

# *Bacterium anitratum* and *Mima polymorpha* infection in Uganda

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**SYNOPSIS** Eleven cases of infection with *Bacterium anitratum* and one with *Mima polymorpha* are described. Microscopically the organisms showed Gram-negative or Gram-variable diplococci with a tendency to form filaments. There appear to be a number of biochemical variants of *B. anitratum*. Serological cross-reactions with the genus *Klebsiella* was found. The antibiotic patterns are given and particular attention is drawn to the resistance of the organisms to chloramphenicol.

In a paper dealing with organisms morphologically indistinguishable from *Neisseria gonorrhoea* in urethral smears, De Bord (1939) proposed a new tribe, Mimeae, consisting of short Gram-negative encapsulated pleomorphic rods, growing well on ordinary solid media from which Gram-stained films show that cells are almost wholly diplococcal in form and identical to the gonococcus in size and appearance. The fermentation reactions of the subgroups varied from acid and gas in some sugars, acid only, and no fermentation. In a later publication De Bord (1942) further characterized the new tribe and described *Colloides*, *Herellea*, and *Mima polymorpha*, which corresponded respectively to the organisms fermenting sugars to give acid and gas, acid only, or not fermenting sugars.

A little later interest was aroused by an organism which by some was considered to belong to Enterobacteriaceae and named B5W (Stuart, Formal, and McGann, 1949), or more commonly *Bacterium anitratum* in view of its failure to reduce nitrate to nitrite (Schaub and Hauber, 1948; Ferguson and Roberts, 1950; Brooke, 1951). This latter organism was considered by some to be identical with the *Herellea* of the tribe Mimeae (Ewing, 1949), but this has recently been disputed (Henriksen, 1963). This group and the relationship to *Diplococcus mucosus*, *Moraxella*, and others have been reviewed by Daly, Postic, and Kass (1962) who give useful references to the French literature.

These organisms have been shown to be of considerable clinical interest. *Mima polymorpha* has thus been incriminated in cases of meningitis (De Bord, 1948; Fred, Allen, Hessel, and Holtzman, 1958; Olafsson, Lee, and Abernethy, 1958; Torregrosa and

Ortiz, 1961), and at times has been mistaken for *Neisseria meningitidis* (Schuldberg, 1953; Waite and Kline, 1959), has caused subacute bacterial endocarditis (Pike, Schulze, and McCullough, 1951), septicæmia (Faust and Hood, 1949; Torregrosa and Ortiz, 1961), Waterhouse-Friderichsen syndrome (Townsend, Hersey, and Wilson, 1954), urethritis (Deacon, 1945; Ino, Neugebauer, and Lucas, 1959), infection of war wounds (Deacon, 1945), and has been found in cases of purulent conjunctivitis and vaginitis (De Bord, 1943), as well as in a number of normal vaginas. De Bord's (1939) claim of a causative relationship with a gonorrhoea-like urethritis appears to have been confirmed and *Mima polymorpha* has been incriminated in many cases of so-called penicillin-resistant gonorrhoea (Svihus, Lucero, Mikolajczyk, and Carter, 1961).

*B. anitratum* has been isolated from cases of septicaemia and osteomyelitis (Torregrosa and Ortiz, 1961), urinary infection (Schaub and Hauber, 1948; Ferguson and Roberts, 1950), and cellulitis as well as from conjunctivae, sputum, chest fluid, the throat, abscess wound, bile, stomach contents, cerebrospinal fluid, and faeces (Ferguson and Roberts, 1950; Mannheim and Stenzel, 1962), and has been thought to cause diarrhoea (Stuart, Formal, and McGann, 1949).

Similarly *Herellea* has been incriminated in cases of acute bacterial endocarditis (Sorrell and White, 1953; Minzter, 1956), chronic synovitis (Ino and Neugebauer, 1956), purulent conjunctivitis (De Bord, 1943), and has been isolated from cerebrospinal fluid following head injury and urine following operation for urethral stricture (Deacon, 1945). Daly *et al.* (1962) describe 18 cases of infection with *Herellea*. The patients were either severely ill from

other diseases, had recently undergone a surgical operation, or suffered trauma. In half the cases the portal of entry appeared to be via an indwelling intravenous polyethylene catheter with resulting septicaemia. Septicaemia was seen in other cases as well. Secondary infection at the site of operation, a subcutaneous abscess following trauma, and a case of pneumonia plus bacteraemia comprised the remainder. The latter authors as well as others use the term *Herellea* as synonymous with *B. anitratum*.

*B. anitratum* grows well aerobically on most media but does not grow anaerobically. This latter feature enables one to suspect the presence of a member of this group of organisms when testing for biochemical reactions, as there may be no acid inside the Durham tube but acid in the medium outside it as described by Sorrell and White (1953) for *Herellea*.

The biochemical reactions of *B. anitratum* as reported in the literature have shown variations within a fairly uniform overall pattern. Thus glucose, usually xylose, arabinose and galactose, and occasionally rhamnose and lactose are attacked with the production of acid but no gas, and usually no reaction occurs with sucrose, maltose, mannitol, adonitol, dulcitol, inositol, inulin, trehalose, raffinose, glycogen, sorbitol, and salicin. H<sub>2</sub>S, indole, and acetyl-methyl-carbinol are not produced, liquefaction of gelatin, urease and methyl red tests are usually negative, citrate is utilized, and nitrates are not reduced to nitrites (Brooke, 1951; Ferguson and Roberts, 1950; Mannheim and Stenzel, 1962; Schaub and Hauber, 1948; Stuart *et al.*, 1949).

The genus *Herellea*, according to De Bord's (1942) definition, 'ferments certain carbohydrates with production of acid only. Nitrates may or may not be reduced'. It is obvious from that definition that the organism cannot be identified biochemically. The species *Herellea vaginicola* produces acid from glucose, mannitol, and dulcitol, is methyl red and Voges-Proskauer negative, citrate and catalase positive, and does not reduce nitrates according to De Bord's definition. In other descriptions of *Herellea* mannitol is usually not fermented and dulcitol fermentation not mentioned. As other biochemical reactions are also in keeping with descriptions of *B. anitratum* it would appear difficult to distinguish between the two as they are described in the literature (Daly *et al.*, 1962; Deacon, 1945; Ino and Neugebauer, 1956; Sorrell and White, 1953).

In contrast to *B. anitratum* and *Herellea*, *Mima polymorpha* does not attack any of the above-mentioned carbohydrates. Neither is citrate utilized, indole or H<sub>2</sub>S produced, nor nitrates reduced to nitrites and the methyl red, and Voges-Proskauer tests are negative (De Bord, 1942; Ino *et al.*, 1959;

Pike *et al.*, 1951; Waite and Kline, 1959), although citrate utilization has been reported (Deacon, 1945; Schuldberg, 1953). Deacon (1945), however, also included organisms which did reduce nitrates to nitrites.

Daly *et al.* (1962), reporting infections due to organisms of the genus *Herellea*, found a number of different serological capsular types, and cross-agglutination was found with a *Klebsiella aerogenes* strain and *Shigella alkalescens*, and to a lesser degree, with some strains of *Escherichia coli* and *Pseudomonas aeruginosa*. Schaub and Hauber (1948) found no cross-agglutination between *B. anitratum* and *Escherichia*, *Aerobacter*, *Eberthella*, *Salmonella*, *Shigella*, *Pseudomonas*, or *Pasteurella*.

The antibiotic sensitivity pattern of these groups of organisms varies from strain to strain, many showing resistance to commonly used antibiotics. They are, however, not infrequently sensitive to tetracyclines and resistant to penicillin (Brooks and Sanders, 1954; Daly *et al.*, 1962; Glick, Moran, Coleman, and O'Brien, 1959; Ino and Neugebauer, 1956; Ino *et al.*, 1959; Mannheim and Stenzel, 1962; Lund, 1954; Olafsson *et al.*, 1958; Pike *et al.*, 1951; Schaub and Hauber, 1948; Sorrell and White, 1953; Torregrosa and Ortiz, 1961; Waite and Kline, 1959).

Similarly the organisms within each group differ in animal pathogenicity, some strains causing death of mice and guinea-pigs following intraperitoneal injection, whilst others do not (Brooke, 1951; Deacon, 1945; Schaub and Hauber, 1948; Schuldberg, 1953; Sorrell and White, 1953).

#### MATERIALS AND METHODS

All but one of the patients in the present series were seen at Mulago Hospital, Kampala, Uganda, the modern 880-bed teaching hospital attached to Makerere College, University of East Africa Medical School, in the period January to September 1963. The one exception was a patient at Rubaga Hospital, Kampala, during the same period. All the bacteriological investigations were performed in the bacteriology laboratory of Mulago Hospital. The organisms were isolated and investigated by conventional bacteriological methods. Motility was examined for both by hanging drop and Craigie tube techniques. Nitrate reduction tests were performed by the method of Bönicke (1962) as well as by Cook's (1950) method. Determination of the sensitivities to chemotherapeutic drugs was carried out on sensitivity test agar (Oxoid) using commercial discs (Multodisc, 25 µg.) Pathogenicity tests were carried out on mice and guinea-pigs by intraperitoneal injection of 0.2 ml. of a heavy bacterial suspension made from an overnight nutrient agar culture. Antisera were pre-

pared in rabbits by repeated intravenous injections of 0.2 ml. of overnight peptone water cultures.

Swabs were taken from the antecubital fossae of 50 randomly selected Mulago Hospital in-patients and from the nose of laboratory staff and of dust in the laboratory and inoculated onto various media to investigate the possibility of obtaining the organisms as contaminants from any of these sources. In no case was *B. anitratum* or *Mima polymorpha* isolated. This is in contrast to the findings of Taplin, Rebell, and Zaias (1963), who found *Herellea* on the skin of 25% and *Mima polymorpha* on the skin of 10% of American subjects.

#### RESULTS

Details of the cases are given in Table I. *B. anitratum* was isolated from blood cultures of five patients with pyrexia of unknown origin, in one case of pyelitis it was grown from a freshly voided mid-stream specimen of urine in numbers greater than

100,000 organisms per millilitre, in two cases it was isolated from the cerebrospinal fluid of children with meningitis, in one case from an infected knee joint, in one case from pus from abscesses, and in one case from urethral discharge. A pure growth was obtained in all except the last case. A profuse almost pure growth of *Mima polymorpha* was obtained from the sputum of a case of pneumonia. Four patients were tested for antibodies against their own organisms, but none were found.

The morphology of both *B. anitratum* and *Mima polymorpha* was characteristic. The diplococcal appearance frequently could not have been distinguished from that of *Neisseria* were it not for the occasional bacillary forms which at times appeared as long filaments. They were Gram negative, or weakly Gram positive, as they did not decolorise as rapidly as most other Gram-negative organisms. Capsules were readily demonstrated by the method of Howie and Kirkpatrick (1934). They grew well on most media aerobically but no growth was obtained

TABLE I  
CLINICAL DETAILS OF THE CASES

Case and Strain No.	Age (yr.)	Sex	Clinical Notes	Remarks	Organism and Source
1. P 853	7	F	Weakness, jaundice 8 days. A case of sickle cell anaemia	Recovered	<i>B. anitratum</i> Blood
2. P 855	30	M	Jaundice, abdominal pain, fever, swelling of legs for 2 weeks, hepatosplenomegaly	Died	<i>B. anitratum</i> Blood
3. P 1110	40	F	Fever, headache 2 weeks	Recovered	<i>B. anitratum</i> Blood
4. P 1119	38	M	Fever, general malaise, loss of appetite 2 weeks	Recovered	<i>B. anitratum</i> Blood
5. P 1198	6	M	Generalized oedema, fever, 3 months, vomiting, 1 week. Primarily a case of kwashiorkor	Died	<i>B. anitratum</i> Blood
6. Q 24	1	F	Fever, headache, neck stiffness, 5 days	Recovered	<i>B. anitratum</i> Cerebrospinal fluid
7. Q 122	1 month	M	Convulsions 2 weeks, neck stiffness	Recovered but subsequently developed a block or cerebral abscess	<i>B. anitratum</i> Cerebrospinal fluid
8. L 3176	30	F	Frequency of micturition, pain in loins 3 days	Recovered	<i>B. anitratum</i> Urine
9. M 724	40	M	Urethritis	Recovered	<i>B. anitratum</i> Urethral discharge
10. N 68	1	M	Acute pyogenic arthritis left knee joint	Recovered	<i>B. anitratum</i> Knee joint
11. M 630	24	M	Abscesses right gluteal, left scapular regions following injection	Recovered	<i>B. anitratum</i> Abscesses
12. SP 500	40 approx.	M	Cough, blood-stained sputum, clinically pneumonia, lung opacities on radiograph	Recovered	<i>Mima polymorpha</i> sputum

anaerobically. No growth occurred on Hoyle's tellurite medium. After overnight incubation on blood agar they formed 2-3 mm. circular, dome-shaped, shiny, whitish-grey colonies with an entire edge. A zone of apparent  $\beta$ -haemolysis (human blood agar) was formed by one strain of *B. anitratum*. On further incubation of some strains the surface of the colonies tended to become granular with small satellite colonies appearing on the surface of the mother colony, probably similar to the daughter colonies mentioned by Schaub and Hauber (1948). The colonies also tended to form grooves and ridges radiating from the centre to the periphery and the edge became undulated. Similar good growth occurred on nutrient and McConkey agar. In the latter case the colonies tended to have a pinkish tinge different from the usual lactose fermentation seen in coliforms. None were motile. In view of the occasional report of motile variants of *B. anitratum* (Halvorsen, 1963; Stuart *et al.*, 1949), smears and sections of some of the strains were examined by electron microscopy. This revealed no evidence of flagellae. The majority of organisms were diplococcal forms with the appearance of a capsule (Fig. 1).

The biochemical reactions of the strains of *B. anitratum* had an overall close similarity. They all formed acid without gas within three days from glucose, L+ arabinose, xylose, mannose, and 10% lactose. None attacked 1% lactose, 1% or 10% sucrose, mannitol, dulcitol, raffinose, glycerol, d-arabinose, rhamnose, aesculin, salicin, inulin, adonitol, or inositol within 30 days. They all grew in citrate medium, they were all catalase positive, they did not form indole, they were methyl red and Voges-Proskauer negative, and they failed to reduce nitrates

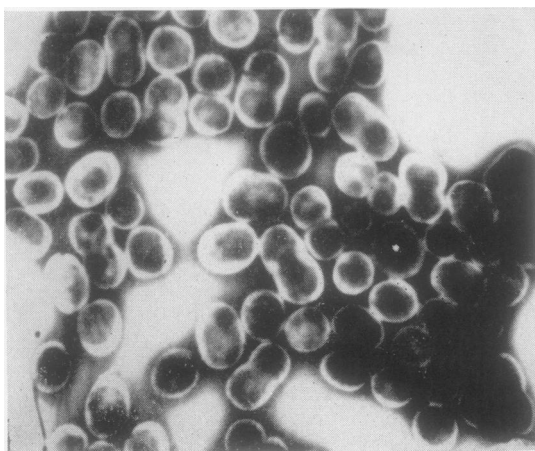


FIG. 1. Negative staining with phosphotungstic acid.

to nitrites. The two strains of *Achromobacter anitratum*, numbers 7363 and 7364, kindly sent to us by the National Collection of Type Cultures (N.C.T.C.), were also found to give these reactions, which are in accord with the majority of descriptions of *B. anitratum*.

With respect to fermentation of maltose, fructose, and galactose, splitting of urea, liquefaction of gelatin and the oxidase reaction, it appears possible to divide the present *B. anitratum* strains into five biochemical groups as shown in Table II. The presence of apparent  $\beta$ -haemolysis on blood agar has been previously encountered (Ferguson and Roberts, 1950), as has gelatin liquefaction (Schaub and Hauber, 1948), acid formation from maltose, and failure to form acid from galactose (Mannheim and Stenzel, 1962). Variants forming acid in rhamnose (Brooke, 1951; Ferguson and Roberts, 1950; Stuart *et al.*, 1949) or not attacking xylose have been reported (Brooke, 1951; Ferguson and Roberts, 1950), which makes a total of at least seven biochemical variants of *B. anitratum*. The formation of acid in l + arabinose and no reaction with d - arabinose in the present study may explain the varying results with arabinose as reported in the literature.

TABLE II  
DIFFERENTIAL BIOCHEMICAL CHARACTERISTICS OF GROUPS OF *B. Anitratum*

	N.C.T.C. 7363 and 7364	P 1110	P 1119	P 1198	Eight Other Strains
Fructose	A <sup>6</sup> & 4	—	A <sup>4</sup>	A <sup>3</sup>	—
Galactose	A <sup>2</sup> & 7	—	A <sup>1</sup>	A <sup>1</sup>	A <sup>1-3</sup>
Maltose	—	—	—	A <sup>13</sup>	—
Urease	—	—	+ <sub>3</sub>	+ <sup>14</sup>	—
Oxidase	—	±	—	—	—
Gelatin	Not tested	+	—	—	—
Haemolysis <sup>1</sup>	—	+	—	—	—
Biochemical groups	1	2	3	4	5

<sup>1</sup> = Human blood agar

— = no reaction

± = weak positive reaction

+ = positive reaction, the figure referring to the number of days required.

A = acid produced, the accompanying figure referring to the number of days taken to produce the acid.

The *Mima polymorpha* attacked none of the above-mentioned sugars, H<sub>2</sub>S, indole, urease and acetyl-methyl-carbinol were not produced, citrate was not utilized, gelatin was not liquefied, oxidase and methyl red tests were negative, and nitrates were not reduced to nitrites, but it was strongly catalase positive.

Anisera were prepared against *B. anitratum* strains P 1119 and L 3176. The former antiserum readily agglutinated strains P 855, P 1110, P 1198,

M 630, M 724, N 68, Q 122, strains 7363 and 7364 of the N.C.T.C. and a strain of Klebsiella isolated in Mulago Hospital Laboratory, but not strain L 3176 or strains of Salmonella, Shigella, Escherichia, Proteus, or Pseudomonas. The antiserum prepared against strain L 3176 did not agglutinate any of the strains agglutinated by the antiserum to P 1119. Strains P 853 and Q 24 died before serological testing was carried out. Thus the serological grouping of *B. anitratum* strains appears to be different from the biochemical one. Among the strains of group 5 in Table II, one strain appears to be serologically distinct whilst all the other strains show serological relationship. However as only incomplete absorption studies were carried out on the antiserum to P 1119 with the various strains, further studies might reveal serological subgroups corresponding to biochemical groups. The apparent serological relationship of some strains of *B. anitratum* with Klebsiella but not with a number of other members of Enterobacteriaceae is noteworthy.

The drug sensitivities of the *B. anitratum* and the *Mima polymorpha* strains are summarized in Table III. The resistance of the organisms to streptomycin

TABLE III

ANTIBACTERIAL DRUG SENSITIVITIES OF *B. Anitratum*<sup>1</sup>

	Sensitive	Resistant
Ampicillin	9	1
Penicillin	—	2
Erythromycin	3	1
Streptomycin	—	9
Tetracycline	3	6
Chloramphenicol	1	10
Sulphonamide	2	7
Furadantin	1	5

<sup>1</sup>Not all strains were tested against all the drugs.

and, with a few exceptions, also to chloramphenicol, tetracycline, and sulphonamides, is striking, as is their frequent sensitivity to ampicillin. The sensitivity to erythromycin agrees with the findings of Ashley and Kwantes (1961) and Mannheim and Stenzel (1962) who reported the majority of strains sensitive to erythromycin, in contrast to Lund (1954) who found the majority resistant to this antibiotic.

The strain of *Mima polymorpha* was sensitive to chloramphenicol, ampicillin, tetracycline, and furadantin, slightly sensitive to streptomycin, and resistant to erythromycin and sulphonamides.

Four of the strains of *B. anitratum* caused death of mice and guinea-pigs after intraperitoneal injection. At necropsy the organisms were cultured from the heart blood and peritoneum. The four remaining strains of *B. anitratum* and the strain of *Mima polymorpha* did not kill the animals.

## DISCUSSION

The findings in the present series indicate that the manifestations of infection with this group of organisms in Uganda are in accord with those seen elsewhere.

It is a feature of most cases in the literature that the evidence for infection by this group of microorganisms is generally limited to isolation of the organisms from clinical specimens. Antibodies to the organisms in the patients' sera appear to have been looked for in a few cases only. Ferguson and Roberts (1950) in a study of *B. anitratum* found the organism in the gastro-intestinal tract at necropsy in a case of lupus erythematosus as well as agglutinating antibodies in the serum to a titre of 1 in 160, whilst Mintzer (1956) found no agglutinating antibodies in a presumed case of *Herellea vaginicola* endocarditis.

In the present series agglutinating antibodies to the corresponding organisms were tested for in four patients with negative results. As the organisms with one exception were obtained in pure culture and there was no evidence of much risk of obtaining them as contaminants, the present cases are thought to be genuine infections.

In several of the septicaemic cases typhoid fever was suspected clinically. In view of the frequent use of chloramphenicol in typhoid fever before bacteriological confirmation is obtained, it might be worth while keeping *B. anitratum* infection in mind when no response is obtained to chloramphenicol.

We are grateful to the Minister of Health, Uganda Government, for permission to publish this paper and to Professor David Allbrook, Dean of the Faculty of Medicine, Makerere University College, for the use of the electron microscope.

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