. Potentials: A: (1) —609, (2) —699, (3) —781 mV. B: (1) —599, (2) —687, (3) —768 mV.

of the acute cadmium maximum is due to phenomena other than the adsorption of maximum-suppressing pyrogens—the explanation advanced by Micka & Kalvoda. We have previously reported the shifting of the electrocapillary zero potential of mercury by charcoal in suspension (Jones & Kaye, 1969) and feel that this provides an explanation of the appearance of the cadmium maximum, in accordance with Heyrovsky's rule (Heyrovsky & Vascautzanu, 1931; Heyrovsky, 1934).

Table 1. *Effect of some pyrogens and gelation on polarographic maxima.* Purified pyrogen solution (5·1 ml) was added to produce a cell concentration of 1 μg/ml.

Maximum suppressor (Cell concentration)	Mean % suppression of the maximum produced by the polarographic electrolysis of:					
	Cd^{++}	Tl^{+}	Oxygen	Co^{++}	Ni^{++}	Pb^{++}
Pyrogen from *Salmonella abortus-equi* (1 μg/ml) ..	—1·0	13·2	3·8	5·0	13·6	—5·5
Pyrogen from *Escherichia coli* (1 μg/ml)	11·7	13·6	0	3·2	17·5	9·7
Pyrogen from *Shigella dysenteriae* (1 μg/ml) ..	6·3	10·7	4·5	17·5	10·5	26·5
Gelatin (1 μg/ml)	26·5	35·0	14·5	49·0	62·7	49·5
Apyrogenic water (5·1 ml)	—6·7	5·0	1·6	—1·2	3·0	4·0

(—ve sign indicates mean increase in maximum height.)

We have also examined the effects of known concentrations of three purified pyrogens on the acute cadmium maximum recorded in the *presence* of charcoal, and on other polarographic maxima recorded in the *absence* of charcoal. Table 1 shows that the cadmium and oxygen maxima are relatively insensitive to pyrogen concentrations of 1 μg/ml, a value which is very high in terms of the rabbit thermal response test, where for example 0·003 μg of the International Reference Preparation is known to elicit a febrile response (Humphrey & Bangham, 1959). For comparison, the results with gelatin, an effective maximum suppressor, are included. All the pyrogens tested are far less effective maximum suppressors than gelatin.

We conclude that the suppression of polarographic maxima does not afford a dependable method of pyrogen detection.

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