R34.17

TETANUS and GAS GANGRENE

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.

Oecology: Soil, faeces, therefore dust. - Habitat probably soil. Clostridia ... they adapt themselves in intestines

Soil + faeces + dust milk + sewage

Morphology: Pleomorphic; identification on morphology alone difficult or impossible.
"Involution forms" common.
Large 3-8 x 0.4-1.2 . striaght or curved: rods.
singly: pairs: chains: bundles.

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

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

Capsules: Cl. welchii in animal body and in media containing serum. & Cl. butyricum capsulated?

2. Staining: Young cultures uniformly Gram-positive; later - very irregular. 3-4 days.

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. oedematiens
Cl. septique

Glucose agar shake cultures: a method of isolation: deep colonies rounded: biconvex: or woolly. Medium often disrupted & blown up c gas.

Blood agar. Haemolysin α or β. and often soluble haemolysin. → Cl. tetani, Cl. welchii, Cl. septique, oedematiens, Cl. chauvoei

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.
Cl. tetani: fir tree - later liquefaction.

Resistance: 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.

Resistance Septique

3. Metabolism - low Oxidation-Reduction Potential required. in medium eg Cooked meat - little oxygen only near top due to unsaturated fatty acids which take up oxygen and glutathione
Biochemical - Reactions irregular - repeat several times to make certain: much gas formed from peptone water or casein water.

Antigenic Structure
Tendency to auto agglutinate
"H" antigen thermolabile - ? 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.
Large dose of antitoxin - intravenously & intramuscular
Botulinum toxin more resistant to heat and acids
80°C. destroys it within ½ hr.
not destroyed by N HCl in 24 hrs.
? only toxin which is absorbed from alimentary canal.
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 ot 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.

Colony.

Stormy fermentation milk 12-48 acid, gas, clot.

Cl. welchii chief agent of gas-gangrene in man and animals.
4 different types of toxin.
may play part in enteritis: appendicitis: puerperal fever: intestinal obstruction.
"B" type - Lamb dysentery (Border region).
"C" " - "Struck" - enteritis in sheep (Kent)
"D" " - entero-toxaemic disease (West Australia) and pulpy kidney of sheep (N.Zealand and Wales).

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

anaerobic. parent colony diffuse - surface film.

Braxy = inflammation of 4th stomach (sheep)
Black disease = liver necrosis.
Blackleg = crepitant flucuating 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

Bacteriophage

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.