Demonstration of pathogenic bacteria in "sterile" inflammatory exudates

RC BRIDGER

From the Department of Microbiology, Godfrey Pathology Laboratory, 137 Kilmore Street, Christchurch 1, New Zealand

SUMMARY One hundred and twenty-seven exudates from inflammatory processes, judged sterile after incubation on standard isolation media, were further investigated. This involved the exclusion of slow-growing strains by a further 48 hours incubation of the primary plates and subcultures from hypertonic broth that had been inoculated concurrently with the initial cultures.

Over 80% of otherwise sterile exudates grew presumptive pathogens only after passage through the hypertonic broth and no further isolations resulted from extended incubation of the primary cultures.

A history of current, or recent, antibiotic therapy commonly accompanied the demonstration of these aberrant strains and clinical remission of symptoms usually followed fresh antibiotic therapy directed solely against the revertant isolates.

The absence of growth of pathogenic organisms on standard media in exudates from non-draining inflammatory lesions is a not uncommon laboratory dilemma. The incidence is significant and steady but because it constitutes a negative finding, figures are seldom reported. An extended period of morbidity in the patient is, however, a general consequence of this type of microbiological failure. Following the successful demonstration of pathogens in a series of apparently sterile nasal sinus exudates,¹ similar methods were applied to all "sterile" exudates processed in the Microbiology Department over a two-year period and a marked increase in the isolation rate of pathogens resulted.

Material and methods

CLINICAL MATERIAL

Clinical material fell broadly into three classes: (i) from localised acute lesions of soft tissue (216); (ii) from infected joints (172); and (iii) from nasal sinus exudates (189). Of those showing significant pus but no growth on primary isolation media, 34 (16%) soft tissue, 25 (15%) joint fluid and 68 (36%) nasal sinus specimens qualified for hypertonic broth passage (see Table 1). All material was aspirated and received either as fluid or as pus in syringes. Nasal sinus specimens were taken either as middle meatal

aspirates,¹ antral washes or as antral pus by direct puncture of the sinus cavity. Samples received on swabs were excluded from the series because the risk of contamination by commensal flora with this type of collection was felt to be unacceptably high.

GRAM STAINS

These were carried out on smears either from swung deposits of joint aspirates or directly from pus.

Table 1 Sources and numbers of exudates requiring hypertonic broth reversion to demonstrate a pathogen

Source	Number
Synovial fluid	25
Breast abscess	10
Peritoneal fluid	
(postop and post-dialysis)	7
Postop joint lesions	4
Ischiorectal abscess	3
Middle ear abscess	2
Parotid gland infection	2
Abscess face	1
Abscess thigh	1
Infected pharyngeal cyst	1
Postop intervertebral disc infection	1
Abscess axilla	1
Fluctuant mass, back	1
Nasal sinusitis	
Middle meatal aspirate	32
Antral wash	17
Antral puncture	19
Total	127

Method of isolation of bacterial pathogens from all exudates.

METHODS

Bacteriological investigation was performed as shown in the Figure. The identity of Gram-negative organisms was confirmed by passage through API-20E and those Gram-positive isolates requiring further identification were treated according to the methods outlined in Cowan and Steel.² The inclusion of Levanthal medium to select for *Haemophilus influenzae* was directed not only at nasal sinus exudates but also at possible strains of this organism causing suppurative arthritis.³

The single case of *Mycobacterium tuberculosis* infection was treated differently from all other exudates for clinical reasons. The lesion was fluctuant, indolent, of several months duration and as the patient had been previously diagnosed and treated for pulmonary tuberculosis, it was decided to culture on to Löwenstein-Jensen medium as well as the standard regimen used for the series.

ANTIBIOTIC SENSITIVITY TESTING

Sensitivity testing of isolates was carried out by standard disc diffusion with inhibition zones meas-

ured against a disc template based on subculture of commercial quality control strains of organisms. The antibiotics of common choice were: penicillin, ampicillin, erythromycin, tetracycline and cotrimoxazole. Additional antibiotics were tested as required and strains of *Staphylococcus aureus* resistant to standard penicillins were tested against methicillin. All strains of *H influenzae* were tested for beta-lactamase production by "Oxoid" beta-lactamase test papers and antibiotic testing of this species was carried out on Levanthal agar.

Results

GRAM STAINS

Where broth reversion was required, characteristic parent-form bacteria were rare in Gram films of primary exudates. Where structures were present, their morphology was generally pleomorphic and their reaction Gram-negative, irrespective of genera. Seventy-three films (58%) showed such structures and the remaining 54 (42%) showed no recognisable structures (see Discussion).

Table 2 Isolates and numbers of pathogens after hypertonic reversion from soft tissues and joints

Isolate	Number
<i>Staph aureus</i>	29
<i>Strep non-haem</i>	6
<i>Peptostreptococcus</i> spp	4
<i>Micrococcus</i> spp	3
<i>Strep faecalis</i>	3
<i>Acinetobacter</i> spp	2
<i>S citreus</i>	1
<i>Mycobacterium tuberculosis</i> *	1
Total	49
No growth	10

*Grown only on Löwenstein-Jensen medium.

Table 3 Isolates and numbers of pathogens after hypertonic reversion from nasal sinus exudates

Isolate	Number
<i>Staph aureus</i>	25
<i>H influenzae</i>	10
<i>Strep pneumoniae</i>	5
β -haem <i>Strep</i> group A	5
<i>Strep non-haem</i>	4
<i>Pseudomonas</i> spp	4
<i>Peptostreptococcus</i> spp	3
<i>Klebsiella</i> spp	2
<i>H parainfluenzae</i>	2
Total	60
No growth	8

ISOLATES

The identity and percentage of each revertant species from soft tissues and joints is shown in Table 2 and those from nasal sinuses in Table 3. Revertants of *H influenzae* were restricted to nasal sinus exudates and only two strains of this species were found to be beta-lactamase producers. Of the 54 revertant *Staph aureus* isolates, none was sensitive to natural penicillins and two were methicillin-resistant.

Discussion

The role of cell wall-damaged strains of bacteria as pathogens has been in contention for many years.⁴⁻¹² The barrier to their universal acceptance in this role has been the lack of clear definitions of the degrees of wall damage and, more importantly, whether such strains revert, replicate and produce toxins in vivo. Recently published work, however, supports their ability to sustain established infections.¹³⁻¹⁷

The fact that some strains return to parent form after a single passage through hypertonic broth is not in doubt. This type of revertant organism conforms to the description of "unclassified wall-defective variants" as defined by McGee *et al*¹⁸ in that they yield vegetative parent bacteria after first

passage through hypertonic media but do not grow in the same media without initial osmotic supplement. It was this type of organism investigated in the study reported here.

Although these aberrant forms were not invariably demonstrable by Gram staining of the initial exudates in this series, it should be noted that this does not exclude their presence.¹⁹ Conversely, Gram stains of positive hypertonic broth cultures always showed some individual organisms that were aberrant in morphology and many that were Gram-variable in reaction but all reverted to parent form and correct Gram reaction at first subculture onto standard solid medium.

The effects of suboptimal doses of antibiotics on bacteria is well documented²⁰⁻²⁴ and the reduced effect even of full doses in a sealed infection also seems proven. Reasons for the latter include: pH changes in such an environment may produce a marked shift to the alkaline side with a consequent reduction in antibiotic activity of some antibacterial agents²⁵; the fact that some antibiotics fail to reach therapeutic levels in a sealed lesion²⁶ and a reduction in the rate of bacterial division may result from the steady loss of trapped bacterial nutrients thus rendering antibiotics ineffectual because only actively dividing organisms are vulnerable to antibiotic action. There are then, a range of antibiotic effects known to alter bacteria morphologically and metabolically, all of which do not necessarily sterilise lesions.

In this series only 17 of the 127 investigated had not been treated with antibiotics for their infection. Of this 17, five had received therapy for unrelated infections during the previous three months and the remainder denied any therapy during the previous year. However, eight of the latter group failed to produce growths in hypertonic medium. The relation between antibiotic challenge and aberrant bacteria would seem very close in this study.

The antibiotic sensitivity patterns of isolates were significant. Strains sensitive to all five drugs of choice comprised only 9% of the total. Resistance to two agents out of five accounted for 35%; to three agents out of five, 24% of isolates; to four agents out of five, 26% of isolates and to all five agents, 6% of the whole series. These generally increased resistance figures would seem to reflect the antibiotic stress to which revertant isolates had been subjected. They would also support the suggestion of Palmer²⁷ that where multiresistant aberrant strains are present in an infection, any completion or discontinuation of therapy allows the re-emergence of that strain in its pathogenic parent form. If this premise is accepted, an extension of the primary infectious process would seem axiomatic.

The ratios of Gram-positive to Gram-negative isolates were noteworthy. The figure of 15.5:1.0 in the soft tissue-joint categories supported the thesis that the generally preferred penicillin group antibiotics may not be adequate to sterilise all enclosed lesions. The nasal sinus category with a ratio of 1.6:1.0 presumably reflects the greater accessibility of this area, through an open ostium during periods of drainage, to changing opportunist flora. Any succeeding exacerbation of infection that closes the ostium by oedema would, of course, produce the same barriers to successful treatment faced by the permanently sealed lesions of soft tissues and joints.

The particularly high incidence of *Staph aureus* in all categories appears to demonstrate the ubiquitous nature of this species, its proven ability to act either as a commensal or a pathogen, the ease of expression of its genetic determinant for beta-lactamase production and the problems of its eradication, even with sensitive strains, by suboptimal amounts of antibiotic directed against its cell wall. A corollary to this concerns the ratios of exudates requiring broth conversion in each category. Two-and-a-half as many hypertonic cultures were needed for sinus exudates as for exudates from other sites. This disparity presumably arises from the multiple treatments required for the type of chronic disease present in nasal sinuses and the consequently greater opportunity for the emergence of cell wall-damaged forms.

The results of therapy directed against revertant pathogens led to an adequate resolution of lesions in 86% of the soft tissue-joints groups. The other 14% were unable to be confirmed due to referral back from specialists to general practitioners. In the nasal sinus group, satisfactory remissions were achieved in 71% of treated patients. The 21% failure rate was due to a variety of reasons and these included: lack of patient compliance with dosage schedules; too short a course of therapy and the decision by some practitioners not to use the antibiotic advised in the laboratory report.

Although it can be argued that adequate drainage of lesions is superior to chemotherapy, this presumes easy accessibility to the lesion for surgical intervention; a completely unimpaired immune response in the patient and also implies an extended period of convalescence.

Given unequivocal signs of an acute inflammatory process, including the presence of significant pus, determined efforts to demonstrate an infective cause should be pursued. It would appear that an initial inability to grow a pathogen does not exclude its presence and that any method likely to increase the yield of organisms should be routinely undertaken. The inoculation of a tube of hypertonic broth with

exudates from any patients showing inadequate response to therapy is a simple and inexpensive addition to the laboratory profile. In the light of the results of this investigation such an approach would seem obligatory, both from a microbiological and a clinical point of view.

I am grateful to Miss Anne Grigor and her staff for technical assistance.

References

- 1 Bridger RC. Sinusitis: an improved regime of investigation for the clinical laboratory. *J Clin Pathol* 1980;**33**:276-81.
- 2 Cowan ST, Steel KJ. *Manual for the identification of medical bacteria*. 2nd ed. Cambridge: Cambridge University Press, 1974.
- 3 Leek JC, Robbins DL. *H influenzae* tenosynovitis. *Ann Rheum Dis* 1980;**33**:530-3.
- 4 Godzeski CW, Brier G, Griffith RS, Black UR. Association of bacterial L-phase organisms in chronic infections (Letter). *Nature* 1968;**205**:1340.
- 5 Charache P. Atypical bacterial forms in human disease. In: Guze LB, ed. *Microbial protoplasts, spheroplasts and L-forms*. Baltimore: Williams & Wilkins, 1968:484-94.
- 6 Barile MF. Variant bacteria and chronic disease. *Hosp Pract* 1968;**3**:41-3.
- 7 Finegold DS. Biology and pathogenicity of microbial spheroplasts and L-forms. *N Engl J Med* 1969;**281**:1159-70.
- 8 Charache P. Cell wall-defective bacterial variants in human disease. *Ann N Y Acad Sci* 1970;**174**:903-11.
- 9 Louria DB. L-forms, spheroplasts and aberrant forms in chronic sepsis. *Adv Intern Med* 1971;**17**:125-42.
- 10 Guinan PD, Neter E, Murphy GP. The significance of L-forms in human renal disease. *J Urol* 1972;**108**:50-3.
- 11 Watanakunakorn C, Glotzbecker C. In vivo behaviour of revertants from *Staphylococcus aureus* L-phase variants compared with the parent strain. *Infect Immun* 1975;**11**:1182-6.
- 12 Watanakunakorn C. Are cell wall-defective microbial variants important in clinical infectious diseases? *J Antimicrob Chemother* 1979;**5**:239-41.
- 13 Bakir M, Attia H, Gad W. L-forms of *Staphylococcus aureus* as a cause of resistant conjunctivitis. *Bull Ophthalmol Soc Egypt* 1978;**71**:237-45.
- 14 Grekova NA, Tolmacheva TA, Vershilova PA. On the pathogenicity of non-stable *Brucella* L-forms and their revertants. *J Hyg Epidemiol Microbiol Immunol (Praha)* 1979;**23**:129-34.
- 15 Gusman BS, Vulfovitch IuV, Kagan GIa. Morphogenesis of the heart lesion in experimental infection with Streptococcal L-forms. *Arkh Patol* 1980;**42**:26-32.
- 16 Salobi Rlu, Berezovskii BA, Golanov VS. Significance of mycobacterial L-forms in the development of pulmonary tuberculosis relapses. *Vrach Delo* 1981;Dec(12):77-80.
- 17 Mavrov II. Isolation of gonococcal L-forms from patients and their role in the pathology of gonococcal infection. *Vestn Dermatol Venerol* 1981;**2**:22-7.
- 18 McGee ZA, Wittler RG, Gooder H, Charache P. Wall-defective microbial variants: terminology and experimental design. *J Infect Dis* 1971;**123**:433-8.
- 19 Cate TR. Cell wall-defective bacteria. In: Lenette EH, Spaulding EH, Truant JP, eds. *Manual of clinical microbiology*. 2nd ed. Washington DC: American Society for Microbiology, 1974:338-43.
- 20 Klainer AS, Perkins RL. Normal and abnormal morphology of micro-organisms. *JAMA* 1971;**215**:1655-7.

- ²¹ Lorian V, Atkinson B. Abnormal forms of bacteria produced by antibiotics. *Am J Clin Pathol* 1975;**64**:678-88.
- ²² Lorian V. Abnormal forms of bacteria. *Mt Sinai J Med* 1976;**43**:762-75.
- ²³ Washington JA II. The effects and significance of subminimal inhibitory concentrations of antibiotics. *Rev Infect Dis* 1979;**1**(5):781-6.
- ²⁴ Lorian V, Waluschka A, Kim Y. Abnormal morphology of bacteria in the sputa of patients treated with antibiotics. *J Clin Microbiol* 1982;**16**:382-6.
- ²⁵ Carenfelt C, Eneroth CM, Lunberg C, Wretling B. Evaluation of the antibiotic effect of treatment of maxillary sinusitis. *Scand J Infect Dis* 1975;**7**:259-64.
- ²⁶ Lundberg C, Malmberg AS, Ivemark BL. Antibiotic concentrations in relation to the structural changes in maxillary sinus mucosa following intramuscular or peroral treatment. *Scand J Infect Dis* 1974;**6**:187-91.
- ²⁷ Palmer DW. Inadequate response to "adequate" treatment of bacterial infection: L-forms and "bactericidal" antibiotic activity. *J Infect Dis* 1979;**130**:725-7.

Requests for reprints to: RC Bridger, Pathology Laboratory, Colston House, 137 Kilmore Street, Christchurch 1, New Zealand.