Postmortem bacteriology: a re-evaluation

J A Morris, L M Harrison and S M Partridge

Updated information and services can be found at:

http://jcp.bmj.com/cgi/content/full/59/1/1

These include:

References
This article cites 32 articles, 8 of which can be accessed free at:
http://jcp.bmj.com/cgi/content/full/59/1/1#BIBL

1 online articles that cite this article can be accessed at:
http://jcp.bmj.com/cgi/content/full/59/1/1#otherarticles

Rapid responses
You can respond to this article at:
http://jcp.bmj.com/cgi/eletter-submit/59/1/1

Email alerting service
Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article

Notes

To order reprints of this article go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to *Journal of Clinical Pathology* go to:
http://journals.bmj.com/subscriptions/

1

Postmortem bacteriology: a re-evaluation

J A Morris, L M Harrison, S M Partridge

....

See end of article for
authors' affiliations
....

Correspondence to:
Professor J A Morris,
Department of Pathology,
Royal Lancaster Infirmary,
Lancaster, LA1 4RP, UK;
Jim.A.Morris@rli.mbht.
nhs.uk

Accepted for publication
19 May 2005
....

Aim: To assess the value of postmortem bacteriology in necropsy practice, with specific emphasis on bacterial invasion of blood and cerebrospinal fluid (CSF).
Methods: A review of published articles on postmortem bacteriology. Studies were selected to cover the full range of necropsy practice including adults, the perinatal period, and infancy. The review covers over 5000 necropsies, mainly in adults, but including 1108 perinatal cases and 468 cases of sudden unexpected death in infancy. Data are available on 4992 blood cultures, 1168 specimens of CSF, and 743 cultures of spleen.
Results: Studies in which careful precautions have been taken to reduce contamination show that approximately two thirds of blood cultures are negative, two in nine yield a single isolate, and one in nine have a mixed growth. The postmortem interval has only a small effect on the isolation rate. A pure growth of a known pathogen has a more than 50% likelihood of being found in association with genuine infection in adults and in the perinatal period.
Conclusions: The main postmortem artefact is contamination, but this can be considerably reduced by careful technique. Agonal spread is less common than is often assumed. Postmortem translocation is not a problem if the body is appropriately stored. A pure growth of a pathogen in blood or CSF should be regarded as a possible contributing factor to death at all ages.

I t is difficult to determine the importance of bacteria isolated from a blood or cerebrospinal fluid (CSF) culture obtained at necropsy. In theory, there are several ways in which a positive result can arise.

(1) Genuine positive: bacteria invade in life and reach the target organ or fluid before death. The mere presence of an organism does not necessarily imply infection and disease because episodes of bacteraemia can occur in life without significant symptoms and without evidence of damage or inflammation. But if bacteraemia occurred before death and was present at death it would be considered to be at least a possible contributing factor. If bacteraemia were to occur in association with symptoms and signs of disease and inflammation then the presence of the organism would be accorded greater importance. A genuinely positive bacterial isolate will usually be a pure growth of a recognised pathogen.

(2) Agonal spread: bacteria invade during the process of dying or during the time when the circulation is artificially maintained by attempts at resuscitation. The concept is that the integrity of the mucosal surfaces is compromised by ischaemia and/or hypoxia, leading to invasion by several different bacterial species. In this case, bacterial invasion is not a cause of death but a consequence of death. The growth will probably be mixed and include both pathogens (potential) and commensals.

(3) Postmortem translocation: bacteria migrate from the mucosal surface into the blood and body tissues after death and after the circulation has ceased, but before the necropsy.

(4) Contamination: the bacteria are introduced into the blood or CSF or organ sample when it is obtained.

Processes 1, 3, and 4 can undoubtedly occur. Bacteria can invade and cause disease; they can also spread in the blood without causing disease; a dead body will putrefy at room temperature as a result of postmortem bacterial growth and tissue invasion; and contamination is a problem when cultures are obtained in life as in death.[1 2] However, the process of agonal spread is a theoretical concept, and it might or it might not occur. In the process of dying, the mucosal surfaces are rendered ischaemic and the performance of the immune defences is compromised; this could lead to increased bacterial invasion, but there is no direct evidence that this does in practice occur.

> ''The process of agonal spread is a theoretical concept, and it might or it might not occur''

Weinstein used a predictive model with multiple variables to assess the results of blood cultures obtained in life.[2] It was found that microorganism identity was an independent predictor. Microorganisms that nearly always ($> 90\%$) represent true bacteraemia or fungaemia include *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli*, other members of the Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Candida albicans*. In addition, *Streptococcus pyogenes, Streptococcus agalactiae, Listeria monocytogenes, Neisseria meningitidis, Neisseria gonorrhoea, Haemophilus influenzae*, members of the *Bacteroides fragilis* group, candida species other than *C albicans*, and *Cryptococcus neoformans* nearly always represent true infection. In contrast, corynebacterium species, bacillus species other than *Bacillus anthracis*, and *Propionibacterium acnes* rarely represent true bacteraemia. Coagulase negative staphylococci are also likely to be contaminants unless associated with catheters or vascular prostheses. Enterococci and the viridans group of streptococci can be found as contaminants and as part of a genuine bacteraemia.

Standards issued by the American Society of Microbiology indicate that the rate of blood culture contamination should not exceed 3%, but in practice most hospital contamination rates are in the order of 4–6%.[1] It is unlikely that

....

contamination rates as low as this can be achieved in necropsy practice.

In this article, we review several postmortem microbiological studies carried out over the past century. The aim is to try to assess the value of the results in diagnostic practice and to estimate the relative contribution of the above four different mechanisms of entry.

HISTORICAL REVIEW

In 1916, Fredette reported a series of 119 cases in which blood cultures were obtained within 30 minutes of death.[3] Blood was obtained from the median basilic vein using a capillary tube and cultured in plain serum broth. A careful aseptic technique combined with iodine to sterilise the skin minimised contamination. Postmortem dissection of the body was undertaken in all cases and Fredette's paper lists the cause of death and the results of blood culture for each case. Of the 119 cases, 77 had no growth on blood culture (65%), 31 cultures grew a single organism (26%), and 11 cultures produced two organisms (9%). In 56 cases the postmortem anatomical diagnosis indicated an infective cause of death; 31 of these cases had positive blood cultures and 25 had negative cultures. In the 63 cases in which the anatomical diagnosis indicated a non-infective cause, 11 cultures were positive and 52 were negative. From these figures, it can be calculated that a positive blood culture as a marker for an infective cause of death in this series had a sensitivity of 55%, a specificity of 83%, a positive predictive value (PPV) of 78%, and a negative predictive value (NPV) of 68%.

Fifteen patients died of lobar pneumonia, a diagnosis that can be made with confidence at necropsy. In seven of these patients, *S pneumoniae* was grown from the blood culture. There were 92 patients in whom the anatomical cause of death was not pneumonia and not meningitis; in two of these, *S pneumoniae* was isolated from the blood and in 90 it was not. Growth of *S pneumoniae* as a marker of lobar pneumonia in this series has a sensitivity of 47%, a specificity of 98%, a PPV of 78%, and an NPV of 92%. Furthermore, of the 15 patients with lobar pneumonia, four had organisms in the blood that were not expected and were apparently unrelated to the cause of death (*Streptococcus salivarius* in three and *Staphylococcus epidermidis* in one). Thus, in 11 of 15 there was no agonal invasion but in four of 15 some form of contamination had occurred.

Streptococcus pneumoniae was isolated from 14 patients; seven with lobar pneumonia, four with pneumonia, one with meningitis, and two others in which its presence did not correlate with the cause of death. The PPV is 86% (12 of 14 true positives).

Streptococcus pyogenes was isolated in nine cases, eight of which were judged to be true positives on the basis of the anatomical findings (PPV of 89%). *Staphylococcus aureus* was isolated in six cases, four of which appeared to be true positives (PPV of 67%). Other streptococci were isolated in 11 cases and four of these were possible true positives. Miscellaneous isolates included lactobacilli (two cases), *S epidermidis* (one case), *Corynebacterium pseudodiphtheriticum* (one case), *Salmonella typhi* (one case), and Gram negative bacillus (one case). With the exception of the *S typhi*, found in a case of typhoid fever, these organisms were probably false positives.

Giordano and Barnes undertook careful bacteriological studies before necropsy in 213 cases.[4] They paid scrupulous attention to technique to reduce the possibility of contamination when cultures were obtained. The surface of the appropriate tissue or organ was seared with a red hot spatula and then fluid or tissue was obtained using a pipette plunged through the sterilised area. Material was inoculated into a

tube of glucose broth for culture. Time from death to necropsy was one to 23 hours but most of the examinations were conducted in under 12 hours. Blood culture was positive in 80 of 206 cases (38%) and spleen culture was positive in 75 of 190 cases (39%).

Hunt and colleagues obtained blood for culture from the pulmonary artery in 567 postmortem cases.[5] The surface of the artery was sterilised by a hot spatula or a flame before obtaining the specimen using a sterile syringe and needle. The postmortem examinations were performed within one to 12 hours of death. One hundred and seventy three cultures were positive (31%).

Burn reported the results of studies in which samples of heart blood, lung, and spleen were obtained from over 136 postmortem cases.[6] He also emphasised the importance of taking stringent precautions to avoid contamination. The surface of the relevant organs was sterilised using a heated broad bladed spatula and then sterile scissors were used to obtain samples for culture. The age range was 0 to 90 years, and the interval between death and necropsy was one to 48 hours (recorded for 122 cases); 63 within one to four hours, 24 within five to eight hours, 15 within nine to 12 hours, and 20 within 13 to 48 hours. Heart blood cultures were positive in 49 of 134 cases (37%) yielding 68 isolates. These comprised staphylococci (13 isolates), *S pyogenes* (10 isolates), *S pneumoniae* (nine isolates), *E coli* (eight isolates), proteus group (eight isolates), α haemolytic streptococci (six isolates), non-haemolytic streptococci (four isolates), *Clostridium perfringens* (three isolates), *S typhi* (two isolates), *P aeruginosa* (two isolates), *H influenzae* (one isolate), and diphtheroids (one isolate). Spleen cultures were positive in 61 of 136 cases (45%) with a similar range of organisms. Lung cultures were positive in 107 of 121 cases (88%). The percentage of positive cultures did not increase with increasing time from death to necropsy.

''The proportion of positive blood samples increased with postmortem interval from 20% to 40% between 0 and 18 hours''

Burn also conducted experimental studies on postmortem bacterial invasion in guinea pigs and rabbits.[7] Bacteria were introduced into the pleural cavity; the animals were then maintained at 10℃, 25℃, or 37℃ for two to 96 hours before necropsy. Samples of heart blood, liver, spleen, and kidney were obtained using the same technique as in the human study. *Clostridium perfringens*, *E coli*, and staphylococci were capable of invading tissues from five to 48 hours after death if the animal was maintained at 25℃. A wide range of other pathogenic and non-pathogenic bacteria failed to translocate from the pleura to abdominal organs in these experiments.

Adelson and Kinney studied 126 cases of sudden unexpected death in infancy (SUDI; age range 10 days to 2 years) in which detailed postmortem examinations were conducted and specimens obtained for bacteriology.[8] Cultures of heart blood, spinal fluid, pharynx, ileum, and lungs were made in each case. Of 120 heart blood cultures, 95 (79%) were sterile, and the other 25 yielded a total of 32 organisms: *E coli* (15 isolates), *Enterobacter aerogenes* (six isolates), *S epidermidis* (five isolates), *S aureus* (four isolates), and α haemolytic streptococci (two isolates). The first 38 CSF specimens were sterile and the rest were not examined. They found no relation between the blood culture results and histological findings in the lungs or elsewhere.

Kurtin reported the results of a necropsy study of 50 patients aged 31 to 85 years.[9] He used a hot spatula to sterilise the surface of the heart, spleen, and lung before obtaining uncontaminated specimens of heart blood, lung, and splenic

tissue. The time from death to necropsy was: less than six hours in nine cases, six to 12 hours in five cases, 12 to 24 hours in 23 cases, and 24 to 48 hours in 13 cases. The heart blood was sterile in 80% and the spleen was sterile in 76%. There was no relation between time to necropsy and sterility. Fourteen of 17 patients with histological evidence of respiratory infection had negative blood and splenic cultures.

Carpenter and Wilkins undertook a retrospective review of 2033 necropsies carried out between 1955 and 1960 in which heart blood cultures and lung cultures had been obtained.[10] The technique involved searing the surface of the left ventricle with a soldering iron and then aspirating heart blood for culture. The surface of the lung was also seared in the same way and then a swab was inserted to obtain tissue. Sixty eight percent of the blood cultures were sterile, 25% yielded a single organism, and 7% yielded two or more organisms. The lung cultures were positive in 68%. Heart blood cultures were positive in 46% of paediatric cases, 37% of cases from surgery, 27% of medical cases, and 15% of patients found to be dead on arrival at hospital. The proportion of positive blood cultures increased with length of hospital stay; from 25% to 40% over a 25 day period (correlation coefficient, 0.77; $p < 0.01$). The proportion positive also increased with postmortem interval from 20% to 40% between 0 and 18 hours (correlation coefficient, 0.74; $p < 0.001$). Positive lung cultures also increased with length of hospital stay and postmortem interval.

"The introduction of tissue procurement centres with sterile morgue facilities allowed a new method of evaluating human postmortem microbiology"

Wood and colleagues reported 62 necropsy cases in which blood cultures were obtained.[11] The age range was 2 months to 90 years, with more than one third over 60 years. At necropsy, the pericardial sac was opened and the surface of the right atrium was seared with a red hot spatula; blood was then aspirated for culture. In this series, blood culture was positive in 44 of 62 cases; 31 cases yielded a single organism on culture, 11 yielded two or more organisms. There were 35 cases in which there was anatomical evidence of infection: 32 of these cases had positive cultures (true positives) and three had negative cultures (false negatives). In 27 cases there was no anatomical evidence of infection; 12 of these cases had positive cultures (false positives) and 15 had negative cultures (true negatives). From these figures one can calculate a sensitivity of 92%, a specificity of 56%, a PPV of 73%, and an NPV of 84%. The authors noted an effect of postmortem interval; the figure for true positives plus true negatives expressed as a percentage was over 83% in cases examined within 15 hours of death but 63% in those examined after 15 hours. The isolates were: *S aureus* (17 isolates; 13/four (13 judged to be true positives and four false positives)), *E coli* (11 isolates; 11/0), enterobacter (eight isolates; four/four), proteus (seven isolates; six/one), pseudomonas (six isolates; three/three), diphtheroids (three isolates; two/one), bacteroides (two isolates; 0/two), *S pneumoniae* (two isolates; one/one), and enterococcus (one isolate; 0/one). From the above, it appears that *S aureus*, *E coli*, and perhaps proteus were the most significant isolates in that they occurred proportionally more often in cases judged to have infection at necropsy.

A retrospective study of septicaemia in premature infants reported the findings in 256 infants with organisms grown from blood cultures.[12] This was from a total intake of 2906 infants of birth weight 1001–2500 g admitted to one hospital. There were 172 isolates that were judged to be significant;

this was based on a known pathogen isolated from more than one site and associated with the appropriate clinical features. In total, 129 isolates were judged to be contaminants; these were either non-pathogens, such as diphtheroids, lactobacilli, or α haemolytic streptococci, or pathogens isolated from a single flask and not associated with clinical evidence of infection. Septicaemia was diagnosed in 158 infants on the basis of blood culture results and the mortality rate was 50%. There were 98 infants with contaminated cultures and they had a mortality rate of 13.3%. The mortality rate was 6.6% in the 2650 infants with no culture taken or negative cultures. Staphylococci were isolated from 66 infants with septicaemia. There were 24 coagulase positive staphylococci (*S aureus*) and these patients had a mortality rate of 38%; the other 42 isolates were coagulase negative (*S epidermidis*) and the mortality rate was 9%. Gram negative rods (such as *E coli*, klebsiella, pseudomonas, and proteus) were isolated from 91 patients, with a mortality rate of 65%. There were 79 deaths from septicaemia; significant organisms were isolated from antemortem cultures alone in 15, from both antemortem and postmortem cultures in 26, and from postmortem cultures alone in 38. The calculated sensitivity of postmortem blood cultures for the diagnosis of septicaemia in this series is 81%. The authors reported the contamination rate in blood cultures obtained in life to be 4% (95% confidence interval, 3.2% to 4.8%).

The introduction of tissue procurement centres with "sterile morgue facilities" allowed a new method of evaluating human postmortem microbiology. O'Toole and colleagues reported their findings in 54 necropsy cases in which 440 tissue samples were obtained.[13] Cases were selected in which there was no premortem evidence of infection. An elaborate procedure was used to clean the skin of the corpse; the staff undertook a "surgical scrub" and "surgical gowning procedure"; the dissection was carried out in a room with controlled airflow; new sterile instruments were used to obtain each tissue sample. The samples were cultured in a range of media to detect both aerobic and anaerobic organisms. The interval from death to necropsy was under 20 hours. In 25 cases there was no growth in the samples obtained. In 29 cases there was growth in one or more sample, yielding 48 different isolates. Most isolates were considered to be external contaminants, such as diphtheroids, α haemolytic streptococci, and *S epidermidis* (26 isolates in total). Isolates thought possibly to be significant included *E coli* (three isolates), *S aureus* (three isolates), *C albicans* (two isolates), *E aerogenes* (two isolates), *P aeruginosa* (two isolates), enterococcus (one isolate), and *Shigella flexneri* (one isolate). There was no growth in 324 (74%) of the 440 samples obtained. Growth in the spleen occurred in 12 of 47 samples obtained; in six cases, the growth was judged to be significant because there was evidence at necropsy of infection. In two cases, the organisms were thought to be external or internal contaminants rather than a consequence of agonal spread. In the remaining four cases agonal spread was a possible explanation.

Minckler and colleagues reported their experience with the collection of tissue samples from 262 surgical operations and 213 necropsies.[14] The techniques of the "sterile necropsy" are as described in the preceding paragraph. A total of 263 tissue samples were obtained at surgery, but 114 were from the gastrointestinal tract, which has a resident flora, and others came from the skin and lung, which are also likely to be contaminated. There were 109 surgical samples of internal organs and 92 were sterile (84%). The equivalent organs obtained at necropsy totalled 738 and 485 were sterile (66%). There were 23 different organisms isolated, four (*E coli*, enterobacter, *S epidermidis*, and *S aureus*) of which accounted for 53.9%.

Roberts assessed the bacteriological results in 100 necropsies.[15] The surface of the relevant organ was sterilised by heat and then heart blood or tissue was obtained. The cultures of heart blood yielded no growth in 63%, in 22% the results were positive and judged to be indicative of genuine infection, and in 15% the results were positive but there was no evidence of infection. The corresponding results for the spleen were no growth in 77%, genuine infection in 19%, and false positives in 4%.

A study of 91 unselected necropsies on individuals aged 7 to 75 years conducted between one and 23 hours after death yielded 39% positive in heart blood cultures and 63% positive in lung cultures.[16] Once again, the surface of organs was sterilised by heat before the sample was obtained.

Dolan and colleagues obtained 211 tissue specimens from 67 necropsies.[17] The patients ranged from 5 to 88 years (mean, 55.1). The specimens were obtained using non-sterile instruments and sent to the laboratory where the capsular surfaces were seared by heat and then tissue was taken for culture. Bacteria or fungi were isolated from 58.3% of specimens. The spleen was positive in 23 of 53.

Wise cultured tissue from the spleen in 192 consecutive necropsies on individuals aged 31 to 91 years.[18] Twenty four of the 192 cultures (12.5%) yielded a growth of organisms: *E coli* in 11, *Proteus* sp. in five, *S aureus* in two, *S epidermidis* in one, *Streptococcus faecalis* in one, *P aeruginosa* in one, and mixed faecal organisms in three. The mean time from death to necropsy was over 24 hours. The splenic tissue was obtained after heat sterilisation of the splenic surface.

"Postmortem bacterial cultures proved useful in the diagnosis of systemic infection in neonatal deaths in a study by Eisenfield and colleagues"

Pryse-Davies and Hurley conducted a retrospective review of 835 perinatal necropsies performed between 1967 and 1976 in which there had been careful bacteriological assessment.[19] The series comprised 130 aborted fetuses over 500 g, 371 stillborn fetuses, 307 neonates dying in the 1st week of life, and 27 neonates dying later in the 1st month of life. A swab of heart blood was obtained from the right ventricle after heat sterilisation of the surface; swabs were obtained from the bronchi after opening the larynx and trachea with flamed scissors; and CSF was obtained by cisternal puncture after wiping the skin with 70% ethyl alcohol. In total, 479 CSF specimens were examined; 58 were positive (12%) yielding 69 isolates. Twenty one percent of 797 heart blood cultures were positive and 41% of 795 bronchial swabs yielded bacterial growth. There were 114 cases with evidence of disseminated bacterial infection; this was defined as a positive blood culture with the same organism at one or more other sites. The most common organisms were "pseudomonads" (33 isolates), *E coli* (24 isolates), staphylococci (14 isolates), *Klebsiella pneumoniae* (14 isolates), streptococci (13 isolates), *P aeruginosa* (five isolates), and *Proteus mirabilis* (three isolates). The 69 isolates from CSF included *E coli* (13 isolates), staphylococci (13 isolates), streptococci (12 isolates), pseudomonads (11 isolates), *K pneumoniae* (eight isolates), *Enterobacter cloacae* (three isolates), and *C perfringens* (two isolates). Varying degrees of histological evidence of meningitis were found in nine cases; in four babies meningitis was considered the primary cause of death. In 47 cases the CSF was positive but there were no histological changes of meningeal inflammation.

Postmortem bacterial cultures proved useful in the diagnosis of systemic infection in neonatal deaths in a study by Eisenfield and colleagues.[20] They obtained blood and CSF from 311 infants; in 293 cases the samples were obtained within two hours of death and 148 of these infants had also had blood and/or CSF cultures in life. In 18 infants only antemortem cultures were obtained. The blood cultures were positive in 38% (118 of 311 cases) and CSF cultures were positive in 16% (51 of 311 cases). In 73 cases, Gram negative organisms were isolated, in 32 streptococcal organisms, in 10 *S aureus*, and in 11 the isolates were mixed. The validity of the postmortem cultures was indicated by:

- Of 44 infants with bacteriological evidence of infection before death, postmortem cultures were also positive, despite antibiotic treatment, in 26. In 25 of the 26 the organism found at necropsy was the same as that found before death.
- In all 43 infants with both bacteraemia and meningitis bacteria isolated from both sites were identical.

Sonnabend and colleagues undertook a careful bacteriological study of 70 babies who suffered from SUDI, aged from 21 to 355 days.[21] Eighty percent were examined between two and 24 hours after death. Specimens for microbiology included heart blood, lung, liver, spleen, kidney, brain, and segments of small and large intestine. Eight of the 70 babies had evidence of overwhelming bacterial infection as the cause of death. This was based on the results of postmortem cultures. They commented that: "the postmortem cultures were of diagnostic value, providing the sole means of identifying the cause of death in eight of the 70 infants, in whom the presence of an infection could be established only after repeated and extensive histological investigations". The causative organisms (nine isolates) were *H influenzae* type b (two cases), *S pneumoniae* (two cases), *S aureus* (two cases), *E coli* (two cases), and group B streptococcus (one case).

A study of SUDI in Avon, UK, between 1987 and 1989, included bacteriological assessment of throat swabs in patients and a comparison group of healthy age and season matched normal infants.[22] Postmortem examinations were conducted on 95 babies affected by SUDI aged 1 week to 2 years. Throat swabs, blood for culture, and CSF were obtained within a median interval of 3.5 hours from the discovery of death (range, 0.25–46). Further tissue samples were obtained when the necropsy was conducted (median time, 25 hours; range, 2.8–73). The blood cultures yielded bacterial pathogens in eight of 95 cases. Spleen cultures were positive for pathogens in four of 94. The CSF was positive for pathogens in one of 95. It is not clear from the report how many cultures contained organisms considered to be contaminants. Comparison of the upper respiratory flora in babies with SUDI and healthy infants showed increased carriage of:

(1) *Staphylococcus aureus*; 24 of 95 in cases versus 12 of 190 (6%) in controls; odds ratio (OR), 5 (95% confidence interval (CI), 2.4 to 10).

(2) Coliforms, 15 of 95 in cases versus two of 190 (1%) in controls; OR, 29 (95% CI, 7.5 to 111).

(3) *Streptococcus pneumoniae*, 10 of 95 in cases versus two of 190 (1%) in controls; OR, 10 (95% CI, 2.9 to 34).

(4) Group B streptococcus, six of 95 in cases versus two of 190 (1%) in controls; OR, 11 (95% CI, 1.9 to 63).

Sadler stresses the value of bacteriological investigations in cases of SUDI.[23] He presents data on 95 necropsies of patients aged 3 days to 29 months, 63 of whom presented as "cot deaths". The protocol included obtaining CSF by cisternal puncture, blood from the subclavian vein, and lung tissue and spleen at necropsy. Nine diagnoses depended on bacteriology results: pneumococcal meningitis and septicaemia (two cases), pneumococcal meningitis (two cases),

meningococcal meningitis (two cases), group B streptococcal meningitis and septicaemia, *H influenzae* pneumonia and septicaemia, and *H influenzae* meningitis. Sixty one specimens of CSF were cultured: 35 showed no growth, six showed significant growth, and 24 grew organisms considered to be contaminants/commensals. Forty five specimens of blood were cultured and only nine showed no growth, five showed significant growth, and 31 grew contaminants/commensals. Twenty one specimens of spleen were examined, 13 had no growth, none had significant growth, and eight grew contaminants/commensals. The degree of contamination found in these specimens is clearly much higher than in the other studies noted above. The median time between death and necropsy was five hours.

The CESDI-SUDI study was a case control study of SUDI carried out in England between 1993 and 1996.[24] There were 456 cases of SUDI and four controls for each case. All cases of SUDI had a detailed necropsy, which included the examination of specimens for bacteriology. Blood cultures were recorded in 287 cases, and 144 (50%) were sterile. Mixed growth was obtained in 130 cases (45%). A major pathogen with corresponding histological changes was found in seven cases; in eight cases there was a single pathogen without corresponding histology. In the latter group the organisms were *S aureus* in three cases, group B β haemolytic streptococcus in three cases, and α haemolytic streptococcus in two cases. There were 279 cultures of CSF, 211 (76%) of which were sterile. There were seven positive cultures with corresponding histological changes in the brain. The organisms were *N meningitidis* (three cases), *H influenzae* type B, *E coli*, group A β haemolytic streptococcus, and *S epidermidis*. In a further 10 patients a single pathogenic organism was grown from CSF without corresponding histological changes in the brain. The organisms listed were *N meningitidis*, *S pneumoniae* (two cases), haemolytic streptococcus (three cases), and haemophilu*s* (two cases). In most of the last group, the organisms were grown from other sites and were associated with respiratory tract infection. In 51 other cases there was a mixed growth or single organisms considered to be contaminants. Thus, blood cultures yielded 15 possibly significant results (5.2%) and CSF cultures yielded 17 positives (6%).

BLOOD–BRAIN BARRIER

The blood–brain barrier was first defined by Ehrlich and his students.[25–27] Analine dyes, such as trypan blue, were injected intravenously into experimental animals. The animals were subsequently sacrificed and it was noted at necropsy that the tissues were stained blue, but this did not occur in the brain or CSF. The dyes combined with albumin in the bloodstream and would pass with albumin through the walls of peripheral capillary vessels, but the vessels in the brain formed a barrier. If trypan blue was injected into the CSF then the brain and CSF were stained blue at necropsy, the peripheral tissues were not.

The capillary endothelial cells in the brain have tight junctions, lack fenestrations, have a paucity of pinocytic vesicles, but contain increased numbers of mitochondria.[28] The tight junctions exclude large molecules such as albumin (molecular weight, 69 000). There are areas of the hypothalamus, the area postrema, and the subfornical and subcommisural organs where the endothelial cells lack tight junctions. This allows diffusion from the plasma to hypothalamic osmoreceptors and the chemoreceptors of the area postrema and the subfornical and subcommisural regions. However, there is free diffusion of molecules as large as inulin (molecular weight 5000) between the ventricular fluid and brain interstitial fluid.

Molecules can be transported across brain endothelial cells by pinocytosis, which is an active process. This can be studied in experimental animals using horseradish peroxidase, which is then visualised by electron microscopy. Hossmann and Olsson studied the effects of ischaemia on vascular permeability in the cat.[29] Evans blue and horseradish peroxidase were injected intravenously and localised by fluorescence and electron microscopy, respectively. Acute complete cerebral ischaemia produced by arterial ligation for 15 minutes to three hours did not lead to extravasation of the tracers. The tight junctions were maintained and transfer of horseradish peroxidase by pinocytosis ceased.

In inflammation, white blood cells cross the endothelial cells by emperipolesis, but large molecules cross by a combination of pinocytosis and or leakage through opening of the tight junctions.[27] The result is that the composition of the CSF in meningitis is closer to that of plasma.

Changes in the composition of body fluids, including CSF and vitreous humour, after death have been reviewed by Coe.[30] In general, total proteins and the albumin to globulin ratio are in the ranges found in life. Thus, after death the blood–brain barrier is maintained, at least in relation to large molecules. Mangin and colleagues obtained samples of CSF within 24 hours of death (three to 24 hours) in 42 necropsies on patients aged 5 to 74 years.[31] The CSF protein was within the normal range in a group of 15 who died suddenly (the agonal period was less than 10 minutes). If death was prolonged over six hours, as in those in intensive care, then the mean CSF protein concentration was raised to 8.3 g/litre. This is presumably a result of circulating cytokines in life causing increased permeability of the brain capillaries.

> "After death the blood–brain barrier is maintained, at least in relation to large molecules"

A change that does occur in CSF composition after death is that the white blood cell count rises.[32 33] The CSF cell count ranged in adults from 1 to 108 $\times 10^6$ cells/litre and in cases of sudden infant death syndrome from 37 to 3250 $\times 10^6$ cells/litre.[32] The cell count rises with time after death; the cells are mononuclear and can be typed as lymphocytes or monocytes in the first 12 hours, but thereafter typing and morphological assessment is no longer reliable. Polymorphs are not seen in the absence of inflammation. The postmortem CSF pleocytosis is probably a result of detachment of the meningeal lining cells.

DISCUSSION

Many of the studies described above used stringent precautions to reduce the possibility of contamination, but it cannot be avoided completely. Wise sterilised the surface of the spleen at necropsy before obtaining a specimen and found only 12.5% (95% CI, 7.5% to 17.5%) positive cultures in 192 cases with a long postmortem interval.[18] Dolan and colleagues by comparison grew bacteria from 23 of 53 (43%; 95% CI, 26% to 61%) splenic samples.[17] The difference was that in Dolan's study the specimens were obtained using non-sterile instruments and the surfaces were subsequently sterilised in the laboratory. This difference is presumably the result of an increased rate of contamination because there is no reason to suppose an increased rate of infection, agonal change, or postmortem translocation in the second study. Even with blood cultures taken in life and with the most careful techniques contamination is always a potential problem.[1 2 34] Buetow and colleagues estimated the contamination rate in over 2000 blood cultures obtained in life as 4% (95% CI, 3.2% to 4.8%).[12]

Table 1 Studies in which stringent precautions were taken to reduce contamination

First author	Date	Age range of patients	Postmortem interval	Sample	N	No growth	Single isolate	Mixed growth
Fredette [3]	1916	4 months to 84 years	<30 min	Blood	119	65%	26%	9%
Giordano [4]	1922	Adults	1–23 h	Blood	206	62%		
				Spleen	190	61%		
Hunt [5]	1929	Adults	1–12 h	Blood	567	69%		
Burn [6]	1934	0–90 years	1–48 h, <12 h in 84%	Blood	134	63%	22%	15%
				Spleen	136	55%		
Adelson [8]	1956	10 days to 24 months		Blood	120	79%		
				CSF	38	100%		
Kurtin [9]	1958	31–85 years	1–48 h, <24 h in 74%	Blood	50	80%		
				Spleen	50	76%		
Carpenter [10]	1964	0–90 years	1–18 h	Blood	2033	68%	25%	7%
Wood [11]	1965	0–90 years	1–48 h <15 h in 65%	Blood	62	29%	50%	21%
O'Toole [13]	1965	Adults	<20 h	Spleen	54	74%		
Minckler [14]	1966	1–90 years	<24 h	Various solid organs	738	66%		
Roberts [15]	1969	Adults		Blood	100	63%		
				Spleen	100	77%		
Koneman [16]	1971	7–75 years	1–23 h	Blood	91	61%		
Wise [18]	1976	31–91 years	Mean 24 h	Spleen	192	87.5%	11%	1.5%
Pryse-Davies [19]	1979	Perinatal, <1 month	37% <24 h, 28% >48 h	Blood	797	79%	17%	4%
				CSF	479	88%	10%	2%
Eisenfield [20]	1983	Neonatal, <1 month	<2 h in 293	Blood	311	62%		
				CSF	311	84%		
Sonnabend*[21]	1985	21–355 days	80% <24 h	Blood, liver, brain, spleen	70	89%	10%	1%
Sadler [23]	1998	0–2 years	Median 5 h	Blood	45	20%		
				CSF	61	57%		
				Spleen	21	62%		
CESDI-SUDI [24]	2000	7–365 days	28% in <24 h, 33% in <48 h	Blood	287	50%	5%	45%
				CSF	279	76%	3%	21%

*The isolation rate is artificially low because not all contaminants are listed.

Studies in which stringent precautions have been taken to reduce contamination show that most blood cultures are sterile (table 1), so that agonal invasion of the bloodstream does not occur as a universal phenomenon, at least in numbers sufficient to produce a positive result.

In the largest series reported by Carpenter and Wilkins[10] blood cultures were negative in 68% (95% CI, 64% to 72%). Results from most of the other studies are in a similar range, and it appears that in adult necropsy practice around two thirds of blood cultures and splenic cultures will be negative if the specimens are taken with care. Carpenter and Wilkins also found a single isolate in 25% (95% CI, 23% to 28%) and mixed growth in 7% (95% CI, 6% to 8%). If mixed growth is equated with the three postmortem artefacts of agonal spread, postmortem translocation, and contamination—and if contamination at necropsy is similar in magnitude to the 4–6% found in life—then agonal change is a rare event. The study by Wise also indicates that agonal spread is rare because he found mixed growth in only 1.5% of cases.[18] Thus, the concept that the entry of several bacterial species at the time of death is inevitable is incorrect. If it occurs at all it is rare. None of the studies deals specifically with the relation between length of resuscitation and isolation rates, but many of the cases in the later studies will have had the agonal phase prolonged for varying intervals by modern resuscitation methods. The rest of the results in the table are broadly in line with the above, although there are exceptions.[11] In the series of Carpenter and Wilkins most single isolates were judged to be causing genuine and significant disease.[10] A minority could represent bacteraemia not causing disease, and some of these episodes could have arisen close to the time of death. Thus, it is possible that a small proportion of episodes of bacteraemia arise in the agonal period, but this is different to the usual concept of agonal spread.

The postmortem interval has only a small effect on blood culture isolation rates. Some of the authors noted no effect,[6 9 19] and some only a small effect,[10 11] but only the

study by Carpenter and Wilkins had sufficient statistical power to answer the question. The authors found that there was a significant increase in isolation rate with postmortem interval, and the best fit regression line fell from 80% sterility to 60% sterility over 18 hours. This observation does not necessarily support the idea of postmortem translocation because it is possible that a small number of organisms present in the bloodstream at the time of death could continue to grow, so that the chance of a positive culture increases with time.

> "The concept that the entry of several bacterial species at the time of death is inevitable is incorrect"

In general, a pure growth of a pathogen in a blood culture is probably associated with genuine infection, but in many of the reports it is not possible to enumerate the relative proportion of true positives and false positives. The report by Fredette is an exception, because he lists the cause of death and the blood culture results for each patient.[3] Some of the causes given are unusual according to modern concepts, but this is the era when lobar pneumonia and puerperal fever killed otherwise young healthy people, and there is good correspondence between the results of culture and the anatomical diagnoses. In this study the isolation of *S pneumoniae, S pyogenes*, and *S aureus* in general indicate significant infection. It is equally important to note that even when a confident diagnosis of lobar pneumonia was made in the era before antibiotic treatment, *S pneumoniae* was isolated in less than 50% of cases in blood taken within 30 minutes of death. Clearly, there are false negative and false positive results. Several authors have tried to correlate antemortem and postmortem blood culture results in an attempt to assess the veracity of the latter.[3 11 12 15 20] There is a degree of concordance in these studies, but if the results differ it does not necessarily imply that the postmortem result is

unreliable; equally, if the results are concordant this does not prove that the postmortem result is correct.

The value of a pathological test is best expressed in terms of sensitivity and specificity. This then allows the NPV and PPV values to be calculated at different levels of a priori probability. We have made calculations where possible of these parameters and they are listed in the historical review. The problem is that there is too much uncertainty concerning the anatomical diagnosis of infection and the number of cases in most studies is too small for reliable estimates. It would be best to calculate sensitivity and specificity for specific bacterial isolates as markers for specific conditions (for example, pneumococcus as a marker for lobar pneumonia), but in general this has not proved possible.

The postmortem bacteriological results in the perinatal and neonatal period are similar to those in adults. Pryse-Davies and Hurley obtained blood cultures in 797 infants in the perinatal age range.[19] There was a single isolate in 17% (95% CI, 14.4% to 20.2%), mixed growth in 4% (95% CI, 2.4% to 5.1%), and sterile cultures in 79% (95% CI, 72.7% to 85.1%). The clinical features were in keeping with the rule that most single isolates of pathogens (> 50%) occurred in association with genuine infection. These results were obtained with standard necropsy practice in which only one third of cases were examined in less than 24 hours. Cultures of CSF yielded mixed growth in 2% (95% CI, 0.9% to 3.6%), a pure culture in 10% (95% CI, 7% to 12.6%), and sterile cultures in 88% (95% CI, 79.5% to 96.3%). Once again, single isolates of known pathogens were concordant with genuine infection. The very low level of mixed isolates in the CSF is probably secondary to contamination, and therefore these results challenge the concept that agonal spread of organisms from the mucosal surface through the blood to the CSF can occur. Eisenfield and colleagues also found a high rate of sterility in CSF samples from 311 neonates.[20] Positive cultures were obtained in 51 of 311 cases (16.4%; 95% CI, 11.9% to 20.9%). Evidence suggested that in at least 43 of the 51 positive cases there was genuine CSF infection; thus, the level of postmortem artefact is close to the minimum level for contamination, and this argues against agonal spread to the CSF.

Interpretation of blood and CSF culture results is of particular importance in SUDI and sudden infant death syndrome. It has long been suspected that disseminated or overwhelming infection has a role in the pathogenesis of sudden death in infancy and microbiological investigation is a standard part of the necropsy protocol.[24] If the specimens are taken with care, most blood and CSF cultures are negative.[8 21] In the few that are positive and indicate genuine infection the diagnosis can often be made with histological examination alone. This has led many workers to become disenchanted with bacteriological investigation and they either neglect to obtain the samples,[24] or take them without adequate care. The result is many contaminated cultures and the view has developed that postmortem microbiology is of little value and the results are difficult to interpret. This can be seen by comparing the level of contaminants in Adelson and Kinney's 1956 study,[8] with that of Sadler in 1998,[23] and the CESDI-SUDI study published in 2000.[24] Adelson and Kinney had no growth in 79% (95% CI, 63.3% to 95%) of 120 blood cultures. The postmortem interval is not stated but is probably over 24 hours in many of the cases. The results indicate that if agonal spread and/or postmortem translocation occur then it can only be in a minority. However, the authors were unsure of the relevance of the positive isolates and this remains the problem in SUDI and sudden infant death syndrome. Is a pure growth of E coli or of S aureus the cause of death, do they indicate bacteraemia unrelated to death, or are they postmortem artefacts? The studies in adults and in neonates suggest that few of the results are

postmortem artefacts and most represent episodes of bacteraemia that may or may not contribute to death. The authors had no growth in 38 CSF samples (0%; 95% CI, 0% to 8%). This result also indicates that agonal spread and/or postmortem translocation from the mucosal surface to the CSF probably does not occur.

Sonnabend and colleagues also undertook a careful bacteriological study of SUDI. They report their results in a different way to most of the other investigations and it is not clear how many cultures had contaminants. The authors were prepared to diagnose the cause of death on the basis of the microbiological results alone. Their assessment depended on the nature of the organism grown and the number of positive sites. Eight of 70 patients were judged to have disseminated bacterial infection. Sadler was also prepared to diagnose the cause of death in SUDI on the basis of a significant pathogen isolated at necropsy in the absence of histological confirmation.[23]

''Interpretation of blood and cerebrospinal fluid culture results is of particular importance in sudden unexplained death in infancy and sudden infant death syndrome''

The study of SUDI by Gilbert et al repays careful analysis.[22] The authors found increased throat carriage of S aureus (OR, 5), coliforms (OR, 29), and group B streptococci (OR, 11) in a comparison with normal healthy age matched infants. In epidemiological studies with the requisite statistical power, ORs of this level would normally be judged as possible causative factors. However, in this case, the authors were cautious because of the possibility of postmortem artefact. In simple terms, they did not trust the microbiological results obtained after death because of the possibility that the bacteria had in some way been introduced during the interval between death and obtaining the specimen. A mechanism by which this could occur, however, is not clear. Harrison and colleagues in a later study found a very low isolation rate of coliforms and other Gram negative bacilli in the nasopharynx of normal healthy live infants aged 0–6 months.[35] Most of these infants slept supine at this age. In later months, many of the infants slept prone; pernasal swabs obtained in the early morning from infants sleeping prone who had a clinical upper respiratory tract infection often contained coliforms and other Gram negative bacilli. These organisms appeared to grow up overnight in secretions pooling in the upper airways when the infants were lying in the prone position. The bacteria disappeared from swabs obtained later in the day when the infants were up and awake and had swallowed the secretions. Thus, the association between coliforms in the upper airways and SUDI is not a postmortem artefact but could be a premortem artefact. The comparison in the study by Gilbert et al was in fact between the early morning upper respiratory tract flora of infants who had been sleeping prone (prone sleeping was prevalent at the time of the study) and had an increased incidence of clinical upper respiratory tract infection compared with normal infants later in the day who had a lower incidence of clinical upper respiratory tract infection. One cannot conclude that the bacterial isolates from the throat swabs are causally linked to SUDI, but ORs of 5, 11, and 29 are usually taken seriously, and they should be regarded as at least candidate organisms for a causal role. The association is biologically plausible because some of the species isolated can produce potent toxins (for example, pyogenic toxins of S aureus and soluble toxins of E coli) that could contribute to death by means similar to those observed in toxic shock syndrome.

There is a barrier between the blood and the brain preventing the egress of large molecules, such as albumin,

and red and white blood cells. The same barrier reduces the chance of circulating bacteria entering the CSF and brain tissue. Thus, even if there is agonal invasion of the body by bacteria, there is a further barrier to invasion of the brain, and in practice it would appear that CSF cultures are reliable if contamination can be avoided. The chance that bacterial isolates are the result of agonal spread or postmortem translocation is low. The examination of CSF for bacterial infection in life includes not only culture but also differential cell counts and the measurement of total protein.[27] The cell count alone is not useful in postmortem CSF samples because mononuclear cells occur normally in the CSF after death,[30 31] but polymorphs indicate inflammation and should be counted. The problem is that the half life of polymorphs in the CSF is short and unless the postmortem interval is also short they are unlikely to be seen. The measurement of CSF protein might help in interpretation because there is evidence that the blood–brain barrier is maintained for up to 24 hours after death.[31]

Our analysis shows that if postmortem cultures are taken with care, most specimens of blood and CSF will be negative, and a substantial proportion of those that are positive will be the result of genuine infection. This applies in the perinatal period, in infancy, and in adults. Establishing that the isolate represents genuine infection is not easy. One useful rule is that a pure culture of a pathogen points to infection, whereas a mixed culture of non-pathogens is more likely to be some form of postmortem artefact. This rule is useful, but is not an absolute indicator, and more corroborative evidence is required. Examination of a CSF specimen taken soon after death would be one form of corroboration, as indicated above. In life, bacteria are cleared from the bloodstream by a variety of processes, including phagocytosis, and this can lead to release of bacterial DNA and RNA. The polymerase chain reaction could then be used to amplify bacterial ribosomal RNA in blood and CSF. Genuine infection, in theory, is more likely to be accompanied by free bacterial specific RNA than is growth secondary to postmortem artefact.[36] The potential of this methodology should be investigated.

CONCLUSIONS

The concept that in the agonal period bacteria of several different species are liable to spread from the mucosal surface into the bloodstream producing mixed cultures is incorrect. Agonal spread in this sense into the blood is rare and into the CSF is very rare. The possibility that a single bacterial species invades in the agonal period is not excluded, but this will depend on factors relating to that organism, such as propensity to invade and density of colonisation, or to local factors affecting its site of colonisation, but not to a general diminution of body defence or a general impairment of mucosal integrity.

Postmortem translocation is only a minor problem if samples are obtained within 24 hours of death, or if the body is maintained at 4°C before necropsy.

The major problem with postmortem samples is contamination, but this can be reduced to levels similar to those for samples obtained in life if stringent precautions are taken.

The importance of a single isolate in blood or in CSF should be analysed as it would be in life. In most cases, a pure growth of a recognised pathogen will be associated with genuine infection confirmed from the clinical picture and by histological changes in tissues. However, the presence of a pathogen in the blood does not necessarily indicate significant disease—this is true in life and presumably also applies in death—but if an episode of bacteraemia occurs before death in a patient in whom the cause of death is otherwise unascertained then the organism should be regarded as at least a possible contributing factor.

Take home messages

- Postmortem bacterial cultures can be useful in assessing premortem bacterial invasion
- The main postmortem artefact is contamination, but this can be considerably reduced by careful technique
- Agonal spread appears to be less common than is normally assumed
- Postmortem translocation is not a problem if the body is appropriately stored
- A pure growth of a pathogen in blood or cerebrospinal fluid should be regarded as a possible contributing factor to death at all ages, but corroborative evidence should be sought using a range of techniques

Confirmation of the significance of an isolate should be sought. This involves the analysis of the clinical history and examination of tissues for evidence of inflammation. In addition, CSF samples should be examined—as in life—with cell count, differential, and protein estimation; however, this will only be of value if the sample is obtained soon after death. Modern molecular techniques should also be used to aid diagnosis. If a bacterial isolate has caused significant disease there will probably be dead bacteria and live bacteria in the circulation, and this can be ascertained by amplifying bacteria specific ribosomal RNA using the polymerase chain reaction.

> "Postmortem microbiology has most to offer when death is otherwise unascertained, but interpretation is at its most difficult in these cases"

Our literature review has been mainly concerned with systemic invasion by bacteria, but the principle that postmortem samples are useful and should be analysed also applies to postmortem mucosal samples. The mucosal flora at necropsy can be compared with community controls and the publication by Gilbert *et al* indicates how useful that approach can be.[22]

Postmortem microbiology has most to offer when death is otherwise unascertained, but interpretation is at its most difficult in these cases. SUDI is a particular problem in that if death is rapid inflammation might not be apparent to corroborate a bacterial isolate. In these cases, samples need to be taken as soon after death as possible and before necropsy. They should be obtained with a full aseptic technique, as in life. The CSF should be submitted for urgent examination as it would be in life. Modern molecular techniques should be used to look for bacterial toxins in blood and CSF and the polymerase chain reaction should be used to detect bacteria specific ribosomal RNA.

. . . .
Authors' affiliations
J A Morris, L M Harrison, Department of Pathology, Royal Lancaster Infirmary, Lancaster, LA1 4RP, UK
S M Partridge, Department of Pathology, Furness General Hospital, Barrow in Furness, LA14 4LF, UK

REFERENCES

1 **Weinbaum FI**, Lavie S, Danek M, *et al*. Doing it right the first time: quality improvement and the contaminant blood culture. *J Clin Microbiol* 1997;**35**:563–5.
2 **Weinstein MP**. Blood culture contamination: persisting problems and partial progress. *J Clin Microbiol* 2003;**41**:2275–8.

3 **Fredette JW**. Bacteraemia in the agonal period. *J Lab Clin Med* 1916;**2**:180–8.
4 **Giordano AS**, Barnes AR. Studies in postmortem bacteriology: value and importance of cultures made postmortem. *J Lab Clin Med* 1922;**7**:538–46.
5 **Hunt HF**, Barrow E, Thompson, *et al*. A bacteriological study of 567 post-mortem examinations. *J Lab Clin Med* 1929;**14**:907–12.
6 **Burn CG**. Postmortem bacteriology. *J Infect Dis* 1934;**54**:395–403.
7 **Burn CG**. Experimental studies of postmortem bacterial invasion in animals. *J Infect Dis* 1933;**54**:388–94.
8 **Adelson L**, Kinney ER. Sudden and unexpected death in infancy and childhood. *Pediatrics* 1956;**17**:663–99.
9 **Kurtin JJ**. Studies in autopsy bacteriology. *Am J Clin Pathol* 1958;**30**:239–43.
10 **Carpenter HM**, Wilkins RM. Autopsy bacteriology: review of 2033 cases. *Arch Pathol* 1964;**77**:73–81.
11 **Wood WH**, Oldstone M, Schultz RB. A re-evaluation of blood culture as an autopsy procedure. *Am J Clin Pathol* 1965;**43**:241–7.
12 **Buetow KC**, Klein SW, Lane RB. Septicaemia in premature infants. The characteristics, treatment and prevention of septicaemia in premature infants. *Am J Dis Child* 1965;**110**:29–41.
13 **O'Toole WF**, Saxena HM, Golden A, *et al*. Studies of postmortem microbiology using sterile autopsy technique. *Arch Pathol* 1965;**80**:540–7.
14 **Minckler TM**, Newell GR, O'Toole WF, *et al*. Microbiology experience in collection of human tissue. *Am J Clin Pathol* 1966;**45**:85–92.
15 **Roberts FJ**. A review of postmortem bacteriological cultures. *Can Med Assoc J* 1969;**100**:70–4.
16 **Koneman EW**, Minckler TM, Shires DB, *et al*. Postmortem microbiology II. Selection of cases for culture. *Am J Clin Pathol* 1971;**55**:17–23.
17 **Dolan CT**, Brown AL, Ritts RE. Microbiological examination of postmortem tissues. *Arch Pathol* 1971;**92**:206–11.
18 **Wise R**. The ''septic spleen''—a critical evaluation. *J Clin Pathol* 1976;**29**:228–30.
19 **Pryse-Davies J**, Hurley R. Infections and perinatal mortality. *J Antimicrob Chemother* 1979;**5**(suppl A):59–70.
20 **Eisenfield L**, Ermocilla R, Wirtschafter D, *et al*. Systemic bacterial infections in neonatal deaths. *Am J Dis Child* 1983;**137**:645–49.
21 **Sonnabend OA**, Sonnabend WF, Krech U, *et al*. Continuous microbiological and pathological study of 70 sudden and unexpected infant deaths: toxigenic intestinal Clostridium botulinum infection in 9 cases of sudden infant death syndrome. *Lancet* 1985;**1**:237–40.
22 **Gilbert R**, Rudd P, Berry PJ, *et al*. Combined effect of infection and heavy wrapping on the risk of sudden unexpected infant death. *Arch Dis Child* 1992;**67**:171–7.
23 **Sadler DW**. The value of a thorough protocol in the investigation of sudden infant deaths. *J Clin Pathol* 1998;**51**:689–94.
24 **Fleming P**, Bacon C, Blair P, *et al*. Sudden unexpected deaths in infancy. In: *The CESDI-SUDI studies 1993–1996*. London: The Stationery Office, 2000:97–112.
25 **Ehrlich P**. *Das Sauerstoffbeduerfnis des Organismus. Eine farbenanalytische Studie*. Berlin: A Hirschfeld, 1885.
26 **Friedmann U**. Blood–brain barrier. *Physiol Rev* 1942;**22**:125–45.
27 **Fishman RA**. Blood–brain barrier. In: *Cerebrospinal fluid in diseases of the nervous system*. London: WB Saunders Company, 1980:43–69.
28 **Oldendorf WH**, Cornford ME, Brown WJ. The large apparent work capability of the blood–brain barrier: a study of the mitochondrial content of capillary endothelial cells in brain and other tissues of the rat. *Ann Neurol* 1977;**1**:409–17.
29 **Hossmann KA**, Olsson Y. The effect of transient cerebral ischaemia on the vascular permeability to protein tracers. *Acta Neuropathol* 1971;**18**:103–12.
30 **Coe JI**. Post-mortem chemistry update. Emphasis on forensic applications. *Am J Forensic Med Pathol* 1993;**14**:91–117.
31 **Mangin P**, Lugnier AA, Chaumont AJ, *et al*. Forensic significance of postmortem estimation of the blood cerebrospinal fluid barrier permeability. *Forensic Sci Int* 1983;**22**:143–9.
32 **Platt MS**, McClure S, Clarke R, *et al*. Post-mortem cerebrospinal fluid pleocytosis. *Am J Forensic Med Pathol* 1989;**10**:209–12.
33 **Wyler D**, Marty W, Bar W. Correlation between the post-mortem cell content of cerebrospinal fluid and time of death. *Int J Legal Med* 1994;**106**:194–9.
34 **Libman E**. On some experience with blood cultures in the study of bacterial infections. *John Hopkins Hospital Bulletin* 1906;**17**:215–18.
35 **Harrison LM**, Morris JA, Telford DR, *et al*. The nasopharyngeal bacterial flora in infancy: effect of age, season, viral upper respiratory infection and sleeping position. *FEMS Immunol Med Microbiol* 1999;**25**:19–28.
36 **Peters RP**, van Agtmael MA, Danner SA, *et al*. New developments in the diagnosis of blood stream infections. *Lancet Infect Dis* 2004;**4**:751–60.