SHORT ARTICLES

ISOLATION OF *YERSINIA PSEUDOTUBERCULOSIS* SEROTYPE V FROM THE BLOOD OF A PATIENT WITH SICKLE-CELL ANAEMIA

JEAN M. BRADLEY AND JOYCE I. SKINNER

Microbiology Department, Royal Free Hospital, Gray's Inn Road, London WC1X 8LF

HUMAN infection with *Yersinia pseudotuberculosis* (*Pasteurella pseudotuberculosis*) occurs in two main forms, namely, a benign and not uncommon mesenteric adenitis in children and young adults (Knapp, 1958; Mair *et al.*, 1960) and a septicaemic form that is rare and tends to occur in older patients. One previous septicaemic infection has been recorded in Great Britain (Macaulay *et al.*, 1967). This paper describes the second such case in this country and the first proven instance in the world literature of human infection with serotype V.

Case Report

A 25-year-old male student from Sierra Leone, who had been in this country for eighteen months, was admitted to hospital on 25 Dec., 1971, complaining of severe limb and back pains of four days' duration. Transient peri-umbilical pain had also been present at the outset. There was no known family history of sickle-cell anaemia.

Although his temperature was normal on admission, it had risen by the following day, at times reaching 40°C. Splinter haemorrhages were present in the fingers of both hands, there was a systolic cardiac murmur, and the edge of the liver was just palpable. There was no abdominal tenderness.

The haemoglobin value on admission was 9.5 g per 100 ml, there were 6500 WBC per mm³, with a normal differential count, but there were 72 nucleated RBC per 100 WBC and the ESR was 56 mm in one hour. Sickle cells were seen in blood films and the sickle-cell test was positive; haemoglobin electrophoresis showed Hb S only, and foetal haemoglobin was 1.6% of the total. Thus the diagnosis of sickle-cell anaemia was established.

Biochemical investigations revealed the following values: serum bilirubin 1.2 mg per 100 ml; alkaline phosphatase 30 KA units per 100 ml; cholesterol 100 mg per 100 ml; total protein 8.2 g per 100 ml; albumin 4.5 g per 100 ml; γ -globulin 1.8 g per 100 ml.

A skull X-radiograph showed no change suggestive of chronic haemolytic anaemia.

Three sets of blood cultures taken on 28–29 Dec. yielded, from all bottles after 24 hours' incubation, an organism with the characteristics of *Y. pseudotuberculosis*. Each set of blood cultures consisted of two bottles of Castaneda's medium (25 ml) and one of Brain Heart Infusion Broth (50 ml), each inoculated with 2–3 ml blood. The organism was found by Dr N. S. Mair to belong to serotype V. Serum taken on 1 Jan. agglutinated a stock strain of type V to a titre of 320, and the patient's own organism to 640. Agglutinating antibody titres, measured at intervals during the following eleven months, showed no significant change. *Yersinia* spp. were not recovered from any of several faecal specimens cultured on a 4% aesculin bile medium.

Treatment was started with 120 mg gentamicin per day in divided doses, by intramuscular injection. All subsequent blood cultures were sterile but by 3 Jan. the patient's haemoglobin had fallen to 6.2 g per 100 ml; there were 5920 nucleated RBC per μ l and many target cells were present. He remained pyrexial. Ampicillin in a dose of 1 g per day was then given by intravenous infusion and gentamicin was introduced into the drip tubing at the increased dose rate of 160 mg per day.

Although the patient was much improved clinically, his temperature continued to rise

Received 27 Nov. 1973; accepted 6 Dec. 1973.

intermittently to 38°C and on 17 Jan. gentamicin treatment was withdrawn and ampicillin treatment alone, at an increased dose rate of 4 g per day, was continued for a further four days. Antibiotic treatment was then discontinued and the patient's temperature remained normal from 27 Jan. onwards. He was discharged, feeling well, on 31 Jan. with a haemoglobin value of 9.4 g per 100 ml, liver function tests having proved normal.

BACTERIOLOGY

The organisms were Gram-negative rods, measuring approximately $2.5 \times 0.8 \mu\text{m}$, with occasional shorter ovoid forms showing bipolar staining. They were motile at 22°C but not at 37°C. Colonies on blood agar were less than 0.5 mm in diameter after 18 hours, but reached 2 mm after 48 hours, the surrounding medium showing slight greenish discolouration. Cultures incubated at 22°C, 30°C and 37°C were similar except that growth at 22°C occurred more slowly, and subsequent tests were performed at 37°C. Growth was apparent on MacConkey agar after 18 hours and on deoxycholate-citrate agar after 48 hours. A nutrient broth culture was uniformly turbid.

In peptone water containing 1% substrate, acid but no gas was produced after 18 hours from mannitol, glucose, maltose, mannose and xylose, and after 72 hours from salicin, glycerol and arabinose. After 14 days lactose, sucrose, dulcitol, dextrin, sorbitol, adonitol and inositol were unchanged.

Tests for β -galactosidase (ONPG), catalase and urease were positive. The organism was methyl-red positive and oxidase negative. It reduced nitrate to nitrite, hydrolysed aesculin, and did not grow in Koser's citrate. Gelatin was not liquefied and tests for oxidation of gluconate and utilisation of malonate were negative. Negative results were also obtained in tests for lysine and ornithine decarboxylases and hydrolysis of arginine. Indole, H_2S and acetoin were not produced. The methods used were those described by Cowan and Steel (1966).

Antibiotic sensitivity was determined by the disk method on Sensitivity Test Agar (Oxoid) inoculated with a suspension of organisms adjusted to give rise to semi-confluent growth after overnight incubation. Sensitivity to ampicillin (10 μg), carbenicillin (25 μg), cephalosporin (5 μg), streptomycin (10 μg), kanamycin (30 μg), gentamicin (2 μg), tetracycline (10 μg), chloramphenicol (25 μg) and co-trimoxazole (25 μg) was similar to that shown by the Oxford staphylococcus (no. NCTC6571). The organism was moderately sensitive to benzyl penicillin (1 unit) and sulphonamide (200 μg), giving rise to inhibition zones greater than 10 mm in width, but smaller by at least 4 mm than the zones given by the Oxford staphylococcus. The organism was judged to be resistant to erythromycin (10 μg), clindamycin (2 μg) and polymyxin (100 units), giving zones of inhibition less than 10 mm wide. Sensitivity to ampicillin and gentamicin was confirmed by the tube-dilution method, the MIC to ampicillin being 1 μg per ml and to gentamicin 0.25 μg per ml.

DISCUSSION

Mollaret *et al.* (1964) collected 17 cases of *Y. pseudotuberculosis* septicaemia from the world literature, noting that alcoholism, liver cirrhosis, diabetes mellitus, and haemochromatosis seemed to be predisposing factors. Marlon, Gentry and Merigan (1971) reported two cases, bringing the total at that time to 23. One of their patients was shown to have haemochromatosis, while the other had polycythaemia vera and was undergoing steroid therapy for continued haemolysis. Marlon *et al.* (1971) in analysing these 23 cases noted that 11 had one or more of the underlying conditions reported by Mollaret *et al.* (1964) and suggested hepatic involvement as the significant common factor.

We have found further reports of infection associated with alcoholism (Dorche, Verneyre and Denis, 1970), chronic venous congestion (Hubbert *et al.*, 1971), polycythaemia and myelofibrosis with haemolysis (Hubbert *et al.*, 1971) and diabetes with haemochromatosis (Yamashiro *et al.*, 1971). Borowsky, Zaremba and Wasiluk (1971) described infection in a 1-year-old girl who was shown at necropsy to have had acute mesenteric lymphadenitis.

Infection is believed to be by the oral route, and usually results in a benign mesenteric

adenitis. Mollaret *et al.* (1964) postulated that there might occasionally be a transient bacteraemia and, more rarely, septicaemia might follow. This is borne out by the high incidence of mesenteric adenitis found at necropsy even in cases where clinical evidence of this was lacking. Marlon *et al.* (1971) thought that the liver might play a critical role in resistance to the spread of infection.

We consider that an important common factor in these cases might be disordered iron metabolism. Iron is essential for bacterial growth (Stephenson, 1949) and various authors have reported an association between the availability of iron and the pathogenicity of certain bacterial species (Martin, Jandl and Finland, 1963; Sword, 1966a; Bullen and Rogers, 1969).

The fact that relatively avirulent strains of *Y. pestis* were rendered fully virulent when experimental animals were treated with iron compounds was reported by Jackson and Burrows (1956). Brubaker, Beasley and Surgalla (1965) demonstrated enhanced virulence of *Y. pseudotuberculosis* in the presence of ferrous iron when organisms were injected intraperitoneally or subcutaneously into rats and mice.

Attention has also been drawn to the part played by iron in the virulence of *P. septica* (Bullen *et al.*, 1968). Bullen, Rogers and Lewin (1971) suggested that the iron essential to the metabolism of *P. septica* is probably not present in the host in sufficient quantities under normal conditions, but if serum transferrin is saturated, free iron may then be available for the organism. Sword (1966b) showed that the serum of animals with experimental *Listeria monocytogenes* infection contained an increased amount of haptoglobulin and transferrin, suggesting a means of increased flow of iron through the host.

Of the 16 recorded cases known to have been associated with underlying disease, no fewer than 12 were associated with conditions likely to increase the availability of iron to bacteria in the blood stream. Thus, five patients had haemochromatosis, two had polycythaemia accompanied by haemolysis, three others had cirrhosis of the liver, one had chronic venous congestion, and our own case had haemoglobinopathy.

We think that the sickle-cell crisis and the infection with *Y. pseudotuberculosis* were related and feel it is possible that the haemoglobin breakdown products potentiated the pathogenicity of the micro-organism.

Most of the isolates from man that have been serotyped have been found to belong to type I. Apart from the culture of Yamashiro *et al.* (1971) somewhat inconclusively designated type I-V, type V has to the best of our knowledge never before been reported in man and is not common in animals. Dr N. S. Mair in a personal communication tells us that five type-V strains collected by him in England were all from guinea-pigs, and that Mollaret's collection of 2145 *Y. pseudotuberculosis* isolates from world-wide sources includes 37 type-V strains, 23 of which were from hares, the others being obtained from rabbits, guinea-pigs, monkeys, a pigeon, a stag, an otter and a partridge. Our patient admitted to having rats in his home and he also owned a cat, but unfortunately co-operation in epidemiological investigations was refused.

SUMMARY

A case of *Yersinia pseudotuberculosis* septicaemia is described in a patient with sickle-cell anaemia. This is believed to be the first reported instance of human infection with serotype V. The possibility that disordered iron metabolism might be the predisposing factor in this and other *Y. pseudotuberculosis* septicaemias is considered.

We are grateful to Dr N. S. Mair for serotyping the strain, for antibody studies and for general interest, and to Dr A. G. Beckett under whom the patient was admitted.

REFERENCES

- BOROWSKI, J., ZAREMBA, M. L. AND WASILUK, L. 1971. Fatal septicaemia due to *Yersinia pseudotuberculosis*. *Br. med. J.*, **1**, 556.
BRUBAKER, R. R., BEESLEY, E. D. AND SURGALLA, M. J. 1965. *Pasteurella pestis*: role of pesticin I and iron in experimental plague. *Science, N.Y.*, **149**, 422.

- BULLEN, J. J. AND ROGERS, H. J. 1969. Bacterial iron metabolism and immunity to *Pasteurella septica* and *Escherichia coli*. *Nature, Lond.*, **224**, 380.
- BULLEN, J. J., ROGERS, H. J. AND LEWIN, J. E. 1971. The bacteriostatic effect of serum on *Pasteurella septica* and its abolition by iron compounds. *Immunology*, **20**, 391.
- BULLEN, J. J., WILSON, A. B., CUSHNIE, G. H. AND ROGERS, H. J. 1968. The abolition of the protective effect of *Pasteurella septica* antiserum by iron compounds. *Immunology*, **14**, 889.
- COWAN, S. T. AND STEEL, K. J. 1966. Manual for the identification of medical bacteria, Cambridge.
- DORCHE, G., VERNEYRE, H. AND DENIS, M. 1970. Un cas de septicémie à bacille de Malassez et Vignal. *J. Méd. Lyon*, **51**, 1347.
- HUBBERT, W. T., PETENYI, C. W., GLASGOW, L. A., UYEDA, C. T. AND CREIGHTON, S. A. 1971. *Yersinia pseudotuberculosis* infection in the United States; septicæmia, appendicitis, and mesenteric lymphadenitis. *Am. J. trop. Med. Hyg.*, **20**, 679.
- JACKSON, S. AND BURROWS, T. W. 1956. The virulence-enhancing effect of iron on non-pigmented mutants of virulent strains of *Pasteurella pestis*. *Br. J. exp. Path.*, **37**, 577.
- KNAPP, W. 1958. Mesenteric adenitis due to *Pasteurella pseudotuberculosis* in young people. *New Engl. J. Med.*, **259**, 776.
- MACAULAY, J. D., WILSON, J. A. C., ABBOTT, J. D. AND MAIR, N. S. 1967. Fatal case of *Pasteurella pseudotuberculosis* infection associated with hepatic cirrhosis. *Br. med. J.*, **2**, 553.
- MAIR, N. S., MAIR, H. J., STIRK, E. M. AND CORSON, J. G. 1960. Three cases of acute mesenteric lymphadenitis due to *Pasteurella pseudotuberculosis*. *J. clin. Path.*, **13**, 432.
- MARLON, A., GENTRY, L. AND MERIGAN, T. C. 1971. Septicæmia with *Pasteurella pseudotuberculosis* and liver disease. *Archs intern. Med.*, **127**, 947.
- MARTIN, C. M., JANDL, J. H. AND FINLAND, M. 1963. Enhancement of acute bacterial infections in rats and mice by iron and their inhibition by human transferrin. *J. infect. Dis.*, **112**, 158.
- MOLLARET, H. H., TEMKINE, J., PRADE, M., PIERON, R., DESTOMBES, P. AND GUILLON, M. C. 1964. Les septicémies humaines à bacille de Malassez et Vignal. *Presse méd.*, **72**, 2671.
- STEPHENSON, M. 1949. Bacterial metabolism, 3rd ed., London, p. 179.
- SWORD, C. P. 1966a. Mechanisms of pathogenesis in *Listeria monocytogenes* infection. 1 Influence of iron. *J. Bact.*, **92**, 536.
- SWORD, C. P., 1966b. Serum protein alterations induced by *Listeria monocytogenes* infections. *J. Immun.*, **96**, 790.
- YAMASHIRO, K. M., GOLDMAN, R. H., HARRIS, D. AND UYEDA, C. T. 1971. *Pasteurella pseudotuberculosis*. Acute sepsis with survival. *Archs intern. Med.*, **128**, 605.