Ninth Annual Biology Colloquium
Saturday, April 10, 1948

NUTRITION

OREGON STATE CHAPTER OF PHI KAPPA PHI
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ROBERT R. WILLIAMS, Sc. D.
Leader of Ninth Annual Biology Colloquium
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THE Biology Colloquium is conducted in a spirit of informal discussion and provides opportunity for participation from the floor. The colloquium is sponsored by the Oregon State Chapter of Phi Kappa Phi with the collaboration of Sigma Xi, Phi Sigma, and Omicron Nu. Sigma Xi assumes special responsibility for the colloquium luncheon. Phi Sigma and Omicron Nu provide afternoon tea. The College Library arranges special displays of the writings of colloquium leaders and notable works on the colloquium theme.

Grateful acknowledgment is made of the cooperation and interest of the several faculties of Oregon State College that are concerned with biology and applied biology, of those biologists contributing to the program, of Chancellor Paul C Packer, President A. L. Strand, and other executives of Oregon State College.

The first Biology Colloquium was held March 4, 1939. Dr. Charles Atwood Kofoid of the University of California was leader, on the theme “Recent Advances in Biological Science.” Leaders and themes of succeeding colloquia have been: 1940, Dr. Homer LeRoy Shantz, chief of the Division of Wildlife Management of the United States Forest Service, theme “Ecology”; 1941, Dr. Cornelis Bernardus van Niel, professor of microbiology, Hopkins Marine Station, Stanford University, in collaboration with Dr. Henrik Dam, Biochemical Institute, University of Copenhagen, theme “Growth and Metabolism”; 1942, Dr. William Brodbeck Herms, professor of parasitology and head of the Division of Entomology and Parasitology, University of California, theme “The Biologist in a World at War”; 1943, Dr. August Leroy Strand, biologist and president of Oregon State College, theme “Contributions of Biological Sciences to Victory”; 1944, Dr. George Wells Beadle, geneticist and professor of biology, Stanford University, theme “Genetics and the Integration of Biological Sciences”; 1945, colloquium omitted because of wartime travel conditions; 1946, Dr. Robert C. Miller, director of the California Academy of Sciences, theme “Aquatic Biology”; 1947, Dr. Ernst Antevs, research associate, Carnegie Institution of Washington, theme “Biogeography.”
Ninth Annual Biology Colloquium

Theme: NUTRITION

Leader: ROBERT R. WILLIAMS, Sc.D., Chairman, Williams-Waterman Fund

Discussion Leaders:

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ROBERT R. WILLIAMS, Sc.D.
Chairman, Williams-Waterman Fund
for the Combat of Dietary Diseases,
New York City.

Chairmen of Sessions:

JOSEPH W. ELLISON, Ph.D.
Professor of History, Head of Department,
Oregon State College (evening session).

MARGARET L. FINCKE, Ph.D.
Professor of Foods and Nutrition,
Head of Department, Oregon State
College (opening session).

FRANCIS J. REITHE, Ph.D.
Assistant Professor of Chemistry,
University of Oregon (first morning session).

JOHN T. VAN BRUGGEN
Assistant Professor of Biochemistry,
University of Oregon Medical School
(second afternoon session).

ROSALIND WULZEN, Ph.D., Sc.D.
Professor of Zoology, Oregon State College
(first afternoon session).

EDWIN A. YUNKER, Ph.D.
Professor of Physics, Oregon State College
(luncheon session).

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(treasurer)
IT IS a great pleasure to return to the campus of Oregon State College where I have formed inspiring acquaintances with several of your faculty so that I feel I am among friends.

The college has made notable progress in research on which I congratulate you warmly. The Williams-Waterman Fund of the Research Corporation of America, which I now have the honor to represent, has as one of its special objectives the stimulation of research in smaller institutions. We think it important that every potential future leader of scientific endeavor should in his formative years be brought into contact with some application of the experimental method to exploration of the still unknown. All too frequently we carry over into our adult lives the notion, formed in childhood, that knowledge is something to be found in books. Maturer thinking later brings a livelier appreciation of our heritage from the past as we realize that the entire content of all books came out of someone’s experience with the world around him. It is the privilege of everyone not only to appreciate what has gone before but also to add something new, wrested by each one of us, from the mysteries of nature herself.

We are fond of pointing to Oregon State College as a school in which the sometimes dull routine of learning books is enlivened by direct appeal to the higher court of nature. Research has inspired teaching here and it has been one of our great satisfactions in the Research Corporation to have aided in a small way by providing some limited funds within the fields in which we operate.

No longer engaged in active, concrete research of my own, I warned Dr. Cheldelin that my remarks here would of necessity be general in character. Yet I think it is no mistake to be general at times—to try to tie our diversities together. The present day volume of current research is so vast that only an alert, retentive mind can read and mentally record what is happening even in a relatively narrow field. For myself, I have given up all hope of gaining or regaining a knowledge of the significant literature of nutrition and metabolism. I live in a fog and fear many other scientists in other fields are having a somewhat similar experience.

However, I have roamed the nutrition woods for a long time and like the pioneer or Indian scout have acquired a familiarity with certain landmarks so I can recognize them through even my own dense private fog. You must each of you have some landmarks too, but mine may add something to your facility in finding your way toward a truly satisfying goal. Certainly the greatest problem of modern science is what to study next and how to relate what one does to the parallel accomplishments of one’s fellows. Science is not ideally a vast catalog of isolated facts but an integrated view of nature.

Accordingly I have chosen for this morning’s topic “The Nutrition of Micro-organisms, Mice and Men.” In treating it I shall assume that many of you devote your prime energies to some other field of science so I shall not hesitate to discuss topics which may seem trite to specialists in nutrition who are in attendance. The importance of these topics is their landmark value and in these values we all can share.

Perhaps the most revealing outgrowth of our acquaintance with vitamins is that many of them, if not all, function as essential components of enzymes. This has been more or less rigidly proved for all of the 8 or 10 B vitamins, and recently vitamin C is gravitating into the same fold. The case for the fat-soluble vitamins is not clear.

What is an enzyme? According to our present knowledge it is a vast protein molecule with certain prehensile appendages capable of seizing other molecules which the organism needs to use. In grabbing these other molecules the enzyme “twists their arms” so that they split in two or become capable, through distortion of their outlines, of combining with some other sort of molecule. Once the enzyme wrecks its will upon the substrate molecule it lets it go and grabs another, repeating the process indefinitely. However, the prehensile fingers of each enzyme are adapted to seize only certain specific molecules which are shaped to its hand.

Most of the essential biochemical processes of all living things are carried out by such mechanisms, as has long been recognized. The new viewpoint contributed by the availability of the B vitamins and our knowledge of their structure is that simple substances which we can make in the laboratory prove to be the prehensile fingers of specific enzymes. Like the artificial hands of our
wounded soldiers they, or simple derivatives of them, are detachable from the body of the enzyme, that is the apoenzyme, which is a protein molecule so huge that we can not hope to reproduce it soon or even to discern its structure. We identify these prosthetic groups in their simple detached forms but we perceive their relationship to the enzyme by the biochemical reactions induced when they are mixed again with the apoenzyme. For example, we dialyse a yeast extract and find that neither the dialysate containing the apoenzyme nor the diffusate containing the prosthetic group, in this case cocarboxylase, is effective in decarboxylating alpha keto acids. Mix the dialysate and the diffusate and the power to decarboxylate is restored.

The term “prosthetic” is a little bit unfortunate as it means an artificial attachment such as an artificial eye or tooth or limb regardless of whether it is functionally effective. Of course, these prosthetic groups of enzymes are not artificial, though they are detachable, and we would have scant interest in them if, like a glass eye, they did not function. Actually the “business ends” of many enzymes are not detachable and so we remain in ignorance of their identities. It is the detachability of the vitamin-containing coenzymes which has opened a whole new chapter of enzymology and has given us the courage to continue to believe in the otherwise imaginative concept that the business end of all enzymes must somehow fit the shape or form of the substrate molecules on which they act.

Without this substantiation of the theory little progress would have been possible in tracing the steps whereby biochemical enzymatic reactions proceed. Hundreds of enzymes coexist in tissue extracts mingling the products of their reactions in a bewildering confusion. Some poisons block certain enzymes selectively and this has helped. More imaginatively, Beadle and his disciples have discovered that artificially produced mutants of Neurospora and other micro-organisms differ from one another in their biochemical capacities. Often it has been possible to show that seemingly simple biochemical reactions take place in a succession of steps. Any one of these steps may be blocked by choice of a mutant which has been deprived of a particular biochemical capacity, e.g., that of making pantothenic acid. Then one can learn that added pantothenic acid will unblock it. In this way a vast amount of information has been built up about biochemical processes, at least in Neurospora, and about the precursors and degradation products of many substances of general biochemical importance. A by-product from our point of view has been a brilliant contribution to the chemistry of genes and the relation of enzymes to them. In many instances a biochemical defect can be traced to a single gene. Dr. Beadle discussed this for you at a past colloquium far more competently than I can.

Another outgrowth of our recognition of certain vitamin derivatives as coenzymes has been the rise of antivitamins as a factor in our theory and in experimental practice. When the structure of the earlier B vitamins began to become known it was often supposed that analogs of them would be partially effective physiologically. This notion pervades a lot of earlier patent literature. Occasionally this idea proved correct for a particular analog, but in general analogs were ineffective. Later Woods noted that sulfanilamide and para-amino benzoic acid act antagonistically and competitively on various bacteria. The bacteriostatic effect of sulfanilamide could be overcome by adding sufficient para-amino benzoic acid to the medium and vice versa. The theory developed that the bacteriostatic effect of the sulfa drugs is due to their being chemical analogs of para-amino benzoic acid, a vitamin required by the organism. The near vitamin is capable of being incorporated in the enzyme system but is unable to perform its function. It is as if a person with an empty eye socket were to swim in a pool in which floated glass eyes and good working eyes. The higher the ratio of glass eyes the lower the chances of picking up a good one because a glass one would presently come to occupy the vacant socket.

This idea of the antagonistic action of a vitamin analog has been borne out in scores of experiments with micro-organisms and with several of the vitamins and their respective analogs. In a number of instances anti-vitamins have also been found to exert an antagonistic effect in animals. There seems little ground for doubting the fundamental soundness of this interpretation.

Indeed this idea became the motif of a vast amount of research aimed at the development of bacteriostatic drugs. Commercially, this has been disappointing. We read a great deal about the relationship between chemical structure and physiological action. However, it is amazing how frequently the new drugs which prove important are the results of lucky accidents. One sets out to make an anti-malarial and winds up with an anaesthetic. Living organisms are such complex structures that prediction of their behavior toward a given chemical is impossible except within rather narrow limits of past experience.
An outstanding question in the field of nutrition today concerns the extent of the occurrence of anti-vitamins in nature. Do effective anti-vitamins occur in foodstuffs? Do they represent a practically important cause of deficiency disease? Dr. Haag will discuss later today a case of what looks like an anti-vitamin occurrence in bracken fern. This case may be little related to the vitamin analogs which I have cited, but I shall leave it to him to interpret his findings in relation to the possible broad significance of the anti-vitamin doctrine in our food economy.

I shall have further occasion before I sit down to say something about the difficulties of my present job which is essentially to try to pick the winners among the great numbers of research projects which involve requests for funds. I should like the gift of divination regarding the importance of developments relating to anti-vitamins which are destined to occur in the next decade.

First, however, I want to discuss nutrition in micro-organisms in its relation to nutrition in higher forms of life. It is difficult to say when the notion arose that metabolism in all living tissues is essentially alike. It received support from the discovery of enzymes of the same or similar character in yeast cells, and in plant and animal tissues. The operation of identical hormones in a wide range of animal life made the concept more plausible. However, facts about the vitamins have dramatically thrust it to the fore. Vitamins which in general are synthesized only by lower forms of life are nevertheless required by animals. Obviously micro-organisms and plants would hardly make these substances for the welfare of the animal world, so it was not, perhaps, surprising that a vast amount of experimental evidence soon accumulated that the vitamins play an essential role, not only in animal life but elsewhere—that is, wherever they occur in cells. Indeed, now there are beautiful microbiological methods for the estimation of each of the vitamins which depend on its stimulation of growth or its promotion of a particular biochemical reaction in a micro-organism.

Dr. Cheldelin will doubtless have much to say about such methods in the course of his address, and few are better qualified to discuss them. Suffice it for me to say that micro-organisms are so convenient to work with and results are so rapidly and inexpensively obtained that an increasing share of the total volume of nutritional experiment now employs them.

The assumption of a community of metabolic processes among all living things is certainly well founded in a general sense. Its fruitfulness has been amply demonstrated. Thus the utility of pantothenic acid to yeast cells and to green plants furnished most of the basis for its approximate isolation and chemical identification and led directly to a recognition of its role in animals. Similarly it was the effectiveness of nicotinic acid in the growth of staphylococcus that suggested its trial in black tongue of dogs and later in human pellagra.

Philosophically I have been surprised at the tacit but well-nigh universal acceptance by biochemists of the principle that a metabolic process discovered in one organism has probably significance for all life. While Pasteur was compelled to fight for decades to gain a hearing for his proposition that human and animal disease is caused by invasions of microscopic organisms, this almost equally important principle has been accepted with so little debate that we are unable even to name definitely the father of the idea. It is amazing how fruitful it has been but we know that it cannot be universally true. Profound biochemical differences exist among the various forms of life.

This brings us again face to face with the problem of picking winners among research projects. Every few months someone comes forward with a discovery that a particular strain of micro-organism requires for its growth some factor not heretofore recognized among the essential nutrients. The missing factor is present in some food-stuff which may appear on our tables among the hors d'oeuvres, and it is proposed to isolate and identify the substance. Is this a good bet and how can one tell?

Needless to say, I shall greatly appreciate your comments on how to discern truly prophetic proposals among the grist of less inspired ones. Some justify themselves on very general grounds, for example, the elaboration of adequate microbiological methods for the determination of each of the essential amino acids in a protein structure. Older methods are so cumbersome that the potential usefulness of the microbiological approach as a tool for further research is obvious. Some, however, have no such general application. Their promise rests upon the hope that a counterpart for the phenomenon discovered in a micro-organism will be found in man or at least in some of the higher animals. What does one do then?

Sometimes I reproach myself for a want of faith. I remind myself that too strong a focus upon a predetermined objective may blind one to
a truly important discovery that may lie around the corner. For example, penicillin would probably never have been discovered by one who set out with the sole objective of discovery of a therapy for pneumonia. It was rather a chance observation in an unrelated field that proved significant. As another example, Beadle's contributions to nutrition grew out of the fact that his interest was in genetics.

I do not approach this question of how to pick a promising research solely from selfish motives. It is not merely that I feel the need of a guiding wisdom in casting votes on applications for grants. As individual scientists, you face the same problem in another guise. In this day of vast and diversified scientific endeavor the opportunities for choice are greatly multiplied. Each question more or less successfully approached raises ten new questions which may tempt you. How can you tell where best to invest your efforts?

The following are some of the thoughts I have found most helpful. I shall appreciate the thoughts of others.

It has been often said that the prime motivation of worthwhile research is curiosity, yet there remains the question what to be curious about. It is no practical answer to try a shot at anything and everything. You, as scientists, have not the time to explore every opening you see and the funds available for research are insufficient for all demands. We must accordingly be selective. One can scarcely justify a careless choice on the ground that useful results often come from unexpected places.

One can choose an investigation which is certain to produce results of value whether the results are positive or negative. Such a choice is relatively safe if the topic chosen has been little explored or has some general significance. It is rather unlikely to lead to unsuspected discoveries, but it will add data to the literature which someone else may use in future with unforeseeable results. I shall never forget the gratification with which we encountered 4-methyl-thiazole-5-carboxylic acid in the literature of 1890. Wohmann, who recorded it, had not the remotest notion of how or when the information would be used, but it saved us months or perhaps years in fixing the structure of thiamine.

There are, however, a lot of data in Beilstein which have found no highly significant use. I prefer, therefore, a research which is not merely data taking, even in a relatively unexplored field. Rather I like to see a research start with a clue. Perhaps one can not tell what it is a clue to, but if it is an unexpected but reproducible observation it offers a good deal of promise as a start. If the observation pertains to or appears to pertain to a subject of great interest or of practical importance the setting for a fruitful research is excellent.

More important perhaps than the choice of topic is the spirit in which it is approached. One can bet with some confidence on a person who has already done a significant research. His past success fairly well proves he has the nose for the significant, though we may have difficulty in defining what we mean by that phrase. It is essential that the investigator keep his eyes open for the unexpected. When an experiment "goes wrong" and the result is contrary to expectations, it is time for a renewed interest. One must believe his results and go to the bottom of them no matter where they lead. There is no substitute for keen observation and curiosity for deviations from predictions.

To revert to my principal theme we should next consider the field of animal experiment. For alliteration I have chosen mice as my example though rats are even more widely used. The moment one tackles experimentally the nutrition of any higher animal, a new scale of difficulty is encountered. The products of many organs pervade the blood stream and through it, all other organs. Isolation of one organ from others can only be partially accomplished, and it is rarely possible to know with finality where a given transformation is occurring or whether the observed result is influenced by events at some other site. However, animal experiment has the great advantage that the findings are more translatable into terms of human physiology than those obtained with micro-organisms or with tissue slices. At the same time the control of variables is vastly better and the cost vastly less than in human experiment.

Animal experiment often gives overall results which can be paralleled quite reliably in human beings. Thus the depletion in fowls of thiamine follows a course recognizably similar to that in human beings, the resulting symptomatology and pathology are essentially similar, and the therapeutic response is clear cut. Similar statements can be made about the causation and cure of scurvy in guinea pigs, rickets in rats and black-tongue in dogs in comparison with the corresponding human diseases.

It is to be noted, however, that one must choose the right animal. Rats are not subject to
scurvy or pellagra so one can not apply to the human case results from animals chosen at random. The task of deciding the extent to which animal results are applicable to human beings is difficult and confirmation by human experiment is indispensable. Yet progress with animals is relatively easy because of the possibility of good control through the use of large numbers. Hence the state of our knowledge of the nutrition of some common animals is always ahead of that in the human field.

When well characterized deficiency disease occurs spontaneously and frequently in human beings conclusive confirmation of its etiology is often possible by reproducing it in animals. However, the human case is usually less well defined because the depletion is rarely as complete or the deficiency so restricted to a single factor as in the experimental animal. Usually there is partial depletion with respect to several factors; often there are concurrent complications perhaps due to infections. The deliberate aggravation of deficiencies in human beings for confirmatory purposes is commonly forbidden both by the wilfulness of patients and by humane considerations. The few notable exceptions, such as the famous Goldberger experiment with pellagra, were possible only under institutional controls and in view of a well crystallized controversy among recognized authorities.

While one can say qualitatively but positively that human beings require thiamine, riboflavin, nicotinic acid, ascorbic acid, etc., it remains somewhat debatable just how much they require. Amounts of each excreted at different levels of intake and the corresponding blood levels achieved afford a notion of the quantitative requirements, but the variations in individual values are large enough to leave substantial debatable regions. The “daily recommended allowances” of the National Research Council frankly admits that the practical answer is a pragmatic one. They merely allow a sufficient margin to care for all cases of reasonable doubt.

A similar degree of uncertainty exists regarding human requirements for calories, amino acids, and inorganic elements.

When one comes to dealing with humanity en masse, as in the application of nutrition to public health, one inevitably enters a region where informed opinion rather than detailed data must govern. To a large extent opinion must rest on the only highly quantitative experiments available, namely, those with animals corrected as well as possible for known species deviations in the human. They must reflect a measure of uncertainty due to the questionable validity of deductions from any other animal species. The difficulty of translating animal results into human terms is well illustrated by the symposium we have recently published under the title “Appraisal of Human Dietaries by Animal Experiment.” Rats on mixed human diets in general do badly especially in the reproductive cycle. The young rat requires a much higher protein level and may well require unknown factors in amounts beyond the range of human needs.

It would be a serious omission at this point to fail to remark upon the relative costs of significant experiments with micro-organisms, mice and men. They stand in the ratios of 1 to 10 to 100 or even higher.

Human diets are often less than ideal in a number of particulars. Which defect is most serious is often debatable and wise choice of corrective measures will depend on feasibility, cost, compatibility with custom, etc., as well as on nutritional considerations.

In conclusion I shall merely mention some of the topics which deserve extensive attention in future research in the human field. We must know a great deal more than we do at present regarding the dependence of the human race upon bacterial synthesis in the gut. There are still some unknown factors to be discovered, particularly those associated with some of the anemias. Perhaps Dr. Lepkovsky will refer to both these topics in his address, and if so I’m sure his remarks will be enlightening.

I am glad to see on our program also Dr. Biddulph’s paper on soil nutrients in relation to human nutrition. Perhaps we have gone vitamin crazy and are neglecting important matters of trace elements. Dr. Fincke’s discussion of the difficult but practically important problem of dental caries and related matters will give a strong human interest to our later discussions, as will Dr. Straumfjord’s expression of the views of a clinician. I do not envy the physician who has to translate our researches into useful human terms much as I admire his courage in attempting it.
Nutrition Research Tools

Vernon H. Chedelin

Dr. Reithel, Ladies and Gentlemen: I am especially pleased to have been placed in this position on today's program, following the excellent introductory remarks which have been presented by Dr. Williams. In the minutes to follow, perhaps we can touch again upon some of the points which he has set forth which relate to the methodology employed in nutrition studies.

I chose for my subject "Nutrition Research Tools" because in the expanding field of nutrition research a number of new techniques of experimental study have appeared during recent years which are of both special and general interest. These have not only made possible more rapid experimentation and have brought the cost of carrying out nutrition research within the budgets of many small laboratories, but have also provided new means of approaching fundamental problems in animal metabolism. After considering the subject in more detail, however, I began to feel that the number of these developments was so large as to make it necessary to limit our discussion somewhat in order to avoid too brief an outline at all points. With your permission, therefore, I should like to dwell at some length upon the developments which have employed micro-organisms as "test subjects," and only mention such other important tools as radioactive indicators, ultramicro chemical assays for vitamins, improved gasometric techniques for microrespiration studies, and the various new techniques of physical measurement, such as electrophoresis of proteins, the use of absorption spectras in analysis, and the use of the ultracentrifuge. The title of this paper might, therefore, be more appropriate if it were listed "Microbiology in Nutrition Research."

One of the most important, and without doubt one of the most widely used tools in nutrition research, has been the microbiological assay, which was designed originally for the determination of vitamins. This work was pioneered by Dr. Roger Williams while he was at Oregon State College and was continued by him and Dr. Snell at the University of Texas. Microbiological assay methods take advantage of the fact that many micro-organisms—yeasts, bacteria and molds—have similar nutritional requirements to our own; if they are placed in growth media lacking in a given vitamin, they fail to grow. This lack of growth may be compared to the growth obtained in the presence of the vitamin, where under proper experimental conditions quantitative growth increments are induced when it is added in graded amounts. These methods are very sensitive; a typical example is given in the graph in Figure 1, showing the response of *Lactobacillus fermenti* to added thiamin. "Optical density," which is a measure of the turbidity of the bacterial culture due to cell division and growth, is plotted against the amount of thiamin per 10 ml culture. As little as 0.01 microgram can be determined with an accuracy of ± 10 per cent. This is even more remarkable when it is remembered that the thiamin contents of most materials range from 5 to 50 parts in ten million, and that a reproducibility of ± 10 per cent therefore requires detection of concentrations of 5 to 50 parts in 100 million. The method is quite specific; other vitamins, amino acids, purines or pyrimidines, etc., are devoid of activity. Also, as may be seen from the graph, the constituent moieties of the thiamin molecule are inactive during the eighteen-hour growth period employed. The growth medium, listed on the next slide (Table 1) is relatively simple. The peptone and casein furr...
nish principally the necessary amino acids; the glucose supplies energy; the sodium acetate buffers the system during the production of lactic acid; uracil and the purines furnish building blocks for nucleic acids; and the salts and vitamins are added to insure an adequate supply of these substances during growth. By employing various organisms, with growth media designed to suit their special nutritional needs, satisfactory test methods for all of the B vitamins have been developed. It is thus possible to obtain a "vitamin profile" of a particular tissue or foodstuff within a few days time.

Microbiological methods are relatively inexpensive. A technician, working with ordinary laboratory apparatus, can readily perform quadruplicate assays on ten to fifteen samples daily, and with the use of automatic pipettes and titrating devices (for titrating acid produced by bacteria such as the lactobacilli), a group of four to six people can analyze hundreds of samples daily. Moreover, since these tiny "cousins" of ours frequently possess nutritional requirements which parallel our own just as closely as do the common laboratory animals, microbiological methods may be considered with some justification to serve as satisfactory reference standards. At least one observation has been made where bacterial assay values are more reliable than those obtained with animals.

Beginning about 1942, a natural extension of microbial methods was made into the amino acid field. Some of the lactic acid bacteria are even more fastidious in their amino acid requirements than are rats or humans, and the proper manipulation of these organisms has provided methods for every one of the naturally occurring amino acids except hydroxyproline. Prominent in this work have been Dr. Dunn's group at UCLA, Dr. Snell and coworkers at Texas and Wisconsin, and Dr. Stokes' group at the Merck Laboratories. Principally as a result of the development of these assay methods, an unprecedented expansion of interest and effort has been directed toward amino acid research; in 1947 over 1,400 articles appeared in Chemical Abstracts dealing with amino acids, whereas in 1937 there were about 750. This expansion has been brought about in at least three ways. First, all that has been said regarding the inexpensiveness, the ease and the rapidity of vitamin assays is equally true of amino acids. In one laboratory, as many as three thousand samples have been analyzed in less than a week by a technical staff of four to six people. Second, until the microbial methods were developed, no truly satisfactory analyses could be made of several of the amino acids, particularly leucine, isoleucine and valine. Third, the demand for amino acids for the compounding of synthetic mixtures became sufficiently great to interest many organic chemists, including groups in some of the pharmaceutical houses, in developing new syntheses or improving old ones for the individual amino acids. The resulting effect upon prices has been very great. Methionine, which a few years ago sold for over a dollar per gram, is now available at ten dollars per pound. Other price drops have been less dramatic, but nonetheless very substantial. With the average cost of amino acids one-tenth to one-fifth of what it was a decade ago, the interest in synthetic animal diets has increased correspondingly.

An interesting example of this broadening of the scope of amino acid studies has been the examination of amino acids in the urine. Urinary excretion measurements have often been employed to obtain an impression of the general health or nutritive state of an individual. In the past, however, amino acid determinations in urine have not been generally feasible, partly because of the lack of reliable methods for all of the amino acids, and especially because they are present in urine only in small amounts. The microbiologic methods, on the other hand, are sufficiently sensitive and specific so that each amino acid may be studied individually. Using these methods, Dr. Wright and coworkers at Sharp and Dohme and Dr. Davidson's group at Harvard have found both with dogs and with humans that the retention of amino acids by the kidney tubules, although very efficient when the blood levels are normal, may fall to only about 85 per cent with certain amino acids when the blood levels are very high (ten or more times the normal). Such high values may be reached when protein hydrolysates or synthetic amino acid mixtures are given intravenously. The amino acids which were found to be lost in greatest amount were threonine and histidine (18 and 15 per cent, respectively) whereas at the other extreme, less than 1 per cent of the arginine was lost. The patterns of retention of the ten "essential" amino acids by the body thus differed from the patterns of administration; threonine, histidine, lysine, tryptophane and phenylalanine were in relatively greater concentration in the urine than in the infusion mixture, whereas arginine, isoleucine, leucine, methionine and valine were in smaller concentration. Finally it was found that when partially digested proteins (e.g., "Amigen," which is a
partial casein-pancreas hydrolysate) were administered, approximately 45 per cent of the polypeptide alpha amino nitrogen was lost into the urine. It would seem reasonable to conclude from this that a completely hydrolyzed protein is probably nutritionally superior to one partially hydrolyzed, although it should not be inferred that an intact protein is also of less value. Rather, it suggests that the polypeptides encountered in some hydrolysates are not of the correct size or configuration to permit the alimentary enzymes to attack them, and further research seems in order to alter these molecular fragments to suit more nearly the purpose for which they were intended. The value of such research may be very great for individuals who must be fed intravenously over prolonged periods.

An interesting possibility, still largely unexplored, for the use of microbiological assays in amino acid and protein research lies in the determination of the biological value of proteins. The past, large groups of animals have been needed in order to evaluate the nutritive qualities of a given protein, where nitrogen "balance" studies have measured the retention of food nitrogen by the animal body. Tetrahymena, a protozoon, can also utilize intact protein, and it seems likely that the growth of this unicellular animal may be able to give us information on the biological value of proteins which will be as valid as those obtained with white rats. Also, the use of microorganisms for the determination of inorganic salts appears attractive. Some work in this direction is being carried on at the present time by Dr. Snell.

A third important use of microbiological tools has been in the discovery and isolation of new growth factors. Of the ten (or eleven) recognized B vitamins, seven of these were found to be necessary for growth of certain microorganisms, and their isolation was made possible by following the various steps with microbial assays. These were: pantothenic acid, pyridoxal and pyridoxamine, biotin, inositol, folic acid, p-aminobenzoic acid, and vitamin B_{12}. Important factors here are the speed of performing assays (16 to 72 hours compared to several weeks usually required with animals), the ease with which a bacterial growth medium may be deprived of the growth essential in question compared to the relative difficulty of removing it completely from animal diets, and the opportunity to select organisms for which a factor in an absolute essential whereas in laboratory animals the same factor often merely stimulates growth or may not be required at all. Examples of the latter are inositol, choline, pyridoxal and pyridoxamine. The tempo of discovery of new growth essentials has been speeded greatly since these methods have been brought into general use, and the increased speed of isolation may be appreciated when it is recalled that folic acid was obtained in pure form less than three years from the time of its discovery, whereas over ten years were spent isolating thiamin (animal assays were used here). It seems likely that it would never have been discovered if laboratory animals were the sole test organisms. Animals (and presumably humans as well) appear to synthesize the larger molecule within their tissues when the vitamin is included in their diet. The conjugate is probably physiologically important, because when animals are deprived of dietary pantothenic acid, the quantity of the conjugate in their tissues does not begin to decrease until after all of the free vitamin has disappeared—in other words, the conjugate is conserved at the expense of the more dispensable free vitamin.

All three of the uses of microbiological methods which we have discussed thus far have been as analytical tools. The next two which I want to take up are tools for studying metabolism, upon which our entire science of nutrition is based.

We are familiar to a greater or less degree with the use of radioactive tracers in studying metabolism. We have learned how a particular amino acid, e.g., glutamic acid, containing a labeled amino group, can be ingested by an animal, can travel throughout the body, and can transfer its labeled amino group to other amino acids. From this we have confirmed and extended the concept of transamination. Similarly, we have verified earlier observations that fat acids are built up from shorter chain acids and that radio-
active (or preferably heavy) hydrogen will migrate rather freely about the tissues. We have concluded, from the work of Schönheimer and others, that tissue constituents are in a dynamic, rather than a static state, and the older concept that tissues are renewed every seven years has been completely abandoned. The value of these labeled compounds for studying metabolism is obviously very great. However, the compounds containing the radioactive tracers must be isolated or at least highly concentrated so that their identity may be stated unequivocally. This is difficult to do with the vitamins, since they are present in such low concentrations, and as a result they have not been subjected extensively to tracer studies.

Insight into vitamin, purine, and pyrimidine metabolism is being gained through the use of another type of "tracer"—the synthetic inhibitor, structurally related to some particular growth factor. The better known inhibitors of this type are those related to sulfanilamide, and are analogs of p-aminobenzoic acid. A few of these are listed on the next slide (Figure 2). All except prontosil are sulfa drugs of current commercial importance.

For several years the principal object in making new sulfa drugs (over five thousand have been synthesized) was to produce superior drugs which would be effective against disease-producing bacteria, yet harmless to the host. Progress in this direction has been very gratifying; but it was not until 1939-40 that sulfanilamide was found to act by reversibly inhibiting growth which had been induced by the vitamin p-aminobenzoic acid. This discovery was set forth by Woods and Fildes in England. They further explained the action of sulfanilamide as being due to its ability to compete with the vitamin for certain centers of activity on the surface of cellular enzymes. In other words, the "prehensile fingers" which Dr. Williams has mentioned as belonging to an enzyme have in this case been sharpened to "hook on" to a particular group of the p-aminobenzoic acid molecule. Moreover, the handle on the hook must be of special size and shape (in this case a benzene ring). Sulfanilamide has the same ring, and its "hook" is enough like that on p-aminobenzoic acid so that it can engage the enzyme protein in a manner similar to that of the vitamin. Once engaged, however, the sulfanilamide molecule is incapable of carrying on normal metabolism, since it lacks the crucial group which the vitamin contains. The enzyme surface, saturated with these "pretender" molecules, can no longer function as a catalyst, and the organism ceases to grow or multiply. The only way in which growth may again be restored is to add more of the vitamin to the system. Inhibition may then occur by a counter-addition of inhibitor; and so on. Inhibition to any desired extent will occur when the two are present in a definite ratio, and the phenomenon has come to be known as competitive inhibition. After 1940, numerous efforts were made to extend the list of synthetic inhibitors to relatives of other growth factors, and by the end of 1947 many hundreds had been successfully produced. The vast majority of these exhibit the same type of competition with the growth factor that we have just observed to exist between sulfanilamide and p-aminobenzoic acid. A few of the principal inhibitors are listed on the next slide (Figure 3) along with the vitamins to which they are related.

During the past four years, efforts have been made to discover the exact role of certain of the vitamins in metabolism through the use of inhibitors, and the full potentialities of these sub-
stances as tools for the study of metabolism are now being recognized. Several laboratories are currently employing these metabolic “tracers”; the more productive groups have been those headed by Dr. Woolley at the Rockefeller Institute, Dr. Shive at Texas, and Drs. Kohn and Harris at Duke, who made the initial observations on the mode of action of p-aminobenzoic acid.

The latter two groups have rather clearly established the principle that a growth factor which competitively counteracts a specific inhibitor can be replaced with greater effectiveness by other compounds which are normally formed from the growth factor. In other words, if growth of an organism is completely inhibited when the inhibitor-to-vitamin ratio is 100:1, replacement of the vitamin by one of the compounds normally formed by the organism from the vitamin will result in a much higher ratio than before, and in some cases the inhibitor may no longer be effective at all. This is explained by assuming that the inhibitor retards only the enzyme system in which it competes, and is incapable of entering into successive biosynthetic reactions within the cell. Viewed in reverse fashion, this concept has also been utilized to identify precursors of growth factors, against which specific inhibitors may be much more effective than against the growth factors themselves.
Two outstanding examples will illustrate the value of inhibitors in determining the course of biological reactions. The first deals with \( p \)-aminobenzoic acid. When the structure of folic acid was announced (see Figure 4) it was noted that \( p \)-aminobenzoic acid was a constituent part of the molecule. This gave rise to the speculation that the two might be interrelated, especially in view of the fact that folic acid had been found to be capable of reversing sulfonamide inhibition in certain organisms, and that certain other organisms had been found to produce more folic acid than normal when they were incubated with large quantities of \( p \)-aminobenzoic acid. Applying the principle just outlined, and using sulfonamides as inhibitors, Dr. Lampen and coworkers at the Lederle Laboratories found that the susceptibility of organisms to sulfa drugs bore no relation to their requirement for \( p \)-aminobenzoic acid. The organisms which could synthesize their own folic acid were inhibited; those requiring folic acid in their growth media were unaffected. Inhibition could of course be counteracted competitively by \( p \)-aminobenzoic acid, but folic acid was much more effective. These observations pointed to \( p \)-aminobenzoic acid as a precursor of folic acid, and they answered the question for the first time as to the lack of correlation between \( p \)-aminobenzoic acid requirement and sulfonamide susceptibility; it was now evident that the action of the sulfonamides was not upon \( p \)-aminobenzoic acid, but upon the synthesis of folic acid from \( p \)-aminobenzoic acid. Thus an organism might synthesize its own supply of \( p \)-aminobenzoic acid, but it would be unable to resist sulfonamides effectively because of the latter's attack upon the conversion system. It was also evident why animals and humans are relatively unaffected by sulfa drugs; for being folic acid-requiring organisms, they would obviously be unaffected by an agent which inhibits the synthesis of a compound which they cannot synthesize anyway! The second example relates to the function of folic acid in metabolism. The ingenuity with which it was developed is an added reason for including it here. First, it was observed that when certain organisms are inhibited by sulfonamides, a yellow-orange compound accumulates in the medium. Upon examination, Dr. Shive found this compound to be 4-aminomimidazole-5-carboxamide, with the structure shown in the next slide (Figure 5). I urge you
NUTRITION

to try to forget the name of this compound soon; the important point is to notice its structure and how it resembles xanthine, also listed on this slide. The resemblance takes on added significance when one considers that xanthine and other purines may aid $\beta$-aminobenzoic acid in overcoming sulfonamide inhibition. Second, the Merck Laboratories have recently reported the isolation of a close relative of folic acid, which they call rhizopterin, which may be a large fragment of the folic acid molecule. It is devoid of the glutamic acid nucleus which is found at the end of the folic acid structure (Figure 4), and in addition it possesses a formyl group attached to the nitrogen atom of the $\beta$-aminobenzoic acid portion. Dr. Shive, evidently surmising that formyl folic acid (Figure 4) might also exist in nature, and from the obvious possibility that it might furnish a carbon atom to be "built into" the xanthine molecule, prepared a material described as formyl folic acid and tested its growth promoting activity. Significantly, the new compound was found to be $20$ to $50$ times as effective as folic acid in overcoming growth inhibition by one of the synthetic folic acid inhibitors ("methylfolic acid"). While the conclusions cannot yet be accepted as bona fide in the absence of supporting evidence from other sources, it appears very likely that at least one of the functions of folic acid may be to catalyze the supply of single carbon units for the building of purines, via formyl folic acid. (The latter compound, being formed from folic acid, would be expected to overcome inhibitors more readily than the vitamin itself.) The purines, in turn, are constituents of nucleoproteins, which are essential to cell formation. Thus, a deficiency of folic acid, with a resulting drop in production of new cell nuclei, would probably be mirrored very soon in a depletion of red blood cells, and the exhibition of anemia in folic acid deficiency thus receives a plausible, if not the only correct explanation. Undoubtedly the picture is not complete, since it fails to take into account the effects of vitamin B$_{12}$, but the correct position of the latter vitamin in the metabolic scheme must await further experimentation. I am convinced that much of the information which is to come will be obtained from further exploitation of synthetic inhibitors. Moreover, the blocking of enzyme systems with various drugs may very likely result in the discovery of new growth essentials, whose presence might otherwise be overlooked.

And now let us examine a slightly different tool—the X-ray mutant.

When Dr. Beadle led this colloquium four years ago, we had the opportunity to view some of the significant contributions which were being made by genetics to the study of metabolism. Our discussion at this time would not be complete without further reference to this remarkable work. You will recall that the mold Neurospora crassa, after being irradiated with X-rays or ultraviolet light, may undergo various mutations. These Dr. Beadle’s group have characterized as being enzyme-linked, that is, each mutation results in the “cutting off” of the prehensile fingers from one of the enzymes, or in some other way damaging or altering it so that it can no longer carry out the particular synthesis for which it was designed. (It is significant that no mutant has yet been observed to have suffered more than one such alteration, and thus it differs from its parent by only one less synthetic enzyme.) Since
the loss of the ability to carry out a synthesis is reflected in the appearance of a new growth requirement, it is easy to find out which synthetic mechanism has been destroyed. Thus, the loss of ability to synthesize nicotinic acid results in the appearance of a nicotinic acid-requiring strain; but once nicotinic acid is supplied, the mutant carries on just as before, indicating that subsequent reactions involving the utilization of this vitamin have not been impaired.

The Neurospora mutants have another important characteristic: even when an enzyme has been damaged so that it can no longer function, the various enzymes which stand before the affected one continue to function. This results in an accumulation in the medium of the compound which can no longer be utilized, and if large quantities of the mold are used, the yield of this metabolic “dead-end kid” may become great enough to permit its isolation in pure form. When this is done, its structure may be determined, and we thus have the identity of a precursor of the compound which must be added to make this mutant grow—in the present example, a precursor of nicotinic acid.

Usually many steps are required to bring about the synthesis of a particular vitamin from the sugar and ammonium salts upon which the organism grows. An irradiation may, therefore, affect any one of several enzymes which precedes the formation of the vitamin, and thus several mutants might be expected, all of which would be related to the eventual synthesis. This has been proven true, and the Pasadena workers have obtained at least six mutants which cannot synthesize nicotinic acid, but in each a different cause prevails: the chain of enzymatic reactions has been broken in different places. The first one (which suffered degradation of an enzyme early in the series) can grow very well on the accumulated products from any of the other five, since each of these stands behind the affected enzyme in the first mutant; and so on. In this way, Dr. Mitchell and coworkers have uncovered a series of events in the synthesis of nicotinic acid in Neurospora, beginning with anthranilic acid, as shown on the next slide (Figure 6). In the parent strain, which has not suffered losses in synthetic powers, each of the arrows represents an enzymic reaction, the presence of which is denoted by its absence in a particular mutant.

This remarkable series of transformations, involving the amino acid tryptophane, as well as the conversion of kynurenine, which is a normal constituent of urine but never before supposed to be of importance except as an end product, into the pyridine type nicotinic acid, furnishes a possible answer to one of the long-standing paradoxes in human and animal nutrition: why the nicotinic acid content of a foodstuff sometimes does not furnish a measure of its pellagra-preventive potency. Milk, which is very low in nicotinic acid, is one of the best pellagra-preventive foods, and corn, with a higher content, is definitely pellagragenic. This work, together with the pioneering experiments with rats by Dr. Elvehjem’s group at Wisconsin, makes it evident that tryptophane, besides functioning as a necessary amino acid for protein synthesis, can also serve as a source of nicotinic acid. When milk and corn are examined for tryptophane content,
their role in pellagra becomes clear; milk is rich in this amino acid, corn is not. A study of metabolic function thus again provides us with basic information to explain the appearance of deficiency syndromes, and the science of nutrition continues to benefit from the application of these research tools.

I believe that the examples which we have considered up to this point have served to emphasize the importance of these research tools to nutrition, and particularly to the study of metabolism. I am further confident that the next few years will see a very rapid extension of our efforts in this direction, for we are still only at the beginning of our efforts to understand the chemistry of life processes.

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<th>Basal Medium for Lactobacillus fermenti.</th>
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<td>Alkalized peptone (± sodium acetate)</td>
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<td>Acid-hydrolyzed vitamin-free casein</td>
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<td>Sodium acetate, anhydrous</td>
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<td>Riboflavin</td>
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<td>Calcium pantothenate</td>
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<td>Nicotinic acid</td>
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Practical Problems in Animal Nutrition
J. R. Haag

FOR over 20 years it has been my privilege to maintain rather close contact with many of the nutritional problems which confront the people of Oregon. During this period the science of nutrition has made phenomenal strides—accompanied by a growing interest in the general theme that our problems frequently involve a chain of events beginning with climate and soil and extending through the crops we grow, the animal products we produce, and finally to their effects upon man.

This theme is a timely one because the science of nutrition has reached a degree of maturity which makes it worth while to re-evaluate many of our findings in the light of this theme. Then too, we have found it to be a useful approach to the problem of selling nutrition research to the layman. Unfortunately the faddist has found it equally attractive as a vehicle for peddling a lot of misinformation about "hidden hunger" while producing bumper crops without attention to their nutritive value. In fact, it was interest in such problems as the relation of soil to regional livestock disorders in the Black Forest of Germany that led to the birth of agricultural chemistry which for many years embraced the application of all of the fundamental sciences to agriculture, including the nutrition of man.

I want now to enumerate some of the problems which we have encountered. When I say we, I include all those who have been or are now associated with me, as well as those in other departments with whom it has been my pleasure to work.

In speaking of Oregon's nutritional problems, we need to keep in mind, first: That our state has a very wide variety of soil and climatic conditions. Rainfall varies from 6 inches to 12 feet. Elevations vary from sea level to snow capped peaks.

The second point is that nutritional disorders, which in pioneer days were strictly regional, under the impacts of education and transportation have become less sharply defined. As an example, I need only remind you that with the wider distribution of sea foods and the use of iodized salt, the once sharply defined goiterous areas are becoming less marked, even for livestock.

Another word of caution has to do with translating the influence of climate and soils on the nutritive value of crops into terms of practical human and animal nutrition.

I might illustrate this point with the influence of sulfur fertilization on the nutritive value of the alfalfa plant. Oregon and Washington are quite unique for the very marked sulfur deficiency of certain soils. An otherwise productive sulfur deficient soil may produce 1.5 or 2 tons of alfalfa hay possessing a pale color and low protein content. The carotene content may also be considerably below normal. The application of sulfur may double the yield, raise the per cent of crude protein and increase the carotene content. It does not follow, however, that sulfur fertilization beyond that needed to produce a normal yield further enhances nutritive value. Yet it is very easy for the layman to come to the conclusion that everyone growing alfalfa should resort to sulfur fertilization to prevent "hidden hunger" in a bumper crop.

About 20 years ago, I became interested in the question of the relation of sulfur deficient soils to the nutritive value of alfalfa protein as measured by its growth—and lactation—promoting properties in rats. As is the case with a number of other legume proteins, the growth-and-lactation-promoting properties of alfalfa proteins are limited by their combined contents of cystine and methionine. Cystine and methionine are the nutritionally useful sulfur-containing amino acids which occur in all biologically adequate proteins. All samples of crude alfalfa protein, regardless of sulfur fertilization history, so far tested, have been improved by additions of cystine or methionine. Peanut meal proteins, on the other hand, are so deficient in methionine, that methionine must be added in order to obtain substantial growth or lactation responses in rats. Whole egg protein possesses most excellent growth-and-lactation-promoting properties. This whole problem is of considerable interest because we still are not sure whether cystine and methionine act as dietary lactagogues, or whether they merely serve to make sulfur deficient proteins biologically adequate in the conventional sense. Some of you are aware that Daggs et al. have presented evidence that cystine is very effective in stimulating lactation in women.
Our access to low sulfur feedstuffs, has among other considerations, led us to a study of the extent to which the amount and kind of sulfur compounds in the ration limit the utilization of non-protein nitrogen by ruminants. Obviously, if the microflora of the ruminant are to synthesize biologically adequate protein from ammonium carbonate or urea, they must also be able to synthesize the sulfur-containing amino acids, cystine and methionine.

Another group of our investigations have variously dealt with the mineral requirements of farm animals. Here we not only need to know requirements and how these requirements are modified by species, age, rate of growth, milk and egg production, but we also need to know how these requirements can be safely and economically met by the use of various crops, plant and animal by-products, and mineral supplements. A very important practical consideration is the extent to, and circumstances under which, farm animals can be depended upon to select mineral supplements to meet their requirements. Here, we still have much to learn. For example, cattle on phosphorus-deficient rations ordinarily crave bone meal, but shun chemically pure tri-calcium phosphate. Cattle fed on low-phosphorus alfalfa frequently do not crave bone meal, but may consume large quantities of di-sodium phosphate.

In addition to the purely nutritional disturbances, such as those induced by deficiencies of calcium, phosphorus, or vitamin D, we have a group of what are sometimes referred to as metabolic disturbances whose causes are not at all well understood. I refer here to such disorders as "milk fever;" "grass tetany," "white muscle" in lambs, etc. Chemically, milk fever is characterized by low blood calcium and is most cleverly explained away as a metabolic disturbance, incident to the stresses of reproduction and lactation. "Grass tetany" is characterized by low blood calcium superimposed upon low blood magnesium. The disturbance is regional, apparently related to diet, but not explained in terms of simple calcium and magnesium deficiency. "White muscle" in lambs is characterized by what appears to be extensive deposits of calcium phosphate in the soft tissues. This disorder also appears to have a dietary background. Then, too, there are a variety of kidney and bladder stones which I suspect will someday be shown to have a nutritional background. Many of these concretions consist of calcium and magnesium phosphates or calcium oxalate, but we are finding a surprising number which consist mostly of silicates. Why an animal should absorb large quantities of silica, excrete it in the urine and then go to the trouble of crystallizing it, is just one more of those unanswered questions.

Another group of studies have dealt with the influence of the feed of the cow on the nutritive value of milk. We have a quite satisfactory regional and seasonal picture of the vitamin A potency of milk fat. Perhaps I should digress here to say that Oregon does not have regions in which cows are known to produce low-calcium milk. I am amazed at how frequently this erroneous notion comes to our attention.

Our experience with the "trace" elements is quite limited. We know a little about the distribution of iodine. Manganese occurs in forages in quantities ranging from 30 to 300 p.p.m. Significant quantities of selenium are known to exist in certain parts of Oregon. Since this element has only been encountered in certain weeds in isolated areas, we have little cause for alarm concerning potential harm to humans and livestock.

During 1945 we undertook a study of the dietary properties of bracken fern because of an unusually serious outbreak of "fern poisoning" in cattle. We soon found that when air-dried fern is incorporated into rations at a level of 40 per cent, death results in about 3 weeks. Subsequent experiments showed that the toxicity of bracken fern to rats can be overcome by feeding supplementary thiamine. After our group had thus quite accidentally discovered that bracken fern owes its toxicity to rats to its anti-thiamine activity, Dr. Williams and the group which he represents very generously contributed to the support of our work. I am most happy, on behalf of myself and associates, to express our appreciation for your generous support.

When high levels of fern, such as 40 to 50 per cent are fed, maximum body weight is reached in 6 to 9 days, and when about 40 per cent of the body weight has been lost, the animals die on about the 20th day. As the level of toxic material is reduced, the day at which maximum weight is reached increases from the 10th to as much as 60 to 100 days. It is under the latter conditions that polyneuritis becomes a frequent symptom. Animals which die in 18 to 20 days do not show polyneuritis. All of these symptoms can be alleviated or prevented with supplementary thiamine. In fact, Dr. Weswig at one time carried some rats through 5 generations on diets containing 25 per cent air-dried fern by means of supplementary thiamine feeding. The
disorder has also been produced in chicks and mice.
All samples of bracken so far tested have proved toxic. These samples include a number of areas and seasons, one sample coming from Pennsylvania.

Another phase of these studies has to do with an attempt to arrive at some of the properties of the causative agent. It appears to be essentially insoluble in acetone, 95 per cent ethyl alcohol, ethyl ether and petroleum ether. Our search for solvents has been rather conspicuously unsuccessful.

It possesses considerable, although not absolute, stability to dry heat for 18 hours at 105° C. The causative agent appears to be inactivated by steaming for 30 minutes—as evidenced by feeding 40 per cent of the dry matter intake as steamed fern for 12 weeks.

Rations prepared to contain 25 per cent of dry matter from the same lot of fern ranked in order of decreasing toxicity as follows: Frozen fresh fern, air-dried fern fed in the moist state, air-dried, and sun-dried. Fresh frozen fern is rendered non-toxic by steaming.

Still another phase of our studies has as its immediate objective the adaptation of chemical and microbiological methods to this problem. This is essential to rapid progress in determining the mode of action of the factor and its distribution in the plant kingdom.

Our first attempts involved the Melnick and Field method in much the same manner as used by Dr. Sealock et al in connection with the fish factor. By this procedure we seem to measure thiamine destruction or interference with the determination of the order of 5,000 to 10,000 micrograms per gram of fern. The agent producing this result is largely heat stable and does not require an extended period in incubation as is the case with the fish enzyme. This method has not been applied to material which has been passed through Decalso. The reaction is so widespread as to raise a serious question as to whether or not we are measuring antithiamine activity. We have recently found that some Scandinavian workers had obtained somewhat similar results.

More recently we have undertaken experiments with the thiochrome procedure on materials passed through Decalso. Here, thiamine destruction is of the order of 500 to 2,000 micrograms per gram of fern. The causative agent shows some heat stability but not as strikingly so as in our rat experiments with steamed fern. A substantial incubation period is essential for maximum thiamine destruction. The rate of destruction increases rapidly as the incubation temperature increases from 0° C. to 60° C.

The problem of equating animal performance against chemical methods is not easy. Very roughly: 1 gram fern destroys 5,000 to 10,000 micrograms thiamine as measured by the Melnick and Field procedure; 1,000 to 2,000 micrograms thiamine as measured by the thiochrome procedure; and about 50 micrograms as measured by rats. The application of our findings to “fern poisoning” in horses and cattle is not yet clear. Horses could be imagined to show symptoms of polynueritis, but in cattle the dominant symptoms include high temperature with severe hemorrhages, terminating in death.

Finally, allow me to say again that whatever merit our contributions may possess is in no small measure due to the loyalty and efforts of those associated with me. I am happy to make this acknowledgment because the joys of accomplishment are concentrated by sharing them with one’s associates.
Interrelations Between Iron and Phosphorus in Plant Nutrition

O. Bioulgh

A study of the functions of phosphorus in biological systems, in particular its function in intermediary metabolism, becomes increasingly fascinating and a great deal of attention is being focused upon it. However, there are so many factors affecting the absorption and translocation of phosphorus that a study of its metabolism did not seem justified prior to an understanding of the mechanics of its supply.

This is particularly true when a method as sensitive as that of radioisotopes is employed. Using this method, either one of two techniques is best followed, i.e., (1) to furnish to the metabolizing cells a uniform flow of the radioisotope during the complete experimental period, or (2) to administer a single "dose" of the radioisotope during a short interval only and then following its flow into and out of the tissues or chemical compounds being studied.

In either event it is necessary to insure an unimpeded flow of the isotopes in question to the tissues being studied. The necessity of this becomes increasingly apparent as one becomes more familiar with the tracer technique.

We are studying as separate projects the absorption of phosphorus from nutrient media; the movement of phosphorus across the cortex of the root and its excretion into the transpiration stream; the pattern of distribution to leaves and other aerial parts; its redistribution from leaves; and the synthesis of some of the major phosphorus-containing organic compounds. I have chosen to present here some of the very interesting effects which iron has upon the uptake of phosphorus and vice versa.

The very low solubility of ferric phosphate would lead one to suspect an interesting relationship between the two. In most nutrient media the phosphorus to iron concentration on a molar basis is maintained at approximately 10 to 1. This puts iron at a disadvantage as it is subject to precipitation with phosphorus and as a result it is rapidly removed from solution. Ferric iron is very rapidly precipitated when introduced into...

Some of the data for this paper are taken from the theses of John H. Rediske and Cyril G. Woodbridge, "The absorption and translocation of iron in the bean plant" and "Studies on the phosphorus metabolism in bean plants." Their cooperation is gratefully acknowledged.
a nutrient solution as the nitrate, chloride, and/or phosphate. Ferric citrate, tartrate, and humate maintain higher concentrations of iron in solution, and ferrous sulfate at pH 4 proved to maintain the highest iron concentration over a 7-day period of any salt which was tried.

The nutrient solutions upon which these precipitation studies were made contained the conventional preponderance of phosphorus over iron. Other solutions, however, were prepared in which the phosphorus and iron concentrations were nearer equality (on a molar basis). This resulted in solutions which were purposely made low in phosphorus, rather than high in iron. Disadvantages would outweigh advantages if this type of solution were used in long-time experiments, but with short-time experiments in which radioisotopes were employed the method gave remarkably consistent and extremely interesting results.

Iron uptake was measured from solutions in which the phosphorus content was systematically varied. The iron content of the trifoliate leaves and the roots was determined separately. Any iron in the leaves has been absorbed and translocated during the experimental period. The iron values for the roots represent total iron associated with the roots, i.e., that which has been absorbed and that which is adsorbed (or precipitated) upon the root surfaces. The results are expressed as percentage gain in iron during the experimental period. The form of iron used was ferric nitrate in which tracer amounts of ferric chloride (radioactive Fe" and Fe"') were incorporated during the experimental period. The pH of the solution was maintained at 6.0. A four-day experimental period (duration of the period in which the radioisotope was available to the plant) was employed, after which the plants were harvested for analysis.

The following points are outstanding: The greatest accumulation of iron by leaves coincides with the least accumulation of iron by the roots.

### Table 2. The Rate of Precipitation of Various Iron Compounds in a Nutrient Solution as Influenced by pH Amounts Expressed as PPM in Solution at the End of Each Period. Phosphorus Concentration of Solutions Was 0.0005 M.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Days</th>
<th>Ferric nitrate PPM</th>
<th>Ferric chloride PPM</th>
<th>Ferric phosphate PPM</th>
<th>Ferric oxalate PPM</th>
<th>Ferric citrate PPM</th>
<th>Ferric tannate PPM</th>
<th>Ferric humate PPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 4.0</td>
<td>1</td>
<td>0.24</td>
<td>0.24</td>
<td>0.088</td>
<td>0.49</td>
<td>0.75</td>
<td>0.55</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.045</td>
<td>0.042</td>
<td>0.046</td>
<td>0.033</td>
<td>0.24</td>
<td>0.24</td>
<td>0.55</td>
</tr>
<tr>
<td>pH 5.0</td>
<td>1</td>
<td>0.022</td>
<td>0.030</td>
<td>0.046</td>
<td>0.013</td>
<td>0.14</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.039</td>
<td>0.032</td>
<td>0.057</td>
<td>0.043</td>
<td>0.13</td>
<td>0.08</td>
<td>0.12</td>
</tr>
<tr>
<td>pH 6.0</td>
<td>1</td>
<td>0.017</td>
<td>0.024</td>
<td>0.042</td>
<td>0.042</td>
<td>0.04</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.019</td>
<td>0.034</td>
<td>0.044</td>
<td>0.044</td>
<td>0.04</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>1</td>
<td>0.021</td>
<td>0.024</td>
<td>0.064</td>
<td>0.064</td>
<td>0.05</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.023</td>
<td>0.031</td>
<td>0.067</td>
<td>0.067</td>
<td>0.06</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.030</td>
<td>0.036</td>
<td>0.072</td>
<td>0.072</td>
<td>0.07</td>
<td>0.07</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.031</td>
<td>0.030</td>
<td>0.073</td>
<td>0.073</td>
<td>0.07</td>
<td>0.07</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Neither K ferricyanide nor K ferrocyanide showed any variations under the above conditions.

This takes place in solutions in which the P:Fe ratio is 1:1 (molar basis). An explanation is offered utilizing the knowledge that the iron ion concentration will be maximum at this point due to the common ion effect. To the left of this point, where iron atoms are in preponderance over phosphorus atoms, the tendency will be toward the formation of a hydrated ferric oxide precipitate. To the right of this point, where phosphorus atoms predominate over iron atoms,
the tendency will be toward the formation of a
t Ferric phosphate precipitate. Iron will be les s
available on either side of equimolar concentra-
tions with phosphorus. The roots reflect the fact
that the precipitate may also form upon them as
as well as upon the walls and bottom of the tanks.

When a similar experiment is carried out
measuring the uptake of phosphorus as a func-
tion of variable iron concentration, a remarkably
similar curve is attained.

Outstanding again is the fact that the greatest
leaf accumulation coincides with the least root ac-
cumulation. This takes place from solutions in
which no iron is present. No precipitate of phos-
phorus appears in this solution to coincide with
the hydrated ferric oxide precipitate in the pre-
vious experiment where iron predominated over
phosphorus.

As the iron concentration of the nutrient solu-
tion increases with respect to phosphorus the up-
take of phosphorus into the leaves decreases.
This is coincident with an increase in the phos-
phorus associated with the roots. Analysis of
the precipitate which formed with increasing iron
concentration confirmed the presence of both iron
and phosphorus.

From an experimental standpoint it appears
quite possible to effect a tie-up, or block, in the
absorption of iron and phosphorus from rela-
tively dilute nutrient solutions. Consequently, it
becomes evident that the root must be regarded
as a medium in or upon which precipitated ma-
terial may collect and remain relatively unavail-
able. Just what the exchange capacity between
root cells and precipitate may be is quite un-
known. It is not unlikely that organic compounds
of plant cell origin are also components of the
insoluble complex.

After having entered the root cells and moved
across the cortex to the xylem the nutrient ele-
ments are translocated upward in the transpira-
tion stream, and deposited in the leaves. These
organs are invariably highest in mineral nutrients,
receiving as they do the supply of nutrients ascen-
ding the transpiration stream. The pathway
backward from the leaves to other organs is the
phloem, and if the element is to be redistributed
it is via this path.

Phosphorus appears to be quite mobile and
moves freely from one part of the plant to an-
other. This is shown by two types of experi-
ments. The first involves a study of the deposi-
tion of radiophosphorus in the plant by the usual
methods of allowing the plant to absorb it from
a nutrient solution. In one experiment a four-
day absorption period was allowed after which
half of the plants were analyzed for radiophos-
phorus in order to show the relative distribution
throughout the plant. The other half of the plants
were then removed to a solution containing no
radiophosphorus ($P_{32}$). After the plants had
produced three additional leaves (i.e., 4 days
later) they were analyzed for radiophosphorus
in order to determine the extent of movement of
the $P_{32}$ from the older tissues to the newer tissues.

These results indicate that phosphorus is
freely mobile and in this instance moved from
older to newer tissue and that the newer tissue
accumulated concentrations of radiophosphorus in
final excess of the tissue which supplied it. This
can occur only when an element is freely mobile
in the phloem tissue.

The second type of experiment involves the
profusion of leaf tissue with a radioisotope and
at subsequent intervals fractionating the tissues
of the plant in an attempt to determine the ex-
tent of migration of the elements from one tissue
to another. It was evident immediately that phos-
phorus was freely mobile in the phloem. It was
possible to determine the daily periodicity in move-
ment and to plot the pattern of the distribution.

Outstanding again is the fact that the greates t
leaf accumulation coincides with the least root ac-
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tively dilute nutrient solutions. Consequently, it
becomes evident that the root must be regarded

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1. Stout, J. W., and D. R. Haagland, "Upward and lateral
movement of salt in certain plants as indicated by radioactive iso-
topes of potassium, sodium, and phosphorus absorbed by roots." 

A strong downward movement can be detected during the daytime, with a peak in the late afternoon. Upward movement from the point of entry into the stem is also evident. The period of minimal movement is during the night hours.

A number of attempts have been made to measure the movement of iron from a leaf using the method employed for phosphorus. Radioactive ferric chloride, nitrate, and citrate have been tried. The latter compound proved most satisfactory in a number of ways. The difficulties encountered in using iron in this type of experiment will not be enumerated here. It is sufficient to say that when concentrations and pH's compatible with leaf tissue were employed it was not possible to obtain samples of tissue remote from the leaf that had concentrations of radioiron much in excess of twice the background count.

Iron is traditionally in the phloem immobile class. Certainly we have encountered no striking examples of mobility, but we prefer to await the availability of a higher specific activity iron before drawing our conclusions.

The degree of mobility of a given element determines its availability for the formation of new protoplasm under certain conditions. If a plant may "stockpile" a given element and draw upon that supply in "lean" times it may weather adverse conditions with fair success if the element is freely mobile, but if an element is not freely mobile the "stockpiling" is of no avail for the element cannot "recirculate" and be drawn upon for metabolic activities when the local supply runs low. Phosphorus is in the class of phloem mobile elements while iron has traditionally been classed in the phloem immobile category.

An additional point of interest is the rapid cure of chlorosis which can be attained by placing chlorotic plants in a phosphorus (and iron) free nutrient medium. The cure is no doubt in part attained by the "freeing" of iron already present within the plant by allowing a "resolubilization" of the iron when the "precipitating" influence of phosphorus is removed. Any iron precipitated upon the roots may also become more available. Only a limited amount of iron is present in a so-called iron free nutrient solution, i.e., .004 ppm. This small trace can, however, be rather efficiently absorbed from low phosphorus solutions. Further work with completely iron free nutrient solutions is necessary in order to conclusively establish the fact that "internal" iron is freed for metabolic process under conditions of reduced phosphorus absorption.

Many problems still center around the extent of mobility of various elements in the phloem tissue, as well as surface relationships between root cells and nutrient environments. It is to be hoped that this paper will serve to arouse questions which may ultimately be resolved by the design and completion of conclusive experiments, rather than to serve as a source of adequate information upon which such questions can be answered.

![Figure 9. Conc. of P³ in bean plants which had received P³ between the 4th to 8th days after experiment began.](image)

![Figure 10. The daily trend in the migration of phosphorus from an "injected" bean leaf.](image)

The Application of Nutritional Results Obtained With Animals to Man

SAMUEL LEPKOVSKY

Introduction
INVESTIGATIONS with human subjects are difficult and very costly. The temptation always exists to apply nutritional findings obtained with animals to man without first seeking confirmation by direct experiments on man even when it is technically possible. A comparison of some of the available results of experiments with animals and man will be made to see to what extent, if any, nutritional findings with animals may be applied to man.

Studies on Protein Metabolism
It has been found that with ordinary diets there is a minimum caloric intake which is essential for the retention of dietary protein. This is about 60 per cent of the caloric requirements of the animal. At this level of caloric intake, dietary protein in amounts necessary for nitrogen equilibrium or less will be completely retained or nearly so. This has been found to hold for the rat (1), dog (2), and man (3). Seemingly, results of this kind obtained with the rat and dog could be applied to man.

The retention of riboflavin is greatly influenced by the protein content of the diet. This is determined by measuring the urinary excretion of riboflavin of animals on high and low protein intakes. On high protein diets, there is a decreased excretion of riboflavin in the urine of the rat (4), dog (4), and man (5). Conversely, on a low protein diet, there is an increased excretion of riboflavin in the urine of the rat, dog, and man. In this respect also, the rat, dog, and man behave alike.

This, however, is not the case for nicotinic acid. On high protein diets, there is a decreased excretion of nicotinic acid in the urine of the dog (4) and man (6), but an increased excretion of nicotinic acid in the urine of the rat (4). Conversely, a low protein diet will cause an increased excretion of nicotinic acid in the urine of the dog and man but a decrease in the excretion of nicotinic acid in the urine of the rat.

There also seems to be differences in the amino acid requirements of rats, dogs, and man. Methionine has received a great deal of study, and it has been found effective in reducing urinary nitrogen in rats (7) and dogs (8, 11) subsisting on low protein diets but is without effect in man (9, 10). Apparently, in rats and dogs on low protein diets, the amino acids arising from the decomposed tissue proteins are effectively supplemented by methionine. This does not appear to be the case in man, either due to a decreased methionine requirement or to an amino acid mixture arising from tissue protein decomposition richer in methionine than that in rats and dogs.

Man's decreased methionine requirements have far reaching consequences, since this amino acid is present in small amounts in many common food proteins, among them the legumes and cereals. Bricker et al determined the biological value of the soy bean and milk proteins in the rat (12) and human (13). The biological values that they found for these proteins are shown in the table.

<table>
<thead>
<tr>
<th>Food protein</th>
<th>Man</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy bean</td>
<td>65</td>
<td>49</td>
</tr>
<tr>
<td>Milk</td>
<td>74</td>
<td>86</td>
</tr>
</tbody>
</table>

It is obvious here that for man the soy bean proteins are almost as good as the milk proteins, but for the rat, the soy bean proteins are much poorer than the milk proteins. These determinations of biological values reflect the lower methionine requirements of man as compared to those of the rat.

Misleading results on the nutritional status of populations could easily be obtained without such information. Populations subsisting in large part on legumes which are low in methionine would be considered as ingesting inadequate proteins if judged by rat studies. Actually, they would probably be getting adequate proteins because of man's low methionine requirements.

Studies on Vitamins
It has long been known that man's vitamin requirements may be different from that of animals. Thus, only man, monkey, and the guinea pig require dietary vitamin C. The other animals synthesize their needed vitamin C.
With respect to nicotinic acid, the requirements of the dog and man are similar (14). The rat (14) and monkey (15) behave alike in that their nicotinic acid needs are very low.

The thiamin requirements of all animals seem to be similar (16) and the symptoms of deficiency seem also to be similar. Thiamin is required for the metabolism of glucose which yields the chemical energy needed for the life of animals. The metabolic importance of glucose is further emphasized by the ability of brain tissue to use only glucose to supply its energy needs (17). Glucose metabolism is apparently so basic to all life that thiamin, which is needed for the normal metabolism of glucose, is consequently needed by all animals metabolizing glucose. The ability of carnivorous and omnivorous animals to synthesize thiamin seems to be limited.

**Man and Monkey**

Man is more closely related to the monkey than to other present-day animals. We almost unconsciously assume that the requirements of monkeys would more closely resemble those of man than those of other animals. This is indeed true for vitamin C, but it is not true for other vitamins. Nicotinic acid deficiency is common in man (14), but it is almost impossible to produce in monkeys (15). Biotin deficiency can be produced in monkeys (18) but so far has not been produced in man (19). It is possible that a common environment may account for similar nutritional requirements and may be as important as anthropomorphous relationships.

**Intestinal Synthesis**

The important role of intestinal bacteria in the nutrition of animals is being emphasized by the vast amount of research on the relation of intestinal bacteria to the dietary needs of animals. The greatest effect of intestinal bacteria is on the dietary needs of the herbivorous animals, especially the ruminant (20), making them largely independent of their environment for their requirements of many vitamins and high quality proteins. The nutrition of other animals is also greatly affected by intestinal bacteria (21), although not to the same extent as in herbivorous animals. The nature of the diet fed the animals will influence the synthetic activity of the intestinal bacteria. Thus on a diet containing dextrin as the carbohydrate source of energy, a coccus was isolated from the intestinal tract of mice which could synthesize folic acid. Another coccus, isolated from the intestinal contents of mice on a diet with glucose as the carbohydrate source of energy, could not synthesize folic acid (22).

Information is accumulating that man is also aided in satisfying his nutritional requirements by bacteria in his intestinal tract. Moreover, in man also, the synthetic activity of the intestinal bacteria is influenced by the character of the food eaten. More thiamin and riboflavin is synthesized by the intestinal bacteria in women subsisting on a diet of natural foodstuffs than on a diet composed of purified ingredients (23). Biotin and folic acid has been shown to be synthesized by the intestinal bacteria in man even on restricted diets (24).

The information on intestinal bacteria and their metabolism throws light on the laxative action of the crude fibre of whole wheat bread. In England, during the war, a high extraction bread (85 per cent) was used and it was one of the factors which was responsible for a decrease in constipation in the population. The decrease in constipation was reflected in a decline in the sale of laxatives (25). Wheat bran by itself is probably constipating. This is so at least in rats. Bacteria-free rats were fed wheat bran and it proved to be constipating. When the wheat bran was mixed with some cecal contents of normal rats, the wheat bran became laxative (26). Apparently, the laxative action of wheat bran is the result of some metabolite or metabolites elaborated by bacteria acting on the wheat bran in the gastrointestinal tract of the rat.

Perhaps the time is not far off when nutritionists will pay as much attention to balancing the diet of the bacteria in the intestinal tract of the animal as to the diet of the animal. Here, too, studies on man's intestinal bacteria will require special attention.

**Studies on Deleterious Compounds**

Phytin has long been known to interfere with calcium metabolism and more especially calcium absorption (27). Long continued studies revealed an unexpected ability on the part of the human subject to adapt himself to the phytin of high extraction bread (28). When put on a diet containing the whole wheat bread, the subjects went into negative calcium balance. Without any change in diet the negative calcium balance progressively decreased until the subjects were in calcium equilibrium, after which they went into positive calcium balance, regaining the calcium lost (28).

The dog, on the other hand, apparently has no such adaptive powers. When phytic acid was
fed to dogs, there was a marked decrease in the absorption of calcium and the decrease in calcium absorption was aggravated the longer the dogs were fed the phytin-containing ration (29). There was no improvement in the absorption of calcium such as occurred in the human subjects.

Phytin, as such, or that in high extraction breads also interfered with the absorption of iron in the human subject (30) but in the dog, the iron of wheat bran was almost completely available for hemoglobin formation (31). Comparisons of this kind must be made cautiously because they are not quite parallel. Absorption of iron was studied in the normal human subject, but the corresponding study was carried out in the anemic dog. There is evidence to indicate that more iron is absorbed when it is needed, as in anemic animals than when it is not needed, as in normal animals (30, 32).

Raw soy beans contain a deleterious compound which decreases the availability of methionine. The deleterious compound is probably a protein which can be destroyed by heat and is thought to be an antitrypsin which has been isolated and crystallized (33).

Animals and man seem to behave similarly in their adverse reaction to raw and heated soy beans. In two well-nourished human subjects, there was about 20 per cent greater retention of nitrogen on the heated soy beans than on the raw (34). Enough methionine had apparently been made unavailable by the deleterious compound in the raw soy beans to render the methionine intake inadequate in spite of the low methionine requirements by the human subjects.

The nutritional state of the human subject must play a role in his reaction to raw soy beans. Many Americans who were Japanese prisoners of war reported that soy beans were unpalatable and caused nausea, vomiting, and diarrhea. They refused to eat them even under the severest condition of starvation (35). It is unknown whether these differences in the reaction to raw soy beans is due to the difference in the nutritional condition of the subjects, differences in the raw soy beans fed or to some other unknown causes.

Conclusions

A consideration of the sketchy and scanty data available of comparative nutritional investigations of man and animals indicates that, while much of the information obtained with animals is applicable to man, it is not safe to apply the results obtained with animals to man until experimentally proved that they do apply.

Bibliography

1. Swanson, P., *Reports*, Food and Container Institute for the Armed Forces.
2. Allison, Jr., *Reports*, Food and Container Institute for the Armed Forces.
6. Sarett, H. P., "Nicotinic Excretion in Man on Low and High Protein Diets."
9. Schwimmer, D., Personal communication.


Nutritional Status of Oregon Population Groups

MARGARET L. FINCKE

F OR many years there have been efforts made to assess the state of nutrition of an individual. Medical examinations have included the height and weight of the subject, the amount of subcutaneous fat, muscle tone, color of the skin and mucous membranes, brightness of the eye and sleekness of the hair. Such examinations are useful, but chiefly in discovering the well advanced cases of malnutrition.

More recently Jolliffe, McLester, and Sherman, reporting for the Food and Nutrition Board of the National Research Council, described the increasing severity of malnutrition as follows:

1. Tissue depletion, an exhaustion of the reserve supplies of the body.
2. Biochemical defects, such as the rise of pyruvic acid in the blood in a thiamine deficiency, thiamine being part of the cocarboxylase molecule needed for the oxidation of pyruvic acid.
3. Functional change, such as the development of night blindness in a vitamin A deficiency.
4. Anatomical lesion; for example, the irreversible change that occurs in rickets resulting in deformity of the bones.

In Oregon State College, in the nutrition laboratory, we have been studying tissue depletion for some time, with the view that if the earliest signs of deficiency were found, conditions might be changed to prevent the more severe stages. Several studies have been made here on ascorbic acid metabolism. It was found that 60 per cent of rural children in a group under investigation showed blood plasma values for ascorbic acid lower than the level of adequacy as suggested by a committee of the American Academy of Pediatrics. On the other hand, hemoglobin values in these same children tended to be high according to current standards. Our study confirmed previous work of Osgood’s on Portland children in this latter respect.

Considerable experience in working with nutritional status of human subjects had been gained in this laboratory when the Extension Women’s Council of Oregon asked us in 1944 to undertake a study of the relation of nutrition to teeth, if they should be able to obtain an appropriation from the Legislature for that purpose. The State Legislature did appropriate $20,000 in 1945 and renewed the grant in 1947 for the present biennium.

At the time when this work was started, few data were available on Oregon populations so far as dental conditions were concerned. It had been found by Nizel and Bibby in examining the teeth of 45,000 men in a camp in Massachusetts that the men from Oregon were among those showing the highest number of decayed, filled, and missing teeth, being surpassed only by the men from Delaware, Maine, Rhode Island, and New York.

The project here was started in the fall of 1946 under the direction of Dr. Clara Storvick, with a staff composed of Dr. Hadjimarkos, dentist, Miss Sullivan, bacteriologist and chemist, and Mrs. Young, who took nutrition histories. We were fortunate in having an advisory committee consisting of Dean Noyes of the University of Oregon Dental School, Dr. Wherry representing the State Board of Health, and Dr. Abbett appointed by the Oregon Dental Association.

Six hundred freshmen at Oregon State College who were residents of Oregon and who had not been in the armed services, served as subjects. The teeth of these students were examined by explorer and by X-ray, and the number of decayed, filled, and missing (DMF) teeth and tooth surfaces counted. Tests were made on the saliva, including lactobacillus acidophilus counts, pH, buffer capacity, ammonia nitrogen, and amylase activity. A one-day dietary record was obtained, together with information on the usual food habits of the subject, including the use of candy and carbonated beverages.

Some of the results are given in the tables. Table 3 indicates that most of the subjects were 18 or 19 years of age, with only a few 16- or 17-year-olds, and a few aged 20 or more.

| Table 3. Number of Freshmen Students at Oregon State College Given Dental Examinations, by Specified Age and Sex Groups. |
|---|---|---|---|---|
| Age in years (last birthday) | 17 or below | 18 | 19 | 20 or above |
| Freshmen students | | | | |
| Number of men | 13 | 172 | 29 | 11 | 235 |
| Number of women | 21 | 214 | 82 | 39 | 347 |
| Number of both sexes | 34 | 386 | 121 | 41 | 582 |
Table 4. Number of DMF Permanent Teeth and Tooth Surfaces by Specified Age Groups of 582 Freshman Students at Oregon State College. Rates Are Expressed per Student.

<table>
<thead>
<tr>
<th>Permanent teeth</th>
<th>Age in years (last birthday)</th>
<th>17 or below</th>
<th>18</th>
<th>19</th>
<th>20 or above</th>
<th>All ages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of DMF teeth</td>
<td>12.00</td>
<td>14.28</td>
<td>16.60</td>
<td>14.68</td>
<td>14.12</td>
<td></td>
</tr>
<tr>
<td>Number of DMF surfaces</td>
<td>22.71</td>
<td>33.51</td>
<td>36.02</td>
<td>36.37</td>
<td>32.46</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 shows the number of DMF (decayed, missing or filled) teeth and tooth surfaces according to age. The DMF incidence increases with age, except for the number of DMF surfaces in the oldest age group. This lower value might be attributed to the few number of cases in this age group, which make it impossible to compare the values.

Table 5. Number of DMF Permanent Teeth and Tooth Surfaces by Specified Age and Sex Groups of 582 Freshman Students at Oregon State College. Rates Are Expressed per Student.

<table>
<thead>
<tr>
<th>Permanent teeth</th>
<th>Age in years (last birthday)</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF teeth</td>
<td>18.00</td>
<td>14.07</td>
<td>13.51</td>
</tr>
<tr>
<td>DMF surfaces</td>
<td>22.23</td>
<td>32.85</td>
<td>30.65</td>
</tr>
</tbody>
</table>

Table 5 separates the men and women so far as DMF teeth and tooth surfaces are concerned. The women showed consistently higher rates than the men at all ages, possibly because women of these chronological ages are older physiologically.

Table 6. Dental Caries Experience (Teeth) of 582 Freshman Students at Oregon State College. Rates Are Expressed per Student of Specified Age and Sex Groups.

<table>
<thead>
<tr>
<th>Permanent teeth</th>
<th>Age in years (last birthday)</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filled</td>
<td>8.77</td>
<td>10.19</td>
<td>10.56</td>
</tr>
<tr>
<td>Required extraction</td>
<td>7.46</td>
<td>6.72</td>
<td>6.16</td>
</tr>
</tbody>
</table>

Table 6 giving the number of filled and extracted teeth, the number requiring extraction and the cavities requiring filling seems to indicate that the women receive better dental care than the men, although both show considerable dental care.

In Table 7 the value of X-rays in detecting cavities requiring filling is demonstrated. Thirty per cent of the cavities were detected by X-ray, only 70 per cent by explorer alone.

That the DMF incidence is high among Oregon State College freshmen is shown in Table 8, which compares the findings of this study with those of studies on similar age groups in Hagerstown, Maryland, in San Francisco, and in New York City. The data from the Oregon study had to be recalculated to include only the decayed teeth found by examination with the explorer, excluding those found by X-ray, in order that they would be comparable with the data from the other studies. It will be seen that the incidence of decayed, filled, and missing teeth among the Oregon students is considerably higher than that of comparable groups in San Francisco, New York, or Hagerstown.

The students were divided according to the geographic division of the state in which they lived. The six districts are: Columbia River Basin, Oregon, Washington, Idaho, California, and New York.

Table 7. Comparison of Cavities Requiring Filling Detected by Means of Clinical and X-Ray Examinations of 582 Freshman Students at Oregon State College. Rates Are Expressed per Student.

<table>
<thead>
<tr>
<th>Total number</th>
<th>Number detected by explorer</th>
<th>Number detected by X-ray</th>
<th>Per cent detected by X-ray</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.30</td>
<td>2.83</td>
<td>1.67</td>
<td>30.56</td>
</tr>
</tbody>
</table>

Table 8. Comparison of Number of DMF Permanent Teeth per Person by Age in Oregon, Maryland, San Francisco, and New York City.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Age in years</th>
<th>17</th>
<th>18</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oregon</td>
<td>13.00</td>
<td>14.28</td>
<td>14.68</td>
<td></td>
</tr>
<tr>
<td>Maryland</td>
<td>7.72</td>
<td>8.72</td>
<td>9.25</td>
<td></td>
</tr>
<tr>
<td>San Francisco</td>
<td>7.4</td>
<td>8.3</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>New York City</td>
<td>7.99</td>
<td>8.69</td>
<td>6.94</td>
<td></td>
</tr>
</tbody>
</table>

*The examination in Maryland, San Francisco, and New York City did not include bite-wing X-rays.*
sin, Blue Mountain area, Central Oregon, the Coast, the Willamette Valley, and Southern Oregon. The students coming from the Coast counties showed the highest DMF rate and those from Central Oregon, the lowest. This was a statistically significant difference, and formed the basis of plans for future work.

Comparison of the analyses of the saliva with the DMF rate yielded some interesting relationships. One theory of caries formation is that erosion of enamel and dentine occurs, caused by acids in the mouth. Another theory is that sugar in some manner contributes to the production of caries, either in conjunction with acid, as a medium for the growth of bacteria or in some other way.

The correlation between DMF incidence and the amylase activity was highly significant (0.460), showing that a higher production of caries occurs with higher formation of sugar from starch in the mouth. Amylase activity is an inverse relation of the time required for a sample of saliva to react with starch-iodine solution in respect to rate of flow. Among different individuals, the time varied from 6 to 1,800 seconds.

Likewise, the relation between DMF and Lactobacillus acidophilus count showed statistical significance. Buffer capacity showed an inverse relationship to DMF, and pH, a positive relationship.

No studies of the relationship of food intake to condition of the teeth were attempted, as it was realized that the nutrition history represented current dietary habits only. It was noticed, however, that 88 per cent drank at least two glasses of milk a day, and 68 per cent drank three or more. Very few used candy and carbonated beverages in large amounts, the averages being three candy bars and three glasses of carbonated beverages a week.

This year the project has been considerably enlarged because of a grant through the Research and Marketing Act of 1947. This provides for research on a regional basis, the western region consisting of Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming. Because a preliminary study had already been made, Oregon was named the key state and the trustee of the funds. Work has been started in Oregon and later will be continued in the other states.

Cooperators in the project are the Experiment Station and the School of Home Economics, the Bureau of Human Nutrition and Home Economics of the U. S. Department of Agriculture, which pays the salaries of several of the workers, and the U. S. Public Health Service, which is supplying a physician and has lent a mobile laboratory with some equipment. The advisory committee of last year was enlarged to include Dr. Erickman of the State Board of Health, Dr. Todd of the University of Oregon Medical School and Dr. Straumfjord appointed by the Oregon Medical Society. County health officers are also cooperating.

The general project consists of a study of the nutritional status of selected population groups of the West. In Oregon, this will be related to the condition of the teeth. The plan includes the examination of 1,000 native born and native reared children 14, 15, and 16 years old, in two areas of the state. In the coastal region, the two counties being visited are Clatsop and Coos, and in Central Oregon, Deschutes and Klamath. These are the two areas chosen in the preliminary study of college freshmen.

The research team, which is in the field now, is under the general direction of Dr. Clara Storvick of this laboratory. In addition, there are a physician, a dentist, three nutritionists, three chemists, two public health nurses, an X-ray technician, a bacteriologist, three research assistants, three laboratory assistants, one combination statistical clerk-apparatus washer, one combination truck driver-apparatus washer, and a statistical consultant; twenty-two in all.

The mobile laboratory consists of a truck and trailer. The trailer is set up for dental examinations, including X-rays, and for the saliva tests. The rest of the team works in schools or county public health offices. Blood samples are taken, some of the analyses done on the spot and the remaining blood is frozen immediately and sent back to Corvallis packed in dry ice.

The examinations comprise general information as to place and date of birth, place of residence from birth to the present, previous medical history, etc.; physical inspection by the physician; dental examination including X-ray; nutrition history; and biochemical tests on the blood including determination of hemoglobin, hematocrit, serum protein, serum vitamin A and carotene, serum ascorbic acid, and phosphatase. As of March 1, 680 children have been examined. All children of those ages, native born and reared and whose parents gave consent, were examined in Clatsop and Coos counties. The team is now in Deschutes County and finally will go to Klamath with the expectation of returning to Corvallis about the middle of May.
When the data have been checked and sent to the statistician for analysis, most of the research workers will go to California, where the project will be in operation next. There the study will be on nutritional status, but in relation to some other problems than teeth.

Analysis of the drinking water in these four counties is also being carried on, for fluoride content, calcium, magnesium and total hardness. Very few fluoride analyses have been reported of waters within the state, and the findings from this laboratory should prove interesting in view of the apparent relation of fluoride content of the water drunk by the child in his early years and the hardness of the teeth.

When all the statistical studies have been made, the work will be prepared for publication. This, then, is a progress report and not a final one.

The study should give a good indication of the state of nutrition of these adolescent children in two areas of the state and will provide a basis of comparison with other areas of the west and with other sections of the country.
Local Factors in Tissue Supply of Vitamin A

JON V. STRAUMFJORD

The organic lesions of vitamin A deficiency affect certain tissues and structures by predilection. The demonstrable histologic derangements resulting from the deficiency are, in general, rather characteristic, yet quite similar structures are affected in variable degree. Even paired organs of the same animal are unequally involved.

The vitamin itself or some complexes or derivatives of it seem to have some role in the function of the cells affected by its deficiency. The variation in the manifestations of the deficiency in cellular aggregates of similar structure can be reasonably attributed to variations in the supply of the vitamin or in the need for it under varying physiologic conditions.

Unfortunately chemical or physical methods fail to give us information about the amount of vitamin A where such information would be most valuable—in the sites where the lesions of its deficiency appear. A consideration, however, of certain physical and chemical properties of vitamin A and the histologic characteristics of the lesions of vitamin A deficiency indicates, I believe, that local factors determine the sites and characteristics of the lesions, that the local tissue supply of vitamin A varies both in normal as well as in diseased tissues.

The ideas here presented are speculative. The generalizations are, I am sure, rather naive oversimplifications. Their only merit may be that they systematize a considerable number of haphazard observations and may indicate a direction which we may go in the pursuit of further information concerning the functions of vitamin A.

In this consideration we are not concerned with the amount of oral intake of vitamin A, its absorption from the intestines, its mobilization from storage deposits or the level of vitamin A in the blood, for these presumably affect all cells equally. We are concerned rather with variations in the tissue supply of vitamin A which are determined by quantitative differences in the local blood supply and by quantitative differences in the "pay load" of its transport after it leaves the blood stream.

With this thought in mind let us look briefly at examples of the lesions of vitamin A deficiency. The microscopic changes in the skin are chiefly an increased thickness of the germinal layer of the skin and increased thickness of the cornified layer. In sites where this is not the normal epithelium, there is replacement of the normal epithelium by an epidermis-like epithelium. The response to vitamin A deficiency is, therefore, epidermatization of areas where such epithelium is abnormal or increased "epidermatization" where the epidermis occurs normally.

The fundamental histologic feature of the lesions is epithelial proliferation with keratinization of the layers furthest removed from the basal layers, both in sites where such an epithelium is normal and also where it is abnormal. This response is universally true whether it occurs in the skin, conjunctiva, cornea, salivary ducts, paranasal sinuses, bronchi, renal pelvis, ureters, bladder, vagina, or cervix.

What is common to all these sites? A characteristic structure is common to all these regions—a cellular organization which provides for a declining gradient of supply of nutrients. The nutrients reach the cells in continually decreasing amounts as the cells are further removed from the surface from which the nutrients come. On the contrary, lesions of vitamin A deficiency do not appear in structures like the renal tubules, which are arranged in a single layer of cells in relation to the surface from which the nutrients arrive. The lesions of vitamin A deficiency and the normal structures affected by the lesions of vitamin A deficiency have a common feature—stratification and orientation with reference to the surface from which the cellular aggregate receives its nutrition.

If all substances are, however, relatively equally restricted, inanition, a general depression of the functions of cells must result. The development of the lesions of vitamin A deficiency depends upon the viability and the responsiveness of the cells affected by the deficiency which in turn are dependent upon the adequate supply of other nutrients. There must be a differential in the nutrient supply—not enough vitamin A and enough of other nutrients. Cellular stratification with surface orientation seems to provide such a differential.

This peculiar association of cellular stratification and the development of the lesions of vitamin A deficiency and the characteristic cellular strati-
fication of the lesions of vitamin A deficiency might lead us to inquire if perhaps stratification
might be operative. Perhaps deprivation of vitamin A is
the "final common path" in cellular stratification
such as the lower motor neurone is the final com-
mon path in the nervous system. If that were
true, processes or factors which hinder the trans-
port of vitamin A to the tissues either under
normal or abnormal conditions or which destroy
vitamin A before it reaches its destination should
lead to the same response—cellular stratification.

Since the epidermis is par excellence a stratified
structure and is subjected to the action of various
physical and chemical agents, and is most
easily observed, a study of the epidermis in rela-
tion to the physical and chemical agents which
might affect the supply of vitamin A to the epider-
smis should reveal a pattern of response (in-
creased stratification) as the result of all agents
which diminish predominantly the supply of vita-
min A. Local mechanical pressure which dimin-
ishes the local blood flow and thereby the supply
of vitamin A should lead to increased stratifica-
tion in the epidermis. Physical and chemical
agents which could destroy vitamin A selectively
when they act upon the skin should likewise lead
to increased stratification or hyperkeratinization.

The physical and chemical properties of vita-
min A which we should examine particularly in
this connection are its fat-solubility and water-
insolubility, its reactivity because of its double
bonds, its sensitivity to heat destruction, espe-
cially in acid medium, and its sensitivity to light.

Let us then examine the effect of these vari-
ous agents upon the skin.

Local mechanical pressure leads to hyper-
keratinization quite like that caused by dietary
deficiency. Such areas, for example, are the isch-
ial tuberosities, the surfaces upon which the
body rests during sleep, in areas of pressure
from brassiere straps, belts, and circular garters
and from shoes. Similar changes result in the
skin from fibrosis of the sub-epidermal tissues as
a result of X-ray burns. The same result is noted
in the skin about chronic ulcers.

The fat-solubility and the water-insolubility
of vitamin A suggests that its diffusion through
extra-vascular regions would be less than that of
water-soluble substances. This might explain
why the necessary differential in the supply of
vitamin A and other nutrients occurs in strati-
fied epithelia.

The application of a fat solvent such as ben-
zeno induces epithelial proliferation experiment-
ally. The solubility of vitamin A in fat may lead
to extraction of vitamin A from the epithelium
of the pilosebaceous follicle and explain the as-
sociation of abnormal amounts of sebum and
acne.

Iodides and bromides, when given internally,
are in part excreted into the pilosebaceous fol-
lle and cause aggravation of acne. Iodides and
bromides readily destroy vitamin A.

When arsenical compounds, such as potas-
sium arsenite, are given medicinally arsenic is
deposited in the epidermis and remains there for
years. In many instances, after a variable length
of time various lesions develop which are char-
acterized by epithelial proliferation and kerati-
ization. Arsenic trichloride forms compounds
with vitamin A and was formerly used in the
Carr-Price reaction.

Farmers and seamen, Texans and Brazilians
who are exposed to sunlight for long periods
show a high incidence of keratoses on the exposed
parts of the body, particularly on the back of the
hands and on the face. Keratoses can be readily
induced in experimental animals by ultraviolet
irradiation. Vitamin A is readily destroyed by
ultraviolet light and the wave length band which
is most effective in producing keratoses is that of
the maximum absorption of vitamin A.

Enormous thickening of the skin of the hands
occurs in workers who use mixtures containing
sulfuric acid for cleaning brass. This acid is said
to have been used to thicken the soles of the feet
of fire-walkers in former times. Vitamin A is un-
stable in acid medium, and while the epidermis
is normally slightly acid (pH 5.5) exposure to
strong acids may hasten still further its destruc-
tion.

Vitamin A is destroyed by heat, especially in
an acid medium. The epidermis of the abdomens
of the Kashmiris, who carry glowing coals in
earth baskets inside their clothing for warmth,
becomes greatly thickened. A learned medical
writer in the early part of the last century re-
ports that: "Among those who accustom them-
selves to long journeys over the burning sands of
Egypt some have had their feet as indurated
with a thick callus as an ox’ hoof, so as to bear
shoeing with iron; and in Siam such persons
have been known to walk with their naked feet
on red hot iron bars."

Thus dietary deficiency in vitamin A, me-
chanical pressure, subepidermal fibrosis, fat sol-
vents, heat, light, iodides, bromides, arsenical
compounds, and sulfuric acid, all agents which
seem quite unrelated when viewed in relation to known physiologic cellular responses, have a common end result when they act upon the epidermis—increased proliferation and keratinization. They also share a common property in their possible diminution or destruction of the local epidermal supply of vitamin A.

It is important, moreover, to note that a certain type of cellular proliferation and differentiation is brought about by deprivation of vitamin A. Vitamin A in this sense is not a growth factor as it is usually thought of but rather a growth restraint factor. I think this should be emphasized because it is largely overlooked.

Many of the lesions mentioned are extremely common in man, so common that if some of them can be prevented by increased oral intake of vitamin A, we must seriously question the prevailing concepts in regard to adequate intake or requirement of vitamin A. The factors mentioned can be classed under the concept of “conditioned deficiency.” How much “conditioning” can be counteracted by increased intake of vitamin A remains to be determined. The very prevalence of these lesions must not lead us to assume that they are the necessary attributes of vigorous health as is being done today in the case of acne and was done in the case of rickets. We may recall with profit that several artists of the 15th century used as models for their Christ Child children with rickets.

The generalization, then, that cellular stratification may mean relative vitamin A deprivation may be useful working hypothesis. It puts into clear relief that possible variety, prevalence, extent and importance of lesions possibly due to local vitamin A deficiency, and furnishes a reasonable explanation for the response of these heterogenous lesions to the administration of large doses of vitamin A.
Applying Nutritional Knowledge to Man

ROBERT R. WILLIAMS

The results of scientific research in nutrition find their application to human problems in a great variety of ways, just as is the case with other scientific findings. Often the modes of application are not foreseeable when the laboratory discoveries are first made. Sometimes many years elapse before practical fruition is realized. You recall that Faraday when asked by a member of Parliament, "What good is induction now you have found it?" replied, "Some day you will be able to tax it." It was quite a prophetic statement as any one in modern electrical industry will tell you, perhaps with considerable feeling.

Nutritional knowledge comes to application through education, through the medical profession, through public health departments, through schools and service organizations such as the Red Cross. The drama of the discovery of the vitamins and their applicability in clinical medicine has, of course, been a tremendous factor in the popular appeal of nutrition. Use of the vitamins in the healing art has been of no small service in itself. The correction of forthright malnutrition has affected many thousands of persons formerly suffering from scurvy, rickets, pellagra, and beriberi, not to mention less defined and more mixed deficiencies which the physician still encounters especially in our city slums and in backward rural communities.

Clinical applications, however, only scratch the surface and exert a relatively negative influence. It is the preventive aspects that must and do engage our prime attention. An excellent measure of the usefulness of the modern knowledge of nutrition can be had at any meeting of the American Dietetic Association. In great contrast to the situation of a generation ago the dietitians of the country as a whole are well informed about the principles of nutrition and no longer follow arbitrary maxims and petty prejudices. Many of them have done animal experiments for themselves.

Less expertly informed but highly influential because of the vast numbers of children whom they reach are the teachers of nutrition in our public schools. In some places and in some measure such instruction extends down into the lower grades. Usually it is fairly well developed in the domestic science courses of our better high schools.

No other subject for adult study has surpassed nutrition in popularity among women's groups throughout the country. Of course, this instruction and discussion is not always discriminative but in the main it is very helpful. Local public health groups, state and county nutrition committees, often in association with federal workers, have been very active in such undertakings especially since 1941. Red Cross groups and staffs of public utilities have also taken leading parts. A major consequence has been strengthening of public support for health programs. One of the most vital measures for immediate results has been the school lunch program in our public schools whereby children get wholesome supplementary food and also learn what food is wholesome. Unhappily many poorer school districts will not make the necessary local contribution necessary to secure federal aid.

Another major channel through which we apply nutritional science is that of industry. All the leading food industries have competent scientific staffs and are quite well aware of the importance of applying nutritional science to their products. A good example is the canning industry. Whereas 20 years ago many nutrient values were sacrificed in the factory process through ignorance of the biochemical reactions involved, today the well trained scientist will not find it easy to make great improvements. The possibilities have been ferreted out and utilized.

Novel food products appear upon the market every month and each adds something to the convenience, the variety, or the economy of our pantries and consequently to the goodness of our dietaries. Frozen foods are an example. There is much competitive advertising of these various wares. This may be socially highly useful, witness the successful introduction of the general use of tomato juice and citrus fruits. Magazine and poster displays often go the limit in commending the virtues of the advertised wares but, thanks in part to the influence of the Federal Food Law, the labeling is truthful and the ethics of the industry is relatively high. Gone are the days when flavored glucose spiced with a dead bee per bottle can be sold as honey. The sterling
scientific character of Nutrition Foundation supported by the food industries is eloquent of the general standard.

Nearly all the available methods for introducing nutritional betterments work best at the higher economic levels. New food products, especially those of superior worth, find their first markets among the well-to-do where the margins of profit are more liberal. Education also succeeds best in intelligent communities which in general are those with adequate incomes. Many of the protective foods are among the more expensive, notably meat and milk. They sell best among groups with better than marginal incomes.

A prime objective of the bread, flour, and cereal enrichment program was to do something for the lowest income groups. The fact that the cereals are the cheapest of all foods and universally consumed in relatively high proportion made them eligible for such a purpose. The fact that cereals are by custom much subject to loss of nutrients by refining made the public as well as the industry welcome an improvement the more readily. Obviously if any staple food product were to be significantly improved it had to be done through the channels of trade and by methods acceptable to the affected industries.

Of course, it has been vigorously pointed out that the thing to do with white flour is to leave it brown. If one could prescribe for the people of the United States that measure would be effective nutritionally. England, through pressure of economic necessity, has prescribed it fairly successfully since 1943 through the latter part of the war and during the present period of austerity. It was politically and practically impossible to prescribe it here or in Canada where wheat milling is a major industry. To make the vending of whole wheat or similar flour generally practicable here it would be quite necessary, in my judgment, to do all milling in small mills scattered about the country near the customers they intend to serve. The flour could then be delivered fresh, palatable and free from weevils at what increase in cost I am not prepared to say. Any warehouse where unrefined flour is long stored presents an almost impossible problem of sanitation. I have seen blending mills run by high grade companies where resifting of long stored "low grade" flours yielded barrels of weevils for each carload of flour shipped.

Artificial enrichment with thiamine, riboflavin, niacin, and iron was welcomed by the great majority of the millers and bakers once they became acquainted with the proposal, its technical feasibility, and potentially low cost. At the outset in 1941 great advantage was taken of the public interest in nutrition in the impending emergency of the oncoming war. Government through the National Research Council and the unit which later became the War Food Administration gave the movement much encouragement. So much so that at first many millers and bakers left the promotion of it entirely to government. Nutritionists, on the other hand, felt that this was the industries’ job and gave their attention to other matters.

The whole thing nearly fell apart in the fall of 1941 and was rescued only by a vigorous appeal both to industry and to nutritionists. Late in 1941 enriched flour was being offered at 2 or 3 cents per retail sack more than unenriched. The higher priced product remained on the grocers’ shelves till the wives of the community were coached to ask for it. Just a little demand based on recognition of superior nutritional value served to double the level of sales in three months time. Since then costs have gone down and price differentials between enriched and unenriched have been generally abolished. The cost of enrichment is now about 1 per cent of the wholesale price of flour.

I need not tell you of tedious weeks of public hearings that were necessary to secure official standards for enriched flour and bread in order that the kinds and quantities of vitamins added to flour might be uniform throughout the country. Otherwise we would have had a competitive race for higher levels among the elements of industry. I need not tell you of the court battles to defend these standards, finally settled only by appeal to the Supreme Court of the United States. A prodigious amount of work went into it to stabilize and rationalize the program and to conciliate all shades of opinion so far as possible.

A major difficulty was that which applies to all food reforms, namely, that they work best at high income levels. Millers were reluctant to add the slightest element of cost to their cheapest flours. The South led by South Carolina met this problem by mandatory legislation requiring enrichment of white flour. This pattern has now been followed by a total of 21 states throughout the country. Vigorous opposition developed among some elements of the dairy industry and the potent dairy lobbies set about to defeat it in many states. The issues are not settled yet though efforts are still continuing. Oregon is still among the states which do not have such legislation. The merit of such legislation is that if enforced it ex-
tends the practice to the cheapest flours of the low income groups and increases the probability of permanence of enrichment in hard times. We have been 80 or 90 per cent successful, but the missing 10 or 20 per cent is the most important percentage pound for pound. Otherwise we would all prefer not to depend on laws.

How much public health benefit enrichment of white flour and bread will bring to the people of the United States must for some time remain a matter of opinion. We have the supporting judgment of many eminent students of nutrition—Sherman, King, Elvehjem, Wilder, Ivy, and others. Some leaders, such as McCollum, Carlson, Morgan, and Lepkovsky, have not joined in support of the program because they feel an effective program based on natural foods would have been possible. They are welcome to try it. We feel they have not the faintest notion of the promotional difficulties but the road remains completely open for such endeavors as they care to make. Enrichment of staple cereals if it can be made universal will at least forthwith prevent the appearance of beriberi and pellagra in our population and limit the occurrence of ariboflavinosis and nutritional anemia. It does so at a per capita cost of about 15 cents per annum and is worth it, even if it merely provides insurance against these specific diseases.

In order to care for this objective it seemed necessary to extend enrichment to corn products because several million people in our South use corn as a staple cereal in partial or complete replacement of wheat. In inaugurating corn enrichment we found the attitude of industry indifferent or hostile. Corn products for southern consumption are produced partly in the Midwest and partly in the South. Midwestern corn meal and grits are degenerated; those of the South are not. The former is an industrial operation in 30 or 40 fairly large sized mills; the latter is mostly a crossroads local industry in perhaps 8,000 tiny mills. The two types of products are competitive and the proportions of each sold is somewhat seasonal and dependent on the size of the southern corn crop. Usually consumption of the two types is roughly equally divided. In general the local miller undersells the distant shipper and there is no love lost between them. This feeling was aggravated by the passage of laws in several southern states requiring enrichment of degenerated corn products but exempting whole corn meal.

Obviously, it was politically and practically impossible to enforce enrichment in the small local mill at least until a mechanism and a supply of enrichment mixture adapted to the little mill could be developed and proved in by extensive trial. It was also obvious that enrichment which omitted whole corn would be a halfway measure.

Accordingly, the Williams-Waterman Fund began to support in 1942 a systematic program of teaching crossroads' millers in South Carolina to enrich their corn products. Dr. E. J. Lease of Clemson College and the Agricultural Extension Service of the State assumed responsibility for the actual work and they have done an extraordinarily effective job under great difficulties. This program is not scientific research in the usual sense yet it has required a good basic understanding of developments in the forefront of nutritional research. In addition it has required grass roots practicality, understanding of human nature, political and business shrewdness. I know few men in science who would be either inclined toward or capable of such a job.

It was first necessary to decide the mechanical means for enriching. It became apparent that any scheme requiring labor in the mill would soon fall into disuse and neglect. An automatic feeder to supply a controlled stream of enriching mixture in proportion to the stream of corn had to be devised. About 10 editions of design were successively tried on corn mills during periods of weeks or months with gradual elimination of faults of operation, excessive wear or undue expense of construction. All these were made in the Clemson shops, and latterly about 500 of the best design have been made there. They sell for $25, representing actual cost.

A supply of enriching mixture was another problem. The mixture had to work in the inexpensive feeder, and it had to be delivered in suitable small packages to the little mills. No commercial firm would undertake it for many little millers only require $10 to $50 worth per year and bookkeeping charges would eat up all their profits. So Clemson College also makes the mixture and ships it C.O.D. by mail all over the state. At first the mixture contained only niacin and iron but as it became apparent that the Food and Drug Administration would frown on any substantial departure from the standard previously adopted for flour the composition was adjusted to conform.

A major problem all along has been how much to subsidize the undertaking. Clearly the less the miller had to pay for feeder or for mixture the more readily could he be persuaded to adopt the practice. On the other hand it would become per-
improvement of it has been aroused by indirect age per capita consumption is only 5 or 6 pounds.

Partly this is due to greater retention of the into-
two-thirds of the vitamins of the whole grain.

Conditions the final milled rice retains half or
sun and milled in the usual way. Under good
rice crop is parboiled by soaking in hot water for
boiling. About a half or two-thirds of India's
version of the ancient Indian practice of par-

converted rice mill association at Sacramento.

At the time I saw it in May 1946 the drying pro-
be somewhat cheaper is practiced by the Cal-

A form of continuous parboiling which may be somewhat cheaper is practiced by the Cal-

In addition to these parboiling operations a
group of 30 or 40 rice millers of the southern
Mississippi Valley have joined in the production
of a rice enrichment premix at New Orleans for
the joint use of all their mills. I shall describe
the process more fully later. It seems probable
that a good share of the rice crop of the United
States will presently escape the nutritional stigma
attached to typical white rice. This is a meri-
torious achievement accomplished by industry
without special fostering by the Food and Nutri-
tion Board or other government agencies.

In watching and encouraging the application
of parboiling and fortification to rice in the
United States I have frankly always had one eye
on the Orient, where nearly half the population
of the globe subsists on rice. The United States
raises and uses less than 2 per cent of the world's
rice crop. It has nevertheless been the world's
best proving ground for innovations in rice mill-
ing due partly to a more progressive technological
spirit but primarily to our high standard of liv-
ing which permits the sale of an improved food product even at some advance in cost. Our rice industry is protected by a tariff of 2 cents a pound and rice has sold here for years for a price a third to a half higher than elsewhere in the world. I offer no defense in general for tariffs but sometimes they have a usefulness in unintended ways. We would have no rice industry without them.

In the summer of 1946 I had opportunity to go to China and the Philippines to restudy the rice problem there. In China disorganization of business due to wild inflation made it impossible to do anything immediately, but in the Philippines the way was open for the inauguration of a large scale experiment in rice fortification which is now under way.

Rice culture in the two countries is quite similar, that of China somewhat better as to tillage, irrigation and yield. Rice seedlings are transplanted from seed beds to the flooded paddies by hand labor which would be prohibitively expensive in this country. I need not dwell on cropping methods though they are vastly important where food calories come largely from rice as in the southern half or two-thirds of China, the southeastern half of India and in the whole of Burma, Siam, Malaya, Philippines, and East Indies. The back-breaking labor of rice growing far surpasses that of any other industry in the world.

Milling methods present similarities and contrasts. In China, at least in the Yangtze Valley, it is the practice to remove the rice hulls on or near the farms and to ship the product as brown rice even from points hundreds of miles back in the hinterland. This saves bulk in shipment. Milling, that is the removal of the bran, however, is done largely near the point of market and consumption. Some milling is done on the boats in transit as they move by sail or river current toward the sea. Much more is done in the borders of the cities. The sole device for industrial milling is the Engleberg huller, the same machine used in the United States, a sort of rotary nutmeg grater which abrades away the exterior surface. Often the huller bears a Chinese label cast in its barrel casting, but it is a Chinese copy of the western machine. Rice for local consumption in interior places may be milled by hand pounding on wood or stone mortars or by a donkey-drawn roller in a circular stone race. These devices, however, are steadily being replaced by steel machines which already mill the rice for the great bulk of population.

The brown rice arrives at its destination well infested with weevils and often in a rancid state. In milling it is the practice to add 3 to 5 per cent of talc to it as it is fed into the hullers. The millers tell you this is necessary as a lubricant or as an abrasive but it is in fact a make-weight and a whitenizer. Precisely the same machine is used elsewhere without talc. The milled rice is very lightly sifted and retains a few per cent of loose bran as well as talc when sold. It is the dirtiest product I have ever seen. By the time the housewife washes it till the washings are clear and picks out a good many of the weed seeds and pebbles she has lost several per cent of the weight and all residual traces of the bran vitamins.

Mayor K. C. Wu of Shanghai, an able and sincere public servant, a graduate of Harvard and an experienced administrator under the government of free China at Chinkiang during the war, was a warm advocate of the use of brown rice. Through municipal government channels brown rice was offered in Shanghai at about two-thirds the commercial price of white. At my first interview with him he expressed his disappointment that there was a negligible demand for it in spite of the fact that all food was very expensive and there was semi-starvation among the poorer elements of the city. One encountered pitiable sights on the Shanghai streets, children scrambling for scraps of food from garbage pails, mothers premasticating solid food for their babies to eke out the milk from their own wasting breasts. Several times newspaper articles about brown rice appeared in the Shanghai papers but in vain. The dirty weevily product in part reflects the decay of industry under war conditions but the public distaste for brown rice is deeper seated and of long standing. Its use has been advocated up and down the Asiatic coasts for nearly 40 years without making any headway. Indeed the increase of machine milling has more than offset the effect of the teaching. Handmilled rice was often allowed to retain some bran to save the housewife part of her labor but domestic preparation is steadily disappearing.

Beriberi is not widely regarded as a disease prevalent in the Shanghai-Nanking area. Further south, especially in Hong Kong, it is more frequently recorded but there are no statistics about its incidence or mortality from it anywhere in China. Observation clearly indicates that the use of vegetables and soybeans is much greater in central China than in the Philippines. Considerable beriberi occurred in prison camps in China during the war as well as in the Chinese army.
but not in starvation areas inspected by UNRRA after the close of hostilities. Half rations of grain supplemented with roots and grasses were the rule in such areas.

Relatively good mortality statistics for beriberi were kept in the Philippines for 20 years before the Japanese occupation. They showed 15,000 to 20,000 deaths annually, about a fourth of them infants. Japan is the only other Oriental country which kept significant figures during the corresponding period. These indicate an annual mortality from beriberi of 10,000 to 20,000 among a population approximately 5 times larger than that of the Philippines. During the war, health statistics among the Filipinos were neglected. In 1946, 45,000 deaths from beriberi are reported from the Philippines. Perhaps the increase is in part really due to aggravation of malnutrition. In part it may reflect less reliable diagnosis. Dr. Juan Salcedo, Jr., the well trained and capable director of the newly formed National Institute of Nutrition, has with our aid finished a six months survey of the incidence of beriberi in the province of Bataan. Detailed figures have yet to be compiled but Dr. Salcedo writes me that 5 to 7 per cent of the population showed clinical evidence of beriberi. The Philippine Department of Health rates this disease as second in importance only to tuberculosis which has risen alarmingly throughout the islands as an aftermath of war.

Rice milling in the Philippines is more highly mechanized than in China and the commercial product is comparable in cleanliness to that in the United States. Rice is shipped from the farms in paddy (unhulled) form to the mills where it is shelled and milled in successive operations. It never travels far nor is it stored in brown form, so the weevil problem is avoided. Milling is done without talc in Engleberg hullers or in pearling cones of the type popular in Europe. Hand milling and hand mortars have disappeared from the well populated coastal areas and brown or undermilled rice is no longer advocated. However, in areas of rice shortage one saw it on sale and in use for want of white rice.

It was our aim both in China and in the Philippines to set up facilities to supply exclusively an improved form of rice to about 100,000 people during a period of two or three years as an experiment and a practical demonstration. It was proposed to conduct a continuing medical survey beginning before introduction of the new rice supply to evaluate the benefits in terms of public health. It was also deemed necessary to inaugurate and to perfect a system of rice inspection, workable under village conditions, whereby rice of inferior nutritional quality offered either by fraud or otherwise might be excluded.

Careful consideration was given in both countries to undermilling and parboiling but neither seemed feasible for trial. A plant to produce parboiled rice for 100,000 people would cost $200,000 to $300,000 and operating charges would be substantial. Moreover shipments of parboiled rice from the United States had encountered serious sales resistance due to popular dislike for it. Within the past three months I have learned of supplies of parboiled rice still in storage from left-over U. S. Army shipments.

On the other hand, Philippine authorities welcomed a trial of fortified rice. By heavily fortifying a small portion of white rice with synthetic thiamine, niacin, and iron, then drying it and coating it with edible fatty acids to prevent washing losses during the customary rinsing of rice one can obtain a suitable premix.

This product mixed with white rice in the proportion of 1 pound to 200 yields a finished product as potent with respect to the added nutrients as brown rice. Riboflavin is not used because it highly discolors the fortified grains which are then liable to be picked out and discarded by the housewife. The machinery for all operations is being provided at a cost of less than $10,000 and the preparation of the fortified rice is starting in all the rice mills of Bataan province (population 90,000). The initial medical survey has been carried out and inspection facilities and personnel have been provided.

The Philippine government could easily be persuaded to adopt the scheme on a wider scale, but we feel the need of practical experience before recommending it. Even the task of mixing A with B in the proportion of 1 to 200 is a stupendous undertaking if it must be done in some hundreds of rice mills with a money incentive for leaving out A altogether. The increment of cost will not be large, perhaps 20 to 25 cents per capita per annum. Nevertheless I suspect the principal obstacle to its country-wide adoption will be the dollar value of the nutrients involved.

In the meantime we feel we have begun a trial which may serve as an important guidepost for other Oriental lands.

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