Chapter 04:

4. Application of Biotechnologies in the Medical Field

4.1. Hormone Production

Biotechnologies applied to the pharmaceutical sector encompass all techniques that use living resources to design and produce active substances.

4.1.1. What is a hormone?

A hormone is a biological substance synthesized by special cells (endocrine cells) and directly secreted into the blood or lymph. There are different types of hormones: peptide, lipid, and steroid hormones (such as cortisol, testosterone…), which are derived from cholesterol. Hormones serve to transmit a chemical signal: they act at a distance, on a tissue or organ different from their site of secretion, which has receptors for the hormone.

4.1.2. Examples of hormone-producing organs and the hormones they secrete:

- **The pituitary gland**: The pituitary is an endocrine gland located in the brain.
    - (ACTH = adrenocorticotropic hormone, oxytocin, vasopressin, prolactin (LTH), which stimulates milk synthesis, etc.)
- **The thyroid**: The thyroid is an endocrine gland that synthesizes and releases thyroid hormones into the bloodstream.
    - Thyroxine (T4) and triiodothyronine (T3). These hormones play a role in basal metabolism, for example by promoting growth and stimulating the consumption of fats and sugars.
- **The adrenal glands**: adrenaline, cortisone, aldosterone, DHEA...
- **The pancreas**: insulin, glucagon
- **The testicles**: testosterone
- **The ovaries**: estrogens, progesterone…

4.1.3. Roles of hormones: growth, reproduction, sleep…

Hormones play various roles in: growth (growth hormone); homeostasis; reproduction and pregnancy (sex hormones); sleep (melatonin)...

4.1.4. Hormones and treatments

Hormones are also used in treatments, for example:

- In oncology, as part of hormone therapy, since certain hormones can play a role in cell growth;

- To treat menopause-related disorders: hormone replacement therapy; and for hormonal contraception.

4.1.5. Plant hormones

By extension, in plant biology, the term "phytohormone" or "plant hormone" is used to refer to molecules such as auxin, gibberellin, cytokinin, ethylene, and abscisic acid, which influence plant development.

4.1.6. Hormone Production

Beyond conventional fermentation techniques, genetic engineering has revolutionized the production of biologically active compounds—particularly recombinant endogenous proteins—and has significantly contributed to the development of safer and more effective therapeutics, such as recombinant human growth hormone (rhGH). Genetically modified organisms (GMOs), including transgenic plants, animals, and recombinant microorganisms, are employed either as biofactories for the synthesis of active pharmaceutical ingredients or as delivery vectors to transport these molecules directly to their target sites within the body.

Naturally occurring hormones—such as progesterone, testosterone, estrone, and corticosteroids—can now be biosynthesized at low cost using microbial cell factories, offering a scalable and economically viable alternative to traditional extraction or chemical synthesis methods.

4.1.7. Human Growth Hormone (HGH)

Human Growth Hormone (HGH), used to treat pituitary deficiencies leading to dwarfism (hypopituitarism), was previously obtained by harvesting pituitary glands from deceased humans. Obtaining a sufficient quantity of the hormone was both difficult and expensive, which severely limited its use. Growth hormone extracted from animals is ineffective in humans.

The gene responsible for growth hormone synthesis was introduced into *Escherichia coli* (E. coli). This genetically modified bacterium now serves as the commercial source of HGH. At one point, approximately 30,000 children in the United States were receiving this hormone.

Thanks to biotechnology, the gene encoding human growth hormone was identified, isolated, and inserted into the genetic code of *E. coli*. This bacterium multiplies rapidly and can produce large quantities of the hormone safely. Initially, researchers extracted the human growth hormone gene from human cells and incorporated it into the DNA of *E. coli* bacteria. Industrial-scale production then takes place in large bioreactors where these genetically modified bacteria are cultivated under carefully controlled conditions to optimize growth.

The bacteria use the human gene to synthesize authentic human growth hormone, which is subsequently extracted and purified for medical use.

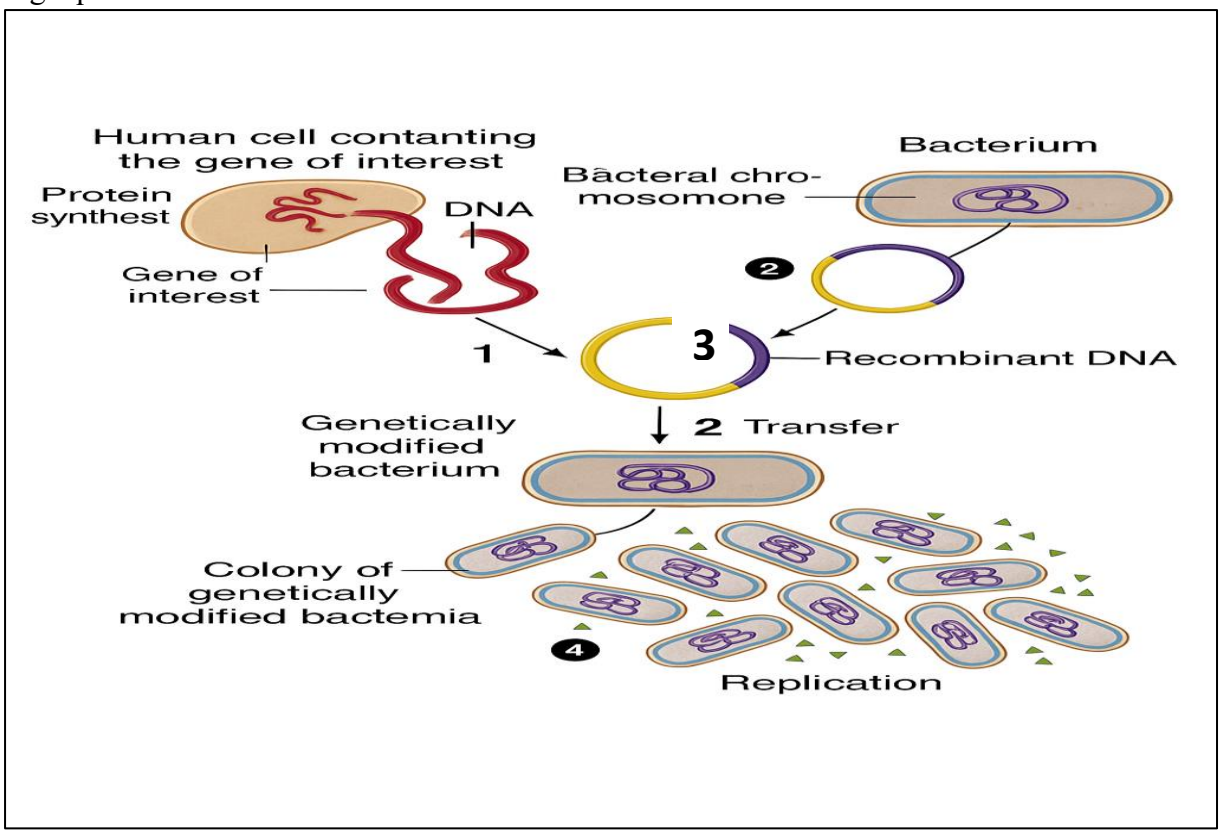
4.1.8. Biotechnology in Action: Recombinant hormone Production Using Bacterial Expression Systems

1- The gene of interest is isolated from the human genome.

2- It is then inserted into a bacterial plasmid.

3- The modified plasmid is subsequently transferred into the bacterium.

4- The bacterium rapidly multiplies into a colony, which produces the desired human protein in large quantities.



4.1.9. Human Insulin:

Insulin is a hormone produced by the pancreas. Insufficient insulin production leads to diabetes. Today, diabetics have access to human insulin produced through genetic engineering. Before insulin was produced using microorganisms, it was extracted from pigs or cattle. Structurally, animal insulin is not exactly the same as human insulin, making it less effective.

Moreover, as the number of diabetes cases has continued to rise, it became necessary to find a method for producing insulin in large quantities and at low cost. First, the insulin-producing gene is isolated. Then, an expression vector—typically a plasmid—is used. This plasmid serves

as the genetic carrier of the gene and is inserted into a host cell, such as the bacterium *Escherichia coli*. The genetically modified bacterium is cultivated in a fermenter where it automatically produces human insulin.

With the development of genetic engineering, scientists turned to microorganisms for insulin production. Since 1984, large-scale commercial production of insulin has been carried out using genetically modified *E. coli* bacteria.

In 1987, insulin production also began using the yeast *Saccharomyces cerevisiae*.

Genetically engineered chloroplasts from tobacco and carrot plants have also been used to express various therapeutic proteins, such as human growth hormone, human serum albumin, interferons, and insulin-like growth factors (IGFs).

4.2. Vaccine Production

Genetically modified antigens are now a reality thanks to advances in biotechnology. These antigens offer several advantages over those extracted from pathogenic bacteria or viruses. Antigens produced by bacteria are less expensive, easier to purify, and free from contamination by other proteins.

4.2.1. Stages of Industrial Vaccine Production

4.2.1.1. Production of the Active Substance

This involves producing an antigen capable of stimulating antibody production by our immune system. This antigen originates from the germ (virus, bacteria, or parasite) that causes the disease and may be:

- A live attenuated germ (live attenuated vaccine: for example, mumps, measles, or BCG tuberculosis);
- An inactivated germ or a fraction of it (inactivated vaccine), or a toxin;
- Recombinant vaccines: Some vaccines are produced through genetic engineering and use an animal cell or yeast to produce the vaccine antigen (e.g., hepatitis B).

Each vaccine type is produced through specific processes, but their manufacturing generally follows the same steps:

1. **Creation of the Germ Bank**:

   This is the starting point of the process. The germ bank gathers viruses or bacteria that must maintain consistent properties to ensure quality vaccines. The germ must be thoroughly characterized, especially to confirm the absence of mutations.

2. **Cultivation and Amplification**:

Culture parameters must be precisely controlled, including duration, temperature, pressure, culture medium composition, germ quantity, aeration, etc. Some growth media are composed of cells, and some cultures are grown in chicken eggs (e.g., for flu or yellow fever).

3. **Harvesting**:

This step involves extracting the produced antigen from the culture medium.

4. **Purification and Concentration**:

This involves removing impurities from the substance and concentrating it through physical processes such as centrifugation.

5. **Inactivation of the Produced Substance (if necessary)**:

Inactivation by heat or chemical agents such as formaldehyde eliminates pathogenicity while preserving immunological properties—i.e., the ability to trigger an immune response without causing disease.

6. **Preparation of Antigenic Valences**:

his step involves combining antigenic substances into a single compound, such as the three types used in the inactivated polio vaccine.

4.2.1.2. Pharmaceutical Formulation

Pharmaceutical formulation results in the final product offered in pharmacies.

1. **Combining Valences for Combination Vaccines**:

Valences are combined, as in the Diphtheria – Tetanus – Poliomyelitis – Acellular Pertussis vaccine.

2. **Formulation**:

Adjuvants and stabilizers may be added: adjuvants enhance efficacy and immune response; preservatives and stabilizers improve the compound's stability. Preservatives may be used in multi-dose presentations.

3. **Aseptic Filling**:

The product is filled into vials or syringes in a sterile (germ-free) manner.

4. **Lyophilization (if needed)**:

This step removes water from the product by converting it to powder, improving stability and preservation.

5. **Packaging**:

This step includes labeling and boxing into batches, each representing a homogeneous set of doses—ranging from 50,000 to 1 million doses per batch, depending on the vaccine type.

6. **Batch Testing and Release**:

Vaccines are subject to dual quality control: by the manufacturer and by an independent authority. Only when both are satisfied are the batches released for distribution.

7. **Delivery of Batches**:

Delivered to pharmacies, hospitals, vaccination centers, etc.

4.2.2. Various Types of Biotechnological Vaccines

4.2.2.1. Vaccines from Yeasts

Against Hepatitis B:

A liver disease caused by the Hepatitis B Virus (HBV). The blood of chronically infected individuals contains a protein particle called HBs. This particle is not toxic but effectively induces anti-Hepatitis B antibody production. This protein has been cloned in *Saccharomyces cerevisiae* and now serves as the antigen source for human immunization.

4.2.2.2. Vaccines from Recombinant Bacteria

- A recombinant *Mycobacterium microti* vaccine increases protection against tuberculosis by inducing a T lymphocyte immune response.
- Ten recombinant proteins from *Plasmodium falciparum* (malaria parasite) are produced by *Escherichia coli*. These proteins generate antibodies in rats.
- *Brucella abortus*, a Gram-negative intracellular pathogenic bacterium, infects animals and humans through ingestion. An antigen from *Brucella abortus* is produced in recombinant *Lactococcus lactis* bacteria—this is the first step towards orally administered live vaccines against brucellosis.
- A study is examining cancer vaccine trials. A recombinant protein combining an enzyme and a tumor-associated antigen is expressed in a recombinant *Escherichia coli* strain. When injected into tumor-bearing mice, the recombinant protein shows anti-tumor activity.

4.2.2.3. Vaccines from Recombinant Viruses

- Research on vaccines against HIV-1 (Human Immunodeficiency Virus type 1) is ongoing. Recombinant measles viruses expressing HIV-1 antigens have been developed, and their immunogenicity has been tested in animals. The aim is to create a pediatric vaccine effective against both measles and AIDS.
- A recombinant Herpes Simplex Virus type 1 (HSV1) is used as a vaccine vector. However, immune response after vaccination is reduced in HSV1-seropositive individuals.

- A recombinant virus expressing a stimulatory molecule infects dendritic cells and stimulates the immune system to combat cancer cell growth. It can be used in cancer immunotherapy.

4.2.2.4. Vaccines from Transgenic Plants

- Vaccines can be produced by transgenic plants. Studies are ongoing to develop an edible vaccine against human papillomavirus (HPV) produced in bananas. These edible or oral vaccines could be a solution for developing countries.
- An oral vaccine against transmissible gastroenteritis virus in pigs has been developed using transgenic plants.
- Transgenic carrots have been engineered to express a new antigen against the measles virus. This antigen could be used in the development of a new vaccine.
- Transgenic tobacco chloroplasts are used to express antigens to develop vaccines against cholera, anthrax, plague, and tetanus.

4.2.2.5. Genetic Vaccines

Molecular biology advances now allow the identification of genes responsible for a microorganism's virulence. Current research favors developing "molecular" vaccines using purified or genetically engineered antigens. These vaccines induce protective immune responses while avoiding side effects from other microorganism components. Tetanus and diphtheria vaccines are now among the most effective and safest.

A genetic vaccine has been developed to protect against venomous snake bites. Alpha-cobratoxin is a neurotoxin found in *Naja kaouthia* (monocled cobra) venom. A modified gene encoding a non-toxic but immunogenic toxin provides protective immunity in mice.