1. **Clostridium (spindle):** Anaerobic or microaerophilic rods, producing endospores, which are usually wider than the vegetative organisms in which they arise - so-called clostridium forms. Generally gram positive; often decompose protein media through agency of enzymes and often ferment carbohydrates. Many species are pathogenic.

Prior to introduction of McIntosh and Fildes' jar during Great War pure cultures very difficult to obtain and only *Cl. tetani* and *Cl. botulinum* well-recognised through very potent exotoxins.

**Ecology:** Soil, faeces, therefore dust. - Habitat: protein + vegetables. They adapt themselves to soil quickly and become very difficult or impossible.

"Involution forms" common.

Morphology: Pleomorphic; identification on morphology alone impossible.

Sporulation: common to all, but varying in facility.
1. equatorial or subterminal: clostridium or club-shaped
2. oval terminal: tennis racquet
3. spherical terminal: drum-stick

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Motility: Nearly all motile: but not *Cl. welchii*.

Capsules: *Cl. welchii* in animal body and in media containing serum.

**Cultural reactions:**
- Rather slow on solid media - often spreading.
  - *Cl. tetani* - for isolation.
  - *Cl. welchii* - low convex with entire edge.
  - *Cl. sporogenes* - may be unbonate with flat periphery.
  - *Cl. oedematosis* - for *Cl. tetani*.

**Glucose agar shake cultures**: a method of isolation: deep colonies.
- Rounded: biconvex: or woolly, *Clostridium botulinum*.
- Blood agar: Haemolysis: or 34, and often soluble haemolysis. *Cl. tetani*.
- Cooked meat: Fluid - turbid. may be gas.
- Proteolytic: digest meat: may turn it black and give nasty smell.
- Saccharolytic: do not digest meat: often turn it pink.

**Gelatin:** growth usually poor.

**Resistances:**
- *Cl. tetani* - fir tree - later liquefaction.
- Very varying resistance of spores to heat, drying and disinfectants.
- Spores of *Cl. botulinum* withstand boiling for 3-4 hours at 105°C. Takes 100 mins. to kill.
- " *Cl. welchii* - killed by boiling in 5 mins.
- " *Cl. sporogenes* - can withstand 5% phenol 8 days.
- " *Cl. tetani* - live for years in dried earth.
3. Metabolism - low Oxidation-Reduction Potential required. in medium.

Biochemical - Reactions irregular - repeat several times to make certain: much gas formed from peptone water or casein water.

Antigenic Structure

H' antigen thermostable - ? type specific
"O" thermostable - ? group specific
Cl. sporogenes, 2 serological groups
Cl. tetani 7
Cl. botulinum 7-8
Cl. welchii wide distribution of receptors.

Toxins: Tetanus. 0.25 mgm. calculated to kill a man
Botulinum 0.0084 mgm. " " " "
Bacterial free filtrate of Cl. botulinum inoculated into sterilized skimmed milk and incubated 4 days at 37°C. increase in toxicity - ? due to more toxin produced by enzyme.

4. Tetanus toxin: one only. 0.00001 cc. of filtrate may kill a mouse. destroyed at 65°C, in 5 mins.
not absorbed from alimentary tract.
can be kept for years dried, in dark at 5°C.
can be modified by formolin.
combines with acid is neutralized by specific antitoxin.

Botulinum toxin more resistant to heat and acids
60°C. destroys it within 1/2 hr.
not destroyed by N HCl in 24 hrs.
only toxin which is absorbed from alimentary tract.
three toxins each from different serological strains.

Toxins of Cl. welchii: Cl. septique: Cl. oedematiens
all destroyed by heat at 70°C. for 30-90 mins.
" " " weak concentrations of acid
lead to gelatinous oedema on inoculation.
4 different types of welchii toxin, each with specific antitoxin
but not all strains produce all 4 toxins.

Pathogenicity: depends almost entirely on toxin production.
Cl. tetani multiplies locally and does not invade
Cl. botulinum of even a parasite
Cl. oedematiens remains almost confined to site of inoculation
Cl. welchii and Cl. septique do invade but only in final stages.

Thermophilic clostridia - spoilage in non-acid canned goods in U.S.A. known as "hard swell".
optimum temperature for growth 50-60°C.
very weak proteolytic action: ferment carbohydrates.
non-pathogenic when fed to rats.

Cl. sporogenes ? not naturally pathogenic
enhances pathogenicity of other anaerobes - e.g. Cl. welchii.
No exotoxin, but broth culture may kill guinea-pig in 1 cc. dose.

Cl. oedematiens a cause of gas-gangrene in man:
causes one type of Braxy in Europe
Black disease in Australia.

Cl. septique gas-gangrene in man
Black leg and braxy in sheep: sometimes blackleg in cattle.
Cl. wolchi is the chief agent of gas-gangrene in man and animals. It may play part in enteritis, appendicitis, puerperal fever, intestinal obstruction.

- "B" type - Lamb dysentery (Border region).
- "C" type - "Struck" - enteritis in sheep (Kent).
- "D" type - entero-toxemic disease (West Australia) and pulpy kidney of sheep (N. Zealand and Wales).

Cl. tetani is the natural pathogen for man and horse. Experimental pathogen for mice, guinea-pigs, and rabbits. Birds resistant.

Braxy = inflammation of 4th stomach (sheep).
Black disease = liver necrosis.
Blackleg = crepitant fluctuating swelling of quarter to death.
An Antigen is any substance that, when introduced parenterally into the animal tissues, stimulates the production of an antibody, and when mixed with that antibody reacts specifically with it in some observable way.

An Antibody is any substance that makes its appearance in the blood serum or body fluids of an animal in response to the stimulus provided by the parenteral introduction of an antigen into the tissues, and, when mixed with that antigen reacts specifically with it in some observable way.

Antigens are usually, but not always, proteins; though many non-protein substances act as partial antigens, or haptens, reacting specifically with the corresponding antibodies, but failing to stimulate antibody-production in vivo.

Antibodies may be regarded as special kinds of serum globulins, endowed with specific chemical groupings that react with specific groupings on the corresponding antigen.

Examples of antigen-antibody reactions:

- Agglutination
- Precipitation
- Lysis (with complement)
- Complement fixation
- Opsonification (to phagocytosis by leucocytes)
- Toxin-antitoxin reaction

All these reactions are fundamentally similar, in that they depend on the specific union of antibody-globulin to the antigen. The antigen may be in solution, or attached to a bacterium, red-cell, etc. The actual reaction observed depends on the nature of the antigen concerned, and on other secondary reagents or factors.

The Antigenic Structure of Bacteria.

Studied by (a) Direct agglutination
(b) Agglutinin absorption
(c) Extraction of antigens from bacterial cells, and their study by precipitation
(d) By (c) associated with chemical methods of analysis
(e) By the study of bacterial variants
Any one bacterial species has many different antigens

Examples

(a) **Typhoid-paratyphoid group**
- Flagellar (H) antigens - type and group
- Somatic (O) antigens - polysaccharide
- Various protein antigens

(b) **Pneumococci**
- Capsular (polysaccharide) antigens - type specific
- Various protein antigens

(c) **Haemolytic streptococci**
- Type specific antigens
- Group antigens (polysaccharide)
- Various protein antigens

**N.B.** Agglutination and agglutinin-absorption tests depend mainly on those antigens that are situated at the bacterial surface.