The Clayton Foundation Biochemical Institute

A Short History

by Roger J. Williams

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I - Introduction

This Institute, integrated with the Chemistry Department of The University of Texas, is of the date of this writing (1965) an association of eight principal investigators along with a substantial number of semi-independent and cooperative investigators and a large number of graduate and undergraduate students, technicians, secretaries and others who contribute in many diverse ways. The role of graduate students is important and the Institute accordingly performs a considerable educational as well as investigational function. The total present staff includes something less than 100 individuals.

The principal investigators are: David J. Cox, Ph.D. (1960) Pennsylvania; Robert E. Eakin, Ph.D. (1942) Texas; Boyd A. Hardesty, Ph.D. (1961) Cal. Tech.; Lester J. Reed, Ph.D. (1946) Illinois; William Shive, Ph.D. (1941) Texas; Alfred Taylor, Ph.D. (1935) Oregon State University (retiring Sept. 1965); Roger J. Williams, Ph.D. (1919) Chicago; and Daniel Ziegler, Ph.D. (1955) Loyola.

The principal investigators in the Institute are autonomous with respect to their own research activities. Since they have been selected on the basis of the appropriateness of their interests, they are free to operate as they wish; there is no disposition to guide or direct their efforts or to curtail or modify their thinking or the expression of their thoughts and opinions. By encouraging each investigator to make his own contributions and earn his own reputation, it has been possible to attract and keep able and highly productive individuals on the staff.

Each of the principal investigators, except Dr. Alfred Taylor whose training was primarily in zoology (physiology), has a professorial appointment in the Chemistry Department. One of the principal investigators, William Shive, is Chairman of the Chemistry Department. In addition to the tie with the Chemistry Department, the Institute has always enjoyed cordial cooperative relations with all related departments and schools in the University.

The policy of helping each independent investigator develop his own program has been successful in promoting their individual advancement. A notable example is one who is no longer on the staff, namely Professor Esmond E. Snell. He gained sufficient eminence in the Institute (1939-45 and 1951-56) to be given a professorship in his Alma Mater, Wisconsin, and later to be made chairman of the Department of Biochemistry at the University of California (Berkeley).

When I first came to the University of Texas in 1939, my research had temporary Rockefeller Foundation support. On Friday, September 13, 1940, Clayton Foundation support was received; the operation was designated an Institute and I served as director, with continuous Clayton Foundation support, from that time until September 1, 1963. This support has been given in a most satisfactory manner; members of the Institute have been able to devote the maximum of their effort to the work that interests them and a minimum to administrative details.

In September 1963, Lester J. Reed became in name and in fact director of the Institute. It is at his instigation and suggestion that this brief story of the Institute and its contributions is being written. It will perhaps serve as a 25-year report to the Clayton Foundation and to inform others of the nature of our objectives and past contributions.

The deepest roots of the Institute go back to the joint interest of Mr. Benjamin Clayton and myself in the fundamentals of the cancer problem. Fortunately there has never been a disagreement between the two of us with respect to (1) our mutual interest in the cancer problem, (2) our recognition that the road to success in the cancer field may be a long one, and (3) the paramount importance of a fundamental approach.

The situation with respect to cancer research may be compared to an imaginary one in which an unlettered inexperienced novice (or group of novices) sets out to repair a complex malfunctioning machine—a watch, an automobile, a radio or a television set. There are two general courses open: (1) to tinker with the machine, make changes here or there with little or no basic understanding, in the hope of stumbling quickly upon the cause of the malfunction; or (2) to take a longer and seemingly less direct course, that of first making a careful scientific study of the machinery in all its parts, before concentrating on a solution to the difficulty.

Cancer being as deep-seated as it is, and the machinery of living matter being as complicated as it is, we have been inclined, justifiably, to take the latter course. We have worked in the direction of learning more and more about the machinery considered to be "in order," as well as that which is obviously out of order, before seriously attempting to correct the malfunctioning involved in malignancy.

The analogy of repairing a malfunctioning machine is satisfactory as far as it goes, but a human organism is incomparably more complicated than any man-made machine. Even a single cell (our bodies are made up of trillions of these) is so complex, particularly as revealed in recent years by electron microscope studies, that it is quite impossible as yet even to name and describe all the working parts. At one time it was supposed that cancer was purely a cellular derangement and that the secret of malignancy lies embedded in the functioning of "the living cell." Now it seems certain that the obscure influences which control differentiation and the interrelationships between cells—the coordination of their activities—are involved.

It has become increasingly evident during the years while the Institute has been in operation that cancer is indeed a most fundamental disease process and not one that could be expected to yield to blind or nearly blind tinkering. A vast amount of background information was and still is essential to an intelligent approach to the disease. From this point of view a fundamental attack

on the cancer problem is, in effect, an exploration in fundamental biochemistry.

Of course everyone concerned with the cancer problem would like to see a quick and happy solution, but without more basic understanding this is not likely to happen. There has never been exhibited in this Institute an impatient desire to get at the matter of an immediate cancer cure nor has there ever been a failure to recognize that any fundamental contribution to biochemistry and nutrition (which is, of course, one of the basic aspects of biochemistry) would inevitably be something of a step toward the ultimate solution of the cancer problem.

The approach we have followed has several advantages. First, we believe it will ultimately lead to treatment and prevention of cancer; second, it has made and now is making possible many other discoveries which may be of far-reaching importance; third, it has a tremendous psychological advantage in that investigators can exploit their intellectual curiosity and gain a feeling of accomplishment when they reach a milestone (even though there may be *many* milestones of many different sizes and shapes). Any investigator who makes a pointed effort to find a cancer cure is likely to develop after a decade or two of failure a feeling of futility and frustration.

There is nothing more fundamental in biochemistry than the amino acids, proteins, minerals, lipids, carbohydrates, vitamins, coenzymes, enzyme complexes, hormones, nucleic acids, viruses, genes and other fundamental units that enter into the machinery of living things. All of these are involved in the cancer problem in a highly significant way, and anyone who contributes to scientific knowledge about any one of these is making a potential contribution to the cancer problem as well as to the understanding of all diseases associated with the malfunctioning of cells. Parenthetically, I believe this includes *all* diseases.

There is nothing in our history that would imply that our Institute's objectives must, in perpetuity or even for a stated period, be centered upon any specific disease or upon any particular approach. It is my hope and expectation that the direction which investigators will take 10 or 20 years from now will be determined by the investigators who from their respective vantage points will be free to use their own best judgments.

Before listing some of the contributions of this Institute, it is desirable to clarify further some fundamental attitudes which have prevailed.

We have consistently promoted independent thinking on the part of members of the Institute. It follows therefore that there can never be in this Institute such a thing as a settled orthodox credo or point of view on which all must agree. There is, in my opinion, no place for orthodoxy in science.

This conviction is based partly on past experience. I am inclined toward the view that whenever a committee of scientists, an editorial board, a review panel, or what not, attempts to *settle* a scientific question, they are not only liable to be wrong as to the settlement, but they are making a basic mistake. They overlook the truth in Orville Wright's saying, "If we all worked on the assumption that what is accepted as true is really true, there would be little hope of advance."

I have personally witnessed in the field of biochemistry two far-reaching examples of "*settled*" points of view which have had to be shortly abandoned. Other such abandonments are in the offing.

As a beginner in research I became interested in the possible role of vitamins in the nutrition of yeast cells. After I had been investigating this area several years intermittently without signal success, I received a friendly letter from a very prominent investigator—an editor—advising me that to investigate vitamins one must work with the nutrition of *animals*, not with yeast. He urged me as a young man not to waste my efforts. This was the orthodox *settled* view at the time. In the light of subsequent developments involving now a generation when most of the findings about vitamins have been and are being made using microorganisms, such a view seems ridiculous.

Another abandonment of orthodoxy has taken place in the field of cancer. Based partly on findings in our laboratory and partly on the extensive investigations of Duran-Reynals, both Dr. Taylor and I became convinced in the early 1940's that in all probability virus-like agents were involved in the production of substantially all cancers. In 1944 I took part in a symposium at Atlantic City where I expounded this view. Dr. Vincent du Vigneaud was the presiding officer and was kind enough to remind the audience that since I had been right in anticipating the vitamin status of pantothenic acid, I quite possibly might be right in my opinion that viruses were involved in the production of cancers. But this was an unorthodox view and was discredited by most cancer investigators for at least a decade. Now it is generally accepted. No one should quarrel with the need for conclusive evidence—this was not on hand at the time of the Atlantic City symposium—but one may quarrel quite justifiably with the thought tenaciously held for many years that the question of the virus origin of cancer had been *settled* in the negative.

What seems unorthodox at one time may be quite proper and respectable at a later date. This has happened repeatedly and will continue to happen. To appreciate all the major contributions of the Institute, it is essential that this *unorthodox* $\rightarrow$ *respectability* process be recognized, because some of the more important contributions of the Institute have been in the realm of advanced ideas, and these do not always find a quick roosting place in scientists' minds. It is of course possible that some of the contributions which I would regard as important may never gain respectability. *It is the inalienable right of every investigator and thinker to be wrong part of the time.* Even ideas which turn out to be erroneous, however, do have value if they provoke thought and stimulate investigation.

Some concept of the work carried on in the Clayton Foundation Biochemical Institute can be gained by perusing the appended titles of the approximately 700 scientific articles (and books) that have been published during its operation. The articles are in most instances technical in nature, and represent the efforts of nearly 300 investigators who are the authors and coauthors. To all of these much credit is due. These citations alone, however, do not fulfill the purpose of this record; to supplement them I have run the risk of selecting what I regard as the more outstanding and provocative contributions and commenting briefly on some of these.

Since it is expected that the material in this short history will be of interest to others than trained biochemists, I have discussed more fully the contributions that are of some general interest and

have abbreviated or omitted discussions related to more highly technical contributions. The treatment of topics is thus necessarily uneven. Certainly the importance of a contribution should not be judged on the basis of the space given to its discussion.

It should be understood that if Dr. Reed or any of my other colleagues were to select for discussion the outstanding contributions of the Institute, the selections would be different from mine. The published papers speak for themselves to the many who have read or will read them. My selected contributions will be grouped according to their nature and considered without regard for strict chronology. The grouping must be imperfect since some contributions do not fit into any single pigeon hole.

II - Contributions Related Primarily to the B Vitamins and Closely Related Substances

1. Determination of structure and synthesis of pantothenic acid. The discovery of this vitamin took place before the Institute was founded, but its structure was announced jointly from the University of Texas and the Merck Laboratories in 1940 (Publ. 3), and it was synthesized with high yield in the laboratories of this Institute at that time.

2. Clearing up of the vexing "bios" problem (Publ. 17). This had presented an enigma for about 40 years.

3. Development and application of microbiological assay methods for riboflavin, pantothenic acid, biotin, nicotinic acid, pyridoxin, inositol, thiamin, folic acid (the vitamin needed to cure the typical nutritional macrocytic anemias) (Publs. 11, 24, 29, 30, 31, 32, 33, 34, 35, 36) and later the anti-pernicious anemia factor (vitamin B_{12}) (Publs. 197, 220), and lipoic acid (Publs. 160, 161, 242).

The use of microbiological assays in the Institute laboratories has been of outstanding importance. Up to March 1952 at least 36 different microbiological assays had been developed and used *in additio*n to those for most of the protein-derived amino acids. A few of these assays proved to be spurious, some were abandoned without complete investigation, but many of them turned out to be assays for new vitamin-like substances or for metabolites of unusual interest.

4. Establishment of the cause of (raw) "egg white injury" in experimental animals and the discovery, purification, and crystallization of *avidin* (Publs. 4, 15, 19, 20, 45).

5. Extensive studies on the distribution of B vitamins in tissues and foods. From these it was first deduced that B vitamins are to be found in all living cells, findings which contributed considerable strength to the concepts then developing and currently accepted concerning the "unity in biochemistry" of all living organisms. (Publs. 37, 60-70).

6. The finding that each type of mammalian tissue, whether from rats, mouse or human, tends to have a characteristic pattern with respect to its content of the various B vitamins (Publs. 62, 63).

7. Isolation of "folic acid" from spinach. We gave this vitamin its name because of its relative

abundance in leaves (Latin—*folium*—leaf). Thirty tons of spinach were processed and samples of highly concentrated folic acid were furnished upon request to 150 or more laboratories throughout the world. This distribution was made possible by a specific Federal grant (Publs. 21, 91, 102).

8. Further contributions relating to pantothenic acid.
(a) In chick "dermatitis" (pantothenic acid deficiency) in which the readily observable symptoms are those where skin and feathers are conspicuously affected, we found general impairment of muscles, kidneys, liver, brain, etc. in spite of the absence of any conspicuous lesions in these tissues (Publ. 12).
(b) Human muscle (the most abundant tissue) was found to be over twice as rich in pantothenic acid as beef, swine or sheep muscle. The ratio of pantothenic acid content to thiamin content is about three times as high in human milk as in cows' milk. Mouse milk (very rich in B vitamins) contains 60 times as much thiamin as human milk but only 15 times as much pantothenate (Publs. 63, 69, 70). The relative abundance of pantothenic acid in *human* muscle and in *human* milk suggests, on the assumption that all this pantothenic acid has an exogenous origin, that human pantothenic acid deficiency is, *a priori*, not improbable.
(c) Pantoyltaurine, the first pantothenic acid antagonist was synthesized (Publ. 25).
(d) When hens and pregnant rats and mice were fed additional pantothenate, the hatchability of the eggs increased and the litter sizes of the rats and mice increased (Publs. 27, 89).
(e) Extra pantothenate (added to a supposedly wholly adequate diet) increased the longevity of mice significantly by 19 per cent (Publ. 551).
(f) Pantothenyl alcohol was synthesized (Publ. 125).
(g) Our Institute collaborated with F. Lipmann to show for the first time the presence of pantothenic acid in coenzyme A (Publ. 176).
(h) Identification of the *Lactobacillus bulgaricus* factor as pantethine (Publ. 291).

9. The discovery of pyridoxal and pyridoxamine, the metabolically active forms of vitamin B_6, and their mode of functioning in metabolism. This monumental and highly technical work merits especial emphasis because of the quality and quantity of the work involved (Publs. 115, 117, 121, 122, 123, 124, 128, et seq.).

10. The isolation of vitamin B_{12} (using assay methods developed in our Institute) from liver preparations used in treating pernicious anemia (Publs. 197, 220). Priority on this contribution cannot be claimed by our Institute because a commercial firm having many times our manpower resources produced the same compound five months earlier. They used an assay method developed at the University of Maryland.

11. Patented process for producing vitamin B_{12}. This, like the pantothenic acid patents, brought into the Institute a substantial supplement to its other funds (U.S. Patent 2,628,186, 1953).

12. The isolation, determination of structure and synthesis of lipoic acid (acetate replacing factor, pyruvate oxidation factor, protogen) with the collaboration of Professor I. C. Gunsalus of the University of Illinois and Eli Lilly and Co. (Publs. 234, 244, 278, 317, 319). By that time (1951) this vitamin-like substance had been investigated intensively in our laboratory for about five years and its isolation was a culmination of this work.

III - Contributions Related Specifically to Cancer

13. Cancers from different species (rat, mouse, human) regardless of tissue of origin, and whether spontaneous or artificially induced, were found to have a resemblance to each other, so far as the content and distribution of B vitamins are concerned. (Publ. 64).

14. The finding that folic acid tends to be in relatively high concentration in cancer tissue (from rats, mice and human) whereas other B vitamins tend to be low. This finding was to have far-reaching effects in other laboratories where many folic acid analogs and presumed antagonists were explored with substantial success and found to be useful agents to combat some forms of cancer, notably leukemia. (Publs. 64, 150, 74-78).

15. The finding that the injection of various individual B vitamins into developing eggs may cause the embryos to develop into abnormally large chicks, or it may inhibit their growth, or it may cause the formation of deformed chicks. (Publ.27).

16. Technique of cultivating cancers in the yolk sac of chick embryos. (Publs.51,96, 114,200,328).

17. Lowered hemoglobin levels associated with cancer growth. (Publs.46,97,145).

18. Evidence for the virus etiology of mammalian cancers. (Publs.80, 118,137,139, 167,170,207).

The literature on this subject is voluminous. Dr. Taylor (Publ. 80) produced mammalian cancers by injecting cell-free material but could not obtain such results regularly and consistently, for reasons which are not wholly obscure in the light of present knowledge. The most conclusive evidence he obtained in support of the cancer-virus idea was the repeated production, by the use of one type of cancer-derived material, of *many different types* of cancers, entirely different histologically from the original cancer from which the cancer-inducing material was derived. This included intraocular cancers in rats produced by administering mouse material. It is difficult to explain these results, which were obtained dozens of times, on any basis other than the presence of virus-like infective agents.

IV - Contributions Related to the Composition, Structure and Functioning of Living Cells, Including Enzyme Studies

19. Protein structure is of paramount importance in biochemistry. The first microbiological determinations of amino acids derived from proteins (Publ. 110) were carried out in our laboratories and these were destined to be widely used in fundamental investigations.

20. Ascending paper chromatography was first used and developed in our laboratory (Publ. 182). This is a widely used tool for fundamental studies.

21. The development and application of competitive analog-metabolite techniques (inhibition analysis) for studying metabolic transformations and the mechanisms involved (Publs. 155, 156, 157, 158, 166, 179, 186, 191,220, et seq.). These pioneering studies have been progressively highly productive in many directions, including the elucidation of biological control mechanisms. Many of the contributions are highly technical and cannot well be discussed here.

22. The initial discovery that biotin functions by participating in processes involving carboxylations (Publ. 180).

23. Identification of 5(4)-amino-4(5)-imidazolecarboxamide as a metabolite and a precursor of purines. (Publ.178).

24. Discovery of formyl folic acid, a functional derivative of folic acid (Publ.189).

25. The isolation of thymidine from liver extracts used in the treatment of pernicious anemia, and the demonstration of its important relationships to vitamin B_{12} and nucleic acid metabolism (Publ.197).

26. Functional interrelationships between purines and vitamin B_{12} (Publ.199).

27. Erythein and apoerythein and their relationship to vitamin B_{12} and to the intrinsic and extrinsic factors which cure pernicious anemia. (Publ.212).

28. Discovery of folinic acids and their first chemical syntheses. (Publ. 211).

29. Widespread investigations (see above items 3, 21, 23, 24, 25, 26, 28) yielded many of the important clues as to how nucleic acids are built up in living organisms. This information is invaluable in the light of recent investigations in the area of biochemical genetics and for understanding normal and pathological growth and development.

30. Isolation and identification of a “cabbage juice factor” as the methylsulfonium derivative of methionine (Publ. 357).

31. Arginosuccinic acid was discovered and identified independently in the Institute laboratories (Publs. 273, 345) but this finding was anticipated in New York by Dr. Sarah Ratner.

32. Discovery of glutamine as an agent which reverses the toxicity of alcohol for microorganisms (Publ. 382), reduces the voluntary consumption of alcohol by rats (Publ. 403); and the finding that this compound appears to be for some human individuals highly effective in reducing their appetite for alcohol (Publs. 464, 686).

33. Contributions to biogeochemistry and to the problem of what chemical elements find usefulness in living matter. (Publ. 574).

34. The discovery of a group of unique enzymes belonging in the general group of oxygenases

(Publs. 670, 678, 679). These are essential for the detoxification by the liver of certain harmful nitrogenous compounds which would otherwise accumulate, and for the inactivation of certain hormones and hormone-like substances which are produced by other tissues. This production of certain substances by one tissue and their inactivation by another is one of the delicate control mechanisms in highly organized types of life.

Other enzymes of this group are essential for the building and degradation of sterols and steroids (adrenal, ovarian and testicular hormones) including cholesterol. The building of cholesterol in the body is an essential process during growth. The continued synthesis of cholesterol in adults can be excessive and unless the mechanisms for degrading it to bile acids keep pace, blood vessel disorders may develop in one's heart, brain, kidneys and elsewhere. Knowledge about these new enzymes is not only basic, it is of the utmost importance in the understanding of and designing of therapeutic controls for a number of metabolic disorders associated with "hardening of the arteries."

35. Possibly the most elegant work contributed by our Institute is that dealing with the multienzyme units—the pyruvate dehydrogenase complex and the α-ketoglutarate dehydrogenase complex. (Publs. 570, 571,572, 628, 666, 684, 688, 694). The make-up of these units (each is distinctive) may be illustrated by the case of the pyruvate dehydrogenase complex which has a molecular weight of about 4.8 million and has in it 42 molecules of enzymes of three different kinds. One type of enzyme has thiamin in it, another has lipoic acid, and another riboflavin.

These multienzyme units have both been obtained in a highly purified state and electron microscope pictures show that they are made up of many subunits clustered together according to a fixed closely fitting pattern. By suitable mild treatment the cluster can be partially or wholly dissociated, and electron microscope pictures of the "pieces" have confirmed that this takes place. Each individual enzyme, however, retains its own particular activity.

Most remarkable of all is the fact that when these individual enzymes are put together in the same solution in the proper proportions under suitable conditions they "fall together" naturally to produce the original multienzyme unit, which functions as originally and which, according to electron microscope pictures, has the same cluster structure as that originally observed before the complex was dissociated.

The implications of these findings which involve multienzyme units from bacterial cells and beef kidney cells, are far-reaching. As a result of these clear-cut demonstrations, it seems highly probable that living cells instead of being bags full of an assortment of enzymes, are highly organized in many particulars and that the enzymes are associated together in a particular way so as to make the machinery of the cell work.

These investigations are a follow-up of the isolation and synthesis of lipoic acid (item 12 above) and constitute an important contribution to our knowledge of how the biochemical machinery of cells operates. It will serve as a model for many later investigations.

V - The Problem of the Origin of Life

36. Evolution of Metabolism (Publ. 659). This paper, in which the author has outlined in considerable detail how the beginnings of metabolism probably antedated by millions of years the beginning of "life" on earth, has received, in terms of requests for reprints, far more attention than any other publication arising out of Institute studies during the 25 years of its existence. It is a publication of great originality and is thoroughly modern in its approach and incorporates keenest biochemical insight.

VI - The Problem of Differentiation

37. Before the mid 1950's there were so many gaps in our knowledge about what goes on within each living cell that it was difficult or impossible to study biochemically what goes on between the cells. Our contributions related to vitamin B_{12}, lipoic acid and the building of nucleic acids helped to fill in these gaps.

About 1955 one of our members seriously attacked the problem of differentiation. This problem is set forth by a consideration of the following facts: Single fertilized egg cells—these are the starting point for every human being and for every mammal—are similar in general make-up to that of a single-celled organism. However, they are also vastly different. When a single-celled organism is in a favorable environment it replicates or copies itself; that is, it produces successively more single-celled organisms just like the original. When, however, a mammalian fertilized egg cell produces "offspring," at first they resemble the parent cell, but the offspring eventually become vastly different in size, shape, composition and function. To illustrate extreme differences in size, some human cells are little pellets about 5 microns in diameter (a micron is 1/25,000of an inch) while some other cells (motor nerve) may be150,000 times this long (30 inches).

The process of becoming successively different after a number of cell generations is called differentiation and takes place in all many-celled organisms, plant and animal. The factors which control what types of cells are produced and how many of each are almost completely unknown. One striking fact is that the cells produced from a human fertilized egg cell are all human cells with the human quota of chromosomes and genes. How this can happen is as yet mysterious. One reason for wishing to understand it better is because when cancer cells develop, there is evidence that the normal control mechanisms have become impaired, and it would be of the greatest help in dealing with cancer if we knew about the basic mechanism and the kind of impairment that develops when the disease takes hold.

The kinds of organisms used by investigators in our laboratories for the study of differentiation include: (1) simple multicellular plants—molds and mosses—in which only a few different kinds of cells are produced; (2) primitive invertebrates, hydra and planarian worms (flat worms) which can in a short time regenerate new parts if these are cut away—embryonic (undifferentiated) cells remaining within these organisms are caused, as a result of the cutting, to differentiate and restore the missing parts; (3) tadpoles whose vital organs during their normal metamorphosis undergo a drastic revision of their biochemical, physiological, and morphological constitution

(they change from a vegetarian-fishlike creature to a carnivorous-terrestrial animal); (4) chick embryos in which development can be studied and altered by the use of specific chemicals.

The findings so far may be briefly outlined: In molds and mosses, chemicals naturally produced by the organisms, as well as synthetic products which resemble them, can markedly affect development, and often their effects depend on their presence at precisely the right stage of development.

In hydra and planaria both the rate and the normalcy of regeneration of the several parts can be altered (and even monstrosities produced) by specific chemical agents. The actions of these compounds are probably fundamentally like those of carcinogens (cancer-producing substances).

In tadpoles at about the mid-point of metamorphosis the synthesis of new ribonucleic acids and deoxyribonucleic acid derivatives takes place first, at a time interval considerably before the enzymes are developed to change the nitrogenous excretion product of tadpoles (ammonia) into urea, the product excreted by adult frogs. During the last portion of the metamorphosis period, the carbohydrate-splitting enzyme—α-amylase—becomes relatively inactive. This is appropriate because of the organism's change in diet. Agents which speed up metamorphosis (e.g. thyroxin) and those which slow it down (e.g. thiouracil) affect the enzyme changes just as they do the anatomical changes.

In developing chick embryos (where a large part of the differentiation occurs during the first five days) it was found first that the idea of Needham, the famous English embryologist, that ammonia excretion takes place first during embryonic development is in error even though it had been accepted for at least twenty years; secondly, that no dramatic changes, qualitative or quantitative, take place in the ribonucleic acid composition of neural tissues, paralleling the sudden appearance of electrical activity (electroencephalograms, brain waves) when the chick embryo brain becomes functional; and thirdly, four different and chemically unrelated compounds (oral carcinogens) affect the proliferation and morphology of liver cells in developing chick embryos and at the same time induce biochemical changes related to nucleic acid metabolism. These changes occur only during the last 5-10 days of the normal 21 day hatching period. The contributions dealing with the subject of development and differentiation include Publs. 422, 436, 461, 462,468, 469, 474, 475, 496, 497, 531, 532, 539, 553, 575,580, 584, 585, 615, 616, 617, 618, 619, 620, and 634.

The process of differentiation is of great interest for many reasons, including those suggested under items 41and 42 in this text. Two closely related animals (or human beings) which have about the same chromosomes and gene patterns may nevertheless have highly distinctive endocrine patterns because the unknown factors which control differentiation may allow in one case the development of a large and active thyroid gland(for instance) and in another a small one.

The subject of differentiation is like a new biochemical continent which is just beginning to be explored.

VII - Contributions Related Primarily to the Broad Applications of Biochemistry

38. We first attacked the problem of the etiology of alcoholism on the hypothesis that it was based upon the interplay between hereditary and environmental influences (Publ. 172). Ensuing investigations led to the publication of a book "Nutrition and Alcoholism" (Publ.239) which was superseded by "Alcoholism: The Nutritional Approach," (Publ. 533) (See also item 32).The ideas set forth in these books are based upon the broader concept of nutrition discussed under item 42, and upon the genetotrophic principle (item 39). In October 1964 the Institute co-sponsored with the Christopher D. Smithers Foundation a symposium in New York on Biochemical and Nutritional Aspects of Alcoholism. A 93-page report of this was published in1965. (Publs. 685-7).

39. The genetotrophic principle (viz., the nutrition of any organism depends upon its genetic background) and the concept of genetotrophic disease (Publs. 203, 213, 222, 225, 354, 406). This latter concept is that disease can result from the failure of any organism to obtain sufficient supply of the nutritional needs peculiar to that organism. Human individuals (who have different genetic backgrounds) must have at least slightly different nutritional needs (quantitatively considered). Nutritional deficiency and disease can therefore result in one individual but not in another even though they consume identical food. Though alcoholism is thought to be an example of a genetotrophic disease, the concept is much broader and its validity does not rest upon its applicability to any specific disease.

40. Exploration of individual metabolic patterns and their relationship to human diseases. (Publs. 246-267, 306, 405, 451).

41. Biochemical individuality. Contributions related to this topic have been (1) in the realm of ideas, (2) in investigations (see, for example, item 40 above) and (3) in collection of material and the publication of a book on the subject (Publ. 414). The basic idea which we have contributed is that since evidence indicates that every individual has his own inborn metabolic characteristics, every application of biochemistry to human beings must take these differences into account, particularly so since the differences between so-called normal individuals often turn out, unexpectedly, to be very large.

Since one of the important objectives of studying biochemistry is to gain the knowledge it may disclose concerning human health and disease, the phenomenon of biochemical individuality should become incorporated into all aspects of the discipline. It should be treated neither as an undesirable "fly in the ointment," nor as a trifling detail.

According to concepts arising directly out of this study, all diseases, including those such as cancer, arthritis, heart disease, alcoholism, schizophrenia and even to a lesser degree infectious diseases, have genetic roots and are related to biochemical individuality. The entire study of pathology is certain to change because of this concept. The knowledge about the genetic roots of disease does not by any means close the door to environmental treatment. Indeed a study of biochemical individuality opens the door to recognizing in advance the disease proneness of individuals (Publ. 591), and the possibility of preventing the diseases, whatever they may be, from taking hold.

42. A Broader concept of nutrition. This contribution in the realm of ideas is based upon many laboratory findings of diverse nature (see above items 5, 6, 8, 15, 21,29, 32, 37, 38, 39, 40, 41). It is particularly worthy of attention and emphasis in the writer's opinion, because applied nutrition at the expert level has remarkable potential and has in general been badly neglected by medical science.

This broader view involves the recognition of the following facts: (1) Cells of all sorts (yeast, bacterial, protozoal, mammalian) are capable of being nourished at many levels of efficiency—the environmental medium may be so deficient as barely to maintain life; it may be of such high quality as to make for maximum well-being or it may be at any point between these two extremes. (2) Satisfactory mammalian nutrition is necessary for every cell and tissue, not merely for "the body as a whole" (The nutrition of human beings studied without cellular perspective is like anatomy studied without recognizing the existence of microscopic anatomy). (3) Different types of body cells have distinctive and different nutritional requirements as has been demonstrated by tissue culture experiments. (4) There are specific nutrients which are required by some somatic cells and not others and not by the body as a whole. Glutamine is one clear-cut example; inositol and lipoic acid may be others. As a consequence of this, the existence of intercellular nutritional symbiosis (the production of specific nutrients by some cells and the requirement of these same nutrients by other cells) must be recognized. This requires extensive exploration. (5) For genetic reasons the nutritional needs of individual human beings(for their bodies as a whole and for their individual cells and tissue) cannot be identical. Actually in many cases they appear to be highly diverse.

This view of nutrition gives quite a different outlook on the prevalence and character of nutritional deficiency. In the medical field nutritional deficiency has traditionally been associated with specific deficiency diseases: scurvy, beri-beri, rickets, pellagra and the like. Our experiments with pantothenic acid deficiency (8 above) showed clearly that impairment in many tissues can take place without overt lesions. In later studies (Publ. 407) we found that baby chicks show nutritional deficiency by decreased growth and lowered food efficiency, as early as 18 hours after they begin eating, if the diet lacks a needed nutrient. This is long before a deficiency disease, in the usual sense, has had time to develop.

We regard identification of human nutritional deficiency with the presence of the symptoms of a recognized deficiency disease as an extremely circumscribed view. We cannot dismiss human pantothenic acid deficiency, for example, as non-existent or unworthy of consideration.

According to the broader view of nutrition, the following are among the effects of nutritional deficiency: (1) decreased growth of young; (2) decreased reproductive ability (see item 8); (3) decreased length of life (item 8); (4) decreased stamina; (5) decreased vigor as evidenced by loss of physical activity and playfulness; (6) decreased food efficiency; (7) impaired appetite; (8) impaired "body wisdom" with respect to food choices (deficient animals consume by choice more sugar and more alcohol than well nourished animals); (9) loss of learning ability;(10) loss of memory, and probably many other losses. None of the above effects is associated with any easily recognized lesions in any specific tissue; yet every one may be highly important in the realm of human health and well-being. The incidence of such human nutritional impairments

may be high.

The broader view of nutrition also encompasses the possibility that individuality in nutrition may be highly significant. Many observations of a miscellaneous nature have been made—many by me personally—which suggest this. For example, oral riboflavin intake in some cases dramatically abolishes extreme sensitivity to light; vitamin A in some cases greatly benefits respiratory difficulties, psoriasis and acne; oral pantothenic acid similarly may benefit allergies or constipation or partially restore the color of grey hair or restore failing memory; nutritional supplements, such as have been recommended in the treatment of alcoholism, have in individual cases relieved headaches, insomnia, high blood pressure, addiction to barbiturates, and surprisingly have rendered innocuous extremely malodorous feet; ascorbic acid in larger than usual doses may in some cases relieve serious difficulties with teeth and gums; oral thiamin has been known to restore in part the color of grey hair or to relieve eyestrain; niacin may relieve arthritic pain; individual amino acid deficiencies may in some individual cases induce personality deterioration, in others loss of mental acuity and in others serious impairment of and loss of teeth; oral glutamine, according to the work of Dr. Shive and his medical collaborators, hastens in some individuals the healing of peptic ulcers. In some of the above miscellaneous cases it seems clear that only certain individuals are affected.

While some of the above observations may be manifestations of the placebo effect (suggestion), many of the results were dramatic or entirely unexpected and in a number of cases well-qualified critical scientists or physicians were involved. The fact that such results as these are obtained inconsistently and have no statistical validity is in line with the possible importance of individuality in nutrition.

Another observation which points to the importance of individuality in nutrition is the well-known but little considered fact that some individuals (the late Winston Churchill is a notable example) can violate all general rules about eating and drinking and yet live magnificently to old age.

This broader concept of nutrition which has developed in our Institute is, I believe, far too little understood. Further information may be found in Publs. 410, 593, 626, 627,and 699.

43. Various attempts have been made in our laboratories to determine the biochemical peculiarities that reside in potential alcoholics and are associated with their vulnerability (Publs. 230, 508, 518, 592). The recent study of Siegel and co-workers (Publs. 672, 673) is probably the most meaningful of these, and strongly suggests that the goal is an attainable one provided the plasma amino acid (and other) patterns are carefully studied and subjected to discriminate analysis using highs peed computers.

VIII - Exploration in the Future

There is no disposition on my part to place the “dead hand of the past” on the Institute or to outline its future activities. That there is enormous room for developments may be appreciated by a consideration of such subjects as differentiation (item 37), the broader concept of nutrition

(item 42), and also the following facts:

(a) We have merely begun to get detailed information about the structure and functioning of living cells. (b) Though striking advances have been made in the elucidation of the mechanisms of inheritance in single-celled organisms (where only replication is involved), biochemical genetics as it applies to mammals and other multicellular organisms (in which differentiation is crucial, indispensable and highly determinative), is still in a dense fog. (c) We are almost totally ignorant as yet as to how hormones work biochemically. (d) A bridge between biochemistry and psychology is, for the most part, yet to be built.

IX - Present Interests of Members of the Institute

David J. Cox. Dr. Cox's interests lie in the field of the physical chemistry of macromolecules (particularly proteins) and the use of peptide models in the study of their structure. In the past decade it has become increasingly evident that proteins, as they occur in living systems, are not merely chains of amino acid residues but are organized so as to have definite spatial structures and interrelationships. Such studies are essential if we are to understand the workings of the machinery of living matter.

Robert E. Eakin. Dr. Eakin's interest is indicated in the discussion of differentiation given under item 37. He is also concerned with the biochemistry associated with the origin and development of life on this planet.

Boyd A. Hardesty. Dr. Hardesty's long term objective is to investigate the difficult but very basic problem of how protein synthesis is controlled in living organisms. Understanding these mechanisms would contribute immeasurably to our insight into the problems of differentiation and cancer with which protein synthesis is intimately associated. Already Dr. Hardesty and his co-workers using a sophisticated approach have made important steps toward the refinement and exploration of protein synthesizing cell-free systems. The results of some of this work will shortly be published.

Lester J. Reed. Dr. Reed's present research interests are suggested by his leadership in the investigation of lipoic acid (item 12) and the multienzyme units (item 35).

William Shive. Dr. Shive's research interests are suggested by the many important contributions made by him and his associates in the areas of intermediary metabolism, biological control systems, the relation between chemical structure and biological activity, and inhibition analysis. See particularly items 10, 11, 22, 23, 24, 25, 26, 28, 29, 30 and 32.

Roger J. Williams. My present interests lie largely along lines suggested by the discussion under item 42—a broader concept of nutrition. In addition we are interested in the fundamental problem of sleep—what is accomplished biochemically when we sleep. This involves pioneering exploratory work; it is much too early for a biochemical theory of sleep. One of the reasons for being interested in this field is because of the tremendous inter-individual variation in sleep patterns, which must be based on biochemical individuality. Sleep is a problem for many

people and we need to understand the basic facts upon which the need for sleep rests.

Daniel M. Ziegler. Dr. Ziegler's interests are in part revealed by the discussion under item 34. One of his longer range objectives is to contribute to our knowledge of how hormones function biochemically.