

Letters to the Editor

Immunohistological demonstration of *Salmonella virchow* in a case of infant death

Salmonella virchow is the third commonest serotype in England and Wales after *Salmonella typhimurium* and *Salmonella enteritidis*. At the Oxford Public Health Laboratory in 1987 *S virchow* accounted for 16 of 206 (8%) salmonella stool isolates but for three of seven (43%) cases of invasive salmonellosis. In England and Wales in 1987 *S virchow* accounted for 7% of all salmonella faecal isolates but for 20% of salmonella blood culture isolates (personal communications, PHLS Communicable Disease Surveillance Centre).

A case of invasive salmonellosis in which *S virchow* was isolated from necropsy specimens is reported. The diagnosis was confirmed by applying the immunoperoxidase technique to tissue sections. A positive control was prepared by infecting a murine macrophage cell line with the *S virchow* isolated from the necropsy samples; the cells were then harvested, pelleted, and routinely processed through formalin fixation and paraffin wax embedding. A standard indirect immunoperoxidase method was used with rabbit anti-salmonella 06:7 (Central Public Health Laboratory, Colindale) diluted 1/320 as the primary antibody, and peroxidase-conjugated sheep anti-rabbit IgG (Serotec, Kidlington) diluted 1/40 as the second stage antibody. These dilutions were determined after checkerboard titration on the positive control. The reaction was developed using diaminobenzidine. Sections of the liver, heart, and middle ear were examined. Appropriate negative controls were prepared by omitting the primary or secondary antibody.

A four month old boy was seen by his general practitioner because of a gastrointestinal illness and an oral rehydration preparation was prescribed. Three days later he appeared in satisfactory condition when seen by a health visitor. He was found dead unexpectedly 10 days after the general practitioner's visit. Postmortem examination showed a well nourished, normally developed infant with some reddening in the nappy area. The only abnormal findings were a slightly reddened tracheal lining, scattered petechial haemorrhages over the pulmonary visceral pleura, and pus in the left middle ear. Gut contents, pus from the left middle ear, and cerebrospinal fluid were cultured and *S virchow* phage type 8 was

isolated from all three samples. Histological examination showed focal myocarditis and focal hepatic microabscesses with a predominantly chronic inflammatory infiltrate. No inflammatory changes of the brain or leptomeninges were seen and this, together with the absence of macroscopic abnormalities, ruled out the possibility of meningitis. The cerebrospinal fluid sample was blood-stained and contamination from the skin flora or from the blood might have occurred. Gram stains of heart, liver, and middle ear sections showed no organisms. With the immunoperoxidase method bacilli were clearly shown in the periportal sinusoidal spaces of the liver (figure) but were not seen in the heart or middle ear.

Non-typhoidal salmonellae are a rare cause of hepatic abscess, infective cholecys-

titis, and subphrenic abscess. Billingham and Slack reported a case of cholecystitis complicated by subphrenic abscess caused by *S virchow*.¹ In the case we describe, although multiple microabscesses were seen in the liver and, with an immunoperoxidase method, we showed the presence of *S virchow* in some of the periportal spaces, no abnormality of the biliary tract was seen at necropsy.

Myocarditis is known to be a possible complication of *S typhi* infections, but it can also occur in association with non-typhoidal salmonellosis. Taha and Peden reported development of congestive heart failure during *S virchow* septicaemia.² Shilkin showed intracellular Gram negative bacilli in a case of *S typhimurium* pancarditis,³ whereas in other reported cases of myocarditis the Gram stain was either negative or

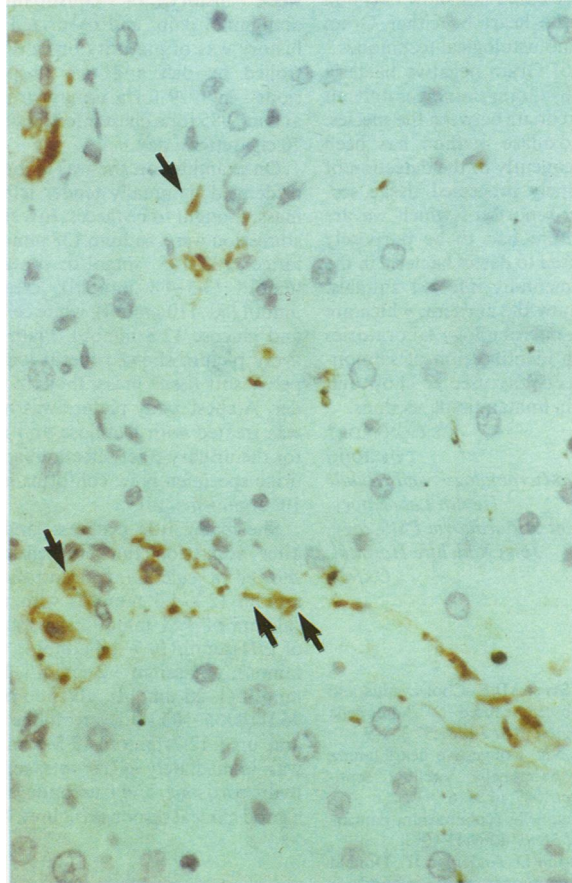


Figure Immunoperoxidase preparation showing labelling of bacilli (arrowed) and bacterial antigen within Kupffer cells in sinusoidal spaces. (Immunoperoxidase with light nuclear counterstain.)

not attempted. We could not show bacilli in our sections of the heart by either Gram staining or immunohistological techniques.

Identification of Gram negative bacteria in tissue sections by Gram staining is difficult and can not differentiate between the species. The immunoperoxidase method has been applied very infrequently to the detection of bacteria in routinely processed tissue sections. In the few reports of which we are aware,^{4,5} the antisera had to be purposely raised and were used to detect bacteria in the tissues of experimentally infected animals. We have shown how the antisera, which are routinely used in microbiology laboratories for the serogroup identification of salmonellae, can be successfully used to show and identify bacteria in human tissue sections.

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Retroperitoneal myxoid liposarcoma presenting with hypercalcaemia

Hypercalcaemia can be an initial presentation of many benign and malignant diseases.¹ Soft tissue tumours can occasionally present with biochemical abnormalities. We present here a case of pelvic liposarcoma presenting with clinical hypercalcaemia in the absence of bony metastases or renal impairment.

A 72 year old woman presented with a five

week history of constipation, vague abdominal pain, and dysuria. Her medical history was of maturity onset diabetes controlled by diet and two hospital admissions—in 1979 for a urinary tract infection and in 1985 for a chest infection. She smoked 20 cigarettes a day.

On examination she was afebrile, with an ill defined minimally tender left iliac fossa mass, thought to be faeces. Investigations on admission were: sodium 138 mmol/l (normal range 132-145 mmol/l); potassium 3.2 mmol/l (3.3-4.8 mmol/l); creatinine 122 µmol/l (60-110 µmol/l); white cell count 16.1 and glucose 12.8 mmol/l. Plain abdominal x-ray picture showed faecal loading and a pelvic soft tissue mass, thought to be bladder. A chest x-ray picture was normal. She was treated with lactulose and amoxycillin for the urinary tract infection (a mid-stream urine specimen grew coliforms greater than 10⁵ organisms/ml).

She made little progress, and four days after admission full biochemical analysis showed a calcium concentration of 3.48 mmol/l (2.1-2.6 mmol/l) with a corrected calcium of 3.51 mmol/l, phosphate activity of 1.04 mmol/l (0.7-1.4 mmol/l), sodium 143 mmol/l, potassium 3.2 mmol/l, bilirubin 10 µmol/l (1-20 µmol/l), alkaline phosphatase 96 IU/l (35-105 IU/l), creatinine 142 µmol/l, and urea 12.7 mmol/l (2.5-8 mmol/l). She was immediately given intravenous fluids, hydrocortisone, and frusemide; she achieved a good clinical response (figure). A bone scan

showed no metastatic disease but an intravenous urogram showed a pronounced bilateral hydro-ureter and hydronephrosis, with a poor bladder outline, consistent with an obstructive uropathy. Pelvic malignancy, probably of gynaecological origin, was diagnosed.

The response to treatment confirmed the suspicion of malignant hypercalcaemia, and due to the good clinical response the anti-hypercalcaemic treatment was withdrawn. Her condition deteriorated, however, mirroring a rise in the serum calcium concentration to 3.58 mmol/l. Despite an initial response to intravenous fluids, steroids, frusemide and calcitonin, she died 21 days after admission.

Other investigations performed were a normal blood film and marrow smear, normal immunoglobulins and a parathyroid hormone concentration of 0.3 (0.2-0.7 µg/l), with a serum calcium concentration of 3.22 mmol/l.

At necropsy there was a diffusely infiltrating pelvic tumour affecting the posterior pelvic wall, bladder, uterus and cervix, with two smaller tumour nodules present on the spleen and colon. There was dilatation of both utero-pelvic/lyceal systems, with early bilateral hydronephrosis. There was a bilateral basal bronchopneumonia. No bony metastases were found and the parathyroids were somewhat atrophic. The other organs were essentially normal. Histological examination showed that the tumour had the

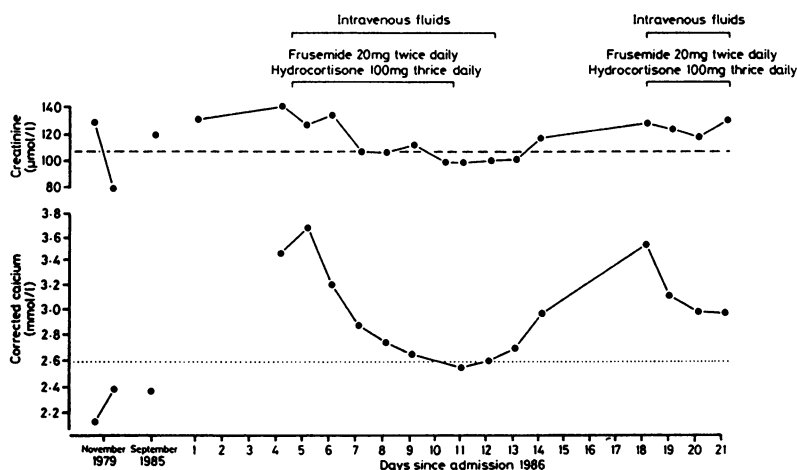


Figure Creatinine and corrected calcium concentrations on previous and last hospital admissions. Dashed and dotted lines represent upper limits of normal for each value, respectively.