BIOPHYSICAL RESEARCH METHODS

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PREFACE

This collective volume is intended to serve as a stimulating but critical guide to that rapidly growing group of scientists who must resort to physical methods of research for the solution of biological, medical, and agricultural problems.

Each chapter provides an authoritative orientation with respect to one general research method. To achieve this end, each chapter covers the following points: (*1*) the fundamental principle of one method, its underlying assumptions, and perhaps a simple mathematical outline of the theory involved; (*2*) the types of problems for which the method offers promise of a unique solution or a helpful approach; (*3*) the demands made on apparatus, materials, and technical skill; (*4*) the limitations of the method in some detail; and (*5*), briefly, its outstanding accomplishments to date. After reading any chapter, a scientist should be able not only to appraise the potentialities of its method with reference to his own biological problem, but also to understand the limiting factors that must be given recognition in the proper design of critical experiments.

The book should prove useful in a wide variety of advanced courses, particularly for collateral reading assignments aimed at the mature student. Each chapter can be read as an independent unit without regard to its position in the volume.

References to commercial sources of equipment and materials have been included by the several authors as a convenience to the reader. Listing of a product and its source does not necessarily constitute an endorsement nor does failure to list a product indicate its inferiority in any way.

My thanks are willingly expressed to the several authors for their kind cooperation at all stages of the work. Others who offered helpful suggestions during the planning stage include: Dr. L. R. Blinks, the late Dr. S. C. Brooks, Dr. C. S. French, Dr. D. R. Goddard, Dr. W. M. Stanley, Dr. Otto Stuhlman, and Dr. Maurice Visscher. Most of the editorial work was accomplished while I was Professor of Physics at Iowa State College. Consequently, I feel most indebted to the Department of Physics at Iowa State College for facilities and secretarial assistance; and particularly to Professors Jay W. Woodrow, Gerald W. Fox and Percy H. Carr for their encouragement. At the Navy Electronics Laboratory, the cooperation of Dr. R. J. Christensen has been very helpful.

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AVOID FRUITLESS EXPERIMENTS

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Few scientists, even on a holiday, would undertake deliberately to conduct a meaningless experiment. But most critical scientists will subscribe, I believe, to the assertion that numerous research articles are either meaningless or destined to be fruitless. Some published evidence exists to support this statement (see Sect. H) and if scientists were less courteous to their fellow workers, there probably would be much more.

The freedom to exercise scientific curiosity experimentally in a laboratory is a priceless heritage of modern science. This freedom, unexploited in ancient times and even denied during the middle ages, should be guarded zealously; it should not be jeopardized by careless indulgence and irresponsibility on the part of some investigators. To be valuable, contemporary research must place increasingly greater stress on the careful analysis, design, and often on the co-operative execution of experimental programs. To point a finger at some of the pitfalls which needlessly embarrass too many naive experimentalists is the aim of this introductory chapter. The writer is conscious of some of its limitations, but would welcome constructive suggestions from any source for its improvement. Specific examples and illustrations particularly are solicited. It is a pleasure to acknowledge my indebtedness to several of the sources mentioned in the bibliography and especially to an inspiring lecture by T. S. Hamilton (*22*).

A. APPROACHES TO IMPORTANT DISCOVERIES

To prescribe what must be done to avoid meaningless or fruitless experiments is relatively easy compared to the task of outlining a procedure for selecting the most meaningful and important problems. Were this not true, science would be much further advanced. No attempt will be made in this chapter to outline any such procedure, for reasons which probably are or will become obvious to the reader very soon. It is felt, however, that the time which could be saved by avoiding fruitless experiments might result in appreciably increasing the number of important discoveries. Some widely held, but conflicting, viewpoints on how to make fundamental discoveries will be presented now for the purpose of background to the fundamental research situation.

1. Accidental Approach

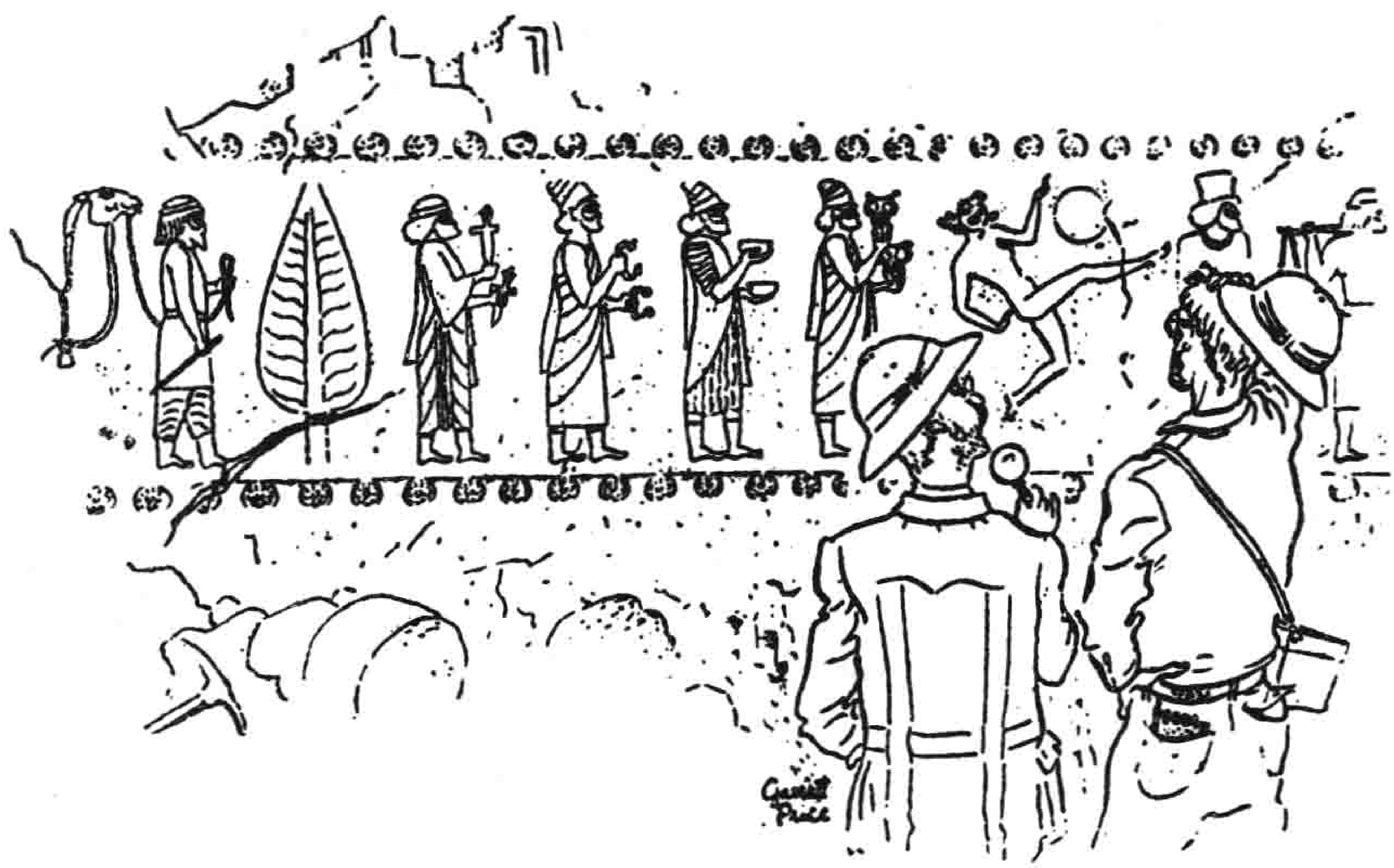
Not infrequently in the past, important scientific discoveries have been made by workers who were not trained to look for them. The idea is rather widespread that great discoveries are largely accidental in nature and that they may happen to almost anyone. The accidental theory of success in research has been strikingly epitomized in a recent remark by an American beer baron. With business booming, the baron had permitted himself the luxury of subsidizing a capable microbiologist, but subject to an annual appropriation. At a year-end conference on the budget, to which the laboratory superintendent had invited the microbiologist, the question of a renewed grant was up for consideration. But the baron quickly settled the matter. "Keep him on," he said with a flourish, "you never know when he might stumble onto something."

To ascribe the tremendous achievements of modern science to an unending series of accidental discoveries not only is placing "Lady Luck" on a very high pedestal but also is unfair to professional scientists. However, whereas an occasional important discovery *may* be accidental, most meaningless experiments are the result of inadequate planning and/or careless execution. While it may be professionally embarrassing for some to admit that an epochal discovery has been made in a backwoods attic laboratory by a novice with crude equipment and a high school education, it would seem downright disgraceful to be forced to acknowledge that many research experiments are not adequately designed by supposedly well trained investigators.

Another type of accidental approach is involved in the contention that experiments that are wrong ultimately lead to the great advances in science. The thought here is that the large amount of careful work required to prove an experiment incorrect results itself in fundamental contributions. This becomes secondarily a hybrid situation which includes incidental elements of the type discussed in the next section. However, the provocative incorrect experiment must be considered accidental—unless eventually efficiency experts deliberately publish such articles in order to stimulate research.

2. Incidental Approach

Important discoveries in science in recent years usually have been made by qualified investigators deliberately engaged in experimental research. Oftentimes it has happened that the important advance was merely a by-product, however, of the original problem under investigation. To this extent, then, the discovery is incidental. If the reader prefers to call it accidental, I believe he will concede that it is a type of accident that does not happen to just anyone. It should occur most frequently to investigators who are alert to the unusual and who are able to perceive quickly the possibilities in the unexpected observation (*cf.* Fig. 1). The fact of X rays, for example, represents a phenomenon that might have been discovered by anyone of perhaps a few hundred experimental scientists at the time, but almost incon-



"Now, I sort of lose the gist of it here."

Fig. 1. An unexpected observation often leads to significant advances. (Courtesy *The New Yorker*.)

ceivably by one of the billion contemporary laymen. According to this approach, great discoveries are favored by the hard work of numerous careful observers who are also resourceful.

3. Deliberate or Direct Approach

A considerable part of the motivation of research workers is doubtless the hope of making a really important discovery. In some scientists this purpose expresses itself as a deliberate, direct attempt. An investigator in this category analyzes every proposed problem primarily on the basis of its possible importance. Much can be said in favor of such a critical attitude. At the least, it prevents a worker from embarking on programs in which he has too little faith, the type undertaken merely with the hope that something worth while will result. The observer who is *looking for* something specific seems to have a greater chance of seeing it than an observer who is *just looking*. An investigator with a strong conviction that the natural world operates on simple laws has a stronger incentive to work doggedly to discover them than an individual who lacks faith in their existence. Absolute devotion to a particular problem, however, must surely end in failure in a great many cases; the number of relatively important discoveries are but a fraction of one per cent of the number of experimental attempts.

The striving for important discoveries, for the "order-of-magnitude" advances, therefore carries a high degree of risk. Some scientists are much better situated than others with respect to assuming this extra hazard. Apart from the mental adjustment and the intestinal fortitude demanded, there is usually an economic or timing factor. This is clearly true with most problems for graduate students. Beginners generally feel the need for early successes, but it can hardly be said that they gamble less than their older colleagues. The student's gamble, however, is often on his professor's judgment rather than deliberately on the problem.

This type of approach doesn't seem well suited to workers who need the frequent stimulation which results from minor successes. The great-gamble type of experiment is not necessarily devoid of by-products and incidental data which are of themselves valuable, provided the time and effort are expended to make them so. But the latter procedure detracts at once from the effort that can be put on the main purpose of the problem or on the next logical attempt at an important discovery. Perhaps few scientists are *entirely* free to pursue their own desires in the matter.

4. Organized or Controlled Approach

In commenting on the influence of Francis Bacon on the scientific revolution, Mees (*4*, p. 81) has stated that "Bacon over-estimated the ease with which scientific knowledge can be obtained, and he fell into an error in which he is followed by many today—the error of believing that scientific research can be organized like an engineering project and that the way to make scientific discoveries is to plan to make them." A great many scientific contemporaries share this view with Mees and are distrustful of too much "direction" of scientific research programs. President Conant of Harvard University has been quoted as saying, "There is only one proved method of assisting the advancement of pure science—that of picking men of genius, backing them heavily, and leaving them to direct themselves."

It should be emphasized that we are discussing approaches to *important* discoveries—without knowing in what direction they lie. The danger inherent in an organized or controlled approach is simply that of concentrating too much effort blindly in too few directions, thereby possibly missing altogether a really fundamental discovery. A strategic break-through on the research frontier would seem to become increasingly probable when many independent investigators are engaged in the search, each on his own initiative. Furthermore, the morale and efficiency of research talent might be lessened tragically by any large scale attempt at organized planning. Now that research scientists have achieved a very considerable measure of success, it is to be expected that efficiency experts will hover about their laboratories to tell them how to plan their experiments—if not, indeed, to control their investigations completely.

B. ANALYZE THE PROBLEM

Scientists resort to experiment when questions or problems arise which cannot be disposed of satisfactorily in any other way. Problems may be relatively trivial so that their experimental solution can be found in a matter of hours and at small cost in terms of time and money; or they may be exceedingly complex so that only a very extensive investigation requiring years of effort can conceivably result in a satisfactory elucidation of the difficulty. A current example of the latter type is the cancer problem. A broad problem such as this can be approached from many angles. There may even be more than one satisfactory solution. I believe that most experimental scientists expect without doubt that a solution will be found eventually. In the meantime numerous aspects of the problem present themselves and it is clear than an unlimited number of experiments could be performed to collect factual data. When ultimately an acceptable scientific answer is found, much of this experimentation will be regarded in all probability as having been quite useless. The bulk of the attempts will not have served any critical function. But how can such meaningless investigations be avoided? I believe that a careful analysis of the problem would do much to eliminate a large percentage of these fruitless experiments.

What constitutes a *careful analysis* of the problem? So many factors are involved that there is no single well marked groove or alley that can be followed blindly. A complete solution of a broad problem may require step-by-step progress through the various stages of what is often called the "scientific method." These stages have been enumerated recently by Northrop (*3*, p. 28) as follows:

(*a*) Discover the basic theoretical root of the problem.

(*b*) Select the simplest phenomenon exhibiting the factors involved in the difficulty.

(*c*) Observe inductively these relevant factors, either by the method of observation, the method of description, or the method of classification.

(*d*) Project the relevant hypotheses suggested by these relevant facts.

(*e*) Deduce logical consequences from each hypothesis, thereby permitting it to be put to an experimental test.

(*f*) Clarify initial problem in the light of verified hypotheses.

(*g*) Generalize solution to the problem by means of a pursuit of the logical implications of the new concepts and theory with respect to other subject matter and applications.

Individual investigators often pursue a very limited phase or aspect of a broad problem; for example, a number of scientists have devoted a lifetime of research to the purely descriptive phase of the cancer problem. This is most essential, but it constitutes only one part of the whole. In any event, an investigator should realize clearly how his individual research may contribute toward a general solution of *the* problem. The failure to view one's experimental work in the light of the broad over-all situation may result in the prosecution of numerous fruitless experiments.

1. Look for Basic Difficulty

You may wish to refer to the theoretical basis of a problem as its heart, its core, or perhaps its quintessence. That a problem exists at all would seem to indicate that a fundamental difficulty of some kind is present. The object is to uncover it, to understand its nature, in order that a solution may be obtained. No attempt will be made to set forth rules as guideposts to the heart of a research problem. Even hints as to how to grope for it cannot be given, but prolonged and serious reflective thought about the nature of the difficulty is highly recommended. A strictly armchair approach probably will not be sufficient. Many problems cannot be analyzed adequately until after extensive preliminary observations of a descriptive type are available. Not infrequently, exploratory investigations of the experimental variety must be conducted before it is profitable to try to lay bare the heart of the difficulty. This information may, of course, have been published already by others and thus be accessible.

In the process of arriving at the theoretical basis of a problem, there may be an intricate interplay between fact and fancy, between ideas and experimental data, between hunches and lucky observations, and between intuition and blunders. Most scientists do not record the tortuous paths traversed in the pursuit of their discoveries. The fact that some famous scientists have been responsible for several basic discoveries discounts the cynical view that scientific progress is purely an accidental process.

External stimulation of the thought processes should not be neglected in an effort to achieve an insight into problematical situations. Apart from the stimulus that derives from germane conversation and from browsing in likely fields of literature, a conscious effort should be made to broaden one's contacts with other, and perhaps remote, domains of scientific endeavor.

2. Focus Attention on Relevant Facts

"It is to be noted that it is the analysis of the problem which provides the criterion for selecting out of the infinite number of facts in the world the few that are relevant," to quote Northrop (*3*, p. 34). To proceed willy-nilly to collect irrelevant facts is not regarded as good scientific procedure, although it may occasionally result in a solution. There is also a possibility that the necessary and relevant facts already exist in the literature. Even after an armchair analysis has led one to the basic root of the problem, there may be several other factors that will have an important bearing on whether a given individual should undertake an experimental investigation. Some of these will be discussed in later sections.

To be most fruitful, experiments should be undertaken only to solve *bona fide* problems. Unless based on a genuine problem, an investigation lacks purpose and directive force. Only chaos can result from an attempt to record all possible facts. Even Charles Darwin, whose chief service to science was the production of a mass of descriptive evidence that evolution has occurred, has said, "How odd it is that anyone should not see that all observations must be for or against some view, if it is to be of any service." It is the problem that determines, for example, with what precision measurements are to be made and how extensive an experiment is to be. The discontinuous nature of experimental data demands that some appropriate interval of time be chosen as a guide in making observations. The value of this interval must be determined by the nature of the problem itself.

In making observations, the scientist selects some of the facts for attention; he does not attempt to record all of them. Scientific facts represent relatively only a very small number of all the facts that could be observed. Although scientists deliberately choose certain facts for observation, the selection must be made on the basis of proper criteria and in an impartial manner according to the unwritten code called *scientific honesty*. The choice is not one of mere caprice nor can it be purely arbitrary in order that the facts may fit into some preconceived scheme. To be accepted as fact, experiments must be reproducible by others.

The ability to distinguish readily the critical factual items in an experimental situation is the mark of a capable scientist. Although good scientists must be opportunists in the sense of making the most of unexpected observations, they must also simulate bloodhounds and not be distracted constantly by irrelevant facts of an inconsequential nature.

3. Discard Meaningless Questions

Scientific experiments are undertaken in order to answer questions concerning nature. To state that an experiment is meaningless either implies failure in securing an unequivocal or satisfying answer to the question, or it suggests that the question itself is without meaning. In a philosophical sense, one can defend the thesis that seriously propounded questions concerning nature always possess meaning. Only when a specific point of view is adopted does it become possible to define questions as meaningless or to assign a scale of values to them. One such point of view has been expounded by Bridgman (*1*, p. 28), who classifies questions as meaningless unless they can be answered by means of "operations." For instance, it means nothing to ask whether a star is at rest or not. To laboratory workers, "operations" connote experimental manipulation and observation. From this standpoint, for example, the question of whether there was once a time when matter did not exist possesses no meaning. Presumably in the same category is the question whether a rabbit and a mouse experience identical sensations to the color red. But all questions that could be answered in terms of operations would be meaningful.

In a narrower sense, many potentially meaningful questions may be meaningless at a given time or to a particular worker because methods or equipment might not exist with which he could conceivably perform the necessary "operations." An example: Are there mountains on the other side of the moon?

A recent discussion of what constitutes meaningful questions with respect to experimental science has been given by Churchman (*5*), who elaborates and extends the concept outlined by Bridgman.

4. Appraise Relative Importance of Problems

Individuals are constantly appraising the relative values of the several experimental sciences and of the more limited fields of investigation within specific branches of research activity. It is only natural that serious consideration be given to the possible significance of a proposed research program and its importance as related to the broader aspects of science and other human endeavor. The task is not an easy one. If history has taught any lesson clearly concerning the ultimate value of research to society, it is the fact that such values are unpredictable in advance. This results in part from the unexpected by-products of experimental studies but in large measure from the autocatalytic nature of cumulative scientific knowledge. Hence, a conscious steering of basic research by society would seem to constitute an unwise procedure, if not an unconscious boomerang. Even scientists, far from claiming omniscience either as individuals or as a group, are not qualified to render *a priori* decisions regarding the eventual value or meaning to society of scientific questions.

Even though society cannot render a verdict in advance as to the ultimate significance of scientific questions, it can often appraise the experimental results. For example, an experiment can be relatively fruitless to society if it constitutes merely a repetition of an earlier published finding or if it is not made available to the public at all, as is true of much commercial research, thus leading to further duplication of effort. Where results are not readily comparable to related investigations and where experiments are not carried to satisfactory and unambiguous conclusions, they may likewise possess little meaning to others. The basic reason for the abundance of meaningless experiments is simply the failure of numerous experiments to supply an unequivocal answer to the question. Results that are presumably satisfying to the investigator himself, at least in some degree, are often unconvincing to scientists generally.

5. Choose Initial Problem Wisely

For the individual contemplating a scientific research career, the choice of a first problem is a matter of extraordinary importance. This first problem often determines the course of a life's work. If it is an unfortunate choice, it may even result in the individual's turning to other fields of endeavor, perhaps forsaking research entirely.

All too often the selection of an initial problem represents the culmination of a haphazard procedure. Not infrequently, the problem derives as a consequence from the choosing of a research professor. Although this method has often worked out to the benefit of the beginner, still it should not be accepted as a routine matter—the percentage of failures is too great. Instead, a very real effort should be made to appraise the relative merits of the various problems under consideration on the basis of specific criteria. The final decision should be deliberate on the part of the individual; it should not be made for him by default or otherwise. In addition to the thoughts expressed under the various subdivisions of this chapter, many of which have a bearing on the selection of a problem, a few comments especially applicable to the beginner may be helpful. Apart from his possible immaturity and lack of experience, a beginner usually suffers also from time limitations. Therefore, a problem should be attractive not only for its own sake but also for its prospect of completion within a reasonable and rather definite length of time.

The following excerpts from an article by Livingston (*23*) are very germane and cover some suggested criteria for judging a proposed research problem for the beginner:

> "There are still those who maintain that any piece of scientific investigation carefully done and published must of necessity bear great fruit in future years, but such views are usually met with in those who do not seriously attempt to keep up with the progress of the current literature of their science.
>
> "The problem chosen should be circumscribed, definite and specific. At the same time, it must be appreciated how this particular problem is related to other similar specific questions, the whole series covering some broad and general field. It frequently happens that a problem which attracts and fascinates a graduate student is far too broad to be rationally attacked, sometimes the mere breadth constitutes an attractive feature and throws a false glamour over the entire proposition. Such a question should be separated into partial questions and these attacked singly. The attempting of too broad and, hence, too indefinite a problem in his earlier years of research has worked lasting injury upon many a man of science.

"The ideal problem for a beginner should be capable of statement, on *a priori* grounds, in the form of several alternatives; all the logically possible answers to the questions may be advantageously erected into hypotheses, and these may be tested in order. This sort of a problem conduces to logical thinking and must leave its sterling mark upon the mind in later years. Furthermore it is economical of energy and time, and the end of the chosen piece of work is more or less clearly in view at the beginning. To bring a problem into this condition requires, of course, a large amount of thought at the outset. . . .

"The satisfactory problem must, of course, be capable of experimental treatment with the knowledge and facilities which are available. The beginner should not be called upon to devote too much time and energy to the devising of methods and the obtaining of apparatus. If he be misled in this he almost surely becomes more interested in the methods than in the results obtained by their employment. This does not imply that the methods to be used should all be familiar to the worker at the start, only that they should be accessible in the literature, so that he need not actually devise them.

"Apparent importance to the science as a whole is a very important criterion in our series. For the best results in all ways, the selected question should be one that interests both the theoretical and the practical worker. . . . The question of the theoretical importance of a given problem is not so easily settled as is that of its practical weight; it requires something of a prophet to judge rightly in this regard. A good way to attack this question is to ask, will any chapter of the science (as it stands at present) be fundamentally altered by the proposed study? . . . A superficial study of a little-known relation is often as important in the development of a science as is a research upon the details of a better-known and already more thoroughly analyzed phase. Such superficial studies are the work of pioneers; they are adapted only to the exceptional beginner in research."

6. Pursue Type of Research That Comes Naturally

Within the framework of the general aim that dominates all scientific work, there may be various subsidiary purposes peculiar to individual types of investigation. Some specific examples are:

(*a*) To test the limits of application of a general theory.
(*b*) To explore a new field for its possibilities.
(*c*) To create an instrument of measurement.
(*d*) To develop or improve some experimental method.
(*e*) To determine constants with a high degree of precision.

A worker who excels in doing experiments of the highest accuracy might not be very successful in exploratory investigations which do not utilize this special talent fully. Scientists accustomed to making