TWO HUMAN INFECTIONS CAUSED BY PASTEURELLA MULTOCIDA

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Pasteurellosis is a disease rarely diagnosed in man. The literature on this subject was reviewed for the first time by Lévy-Bruhl (1938), and the cases reported up to that time were subjected to a critical analysis by Regamey (1938, 1939). Schipper (1947) included in his review all the cases on record except those described by Tomić-Karović and Ivanović (1944) and Ory, Jackson, and Finland (1946). Since this publication cases have been described by Appelbaum and Gelfand (1947), Cappellato (1947), Kapel (1947), Svendsen (1947), and Bartley and Hunter (1947). Recently Needham (personal communication) has isolated 36 strains from 28 patients, nearly all of whom were suffering from bronchiectasis. Although the disease is of wide prevalence, 30 out of the 36 cases not included in the two reviews mentioned above were found in the United States, and of the remainder two occurred in Italy and one each in Denmark, Norway, England, and Yugoslavia. In his monograph Regamey (1939) divided the cases of human pasteurellosis into three groups; those due to animal bite, those following trauma, and those in which the mechanism of infection could not be determined. A survey of all the cases recorded up to date shows that approximately 50% followed an animal bite, 10% were post-traumatic, and in the remainder the route of infection was quite obscure. In this connexion it is of interest that all the cases of cryptogenic origin were related to disease of the respiratory tract, implying a strong affinity of the causal organism for the air passages, while the frequent occurrence of meningitis following trauma to the face or head suggests that the organism may occur as a commensal in the nasal cavities or cranial sinuses.

As already mentioned, Tomić-Karović and Ivanović isolated *Past. multocida* from a human subject in 1944, and the two cases described below thus represent the second and third isolations of this micro-organism from human sources in the Microbiological Institute of the Medical Faculty of Zagreb.

Case Reports

Case 1.—A farmer aged 38 was admitted to hospital on November 11, 1950, with a seven-years history of lung disease. Before admission he had been treated for tuberculosis, but *Mycobacterium tuberculosis* had never been isolated from the sputum. While in hospital a diagnosis of bronchiectasis was established, and on five occasions *Past. multocida* was isolated from the sputum. Although the organism isolated was very sensitive to penicillin *in vitro*, parenteral and intrabronchial administration did not produce any noticeable effect on the disease process, but on his discharge on January 29, 1951, his clinical condition was thought to be somewhat improved. Before his illness he had been bitten by a dog, had consumed diseased poultry, and had been in contact with mice and rats—factors which may have initiated infection with *Past. multocida*.

Case 2.—A nurse aged 31 was admitted to hospital on January 9, 1951, with a history of a febrile illness characterized by purulent nasal discharge and meningeal symptoms of nine days' duration which had been preceded by a common cold. For six years previously the patient had suffered from a nasal disorder accompanied by discharge of greenish-yellow pus, and this had begun while she was employed as a farm worker. Parenteral and intrabronchial administration of penicillin was started after admission to hospital, but no remission of symptoms resulted. After the discovery of a suppurative condition in both maxillary sinuses a bilateral radical operation was performed. Symptoms disappeared rapidly and the patient was discharged five days later, on February 1. From the pus obtained from the maxillary sinuses a pure culture of *Past. multocida* was obtained. Histological examination of the nasal mucosa showed typical inflammatory changes with abundant plasma cells, lymphocytes, and degenerate polymorphs. Five months later, although the nasal discharge was very slight, *Past. multocida* was again isolated from the exudate.

Bacteriological Investigations

Both strains of *Past. multocida* showed almost identical characters, and it is sufficient to give one description, mentioning only the differences which were observed. In Case 1 the organism was isolated in almost pure culture, and in Case 2 the primary growth was quite free from contaminants.

Morphology.—In direct smears from Case 1 Gram-negative rods about $2\ \mu$ in length were seen. On culture on semi-solid media shorter almost coccoid forms were found. Bipolar staining was not observed until after several subcultures in a fluid medium. In smears prepared from the organs of animals inoculated with cultures, micro-organisms showing typical bipolar staining were found.

Cultivation.—The material was cultivated in blood-agar plates at 37°C . and growth appeared after 24 hours. At room temperature growth was much less abundant, and on ordinary agar it grew much less vigorously. The colonies, which were about 0.5 mm. in diameter, were raised, opaque, bluish-grey in colour, with a sharply defined edge, and the growth had a recognizable odour. On ordinary blood agar no haemolysis took place, but the medium developed a rather brownish coloration; when the proportion of blood was reduced to 3% partial haemolysis was observed. Growth in glucose agar was slow and resulted in a slimy deposit at the bottom of the tube. Neither gelatin nor coagulated serum was liquefied, and motility was not observed at either 22°C . or 37°C .

Biochemical Reactions.—The following carbohydrates were fermented without gas production: mannitol, saccharose, glucose, arabinose, galactose, and laevulose. Dulcitol, sorbitol, salicin, aesculin, inositol, maltose, lactose, raffinose, xylose, dextrin, starch, and glycerol were not attacked. The indole reaction was strongly positive, and H_2S was produced in small quantities after prolonged incubation. The catalase reaction was also positive, and nitrates were reduced with the production of ammonia. The methyl red and Voges-Proskauer reactions were negative, citrates were not utilized, and inhibition of growth took place in bile-containing media. Reduction was noted in milk containing 1% methylene blue, but no change took place in milk containing brom-cresol purple.

Pathogenicity.—The strains isolated from Case 1 were more pathogenic for laboratory animals than those from Case 2. Mice, guinea-pigs, and rabbits died within 24 hours of inoculation, and at necropsy showed pleural and peritoneal exudate and congestion of internal organs, while with cultures from Case 2 death did not occur until two to four days after infection. In both cases, however, the organisms could be isolated from the heart blood, peritoneum, liver, and spleen. Neither of the strains was pathogenic for pigeons by the subcutaneous route.

Sensitivity Tests.—Selected strains isolated from both patients were tested for sensitivity to penicillin, streptomycin, and sulphadiazine by the Moersch-Lund (1949) method and in the case of penicillin by dilution technique also. The first strain isolated from Case 1 was very sensitive to penicillin (+++), less so to sulphadiazine (++), and resistant to streptomycin. The second and third strains, isolated during treatment with penicillin, were rather less sensitive to penicillin (++), and those obtained after treatment ceased (32 million units) showed a similar sensitivity. The first strain derived from Case 2 showed a similar sensitivity to penicillin (+++). The minimal inhibiting concentrations of penicillin were estimated by the usual dilution technique. These were: 0.4 unit for the first strain, 1 unit for the second isolation, and 5 units for the third. The strain isolated from Case 2 was inhibited by 0.3 unit.

Serological Tests.—Limited serological studies were carried out. Serum taken from the first patient about five weeks after admission to hospital showed an agglutinating titre of 1/40 against the homologous organism. Normal control sera gave negative results. No complement-fixation tests were carried out. In the case of the second patient, a serum specimen taken five months after operation gave quite negative results in both agglutination and complement-fixation tests.

Discussion

Although the organisms isolated conform to the general characters of *Past. multocida*, their precise relationship to the disease processes present in the patients is less certain. In Case 1 it is impossible to say whether the infection with *Past. multocida* preceded the bronchiectasis or vice versa, and the situation is made more difficult by the progressive character of this disease, irrespective of the presence or absence of the causative agent. The second case is very similar to that described by Bartley and Hunter (1947), and seems to be the second example in the literature of sinusitis due to an organism of the *Pasteurella* group. These authors describe a case of frontal sinusitis of long duration from which *Past. multocida* was isolated which did not react to penicillin but responded to operation. It seems reasonable to assume that in both these patients the cause of the sinusitis was *Past. multocida*. Determination of the source of the infection is also a difficult problem. In some instances it has been possible to establish a connexion with the bite of an animal, but in the absence of any such significant event in the patient's history there remain as guides only the biochemical characters of the organism isolated and the species pathogenicity. Pestana and Rugai (1943) stated that 96.88% of strains isolated from birds fermented arabinose, while those from mammals failed to do so. Stamatin (personal communication, 1951) stated that avian strains ferment arabinose but not xylose, while mammalian ferment xylose but not arabinose. The first patient had a history of animal bite and of contact with diseased poultry and with rats and mice before the onset of symptoms, thus giving a wide but vague range of possible sources of infection, but in Case 2 no such data were provided. Moreover, although the fermentation reactions in both cases are of the avian type, the strains were avirulent for pigeons. In the reports in the literature of *Past. multocida* isolated from human beings the strains obtained cannot readily be classified into two fermenting groups, for some fail to ferment both arabinose and xylose (Regamey, 1938, 1939; Le Chuiton *et al.*, 1939; Cooper and Moore, 1945), while others (Bartley and Hunter, 1947; Svendsen, 1947) attack both these carbohydrates. Stamatin's classification, therefore, although applicable to strains obtained from birds and mammals, cannot necessarily be applied to those isolated from cases of human pasteurellosis.

Summary

Two cases of human pasteurellosis, one with bronchiectasis, the other with maxillary sinusitis, are described from which almost identical strains of *Past. multocida*

were isolated. Although the organisms were sensitive to penicillin *in vitro*, penicillin treatment was without success. Detailed descriptions of the strains and a discussion of the literature on the subject are given.

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IMPROVEMENT OF HEARING IN MALFORMATION OF THE EXTERNAL AUDITORY MEATUS AND MIDDLE EAR

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Congenital malformation of the pinna, auditory meatus, and middle ear is a comparatively uncommon condition which presents a functional as well as an aesthetic problem. The surgical techniques for reconstruction of the pinna are well recognized and regularly practised, but the measures for improving the hearing are perhaps not so readily appreciated.

In most cases of malformation of the external meatus and middle ear the internal ear with its contained organ of hearing is intact. The absence of any conducting mechanism reduces the hearing to practically a non-existent level. If a new meatus can improve hearing for speech to about 3 feet (90 cm.), the addition of a window in the bony external canal, as in the fenestration operation for otosclerosis, may produce much better hearing. Such procedures have already been reported by Ombredanne (1944, 1947) and others, and the following case record confirms their value.

Case Report

A boy aged 5 was first seen at the Hospital for Sick Children on April 21, 1951. The outward appearance was as shown in the photograph. The pinna was malformed on the left side and the external auditory meatus absent. The right ear was normal. There was no perception of the spoken voice in the left ear with the right meatus occluded. So far as could be ascertained bone conduction by fork-testing was normal on the left side, but the child was not a good witness. X-ray investigation revealed the presence of semicircular canals.

It was thought justifiable in this case to attempt the restoration of hearing in the left ear by a modified fenestration technique. The matter was fully ex-

Photograph showing malformed pinna and absence of external auditory meatus.