

## 1. ACTION OF METRONIDAZOLE

Metronidazole is a drug which was initially developed to treat infections caused by *Trichomonas vaginalis* - a parasitic protozoan. Later it was found to be active against intestinal amoebae and *Giardia* and subsequently its selective toxicity for anaerobic bacteria was recognised. It is one of a group of nitroimidazole compounds.

Its antimicrobial action involves:

1. Diffusion into the cell.
2. Activation of the drug.
3. Action on cell DNA.

The selective action of metronidazole against anaerobes is explained by the fact that activation requires reduction of the nitro group and aerobes and facultative anaerobes do not have enzymes which can produce a low enough redox potential to do this. The important enzymes are those involved in ketoacid metabolism (e.g. those required for oxidative decarboxylation of pyruvate). In anaerobes the electron transport enzymes are ferredoxin and similar proteins ( $E_0 =$  about -400mV) which are able to reduce metronidazole. Pyruvate metabolism in aerobic organisms and in mammalian cells involves pyruvate dehydrogenase with NAD/NADH coenzymes ( $E_0 =$  -320 mV). These are unable to activate metronidazole.

It has been shown that, once reduced, the drug binds to DNA under anaerobic conditions and causes strand breakage and degradation of the molecule. It also inhibits an important DNA repair enzyme. These effects lead to cell death within 2-3 generations. Naturally acquired resistance to metronidazole is extremely rare.

## 2. USE OF GAS-LIQUID CHROMATOGRAPHY IN IDENTIFICATION OF ANAEROBIC INFECTIONS

Although conventional methods of identification such as use of a Gram-stained film, colonial appearance and utilisation patterns of carbohydrates are of value in identifying anaerobic bacteria, they are not sufficient on their own to allow accurate identification of all species.

The obligate anaerobic bacteria differ from aerobes by producing a variety of volatile fatty acids, short chain carboxylic acids and low molecular weight alcohols as products of the metabolism of carbohydrates and amino acids. The production of volatile fatty acids in particular is characteristic of obligate anaerobes and these can be detected and identified quickly and easily by gas-liquid chromatography, though this technique is rarely used routinely.

Gas-liquid chromatography has two main applications in routine anaerobic bacteriology work:

1. Identification of pure cultures grown *in vitro*. Because the different genera and species of anaerobes vary in the range of volatile fatty acids produced and because these patterns of metabolic end products are stable and reproducible, they are of value in identification.
2. Detection of anaerobes in clinical material. Anaerobes produce their characteristic fermentation products *in vivo* as well as *in vitro*. Thus the detection of volatile fatty acids in pus is a rapid method of detecting anaerobic infection. By contrast pus from infections due to aerobes or facultative anaerobes contains either no detectable volatile fatty acids, or acetic and propionic acids only.

## 3. GAS GANGRENE

This occurs when spore-forming anaerobes of genus *Clostridia* infect severely damaged skeletal muscle. The lesions become infiltrated with gas bubbles (predominately H<sub>2</sub>) produced as a product of bacterial fermentation. Toxins cause massive necrosis of muscle fibres into which the organisms spread.

The *Clostridia* (see below) are found as saprophytes in soil and in the alimentary tracts of man and animals. Some species are opportunistic pathogens. Infections are most likely to be dangerous when the blood supply is poor and/or the wound is infected with facultative anaerobes such as *E. coli*, *Staphylococci* and *Streptococci* which further lower the redox potential. Gas gangrene can develop in 6-72 hours after infection. Treatment is radical surgery, antitoxin therapy, antibiotics and nursing in a hyperbaric, oxygen-rich atmosphere (>3 atmospheres).

The important gas gangrene bacteria are *Clostridium perfringens* (syn.: *Clostridium welchii*), *Clostridium septicum* and *Clostridium oedematiens* (syn.: *Clostridium novyi*). Each produces its own array of toxins. Identification of the organisms present in a gangrenous wound is important if the correct antitoxins are to be given. In practice *Clostridium perfringens* is found in about 70% of cases. Mixed infections are also common. Gram-negative facultative anaerobes may cause gassy cellulitis resembling gas gangrene. Quick and efficient differential diagnosis is required if the patient is to be spared the radical surgery which only clostridial gas gangrene calls for.

**4. CLOSTRIDIA** are Gram-positive spore-forming anaerobic rods. The species are differentiated by :

1. Colonial phenotype.
2. Shape and position of the spore.
3. Metabolic tests: carbohydrate fermentations; volatile fatty acid production (by GLC).
4. Immunological recognition of toxins and somatic antigens.

The species examined in this class are :

*Clostridium sporogenes*. "Medusa-head" colony. Opaque. Haemolytic. The oval spores are sub-terminal and bulge the vegetative cell. Markedly proteolytic (hence smell of H<sub>2</sub>S from putrefying flesh). Non-toxigenic.

*Clostridium perfringens*: Flat, almost circular colony. Haemolytic, with two zones of lysis, due to the  $\alpha$  and  $\tau$  toxins. Produces oval, central spores (found *in vivo*, but only seen on special medium *in vitro*). Many toxins. The species is separated into Types A-->E depending upon toxin spectra. Type A is the usual gas gangrene organism in man. Types B-->E are opportunist pathogens in sheep and cattle. Type C is a rare cause of lethal necrotic enteritis in man.

#### Toxins of Type A *Clostridium perfringens*

1. Most important is  $\alpha$  toxin (lecithinase, or phospholipase C) which is Ca<sup>++</sup> or Mg<sup>++</sup> dependent. It splits lecithin into diglyceride and phosphorylcholine. The toxin is lethal, necrotising and haemolytic.
2. A collagenase, hyaluronidase and DNAase are also formed.
3.  $\tau$ -toxin : causes haemolysis on horse blood agar. Theta-toxin also cross-reacts antigenically (as it has epitopes in common) with streptolysin O and with tetanolysin.

*Clostridium tetani*. A highly motile organism, the colonies are surrounded by wispy swarms of bacteria. It has round, terminal spores. No common sugars are metabolised. Tetanus toxin is a protein monomer of 68,000 daltons. It blocks spinal inhibitory synapses giving local or generalised muscle spasm. Death is due to respiratory muscle spasm (remember "tetanic contractions" from your work in physiology - where muscle never rests).

Other pathogenic *Clostridia* include *Clostridium botulinum*, one of the organisms that can cause toxic food poisonings. *Botulinum* toxin is one of the most dangerous toxins known. It is formed

when foodstuffs, contaminated with *Clostridium botulinum* from soil or faecal material, cool slowly under anaerobic or microaerophilic conditions such as found in the depths of stews, fish dishes and in home-preserved jars of meat and vegetables or in tins of food that have been perforated and contaminated. The preformed toxin is ingested when these foodstuffs are eaten cold or when inadequately heated. Thorough re-heating to the boiling point denatures the toxin and renders the food harmless. The spores do not germinate in preserved fruit where the pH is less than 4.5. Occasionally spores may germinate in alkaline water or swamps, and toxin is then formed. Birds on the Norfolk Broads were poisoned by botulinum toxin during a drought and heat wave which occurred during the summer of 1976.

## **5. ANAEROBIC CULTURES:**

An airtight jar containing a catalyst is now commonly used to grow anaerobic bacteria in conjunction with a "Gaspak". The latter is a disposable H<sub>2</sub> generator which allows all the oxygen in the jar to be combined with the hydrogen. CO<sub>2</sub> is formed as well; many bacteria grow better in increased CO<sub>2</sub> concentrations.

Anaerobic cabinets with piped gases are used in many laboratories.