Biomedical Research Methods

"An integrated approach using chemical, mathematical and computer simulations; in vitro tests; whole animal models; and human epidemiological studies and clinical trials is currently the best approach to advance science, develop new products and drugs, and treat, cure and prevent disease."

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In nature, the living, biological world is connected with the nonliving, physical world. In science, there is a similar link between biology and chemistry — both of which are essential to understanding the world around us. While we look to biology to describe life processes and living organisms, we rely on chemistry to explain these processes in terms of ions and atoms. All biological processes, including photosynthesis and digestion, are chemical reactions involving changes in molecules made up of atoms. As chemistry has revealed, all things are composed of atoms from less than 110 different elements.

Just as the sciences must be linked to understand the world, biomedical research techniques must be linked to understand truly the life process. Therefore, as scientists look for clues and cures to human and animal disease, they use a combination of highly interdependent, state-of-the-art methods such as:

*Chemical, mechanical, mathematical and computer simulations* prove most useful in the preliminary research stages where scientists can stimulate ideas about new research directions.

*In vitro tests* are experiments performed in laboratory containers using tissues or cells. These tests are most useful during the early and intermediate research stages to study a single effect of a substance in isolation.

*Nonhuman animal models* provide the most reliable and complete data on the functioning of a living system, and they offer the best indicator of how humans will react to a new drug or medical procedure.

*Human studies* involve taking laboratory data on the safety and effectiveness of new vaccines or medicines and evaluating them in carefully staged clinical trials using informed human volunteers.

*Epidemiological studies* are another type of human study. These studies look at occurrence and distribution of disease in a population.
Chemical, Computer and Mathematical Models

Introduction to the Models: Biomedical research is increasingly incorporating chemical, mathematical, computer and mechanical methods to understand and simulate living systems. These approaches are being applied in studies at all levels of biological organization, from interactions among molecules to interactions among major organ systems. While these models represent a simplified version of reality, they are nonetheless helpful in understanding complicated biological principles and dynamics, particularly in the preliminary research stages when scientists are sorting out fundamental questions. In addition, they often provide ideas about new research directions to pursue.

Examples of chemical, computer and mathematical models include:

- **Chemical models:** Analytical chemistry tests detect a substance or measure its potency and are useful in developing vaccines, prescription drugs and vitamins. Some of these methods are based on the selective binding that occurs between a particular substance and its antibodies. For example, in an assay for botulism toxin, which traditionally required up to 200 mice, antibodies obtained from rabbits are modified so the binding of the toxin can be easily detected. In four weeks, one rabbit can produce enough antibody, with little discomfort, to perform tests that would otherwise require thousands of mice.

- **Mathematical and computer models:** Advances in computer technology have led to sophisticated mathematical models that can predict biological responses on the basis of a chemical’s structure and “activity” in an organism. Using this approach, the biological effects of chemicals can be quantified and correlated with a chemical’s biochemical properties, composition and structure. When a new and somewhat similar compound is developed, it can be compared to the database of characteristics for known compounds. This allows researchers to predict a new chemical’s likely biological properties.
Chemical, Computer and Mathematical Models (continued)

Researchers are applying this approach to the human immunodeficiency virus (HIV), the virus that causes AIDS. Powerful computer programs and structural information about HIV’s key proteins are being used to design highly specific anti-HIV compounds. Investigators announced that they have clarified the structure of a key protein manufactured by the simian immunodeficiency virus (SIV), which causes an AIDS-like illness in monkeys.

Structural information on this SIV protein has been generated as three-dimensional, computer-generated portraits showing the exact locations of its constituent atoms.

Strengths of Models: Models increase the speed and efficiency with which data can be studied and processed. Using pattern recognition programs enables scientists to compare characteristics of one compound with another.

The simpler the system being modeled, the more accurate the results.

Models also provide scientists with the capability of extrapolating data from high-dose experimental exposures to low-dose exposures. They can also extrapolate data from animals to humans.

As a result, models may reduce the number of animals required for research.

Limitations of Models: While computers are valuable research tools, they cannot replace laboratory testing. Computers do not “generate” data, they only “process” what has been entered.

Computer models rely on existing data. Therefore, their reliability is a function of how well the organism or system being simulated is defined. When a computer model is applied to a new area of research, the results must be validated by extensive testing in complex living systems. Computer models can replace living systems only after they are found to simulate real life.

In addition, sophisticated computer equipment and software are sometimes prohibitively expensive.
In Vitro Studies

The term *in vitro* literally means "in glass." Biomedical research scientists use the term when referring to any biological process or reaction that takes place in an artificial environment.

The cells or tissues studied originally come from a living organism such as a plant, a person or another animal. These studies may include an assortment of living systems—bacteria, cultured animal cells, fertilized chicken eggs or frog embryos, to name a few.

Scientists use cell cultures, isolated tissues and organs extensively in the early and intermediate stages of biomedical research. Many potential remedies are first tested *in vitro* to discover how they interact with cells and tissues. *In vitro* studies are an essential part of medical research.

Examples of *in vitro* studies:

- **Ames test for mutagenicity:** A compound is tested first in bacteria for its ability to cause mutations, which signal that a compound could cause cancer. However, not all cancer-causing substances cause mutations, and not all mutagens cause cancer. This means more extensive testing is necessary.

- **Pregnancy tests:** At one time, rabbits were injected with the urine from pregnant women and then put to sleep in order to examine their ovaries, which would determine if the woman was pregnant. That practice led to the common expression, "the rabbit died," which meant the woman was pregnant. Now, *in vitro* pregnancy tests offer the convenience and privacy of at-home testing without the use of a rabbit.

**Strengths:** *In vitro* tests allow scientists to study a single effect of a substance in isolation, without the interference from other biological phenomena such as hormones, enzymes and immune responses.

They can also be significantly less expensive, less time-consuming, more accurate and more readily controlled than *in vivo* (whole animal) systems. By enabling complete control of temperature, acidity, oxygen levels and environmental conditions of cultured cells, *in vitro* studies yield more precise results.
In Vitro Studies (continued)

Cell cultures often, although not always, contain cells that can replicate themselves in a liquid medium in a laboratory container. New techniques have been refined that allow individual cells or pieces of tissue to grow for long periods, in fact decades.

In vitro tests are critical to the study of viruses, which grow only in living cells. These viruses are grown and attenuated, or weakened, to provide the killed or weakened viruses needed to produce vaccines.

Limitations: The biochemical process leading from chemical exposure to toxic effect is so complex that it cannot be duplicated in vitro. In the study of cancer, for instance, which we now know is a multistep process, the steps cannot be put together in vitro in a way that accurately duplicates the process in the whole organism.

Cells grown in a culture are not exposed to other functions taking place in a living organism; for example, there is no regular pumping of blood and interstitial fluid, and there is no nervous system or endocrine gland adjusting the metabolism of cells.

In addition, cells do not metabolize toxins in culture the same way they do in the whole body. The variety of cells, tissues and organs just cannot be represented as they exist in the body. (More than 200 different cell types exist in the human body.)

It is also difficult to maintain differentiated cells in a culture since the cells tend to become unspecialized after a short time, losing the characteristics of the organ or tissue from which they were taken.

As a result, cell cultures cannot generate sufficiently reliable data about how a substance affects a complex interactive system made up of millions of cells, thousands of enzymes, hundreds of biological messengers and dozens of organs. For example, blood pressure medications cannot be tested without the presence of blood pressure.
Nonhuman Animal Models

Introduction: For ethical and other reasons, it is often not feasible to conduct experimental studies of disease and its treatment on humans. Instead, animal models are used. Animals provide the best known surrogate for humans in the laboratory.

Although diseases and drug reactions vary between humans and nonhuman animals, the similarities outweigh the differences. Scientists strive to develop a clear understanding of how animal species differ. By combining what they know about the differences with knowledge of our biological similarities, scientists can apply results from animal experiments to the human condition.

Animals are a vital research model because they provide a whole, complex living system that can interact and react to stimuli much as humans do. They give us an indication of how people may recover from a new surgical technique or respond to the long-term effects of a new medicine. They permit us to discover how a drug, chemical or environmental factor interacts with different organs and systems and the different routes a substance may take when swallowed, inhaled, injected, absorbed and excreted.

Strengths: Animal models provide an ethical alternative to the use of humans in experimental studies in the search for treatments, cures and prevention of diseases and disabilities.

They also provide a whole, integrated biological system and are the best surrogate for the complexities of the human system. Human and nonhuman animal similarities far outweigh their differences. Animals share the same structures (cells, tissues, organs and systems) as humans, and they function in much the same way as humans.

By using animal models, scientists can design experiments in which they can control for more variables than with humans.

Limitations: Because animals are not identical to humans, the results from experimental animal studies must be extrapolated to humans.

In addition, a whole-animal model introduces more variables than, for example, a cell or tissue culture.
Nonhuman Animal Models (continued)

Research animals are also expensive to purchase, house, feed and provide with veterinary care.

The use of animals in research is governed by an extensive, time-consuming and costly system of federal regulation.

Examples of animal models include:

**Cystic fibrosis (CF):** A mouse has been genetically engineered to provide the first animal model for CF. This breakthrough will allow quicker exploration of new treatments for the deadly disease.

**Accidents:** Primates have been used to develop ways to restore muscle function to human limbs paralyzed by spinal cord damage.

**Heart disease:** Dogs have played a crucial role in developing prosthetic devices for replacing congenitally defective heart valves. The pig is an excellent model for evaluating ways to prevent blood vessels from narrowing (restenosis) after they have been opened using a nonsurgical procedure. Rabbits have been important in the study of hypertension (high blood pressure) and atherosclerosis (hardening of the arteries).

**Vaccines and infectious diseases:** A variety of animal models, from rodents to primates, have been indispensable in developing and safety-testing vaccines.

**AIDS:** The recent development of a monkey model is a major advance for vaccine and therapy studies of the AIDS virus.

**Hepatitis B and C:** Woodchucks infected with the woodchuck hepatitis virus (WHV), a virus closely related to the human hepatitis B virus, develop severe hepatitis and liver cancer. They are an ideal model to test new drugs and other strategies to treat hepatitis B in humans.

**Anthrax:** Rabbits exposed to anthrax spores develop a disease similar to humans. The guinea pig is also a useful model for studying the effectiveness of anthrax vaccines.

**Wound healing:** Pigs are one of the best models for studying the healing process because the repair process is similar to the process in humans.

**Leprosy (Hansen’s disease):** Naturally occurring leprosy is found in wild armadillos. It is still impossible to grow the bacteria that causes the disease in vitro so armadillos are the only source of organisms for study and vaccine preparation.

**Diabetes:** Dogs and humans share similar complications of diabetes, and researchers are using dogs to transplant the insulin-producing islet cells of the pancreas to reverse the disease in humans.

**Cancer:** Scientists have developed mice with leukemia, breast cancer and many other types of cancer, allowing new treatments to be tested.
Human Clinical Trials

Introduction: Human clinical trials are an important component of the biomedical research process and are most often used in developing prescription drugs. Even after a promising new drug has undergone extensive laboratory research and testing, scientists still need actual human data from controlled studies to answer two key questions: Is the drug biologically active in humans? And, is it safe in humans?

There are three major phases of clinical trials that begin after a pharmaceutical firm files an Investigational New Drug (IND) application with the Food and Drug Administration (FDA). In the IND, a pharmaceutical firm shows the results of laboratory testing and explains how the drug is made.

In Phase I clinical trials, researchers determine a drug’s interaction with the human system, including how it is absorbed, distributed, metabolized and excreted, and the likely duration of its therapeutic effect. This phase involves a small number of healthy volunteers and takes approximately one year.

Phase II trials use controlled tests that help determine a drug’s effectiveness. These studies involve 100 to 300 volunteer patients. Simultaneous animal and human tests are also conducted at this stage as researchers continue to assess the safety of the drug. This phase takes approximately two years.

Phase III trials are conducted to confirm the results of earlier efficacy tests and further identify any adverse reactions. Clinical testing at this point is extensive, involving 1,000 to 3,000 volunteer patients in medical clinics and hospitals. This phase takes approximately three years.

After human clinical trials are completed, firms file a New Drug Application (NDA) with the FDA. The NDA is a comprehensive statement of the information on: drug structure, the scientific rationale and purpose of the drug therapy, pre-clinical animal and other laboratory study results, all human clinical testing results, drug formulation and production details and the company’s proposed labeling. This takes approximately 2.5 years to complete.

Currently, it takes approximately 12 years from initiation of animal and other laboratory studies through all phases of clinical trials and submission of data to the FDA for approval. For each new medicine approved, the cost is hundreds of millions of dollars.
Human Clinical Trials (continued)

Example: The first human trials of a hormonal growth factor called stem cell factor (SCF) indicate that cancer patients treated with SCF recover more quickly from standard chemotherapy than would otherwise be expected.

Strengths: Human clinical trials provide actual human data on the efficacy and safety of promising new drugs.

Limitations: Ethical and moral considerations limit the extent to which human volunteers can be used as test subjects for a potential new drug.

Such trials also require extensive pre-clinical testing before they can be conducted.

In addition, numerous variables, which may affect the test data, are introduced whenever humans are used as test subjects. These include genetic makeup, exposure to other chemicals, disease history, etc.

Source: Joseph Dirnasi, Tufts University
Epidemiological Studies

Epidemiology is the study of disease incidence and its distribution in a population. To prevent diseases, we need to know how they are caused. By putting together data on which people get particular diseases and in which countries, epidemiologists try to determine how disease may be spread.

Epidemiological studies may be divided into three general types: experimental, descriptive and observational. *Experimental epidemiology* is the human equivalent of animal testing — providing or withholding a substance to determine its toxic or beneficial effects. Such studies are greatly limited by ethical and legal considerations as well as the difficulties involved in securing the cooperation of a large number of people.

*Descriptive epidemiology* analyzes data on the distribution and extent of health problems or other conditions in various populations, trying to find correlations among characteristics such as diet, air quality and occupation. Such comparisons are frequently made between countries and smaller geographic regions.

*Observational epidemiology* uses data derived from individuals or small groups. Data are evaluated statistically to determine the strength of association between a particular variable and disease. In cohort studies, a well-characterized and homogenous group is studied over time. In case-controlled studies, a control group is selected retrospectively based on variables thought to be relevant to the effort. Both methods rely on accurately predicting the important variables. They are subject to various selection biases.

This information gathering occurs “after the fact” and, as in the case of cancer, can occur many years after exposure. Thus, epidemiological studies do not demonstrate a direct cause and effect, but instead, establish a statistically significant association between exposure to causative factors and disease or ill-health effects.

Epidemiological studies have been in use for hundreds of years. Some examples are:

- In 1775, Sir Percival Pott of England reported that chimney sweeps had a very high incidence of scrotal cancer. His report may have been the first epidemiological study.

- Early in this century, epidemiological studies established that the 14th century Black Plague was spread from rats to humans via fleas.
Epidemiological Studies (continued)

- In 1854, Dr. John Snow of London identified the source of a cholera outbreak to a contaminated water pump.

- In 1976, an outbreak of Legionnaires’ Disease was traced to a bacterium that flourished in static water supplies such as in air conditioning units and was spread in tiny droplets. Effective cleansing and purification prevented further cases.

- Epidemiological studies have linked particular occupational settings such as asbestos plants and coal mines to various lung diseases.

- The Hammond-Horn Smoking Study (an analytic epidemiologic study undertaken by the American Cancer Society in 1952) decisively demonstrated the effect of cigarette smoking on death rates from cancer and other diseases.

**Strengths:** Epidemiological studies offer scientists a direct opportunity to study the effects in humans exposed to chemicals and disease-causing organisms.

These studies are also useful in identifying patterns in disease or injury distribution. These patterns may be traced to causative factors.

**Limitations:** A major disadvantage of epidemiological studies is that considerable human exposure can take place before a toxic effect is detectable, particularly in the case of diseases like cancer that take many years to develop.

It is also difficult to demonstrate a direct cause-and-effect relationship between a specific exposure and disease.

In addition, there are limited methodologies to measure or verify such things as an individual’s prior exposure, route of exposure or extent of exposure to a causative agent.

There is also difficulty in identifying control groups or “unexposed” populations, e.g., the groups who can provide the data necessary for comparison.

Privacy must also be considered, preventing the collection of additional data that would be useful to understanding the disease under study.

Epidemiological studies can also be quite expensive to conduct.