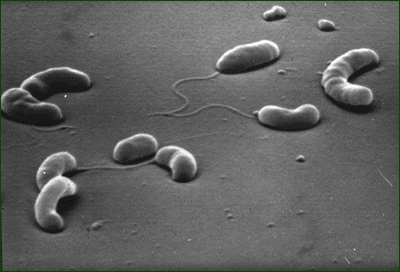
**Lec 6:**

**Cholera Toxin ( CT )**

**The causative agent of cholera**

**Man usually contracts cholera from water and food which had been contaminated by feaces of cholera victim .**

**The causative bacteria is Vibrio cholera .**

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**The injested bacteria multiply in the alimantay tract and after 2 – 5 days cause the sudden onset of :**

**nausea , vomiting , acute diarrhoea watery non – bloody ( rice water stool ) .**

**In sever cases , the lose of fluids from the gut in the from of liquid referred to as (( Rice water stool )) , may be as great as 20 Liters perday i.e. , 1L each 1 hour , leading to dehydration . Death may occur with in few hrs .**

**Without a dequate therapy , the mortality rate about 60%.**

**Mortality reduced to 1% by intravenous fluid replacement .**

**Cholera toxin ( CT ) causes diarrhea by stimulating adenylate cyclase activity in the intestinal tract .**

**Structure of CT**

**Cholera toxin and E. coli heat labile toxin ( LT ) consist of a single catalytic A subunit and 5 B subunits .**

**CT is an aligomeric protein of 84 KDa composed of one A subunit ( 29 KDa ) and 5B subunit ( 11. , KDa each ) .**

**CT A is synthesized as a single protein that is later proteolytically nicked to produce 2 polypept idees :**

**CTA1 and CTA2 which remain covalently linked by single disulfide bond ( s – s ).**

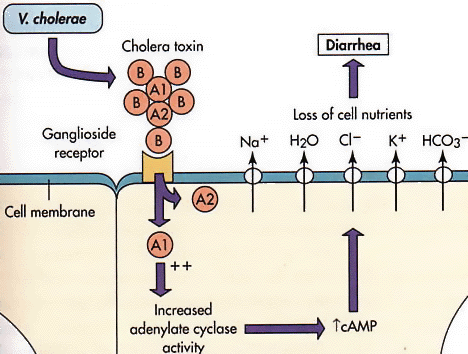
* **The five B subunits form a ring structure ( a ) , into which the A subunit partially insert s ( b ) .**

**Mode of entry :**

**To enter the host cell ,**

* **CT most first binds to gangliosides , B subunits bind to specific receptors.**
* **Induces a conformational changes in these subunits and their insertion into the membrane.**
* **Create a hydrophploic chanal through which the A subunit can diffuse into the cell.**
* **Here , thiol agents reduce the disulfide bonds , allowing fragment A1 to diffuse into the cytoplasm and activate the adenylate cyclase.**

**Binding of toxin to the receptors on the surface of human ileal mucosal cells is followed by entry of fragment A1 which interact with adenylate cyclase complex on the basal membrane inhibiting the GTPase mediated turn off the cyclase , increased c AMP levels .**

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**CT : Cholera Toxin .**

**Amp : Adenosine Mono phosphate .**

**AC : Adeuylase cyclase .**

* **The receptor for CT is the glucolipid ganglioside GM1 .**
* **The six subunit holotoxin is internalized by receptor mediated endocytosis ( RME ) .**
* **The A subunit is proteolytically nicked to generate fragment A1 , which carries the enzymic activity and delivered to the cytoplasm**
* **A1 fragment is an NAD – dependent ADP – ribosyltransferase .**
* **CT adds the ADP – ribose moiety to the - subunit of G. protein .**
* **G protein is a heteromeric protein which involved in stimulation of Adeuylate cyclase ( AC ) .**
* **As a result , cyclic AMP ( cAMP ) increase there by activity of protein kinase A , which inturn leads to supranormal phosphorylation of chloride channals in apical epithelial cell membrane .**
* **Chloride secretion is stimulated while sodium absorption is inhibited leading to serious disturbance influids and electrolytes leading to manifestation of diavrhoes .**

**Thermolabile enterotoxin of E. coli E coli heat toxin**

**( LT )**

**Immunogenic toxin consisting of 2 proteins :**

1. **MW = 11.500 dal. ( same size as ( Y ( B ) sub. )**
2. **MW = = 25.500 dal. ( some size to fragment A . )**

**LT is antigenically similar to CT so that antibodies prepared against either cross neutralize both toxic activities .**

**Like CT , LT possesses adenylate cyclase activity stimulation .**

**LT differs functionally from CT in itʼs lacking of fragment A2 - like moiety .**

**Unlike CT ganglioside ( GM1 ) does not inactivate the LT , this suggests different binding site on gun epithelium .**

**Assay of Enteroxins**

1. **Ligated segments of the small intestine in the living rabbit .**
2. **Assayed by determination their ability to activate adenylate cyclase in number of tissue cell lines.**