1. Biopharmaceuticals

- Pharmaceutical substances: Medicinal therapy
  - Traditional pharmaceutical sectors:
    - Chemical-based drugs: chemical synthesis
    - Extraction or isolation from biological sources

- Biopharmaceuticals: A class of therapeutic agents produced by modern biotechnological techniques, like recombinant DNA, protein engineering, and hybridoma technologies etc. (used in the 1980s)
  - Nucleic acids used for gene therapy and antisense technology
  - Proteins for in vivo diagnostics

- A Protein or nucleic acid-based pharmaceutical substances used for therapeutic or diagnostic purpose, which is produced by modern biotechnological techniques

- Form the backbone of medicinal agents in the modern biotech era
Before 20th century: Naturally occurring substances: Medicinal herbs
- Digitalis to stimulate heart muscle
- Quinine for malaria
- Pecacuanha for dysentery
- Mercury to treat syphilis

- Synthesis of aspirin in 1895 by Bayer

- Chemical synthesis of artificial dyes proved to be therapeutically useful → Foundation of Bayer and Hoechst
1930s; Beginning of pharmaceutical industry
- landmark discovery and synthesis of sulpha drugs against bacterial infections from the red dye, Prontosil rubrum (Fig. 1.1)
- large-scale industrial production of insulin

1940s: Industrial-scale production of penicillin
→ boosting the development of biopharmaceutical industry

1992: Taxol approved by FDA for ovarian, lung, breast cancer
Taxol from the bark of the Pacific yew tree by Bristol-Myers-Squibb (BMS)
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출처: Randy Moore et al., 식물학, 2판 Dubuque, IA: Brown, 1998. 표 2.2 p.37
Morphine

Opium poppy
• Foundation of the leading pharmaceutical companies
  - Ciba Geigy, Eli Lilly, Wellcome, Glaxo, Roche

✓ **Current pharmaceutical industry**
  - 10,000 pharmaceutical companies
  - 5,000 substances are used routinely in medicine
  - 500 potential drugs under clinical trials
  - $ 200 billion market
Story of Taxol

- Identified in 1967 by NCI from the bark of the Pacific Yew tree (*Taxus brevifolia*): 주목 나무
- Used by American Indian for treatment of inflammation
- Developed as anti-cancer agent by Bristol-Myers-Squibb (BMS)

Paclitaxel
1955: Plant screening project by NCI to discover new anticancer agents → screening of 35,000 plants

1967: Identification of cytotoxic ingredient from the bark of Pacific yew tree → Taxol (Generic name)

1969: 10 g of pure compound from 1,200 kg of bark

1979: Mechanism of action in leukemic mice → inhibition of cell division by stabilization of microtubules

1984: Phase 1 clinical trial → problem of supply

1988: Phase 2 clinical trials
A remarkable response rate of 30% in patients with refractory ovarian cancer
• All the ovarian cancer and melanoma cases in the US: destruction of 360,000 trees annually ➔ Ecological concerns about the impact on yew populations

• 1989: Cooperative Research & Development Agreement for practical and financial supports from a company
  - The NCI was thinking, not of collaboration, but of a hand-over of taxol (and its problems)

• 1989: BMS selected as the partner
  ➔ Investment of $100 million ➔ successful development
  ➔ Generic name was changed to Paclitaxel from taxol

• 1992: FDA approval
  Five years exclusive marketing right to BMS for a non-patentable item: What is patentable?
- Effective for cancers like ovarian, breast, and lung

- **1991: Controversy about the deals and Congressional hearings:**
  - Trade name: TAXOL
  - Assignment of rights

- **Currently produced by plant cell culture technology developed by Phyton Biotech., Inc**
  - The use of Taxus cell line in a large fermentation tank
  - Annual sales: $2-3 billion

- **Solvent used for dissolving taxol → Toxicity**
  - Conjugation with albumin: approved by FDA in 2005
Lessons from the TAXOL story

• What contributions did Biotech make?
• Why is a patent important?
• Others?
History of Penicillin

✔ Modern era of antibiotic discovery
✔ Inhibiting the formation of peptidoglycan cross-links in the bacterial cell wall: Inhibition of DD-transpeptidase

Penicillin G (Benzyl penicillin)
R = benzyl group
Discovery and development

✓ Alexander Fleming: tried to isolate the bacterium, *Staphylococcus aureus* by growing it on the surface of nutrient at St. Mary's Hospital in 1928

→ **Breakthrough in the antibiotic history**

- He noticed that no bacteria grew near the invading substance in the contaminated plate: The cell killing must be due to an antibacterial agent
  - **Not a failed experiment, but a meaningful finding**

- Identification of foreign particles as common mold of the *Penicillium* genus (later identified as *Penicillium notatum*)
Recovery and test of a tiny quantity of secreted material using the crude extraction methods: powerful antimicrobial activity and named "penicillin".

- The discovery laid essentially dormant for over a decade.

World War II resurrected the discovery: desperate demand an antibiotic with minimal side effects and broad applicability.

- Howard Florey and Ernst Chain of Oxford: rebuilt on Fleming’s observation.

- They produced enough penicillin to treat some laboratory animals: Treat of a London policemen for a blood infection.

→ Great efficacy against infection.
The supply of penicillin was exhausted
- Need a process to make large amounts of penicillin
- **Process development** required engineers, microbiologists, and life scientists
- Approached pharmaceutical companies in the USA like Merck, Pfizer, Squibb, and to develop the capacity to produce penicillin at large amount

- **First attempt**: chemical synthesis of penicillin because of a great deal of success with other drugs
  - *Chemical synthesis*: proved to be extremely difficult
  - *Fermentation process*: an unproved approach
The War Production Board appointed A.L. Elder to coordinate the activities of penicillin producers to greatly increase the supply of penicillin in 1943.

- Commercial production of penicillin by a fermentation process

**Problems:** very low concentration (titer) of penicillin

- In 1939, the final concentration of penicillin in broth: \( \sim 0.001 \text{ g/L} \)

- Low rate of production per unit volume: Low productivity
  → very large and inefficient fermentors

- Difficult with product recovery and purification
  - Fragile and unstable penicillin → constraints on recovery and purification methods
✓ Major contribution to the penicillin program by NRRL

- Development of a corn steep liquor-lactose based medium → ten-fold increased productivity

- Isolation of a new strain (> few hundreds) : Penicillium chrysogenum

- Other hurdles : Manufacturing process
  - Growth of the mold on the surface of moist bran
  - Growth of the mold on top of a liquid medium ; requires many milk bottles → Bottle plant → long growing cycle and labor intensive
✓ **Submerged fermentation process**: Challenges
  - Mold physiology: productivity vs conditions
  - Reactor design: reactor size and configuration, **oxygen supply** (low solubility of oxygen, viscosity, mixing, mass transfer), heat removal, agitator design, mechanical sealing, decontamination,

✓ **Product recovery/purification**: pH shift and liquid-liquid extraction

✓ **First plant for commercial production by Pfizer**
  → **100,000 gal scale** in 1945

✓ Nobel prize in 1945 for three scientists
Accomplishment required a high level of multidisciplinary work
Ex) Merck assigned an engineer and microbiologist together to each aspect of the problem

Continued progress with penicillin fermentation through physiology, metabolic pathway engineering, mold genetics, process control, reactor design:
- Increase from 0.001 to ~100 g/L

Production of penicillin derivatives with greater potency: Antibiotic resistance
- Semi-synthetic antibiotics
- Protein engineering to design relevant enzymes:
  More economically feasible process
Biosynthesis of Penicillin G in *Fungus*

\[ \text{L-Amino-adipic acid} + \text{L-Cysteine} + \text{D-Valine} \]

\[ \rightarrow \text{ACV-Tripeptide} \]

\[ \rightarrow \text{Isopenicillin N} \]

\[ \rightarrow \text{Cephalosporines} \]

\[ \rightarrow \text{Penicillin G} \]
Penicillin G

Penicillin nucleus (6-APA)

Penicillin F

Enzymatic process

Protein Engineering

Derivatives (rational design)
Animal test
Clinical trials (Phase I, II, III)

New antibiotics with greater potency
Lessons from the penicillin story

• Penicillin process established a paradigm for bioprocess development and biotechnology

• Analysis of the failed experimental results in a critical way:
  Curiosity leads to a creative and original idea

• The development of biological process requires a high level of multidisciplinary work
Emergence of antibiotic-resistance pathogens:

- Genes can be transferred between bacteria in a horizontal fashion by conjugation, transduction, or transformation
- A gene for antibiotic resistance that had evolved via natural selection can be shared
- Evolutionary stress such as exposure to antibiotics selects for the antibiotic resistant trait.

Superbug: a bacterium with several resistance genes
- MRSA (Methicillin-resistant *Staphylococcus aureus*)
- VRSA (Vancomycin-resistant *Staphylococcus aureus*)

Major cause: misuse and overuse of antibiotics
Prevention

• Rational use
• Alternative therapy
  - Phage therapy
    Currently used for curing the animals infected by pathogens
  - Others ?
Traditional Pharmaceuticals of Biological Origins

✓ Pharmaceuticals of animal origin: Table 1.10
- Protein-based drugs are produced by recombinant DNA technology
- Non-proteinous pharmaceuticals like steroid hormones, corticosteriods, prostaglandin are produced synthetically

✓ Pharmaceutical substances of plant origin: Table 1.16
- Estimated 3 billion people worldwide continue to use traditional plant medicines as their primary form of healthcare
- Directly extracted
- Direct chemical synthesis
- Identification of effective components from plant “lead drug”
- Chemical families of plant-derived medicines:
  - alkaloids, Flavonoids, terpens, terpenoids, steroids, coumarines, quinines, salicylates, and xanthines
Pharmaceutical substances of microbial origin
- Produce a wide variety of secondary metabolites with potential therapeutic applications
- Antibiotics: the greatest positive impact on human healthcare
  low molecular mass microbial secondary metabolite
- ~10,000 antibiotic substances isolated and characterized
- More than 100 antibiotics available on the market

Major families of antibiotics (Table 1.17)
- β-Lactams (Fig. 1.14): penicillins and cephalosporins
  - Characteristic β-Lactam core ring structure (Fig. 1.14)
  - Inhibits the synthesis of peptidoglycan
  - Semi-synthetic derivatives from 6-APA and 7-ADCA
    enzymatic removal of a natural penicillin side chain followed by addition of novel side chains
Structure of B-lactam antibiotics

Penicillin

Cephalosporin
- **Tetracyclines**: characteristic 4-fused-core ring (Fig.1.16)
  - A broad-spectrum polyketide antibiotics produced by the *streptomyces*
  - inhibit protein synthesis by binding to the 30S subunit of microbial ribosomes
  - widespread use due to broad spectrum against Gram-negative and Gram-positive
  - Derivatives (Table 1.18)
Aminoglycosides: (Table 1.19)
- Cyclic amino alcohol to which amino sugars are attached
- Exclusively produced by the genus *Streptomyces* and *Micromonospora*
- Inhibit protein synthesis by binding to 30S and 50S ribosomal subunits
- Amikacin, gentamicin, kanamycin, neomycin, paromomycin, streptomycin, tobramycin
Macrolides:
- core ring structure containing 12 or more carbon atoms
- predominantly produced by *Streptomyces*

Ansamycins:
- a core aromatic ring structure
- produced by *Actinomycetals*
- rifamycine; effective against Gram-positive and mycobacterium (*Mycobacterium tuberculosis*)
The age of Biopharmaceuticals

✓ Biomedical research since the 1950s:
  → Naturally occurring proteins having therapeutic efficacy: interferons, interleukins, EPO, insulin etc.
  → Widespread medical application was limited due to the tiny quantities and consequently high cost
  → The advent of recombinant DNA technology and biotechnology
  → Mass production and cheap supply: Benefits to humans

✓ Advances in biosciences: Understanding of the underlying mechanisms for diseases
  → Development of new therapeutic proteins with greater efficacy

Era of biopharmaceuticals
Recombinant DNA technology : Impact on many areas

Four impacts on the production of protein production

- Overcomes problem of source availability
- Overcomes problems of product safety :
  ex) Transmission of blood-borne pathogens like hepatitis B and HIV via infected blood products
  Transmission of Creutzfeldt-Jacob disease to persons from receiving human growth hormone preparation from human pituitaries
- Provides an alternative to direct extraction from inappropriate sources
  ex) Purification from urine : Fertility hormone (FSH), hCG, and Urokinase
- Facilitates the generation of engineered therapeutic proteins displaying some clinical advantages over the native ones
Impact of DNA technology on the bio-industries

- Foundation of start-up biotech companies in 1980s

- Strategic alliance:
  - Between start-up and pharmaceutical companies
    - **Start-up company**: Significant technical expertise, but lack of experience in drug development process and marketing
    - **Big company**: slow to invest in biotech R &D
      - ex) Genentech and Eli Lilly
      - Development of recombinant human insulin
      - Clinical trials and marketing by Eli Lilly (Humulin)
    - **Merger of biotech capability with pharmaceutical experience → Biotech sector**
✓ Development of new biopharmaceuticals
• High Risk (low success rate), but High Return
  - Long term: 6 ~ 10 years
  - High cost: ~ $200-500 million
  - High return: ~ $1-2 billion per single drug for 20 yrs

• Major biopharmaceutical companies: Table 1.6
Initial biopharmaceuticals: simple replacement proteins

Large-scale production by using recombinant DNA tech. → Economic feasibility and engineering concept

The vast majority of recombinant proteins are produced in *E. coli*, *S. cerevisiae* or in animal cell lines (Chinese hamster ovary (CHO) or baby hamster kidney (BHK) cell lines).

→ Requiring the development of bioprocess engineering and biotechnology for commercial production

- Protein engineering,
- Process control and optimization
- Cell culture engineering
- Purification process etc.
The use of modern biotechnology such as protein engineering in conjunction with an increased understanding of structure-function relations of protein facilitated development of more potent therapeutics.

Patent protection for many first-generation biopharmaceuticals (human growth hormone, insulin, EPO, interferon, granulocyte colony stimulating factor etc..) has now come/is coming to an end.

Most of these therapeutics have an annual market value in excess of $ 1 billion.

Generic drug

✓ Produced and distributed without patent protection

✓ Bioequivalent to the brand name counterpart with respect to pharmacokinetics and pharmacodynamics

✓ Identical in safety, efficacy, dose, strength, route of administration, intended use

✓ Generics also go through a rigorous scientific review to ensure both safety and efficacy
✓ Benefit to consumers and insurance companies: Lower price

- **Generic manufacturers**: no burden of proving the safety and efficacy of the drug through clinical trials, since these trials have already been conducted by the brand name company.

- Only need to prove that their preparation is bioequivalent to the original drug to gain regulatory approval.
  - Production at a much lower cost
  - Competition among manufacturers
Pharmacology

✓ Study of the properties of drugs and how they interact/affect human body

✓ Pharmacodynamics: the study of the biochemical and physiological effects of drugs on the body, the mechanisms of drug action, and relationship between drug concentration and effect

✓ Pharmacokinetics: the fate of substances administered externally to a living organism including absorption, distribution, metabolism, excretion
Bio-similar (Bio-generics)?

✓ **Small-molecule drugs (generic drug)**: generic form can be marketed if their therapeutic equivalence to the original drug is proved
   → pharmaceutical equivalence (identical active substance) and bioequivalence (comparable pharmacokinetics)
   → no clinical efficacy and safety test

✓ **Therapeutic proteins**: the generic approach can not be applied to copies of therapeutic proteins because of complexity
   → impossible to prove two protein products to be identical
   → comprehensive clinical data: clinical equivalence (safety and efficacy)
   → approval by regulatory authority → marketing
✓ Approval and regulation
• Bioequivalence to the original drug
  - Bioequivalence, however, does not mean that generic drugs are exactly the same as their original counterparts, as some differences exist
• An applicant files an Abbreviated New Drug Application (ANDA) with demonstration of therapeutic equivalence to a previously approved drug
• FDA launched the Generic Initiative for Value and Efficiency in 2007 to increase the number and variety of generic drug products available.
✓ **Brand-name drug companies**: a number of strategies to extend the period of market exclusivity on their drug, and prevent generic competition: ever-greening (ex) EPO
Future prospects in biotech industry

• Technology development in many areas like genomics, proteomics, high throughput screening will have a great impact on the development of biopharmaceuticals (therapeutic proteins)

• These technologies will identify new drug target and facilitate the development of new biopharmaceuticals
HW # 1 : Due February 22, 2012

1. Please describe what is a “patent” and requirements for it.

2. Please state a way of introducing a mutation into specific position of a protein.

Do not copy and paste from internet !!
Narrow spectrum penicillins
- benzathine penicillin
- benzylpenicillin (penicillin G)
- phenoxymerthylpenicillin (penicillin V)
- procaine penicillin

Narrow spectrum penicillinase-resistant penicillins
- methicillin
- dicloxacillin
- Flucloxacillin

Narrow spectrum β-lactamase-resistant penicillins
- Temocillin

Moderate spectrum penicillins
- amoxicillin
- Ampicillin

Broad spectrum penicillins
- co-amoxiclav (amoxicillin+clavulanic acid)

Extended Spectrum Penicillins
- azlocillin
- carbenicillin
- ticarcillin
- mezlocillin
- piperacillin
Beta-lactam antibiotics