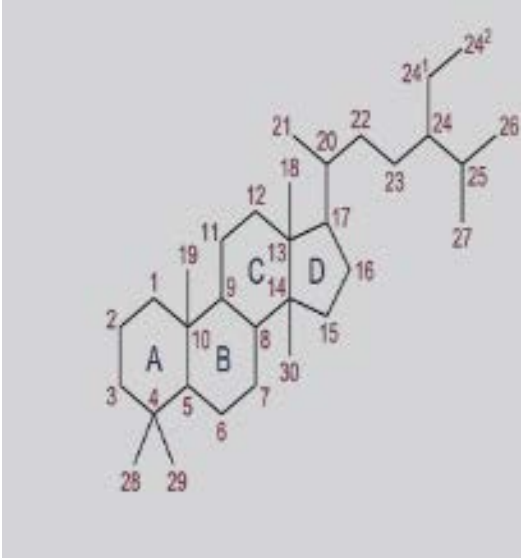
Steroids biotransformation

By – Dr Ekta Khare

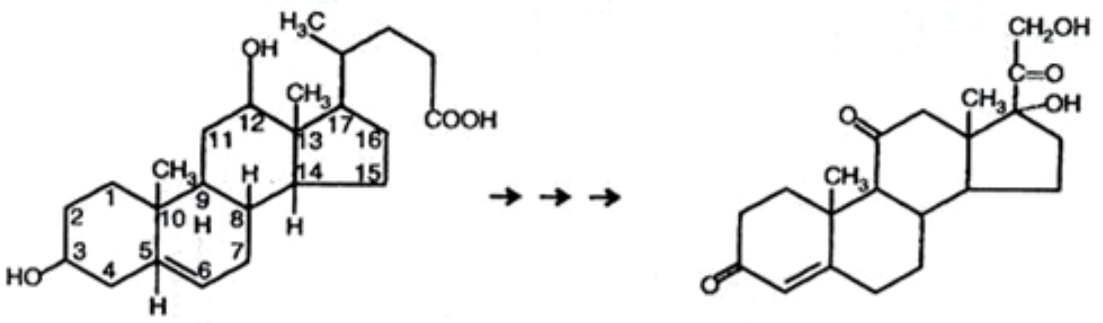
Steroids

- Steroids represent a class of natural products with diverse therapeutic properties. Steroids are also known as cyclopentonoperhydrophenanthrene.
- Steroids are organic molecules with a characteristic molecular structure containing four rings of carbon atoms synthesized in steroidogenic tissues.
- About more than 250 steroids and its derivatives exist in:
  - Insects (eg. Ecdysteroids)
  - Plants (eg. Phytosterols, diosgenin)
  - Vertibrates (eg. Cholesterol; corticosteroids: glucocorticoids, mineralocorticoids; sex hormones: androgens, estrogens; bile acids)
  - Fungi (eg. Ergosterol, ergosteroids)
- It has been observed that minor changes in the molecular structure of steroids can affect their biological activity.
- Hence numerous research have been conducted to improve the activity of existing steroid compounds and to synthesize novel steroidal compounds with pharmacological activity, and thus the most significant area of these research is the transformation of steroids using biocatalysts.



Transformation of steroids

- Transformation of steroids means conversion of precursor steroids to important drug intermediates and further conversion of these intermediates to active compounds by simple chemical or microbial processes.
- The chemical synthesis and transformations of steroids requires multiple steps and makes the use of reagents that have health risks and cause serious environmental disposal problems.
- **Example: Cortisone steroid can be synthesized chemically from deoxycholic acid. This process requires 37 steps many of which must be carried out under extreme condition of temperature and pressure with the resulting product costing over $200 per gram.**

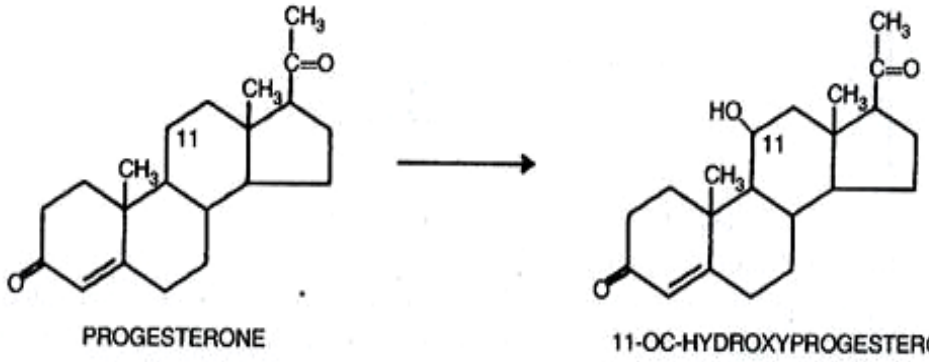


DEOXYCHOLIC ACID

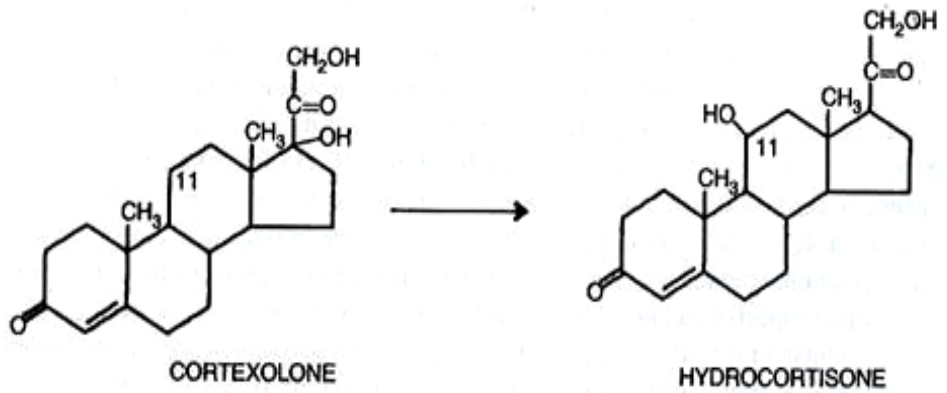
CORTISONE

Biotransformation

- Biotransformation could be defined as the modification of an organic compound into a recoverable product by chemical reactions catalysed by enzymes originating from a biological system.
- The biotransformation of steroids is one of the most important microbial processes that are highly regio- and stereospecific, involving chemical modifications (e.g. oxidation, reduction, hydrolysis, isomerisation, epoxidation, etc.) to the parent steroid which are catalysed by the microbial enzymes.



PRODUCTION OF 11-OC-HYDROXY PROGESTERONE BY HYDROXYLATING PROGESTERONE THROUGH *RHIZOPUS ARRHIZUS*



HYDROXYLATION OF STEROID CORTEXOLONE THROUGH *CUNNINGHAMELLA BLAKESLEEANA* OR *CURVULARIA LUNATA* FOR PRODUCTION OF STEROID HYDROCORTISONE

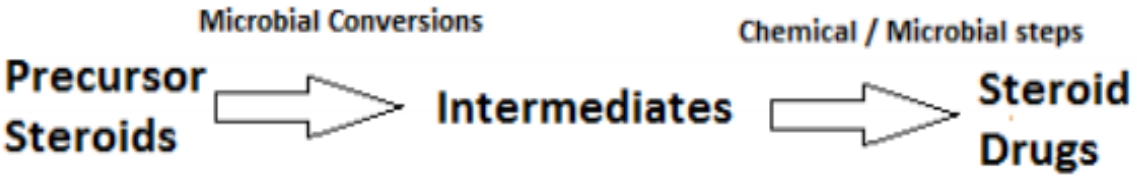
Fig. 22.5. Steroid Biotransformation

Types of transformations catalyzed by microbial enzymes

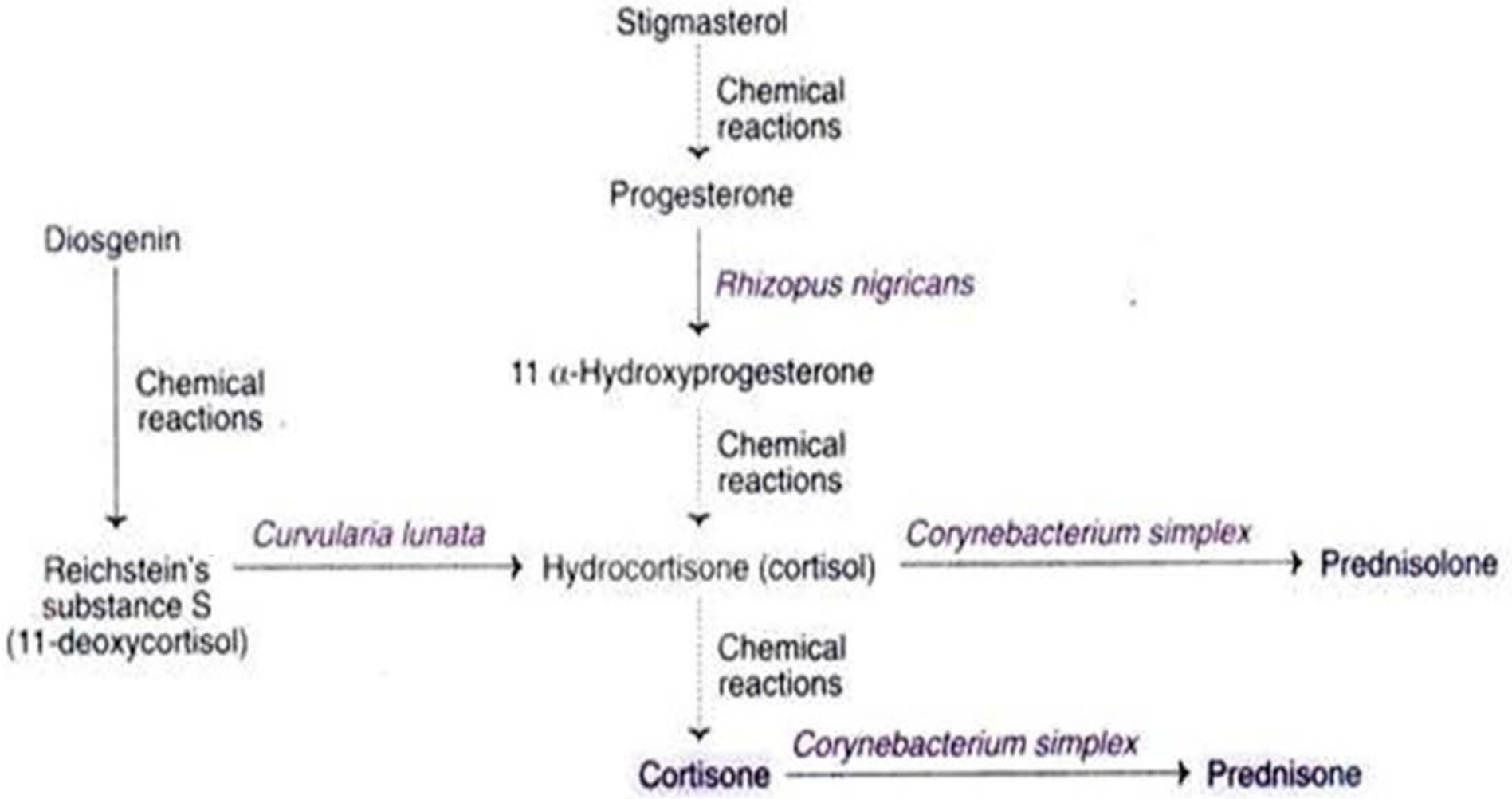
- Oxidation
  - Hydroxylation
  - Dehydrogenation. – Epoxidations
  - Oxidation to ketone through hydroxylation
  - Ring A Aromatization
  - Degradation of steroid nucleus
  - Oxidation of alcohols to ketone: 3β-OH to 3keto
  - Side chain cleavage of steroids
  - Decarboxylation of acids
- Reduction
  - Double bond
  - aldehyde and ketone to alcohol
- Hydrolysis
- Isomerization
- Resolution of racemic mixture
- Other reactions
  - Aminations
  - Enolization of carbonyl compounds
  - Esterification.

General scheme for steroid drug production

- The production of steroids, entirely by bio-transformation reactions is not practicable. Therefore, microbial transformation along with chemical reactions is carried out.
- Steroid drugs are synthesized by chemical or microbial routes, both of which involve conversion of steroid precursors to drug intermediates and final conversion of intermediates to active drugs.



The major steps involved in the combined chemical-microbial transformation of steroids (Example 1)



The major steps involved in the combined chemical-microbial transformation of steroids (Example 1)

- Stigma sterol extracted from soybeans or diosgenin isolated from the roots of the Mexican barbasco plant can serve as the starting material.
- Stigma sterol can be chemically converted to progesterone which is subjected to bio-transformation to form 11 α-hydroxyprogesterone by the microorganism, *Rhizopus nigricans*. Cortisol (hydrocortisone), produced from 11 α-hydroxy-progesterone by chemical reactions, undergoes microbial transformation (*organism-Corynebacterium simplex*) to form prednisolone.
- Further, cortisone formed from Cortisol can be subjected to biotransformation by *Corynebacterium simplex* to produce prednisone.
- When diosgenin is used as the starting compound, substance S can be produced by chemical reactions which can be converted to Cortisol by biotransformation with the help of the microorganism *Curvularia lunata*.

The major steps involved in the combined chemical-microbial transformation of diosgenin (Example 2)

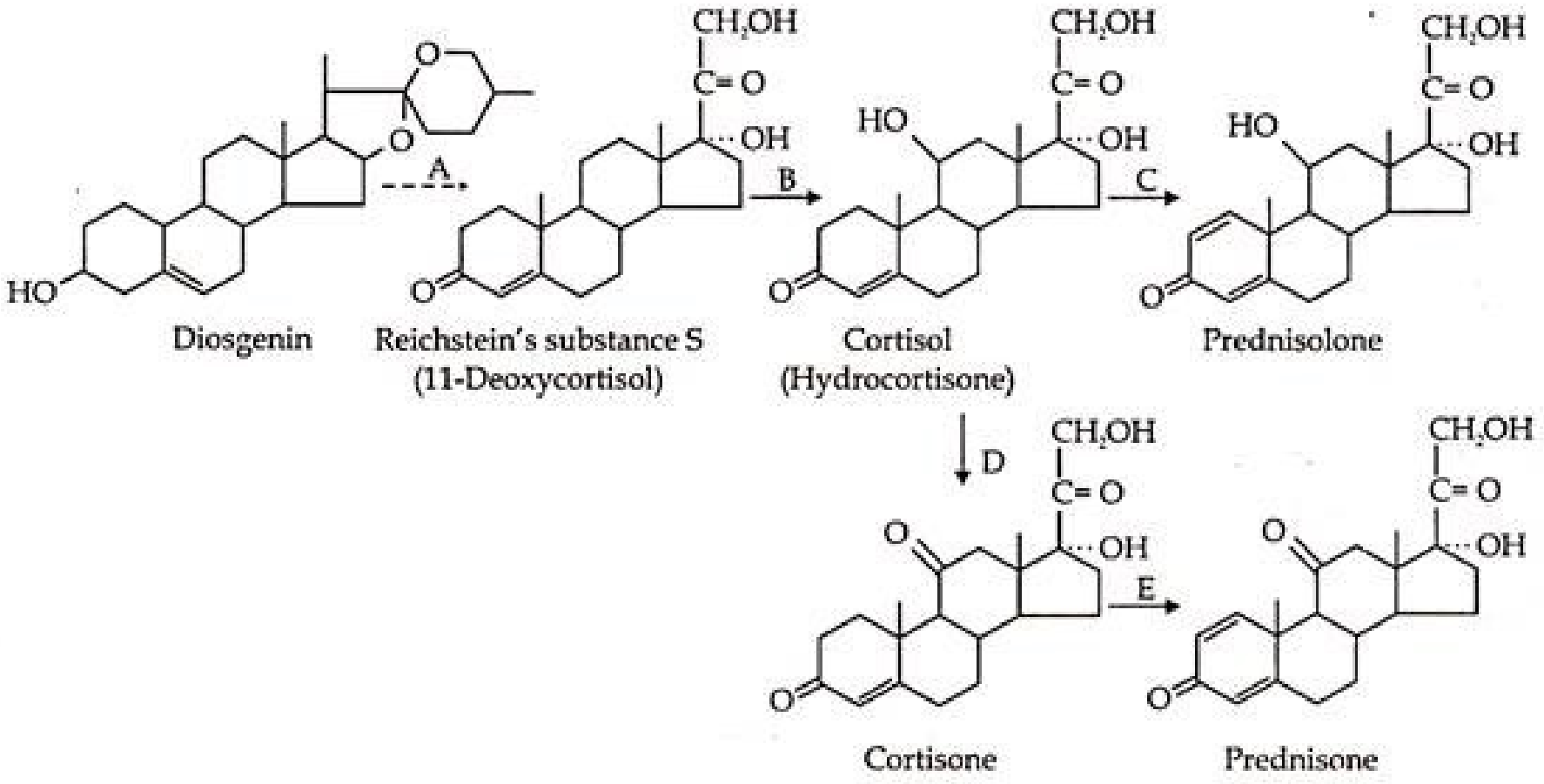
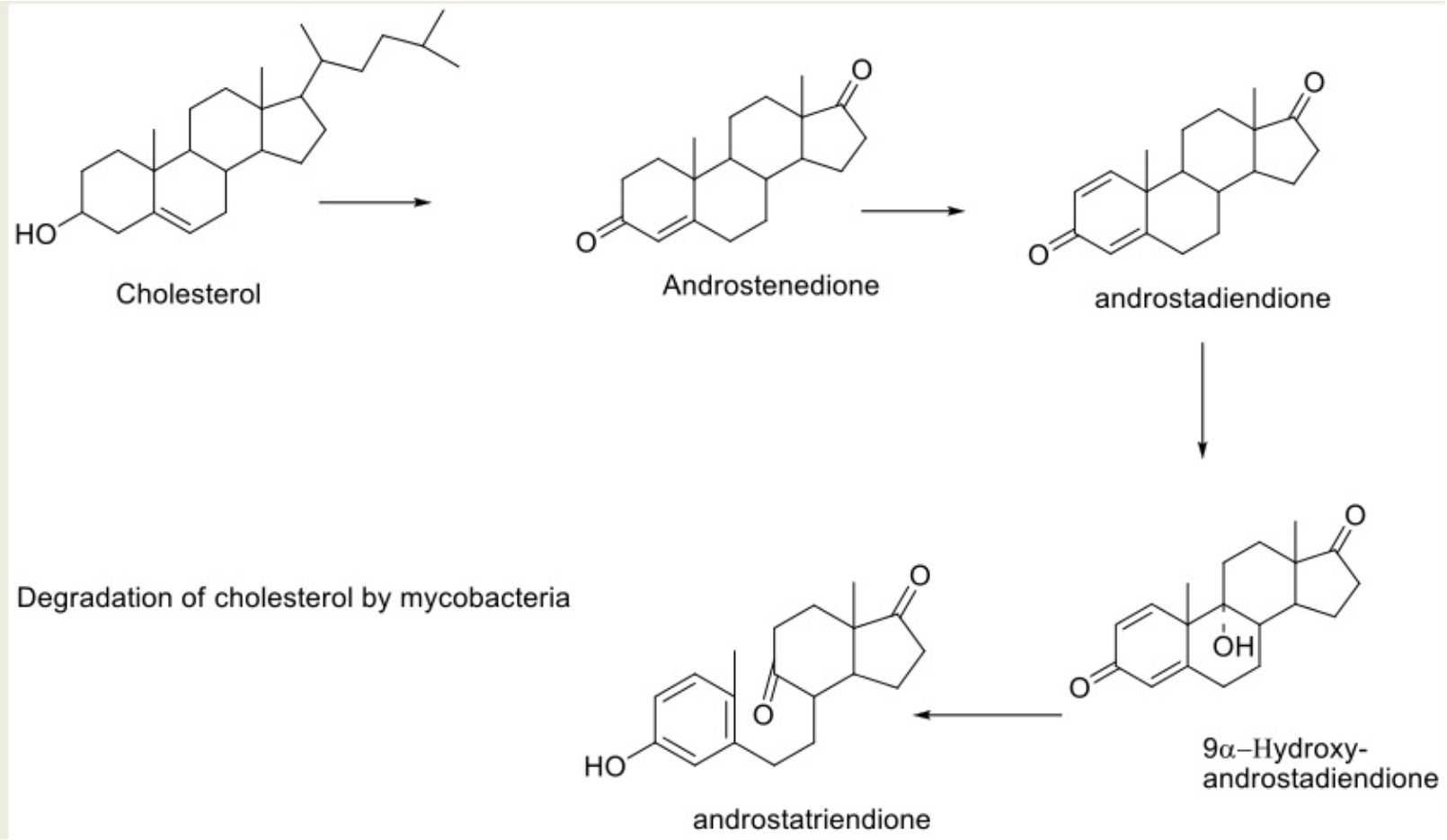


Fig. 17.5: Production of cortisol, cortisone and the 1-dehydro compounds from diosgenin via Reichstein's substance S (A, several steps; B, 11β-hydroxylation with *curvularlia lunata*; C, 1-dehydration with *Corynebacterium simplex*; D, chemical oxidation; E, 1-dehydration with *Corynebacterium simplex*)

Microbial biotransformation

- **Certain commercially important steroids (e.g. androstendione, androstadiendione) can be produced directly from cholesterol by biotransformation**



Industrial production

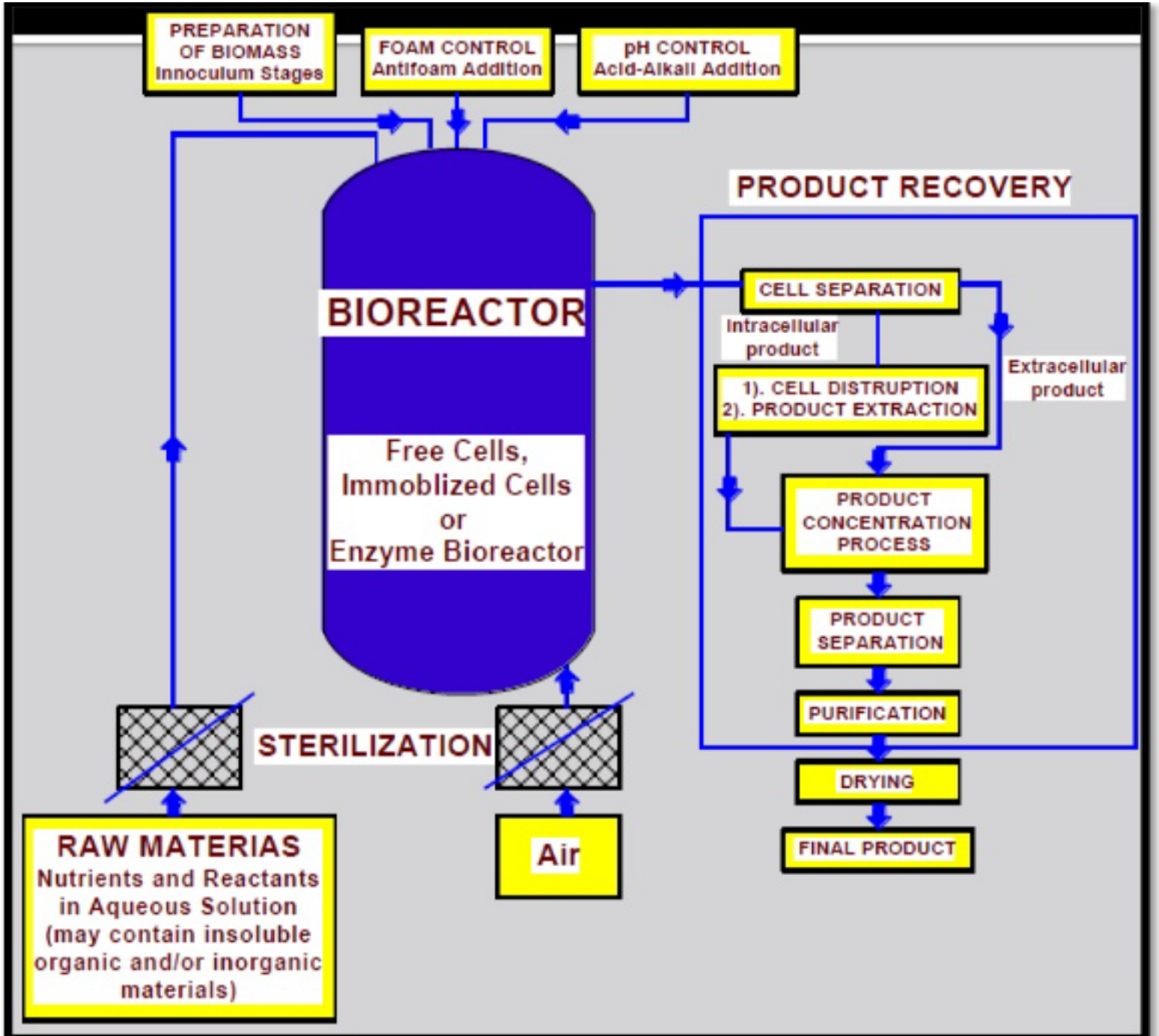
- Vegetative cells, spores, resting cells, enzymes, and immobilized cells/enzymes are generally used for microbial transformation.
- In process with growing cultures, the strain used is cultivated in a suitable medium and a concentrated substrate solution is added after suitable growth of the culture (6-24h).
- A variant of this procedure is to use a very large inoculum and to add the concentrated substrate immediately without allowing for a growth period.
- Emulsifier such as Tween or solvents (water-miscible and low toxic (ethanol, acetone, dimethyl formamide, dimethyl sulfoxide) may be used to help solubilise poorly soluble compounds.
- For the biotransformation of lipophilic materials it is possible to employ a **polyphase system**. The aqueous phase containing the cell material or the enzyme is overlayed with the water immiscible fluid phase in which the substrate has been dissolved. The substrate passes slowly into the aqueous phase and as the transformation reaction proceeds, the product passes back into the solvent phase. In some cases, the actual transformation occurs at the interface of the aqueous and solvent phases.

...Industrial production

- Transformation reactions in large-scale equipment are carried out under sterile conditions in aerated and stirred fermenters, the conversion process being monitored chromatographically or spectroscopically.
- The process is terminated when a maximum titer is reached.

Purification of product

- The end products of transformation reactions are found extracellularly and may occur in either dissolved or suspended form.
- The cell material is then washed repetitively with water or organic solvents in order to detach the reaction product which can be adsorbed to the cells.
- Depending on the solubility of the product, recovery is performed by adsorption to ion exchangers, by precipitation as the calcium salt, by extraction with appropriate solvents or for volatile substances, by direct distillation from the medium.


PREPARATION OF BIOMASS Innoculum Stages
FOAM CONTROL Antifoam Addition
pH CONTROL Acid-Alkali Addition
PRODUCT RECOVERY
BIOREACTOR
Free Cells, Immoblized Cells or Enzyme Bioreactor
CELL SEPARATION
Intracellular product
Extracellular product
1). CELL DISTRUPTION 2). PRODUCT EXTRACTION
PRODUCT CONCENTRATION PROCESS
PRODUCT SEPARATION
PURIFICATION
DRYING
FINAL PRODUCT
STERILIZATION
RAW MATERIAS
Nutrients and Reactants in Aqueous Solution (may contain insoluble organic and/or inorganic materials)
Air

Questions

- Write an assay on steroid biotransformation.
- Why microbial transformation is better over chemical transformation?
- What are the type of chemical reactions involve in biotransformation of steroids?
- Explain the scheme of biotransformation of steroids giving examples.
- Write short note on industrial process of steroids production.