# ACUTE INFLAMMATION AND ABSCESS FORMATION DUE TO A DIPHTHEROID BACILLUS

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IN October 1944 a diphtheroid bacillus was isolated which was studied because of the unusual clinical features of the case from which it was derived. Human infections caused by proved pathogenic diphtheroid bacilli are sufficiently rare to warrant recording, although in this case the investigation could not be completed owing to the bacillus losing its virulence.

## HISTORICAL

Infections caused by *Corynebacterium ovis* (Preisz-Nocard), *Corynebacteria pyogenes*, *renalis* and *equi*, and by *Listerella monocytogenes* will not be considered in this review as the organism was proved not to be any of these varieties.

Two groups of other pathogenic diphtheroids can be distinguished in the literature: those isolated from the human and those from the animal host. The latter group will be summarised first.

*Animal Infections.*—The first to describe an infection of mice by diphtheroids was Kutscher [1] in 1894. The causative organism produced nodules in the internal organs. The mice succumbed after a few days, but guinea-pigs, rabbits, cats, dogs and fowls were not affected.

In 1901 two laboratory infections were recorded. One, by Bongert,[2] whose strain was also very infective for mice and produced numerous small abscesses in various organs; but his strain was Gram-negative on isolation and only old cultures exhibited Gram-positivity. Rats, guinea-pigs, rabbits, pigeons, fowls and sheep were immune. His organism produced a toxin which was lethal for mice. Klein [3] in the same year reported the isolation of a Neisser-positive strain from rats, which was also pathogenic for guinea-pigs. Injected subcutaneously only a local infection resulted. The infection was not influenced in any way by 600 units of diphtheria antitoxin.

The strain of von Holzhausen [4] obtained from mice remained virulent for one year though cultured on artificial media. The organism was pathogenic for mice only, guinea-pigs, rats, rabbits and dogs remaining unaffected. Neither toxins nor local abscesses were produced, the mice dying of a septicæmia.

*Human Infections.*—One of the earliest recorded human infections due to a pathogenic diphtheroid bacillus is the case described by De Witt.[5] His patient was a girl of sixteen who suffered from a

generalised infection by a Gram-positive, Neisser-positive, but motile bacillus. This was pathogenic for guinea-pigs and also produced a toxin. The patient's blood agglutinated the organism up to a serum dilution of 1 : 50.

Parker,[6] in 1922, claimed to have isolated pathogenic diphtheroids frequently from the middle ear of cases suffering from otitis media, especially after scarlatina. His diphtheroids were Neisser-positive, required blood for their growth and caused a toxæmia in guinea-pigs, rabbits and mice. Diphtheria antitoxin (500 units) had no beneficial effect.

Gilbert and Stewart [7] recorded the isolation of a diphtheroid bacillus from the throat which caused necrosis in the guinea-pig after subcutaneous injection, and produced abscesses in liver and omentum after intraperitoneal injection. The organism produced a toxin which was only partially neutralised by diphtheria antitoxin. In a later paper [8] they suggested that the organism might be spread by infected milk.

Mair,[9] in 1928, published a note on a "strain of *B. diphtheriæ* showing unusual virulence for guinea-pigs," and his strain was included in the larger and very thorough study of Barratt [10] in 1933. Mrs Barratt was able to show that there are "aberrant" strains of *C. diphtheriæ* which will kill rats in 1-2 days after intraperitoneal injection and which are sometimes partly neutralised by the antitoxin of *C. diphtheriæ*, and sometimes by that of *C. ovis*. To quote her seventh conclusion: "Wherever the characters of the aberrant strain differ culturally or biologically from those of *C. diphtheriæ*, they approach, if not completely, those of *C. pseudotuberculosis ovis* (bacillus of Preisz-Nocard)."

The last paper relating to this subject which I was able to find was that of Barber, Guiseppi and Knott [11] in 1937, who record two cases, one of them previously published in 1920.[12] The first case was a subcutaneous infection by a diphtheroid bacillus which caused abscesses in the guinea-pig after subcutaneous injection, while another animal was protected against infection by diphtheria antitoxin. The organism was agglutinated both by the patient's and the guinea-pig's serum. The strain isolated from the second case caused necrosis after subcutaneous injection of the guinea-pig, but the animal could not be protected by diphtheria antitoxin.

## Investigations on the Isolated Bacillus

*Patient's History.*—The patient was a boy of 13 years, who was first seen by his doctor on 3.10.44. The latter found a pleural rub on the left side; the temperature was raised to 101·4° F., with a pulse rate of 100 and respirations 30 per minute. The patient was put on a course of sulphathiazole. The temperature fell and the pulse and respiration became normal. He was allowed to get up. On

7.10.44 the doctor was called in again. He found the boy very distressed in his breathing, the temperature subnormal and there were no signs of air entry over the left lung. In this condition the patient was admitted to the Ear, Nose and Throat Department of the Royal Infirmary, Edinburgh. Here it was found that the inspiratory distress was due to a complete right-sided paralysis of the larynx. There was a large bulge coming from the right sub-epiglottic region with an intensely red surface, but *not* covered by a membrane. A tracheotomy was performed. During the operation the cause of the swelling, which was bulging into the trachea and partially blocking it, was found to be an abscess with free pus, situated between the lower border of the cricoid, trachea and posterior sheath of the thyroid. The patient made an uneventful recovery. No growth was obtained from the pus of the abscess, though the direct films showed clusters of Gram-positive filaments which appeared to show branching. But cultures from the larynx, taken through the tracheotomy opening from the highly inflamed mucous membrane yielded a growth of diphtheroid bacilli on four different occasions (the last on 23.10.44). The first culture was pure, those obtained later mixed with *Staphylococcus aureus*, and later still with hæmolytic and non-hæmolytic streptococci.

*Source of Infection.*—Immediately a search was instigated as to the source of the boy's infection. It appeared that some six weeks ago he and a friend had acquired two rabbits, the larger of which had died three weeks before his illness. His friend and the surviving rabbit were healthy and no similar bacillus could be obtained from the latter.

*Description of Bacillus.*—The organism was a short Gram-positive bacillus measuring $2 \times 0{\cdot}3\ \mu$. It was non-motile, not spore-bearing, with a tendency to club formation, especially in older cultures, and also to branching. In fluid cultures the organism assumed a coccoid form. With the Neisser-stain polar bodies could be demonstrated in about 30 per cent. The bacillus was not acid-fast. It was a facultative anaerobe and grew best at 37° C. on all ordinary media including the tellurite plate, on which the colonies were large, smooth and black. No growth, however, was obtained on MacConkey's medium. On agar and Löffler slopes an abundant growth was obtained, smooth, glistening, slightly cream coloured, not unlike a staphylococcal growth.

Gelatin and coagulated serum were not liquefied. No hæmolysin against human and sheep red blood corpuscles was produced on solid or in fluid media. Nitrates were not reduced and no formation of $H_2S$ occurred. Indol was not produced and the phosphatase reaction was negative.[13] Litmus milk was not changed.

In Hiss' serum medium the organism fermented glucose, maltose, saccharose, lævulose, galactose and mannose with formation of acid only. Lactose was slightly fermented. No change occurred in

dextrin, starch, xylose, raffinose, inulin, mannite, salicin, dulcite and glycogen.

Its sensitivity to penicillin was about half of that of the Oxford *Staphylococcus aureus* strain.

The organism was readily emulsifiable and could be stored for a fortnight or longer at room temperature and in the refrigerator.

The principal characters of this bacillus are set forth in the Table and compared with those of the organisms of the same group.

*Serology.*—The patient's serum as well as the serum of the surviving rabbit failed to agglutinate the bacillus. Too much stress need not be laid on this as the infection was not a generalised one (*cf.* Hughes [14]). Twelve " normal " human sera, chosen at random, did not agglutinate the diphtheroid bacillus either.

Two rabbits were immunised by intravenous injections with live bacilli, and after six injections, given twice a week, their sera agglutinated the diphtheroid up to a serum dilution of 1 : 6400 at 37° C., and better at 56° C.

The following strains, obtained from the National Collection of Type Cultures, were not agglutinated by this serum. Three different strains of *C. ovis*, *C. flavidum* (No. 764), *C. murium* (No. 949), *C. murisepticum* (No. 807A), a diphtheroid strain type X (Daniels, No. 1929) and a *C. ovis* strain received from Dr Lovell. Of a great variety of diphtheroid bacilli, isolated from various sources, none was agglutinated by this serum. In addition, a Listerella strain, obtained from Professor Webb, was also not agglutinated.

*Animal Susceptibility.*—Rats, rabbits and cockerels, injected subcutaneously, intramuscularly, intraperitoneally, and/or intravenously with large numbers of live bacilli ($\frac{1}{2}$-5 slope cultures after 18-24 hours' incubation) proved to be refractory. It may be noted here that the blood picture in rabbits showed an increase in polymorphs, but no increase of mononuclears. Guinea-pigs were also immune, except for one animal which received the exceptionally large dose of 5 slope cultures intraperitoneally and died after 48 hours. No Straus reaction was observed. The post-mortem findings in this animal were those of a generalised purulent peritonitis and toxæmia. The organism was recovered in pure culture from the peritoneal fluid and the heart blood.

Otherwise mice proved the only susceptible animal, injection of $\frac{1}{2}$ slope culture proving invariably fatal within 24-48 hours after intraperitoneal or intramuscular injection. After subcutaneous injection the mice survived. The post-mortem findings were usually a purulent peritonitis after intraperitoneal injection, while twice after intraperitoneal as well as after intramuscular injection abscesses in the omentum were observed. Once a small liver abscess (3 mm. in diameter) was found, from which the organism was recovered in pure culture. The organism was recovered from the heart blood in all cases. No Straus reaction was obtained, and the adrenals never showed hæmorrhages.

| | Growth on Ordinary Media. | Type of Colony. | Colour of Culture. | Pathogenicity to Animals. | Adrenal Hæmorrhages. | Straus Reaction. | Protection by Diphth.-antitoxin. | Auto-agglutination. | Production of Hæmolysins. | Reduction of Nitrates. | Liberation of $H_2S$. | Indol Formation. | Fermentation of Milk. | Liquefaction of Gelatin & Serum. | Neisser Stain. | Production of Phosphatase. | Fermentation of: Glucose. | Maltose. | Saccharose. | Dextrin. | Starch. | Glycogen. | Lactose. | Xylose. | Raffinose. | Inulin. | Mannite | Salicin | Dulcite. | Mannose. | Lævulose. | Galactose. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. diphtheriæ* . . | + | Opaque discs, granular | Greyish-white | Guinea-pig, rabbits, birds | + | − | + | − | ± | ± | ... | − | − | − | + | − | + | ± | − | ± | ± | − | ... | ... | ... | − | ... | ... | ... | + | ± |
| *C. pyogenes* . . . | − | Minute, dew-drop like | Smoky-brown or bluish-white | Domestic animals: rabbits when injected intravenously | ... | ... | ... | + | + | − | − | ... | AC | + | − | + | + | + | ± | + | ... | − | + | ± | − | − | − | − | ... | + | + | + |
| *C. ovis* (Preisz-Nocard) . | ± | Small, thin, dry or large, friable | Greyish-white or creamy-orange | Guinea-pig, rats, rabbits | − | + | ± | + | ± | − | − | − | − | ± | + | ± | + | + | ± | ± | ... | − | − | ... | − | − | − | − | − | + | + | + |
| *C. renalis* . . . | + | Moist, small raised | Greyish-white or cream | Cattle | ... | ... | ... | + | − | − | − | ... | AC | ± | + | ... | + | − | − | ... | ... | − | − | ... | ... | ... | − | ... | ... | ± | ± | ... |
| *C. equi* . . . | + | Viscid, small | Red | Horse | ... | ... | ... | + | − | + | − | ... | ... | ... | ... | ... | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Aberrant diphtheria bacilli (Barratt) | + | Small, thin or opaque, thick | Colourless, later cream | Guinea-pig, rats | ± | ... | ± | ... | ... | ... | ... | ... | ... | + | + | ... | + | + | − | ... | + | ... | ... | ... | ... | ... | − | ... | ... | ... | ... | + |
| *C. ulcerans* (Gilbert and Stewart) | + | As *C. diphtheriæ* | | Guinea-pig, rabbits | − | ... | ∓ | ... | ∓ | ... | − | − | − | + | + | ... | + | + | − | + | ... | ... | − | ... | ... | ... | − | ... | ... | ... | ... | ... | ... |
| *C. murium* (Kutscher) . | + | Small, round | Whitish-grey | Mice | ... | ... | ... | ... | ... | ± | − | − | ∓ | − | ... | ... | + | + | + | − | ... | ... | − | − | ... | ... | ... | ... | ... | ... | ... | − |
| *C. murisepticum* (von Holzhausen) | + | Small, thin | Colourless | Mice | ... | ... | ... | ... | − | ... | + | − | A | ... | − | ... | + | + | + | ... | ... | ... | + | ... | ... | + | + | ... | ... | ... | + | + |
| *Listerella monocytogenes* | + | Very small, dew-drop like | Transparent, later whitish | Rabbits, guinea-pigs, mice, sheep | ... | ... | ... | − | ∓ | + | ... | ... | A sl. | − | ± | + | + | ± | ± | ... | ... | ± | ± | − | − | − | − | ± | − | ... | ... | − |
| *C. flavidum* . . . | + | Wrinkled | Yellow | Laboratory animals | ... | ... | ... | ... | ± | − | ... | − | A sl. | − | ... | ... | + | + | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| Own strain . . . | + | Round, moist, viscid | Cream | Mice, feebly for guinea-pig | − | − | − | − | − | − | − | − | − | − | + | − | + | + | + | − | − | − | + sl. | − | − | − | − | − | − | − | + | + |

To illustrate the post-mortem findings the following histological reports are given :—

1. Two mice, injected intraperitoneally with ½ slope culture. Death occurred within 48 hours. Both mice show essentially the same changes. The most definite finding is an exudate on the peritoneal surfaces of liver and spleen. This contains polymorphs and mononuclears, necrotic cells and very large numbers of bacteria ; little, if any fibrin. The bacteria are short, Gram-positive rods ; a few forms show bipolar granules when stained by modified Neisser method.

The liver parenchyma shows parenchymatous degeneration. A superficial strip of liver tissue over a small area in mouse II is necrotic. Gram-positive bacilli are found singly and in groups throughout the sections, some in von Kupffer's cells, some apparently lying free.

The spleens and lungs show only active congestion with a little hæmorrhage in the lung of mouse I, and some œdema in the lung of mouse II. A few Gram-positive bacilli were seen in both spleens, none in the lungs.

None of the sections shows abscesses or areas of focal necrosis.

2. Mouse, injected intraperitoneally, death within 48 hours. The spleen apart from perhaps slight congestion of the pulp, is normal. In particular no abscesses are present within its substance, but a fair number of such lesions are adherent to the outer aspect of its capsule.

The liver shows slight irregular congestion and slight cloudy swelling. No abscesses occur in its tissue, but two or three small suppurating lesions occur immediately to its capsule.

The abscesses on the surface of the spleen and liver contain large numbers of Gram-positive and Neisser positive bacilli.

3. Mouse, died two months after intraperitoneal injection with 1/15 slope culture.

Both specimens are portions of fibro-fatty tissue (omentum ?). Very extensive areas of necrosis are present in the two specimens with moderate round cell infiltration at the periphery of the necrotic material. Numerous Gram-positive and Neisser-positive, very pleomorphic bacilli are present.

*Toxin Production.*—When freshly isolated the bacillus produced a filterable toxin. After three weeks' growth in broth 1 c.c. of the filtrate injected intraperitoneally killed mice within 24 hours. No other animal proved to be affected, even after injection of 5 c.c. The mice could not be protected by 500 units of diphtheria antitoxin.

It was decided to execute similar experiments with *C. ovis* antitoxin. Unfortunately, by the time a virulent strain of *C. ovis* had been procured and a toxin and subsequently an antitoxin had been produced, the bacillus, subcultured on artificial media, appeared to have lost its virulence as well as its powers of toxin production. The original toxin, though kept in the refrigerator, had deteriorated also by that time.

## Discussion

Though the organism was not grown on culture from the pus of the patient's abscess—of which only one specimen was received—it was found to be present in large numbers in the highly inflamed

tracheal mucosa. The same phenomena of branching and cluster formation, as seen in the original pus, were observed in old cultures. It can, therefore, be assumed that this diphtheroid bacillus was the cause of the abscess formation. This opinion is strengthened by the fact that the organism, when injected into mice, caused the formation of small abscesses of varying sizes, though admittedly frequently only of microscopical dimensions. However, larger abscesses did occur.

Identification of this diphtheroid bacillus offered difficulties. It was not a diphtheria bacillus, as, besides other criteria, no protection was obtained by the administration of diphtheria antitoxin.

The possibility of its being a Listerella could be ruled out on the ground of a too luxuriant growth, non-pathogenicity for rabbits and absence of motility, and no production of monocytosis, while serological reactions were negative.

As the bacillus grew well on ordinary media the possibility of it being a *C. pyogenes* strain need not be considered.

It was equally not possible to regard it as a *C. ovis* strain, as it possessed little pathogenicity towards guinea-pigs and none towards other laboratory animals, produced no Straus reaction and, *in vitro*, showed no auto-agglutination, and other negative qualities.

The literature as regards *C. murium* (Kutscher) is scanty. The main objection against its being regarded as a murine strain is the fact that it proved pathogenic for the guinea-pig, though only in very large numbers.

In all probability, if the last objection is recognised, this diphtheroid bacillus represents an " aberrant " diphtheria strain; for this suggestion, unfortunately, the last and most valid proof is missing—the protection by *C. ovis* antitoxin.

The bacillus has been added to the National Collection of Type Cultures and has been given the number 6965.

## Summary

A diphtheroid bacillus is described, isolated from the tracheal mucosa of a boy suffering from an extra-tracheal abscess formation, which proved to be virulent for mice, feebly virulent for guinea-pigs, and produced a toxin which was not neutralised by diphtheria antitoxin.

Though conclusive proof could not be brought forward, various properties indicate that the organism has to be regarded as belonging to, or being related to, the " aberrant " diphtheria bacilli.

I wish to thank Dr W. R. Logan, bacteriologist to the Infirmary, for his constant help and advice, Dr St John-Brooks for kindly sending me different strains from the National Collection of Type Cultures, and Dr Lovell for his *C. ovis* strain. Thanks are also due to Dr Simson Hall for his permission to make use of the clinical notes, and to Dr R. F. Ogilvie for his permission to quote the histological reports. I also wish to acknowledge my indebtedness to Professor Webb for his co-operation.

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