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**ACTION OF CHOLERA TOXIN**

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Cholera toxin, a potent activator of adenylate cyclase in almost all types of eukaryotic cell, is made up of two components: one subunit A (itself two polypeptide chains, A1 (mol. wt 22 000) and A2 (5 000)), and five subunits B (11 500). Subunit B binds to ganglioside GM1 on the outer membrane of susceptible cells, and, in some way not understood, this allows the A1 chain to catalyse a presumably cytoplasmic reaction: the ADP-ribosylation of a GTP-binding protein in the cyclase complex. Toxin in which the subunits had been covalently cross-linked remained active. Proteolytic fragments of the toxin were active, but experiments using 3H-labelled toxin suggested that they were not formed in vivo enough to account for the activity. Toxin bound to nylon strips was not active even with lysed cells. Pigeon erythrocytes whose membranes had been artificially depleted of cholesterol, so increasing their fluidity, showed a much shorter than usual lag phase before cyclase activity increased. These and other observations suggest that the entry of some active part of A1 may be a comparatively non-specific process. The activity of the toxin in rat liver membranes has been studied. No cytoplasmic components are required. Studies with 32P-NAD show that only a few proteins are ADP-ribosylated; the molecular weights of the major bands were 43 000 and 49 000. Reconstitution experiments suggested that these were regulatory, not catalytic components. The reaction is not reversible. In a solubilized system, the toxin seems to act as an inhibitor of endogenous ADP-ribosylation.

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**RECONSTITUTION OF HORMONE RECEPTOR FUNCTION USING SOLUBILIZED COMPONENTS.**

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Our earlier studies demonstrated that hormone receptors can be transplanted from one adenylate cyclase system to another by membrane fusion. By this means it was revealed that the many different hormone receptors which activate adenylate cyclase systems are all interchangeable. The ability to couple the hormone receptor to a foreign adenylate cyclase permitted chemical and physical manipulations of the receptor even if these inactivated the other components of the adenylate cyclase system. It thus became possible to work out the solubilization of a β-adrenergic receptor which would not only demonstrate ligand binding but would actually function in activation of adenylate cyclase when implanted in a naive system. Recently the β-adrenergic receptor (R) and the guanyl nucleotide binding component (G) from turkey erythrocytes were obtained in separate soluble fractions. R and G could be recombined at desired ratios to produce a hormone responsive system. In presence of hormone and Gpp(NH)p a persistently active G (G*) was produced. The amount of G* formed was measured by the extent of adenylate cyclase activation which it produced when implanted in a membrane containing the catalytic unit of the enzyme. Kinetic studies indicate that the encounter of R with G, in the membrane, occurs much faster than the R induced activation of G. The interaction of G and G* with R is being further studied in the hormone responsive reconstituted system.