by a simple penicillin disc test is recommended. Any strain growing up to, or producing only a small zone of inhibition round a 1 µg disc should be tested for β-lactamase production by the chromogenic cephalosporin test (O‘Callaghan et al., 1972).

Fortunately these strains are at present sensitive to a variety of other antibiotics such as tetracycline, spectinomycin, kanamycin, cotrimoxazole and a new cephalosporin, cefuroxime. From the reports so far available, spectinomycin in a dose of 2 g seems to give very good results. It has a very rapid bactericidal effect and the MIC of almost all strains is within the range of 5 to 20 µg/ml. A dose of 2 g gives serum levels of 100 µg/ml. Resistance to spectinomycin can be produced in vitro but naturally resistant strains seem to be very rare; Reyn et al. (1973) have reported one such random isolate and another which became resistant during treatment; both had MICs of over 480 µg/ml. Because of this, it seems prudent to restrict the use of spectinomycin as far as possible to the treatment of patients shown to be infected with β-lactamase producing strains, their recent sexual partners (who should be treated on epidemiological grounds without waiting for bacteriological confirmation) and patients who have failed to respond to penicillin, ampicillin or tetracycline. Rapid identification of β-lactamase-producing strains, effective treatment and thorough contact tracing measures should minimize the risk of these strains establishing themselves widely in the community.

A. E. WILKINSON
Venereal Disease Reference Laboratory
London Hospital, London El, England

References

Nutrient depletion and antibiotic susceptibility

Recently there have been many developments regarding the important role of the bacterial envelope in resistance to drugs and to in vivo body defence mechanisms (Costerton & Cheng, 1975). In particular, it has been established that when faced with the lack of an essential nutrient a dividing bacterium will manufacture an envelope characteristic of the particular depletion. The effect of different nutrient depletions, in addition to effects on general biochemistry, is to give rise to cultures with radically different envelopes (Ellwood & Tempest, 1972; Holme, 1972). This phenomenon is particularly evident with respect to the outer membrane of Gram-negative bacteria (Brown & Melling, 1969; Brown, 1975). The implications of this basic work are only slowly being realized in terms of antibiotic assay or disinfectant testing or indeed any Pharmacopoeia tests for preservative efficacy. Furthermore, it seems probable that the physiological state of bacteria in certain in vivo situations will be influenced by nutrient
depletion. Thus a major component of 'biological variation' may possibly be reduced significantly. The influence on resistance of inoculum history in general has been reviewed elsewhere (Farwell & Brown, 1971). This article is mainly on the importance of the bacterial envelope in the light of recent work.

These effects of nutrient depletion in altering envelope structure have been greatly underestimated. Overnight broth cultures have been used in drug resistance studies for so long that these oxygen depleted cultures have come to be regarded as 'normal'. Which nutrient finally limits growth in 'nature'? There is virtually no published data on this question. Indications are that bacterial growth in ponds and rivers may be limited by carbon, nitrogen or phosphorus depending upon availability of, for example, sewage or fertilizer (personal communication, Dr C. M. Brown, University of Dundee). In the case of the hospital or factory environment the situation is also relatively unknown. However, in the in vitro situation two facts do seem clear. First, the vast majority of the world's potential bacterial contamination must be nutrient limited. Secondly, although lack of oxygen may be a common limitation, it is only one of many possibilities. A conceptual error has been the belief that the use of chemically defined media necessarily defines a culture of bacteria. It is commonly the case that the nature of the growth-limiting nutrient is not known. Furthermore, this would appear not to have been considered. The determination of which nutrient is depleted first, and at what population size, requires empirical study. It may also be that a culture may be in a stationary phase under the influence of lack of more than one essential nutrient (Brown & Melling, 1969). It could also be that, where such measurements have not been made, a small variation in contamination of medium constituents might tilt the balance so that one essential nutrient is depleted first rather than another. Despite advances in the reproducibility of in vitro testing techniques (Kelsey & Maurer, 1974), no specification was made of the nature of the nutrient(s) depleting the growth of the test cultures. These factors have recently been reviewed in detail elsewhere (Melling & Brown, 1975).

Another potentially significant variable is that of growth rate. Bacterial doubling times during an infection may be slow and of the order of 20 to 24 h (Meynell & Subbiah, 1963; Eudy & Burroughs, 1973). A crucial technique for controlling growth rate is that of continuous culture described in general in numerous reviews (Tempest, 1970) and recently with respect to antibacterial studies (Dean, Ellwood, Melling & Robinson, 1977). Under equilibrium (steady state) conditions in a chemostat the cells are reproducing at a constant rate in an invariant environment, unlike a batch culture where there is constant change. Using this technique, significant effects of growth rate and specific nutrient limitation on the sensitivity of P. aeruginosa to drugs (Finch & Brown, 1976) and to phagocytosis or leucocyte cationic protein (Finch & Brown, 1976) have been shown. In view of the general importance of these influences, it is perhaps surprising that no pharmacopoeial or other official test in any country defines inocula by specific nutrient growth limitation either in batch or continuous culture. Nevertheless, the enormous phenotypic variation of potential microbial contaminants, together with lack of knowledge about these phenotypes, makes the task of designing in vitro tests daunting.

It is worth noting, in passing, that a variety of specific nutrient limitations induced sporulation in Bacillus megaterium and B. stearothermophilus such that the spores reproducibly had chemical, physical and biological properties characteristic of the particular limitation imposed (Hodges & Brown, 1975; Lee & Brown, 1975). Hopefully, this may lead to enhanced reproducibility of spore crops for monitoring of sterilization processes and for antibiotic assay.

In the in vivo situation there is evidence that iron is normally growth limiting in serum (Bullen, Rogers & Griffiths, 1974) and that infection may elicit the response of a drop in the concentration of iron available to invading organisms (Sussman, 1974). A similar in vivo growth limiting possibility has been suggested for inorganic phosphate or even zinc (Weinberg, 1974). There is little published work on the effects of iron limitation on bacterial structure and resistance (Light & Clegg, 1974). Conceivably slow growing, iron limited chemostat cultures may prove more relevant for some purposes than ‘overnight’ broth cultures and possibly even more reproducible.

One may speculate about the nature of the growth limiting nutrient of bacteria in various localized sites in vivo but little empirical information is available. Ellwood & Hunter (1977) have discussed the resemblance between the mouth and a chemostat for oral bacteria. Little is known about growth limitation in urine, although Anderson (1976) has pointed
out the critical differences of urine pH, osmolality and constituents from laboratory media which can influence the physiological state of the bacteria and drug resistance. Broughton, Anderson & Bowden (1968) reported on the fall in serum magnesium in a proportion of patients with burns. The magnesium concentration in the burns tissue was not reported but the practice of saline baths probably would have even further reduced magnesium levels in the burn and possibly influenced growth of bacteria. The reasons for the appearance and persistence of mucoid forms of *Pseudomonas aeruginosa* in clinical sources has been of importance to many microbiologists (Doggett, 1969). Environmental factors must be critical since they are rarely isolated from patients in general and usually revert to non-mucoid forms *in vitro* (Govan, 1975, 1976).

The precise nature of the environment of microorganisms in most infections is not known, especially in terms of nutrient limitation. The latter environmental circumstance profoundly influences the structure and resistance properties of the cell envelope, particularly for Gram-negative bacteria. It is easy, but perhaps futile at present, to speculate about the influence of the environment in bacterial infection of various sites, of the influence of various diseases or of mixed infections on available bacterial nutrients. With the possible exception of iron, almost no data exists about the influence of growth rate and specific nutrient depletion on bacterial resistance to drugs and body defence mechanisms. When more data is available it will be possible to produce physiologically defined and more relevant inocula to mimic the *in vivo* situation.

**M. R. W. BROWN**

*Department of Pharmacy*

*University of Aston in Birmingham*

*Birmingham, England*

**References**


Hodges, N. A. & Brown, M. R. W. Properties of *Bacillus megaterium* spores formed under conditions of nutrient limitation. In *Spores VI*
Rapid sensitivity tests

The two symposia on Rapid Methods and Automation in Microbiology presented a somewhat bewildering array of potential techniques for rapid determination of microbial sensitivity to antibiotics. The methods described vary from the simple (and cheap) to the highly complex. The simplest approach used multi-well trays and replicators. It certainly allows screening of many strains with ease and economy (Newson, 1975) and with correct inoculum size might be adaptable for rapid determinations, and may add sets of single dilutions for sensitivity tests later. Dynatech sell the MIC 2000—a machine which lets the user fill trays with 96 solutions of his own choice and includes inoculating and reading modules. Considerable capital outlay is required for the system, which depends on the user for accurate dilutions, but is very flexible and allows simultaneous MICs and identification tests.

Full automation of sensitivity tests might provide more rapid, standardized results with minimal operator involvement, but requires complex machinery. Isenberg et al. (1971) described a prototype system based on exposure of known numbers of bacteria to antibiotics for at least five generation times—then estimating growth in a particle counter. The apparatus required was very bulky; subsequent machines have used optical sensors.

Praglin et al. (1975) described the Autobac (Pfizer) at the First Symposium, and it has now been evaluated in several countries. A cassette with 12 antibiotic-containing wells and a blank is charged with a standardized inoculum, and incubated. When the blank growth is adequate (usually 3 h) the cassette is placed in a light scatter system that scans each well and computes sensitivity patterns from pre-determined 'break-points'. A collaborative study in the USA (Thornsberry et al., 1975) found that Autobac and Kirby Bauer results agreed 91-5% of the time (with 17 agents); only nitrofurantoin was markedly discordant (77% agreement). However, Waterworth (1976) noted a discrepancy with erythromycin, and a lack of reproducibility with ampicillin; also that strains of pseudomonas took 5 h or more to test. Nevertheless Matsen, Krall & Saxon (1977) showed that 13-7% of the patients they studied benefited by a shorter stay in hospital obtained by same-day results.

The Akro-Medic/Abbott MS 2 (Spencer et al., 1977) is a more automatic system—still in prototype form. Once the reservoir in a multi-well cassette has been inoculated and the whole placed in the incubator/analyser module the test is automatic. Growth is monitored every 5 min by light emitting diodes and optical sensors which feed a Motorola microcomputer with information. When the inoculum reaches log-phase growth it is sucked into the test and blank wells and monitored. The computer can follow 56 tests (i.e. 560 wells) at a time. When control growth is adequate that in the antibiotic wells is compared and sensitivity test results are computed. This highly sophisticated unit is still undergoing 'in house' tests, but will also be being evaluated in a multi-centre trial. Mitchell, Johnston & Curtis (1977) assembled a continuous-flow system for tests on rapid-growing clinical isolates. They used an automatic sampler with a peristatic pump which

(Leading articles)