Antibiotic sensitivity testing by flow microcalorimetry

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Summary. The proposed flow microcalorimetric method for the diagnosis of bacteriuria has been extended to include antibiotic sensitivity testing. Sensitive organisms rapidly (4–8 min) show thermal responses to the added antibiotics over the normal range of concentrations (1×, 2×, MIC value).

Flow microcalorimetry has recently been proposed as a rapid screening technique in the diagnosis of bacteriuria[1-3]. The microcalorimetric technique involves detection of the heat evolved by metabolizing microorganisms since it has been shown[4] that there is a direct correlation between this heat evolution and cell numbers.

Interference with metabolism by some modifier, e.g. an antibiotic, has been demonstrated either to alter the heat evolution rate or to reduce heat evolution to zero. This result has led to the development of assay procedures for polyene antibiotics[5,6]. There also exist a number of qualitative accounts of the interaction of antibiotics with microorganisms as recorded calorimetrically[7-11]. However, only one report exists on antibiotic sensitivity testing in a clinical situation[12]. Here, organisms cultured from infected urines were examined microcalorimetrically for their sensitivity toward a range of antibiotics. The technique described was time-consuming, laborious and not easily automatable.

Routine bacteriological laboratories report not only the number and species of bacterial strains in urine specimens but also the sensitivity of the infecting organism toward a spectrum of antibiotics. Conventionally, such routine investigations involve the agar plate diffusion technique which requires overnight incubation (16–18 h). This paper reports the extension of the microcalorimetric technique for the diagnosis of bacteriuria to the rapid determination of antibiotic sensitivity.

Methods and materials. The minimum inhibitory and bacteriocidal concentrations (MIC and MBC) were determined by the broth dilution technique[13].

Calorimetry. The design[14] of the LKB flow microcalorimeter (type 10700-1, LKB Produkter, S-161 25 Bromma, Sweden) and its operation[4] are as described previously.

Presentation of sample to calorimeter. A 250 ml conical flask containing 100 ml of a semi-defined medium[15] was incubated at 303 K in a shaking water-bath. This medium was pumped (LKB Perpex Peristaltic Pump, 48 ml h^{-1}) through the microcalorimeter and the outflow returned to the incubation flask. An instrumental baseline was established by pumping this medium through the calorimeter before inoculating with microorganisms. 1 ml of an overnight culture of the test organism was added to the flask and after 50 min growth a heat effect equivalent to a 40% deflection of the chart recorder (10 μV f.s.d., Philips PM 800) was achieved. This deflection corresponded to a bacterial concentration of 2.0×10^7 cells ml^{-1}. At this point, the appropriate antibiotic was introduced into the incubation flask in a small volume (2 ml) of distilled water and vigorously mixed with the culture.

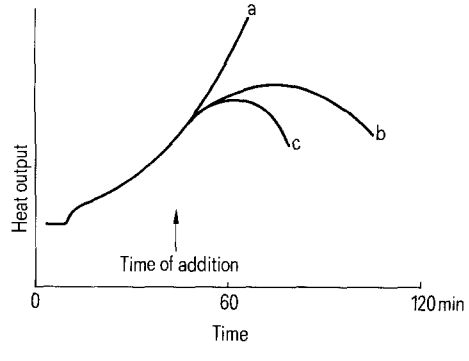
Bacterial strains. 2 strains of *Escherichia coli* which had previously been isolated from urine specimens were used. The MIC of these 2 strains against the antibiotics under test is shown in the table.

Antibiotics. Standard solutions of ampicillin, streptomycin, chloramphenicol, tetracycline and sulphadimidine were made from the pure materials.

Results and discussion. In the absence of added antibiotic, the heat output rate of growing cultures of both strains of *E. coli* increases exponentially. This is consistent with exponential growth of the cultures. When antibiotics are added to cultures, a number of effects may be observed, according to the nature and concentration of antibiotic. The figure shows the effect of various concentrations of ampicillin on the heat output of growing cultures of *E. coli* 1. At 1 μg ml^{-1} there is no effect; at 2 μg ml^{-1} there is, at first, a reduction in the rate of increase followed after 20 min by a decline in heat output and at 5 μg ml^{-1} there is a decline in heat output after 8 min. With *E. coli* 2 (results not shown), 5000 μg ml^{-1} had no effect, whereas 10,000 μg ml^{-1} causes the heat output rate to become static, and then, after 30 min, to decline.

The significant features of these results, in the present context, are the rapid response times and the level of ampicillin concentrations required to produce response. For both strains, this concentration is equivalent to the MIC determined by the tube dilution method (see table).

Results essentially similar to those for ampicillin were observed with both strains of *E. coli* with streptomycin, chloramphenicol and tetracycline. In all cases, one half of the MIC produced no or only marginal effect on the heat output, whereas the MIC level either prevented any further increase or caused a decline in heat output. The times required to produce these responses at the MIC levels were less than for ampicillin and varied from 8 min to 4 min. Response times of less than 4 min could not be determined



The effect of various concentrations of ampicillin on the heat output of exponentially growing cultures of *E. coli* 1. a: Control, 1.0 μg ampicillin; identical curves. b: 2.0 μg ampicillin. c: 5.0 μg ampicillin.

Antibiotic MIC's against *E. coli* 1 and 2

Antibiotic	MIC (μg ml^{-1}) *E. coli* 1	*E. coli* 2
Ampicillin	1.0	10,000
Streptomycin	10	128
Chloramphenicol	256	128
Tetracycline	0.5	64
Sulphadimidine	32	> 33,000

as this is the time required to pump the suspension from the culture vessel to the calorimetric chamber.

However, sulphadimidine (which was only used with *E. coli* 1) had no effect on heat output at the MIC level and even at a concentration of 30× MIC had only a slight and transitory effect. This may reflect the relatively high bacterial concentration used in the calorimetric experiments (2×10^7 cells ml^{-1}) compared to that used in the tube dilution method for determining MIC's (2×10^5 cells ml^{-1}). (This apparently large difference in the bacterial cell concentrations, however, had no effect on the response to MIC's of the other antibiotics.) The dependence of sulphadimidine MIC determinations on inoculum density has been reported[13]. It is possible that if a calorimeter able to monitor heat outputs from a population as small as 10^5 cells ml^{-1} were available, a better correlation could be obtained. It is also well-established[13] that some bacterial culture media contain sulphadimidine inhibitors (p-amino benzoic acids) which interfere with the determination of sensitivity to sulphadimidine, using standard disc diffusion techniques. This problem is usually overcome by the incorporation of lysed blood into such media. It is possible that the medium used in the work reported here contained such inhibitors.

The results, therefore, indicate there are measurable changes in heat output by metabolizing bacteria when exposed to concentrations of most antibiotics equivalent to the MIC as determined by the tube dilution method. Moreover, these changes occur within 8 min or less after exposure of the culture to the antibiotic and therefore suggest that this technique could be adapted for the rapid assessment of sensitivity of bacterial isolates to particular antibiotics in the diagnostic microbiology laboratory.

Furthermore, the flow microcalorimeter is easily automated, and, if a multi-channel device were available, a large volume handling system could be devised with a rapid through-put potential.

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15 Medium constituents (g l^{-1}): glucose, 1; K_2HPO_4, 4.2; NaH_2PO, 3.9; peptone 1; yeast extract, 0.2; $MgCl_2$, 0.13; $CaCO_3$, 0.0003; $FeSO_4\cdot7H_2O$, 0.007; $MnCl_2\cdot4H_2O$, 0.001.

Anticonvulsant action of YG 19-256 in baboons with photosensitive epilepsy

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Summary. YG 19-256, 4-(1,3,4,9b-tetrahydro-5 methyl-2H-indeno[1,2-c]pyridine-2-yl)-2-butanone methane sulphonate, 1-3 mg/kg i.v., abolished or reduced photically-induced myoclonic responses for 1.5-6.5 h in baboons, *Papio papio*, without producing signs of acute neurological toxicity.

YG 19-256 is an indenopyridine derivative (figure 1) that inhibits aggressive behaviour in rodents and primates without producing generalized sedation[2,3]. Preliminary test in mice indicate that YG 19-256 protects against the tonic-extensor component of electro-shock induced convulsions (ED 50=25 mg/kg, i.p.), but not against seizures induced by i.v. pentylene-tetrazol[4]. We are reporting that YG 19-256, at dose levels that do not produce motor signs of neurological toxicity, prevents epileptic responses induced by intermittent photic stimulation (IPS) in baboons, *Papio papio*, from the Casamance region of Senegal that spontaneously show photosensitive epilepsy[5,6].

Methods. Experiments were performed in 5 baboons that were moderately or highly photosensitive (i.e. they responded to IPS with generalized myoclonus that was stimulus-limited or self-sustaining, or with a full tonic-clonic seizure). Animals were chronically implanted with epidural electrodes to permit artefact-free EEG recording, and were seated in a primate chair for each acute experiment with a standardized exposure to IPS before, and at fixed intervals from 15 min to 6.5 h after, administration of YG 19-256. In 2 animals the natural syndrome of photically-induced responses was enhanced by the prior administration (175 min earlier) of DL-allylglycine, 180 mg/kg, i.v.[7]. YG 19-256 was administered i.v. as the methane sulphonate, dissolved in sterile saline.

Results. Baboons remained behaviourally alert after YG 19-256, 1-3 mg/kg, but with diminished teeth-baring responses to threatening gestures. Mydriasis and some slowing of EEG background rhythms were noted 3-10 min after YG 19-256, 3 mg/kg. This slowing was maximal after 10-60 min. Motor signs of neurological toxicity of the kind observed after hydantoin or carbamazepine (e.g. nystagmus and ataxia)[7] were not seen at any time after YG 19-256.

In 2 baboons showing generalized myoclonus, but not seizures, in response to control rests with IPS, YG 19-256, 1