PLANTS IN PERSPECTIVE

A LABORATORY MANUAL OF MODERN BIOLOGY

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W. H. FREEMAN AND COMPANY

San Francisco and London

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PLANTS IN PERSPECTIVE

Preface

Many traditional introductions to laboratory work with plants emphasize a compartmentalized approach to higher plant anatomy and physiology, followed by a rather lengthy survey of the plant kingdom. Our intention in preparing this manual has been, on the other hand, to utilize recent advances in biology to attempt a more unified treatment of the basic properties and principles relating to plant life, and to introduce more experimentation into the elementary work.

This emphasis on experimentation is perhaps not in accord with the current tendency to minimize the laboratory aspect of introductory courses. However, this manual has resulted from the conviction of the authors that laboratory experience assures a more interesting course and also that a true appreciation for biological science is most likely to develop in students who have worked with and manipulated living organisms in reasonably sophisticated experiments. An appreciation of the current rate of scientific progress and the level of our understanding of biological phenomena also can be more effectively transmitted to students if they participate in appropriate laboratory experiments.

The first version of the manual was written several years ago for use in a large, one-semester course in general botany. During subsequent revisions, a concept of evolution based on recent advances in genetics naturally and perhaps inevitably emerged more and more prominently as the central theme. The structure, properties, and importance of DNA, the characteristics of viruses, and the induction of mutations in microorganisms are emphasized relatively early in the manual and provide a basis for understanding the evolutionary process. These topics are not simply appended to the traditional material, but furnish the basic knowledge upon which a perspective on biology is developed.

Our approach has necessitated the omission of some of the material covered in traditional manuals. In particular, the examination of prepared slides, preparation of drawings, and study of life cycles have been de-emphasized. We believe that the stress on fundamental principles and on the interrelations of structure and function permitted by recent discoveries makes it possible to eliminate much detail and still give the student a better understanding of the nature of plant life, as well as of the progress being made in biology today.

During the development of the manual, a more general appreciation of the fundamental importance of recent advances in biology has taken place, making our approach appear considerably less radical than when first projected. Indeed, some might now prefer an even sharper break with the traditional material through a stronger polarization around DNA, RNA, and modern genetics in general. In the flush of enthusiasm resulting from the important triumphs of molecular biology, and particularly the establishment of the structure of DNA, some have felt that the main currents of research have left many of the classical areas hopelessly stranded in brackish backwaters. Others have felt that the physicists and chemists who in increasing numbers have taken up biological problems do not fully appreciate the complexities of living systems at higher levels of organization and interaction. Our belief is that the extreme of either position does not represent the actual situation. We believe that a major revolution is indeed taking place and that all areas of biology are being affected by it. The long-standing disciplines of morphology and taxonomy, for example, far from being worked-out and exhausted, will be due in their turn for a tremendous surge of interest and a period of rapid advancement when the necessary groundwork has been laid at lower levels of organization.

Thus we believe that the near future will see the rejuvenation of the classical areas, as old problems are recast and restated, and approached with new techniques. In emphasizing the significance of recent advances in molecular biology and attempting to communicate to beginning students the excitement which the molecular approach has engendered in virtually all biology, and in affirming the importance of the classical areas while approaching them from what we hope is a fresh and more unified point of view, the present manual is an effort to anticipate this trend.

It is a pleasure to acknowledge the helpful suggestions of our colleagues in the Department of Botany at the University of Wisconsin and the advice of the many graduate teaching assistants who used this manual during several years of preparation and modification.

July 1963

Eldon H. Newcomb
Gerald C. Gerloff
William F. Whittingham

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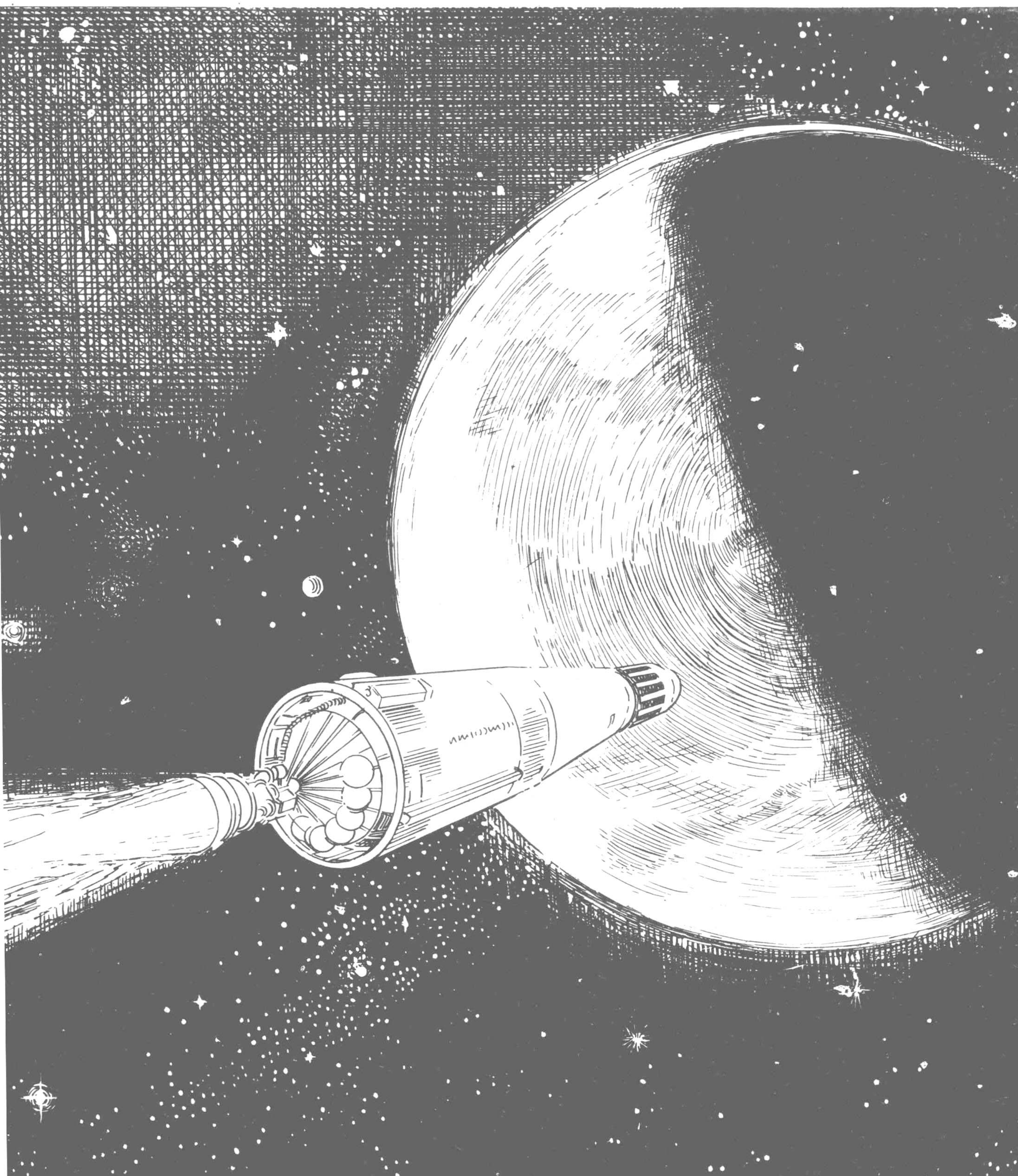
I

THE ADAPTABILITY OF LIFE

Much has been written about the felicitous qualities of our planet which have made it so ideal a place for the nurture of life: a favorable position, as the third most distant member of the solar system, for the reception of the radiation which provides the energy for life; the abundance of water, upon the remarkable properties of which the origin and evolution of the living state have been dependent; a temperature range that permits water to exist as a liquid over much of the globe's surface; and an atmospheric blanket, which provides the gases from which elements are extracted by the living substance for building and for propagating itself, and which screens out much of the solar and cosmic radiation so inimical to life. And yet, what appears as the remarkable suitability of conditions on the earth for the existence of life has come about, we realize, through an evolutionary process that has been possible because of these particular conditions. Other conditions on some other world doubtless would seem no less well suited to the requirements of the living forms which had evolved there.

Now, some three billion years since the origin of life, our planet is populated by a million and a half species of living forms. The immense variety in the appearance, structure, and behavior of these organisms reflects the fact that in the long course of evolution life has become adapted to exist in virtually every conceivable environmental niche on the earth's surface. The lush vegetation and spectacular birds and insects of the tropics, the bizarre creatures of the ocean depths, the tenacious seaweeds of the coasts, the lichens of arctic regions, the leathery plants of the deserts—all bear striking testimony to the capacity of the living substance for progressive change, and to the effectiveness of the mechanism by which it evolves.

There is a unity underlying the diversity, however. Unrelated as they may seem, the varied adaptations are in fact modifications, suited to different environments, of basic, underlying patterns of organization and function; and on analysis they are found to provide the means of satisfying the same basic requirements of life that are possessed by all organisms. Thus plants adapted to extremely arid environments are like all other organisms in requiring water as the matrix of life processes, and many of the features which seem to set them so far apart from other plants are in fact adaptations which aid in obtaining and retaining water. Similarly, all organisms are carbonaceous; all have somewhat similar requirements for elements present at the earth's surface in gaseous and mineral form; all are confined to existence within a narrow range of temperature and within certain limits of acidity and alkalinity. These common requirements arise from the nature of the living material itself, which is fundamentally similar in its structure and chemistry in all organisms.



EXERCISE 1

Where Can Life Exist?

The dawn of the era of space flight and the imminence of exploration of the nearby planets has given a new interest and sense of immediacy to the question of the existence of life elsewhere in the solar system and galaxy. Recently a number of scientists have presented reasons for believing that the possession of planets, many of which might have conditions suitable for the evolution of life, may be a common rather than an unusual feature of stars, and that life itself, far from being a unique event confined to our earth, may be of frequent occurrence in the universe. The discovery of life elsewhere in the solar system—most promisingly on Mars—and the analysis of this living matter and its comparison with the life of our own planet, would have enormous impact on biological theory.

It has been pointed out by biologists who are planning the future explorations for life beyond the earth that the recognition of living systems may prove to be no easy matter. Although these may be obviously analogous to earthly organisms, on the other hand they may differ radically in chemical composition and properties from life as we know it; a decision as to whether they are living may require a reappraisal of the definition of "life" in terms of the chemical composition of their planet. We may well ask, at this point, whether there is a set of indispensable attributes shared by all life on the earth. If so, which of these would we require matter from another planet to exhibit before we would decide that it was "alive"?

In this exercise some organisms are considered which possess quite surprising characteristics. They seem to depart radically in some ways from the organisms of our everyday acquaintance, and the differences appear to involve some of the basic requirements of life. They are introduced here because they may provoke us to question whether there is in fact a unity beneath life's diversity, and may give us a more sophisticated point of view in our consideration of the attributes of life.

There *are* characteristics which are believed to be common to all living material and which can be used to define the living state as biologists now understand it. These will be considered in subsequent exercises.

LABORATORY PROCEDURE

A. Life on the Earth

Observe and take notes as suggested in the following outline. Work in pairs in conducting the experiments.

1. An Environment at High Temperature

We are all familiar with the fact that life exists within the temperature range at which water is a liquid, and that most organisms cannot long survive a temperature near the boiling point of water. There are **thermophilic** or "heat-loving" algae, however, which can not only survive in water not far below the boiling point, but actually thrive and grow in it. Among these are the algae which are responsible for the bright yellow, orange, and blue-green colorations of the hot springs and pools of Yellowstone National Park (Figure 1-1). These algae are found in water as hot as 163°F, a temperature that would kill most forms of life. In hot sulfur springs there are also species of bacteria that can grow in this temperature range.

Observe the thermophilic algae or bacteria on display. One of these is *Cyanidium caldarium*, a blue-green alga which is remarkable because it is both heat- and acid-tolerant. It is the predominant species of alga in acidic hot springs, and often carpets the sides and bottoms of these pools with a bright blue-green layer. Make notes below on the appearance of the thermophilic organisms and the conditions under which they are being grown.

Figure 1-1. Thermophilic algae live in Yellowstone Park hot springs at temperatures as high as 163°F.

2. A Highly Acid Environment

Most organisms can survive only in environments which are not strongly acid. *Thiobacillus thio-oxidans*, however, is able to survive in surprisingly acid solutions. It is a bacterium which lives in soil and in the interstices of the damp concrete of walls, statues, and other constructions. It oxidizes sulfur in its vicinity to sulfuric acid, and is able to live in a 5% solution of this acid!

In determining the approximate degree of acidity of the medium in which the bacterium is growing, it is helpful to be acquainted with the notation used by scientists when they make such measurements. The degree of acidity or alkalinity of a solution is determined on a **pH scale.** Acidity is actually due to hydrogen ions (H^+), while basicity or alkalinity is due to hydroxyl ions (OH^-). In acid solutions (any *p*H below 7.0), H^+ ions are more numerous than hydroxyl ions, while the reverse is true in alkaline solutions (any *p*H above 7.0).

The *p*H is defined as the logarithm of the reciprocal of the hydrogen ion concentration expressed in grams per liter. Thus the *p*H of a solution in which the H^+ ion concentration is 1/100,000 g/liter would be 5.0, and the *p*H of a solution containing 1/10,000 g of H^+ ion per liter would be 4.0. The lower the *p*H value, the greater is the actual H^+ ion concentration. Since the *p*H scale is logarithmic, a solution with a *p*H of 4.0 is 100 times as acid as one with a *p*H of 6.0. Similarly, a solution with a *p*H of 8.0 is 10 times as alkaline as is a solution of *p*H 7.0.

The *p*H can be determined approximately by the use of "*p*H paper," which contains a dye giving a characteristic color response at a particular *p*H. Both wide-range and narrow-range papers are available; the former are useful over nearly the entire *p*H range from 0 to 14, but are not so exact as the latter, which change color over a range of 1–2 *p*H units. One type of wide-range paper in common use is deep red at *p*H 1, light red at 3, orange at 5, yellow-green at 7, deep green at 9, and blue at 11.

(1) Observe a culture of *Thiobacillus thio-oxidans* growing in the presence of sulfur, from which it forms sulfuric acid. Test the acidity of the medium with a small piece of wide-range *p*H paper. The paper will turn red if the medium is highly acid (*p*H 2 or 3). Also test a similar flask of medium containing sulfur which has not been inoculated with the bacterium. An orange color indicates a *p*H in the vicinity of 5, while a yellow or yellow-green color indicates a *p*H closer to neutrality. Record the results of your tests in Table 1-I.

TABLE 1-I

Comparison of pH Values of Solutions Supporting Plant Life.

SOLUTION	COLOR OF *p*H PAPER	*p*H (*approx.*)
Culture medium containing *Thiobacillus*		
Culture medium not inoculated with *Thiobacillus*		
______ water		
______ water		
______ water		

(2) Using the wide-range paper, test the *p*H of one or more samples of water available in the laboratory in which algae and other familiar water plants are growing, such as lake, pond, and aquarium water. Record your results in Table 1-I. Roughly, how many times as acid is the solution in which *Thiobacillus thio-oxidans* is growing compared with the lake or pond water containing a wide variety of living forms?

3. *An Environment Without Oxygen*

The plants and animals with which we are most familiar require gaseous oxygen and cannot remain alive for long without it. Certain kinds of bacteria, however, can exist in the complete absence of oxygen, and some of them are even *poisoned* by oxygen. The bacteria which can grow only in the absence of oxygen are termed **obligate anaerobes.** One of these, *Desulfovibrio desulfuricans*, has been isolated from hot artesian water, and can apparently grow deep in the earth in hot water under pressure.

Clostridium is an important genus of bacteria, all members of which grow only in the absence of oxygen. The majority of these are harmless, but the group also includes the organisms producing tetanus (lockjaw), gas gangrene, and botulism (a type of food poisoning).

Certain other bacteria which grow only in the absence of oxygen are photosynthetic, and require light in the manufacture of food. They include red bacteria in the genus *Chromatium* and green bacteria in the genus *Chlorobium*.

(1) Observe cultures of species of *Clostridium* or *Chromatium* on display in the laboratory. Two cultures of the organism should be compared, one maintained in a sealed atmosphere of nitrogen gas and the other kept in contact with air after inoculation. Indicate the relative amount of growth in the cultures.

(2) List some environments in which organisms might be expected to occur even though these environments have little or no gaseous oxygen.

4. *Environments With a High Salt Concentration*

Many plants, especially algae and bacteria, are adapted to life in the oceans, and in fact cannot live in fresh water. These plants thus exist and thrive immersed in water which has a total concentration of salts of about 3.5%. This is, of course, a much higher salt concentration than that which surrounds fresh-water plants or is present in the soil water with which roots of most land plants are in contact.

There are also salt-tolerant land plants, the halophytes (from Greek *halos*, salt), some of which grow in saline marshes along the ocean coasts and others in inland alkali flats like those near Great Salt Lake, Utah. Fleshy leaved saltbushes such as greasewood and glasswort are believed to have evolved from desert forms. They can be seen growing in the salt flats of the western United States, where the salt concentration is much higher than 3.5%; here white deposits of solid salt encrust the ground and the plants themselves. Many of these plants are **obligate halophytes:** they *require* a high salt concentration.

A few molds are known which occupy a special niche in our own surroundings, since they are able to grow even more luxuriantly in the presence of very high concentrations of salt than they are under low-salt conditions.

(1) Inspect tomato or bean plants which have been watered for 1–2 weeks with 3.5% salt solution. Record your observations.

(2) Examine cultures of the mold *Aspergillus glaucus* growing on a medium containing 20% NaCl. Compare the growth of this species of *Aspergillus* and of other species of molds on this medium and on a medium from which NaCl has been omitted. Record your observations.

5. *An Arid Environment*

Most plants with which we are familiar grow either in water or in soil in intimate contact with water and dissolved minerals. Next examine some plants that grow in environments where the availability of water and mineral salts would appear to be severely restricted.

(1) Lichens growing on rocks. A lichen consists of an alga and a fungus growing in intimate association as a single plant body. How do these lichens obtain water and minerals from their surroundings?

(2) "Spanish moss" growing suspended from a branch or wire. This is a flowering plant related to the pineapple. It can grow suspended from tree branches and even telephone wires. How do you think water and minerals are obtained?

(3) Cacti and other desert-inhabiting flowering plants. List some of the adaptations which fit these plants to their normal environment.

ENVIRONMENTS OF THE PLANETS

Earth	Surface temperature varies between −71° and +57°C. Atmosphere contains 79% N_2, 20% O_2, 0.03% CO_2, and small amounts of other gases; variable amounts of water vapor present.
Mercury	One side always toward the sun and very hot (+277°C). Temperature of side away from sun close to absolute zero (−234°C). No atmosphere.
Venus	Surface temperature in excess of 400°C. Atmosphere has little O_2, but high CO_2 content. Surface hidden by a continuous, heavy cloud blanket. Rotation time 10–30 days.
Mars	Surface temperature cooler than that of the Earth, varying between −101° and +30°C. Atmosphere rarefied, with atmospheric pressure equivalent to the pressure 10–11 miles above Earth's surface. So far only CO_2 detected in the atmosphere, but very small amounts of water and O_2 are assumed to be present; principal component assumed to be N_2. High intensities of ultraviolet radiation occasionally penetrate to the surface. Length of day nearly the same as on Earth.
Jupiter, Saturn, Uranus, Neptune	Very thick atmospheric envelopes containing hydrogen, helium, methane, and ammonia. Surface temperature believed to be quite low (below about −150°C).

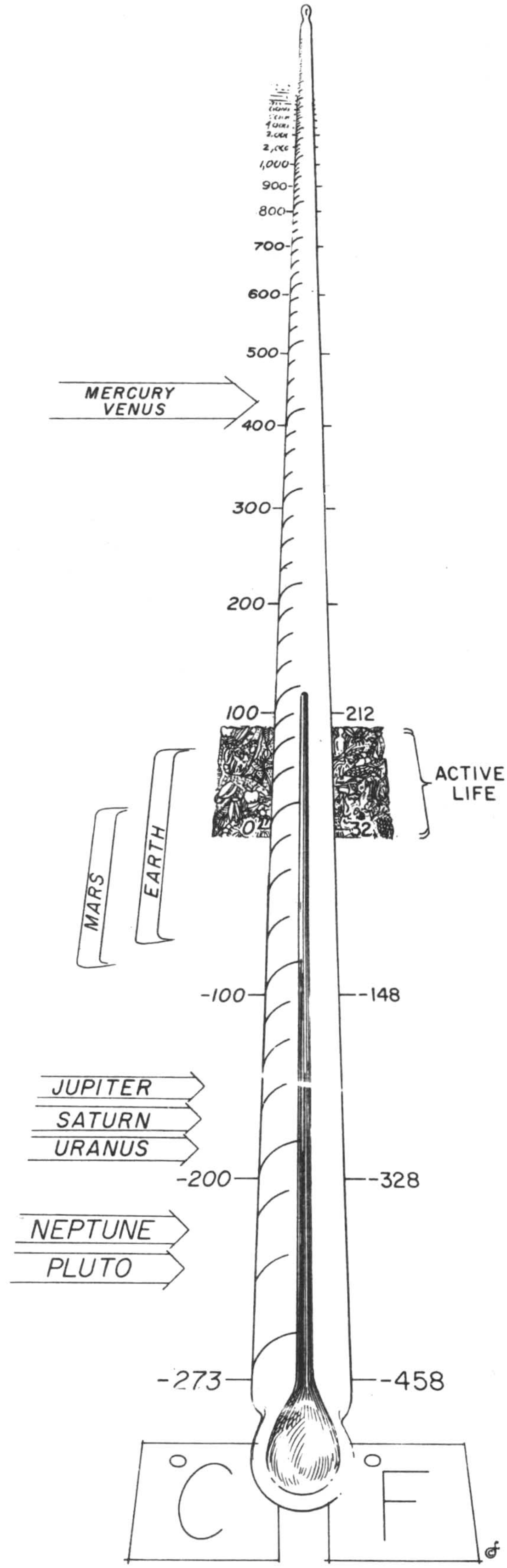


Figure 1-2. The planetary thermometer.

B. Life on Other Planets

Study both Figure 1-2, of a planetary thermometer, and the table of planetary atmospheres, and then answer the following questions.

(1) On which planet other than the earth do you think life is most probable? Why?

(2) Of the organisms observed in this exercise, which do you think would have the best chance of survival if introduced onto the surface of Mars?

(3) Would a bean plant be capable of growing on Mars? Discuss.

(4) What are some characteristics of planets—other than those discussed in this exercise—which might have a major influence on the occurrence or characteristics of living matter?

(5) What is "evolution"? If there is life on another planet in our solar system, do you think that it has undergone evolution? Why or why not?

QUESTIONS FOR DISCUSSION

1. It is believed that oxygen was absent from the atmosphere when life arose on earth, and that life evolved in its early stages under anaerobic conditions. Which of the organisms considered in this exercise might have been able to survive in that environment?

2. Do you think the algae of Yellowstone's hot springs may be similar to earlier forms of life which were common when active volcanism was widespread? Why or why not?

3. The chlorophyll in *Cyanidium caldarium* is rapidly destroyed if exposed to the concentration of acid in which this organism can grow. What does this suggest about the mechanism by which this alga is able to survive in a highly acidic environment?

4. In England, early in World War II, the National Fire Service hoses, which had linings of vulcanized rubber, suffered a curious form of corrosion of the linings, identified as damage due to acid. Make a guess as to the source of the trouble, and suggest a simple remedy.

5. It is occasionally desirable to reduce the alkalinity of a soil. Considering the activities of one of the organisms discussed in this exercise, what could you apply to the soil to accomplish this?

6. What is the salt concentration of human blood? How does this concentration compare with that of seawater?

7. What is the *p*H of human blood? of the human stomach contents? What is the *p*H range of soils in which crop plants are grown?

8. Was the earth's atmosphere at one time more nearly like the present atmosphere of any of the other planets?

9. Has study of the organisms considered in this exercise altered your previous views about the nature and properties of living matter? Discuss.

Figure 2-1. A few examples of the various ways in which plants obtain energy for life processes and of different types of associations among plants.

EXERCISE **2**

Energy: A Requirement for Life

Animals that move and maintain a body temperature higher than their surroundings obviously require a source of energy for these functions. In less obvious ways, all living organisms utilize energy to sustain the chemical reactions and complex organization which distinguish life from the surrounding inanimate matter.

Although the need for energy and the capacity to utilize it are features basic to all living forms, this does not mean that every organism obtains this energy by the same means. The competition for an energy source in the biological world is keen, and evolution has provided various mechanisms in organisms for obtaining energy (Figure 2-1). In this exercise some of the different ways in which organisms satisfy their energy demands will be demonstrated.

It is common knowledge that sunlight is the primary energy resource for all organisms, including human beings. However, light energy cannot be utilized directly to power cellular activities. It first must be converted to chemical energy in the form of sugar or other compounds of carbon. This is accomplished in **photosynthesis.** A few bacteria obtain energy for the formation of food (high-energy carbon compounds) through the oxidation of elements, such as sulfur and iron, or simple compounds, such as hydrogen sulfide (H_2S) and methane (CH_4). These are **chemosynthetic** plants. The photosynthetic and chemosynthetic organisms together are known as **autotrophs**— self-sufficient organisms capable of producing food by utilizing the energy from sunlight or from simple chemical substances in their environment.

Heterotrophic organisms cannot manufacture their own food, but are dependent on autotrophs for the carbon compounds that serve as a source of energy. **Saprophytes** are those heterotrophs which obtain food from nonliving material in their environment. The many bacteria and fungi which bring about the decay of animal and vegetable remains belong in this group.

Other heterotrophs obtain part or all of their food directly from the living tissues of other plants or animals with which they are intimately associated; these are **parasites.** The association obviously is beneficial to the parasite, but in many cases is to some degree detrimental to the host from which the parasite obtains food.

When two organisms occur in close physical association, deriving mutual benefit from the association, they are said to exist in a **symbiotic** relationship.

Insectivorous plants comprise an interesting group of organisms which obtain most of their food by photosynthesis, but by various devices also capture insects. To the extent that they rely on the insects as a food source, they are carnivorous.

There are many examples of close associations between organisms in which one (an **epiphyte**) grows in close association with a larger plant but the two are only loosely attached so that the epiphyte is not dependent on the host plant for food. Orchids growing on the limbs of tropical trees are epiphytes.

It should be realized that organisms do not always fall sharply into one of the above categories. Depending on conditions, some organisms may be either parasitic or saprophytic. For example, some bacteria which are parasitic in the animal body may be cultured in the laboratory on a nonliving medium. The degree to which an association of organisms is beneficial or detrimental to the participants also is often difficult to determine.

LABORATORY PROCEDURE

The following organisms, or other suitable ones, will be available to illustrate different modes by which energy is obtained. Examine each of them, classify it according to the means by which it obtains energy, and record your observations in Table 2-I.

Mushrooms
Purple bacteria
Green or blue-green algae

TABLE 2-1. *Classification of Organisms by Energy Source*

ORGANISM(S)	CLASSIFICATION BASED ON ENERGY SOURCE	REMARKS

Nitrogen-fixing bacteria in clover root nodules
Lichens
Spanish moss on a tree branch
Dodder
Wheat rust or powdery mildew
Sundews and pitcher plants
Fungus gardens from termite mounds
Tomato or bean plants

In most cases, the color of a plant is a reliable guide in determining whether it is primarily autotrophic or heterotrophic. If you are undecided about some of the plants, study of their association with their environment may prove helpful. For example, if a plant is growing upon another plant, does it send appendages down into the host, or is it only superficially attached or not attached at all? If a non-green plant is growing from the ground, can it be traced underground to a point of attachment with a living host or with dead matter? Examination with a hand lens and a dissecting microscope will aid you in determining these points.