LANDMARKS OF A HALF CENTURY OF NUTRITION RESEARCH

A symposium

honoring

DOCTOR AGNES FAY MORGAN'S

FIFTIETH ANNIVERSARY

at the

University of California

Berkeley, California

MAY 8, 1965

THE JOURNAL OF NUTRITION

SUPPLEMENT 1, PART II OF VOLUME 91, NUMBER 2, FEBRUARY 1967

PUBLICATION OF SUPPLEMENTS

The Journal of Nutrition is pleased to consider for publication as a supplement those manuscripts of scientific merit but of too great length to include in its regular issues. Such papers are subject to the same procedures of review as those submitted for regular publication and must conform to style as outlined in the Guide for Contributors. The full cost of publication is borne by the author. A more complete statement concerning supplements appears in volume 52, Supplement 1, April 1954.

RICHARD H. BARNES, *Editor*

Printed in the United States of America at
THE PRESS OF THE WISTAR INSTITUTE

CONTENTS

FOREWORD

This supplement to the *Journal of Nutrition* contains the Proceedings of a Symposium, "Landmarks of a Half Century of Nutritional Research" held May 8, 1965, at the University of California, Berkeley to commemorate Dr. Agnes Fay Morgan's 50 years of nutritional research.

During this period Dr. Morgan has witnessed, and contributed to many significant advances in the field of nutrition. Her efforts have included studies of a number of the fat- and water-soluble vitamins; studies on factors responsible for beneficial as well as deleterious changes in protein quality; investigation into mineral metabolism and to a lesser extent, interrelationships of lipids and other nutrients. In addition, she has applied the knowledge obtained from basic nutritional research by initiating and coordinating programs designed to investigate the nutritional status of certain socio-economic and age groups in the United States.

As a result of these broad interests in nutrition, the program and speakers were selected, as much as possible, to provide a perspective of the past, present and anticipated future contributions of research in these areas of nutrition.

It is hoped that from these discussions, the reader will gain insight and appreciation of the vast amount of work that has been accomplished, yet be stimulated by the challenge of the many problems still to be resolved.

RICHARD L. LYMAN

Agnes Fay Morgan — *Her Career in Nutrition*

"Professor Morgan was born in Peoria, Illinois, May 4, 1884. She received the B.S. degree in 1904, the M.S. in 1905, and the Ph.D. degree in Chemistry in 1914, all from the University of Chicago. She served as instructor in chemistry at Hardin College from 1905 to 1907, as teaching fellow at the University of Montana in 1907 and as instructor in chemistry at the University of Washington in Seattle from 1910 to 1913. After majoring in physical and organic chemistry and obtaining the doctorate in 1914, she was appointed Assistant Professor of Nutrition at the University of California in 1915. She was promoted to Associate Professor of Household Science in 1919 and to the Professorship in 1923. When in 1938 the Department of Home Economics was established, her title was changed to Professor of Home Economics and she was named Chairman of the Department and Biochemist in the Agricultural Experiment Station, positions which she held until her retirement in 1954. She is a member of the American Association for the Advancement of Science, the Society of Biological Chemists, the American Institute of Nutrition, Phi Beta Kappa, Sigma Xi, and many other organizations.

"When Dr. Morgan came to the University of California, the field of nutrition was in its infancy. Only a few programs of study in the field were being offered in this country, and there was a great dearth of information. However, Professor Morgan organized the material at hand and in 1915 taught the first scientific human nutrition course at the University of California. She also embarked on a program of research which with effective and distinguished teaching resulted in a department which is world famous. Professor Morgan early became interested in vitamin analyses of processed foods, and a great many processed foods are more nutritious today because of her researches. She was the first to observe the effect of a commonly used food preservative, sulfur dioxide, on the vitamin content. She demonstrated that sulfur dioxide had a protective effect on vitamin C and a damaging effect on thiamine. Many publications appeared on the vitamin content of important California-grown foods and the effects of processing thereon. This concern with human nutrition is also evidenced by her early studies on the effect of small supplementary feedings on the growth of school children.

"Professor Morgan also became interested in the effect of heat on the nutritional value of proteins. She was among the first to demonstrate that heat can destroy a part of this value due chiefly to loss of lysine

availability and devoted much effort to studies designed to discover the mechanism of this heat damage. Professor Morgan has made significant contributions in studies involving the interrelationships between vitamin and hormone activities. She has been particularly interested in the relationship between vitamin D and the parathyroid secretion, between vitamin A and carotene and thyroid secretion, and between riboflavin and pantothenic acid and the secretions of the adrenal gland. She was the first to demonstrate that a deficiency in pantothenic acid can cause severe damage to the adrenal gland and that the administration of vitamin D can produce marked effects on the physiological activity of parathyroid extracts. This latter fact has very recently been 'rediscovered' by others. She also demonstrated in 1940 that a vitamin deficiency (pantothenic acid) can produce greying of hair. One of her later interests was the evaluation of nutritional status and in 1959 she published a bulletin, 'Nutritional Status, U.S.A.' (California Agricultural Experiment Station Bulletin 769) which reviewed and collated 179 publications on this subject by all the experiment stations. This was a California Experiment Station 'best seller.'

"About three-quarters of the more than 150 research publications of Professor Morgan deal with the four subjects just described, and these publications have come in a steady stream. However, almost every year one or more publications on other aspects of problems in nutrition have appeared. This provides evidence of a very broad interest in the problems associated with nutrition and food chemistry and a constant probing into new areas of research. During World War II she spent four years working under the Office of Scientific Research and Development to improve methods of food dehydration, with special reference to quality and nutritive value. Professor Morgan has not only demonstrated distinguished research ability, but by training many men and women in the methods of research, she has also made a signal contribution to the science of nutrition and to the teaching of home economics. These persons are now working in many important positions in this country. Professor Morgan had an additional marked influence on home economics on a national scale because of her membership for 16 years on the Committee on Experiment Station Organization and Policy. From 1946 to 1950 she served as the only woman on the Committee of Nine selected from the experiment stations to give guidance to the national program of research. Additional recognition of Professor Morgan's outstanding accomplishment came when she was awarded the 1949 Garvan Medal of the American Chemical Society in recognition of her distinguished service to chemistry.

"Professor Morgan was elected University of California Faculty Research Lecturer for 1950-51. In 1954 she received the Borden Award from the American Institute of Nutrition; she received the first research award of the Society of Medical Friends of Wine in 1962 for her work on the vitamins in wine; she was the first woman Fellow elected by the

American Institute of Nutrition in 1959; and she received the LL.D. degree from the University of California in that same year. Since her retirement in 1954, she has published 22 research papers, 5 abstracts, and 15 professional and review papers.

"In 1964 Professor Morgan was awarded the Phoebe Apperson Hearst Gold Medal from the *San Francisco Examiner* as one of the ten outstanding Bay Area Women of 1963."

Quoted in part from the Report of Committee on Faculty Research Lecture, 1950-1951. *The Faculty Bulletin,* University of California, p. 41, 1950.

Reminiscences on the Discovery and Significance of Some of the B Vitamins

PAUL GYÖRGY
Professor Emeritus of Pediatrics,
University of Pennsylvania, Philadelphia, Pennsylvania

It is difficult for one to realize the integrated nature of passing time. Past events appear as more or less independent pictures but not as part of a continuum and it requires much effort to bring them back into life. Scientific progress and its particular way stations are no exceptions. In addition, old recollections have their melancholy undertone since one becomes suddenly aware of the years passed.

In this regard, today's Symposium is a rather happy occasion. We are assembled here to honor Dr. Agnes Fay Morgan's 50th Anniversary at the University of California.

For me it is a professional and special personal honor to have been selected as the first speaker at this Symposium.

I met Dr. Morgan first in 1937, when at the invitation of a great old man of California, known certainly to the older local generation of this audience, Dr. Grant Selfridge, I paid my first visit—together with my wife—to the beautiful State of California. I met here in 1937 not only Grant Selfridge and Dr. Morgan, but also Dr. Herbert Evans, Dr. Gladys Emerson, Dr. Samuel Lepkovsky, Dr. I. L. Chaikoff and – last but not least – Dr. Thomas Jukes.

My first introduction by Dr. Selfridge to Dr. Morgan was in the old Institute of Life Science. The phenomenal development from the old Institute to the new Center of Nutritional Science must be credited mainly to Dr. Morgan, the important new additions under the auspices of the new "landlord," Dr. G. M. Briggs notwithstanding.

In reading Dr. Morgan's biography, I was especially intrigued by her achievement in research on wine. She has the honor of being the first recipient of the Research Award from the Society of the Medical Friends of Wine and being an honorary member of the same Society. She received in 1964 the Phoebe Apperson Hearst Gold Medal from the *San Francisco Examiner* as one of the ten outstanding Bay Area Women in 1963.

In coming to the more scientific contacts, both Dr. Morgan and I are recipients of the Borden Award of the American Institute of Nutrition. Our most important, sometimes even competitive, scientific work was centered around the vitamin B complex, the chief theme of this presentation.

The unraveling of the vitamin B complex was one of the most intriguing chapters in the rapid development of vitamin research. It was my good fortune to participate in the study of vitamin B_2 from 1929 onward, practically at the start of an exciting and personally highly rewarding journey.

In 1927, the British Committee on Accessory Food Factors had distinguished two separate components of the vitamin B complex: 1) vitamin B_1, the antineuritic factor; and 2) vitamin B_2, the antipellagra factor. The same committee defined vitamin B_2 as "the more heat stable, water soluble dietary factor recently described and named P-P (pellagra preventive) factor by Goldberger, Wheeler, Lillie and Rogers (1926) and found necessary for maintenance of growth and health and prevention of characteristic skin lesions in rats and considered by the latter workers to be concerned in the prevention of human pellagra."

In 1929 we proceeded with the chemical isolation of so-called vitamin B_2, in collaboration with Professor Richard Kuhn and Dr. Th. Wagner-Jauregg. For assay purposes, we used the growth-response of rats fed a purified supplemented diet, containing cod liver oil as source of vitamin A and D and an alcoholic extract of wheat as the source of vitamin B_1. Modern research workers, accustomed to microbiologic tests which give an answer in 20 to 48 hours and enable the simultaneous assays of scores of test substances, should be impressed by the fact that each assay for vitamin B_2 in rats required a testing period of 3 to 4 weeks, with a corresponding number of prepared experimental animals.

During the course of the isolation of "vitamin B_2" it was our collaborator, Dr. Wagner-Jauregg who first noted that all concentrates which proved to be active when used as supplement to a vitamin B_1 concentrate were colored and showed an intensive green-yellow fluorescence in direct proportion to their biological effect. Exposure to visible light destroyed the growth-promoting activity of these concentrates (1). But the obvious working hypothesis which identified vitamin B_2 with a yellow-green pigment soon met serious difficulties. To the great disappointment and despair of the chemist, the concentrates became biologically inactive in the rat growth test as they were further purified, and became more highly colored. Here, the biologist and animal experimentalist came to the rescue of the chemist (2). It was shown that, by supplementing the diet with a yeast concentrate from which all colored material has been removed by absorption, the biologic activity of the colored preparation was restored (fig. 1). Thus, what was at first sight disappointing, opened the gate to important new assaults on the problem of vitamin B_2 complex.

But first the green-yellow fluorescent substance had to be isolated. This was readily achieved as the result of the cooperative study undertaken with Richard Kuhn and Th. Wagner-Jauregg (3–6). This new substance is now called riboflavin. Inasmuch as riboflavin was the first member of the vitamin B_2 complex isolated and identified,

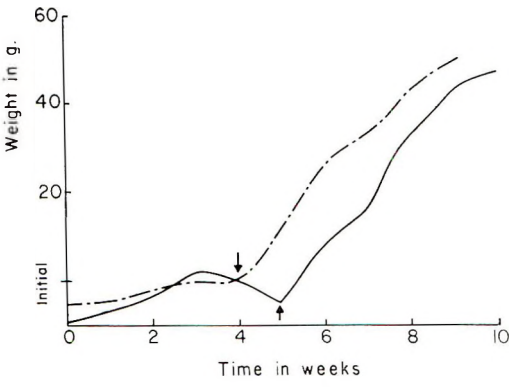


Fig. 1 Apart from the basal diet both animals received 3 pigeon doses of vitamin B. In addition to this, the animal — received 10 μg lactoflavin, and animal —·— 1 ml Peter's eluate daily (equivalent of 10 g of fresh baker's yeast). At ↑ the animal — was given 1 ml of Peter's eluate also, and at ↓ the animal —·— 10 μg lactoflavin. An increase in weight was only produced by the combined administration of vitamin B_1 + lactoflavin + Peter's eluate (2).

it is not surprising that it is still often called vitamin B_2, without reference to the comprehensive character of the original term, vitamin B_2.

Riboflavin was the first vitamin recognized as part of an enzyme system. Thus, it acts not only as a vitamin but also as a proenzyme, as a prosthetic group of the flavoenzymes (6). The yellow pigment isolated previously by Warburg and Christian (7) from their "yellow" enzyme was a photo-derivative of riboflavin and was inactive as a vitamin. Riboflavin bridged the gap between an essential nutrient and cell enzymes and cellular metabolism. In biochemical research it represented a special milestone. Today, with the general acceptance of this idea, it is not surprising that water-soluble vitamins have been found to be essential parts of enzyme systems.

Crystalline vitamin B_1 became available in 1933. The isolation of riboflavin in pure form gave us the key for a real breakthrough in the investigation of the vitamin B_2 problem. It was shown (2, 8) that after a few weeks on a diet free from the whole vitamin B complex and supplemented with required amounts of vitamin B_1 (thiamine) and riboflavin, young rats showed a reduced growth rate and developed a scaly symmetric dermatitis that was most pro-

nounced on the peripheral parts of the body (paws, ears, snout). Inasmuch as the distribution of these cutaneous lesions somewhat resembled the skin lesions in human pellagra, the dermatitis seen in rats receiving a vitamin B-free diet supplemented only with pure thiamine and riboflavin was first called pellagra-like, "without prejudice as to their identity or nonidentity with human pellagra . . ." (2). This observation furnished exact proof for the conclusion that vitamin B_2 was not a single vitamin but a complex in itself, with riboflavin its first definitely established member. In order to avoid confusion with previously discussed but not properly identified factors, like vitamins B_3, B_4, B_5 (and Y) our "rat pellagra factor" was named vitamin B_6 (8).

Further investigations (9–11) led to the differentiation of riboflavin and vitamin B_6 from the specific pellagra preventive factor (P-P) of Goldberger and his associates, and definitely established the separate existence of these three members of the vitamin B complex. The designation "pellagra-like dermatitis" of vitamin B_6 deficiency has been changed to "rat acrodynia," again on this basis of the distribution of the cutaneous changes, without any reference to human acrodynia (9).

The isolation of pure crystalline vitamin B_6 was first reported by Lepkovsky (12) barely 4 years after the recognition of this specific member of the vitamin B_2 complex. Independently but slightly later, several other groups (13–16) also reported the isolation of vitamin B_6. It is perhaps appropriate to recall the fine, quite unusual, friendly gesture of Dr. Lepkovsky, who knowing my active interest in the isolation of vitamin B_6, advised me early in 1938 that he and Keresztesy were ready to submit independently their papers on the successful isolation of vitamin B_6 for publication. This enabled me, with some accelerated urgency, to make ready and submit my own publication in time.

Within a year the exact chemical structure of vitamin B_6 as a pyridine derivative had been elucidated (17, 18). The term pyridoxine, proposed by us (19) for this compound, has received general acceptance.

At this stage of the historical development, microbiological research entered the scene. Credit is due to Snell and his associates (20) for first recognizing the existence of other forms of pyridoxine, i.e., pyridoxal and pyridoxamine. It became customary (21) to speak of vitamin B_6 as a subgroup of the vitamin B_2 complex, with pyridoxine, pyridoxal and pyridoxamine as its particular chemical representatives.

In 1936, it had been shown by Lepkovsky, Jukes and Krause (22) that rats need, in addition to riboflavin and vitamin B_6, a third, mainly growth-activating fraction of the vitamin B_2 complex. In growing chicks under similar experimental conditions severe, progressive dermatitis was also observed (23). This factor was called the "filtrate factor" (24), owing to the outstanding chemical properties revealed during the course of preliminary attempts at its purification. In 1939, this "filtrate factor" was recognized to be identical with pantothenic acid, which was known since 1933 as a specific, vitamin-like growth factor for yeast (25). It was Dr. Morgan who, with her associates had first shown that deficiency of the "filtrate factor" produces in rats depigmentation of the fur, "achromotrichia" or graying in piebald or black animals (26, 27). Several publications followed from other laboratories confirming Dr. Morgan's observation (14, 19, 28, 29). Working with albino rats, we reported first only "rusting" of the fur. At my request, Dr. Morgan had kindly sent us the strain of black rats used in her experiments on nutritional achromotrichia: a shining example of her cooperative spirit. We were then able to show that a highly purified concentrate of pantothenic acid, obtained through the courtesy of the late Dr. Subbarow (30), and soon after that also pure synthetic pantothenic acid prevented and cured nutritional achromotrichia in rats and mice (31).

In pursuing further the history of the vitamin B_2 complex, one more interesting although not an absolutely direct contact between Dr. Morgan's research and my own should be mentioned. This refers to the important series of publications by Dr. Morgan and her collaborators on the effect

of vitamin deficiencies on adrenocortical functions, superbly summarized in her review in *Vitamins and Hormones* (32). In 1937 we reported on the high incidence of adrenal hemorrhage and on other hemorrhagic manifestations as well as panmyelophthisis in rats and related it to deficiency of the filtrate factor or to one of its constituents (33). Later, Daft and Sebrell (34) had proven conclusively that the adrenal hemorrhage could be prevented by pure pantothenic acid. We were able to prevent not only adrenal hemorrhage but all other manifestations of hemorrhagic diathesis and of aplastic anemia by pantothenic acid. In close relation to the views of Dr. Morgan, "one could . . . look upon adrenal hemorrhage and adrenal necroses as the 'stage of exhaustion' during the course of an alarm reaction. Its presence, in rats kept under special dietary conditions, might conceivably indicate a close relationship between pantothenic acid and functional state of the adrenal cortex" (35).

Riboflavin, vitamin B_6 and pantothenic acid were the main representatives of the vitamin B_2 complex which were of common interest to Dr. Morgan and me. I will, therefore, refrain from discussing other remaining members of the B_2 complex, even biotin, which occupied many years of my research activity.

Dr. Morgan's studies on nicotinic acid bring me, via a roundabout way, to my major present interest, i.e., through tryptophan to amino acids and then to protein and finally to protein deficiency. Lack of protein is today perhaps the leading nutritional problem in the world. Dr. Morgan's bibliography contains publications on supplementary feeding of underweight children which appeared many years ago (36–39). This problem is still with us! On an immense scale, we need a new generation to get at it on a global basis! The example of Dr. Morgan and her dedication to this and many other problems of nutrition, some of which I had the honor to discuss today, should be the shining beacon to follow.

LITERATURE CITED

1. György, P. 1935 Vitamin B_2 complex. III. The inactivation of lactoflavin and vitamin B_6 by visible light. Biochem. J., *29:* 767.
2. György, P. 1935 Vitamin B_2 complex. I. Differentiation of lactoflavin and the rat antipellagra factor. Biochem. J., *29:* 741.
3. György, P., R. Kuhn and Th. Wagner-Jauregg 1933 Vitamin B_2. Naturwissenschaften, *21:* 560.
4. György, P., R. Kuhn and Th. Wagner-Jauregg 1933 Vitamin B_2. A review. Klin. Wochenschr., *12:* 1241.
5. György, P., R. Kuhn and Th. Wagner-Jauregg 1934 Distribution of vitamin B_2 in the animal body. Z. Physiol. Chem., *223:* 21.
6. György, P., R. Kuhn and Th. Wagner-Jauregg 1934 Flavins and flavoproteins as vitamin B_2. Z. Physiol. Chem., *223:* 241.
7. Warburg, O., and W. Christian 1932 A second oxygen-transfer enzyme and its absorption spectrum. Naturwissenschaften, *20:* 688.
8. György, P. 1934 Vitamin B_2 and the pellagra-like dermatitis in rats. Nature, *133:* 498.
9. Birch, T. W., P. György and L. J. Harris 1935 The vitamin B complex. Differentiation of the anti-black tongue and the "P-P" factor. Biochem. J., *29:* 2830.
10. Koehn, C. J., Jr., and C. A. Elvehjem 1936 Studies on vitamin G (B_2) and its relation to canine black tongue. J. Nutr., *11:* 67.
11. Elvehjem, C. A., R. J. Madden, F. M. Strong and D. W. Wooley 1937 Relation of nicotinic acid and nicotinic acid amide to canine black tongue. J. Amer. Chem. Soc., *59:* 1767.
12. Lepkovsky, S. 1938 Crystalline factor 1. Science, *87:* 169.
13. Keresztesy, J. C., and J. R. Stevens 1938 Vitamin B_6. J. Amer. Chem. Soc., *60:* 1267.
14. György, P. 1938 Crystalline vitamin B_6. J. Amer. Chem. Soc., *60:* 983.
15. Kuhn, R., and G. Wendt 1938 The antidermatitic vitamin of yeast. Ber. Dent. Chem. Ges., *71B:* 780.
16. Ichiba, A., and K. Michi 1938 Crystalline vitamin B_6. Sci. Papers Inst. Phys. Chem. Res. (Tokyo), *34:* 623.
17. Harris, S. A., and K. Folkers 1939 Synthesis of vitamin B_6. J. Amer. Chem. Soc., *61:* 1245.
18. Kuhn, R., K. Westphal, G. Wendt and O. Westphal 1939 Synthesis of adermine. Naturwissenschaften, *27:* 469.
19. György, P., and R. E. Eckhardt 1939 Vitamin B_6 and skin lesions in rats. Nature, *144:* 512.
20. Snell, E. E., B. M. Guirard and R. S. Williams 1942 Occurrence in natural products of a physiologically active metabolite of pyridoxine. J. Biol. Chem., *143:* 519.
21. Snell, E. E. 1953 Metabolic significance of B vitamins: Symposium: summary of known metabolic functions of nicotinic acid, riboflavin and vitamin B_6. Physiol. Rev., *33:* 509.

22. Lepkovsky, S., T. H. Jukes and M. E. Krause 1936 The multiple nature of the third factor of the vitamin B complex. J. Biol. Chem., *115:* 557.
23. Lepkovsky, S., and T. H. Jukes 1935 The vitamin G requirement of the chick. J. Biol. Chem., *111:* 119.
24. Lepkovsky, S., and T. H. Jukes 1936 The distribution of the "filtrate factor" in certain feeding stuffs. J. Biol. Chem., *114:* 109.
25. Williams, R. J., C. M. Lyman, G. H. Goodyear and J. H. Triesdall 1933 "Pantothenic acid," a growth determinant of universal biological occurrence. J. Amer. Chem. Soc., *55:* 2912.
26. Morgan, A. F., B. B. Cook and H. G. Davison 1938 Vitamin B_2 deficiences as affected by dietary carbohydrate. J. Nutr., *15:* 27.
27. Morgan, A. F., and H. D. Sims 1940 Graying of fur and other disturbances in several species due to a vitamin deficiency. J. Nutr., *233:* 250.
28. Lunde, G., and H. Kringstad 1939 Changes of the fur of rats produced by lack of certain factors of the vitamin B complex. II. Z. Physiol. Chem., *257:* 201.
29. Nielsen, E., J. J. Oleson and C. A. Elvehjem 1940 Fractionation of the factor preventing nutritional achromotrichia. J. Biol. Chem., *133:* 637.
30. György, P., C. E. Poling and Y. Subbarow 1940 The factor curative of nutritional achromotrichia. J. Biol. Chem., *132:* 789.
31. György, P., and C. E. Poling 1940 Pantothenic acid and nutritional achromotrichia in rats. Science, *92:* 202.
32. Morgan, A. F. 1951 The effect of vitamin deficiencies on adrenocortico function. Vitamins Hormones, *9:* 161.
33. György, P., H. Goldblatt, F. R. Miller and R. P. Fulton 1937 Panmyelophthisis with hemorrhagic manifestations in rats on nutritional basis. J. Exp. Med., *66:* 579.
34. Daft, S., and W. H. Sebrell 1939 Hemorrhagic adrenal necrosis in rats on deficient diets. U. S. Public Health Rep., *54:* 2247.
35. György, P. 1948 Hemorrhagic manifestations observed in experimental deficiency of pantothenic acid, choline and cystine. Ann. N. Y. Acad. Sci., *49:* 525.
36. Morgan, A. F., G. D. Hatfield and M. A. Tanner 1926 Comparison of effects of supplementary feeding of fruits and milk on the growth of children. Amer. J. Dis. Child., *32:* 839.
37. Morgan, A. F., and M. A. Tanner 1927 Supplementary feeding of school children. Amer. J. Dis. Child., *33:* 404.
38. Morgan, A. F., and L. Warren 1928 Stimulation of growth of school children by small supplementary feeding. Amer. J. Dis. Child., *36:* 972.
39. Morgan, A. F., and M. M. Barry 1930 Underweight children. Increased growth secured through use of wheat germ. Amer. J. Dis. Child., *39:* 935.

The Paths to the Discovery of Vitamins A and D

ELMER VERNER MCCOLLUM
Professor Emeritus of Biochemistry
Johns Hopkins University, Baltimore, Maryland

In 1907, on completing my studies at Yale I was, by the standards of the period prepared to undertake investigations in organic and biological chemistry. I wanted an academic position. The only opportunity I found was an instructorship in Agricultural Chemistry in the College of Agriculture at the University of Wisconsin. I was to work principally in the Experiment Station under the direction of Professor E. B. Hart. The project was to find the cause or causes of the malnutrition manifested by cows which had been restricted through most of their period of growth to rations derived solely from single plant sources, viz., the wheat, oat, and corn (maize) plant. Malnutrition was severe in the wheat-fed cows. They were inferior in appearance, had become blind, and delivered very premature, undersized, dead calves. The oat-fed cows looked better, their eyes appeared normal, and though they carried their calves to term, none survived longer than a few hours. In marked contrast the corn-fed cows were in fine condition and produced vigorous calves. Chemical analysis had shown that the rations were of equivalent nutritive value. Obviously the chemical method of analysis was unreliable as a guide to nutritive values.

I had never analyzed a food, nor conducted an animal experiment. I set to work to educate myself by reading books, journal articles and bulletins. After consultation with Professor Hart, I began to make extensive analyses of the blood, urine, milk and feces of the cows in hopes that the data secured might furnish a clue to the cause or causes of their contrasting condition.

The sources of information available to me afforded no assistance for the solution of my problem. I decided to examine all abstracts of scientific publications in recent decades relating to the chemistry of plant and animal substances, physiology, and nutrition studies with men and animals to seek facts or suggestions as to what I might do next. These were available in Maly's *Jahres-Bericht Uber die Fortschritte der Thier-Chemie*. There were 37 volumes covering the publications from 1870 to 1907. I bought the file, and of evenings leafed the pages of every volume, studying all abstracts with care. My knowledge of biochemistry was greatly enlarged. While thus engaged my mind was alert to discover ideas which suggested philosophic insight.

In the volume for 1880 I found a description of the experiments of N. Lunin, a Russian student of the distinguished Professor C. von Bunge at Dorpat. Lunin restricted mice to a diet of isolated, purified protein (casein), carbohydrates, fats and an inorganic salt mixture made in imitation of the ash of milk. The mice failed rapidly and all were dead within 21 days. Mice, to which he gave only milk, were in good condition and were lively at 60 days. He concluded that milk contained unidentified nutrients not hitherto suspected. In other volumes I found descriptions of similar experiments with "purified" food-stuffs described by 13 other investigators. All reported that their animals, mostly mice, failed rapidly and died when confined to such diets. I took notes on these studies, and concluded that the most important problem in nutrition was to discover what was lacking in diets containing only "purified" constituents.

Another result of this study of the experiments of earlier biochemists was the discovery that no one had attempted to investigate the degree of completeness of individual natural food substances such as leaf, seed, tuber, root or fruit, as sole source of essential nutrients for an animal. Such an inquiry would be the simplest possible type of nutrition investigation. If in any case the animals failed nutritionally it should be possible to systematically study the problem of what was lacking by supplementing the food with single or multiple known substances . . . If this approach was not successful the addition of very small amounts of one or another natural food, should reveal where the missing unidentified nutrient was to be found. From this reasoning the thought occurred that animal feeding studies with two-source diets, one constituent small, the other large, should reveal which foods made good the deficiencies of each other. Similar studies, I concluded, should be made using single type-animal derivatives – muscle, liver, kidney, etc. Nothing of this type of study had ever been attempted. Reflecting on these ideas, I saw a vista of great promise for revealing new knowledge of the biochemistry of nutrition.

It also occurred to me that experiments should be made with small animals. They grew to maturity in a short time, reproduced, reared young, and had a short lifespan. They ate little, so one could afford to do the necessary chemical work on the diets. The life history of the animals could be observed.

While engaged in these speculations I became convinced that the project we were engaged in with cows, fed highly complex chemical rations could not lead to the discovery of anything of importance and that I was wasting my time.

I was in a predicament, because Professor Hart was elated by his cow experiment and it had been given wide publicity. Animal husbandrymen were enthusiastically discussing it. The discovery of what was wrong with the wheat ration would reflect great credit on us. But I was 28 years old. It was imperative that I accomplish something of scientific worth to gain advancement and establish myself as a productive scientist. It was dishonest for me to continue accumulating analytical data which I considered worthless, so it was imperative that I divulge my thoughts and take the consequences.

Professor Hart was astonished and offended at my pronouncement on the cow project. He was contemptuous of my suggestion that we turn to the rat as an experimental animal. I was at fault in wanting to abandon so soon the project on which I had agreed to work. Our interview was brief and stormy.

Two days later I told about my speculations to Emeritus Professor Steven M. Babcock. He responded enthusiastically, saying that mine was the best suggestion he had ever heard of for study of foods and nutrition. He took me to the office of Dean H. L. Russell and asked me to tell him of my ideas. Dr. Russell's answer was an emphatic "no." The rat was a farm pest and it would never do to spend Federal and State funds on experiments with rats. Dr. Babcock was the most honored man in the College of Agriculture, and because he insisted that he wanted to know what could be learned by my plan, I was meekly permitted to set up my rat colony. No formal project was made of my enterprise, and no funds were allocated for its support.

I made cages out of boxes in which supplies came to the laboratory. I needed quarter-inch wire netting for one side, and placed on Professor Hart's desk a requisition for two dollars' worth. He declined to sign it and I bought it out of my $1,200 annual salary. I caught wild rats and tried to use them, but they were so frightened and ferocious that I discarded them and bought, at my own cost, a dozen albino rats from which I built up my colony.

The ingredients of my diets were prepared incidentally while doing analytical work on the cows, but I cared for my rats outside of regular work hours. Starting with enthusiasm I soon realized that although most of my projected experiments would be terminated in a short time because of failure of the little rats to survive on my "purified" diets, much time would be required to complete the several hundred

tests on formulas I had devised. It would take several years to accumulate sufficient data to enable me to make any important conclusions. I doubted I should be permitted to continue so long without justifying myself by results.

In July 1909, I had the good fortune to have Miss Marguerite Davis ask me to take her as a student in biochemistry. She had just graduated at the University of California at Berkeley, and had come to Madison to live with her father. I gave her a place in the laboratory and assigned some exercises, and from time to time stopped to talk with her. Before long I told her what I was trying to do with my rat colony. She was enthusiastic about the project and volunteered to take care of the rats. I at once began to teach her rat housekeeping, and began the construction of more cages. Before long we were carrying on ten or more times as many experiments as I alone could manage. It was due to her interest and loyalty to the enterprise that we made important discoveries.

It had been proposed that certain farm rations which failed to give satisfactory results were deficient in certain organic phosphorylated compounds, e.g., phosphoproteins, lecithin, cephalin or nucleic acids. I was able to demonstrate that rats could synthesize all such compounds using inorganic phosphates as source of phosphorus —an important discovery at that time.

At the outset of my rat experiments I was aware of the great differences in the amino acid make-up of different proteins, as had been shown by chemists. Hopkins, in 1906 had demonstrated the indespensability of tryptophan. More than a year after I started work with rats and the "purified" diet, Osborne and Mendel began work with rats to study the comparative values of proteins of widely different chemical composition. They included in their diets a single isolated protein and 28.3% of dried whey from which lactalbumin had been removed by coagulation. This supplied sufficient of all unidentified nutrients to make their growth studies successful. They interpreted their results without considering unidentified dietary essentials. They dramatized the significance of individual amino acids, confirming the differences shown by chemists.

Our first highly important discovery was that a certain diet which I supposed to contain only "purified" substances, was able to support growth and apparent well-being for a few weeks when either butter fat or the fats of egg yolk were included, but when the fat was supplied by lard or olive oil, the rats soon failed nutritionally and manifested an eye condition characterized by swollen lids, ulceration of the cornea, and destruction of the eye. We called this nutrient fat-soluble A. It is now known as vitamin A. Later I was to learn that the diet, which contained commercial milk sugar, was inadequate to support life when the milk sugar was recrystallized. Furthermore the mother liquor from crystallization of lactose was of significant value in improving the condition of the little rats. It became evident that we had proven that there was a water-soluble as well as a fat-soluble unidentified essential nutrient. This we confirmed in our studies of the dietary deficiencies of polished rice. At the outset of our studies I was not acquainted with the work of Eijkman and of Grijns on the experimental production of polyneuritis in birds by restricting them to a diet of polished rice. But the "toxicity" theory of Eijkman and the "deficiency" theory of Grijns had been debated by medical writers for two decades. We independently discovered what I believed at the time to be the antiberi-beri substance.

The discovery of a fat-soluble indispensable nutrient (vitamin A) aroused great interest among the biochemists and physiologists. Hitherto all fats had been considered as sources of energy only and were believed to be alike on an equal caloric basis. We described the eye conditions and published pictures of rats exhibiting the disorder. We extended our studies and found that fats from liver and kidney, and ether extract of the leaf of a plant supplied the new nutrient. The earliest study of the pathological changes in the eye condition was made in my laboratory by Dr. S. Mori, a Japanese ophthalmologist, who saw pictures of our rat's eyes in the new deficiency state, and spent a year making histological

studies of the eye, the lachrymal, harderian and mibomian glands. I prepared for him rats in several stages of deficiency of vitamin A, and he concluded that keratinization of epithelial cells, of whatever type of specialization as to function, was the primary cause of the pathological manifestations of the eyes. Keratinization destroyed the function of the tear glands, with consequent stagnation of tears in the conjunctival sac. This permitted overgrowth of the eye and inner surfaces of the eyelids by microorganisms, ulceration of the cornea and destruction of the eye.

That liver fats contained the new nutrient soon brought to light that a condition long known as night blindness was cured by eating liver. In ancient Egypt the household remedy for night blindness was eating liver of a black cock. In Newfoundland, where nightblindness was common in 1912, the belief prevailed that fishermen whose eyes were exposed to the glare of sunlight on water, who could not see in twilight or darkness, were cured by eating the liver of a sea gull. Within a few years it emerged that vitamin A is essential for regeneration of light sensitive visual purple of the retina. The discovery of vitamin A induced an increasing number of scientists to attempt its isolation, chemical identification and synthesis. Its functions other than those relating to the eye invited inquiry by biochemists, physiologists and pathologists. After half a century it is still a prominent subject for investigation.

Examination of the photographs which we published showed that by the end of 1916, when we had completed application of what I called a biological method for analysis of a foodstuff, we had produced several kinds of dietary deficiency diseases. We had completed over 1,600 experiments. The kinds of information secured about the dietary properties of single seeds, tubers, root vegetables, alfalfa leaf, wheat germs, refined wheat flour and cornmeal, constituted an array of information of an entirely new kind. We had also shown as a source of nutrients the superiority of liver and kidney over muscle, and the extent to which gelatin supplied amino acids which were not optimally abundant in the protein mixture in several seeds.

Among the effects produced by certain of our diets, were strongly contrasting appearance of the rats, loss of hair on different parts of the body, scaliness of the skin and in others dermatitis. Abnormalities of body form, posture, stunted size, sudden decline and death of rats which had grown well for a time; in some, fertility was decreased; some were sterile; others gave birth to dead or of living, but nonviable young, etc. Many had the eye disorder already mentioned.

It was evident to me that here was pathology of several types which was caused by deficiencies or excesses, or unfavorable quantitative relations among the nutrients already known. My approach to this study was that of a chemist, and I was keenly aware of my inability to interpret the meaning of symptoms. I carried with me photographs of rats with contrasting symptoms and showed them to physicians, pathologists and veterinarians. I explained the nature of the faults in certain diets, but in most cases the defects in the diets were a mystery. None of the people contacted were interested and they gave me little or no assistance.

My hope at the outset that my comprehensive project for studying the properties of foods might assist in explaining the cause of the malnutrition of the wheat-fed cows, was realized. When we discovered that the leaf of the plant is a far superior source of essential nutrients than the seed, reflection brought to memory that we had not fed the leaf of wheat to the cows. We did not grow wheat on the experiment station farm except in plots for testing varieties for their performance in the short Wisconsin summer. We fed purchased wheat, wheat gluten and wheat straw secured from neighboring farmers. In threshing, the whirring teeth of the cylinder beat the wheat leaves to bits too small to be picked up with a pitchfork, so when the farmers brought us straw, they left the leaves on the farm. The two most serious deficiencies in the wheat plant rations were calcium and vitamin A.

Our experiments were described in a series of papers in scientific journals and attracted much favorable comment. As evidence of this, an invitation was extended

to me to give a lecture before the Harvey Society in New York on January 17, 1917, on "The Supplementary Relations Among Our Common Food-stuffs." The following day Professor William H. Howell offered me the professorship in chemistry at the newly established School of Hygiene and Public Health at the Johns Hopkins University. I accepted and moved my rat colony to Baltimore, leaving about 100 behind, which formed the basis of the colony with which Professor Hart and his associates accomplished highly meritorious investigations. Our work led to the setting up of rat colonies in other institutions. The rat became highly popular as a subject for nutrition studies.

At the Johns Hopkins Medical School I was fortunate in securing excellent collaboration of Drs. Edwards A. Park and Paul G. Shipley which resulted in the discovery of a second fat-soluble vitamin, whose function was directing bone growth along normal lines even when the diet was of unfavorable composition. We had observed little rats on a certain diet to exhibit deformity of the thorax characterized by buckling of the ribs at the costochondral junctions, and nodes due to callus formation at spontaneous fractures. The condition resembled the so-called "pigeon breast" seen in human rickets. I believed that the condition was experimentally induced rickets. Dr. John Howland, Pediatrician-in-Chief, asked me if I thought anyone had ever produced rickets experimentally in an animal. I showed our deformed rats to him and he confirmed my belief that the condition was rickets. We arranged a collaborative research. Drs. Park and Shipley were to describe the histology of bones of such animals as I should prepare with a view to finding the nature of the dietary defect. Two technicians were employed to cut sections and differentially stain the region of growing bones. I at once set about planning a series of more than 300 modifications of the incidentally discovered rickets-producing diet. Little rats restricted to these formulas were prepared with a view to studying how growing bones responded to a long series of diets which induced one or another kind of malnutrition. Many diets were devised after listening to descriptions of the histological details of bone sections, such as abnormalities in appearance of cartilage as it changed from resting to vesicular type, distribution of blood capillaries, presence or absence of bone trabeculae, and presence or absence of osteoid tissue, etc. After hearing such comment, I studied the diet and planned another intended to accentuate or to alleviate certain tendencies of growing bones to deviate from normal. In this way we learned how to induce severe and acute rickets in a short time. One, diet 3413, was exactly suitable for preparing little rats for testing their response to an anti-rachitic agent, and served as a method of quantitative assay of any substance for its potency in preventing or curing rickets. We thus clearly demonstrated the existence of a hitherto unknown nutrient of great significance in directing the processes of bone growth.

In 1918, Mellanby had tentatively concluded that the protective power of certain fats and oils against rickets might be assigned to vitamin A. To examine this question we treated a sample of cod liver oil, which not only prevented or cured rickets, but also prevented or cured the eye disease due to vitamin A deficiency, by passing heated air bubbles through it to destroy vitamin A. This treated oil was still effective in preventing or curing rickets. We called the new nutrient vitamin D. This discovery was announced in 1922. Owing to the high reputation of Dr. Park, the medical profession immediately began to prescribe a source of vitamin D for infants and children. This rapidly led to the virtual disappearance of rickets in children throughout the world.

Employing our "line-test" in rats fed diet 3413 we confirmed the observation of others that sunlight which had not passed through glass, had anti-rachitic effects. Bills subjected many liver oils of fishes to this assay method, and discovered the extremely high potency of the liver oil of tuna. Steenbock, using a rickets-producing diet, discovered that irradiation of certain foods conferred on them anti-rachitic properties.

The rat has had a long history of evil deeds, including the nourishing and distribution of rat fleas, which transmitted

bubonic plague. It has been the agent of rat-bite fever and has caused immense destruction of young poultry, and of feed grains about farmyards. Its past is a history of evil not equaled by any other animal. But its introduction as a subject for nutrition investigation, gave it opportunity to contribute enormously to human welfare. When placed in carefully designed situations where its physiological status depended on its response to diets, faulty in one or another respect, it has answered more questions about essential nutrients and their roles in metabolism, than has any other animal. Caged and used intelligently the rat has at last conferred very great benefits on mankind.

The Fatty Acid Story—Lessons and Expectations

WENDELL H. GRIFFITH
Director, Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, Maryland

To participate in the honoring of such a distinguished fellow scientist is a prized privilege. This symposium marks a stepping stone in the career of Dr. Morgan. It is neither a beginning nor an end. It is a commemoration of an unusual period of service to the University of California and to science.

The topic which I will deal with is fatty acids; hence it is proper to refer to that French chemist of another era, Michel Eugene Chevreul (1786-1889), who first determined the constitution of fats and first identified cholesterol. Because Peoria, Illinois, is some distance from France and because Dr. Morgan was only 5 years old when Chevreul attained the handsome age of 103, it is not likely that she remembers him as a person. What is material, however, is the evidence that today's observance highlights stepping stones along the Morgan pathway which, like Chevreul's, also stretches on and on into the future.

It is my assignment to speak of a half century of research in lipid chemistry and nutrition. Necessarily, this cannot be a systematic review of lipid biochemistry but it can touch on some of the major research contributions in the lipid field.

What are the landmarks in the story of the fatty acids? The recognition of the dietary indispensability of certain polyenoic acids was clearly a notable discovery. Another was the identification of coenzyme A and the clarification of its functions in the metabolism of fatty acids. Opinions will differ regarding the priority of other developments, among which are the following: a) the establishment of adipose tissue as a tissue which is active metabolically rather than inert and which provides fatty acids for the satisfaction of energy requirements under neural and hormonal control, with epinephrine and norepinephrine as principal regulators; b) the proof of the specific role in energy transfer of molecules possessing energy-rich bonds like adenosine triphosphate, S-adenosylmethionine, and coenzyme A; and c) the demonstration of the role of choline in transmethylation and in the prevention of the fatty liver.

My comments will deal with essential polyenoic acids, with coenzyme A, and with choline. First, however, recognition must be given to improved laboratory procedures. Earlier methods of separation and analysis of fatty acids involved fractional crystallization and distillation and the determination of various "numbers" such as the iodine and saponification numbers. To these methods have been added the determination of conjugated double bond systems by alkaline isomerization and ultraviolet spectroscopy, the techniques of microanalysis, and, most importantly, chromatographic analysis in all of its forms, including thin-layer and gas-phase chromatography. Isotopic labeling of fatty acids has been of the greatest importance.

Additionally, one would be remiss not to recognize an achievement of a quite different type which has been and will continue to be a powerful force in the continued development of the fatty acid story. This is, truly, a monumental work, the three volumes on the chemistry and biochemistry of the lipids by the late Harry J. Deuel, Jr.

To give recognition to those who established the science of nutrition we may quote from Graham Lusk's preface to the third or 1919 edition of *The Science of Nutrition*: "It is, furthermore, a privilege to recognize the great influence which a

personal acquaintance with such men as F. G. Benedict, S. R. Benedict, Cathcart, Chittenden, Cremer, Dakin, Folin, Halliburton, Hopkins, Kossel, Levene, Magnus-Levy, Lafayette Mendel, Friedrich von Muller, von Noorden, Rubner, E. Voit, and Zuntz has had upon the conceptions of the subject of nutrition as set down in this book." The book, of course, is dedicated to Carl von Voit. That the period following 1915 was a period of transition was clearly evident to Lusk. In his text Lusk presented the relation of body fat to the combustion of tissue protein during starvation, the evidence for the beta-oxidation of fatty acids, the detailed proof of the conversion of glucose into fat, the minimal specific dynamic action of fat, and the characteristics of the respiratory quotient during the combustion of fat, as well as the findings on the newly discovered vitamins. At the end of the chapter on the nutritive value of foods, Lusk rather cautiously noted the probability that energy metabolism could no longer be the main attraction in the field of nutrition research. He wrote, "It is evident from the material presented in this chapter that the science of nutrition includes something more than the production of energy from fat, carbohydrate and protein. There must be certain salts and certain qualities of protein in the diet, and there must be minute amounts of 'vitamins.' The chemical composition of the latter will some day be known, even as the chemical composition of epinephrine is known."

Ten years later, Lusk's forecast had been fulfilled only in part. Among all the water-soluble vitamins known today, only the antineuritic factor had been isolated, in 1926 by Jansen and Donath, and its structure had not yet been demonstrated by R. R. Williams. Although the occurrence of at least 3 fat-soluble vitamins in the nonsaponifiable fraction of various fats was a matter of record, the conversion of beta-carotene to vitamin A was unproved, the differing antirachitic activity of irradiated ergosterol and of irradiated 7-dehydrocholesterol remained a source of confusion, and little was known about the antisterility factor. Under these circumstances it is not surprising that a question was raised regarding the requirement of dietary triglyceride. Despite its importance as a source of energy and as the primary storage form of excess calories entering the body either as fat or as carbohydrate, was fat per se really essential? Did it have any other major function than to serve as a carrier of fat-soluble vitamins, assuming, of course, an adequate supply of dietary calories from nonlipid sources? It was in this period of uncertainty that Evans and Burr in 1926 and Burr and Burr in 1929 and 1930 reported their findings on polyunsaturated fatty acids (1–3).

The Burrs found that rats fed a fat-free diet grew almost normally for 4 to 6 months, then there was growth failure and death, with death resulting from severe renal degeneration. The growth failure, dermatitis, and inability of the skin to prevent loss of water were not prevented by the antixerophthalmic, antirachitic, or antisterility factors, or by individually tested saturated fatty acids but were prevented or cured by corn oil, linseed oil, cod liver oil, and by linoleic acid. Accordingly, it was concluded that warm blooded animals in general cannot synthesize appreciable quantities of linoleic acid. Subsequent studies have added to these initial findings. Arachidonic acid is now recognized as the important member of the group of essential polyenoic acids although linoleic acid remains as the primary dietary precursor of both the linoleic and arachidonic acids of tissues. Essential fatty acid deficiency has been produced also in the mouse, dog and chick (4, 5) and in young swine (6). Linoleic acid was effective in preventing dermatitis and in restoring altered polyenoic levels in the plasma of infants fed a diet low in fat and in essential fatty acids (7).

Wesson and Burr observed that the basal energy metabolism of deficient rats was increased (8). This important finding has been extended by the demonstration of a 25% increase in the liver cytochrome oxidase activity (9) and by greater fragility of liver mitochondria (11). The significance of these results is greatly enhanced by the work of Fleischer and co-workers who have demonstrated a phospholipid requirement in each of 3 complexes of the electron transport

chain from succinate to oxygen (12–14). Their findings depended on the removal of lipids from mitochondria by a mild technique that left the mitochondria intact. These organized particles were then unable to catalyze electron transfer unless phospholipid was restored. The data suggested that the requirement of phospholipid depended on its ability to form micelles that acted as bridges between hydrophilic and hydrophobic areas and between functional groups.

The feeding of linoleic acid or of linolenic acid to depleted animals is followed by increased tissue levels of dienoic and tetraenoic acids and of pentaenoic and hexaenoic acids, respectively (15, 16). Continuation of a fat-free diet results in the accumulation of 5-,8-,11-eicosatetraenoic acid, a dehydro-arachidonic acid (17). Of particular interest from the viewpoint of the relation of chemical structure to function is the observation that the several polyenoic acids having full or partial activity as essential fatty acids have double bonds at the "6" and "9" positions, counting from the terminal methyl carbon of the fatty acid chain (18). Table 1, which is modified from that of Mead (17), indicates that there are 4 families of noninterconvertible mammalian polyenoic fatty acids arising from linolenic, linoleic, oleic, and palmitoleic acids by 2-carbon additions and dehydrogenations. The great importance of the 6-9-dienoic structure is clear, as is the implication that substitution of arachidonic acid in phospholipids by polyunsaturated acids not having the 6-9 unsaturation may give rise to structural interference and abnormal metabolism. At the present time only a beginning has been made in the understanding of the relationship between the phospholipid fatty acids and the structure, permeability, and functions of cellular and vascular membranes. It is clear, however, that the very existence of certain lecithins and other complex lipids in tissues is dependent on a dietary supply of essential polyenoic acids.

TABLE 1

Families of fatty acids in mammalian tissues and requirement of double bonds for essential fatty acid activity[1]

	Double bonds in fatty acid[2]	Double bonds in 20-carbon product[3]
Linolenic	3 – 6 – 9	3 – 6 – 9 – 12 3 – 6 – 9 – 12 – 15
Linoleic	6 – 9	6 – 9 – 12 6 – 9 – 12 – 15[3]
Oleic	9	9 – 12 9 – 12 – 15
Palmitoleic	7	7 – 10 – 13

[1] Modified from J. F. Mead. Federation Proc., *20*: 952, 1961.

[2] Location of double bonds with numbering from methyl carbon.

[3] Arachidonic (5-,8-,11-,14-eicosatetraenoic).

Reference has been made to the lengthening and desaturation of fatty acid carbon chains. Such reactions had been taken for granted in connection with the synthesis of the primary triglycerides of animal tissues from carbohydrate. The first unequivocal experimental proof of these changes was provided by Schoenheimer and his coworkers, starting in 1936, through the use of deuterium-labeled fatty acids (19). A number of years were to pass before it was known that these chemical changes were dependent on the formation of acyl derivatives of coenzyme A (20, 21). This is the coenzyme for most, if not all, of the acetyl-transferring systems, including the acetylation of choline to acetylcholine.

The free-SH group is the principal site of reactivity in the CoA molecule. It is readily acetylated in the presence of ATP. The acyl-mercaptide linkage is an energy-rich bond and the acetyl group is aptly described as "active acetyl" or "active 2-carbon fragment." The lengthening and shortening that occur in the synthesis and in the degradation of fatty acid chains, respectively, involve these 2-carbon fragments. The idea of metabolism of 2 carbons at a time goes back to the studies of Knoop reported in 1904 and to his theory of "beta oxidation" (22). He was among the first to study metabolic reactions by means of tagged or labeled molecules, viz., phenyl-substituted fatty acids (table 2). After feeding these to dogs the primary catabolic product was benzoylglycine if the fatty acid contained an odd number of carbons and

TABLE 2

Basis of Knoop's theory of beta-oxidation of fatty acids[1]

Phenylformic acid (benzoic)		
Phenylproprionic acid	→→→	Benzoylglycine
Phenylvaleric acid		(Hippuric acid)
Phenylacetic acid	→→→	Phenacetylglycine
Phenylbutyric acid		(Phenaceturic acid)

$$C_6H_5CH_2CH_2CH_2CH_2COOH \rightarrow C_6H_5COOH + 2CH_3COOH$$
$$C_6H_5CH_2CH_2CH_2COOH \rightarrow C_6H_5CH_2COOH + CH_3COOH$$

[1] Knoop, F. Beitr. Chem. Physiol. Pathol., *6*: 150, 1904.

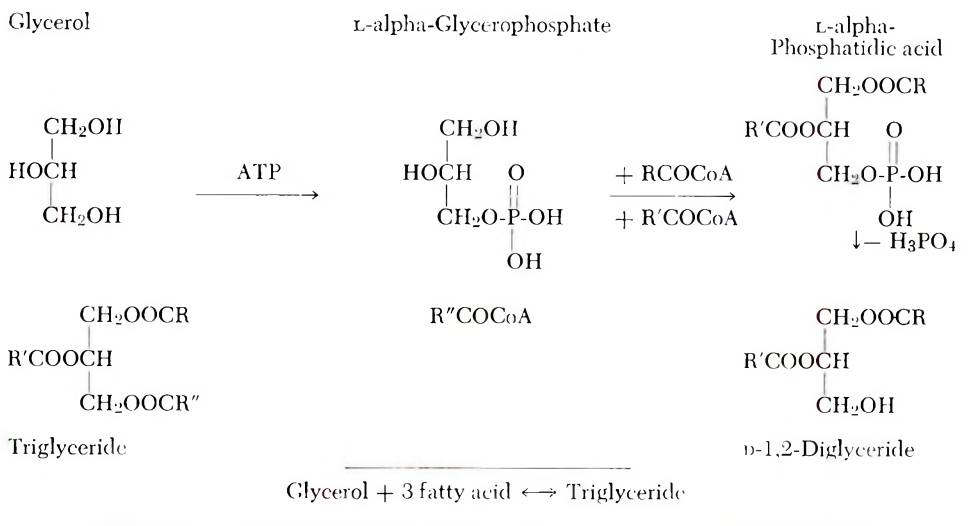
phenacetylglycine if an even number. This result was explicable only if the oxidative shortening of the fatty acid side chain occurred 2 carbons at a time. Dakin contributed to the theory of beta-oxidation by showing the presence of the beta-hydroxy derivative in the urine after the feeding of phenylpropionic acid (23). The beta-oxidation concept was most useful and was correct in principle even though certain related interpretations proved erroneous. For example, in pre-insulin days severe ketosis and acidosis, characterized by the accumulation of acetoacetate in blood and urine, were unavoidable consequences of severe diabetes mellitus. Great efforts were made to preserve the "ketogenic-antiketogenic balance" which was based on the belief that the simultaneous oxidation of glucose was a prerequisite for the complete oxidation of the terminal 4 carbons of a long-chain fatty acid after shortening of the chain according to Knoop's theory. The involvement of glucose in the oxidation of acetoacetate was believed to be direct and led to Macleod's statement, ". . . fat is incompletely consumed if carbohydrate fires do not burn briskly enough." Now, it is known that acetoacetate accumulates in the untreated diabetic because of the acceleration of fatty acid catabolism in the absence of carbohydrate as an energy source. Nevertheless, the correlation of the effects of diabetic ketosis with these earlier hypotheses was surprisingly good.

Coenzyme A is essential in the transfer of fatty acids in the form of an acyl derivative (RCOCoA) to glycerol in the synthesis of triglyceride. Table 3 shows the series of reactions described by Kennedy in the formation of a fat molecule from glycerol and 3 molecules of acyl coenzyme A, each representing a different fatty acid (24). The D-1, 2-diglyceride is also an intermediate in the biogenesis of lecithin in mitochondria. In this instance (table 4) cytidine diphosphate choline, formed by the interaction of cytidine triphosphate and phosphorylcholine, transfers the phosphorylcholine moiety to the diglyceride (25). Cytidine derivatives are also intermediate in the biogenesis of phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, inositolphosphatides, sphingomyelins, and plasmalogens (26, 27).

The biogenesis of complex lipids such as the phospholipids is dependent either on a dietary or on an endogenous supply of all unit components, including choline. In retrospect, it is understandable that fatty acids should be channeled into liver triglyceride and that this fat should accumulate in the liver if there is absence of choline-containing phospholipids. Consequently, it did not appear unusual that Best and his co-workers in 1932 were able to demonstrate a lipotropic or fatty liver-preventing effect of choline in experiments which arose directly from studies of the action of the newly discovered insulin (28). More surprising was their observation that casein also exhibited lipotropic properties (29), a result confirmed by Channon and Wilkinson (30). Soon thereafter cystine was found to be anti-lipotropic (31, 32) and methionine lipotropic (33). At this

TABLE 3

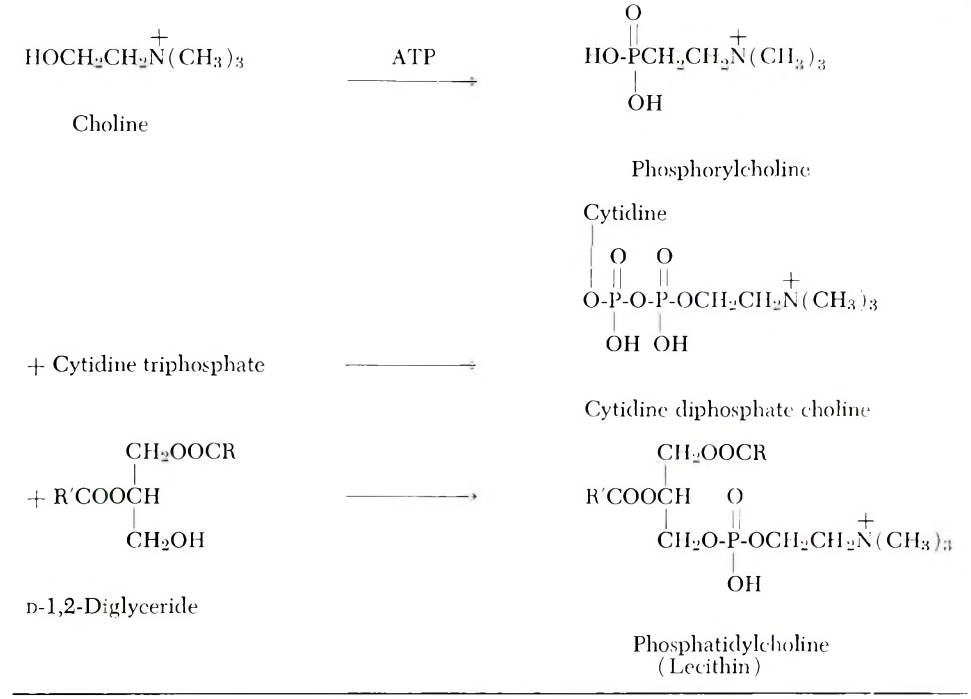
Synthesis of triglycerides[1]



[1] Weiss, S. B., and E. P. Kennedy. J. Amer. Chem. Soc., *78*: 3550, 1956.

TABLE 4

Synthesis of phosphatidylcholine (lecithin)[1]



[1] Kennedy, E. P. Amer. J. Clin. Nutr., *6*: 216, 1958; and Kennedy, Federation Proc., *20*: 934, 1961.

time no information was available on the phenomenon of transmethylation, nor was there any real hint regarding the *de novo* synthesis of methyl groups.

The stimulation to investigate the specific effects of amino acids on the development of the "fatty liver" resulted from hearing H. J. Channon discuss the influence of cystine at a meeting of the British Biochemical Society at University College, London, early in 1937. The decision to use young rather than adult rats was based on the hope that the additional requirements of both choline and amino acids for growth might permit a clearer definition of their interrelations. Accordingly, fibrin, casein, and ovalbumin were selected as proteins that supplied a superior mixture of amino acids and the effectiveness of the diet was tested first with growth and not with choline deficiency in mind. Dried hog liver (2%) and powdered brewer's yeast (5%) were added to these original diets to ensure, if possible, the provision of trace nutrients.

Two unexpected findings resulted from this experiment (table 5) (34). Extra liver fat was found in every rat in the absence of added choline, despite the significant amounts of choline provided by the liver and yeast supplements. Furthermore, the extent of the fatty liver was proportional to the level of dietary protein and to the rate of growth. The results demonstrated clearly that weanling rats were indeed likely experimental animals for these studies on choline.

Following this demonstration, a similar diet was prepared in which the choline-containing liver and yeast supplements were replaced by supplements containing less choline. Autopsy of a group of weanling male rats after a 10-day period on the modified diet showed greatly enlarged, purplish-red kidneys with hemorrhagic capsules in 30 of 40 rats. No such lesion was observed in a group fed the same diet supplemented with choline chloride. Methionine and betaine were also effective in place of choline. The renal lesion was called "hemorrhagic degeneration" because of the unmistakable evidence of subcapsular bleeding and because of the hemorrhagic appearance of the cortex of the kidney. The lesion was wholly prevented by supplements of choline too small to affect the deposition of liver lipids (35).

The biochemical cause of the renal lesion remains unknown. A clue may be afforded by the observation that a significant lowering of the levels of linoleic and arachidonic acids in the renal phospholipids occurs during the acute phase (table 6). These animals were fed the same low-choline diet, and the kidneys were grouped according to their gross appearance, as normal, hemorrhagic, or partially recovered. It may be inferred that the lack of choline impairs the synthesis of phospholipids containing polyenoic acids.

During this period du Vigneaud and his collaborators were establishing the concept of transmethylation or transfer of intact

TABLE 5

Production of fatty livers in weanling male rats[1, 2, 3]

Total dietary protein[4]	Added choline chloride	Gain in wt	Liver lipids	
%	%	*g*	*g*	%
5	0	21	1.02	27.5
5	0.5	38	0.18	5.4
10	0	59	1.50	27.3
10	0.5	65	0.21	4.7
15	0	67	2.32	30.6
15	0.5	71	0.28	6.0

[1] Griffith, W. H., and N. J. Wade. J. Biol. Chem., *131*: 567, 1939.
[2] Period: 30 days.
[3] Supplements: cod liver oil, 5%; whole dried hog liver, 2%; powdered brewer's yeast, 5%.
[4] Dietary protein: fibrin-casein-dried egg white (2:2:1).

TABLE 6

Renal fatty acids in choline-deficient rats[1]

Appearance of kidney	Normal	Hemorrhagic	Partially recovered
Kidney weight, % of body wt	1.16	1.84	1.50
Total lipid, % of kidney	3.97	3.20	3.10
Distribution of fatty acids, % on molar basis			
Palmitic	28	34	30
Stearic	20	19	18
Oleic	19	26	21
Linoleic	13	9	9
Arachidonic	26	13	22

[1] Fewster, M. E., J. F. Nyc and W. H. Griffith, unpublished data.

methyl groups (36). Choline and creatine, containing deuterium-labeled methyl, were isolated from the carcasses of rats fed a choline deficient diet supplemented with methionine containing deuterium in the sulfur methyl. In addition, the transfer of methyl from choline to methionine occurred if labeled choline and homocystine replaced methionine in the diet. This impressive evidence of transfer of intact methyl was given additional support by the experiment in which choline was isolated after feeding rats doubly labeled, intramolecularly labeled methionine. Within experimental error the same ratio of ^{14}C to deuterium was found in choline and and creatine methyl as in the sulfur methyl of the administered methionine (37).

The discovery of the energy-rich sulfonium derivative of methionine, S-adenosyl-L-methionine, by Cantoni added importantly to the understanding of the mechanism of transmethylation (38). Interest in the identity of the individual methyl acceptors in the production of choline from aminoethanol has been increased by Greenberg's demonstration of an enzymatic system which converts phosphatidylaminoethanol to lecithin, with monomethyl and dimethyl phosphatides as intermediates and with phosphatidylaminoethanol formed by the decarboxylation of phosphatidylserine (39, 40). Each of the 3 methylations is believed to involve transmethylation from the S-adenosylmethionine. Other workers have confirmed the methylation of phosphatidyl derivatives (41–43).

There was no reason in 1940 to doubt the dietary indispensability of labile methyl and the dependence of phospholipid metabolism on a supply of such methyl for the synthesis of choline. The ease with which choline deficiency was produced and the ease of prevention by methionine or by choline made the concept of a dietary deficiency of labile methyl very plausible. More recent findings, however, have made it necessary to revise this concept. The animal organism does have the ability to synthesize the methyl found in choline and methionine provided the diet contains adequate amounts of required nutrients, including folic acid and cobalamin. Thus, in the decade following the discovery of what was believed to be a new dietary essential, labile methyl, and a new metabolic process of transfer of an intact methyl group, irrefutable evidence has shown that labile methyl is of both exogenous and endogenous origin (44, 45). The misconception of 1940 is only partly understandable on the basis that nothing was then known of folic acid and of its relation to the metabolism of formate or of cobalamin and of the formation of labile methyl through a cobalamin intermediate. Transmethylation remains, however, as a proven mechanism of transfer of methyl, a transfer process that appears to be independent in many instances, at least, of the nutrients that control methyl synthesis from formate.

Notwithstanding, one cannot assume that the books are closed on this problem. Table 7 shows an attempt to demonstrate *de novo*

TABLE 7

Lipotropic action of cobalamin in weanling male rats fed on a low choline diet[1, 2, 3]

Gain in wt	Liver		Kidney		Renal lesions	Supplement
g	*g*	% body wt	*mg*	% body wt	%	
27	6.7[4]	8.3	1025	1.30	90	cobalamin omitted
44	7.4[5]	7.7	905	0.94	17	—
45	5.2	5.6	919	0.99	17	methionine
31	6.6	8.4	919	1.17	40	glycine
54	8.9	8.6	983	0.96	25	glutamic acid

[1] Griffith, W. H., unpublished data.
[2] Period: 14 days.
[3] Diet: 8% casein + cystine + all essential amino acids except methionine.
[4] Liver lipid %, 26.4
[5] Liver lipid %, 27.1.

synthesis of choline. Young rats in groups of twelve were fed a diet low in choline and in methionine but containing all required nutrients including folic acid and cobalamin. The deposition of liver fat and the incidence of the renal lesion were increased by the omission of cobalamin, but these animals were far from normal and a choline requirement remained despite the presence of folic acid and cobalamin. It may be that the rate of *de novo* synthesis of choline in the weanling rat on a low choline, low methionine diet is inadequate to prevent the accumulation of the liver fat.

The title of this paper includes "lessons and expectations." Lessons are clear, expectations less so. Experimental science is describing the many reactants and catalysts that account in bewildering details for reactions which were known 50 years ago only in their overall nature. The concepts supporting the activity of fatty acids as fuels under the control of neurohormones, as components of complex indispensable lipids, or as components of acyl coenzyme A represent astounding increments of knowledge. But, how is this knowledge being put to use in the betterment of human welfare? Certainly not as effectively as one might hope. It is discomfiting to realize that Lusk might have made pronouncements in 1915 on the relation of fat to obesity or on the relation of fat to coronary artery disease which would have been useful as many of those we make today. How does it happen that there is so much acceptance of a direct relationship between dietary fat and obesity when we know that obesity is dependent only on the *balance* between total ingested calories of any and all types and caloric *requirements* as modified by genes, by activity, by environment, and by disease? How does it happen that there is so much acceptance of a direct relationship between dietary fat and coronary artery disease when such a direct relationship has yet to be proved? Can one truthfully say that all that Lusk knew about fat and all that has been learned since his time is really inconsequential, that the occurrence of fat in milk is an unfortunate mistake of nature, that fat is injurious, and that all of the biochemical systems for the metabolism and storage of fat are really intended as measures to remove a noxious material? How will future science historians appraise this era in which the major responsibility for a distressingly high mortality from coronary artery disease is placed on one or more normal constituents of the body rather than on the *whole man,* on his inheritance, on his mode of life, on the sum total of factors that determine how he adjusts to an environment which he does not understand completely and has learned to control only in part?

Perhaps what we need is a change of perspective, a new perspective arising from a deeper appreciation of the past and a broader outlook into the future. A thoughtful analysis of the progress of knowledge of food and of metabolism brings the realization that the earlier nutritional findings

contributed immeasurably first to physiology, then to biochemistry, and now to molecular biology. The fundamental significance of such research requires that investigators of the science of nutrition must be conscious of a responsibility for high standards in research that should include metabolic interrelationships in the whole organism. The need to know the details of the *intracellular* conversion of carbons and hydrogens of fatty acids to carbon dioxide and water diminishes not one whit the need to identify and to understand the mechanisms available in the *multicellular* body for the provision of highly selective chemical compounds that are to be used in synthesis or as fuels in the individual cells. This is a selection that may well vary with age, sex, familial background, temperament, with all of the many vicissitudes of life. Perhaps, like Roger Williams, we should pay more attention to the reasons for biochemical differences between so-called normal individuals, differences that frequently are greater than those between groups of normals and abnormals.

The world at large has come so easily to regard nutrition as synonymous with a dietary regimen. We who know that it is more than this must redefine—at least for the public and for scientists also—what it is that concerns us. It is the relation of what one eats to what happens to him. Human nutrition is a science that relates food, as a building material of tissues, as a fuel, and as a regulator of metabolism to the survival of dissimilar individuals—men, women, and children—in environments that also differ markedly in the requirements of nutrients which, insofar as they are applicable, help resist disease, support physical endurance, assist neuromuscular coordination, and aid neurological and psychological stability. We need to move quickly and decisively from the concept that nutrition is concerned with a well-tasting mixture of foodstuffs to the concept that the science of nutrition provides a mixture of foods, well-tasting to be sure, that is in such quantity and in such proportions as to least burden the body and with the quantities and proportions adjusted to the age, sex, physical activity, genes, state of health, and environmental situation of each individual. As of 1965, may this be our goal—our expectation.

LITERATURE CITED

1. Evans, H. M., and G. O. Burr 1926 A new dietary deficiency with highly purified diets. Proc. Soc. Exp. Biol. Med., *24:* 740.
2. Burr, G. O., and M. M. Burr 1929 A new deficiency disease produced by the rigid exclusion of fat from the diet. J. Biol. Chem., *82:* 345.
3. Burr, G. O., and M. M. Burr 1930 The nature and role of the fatty acids essential in nutrition. J. Biol. Chem., *86:* 587.
4. Holman, R. T. 1954 The Vitamins, vol. 2. Academic Press, New York, p. 292.
5. Wiese, H. F., W. Yamanaka, E. Coon and S. Barber 1965 Lipid changes in skin of puppies in relation to dietary fat. Federation Proc., *24:* 438.
6. Sewell, R. F., and L. J. McDowell 1965 Observations on the essential fatty acid requirement of young swine. Federation Proc., *24:* 438.
7. Hansen, A. E., M. E. Haggard, A. N. Boelsche, D. J. D. Adam and H. F. Wiese 1958 Essential fatty acids in infant nutrition. III. Clinical manifestations of linoleic acid deficiency. J. Nutr., *66:* 565.
8. Wesson, L. G., and G. O. Burr 1931 The metabolic rate and respiratory quotients of rats on a fat-deficient diet. J. Biol. Chem., *91:* 525.
9. Kunkel, H. O., and J. N. Williams, Jr. 1951 The effects of fat deficiency upon enzyme activity in the rat. J. Biol. Chem., *189:* 755.
10. Klein, P. D., and R. M. Johnson 1954 Phosphorus metabolism in unsaturated fatty acid-deficient rats. J. Biol. Chem., *211:* 103.
11. Levin, E., R. M. Johnson and S. Albert 1957 Mitochondrial changes associated with essential fatty acid deficiency in rats. J. Biol. Chem., *228:* 15.
12. Fleischer, S., G. Brierley, H. Klouwen and D. B. Slautterback 1962 Electron transport system. XLVII. The role of phospholipids in electron transfer. J. Biol. Chem., *237:* 3264.
13. Fleischer, S., A. Casu and B. Fleischer 1964 A phospholipid requirement for DPNH oxidation. Federation Proc., *23:* 486.
14. Fleischer, S. 1964 The role of lipids in mitochondrial structure and function. Sixth International Congress on Biochemistry, New York, Abstracts, p. 605.
15. Witten, P. W., and R. T. Holman 1952 Polyethenoid fatty acid metabolism. VI. Effect of pyridoxine on essential fatty acid conversions. Arch. Biochem. Biophys., *41:* 266.
16. Reiser, R. 1950 The biochemical conversions of conjugated dienoic and trienoic fatty acids. J. Nutr., *42:* 325.

17. Mead, J. F. 1961 Synthesis and metabolism of polyunsaturated acids. Federation Proc., *20:* 952.
18. Thomasson, H. J. 1954 Stearolic acid, an essential fatty acid? Nature, *173:* 452.
19. Schoenheimer, R. 1942 The Dynamic State of Body Constituents, Harvard University Press, Cambridge, Massachusetts.
20. Lipmann, F., and N. O. Kaplan 1946 A common factor in the enzymic acetylation of sulfanilamide and of choline. J. Biol. Chem., *162:* 743.
21. Lipmann, F., N. O. Kaplan, G. D. Novelli and L. C. Tuttle 1950 Isolation of coenzyme A. J. Biol. Chem., *186:* 235.
22. Knoop, F. 1904 Der Abbau Aromatischer Fettsauren im Tierkoper. Beitr. Chem. Physiol. Pathol., *6:* 150.
23. Dakin, H. D. 1909 The mode of oxidation in the animal organism of phenyl derivatives of fatty acids. IV. Further studies on the fate of phenylproprionic acid and some of its derivatives. J. Biol. Chem., *6:* 203.
24. Weiss, S. B., and E. P. Kennedy 1956 The enzymic synthesis of triglycerides. J. Amer. Chem. Soc., *78:* 3550.
25. Kennedy, E. P., and S. B. Weiss 1955 Cytidine diphosphate choline: a new intermediate in lecithin biosynthesis. J. Amer. Chem. Soc., *77:* 250.
26. Kiyasu, J. Y., and E. P. Kennedy 1960 Enzymic synthesis of plasmalogens. J. Biol. Chem., *235:* 2590.
27. Kennedy, E. P. 1961 Biosynthesis of complex lipids. Federation Proc., *20:* 934.
28. Best, C. H., and M. E. Huntsman 1932 The effects of the components of lecithin upon deposition of fat in the liver. J. Physiol., *75:* 405.
29. Best, C. H., and M. E. Huntsman 1935 The effect of choline on the liver fat of rats in various states of nutrition. J. Physiol., *83:* 255.
30. Channon, H. J., and H. Wilkinson 1935 Protein and dietary production of fatty livers. Biochem. J., *29:* 350.
31. Channon, H. J., M. C. Manifold and A. P. Platt 1938 The action of cystine and methionine on liver fat deposition. Biochem. J., *32:* 969.
32. Beeston, A. W., and H. J. Channon 1936 Cystine and the dietary production of fatty livers. Biochem. J., *30:* 280.
33. Tucker, H. F., and H. C. Eckstein 1937 The effect of supplementary methionine and cystine on the production of fatty livers by diet. J. Biol. Chem., *121:* 479.
34. Griffith, W. H., and N. J. Wade 1939 Choline metabolism. I. The occurrence and prevention of hemorrhagic degeneration in young rats on a low-choline diet. J. Biol. Chem., *131:* 567
35. Griffith, W. H. 1958 Renal lesions in choline deficiency. Amer. J. Clin. Nutr., *6:* 263.
36. du Vigneaud, V., J. P. Chandler, A. W. Moyer and D. M. Keppel 1939 The effect of choline on the ability of homocystine to replace methionine in the diet. J. Biol. Chem., *131:* 57.
37. du Vigneaud, V., J. R. Rachele and A. M. White 1956 A crucial test of transmethylation *in vivo* by intramolecular isotopic labeling. J. Amer. Chem. Soc., *78:* 5131.
38. Cantoni, G. L. 1953 S-Adenosylmethionine: a new intermediate formed enzymically from L-methionine and adenosine triphosphate. J. Biol. Chem., *204:* 403.
39. Pilgeram, L. O., R. E. Hamilton and D. M. Greenberg 1957 Some factors influencing phosphatidylcholine formation. J. Biol. Chem., *227:* 107.
40. Bremer, J., P. H. Figard and D. M. Greenberg 1960 Biosynthesis of choline and its relation to phopholipid metabolism. Biochim. Biophys. Acta, *43:* 477.
41. Gibson, K. D., J. D. Wilson and S. Udenfriend 1961 Enzymic conversion of phospholipide ethanolamine to phospholipide choline in rat liver. J. Biol. Chem., *236:* 673.
42. Borkenhagen, L. F., E. P. Kennedy and L. Fielding 1961 Enzymic formation and decarboxylation of phosphatidylserine. J. Biol. Chem., *236:* PC28.
43. Artom, C. 1964 Methylation of phosphatidylmonomethylethanolamine in liver preparations. Biochem. Biophys. Res. Commun., *15:* 201.
44. Schaefer, A. E., W. D. Salmon and D. R. Strength 1949 Interrelationship of vitamin B_{12} and choline. I. Effect on hemorrhagic kidney syndrome in the rat. Proc. Soc. Exp. Biol. Med., *71:* 193.
45. Stekol, J. A., S. Weiss, E. I. Anderson, P. T. Hsu and A. Watjen 1957 Vitamin B_{12} and folic acid in relation to methionine synthesis from betaine *in vivo* and *in vitro*. J. Biol. Chem., *226:* 95.

Studies on Nutritional Factors in Mammalian Development[1]

LUCILLE S. HURLEY
Professor of Nutrition, Department of Nutrition, University of California, Davis, California

It is a special pleasure for me today to be able to contribute some small part to the honoring of my professor, Dr. Agnes Fay Morgan, who has had a most important influence on my life and continues to be a source of inspiration.

Much of the work I shall discuss saw its beginnings in the investigations of distinguished researchers who are present today, both as speakers and as members of the audience. This example of the continuity of our science provides a fitting tribute to our guest of honor and to her dedication to the future of nutrition research.

My subject, the role of nutritional factors in mammalian embryonic development, fits appropriately in the context of the theme of this symposium, because it is during the time-span being discussed today that research on this subject began. Systematic studies of the influence of nutrition on the prenatal development of mammals can be said to have begun with the observations of Hale about 1935 (1).

Before this time, a considerable number of experiments had been carried out with avian and amphibian eggs, and it was recognized that environmental factors could influence the development of these embryos (2, 3). Yet even in these species, little thought was given to the role of nutrition in embryonic development, and as far as mammalian embryos were concerned, genetics claimed the day. It was thought that the mammalian embryo was too well-protected by the maternal organism to be affected by environmental influences, and congenital abnormalities, that is, abnormalities existing at birth, were generally considered to be hereditary (4).

The first experimental evidence that a change in the environment could disrupt the normal development of a mammal appeared about 1935 when Hale, working in Texas, reported that pigs born to vitamin A-deficient sows had malformations including cleft lips and missing eyes. Hale (1) provided convincing evidence that the malformations were not hereditary. Thus, the first clear proof that an environmental factor could produce congenital malformations came from nutrition research.

Hale's experiments provided valid, although accidental, proof of the influence of a nutritional deficiency on development. The work of Warkany and his colleagues, however, was the first experimental use of nutritional deficiencies in a deliberate attempt to study their effects upon the development of mammalian embryos. In the early '40's, Warkany published a series of papers reporting the production of congenital malformations in rats when the maternal diet was deficient in riboflavin. These malformations consisted almost entirely of skeletal anomalies, including shortening of the lower jaw, fusion of the ribs, syndactyly, and cleft palate (5).

Important contributions to this subject were made by a former associate of Dr. Morgan's, the late Dr. Marjorie M. Nelson, whose premature death two and one-half years ago was a great loss. Dr. Nelson was the first to use antimetabolites as a means

[1] Part of the work described here was assisted by Public Health Service Research Grants A-1340 and HD 00429, and National Science Foundation Grants G-7088 and GB-2316.

of inducing nutritional deficiencies very severely and acutely in order to study their effects upon embryonic development. The effects of folic acid deficiency, as produced by x-methyl pteroylglutamic acid were extensively and systematically studied in this way (6–10). Dr. Nelson and her co-workers also studied pantothenic acid deficiency in pregnant rats, and found that various abnormalities, including exencephaly, urogenital anomalies, and necrosis of the paws resulted under these conditions (11).

And now I should like to discuss with you some of our own work. In this connection, I wish to acknowledge the valued collaboration of my colleagues and students, particularly Drs. C. W. Asling, Gladys Everson, and Paola Timiras.

One of the nutrients which we have studied in relation to fetal development is pantothenic acid. I had been interested in this vitamin since the days of my dissertation research as a student of Dr. Morgan's. Since Nelson and also Giroud of Paris (12) had shown that congenital malformations occurred in offspring of pantothenic acid-deficient rats, I was interested in investigating the biochemical relationships between pantothenic acid and coenzyme A during development, as well as the influence of a deficiency of the vitamin upon the offspring.

The rat fetus, however, because of its small size, is a difficult animal to work with for chemical studies. We therefore used the guinea pig. In the first aspect of the study, the changes in the levels of free pantothenic acid, bound pantothenic acid, and coenzyme A in the liver were determined during fetal and post-natal development. The results of this study are summarized in figure 1.

The concentrations of these compounds remained almost stationary from the 33rd to the 58th day of gestation. At 58 days of gestation, a sharp rise in bound pantothenic acid and in coenzyme A occurred which reached its maximum at 4 days after birth. These results suggested that the critical period with respect to pantothenic acid and coenzyme A in the developing guinea pig is in the period shortly before and after birth (13).

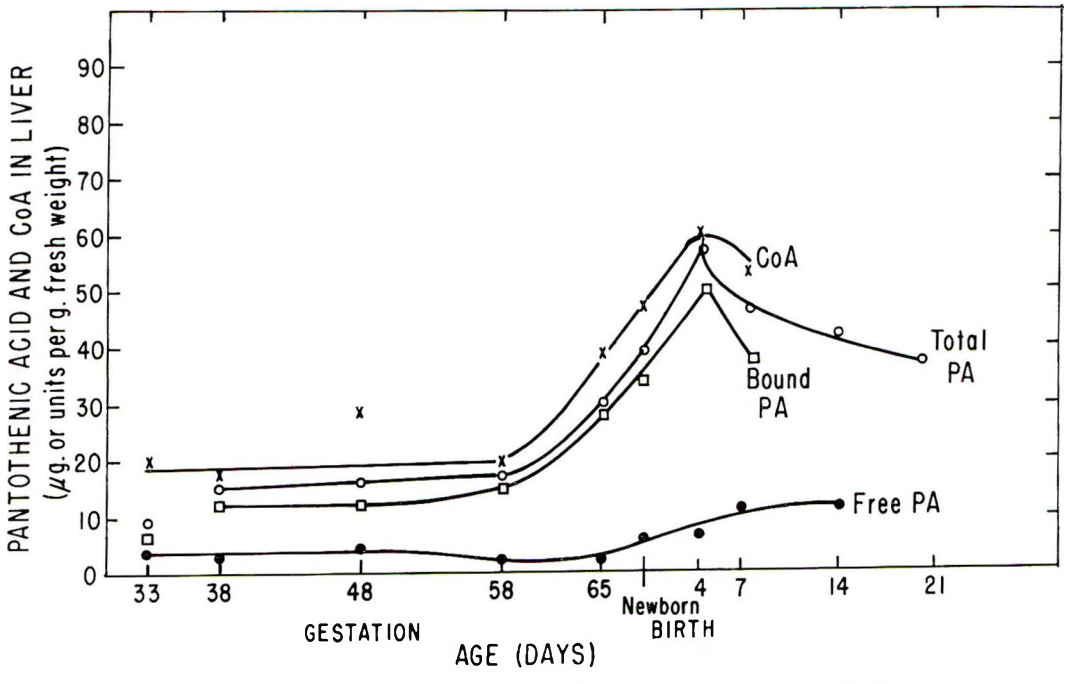


Fig. 1 Pantothenic acid and coenzyme A in livers of developing guinea pigs. Each point represents the mean of from 2 to 9 fetuses or young guinea pigs, in most cases, 5. (Hurley, L. S., and N. Volkert. Biochim. Biophys. Acta, *104:* 372, 1965.)

In the next phase of this work, the effect of pantothenic acid deficiency during pregnancy upon the development of the offspring was studied (14). Preliminary experiments indicated that the pregnant guinea pig could not withstand long periods of a deficiency of this vitamin. A transitory deficiency period of one week was therefore chosen.

In this experiment, pregnant guinea pigs were transferred from a complete synthetic diet to the pantothenic acid-deficient diet for a period of one week during various periods of their pregnancies. One week of deficiency resulted in a reduction in the number of young born alive, and an increase in the number of litters aborted. This appeared to be especially severe when the deficiency period was the ninth week of gestation. (Gestation in the guinea pig is about 70 days, or 10 weeks.)

The effects of the transitory deficiency were also studied by measuring the levels of pantothenic acid and fat in the livers of the offspring (see fig. 2). The liver fat level of the normal newborn was found to be about 10 times higher than that of the adult, but by 7 days of age it had decreased almost to the adult value. In contrast, liver pantothenic acid concentration in the normal newborn was about 60% of the adult value, but rose during the first 7 days after birth.

A dietary deficiency of pantothenic acid during the 10th week of gestation appeared to produce a significant rise in the liver fat of the newborn. At the same time, the

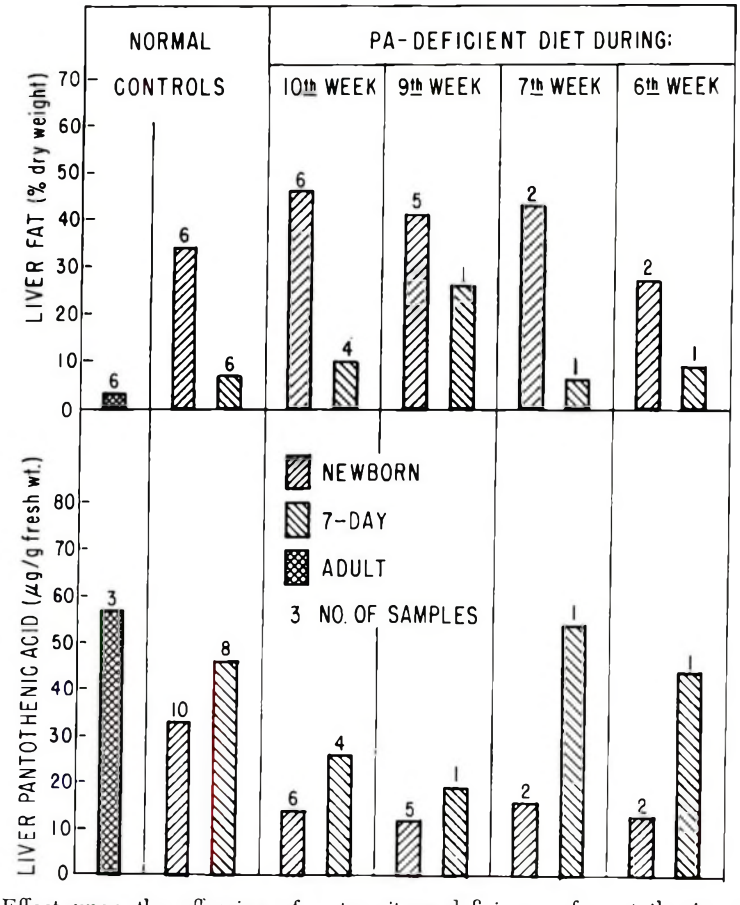


Fig. 2 Effect upon the offspring of a transitory deficiency of pantothenic acid during gestation in guinea pigs. Each bar represents the mean of several samples, the number of which is shown above the bars. (Hurley, L. S., N. E. Volkert and J. T. Eichner. J. Nutr., 86: 201, 1965.)

liver pantothenic acid level of this group was lower than normal, both at birth and at 7 days of age. Young whose mothers had received the deficient diet earlier than the 10th week showed no significant changes in liver fat, but did have low pantothenic acid levels.

The results of this experiment are in accord with our findings on changes in pantothenic acid and coenzyme A levels in fetal tissue. These works together suggest that the greatest need for pantothenic acid during fetal development of the guinea pig is in the period shortly before birth, possibly from about 58 days of gestation to term. It is of some interest that nutritional deprivation coming at the very end of pregnancy should have a demonstrable effect upon the offspring. This is in contrast with many experiments on teratogenic effects of nutritional factors, but this is not the only nutrient which has effects upon development relatively late in intrauterine life (15, 16).

Another nutrient with which we have been concerned is the trace element manganese. I am very pleased to describe this work today in the presence of Dr. McCollum, because of his early contributions to nutritional knowledge of this element. Orent and McCollum (17) reported in 1931 that the offspring of rats given a diet lacking in manganese were weak and did not survive. The early interpretation was that the deaths occurred because the females failed to nurse their young, and I understand that newspaper reports of the day hailed the discovery of "the mother-love factor." Daniels and Everson (18), however, showed that the high death rate was due to debility of the young themselves, since manganese-deficient mothers were capable of raising foster control young, although their own offspring died. Shils and McCollum in 1943 (19) showed further that the deficient offspring which survived exhibited an ataxia characterized by incoordination, lack of equilibrium, and retraction of the head.

It was actually known since 1939, when Dr. Leo Norris reported on his experiments with chickens, that a maternal dietary deficiency of manganese results in defective offspring which are ataxic (20). Various investigations were subsequently carried out in search of a lesion, either morphological or biochemical, which could account for this abnormal condition; but neither histological studies of the nervous system nor assays of various enzymes revealed the nature of the congenital defect resulting in ataxia (19, 21–23). It was to this question that we addressed ourselves.

Time does not permit me to present a detailed story. I can only attempt to show you a few of the parameters we have studied. Our experiments have been conducted with both rats and guinea pigs. In both species there is poor survival of the offspring of manganese-deficient females, and a high incidence of ataxia in the survivors (15, 24, 25). There is also a pronounced delay in the development of body-righting reflexes (26). These have been studied in two ways. In one test, the time required for the animal to turn from its back to its feet was measured; in the other, the ability of the animal to right itself in air was tested. In both tests, the manganese deficient young showed a marked delay in the development of reflexes responsible for body righting reactions.

The survival and the incidence of ataxia in the offspring were influenced by manganese supplementation during gestation. Table 1 shows that survival of manganese-deficient rats to 28 days of age was very low, only 11%, and 81% of these were ataxic. If, however, the mother was given the manganese-supplemented ration for only 24 hours on day 14 of gestation, ataxia was completely prevented. If supplementation was given on day 18, it had no effect at all on the ataxia, and this was true even if the control ration was continued from day 18 of gestation until weaning (15, 25). Thus, the congenital ataxia of manganese-deficient rats was due to an irreversible defect occurring between day 14 and day 18 of gestation.

The delayed development of the righting reflexes as well as the behavior of the animals led us to examine the vestibular portion of the inner ear. This was first done by examination of the skulls in alizarin-stained specimens. We found that

TABLE 1

Survival and incidence of ataxia in manganese-deficient young

Treatment	No. litters	Young		
		No. born alive	Survival to 28 days	Ataxia
			%	%
Mn-supplemented entire period	61	406	54	0
Mn-deficient entire period	30	191	11	81
Mn-supplemented only for 24 hours during gestation on:				
day 14	23	172	51	0
day 16	29	186	51	62
day 18	36	220	39	84

there was a marked delay in the ossification of the otic capsule, the bony covering surrounding the inner ear (27). In addition to the delayed development of ossification, there were actual malformations of this structure. Histological examination of the inner ear showed that there was a progressive degeneration of the neural epithelium of the semi-circular canals in the deficient rats. This degeneration was extreme by 4 days of age, but was already present at birth (28).

Thus, we were able to show that manganese deficiency during gestation in rats resulted in a defect of the inner ear consisting of gross malformations of the vestibular apparatus and histological damage to its neural epithelium. This defect would account for at least some of the abnormal symptoms of manganese-deficient offspring. Other manifestations of the deficiency, however, such as tremor, do not appear to be related to vestibular function. We were therefore interested in investigating another aspect of the nervous system.

In one experiment, the electroshock threshold, the amount of current in milliamperes required to produce convulsions, was measured (see table 2). By means of appropriate supplementation with manganese, the two variables of congenital ataxia and manganese deficiency were separated; this allowed the measurement of response to electroshock in manganese-supplemented and manganese-deficient rats, either with or without ataxia (15, 25). The threshold for convulsive seizures was significantly lower than normal in the two manganese-deficient groups, indicating that brain excitability or convulsability was increased in manganese-deficient rats regardless of the presence or absence of ataxia. Thus the level of manganese in the body appears to be important in determining the susceptibility of an animal to convulsive states (29).

Another system which is profoundly affected by manganese deficiency is the skeleton (see fig. 3). In deficient animals, there is disproportionate growth of the skeleton. This is already apparent at birth, and involves shortening of the long bones, and doming of the skull. There are also curvatures of radius and ulna, ulnar deviation of the forepaws, scoliosis and kyphosis (30–32). In addition, a disorder of the knee joint occurs in which there is abnormal development of the tibial epiphysis (33).

TABLE 2

Electroshock threshold in normal and manganese-deficient rats

Group	No. rats	EST[1]
		ma
1 Not deficient, not ataxic (Mn^+ controls)	14	19.50
2 Mn-deficient, not ataxic (supplemented day 14)	12	14.58[2]
3 Mn-deficient, ataxic (Mn^-)	13	16.42[2]
4 Not deficient, ataxic (supplemented day 18 and thereafter)	6	19.08

[1] Electroshock threshold in milliamperes.

[2] $P < 0.001$ as compared with Mn^+ control (Student's *t* test).

Fig. 3 Whole-body roentgenograms of 7-month-old manganese-supplemented and manganese-deficient rats. Note stunting of growth, curvatures of radius and ulna, ulnar deviation of forepaws, scoliosis, flared lower pelvis and thickening of upper portion of tibia in the deficient animal; in inserts (lateral view of thoracolumbar junction) note kyphosis. (Asling, C. W., and L. S. Hurley. Clin. Orthop. 27: 213, 1963.)

Various aspects of the skeletal as well as the vestibular abnormalities suggested that manganese played a role in cartilage metabolism. Analyses of mucopolysaccharide precursors substantiate this view (see table 3). In epiphyseal cartilage from manganese-deficient rats, the levels of hexuronic acids, glucosamine, and galactosamine were significantly reduced, both at birth and at 28 days of age. Similar effects were seen in guinea pigs (34).

Manganese-deficient animals also show abnormal electrocardiograms (35) and we have found abnormal oxidative phosphorylation in isolated liver mitochondria.[2]

Thus, there is a spectrum of abnormal changes resulting from a congenital deficiency of manganese. These include physiological changes, such as ataxia, abnormal

[2] Kagawa, Y. 1962 Some biochemical properties of mitochondria from normal and manganese-deficient rats. Master of Science Thesis, University of California, Davis.

TABLE 3

Mucopolysaccharide precursors in epiphyseal cartilage in rats

Age	Diet	No. rats	Hexuronic acids	Glucosamine	Galactosamine
			% dry wt	*% dry wt*	*% dry wt*
Newborn	Mn^+	10[1]	11.3	0.51	7.10
	Mn^-	10[1]	7.85[2]	0.45[2]	4.35[2]
28 Days	Mn^+	4	3.30	0.23	2.18
	Mn^-	5	2.37[2]	0.17[2]	1.26[2]

[1] In 2 pooled samples of 5 animals each.
[2] $P < 0.02$ as compared with Mn^+ Control (Student's t test).

brain function, and electrocardiogram abnormalities. There are also morphological changes, such as those seen in the inner ear, and skeletal abnormalities. And, finally, there are biochemical changes, such as decreased levels of mucopolysaccharide precursors, and disturbed oxidative phosphorylation in mitochondria.

The third and last nutrient which I would like to discuss is zinc. We became interested in zinc because of the similarity of some signs of zinc deficiency to those of manganese deficiency. In order to study the influence of zinc deficiency on embryonic development in rats, it was necessary first to establish that a severe deficiency state could be produced. This was accomplished by the use of a diet containing soybean protein, and by stringent elimination of sources of zinc contamination from the environment.

Under these conditions, animals receiving the zinc-free diet (0 ± 2 ppm by X-ray fluorescence analysis) from weaning showed almost no growth, and developed signs of severe zinc deficiency. These included alopecia, dermatitis, and a hunched, almost kangaroo-like posture (fig. 4). When these extremely abnormal animals were supplemented with zinc, their growth rate immediately rose and quickly reached that of the controls (fig. 5). In addition, all outward signs of zinc deficiency disappeared, and the animals became perfectly normal in appearance.

Under these extreme conditions, almost no reproduction was possible. The females showed severe disruption of the estrus

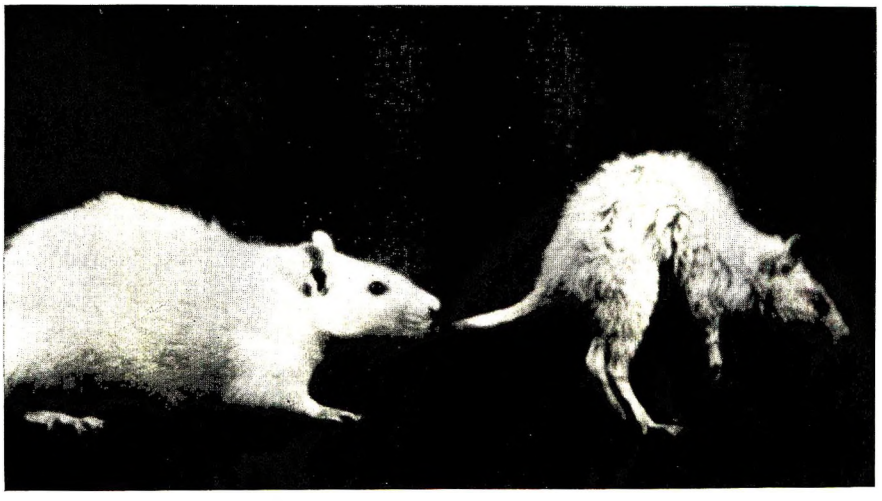


Fig. 4 Normal and zinc-deficient female rats after seven weeks on a purified diet containing either 40 or 0 ppm of zinc. Note ruffled hair, dermatitis, abnormal posture, and depressed growth in animal on right.

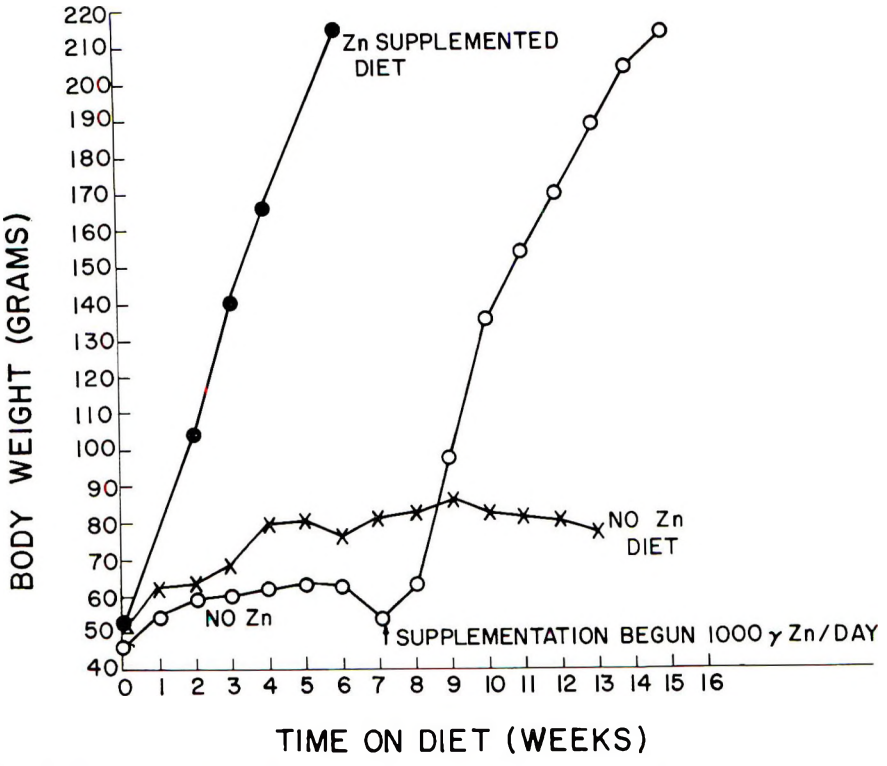


Fig. 5 Examples of growth curves of rats illustrating failure of growth in zinc-deficient animals, and rapid response of growth to oral supplementation with zinc.

cycles; in most cases no mating took place. Therefore, in order to study the effect of zinc deficiency on prenatal development, it was necessary to induce a less extreme form of deficiency which would permit reproduction to occur.

This was successfully accomplished by the procedure of maintaining the females on a marginally deficient diet until maturity. This diet did not produce signs of zinc deficiency in the animals. At maturity the rats were mated with normal males fed a stock ration, and were given either the extremely deficient ration containing no zinc, or the zinc-supplemented ration. In order that we could examine every living fetus, the animals were delivered by Caesarean section on day 21 of gestation, thus preventing the eating of defective young by the mother.

Table 4 shows that the rats which received the zinc-deficient diet lost rather than gained weight during pregnancy, and less than half of them had living young at term. The zinc-deficient females also had a smaller number of young per litter than did the controls, and the young were less than half the normal body weight. In addition, almost all of them, 98%, showed gross congenital malformations.

We also looked at all implantation sites in the uterus to have information on resorptions. Table 5 shows that in the deficient females, all but one of 129 implantation sites were affected. That is, the implantation site showed either a resorbed conceptus, or it had given rise to a malformed fetus. Thus, 99% of the implantation sites in deficient females were affected, as compared with 2 to 3% resorptions in the normal rats.

In figure 6, the appearance of these fetuses is shown. There is peculiar shaping of the head, clubbed feet, fused or missing digits, short lower jaw. Figure 7 shows an example of extreme syndactyly (fused or missing digits). Seventy-four percent of the deficient fetuses had this anomaly.

TABLE 4

Reproduction in zinc-deficient rats

No. rats	Net body wt change during gestation	Rats with living young (day 21)		Living young day 21				
				Total no.	Avg/litter	Body wt	Abnormal	
	g	No.	%			*g*	No.	%
				Stock diet				
5	+98	5	100	59	11.8	5.6	0	0
				Zn-supplemented controls				
12	+56	12	100	122	10.2	5.5	0	0
				Zn-deficient				
16	−19	7	44	43	6.1	2.1	42	98

TABLE 5

Implantation sites in zinc-deficient rats

Group	Implantation sites				
	Total no.	No. resorbed	No. abnormal fetuses	Total affected	
				No.	%
Stock	60	1	0	1	2
+Zn controls	126	4	0	4	3
Zn-deficient	129	86	42	128	99

Fig. 6 Fetuses at term from rats fed zinc-supplemented ration (on far left) or zinc-deficient ration. Note small size, abnormally shaped heads, clubbed feet, short lower jaw, and short or absent tail.

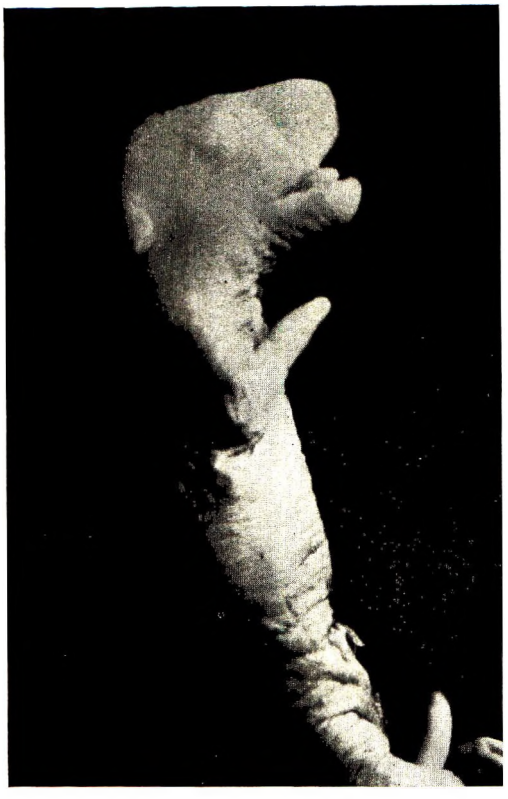


Fig. 7 Extreme syndactyly (fused or missing digits) in a fetus from a zinc-deficient rat on day 21 of pregnancy.

A striking proportion of the deficient offspring, 84%, had brain abnormalities including hydranencephaly. In this condition, there is enlargement of the ventricles, as well as a lack of development of the cerebral cortex.

The congenital malformations produced by zinc deficiency were varied and occurred in high incidence (table 6). A large number of skeletal defects were seen, in incidences ranging from 19% for short or missing lower jaw, to 81% for curly or stubby tail. A high incidence of soft tissue malformations were also seen, including hydrocephalus, small or missing eyes, hernias, and heart, lung, and urogenital abnormalities (36).[3] Since the anomalies occurred in such high incidence, I believe we have a good system in which to attempt an elucidation of the biochemical and enzymatic mechanisms by which these profound disruptions of normal embryonic development were brought about.

In conclusion, I have presented three examples of the interaction of nutrition and embryonic development. By chance, the three examples also illustrate three different aspects of the general study of this subject. In the case of pantothenic acid, we saw a manifestation of biochemical changes. In zinc deficiency, on the other hand, severe morphological alterations were obvious. With manganese deficiency, it was primarily physiological abnormalities which first attracted our attention.

The study of the role of nutritional (or indeed any environmental) factors can be approached through any of these three avenues. To provide a meaningful explanation, however, in relation to modern concepts of biology, all three of these avenues must be brought together and the influence of nutritional factors on the embryo must be understood in terms of the correlation of morphological, physiological, and biochemical events in development.

TABLE 6

Types and incidence of gross congenital malformations in zinc-deficient fetuses[1]

Malformation	%
Cleft palate	26
Short or missing mandible	19
Scoliosis or kyphosis	70
Clubbed forefeet	49
Clubbed hindfeet	51
Fused or missing digits	74
Curly or stubby tail	81
Hydrocephalus or hydranencephalus	84
Small or missing eyes	44
Herniations	40
Heart abnormalities	26
Lung abnormalities	56
Urogenital abnormalities	46

[1] 43 fetuses examined.

[3] For data published since this review was prepared, see Hurley, L. S., and H. Swenerton, Proc. Soc. Exp. Biol. Med., *123:* 692, 1966.

LITERATURE CITED

1. Hale, F. 1937 The relation of maternal vitamin A deficiency to microphthalmia in pigs. Texas J. Med. *33:* 228.
2. Landauer, W. 1961 The hatchability of chicken eggs as influenced by environment and heredity. Monograph 1. University of Connecticut Agr. Exp. Station, Storrs, Connecticut.
3. Willier, B. H., P. A. Weiss and V. Hamburger, eds. 1955 Analysis of Development. W. B. Saunders Company, Philadelphia.
4. Warkany, J. 1965 Development of experimental mammalian teratology. In: Teratology, eds., J. G. Wilson and J. Warkany. University of Chicago Press, Chicago.
5. Warkany, J., and E. Schraffenberger 1943 Congenital malformations induced in rats by maternal nutritional deficiency. V. Effects of a purified diet lacking riboflavin. Proc. Soc. Exp. Biol. Med., *54:* 92.
6. Nelson, M. M., and H. M. Evans 1949 Pteroylglutamic acid and reproduction in the rat. J. Nutr. *38:* 11.
7. Nelson, M. M., H. V. Wright, C. W. Asling and H. M. Evans 1955 Multiple congenital abnormalities resulting from transitory deficiency of pteroylglutamic acid during gestation in the rat. J. Nutr., *56:* 349.
8. Nelson, M. M., H. V. Wright, C. D. C. Baird and H. M. Evans 1956 Effect of 36-hour period of pteroylglutamic acid deficiency on fetal development in the rat. Proc. Soc. Exp. Biol. Med., *92:* 554.
9. Monie, I. W., M. M. Nelson and H. M. Evans 1954 Abnormalities of the urinary system of rat embryos resulting from maternal pteroylglutamic acid deficiency. Anat. Rec., *120:* 119.
10. Monie, I. W., M. M. Nelson and H. M. Evans 1957 Abnormalities of the urinary system of rat embryos resulting from transitory deficiency of pteroylglutamic acid during gestation. Anat. Rec., *127:* 711.
11. Nelson, M. M., H. V. Wright, C. D. C. Baird and H. M. Evans 1957 Teratogenic effects of pantothenic acid deficiency in the rat. J. Nutr., *62:* 395.
12. Giroud, A., J. Lefebvres, H. Prost and R. Dupuis 1955 Malformations des membres dues à des lésions vasculaires chez le foetus de rat déficient en acide pantothénique. J. Embryol. Exp. Morphol., *3:* 1.
13. Hurley, L. S., and N. E. Volkert 1965 Pantothenic acid and coenzyme A in the developing guinea pig liver. Biochim. Biophys. Acta, *104:* 372.
14. Hurley, L. S., N. E. Volkert and J. T. Eichner 1965 Pantothenic acid deficiency in pregnant and non-pregnant guinea pigs, with special reference to effects on the fetus. J. Nutr. *86:* 201.
15. Hurley, L. S., G. J. Everson and J. F. Geiger 1958 Manganese deficiency in rats: congenital nature of ataxia. J. Nutr., *66:* 309.
16. Chamberlain, J. G., and M. M. Nelson 1963 Congenital abnormalities in the rat resulting from single injections of 6-aminonicotinamide during pregnancy. J. Exp. Zool., *153:* 285.
17. Orent, E. R., and E. V. McCollum 1931 Effects of deprivation of manganese in the rat. J. Biol. Chem., *92:* 651.
18. Daniels, A. L., and G. J. Everson 1935 The relation of manganese to congenital debility. J. Nutr., *9:* 191.
19. Shils, M. E., and E. V. McCollum 1943 Further studies on the symptoms of manganese deficiency in the rat and mouse. J. Nutr., *26:* 1.
20. Norris, L. C., and C. D. Caskey 1939 A chronic congenital ataxia and osteodystrophy in chicks due to manganese deficiency. J. Nutr., *17*(suppl.): 16.
21. Caskey, C. D., L. C. Norris and G. F. Heuser 1944 A chronic congenital ataxia in chicks due to manganese deficiency in the maternal diet. Poultry Sci., *23:* 516.
22. Hill, R. M., D. E. Holtkamp, A. R. Buchanan and E. K. Rutledge 1950 Manganese deficiency in rats with relation to ataxia and loss of equilibrium. J. Nutr., *41:* 359.
23. Van Reen, R., and P. B. Pearson 1955 Manganese deficiency in the duck. J. Nutr., *55:* 225.
24. Everson, G. J., L. S. Hurley and J. F. Geiger 1959 Manganese deficiency in the guinea pig. J. Nutr., *68:* 49.
25. Hurley, L. S., and G. J. Everson 1963 Influence of timing of short-term supplementation during gestation on congenital abnormalities of manganese-deficient rats. J. Nutr., *79:* 23.
26. Hurley, L. S., and G. J. Everson 1959 Delayed development of righting reflexes in offspring of manganese-deficient rats. Proc. Soc. Exp. Biol. Med., *102:* 360.
27. Hurley, L. S., E. Wooten, G. J. Everson and C. W. Asling 1960 Anomalous development of ossification in the inner ear of offspring of manganese-deficient rats. J. Nutr., *71:* 15.
28. Asling, C. W., L. S. Hurley and E. Wooten 1960 Abnormal development of the otic labyrinth in young rats following maternal dietary manganese deficiency. Anat. Rec., *136:* 157.
29. Hurley, L. S., D. E. Woolley, F. Rosenthal and P. S. Timiras 1963 Influence of manganese on susceptibility of rats to convulsions. Amer. J. Physiol., *204:* 493.
30. Hurley, L. S., G. J. Everson, E. Wooten and C. W. Asling 1961 Disproportionate growth in offspring of manganese-deficient rats. I. The long bones. J. Nutr., *74:* 274.

31. Hurley, L. S., E. Wooten and G. J. Everson 1961 Disproportionate growth in offspring of manganese-deficient rats. II. Skull, brain and cerebrospinal fluid pressure. J. Nutr., *74:* 282.
32. Asling, C. W., and L. S. Hurley 1963 The influence of trace elements on the skeleton. Clin. Orthop., *27:* 213.
33. Hurley, L. S., and C. W. Asling 1963 Localized epiphyseal dysplasia in offspring of manganese-deficient rats. Anat. Rec., *145:* 25.
34. Everson, G. J., W. deRafols and L. S. Hurley 1964 Manganese deficiency in guinea pigs related to ground substance defect and electrolyte balance. Federation Proc., *23:* 448.
35. Parker, H. R., G. J. Everson, R. Shrader and L. S. Hurley 1964 Electrocardiological changes in offspring of normal and manganese deficient guinea pigs. Federation Proc., *23:* 292.
36. Hurley, L. S., and H. Swenerton 1965 Congenital malformations resulting from zinc deficiency in rats. Federation Proc., *24:* 568.

Building Blocks and Stepping Stones in Protein Nutrition

RUTH LEVERTON
Assistant Administrator, Agricultural Research Service, U. S. Department of Agriculture, Washington, D. C.

Fifty years ago an impressive amount of information was available about protein. More was known about its nutritional significance than about either fat or carbohydrate, except as sources of energy.

A scientist beginning his study and search of the literature about 1915 would have found that eighteen of the amino acids had been isolated from hydrolysates of protein. Only two of nutritional value remained to be discovered—methionine and threonine.

It was recognized that a relation existed between the chemical structure, the amino acid content, and the nutritive value of a protein. The exact relationship, however, had not been described and quantified.

Willock and Hopkins (1) had established that 1) zein as the only nitrogenous constituent of a diet was unable to maintain growth in young mice; 2) the addition of tryptophan did not make zein capable of supporting growth but did prolong survival; and 3) the addition of tyrosine had no effect. The scientists wondered if adding lysine would have helped but they had not tried it.

In 1915 Osborne and Mendel (2) reported on their experiments with zein. They confirmed the ability of zein plus tryptophan to maintain life and announced that zein plus tryptophan plus lysine supported growth. The growth charts first presented by these investigators have become classic illustrations and are still of value in textbooks used today.

Thomas (3) had introduced the concept of the "biological value" of proteins. He proposed expressing it in terms of the percentage of digestible nitrogen from a test food that was retained by the adult body. Later Mitchell (4) would apply the technique for growing rats and then (5) make it more precise by paired feeding. Today the biological value of proteins is the keystone of most of our efforts to achieve a well-fed world.

By 1915 Osborne and Mendel (6) had prepared a protein-free milk that contained the sugar and salts of milk, together with yellow-green pigments, and small amounts of unidentified substances of unknown value. This made it possible to compound a nearly purified diet for rats and opened the way to systematic study of the nutritive value of different proteins. An example of the results from such studies is the report of these investigators in 1915 (2) that the efficiency of casein for the growth of rats was greatly improved by the addition of cystine. They used the term "limiting factor" in referring to the fact that different amounts of casein, lactalbumin, and edestin were required to give comparable growth in rats (7). They also introduced the concept of "protein efficiency ratio" as the grams of weight gain per gram of protein intake.

Even in 1915 the undergraduate who was interested in or obliged to learn of protein metabolism had considerable to comprehend. Much was known of protein nutrition and the protein content of common foods. The specific dynamic action of various foodstuffs had been measured by Rubner (8) who reported that protein increased metabolism by 40 per cent. In 1958 Swift and his associates (9) were to

report that this increase was not more than 5 per cent.

In 1911 Dr. Henry C. Sherman published his textbook (8), *Chemistry of Food and Nutrition*. Between 1911 and 1952 this book would be extended to a total of eight editions and remain an authoritative source long after 1952. From the 1911 edition the student would have learned among many other things about protein that 1) muscular activity does not require protein, 2) carbohydrates and fat are protein sparers, 3) the fuel value of the diet has great influence on protein metabolism, 4) within wide limits the body adjusts its protein catabolism to its protein supply, and 5) the protein content of the previous diet has a definite influence on the response of a person to an experimental level of protein intake.

In 1912 Dr. Mary Swartz Rose published her *Laboratory Manual of Dietetics*, another book (10) that was to serve through many revised editions. She described the types of proteins as those able to support growth, those able only to maintain life, and those which could do neither. The previous year Osborne and Mendel had discussed two groups of proteins—those adequate and those inadequate for growth—and had referred to them as complete and incomplete. In Sherman's 1918 edition (11) he began using the classifications of complete, incomplete, and partially complete.

In the preface of her book Dr. Rose said, "Investigations into the quantitative requirements of the human body have progressed so far as to make dietetics to a certain extent an exact science, and to emphasize the importance of a quantitative study of food materials." Then, using Atwater's data on composition, she calculated the protein content (also the energy value and content of carbohydrate, fat, and 8 minerals) of customary measures and servings of 116 common foods.

Since 1915 this and much more information about the proteins has served as a springboard for extensive research into the building blocks of protein.

The discovery of methionine in 1922 (12) and of threonine in 1935 (13) made possible the experimental work that led to identification of the amino acids which are essential for maintaining nitrogen equilibrium in man.

Then the quantitative requirements of these essential amino acids were determined for men by Rose and his associates (14), and for women by several scientists working at three universities and supported in part by contracts of USDA (15–17) and for infants by Albanese (18) and by Holt (19) and their associates.

In addition to learning the kinds and amounts of amino acids needed by the human body, it was demonstrated, first with rats (20) and later with human subjects (21), that the body also needed to have these essentials present simultaneously for adequate protein replacement or synthesis. Apparently the body had no ability to store individual amino acids for use at a later time in the synthesis of tissue proteins.

With the knowledge of the quantitative requirements for each essential amino acid, the amino acid content of foods, and the biological value of many proteins, the urge to express relationships, preferably in numerical terms, was great and so were the possibilities.

In the 1950's the concept of a desirable pattern of essential amino acids became one basis for the nutritional evaluation of protein (22). The amino acid content of individual foods and of food combinations could then be evaluated by comparison with such a pattern. The "protein score" was developed to express the extent to which a food or food combination supplies the limiting amino acid as compared with a quantitative pattern of essential amino acids. An "Essential Amino Acid Index" was proposed by Oser (23) as another means of expressing the overall nutritive value of proteins. It is the geometric mean of the ratios of the essential amino acids in the food relative to their content in a highly nutritive reference protein, i.e., whole egg. Also, a reference protein, one of high biological value such as in milk and egg, was suggested as the common denominator for expressing protein requirement (22).

Progress has been made along many lines to increase our understanding of the amino acid components of protein. As a nutri-

tionist, however, I am especially concerned with the conduct of research and the translation of at least a fraction of the research findings into guidelines for food production and food selection which will maintain and improve nutritional well-being of population groups the world around. In the foreseeable future people will not be eating mixtures of amino acids with purified forms of other nutrients under controlled conditions of heredity and environment. They will be eating foods, probably in widely varying proportions and of many different kinds.

One of the chief goals of nutrition research is to understand human needs and secure a sound basis for recommending dietary intakes. During the last 50 years, advances in this phase of protein nutrition have been meager.

In 1911 in the first edition of *Chemistry of Food and Nutrition*, Sherman (8) recognized the controversy that existed about whether protein intakes should be generous as indicated by customary diets of the well-to-do population or limited as indicated by the well-being of Chittenden's subjects, or at some midpoint.

The recommendations for protein intake were usually related to energy needs and the basis was 3,000 kcal for a 70-kg man. Voit, Playfair, and Gautier suggested 16 per cent of the daily calories should come from protein. This amounted to 118 g for a 3,000-kcal diet. Atwater suggested 15 per cent of the calories; Langworthy, 12 per cent; and Chittenden, 8.5 per cent. After considering the various arguments and evaluating the evidence, Sherman suggested in his 1911 edition (8) that 100 g of protein daily for an average diet was "enough not only for equilibrium but such reserves as we are accustomed to carry." However, when a low protein intake was needed for physiological or economic reasons, he suggested 75 g daily.

Rose (10), in her *Laboratory Manual of Dietetics* of 1912, used the lower amount and considered 1 g of protein per kg of body weight as an adequate protein supply, a figure still in use in 1965.

In his second edition of 1918 (11), Sherman presented most of the material that we are still using today as a basis for recommending levels of protein intake for adults. Using nitrogen output as an indication of requirement, Sherman summarized data from 86 experiments on 41 healthy adults (37 men and 4 women) studied by 20 independent investigators. Calculated to a basis of 70 kg, the average amount of protein needed for nitrogen equilibrium was 49.2 g daily. The extremes were great, from 20.0 to 79.2 g. In this 1918 edition, Sherman also introduced the subject of the difference between minimum requirement and standard allowance. He proposed that there was a rational basis for a protein allowance being 50 per cent above actual requirement. Thus the daily protein allowance was calculated as 50 g plus 50 per cent or 75 g for a 70-kg man.

The justification for expressing the protein need as relatively constant percentage of the energy need—10 to 15 per cent of the calories—at every age is also given in this 1918 edition (11). This is still used almost unchanged in many discussions of recommended intakes.

By 1927 in Sherman's third edition (24), the experiments used as a basis for calculating protein requirement had increased from 86 to 109, the number of investigators from 20 to 25, and the number of subjects from 41 to 47. The average amount of protein needed daily for nitrogen balance was 44.4 g per 70-kg man. Increased by 50 per cent, the standard allowance became 70 g of protein, or 1 g per kg.

In 1933 in his fourth edition (25), Sherman explains that "In the desire to avoid any danger of arbitrary selection of data, (he) probably erred in the direction of including some experiments which gave misleadingly high results because of too short periods on the low protein diets. The best data would probably yield an average result not far from 0.5 gm protein per kg of body weight per day for normal adult maintenance after allowing a reasonable period of adjustment to such a low protein diet." This figure of 0.5 g/kg is still used as a base line in calculations of protein requirement.

At midpoint in these last 50 years, the Food and Nutrition Board of the National

Research Council was established (a successor to the Committee on Foods and Nutrition). One of its first concerns was to define recommended daily allowances for specific nutrients. From the preliminary release in May, 1941, and the first publication in 1943, through the sixth revision in 1963, the recommended dietary allowance for protein has remained at 1 g per kg for the adult man (26). The comment is usually added that this amount is known to be generous. Until the 1953 revision the allowance for the 56-kg woman was 60 g. In that revision the Board reduced the weight of the standard man from 70 to 65 kg and of the woman from 56 to 55 kg. In the next edition in 1958, however, the standard man had grown 5 cm taller and returned to a 70 kg weight, and the standard woman was 6 cm taller and her weight was extended to 58 kg. Thus protein allowances were and still are 70 g and 58 g, respectively. The increase for pregnancy has dropped from approximately 25 to 20 g. The increase for lactation has remained at 40 g with but one exception—in 1953 when the increase for the 55-kg woman was 45 g.

The discussion of protein allowances for adults in the publications of recommended dietary allowances has increased from 12 lines with 3 references in the 1945 edition to 102 lines, 1 table, and 23 references in the 1963 revision. But the recommended amount is still the same—1 g/kg which, according to the Board, ". . . does not appear to be excessive, although it may well prove to be at least twice the minimal requirement." In the 1963 revision, however, an attempt was made to take into consideration the biological value of the protein supply.

The constancy of the recommended allowance of 1 g/kg need not be taken as evidence of lack of careful consideration of the facts available. Rather it suggests the dependability of results of carefully conducted studies and the thorough evaluation of such results by knowledgeable investigators.

The greatest need in the field of protein nutrition today is for studies of "typical proteins in typical diets." This is, of course, an oversimplification of wording but the message is there. The use of purified diets has provided us with building blocks of fundamental facts. The feeding of a single food or a single protein for the study of the protein need of the subject and the value of the food in meeting this need has provided us with important stepping stones. But in practice human needs must be met by a combination of foods.

Current emphasis on the study of ever smaller discrete units of metabolic activity gives us fundamental facts about isolated cells and tissues. But the practicing nutritionist must have facts for whole people, with their heredity already determined, living in their current environment, reacting as whole organisms, and eating food.

Our task is to cope with the problems of protein nutrition today, wherever they exist —in faraway developing countries, in pockets of poverty or ignorance in this country, or in our affluent society prone to obesity and heart disease.

We have an obligation to see that these much needed facts are among the nutrition landmarks of the next half-century.

LITERATURE CITED[1]

1. Willock, E. G., and F. G. Hopkins 1906 The importance of individual amino acids in metabolism: observations on the effect of adding tryptophan to a diet in which zein is the sole nitrogenous constituent. J. Physiol., *35:* 88.
2. Mendel, L. B. 1915 Nutrition and growth. J. Amer. Med. Ass., *64:* 1539.
3. Thomas, K. 1909 Biological value of nitrogenous substances in different foods. The question of the physiological protein minimum. Arch. Anat. Physiol., *219:* 302.
4. Mitchell, H. H. 1924 A method of determining the biological value of protein. J. Biol. Chem., *58:* 873.
5. Mitchell, H. H., T. S. Hamilton, J. R. Beadles and F. Simpson 1945 The importance of commercial processing for the protein value of food products. I. Soybean, coconut and sunflower seed. J. Nutr., *29:* 13.
6. Osborne, T. B., and L. B. Mendel 1911 Feeding experiments with isolated food substances, publ. 156, part 2, Carnegie Institution of Washington, p. 53.

[1] The reader is referred also to a valuable critical review: Holmes, E. G. 1965 In: World Rev. Nutr. Diet., *5:* 238. An appraisal of the evidence upon which recently recommended protein allowances have been based (about 100 references).

7. Osborne, T. B., and L. B. Mendel 1916 A quantitative comparison of casein, lactalbumin and edestin for growth or maintenance. J. Biol. Chem., *26:* 1.
8. Sherman, H. C. 1911 Chemistry of Food and Nutrition, ed. 1. The Macmillan Company, New York.
9. Swift, R. W., G. P. Barron, K. H. Fisher, C. E. French, E. W. Hartsook, T. V. Hershberger, E. Keck, T. A. Long and N. D. Magruder 1958 Effect of high versus low protein equicaloric diets on the heat production of human subjects. J. Nutr., *65:* 89.
10. Rose, M. S. 1912 A Laboratory Hand-book for Dietetics. The Macmillan Company, New York.
11. Sherman, H. C. 1918 Chemistry of Food and Nutrition, ed. 2. The Macmillan Company, New York.
12. Mueller, J. H. 1921 A new sulfur-containing amino acid isolated from casein. Proc. Soc. Exp. Biol. Med., *19:* 161.
13. McCoy, R. H., C. E. Meyer and W. C. Rose 1935 VIII. Isolation and identification of a new essential amino acid. J. Biol. Chem., *112:* 283.
14. Rose, W. C. 1949 Amino acid requirements of man. Federation Proc., *8:* 546.
15. Jones, E. M., C. A. Baumann and M. S. Reynolds 1956 Nitrogen balances of women maintained on various levels of lysine. J. Nutr., *60:* 549.
16. Leverton, R. M., M. R. Gram, M. Chaloupka, E. E. Brodovsky and A. Mitchell 1956 The quantitative amino acid requirements of young women. J. Nutr., *58:* 59, 83, 219, 341, 355.
17. Swenseid, M. E., I. Williams and Max S. Dunn 1956 Amino acid requirements of young women based on nitrogen balance data. I. The sulfur-containing amino acids. J. Nutr., *58:* 495.
18. Albanese, A. A. 1959 Protein and Amino Acid Nutrition. Academic Press, New York, p. 419.
19. Holt, L. E., Jr., P. György, E. L. Pratt, S. E. Synderman and W. M. Wallace 1960 Protein and Amino Acid Requirements in Early Life. New York University Press, New York.
20. Geiger, E. 1947 Experiments with delayed supplementation of incomplete amino acid mixtures. J. Nutr., *34:* 97.
21. Leverton, R. M., and R. M. Gram 1949 Nitrogen excretion of women related to the distribution of animal protein in daily meals. J. Nutr., *39:* 57.
22. Food and Agriculture Organization of the United Nations 1957 Protein requirements. Report of the FAO Committee, 1955. FAO Nutritional Studies no. 16, Rome.
23. Oser, B. L. 1951 Method for integrating essential amino acid content in the nutritional evaluation of protein. J. Amer. Diet. Ass., *27:* 396.
24. Sherman, H. C. 1927 Chemistry of Food and Nutrition, ed. 3. The Macmillan Company, New York.
25. Sherman, H. C. 1933 Chemistry of Food and Nutrition, ed. 4. The Macmillan Company, New York.
26. National Research Council, Committee on Food and Nutrition 1941 Recommended Daily Allowances; revised 1943, 1945, 1948, 1953, 1958 and 1964. National Academy of Sciences–National Research Council, Washington, D. C.

The Relation of Nutrition to Cellular Biochemistry

THOMAS H. JUKES
Professor in Residence,
Medical Physics, Space Sciences Laboratory, and
Department of Nutritional Sciences,
University of California, Berkeley, California

We are here today to honor Dr. Agnes Fay Morgan and to mark the fact that she continues to make many contributions to research over a long period of years. It may be appropriate to comment briefly on changes in the field of nutrition that have taken place during the time embraced by her scientific career. We shall also, as the title of this talk indicates, point out certain interrelationships between nutrition and cellular biology.

Nutrition half a century ago was an exploratory field, filled with urgent and unanswered questions. Some of the excitement of those days was described by Paul de Kruif in his book *Hunger Fighters.* Beriberi was one of the world's major diseases. The solution to this problem was provided by the synthesis of thiamine and its manufacture on a large scale as a chemical commodity. Pellagra was common in the southern U.S.A. Its numerous victims were in the hospitals and the insane asylums. In 1937 it was found that nicotinic acid deficiency was a primary cause of pellagra. This finding, together with a general improvement in the food supply, has led to pellagra becoming an extremely rare disease. Protein deficiencies were imperfectly understood; the list of essential amino acids was incomplete; the words methionine and threonine had not yet been coined. Simple goiter, due to iodine deficiency, was common in inland areas. The introduction of iodized salt by Marine and Kimball in 1917 overcame the deficiency. It was against this background that forty years ago we read Professor Elmer McCollum's book, *The Newer Knowledge of Nutrition,* first published in 1918, and saw before us in its pages new horizons of uncharted scientific territories.

Today most of these territories have been crossed and criss-crossed. The medical profession has come to have an increasing role in the field of nutrition; perhaps this shows that nutrition has changed from an exploratory science into a technological branch of public health practice. Such a change affects some of us nostalgically, but we recognize the benefits that have accrued to the public at large during this metamorphosis.

The place of nutrition in the history of evolution of living organisms is fascinating to contemplate and to conjecture upon. Let us turn back the clock, not for 50 years as some of the previous speakers have done, but for about 3 billion years into the realm of speculation. The interest centers on the warm anaerobic culture broth that we presume to have filled the early terrestrial oceans. Here dwelled the archetypal organisms. Their food supply had been made for them by chemical reactions in which more complex compounds were formed from simple starting materials such as cyanide, ammonia, methane, formaldehyde and water. This conclusion is reached from the experiments of Miller, Fox, Orò, and others who have shown that these simple precursors under the action of heat, ultraviolet light and electrical discharges can produce small amounts of amino acids, nitrogenous bases, the precursors of carbohydrates, and other ingredients used in the formation of protoplasm. These food substances in the primitive oceans were used by the early organisms and were broken down in energy-yielding reactions. The supply of nutrients therefore dwindled and the first nutritional

deficiencies appeared, due to which many forms of life must have died and become extinct. A few survived; they were organisms that by a piece of evolutionary good fortune had developed a procedure for making an enzyme system that carried out the final step in a biosynthetic process. Let us suppose, for example, that the supply of phenylalanine became used up, but that phenylpyruvic acid was still available. Obligate phenylalanine-requiring organisms would disappear. Organisms that could convert phenylpyruvic acid to phenylalanine, however, would survive. Their descendants live today in the plant kingdom.

We now leapfrog a billion and a half years and find ourselves in a world of green plants. The terrestrial atmosphere consisted of nitrogen, oxygen, and carbon dioxide, just as it does today. A new form of life was emerging. Unlike the archetypal form, it was parasitic rather than saprophytic. It was the animal kingdom. Like the early saprophytes, its members lack the genetic ability to make the pigments and enzymes needed for utilizing solar energy in the synthesis of key nutrients. However, the parasitic species survive and thrive, despite this lack, by preying on their more industrious and self-sufficient neighbors. Our imaginary journey now brings us back to the present day when mankind has circumvented some of his parasitic needs by discovering how to make essential nutrients in the test tube and the chemical factory.

It was the study of nutrition that led chemists to some of these discoveries. Let us select some examples, and illuminate them with modern developments.

Twenty, and only twenty, amino acids participate in the biological synthesis of protein. The absence of any one of these amino acids can bring protein synthesis to an abrupt halt by stopping the formation of polypeptide chains at points where the genetic message calls for the insertion of the missing amino acid. If a young animal is placed on a diet containing no tryptophan or lysine, its growth will cease immediately because animals cannot make these two amino acids. It was found by Osborne and Mendel in 1912 that rats stopped growing on a diet based on the corn protein zein, and that growth was restored by adding lysine and tryptophan. By means of this experiment, and others like it, the 20 amino acids needed for protein synthesis were enumerated and identified and their places in cellular biochemistry became perceived. Not all of the twenty need be provided in animal diets, but all of them are needed for the translation of the genetic message into protein molecules. Other amino acids, such as hydroxyproline, are also present in certain proteins, but no member of this second group is needed in the diet. All of the 8 or 10 amino acids necessary in the diets of animals are members of the "group of 20," of course.

The experimental design used by Osborne and Mendel was employed in the isolation and identification of other amino acids and of other nutritionally essential substances which are obtained by parasitic and saprophytic organisms from other living or dead forms. In some cases bacteria were used in the growth tests. Thus Mueller used the growth of hemolytic streptococci to pinpoint an unidentified nutrient that proved to be methionine. Rose, Meyer, McCoy, Carter and their collaborators fractionated casein and used rat growth tests like those of Osborne and Mendel to discover and synthesize threonine. By such methods the list of ingredients was assembled for today's science of chemical genetics. We can now write the code that shows the means by which all 20 of the amino acids are arranged in the genetically determined polypeptide sequences that make protein molecules. For the past century proteins have been appropriately the center of attention in the nutritional sciences, and in the last 15 years it has been realized that the order in which amino acids are arranged in proteins is written in the basic language of genetics—the amino acid code. This is shown in table 1, which lists the sequences of ribonucleic acid (RNA) bases that specify the codes for the 20 amino acids. There are 64 possible ways in which the RNA bases uracil, cytosine, adenine and guanine (U, C, A, and G) can be arranged in groups of 3. Sixty-one of the groups specify amino acids, 2 of the other 3 denote the gaps or intervals that mark the ending

TABLE 1

The amino acid code[1]

UUb phenylalanine	CUd leucine	AUb isoleucine	GUd valine
		AUA isoleucine	
UUe leucine		AUG methionine	
UCd serine	CCd proline	ACd threonine	GCd alanine
UAb tyrosine	CAb histidine	AAb asparagine	GAb aspartic acid
UAe gaps	CAe glutamine	AAe lysine	GAe glutamic acid
UGb cysteine	CGd arginine	AGb serine	GGd glycine
UGA unassigned			
UGG tryptophan		AGe arginine	

[1] U = uracil; C = cytosine; A = adenine; G = guanine; b = U or C; d = U, C, A, or G; e = A or G.

of one protein molecule and the beginning of another, and one has no known function.

These findings are a great landmark in biology. The solving of the coding problem was the work of many laboratories, prominent among which are those of Nirenberg, Ochoa, Brenner, Khorana and Doty. The conclusions were aided greatly by the findings with single-amino-acid mutations in proteins including, among these, those made in the laboratories of Ingram, Fraenkel-Conrat, Wittmann, Yanofsky and Garen.

Some experiments in our laboratory show that nutrition can be related to the synthesis of deoxyribonucleic acid (DNA) in microorganisms. Recent research by Dr. Hiroshi Yoshikawa has involved the use of classical nutritional approaches in the identification of a factor concerned with the synthesis of DNA by the organism *Bacillus subtilis*. Dr. Yoshikawa reported these results at a conference on the Berkeley campus, and has given me permission to quote from them. The assay procedure, rather than making use of animals or microorganisms for growth tests, was carried out with genes.

Bacillus subtilis was used by Yoshikawa and Sueoka (1) in the mapping of genetic loci on the bacterial chromosomes. They adapted the discovery of Spizizen (2) that *B. subtilis* was transformable by its own DNA. The term *transformation* in this context refers to the uptake by recipient bacteria of genetic determinants carried by a small piece of exogenous DNA extracted from a donor cell. Genetically defective mutants of the organism may be restored to normality by incubation with DNA prepared from wild type *B. subtilis*. The DNA penetrates the cell walls of the deficient cells, carrying with it the genetic information for the synthesis of substances, such as adenine and methionine, which the defective mutant cannot make. The intruding DNA is incorporated into the chromosome of the recipient cell and from then on the formerly-inadequate mutants can make their own adenine and methionine. Up to this point the story is an old one.

Yoshikawa and Sueoka, however, went a step further, and made their measurements quantitative to the extent that they were able to measure the numbers of adenine-producing and methionine-producing genes in a sample of wild-type DNA. Equal numbers of each gene were present in DNA obtained from resting cells. However, when the cells were growing rapidly, the DNA contained twice as many "adenine" genes as "methionine" genes. Clearly the chromosomes were doubling in unison, and the "adenine" gene was made at the front end of a linear structure which contained a fork as depicted in figure 1.

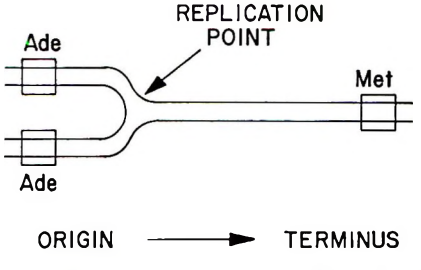


Fig. 1 Diagrammatic representation of normal chromosome replication in *Bacillus subtilis*.

The rapidly growing wild-type cells had been cultured on a simplified medium of salts, glucose, casein hydrolysate and tryptophan. This corresponds to a marginal diet as used in nutritional studies with animals. What would happen if the cells were grown in a crude enriched medium? This was tried. The enriched medium contained beef extract, yeast extract, peptone, dextrose and salts. The DNA from cells on this medium was purified as before and mixed with the defective cells, but this time the results were different and unexpected. The DNA now contained 4 times as many *adenine* genes as *methionine* genes (3). This is shown in figure 2. This meant that a second reproductive cycle had been initiated before the first cycle had been completed. Note that the replication of DNA, as shown by the adenine: methionine genetic ratio, definitely preceded cell duplication, as shown by optical density. The model diagram for this accelerated replication is shown in figure 3.

The factor responsible for this "stepped-up" rate of DNA duplication was evidently a nutritional substance that was absent from

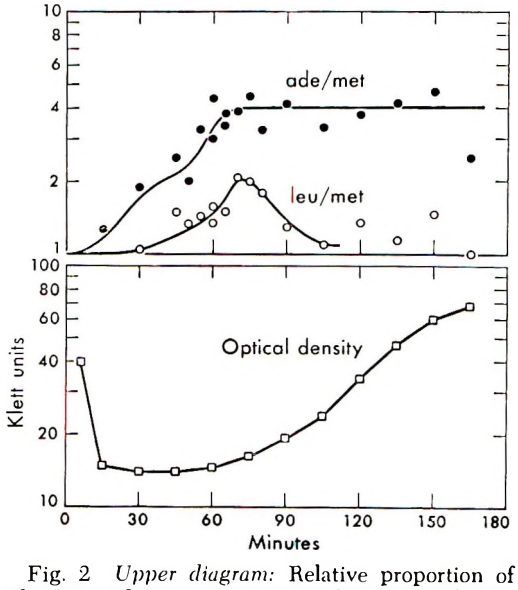


Fig. 2 *Upper diagram:* Relative proportion of adenine-producing genes to methionine-producing genes, plotted against time, during rapid growth of *Bacillus subtilis*. *Lower diagram:* Changes in optical density caused by germination of *Bacillus subtilis* spores followed by rapid growth of vegetative cells plotted against same time scale as upper diagram.

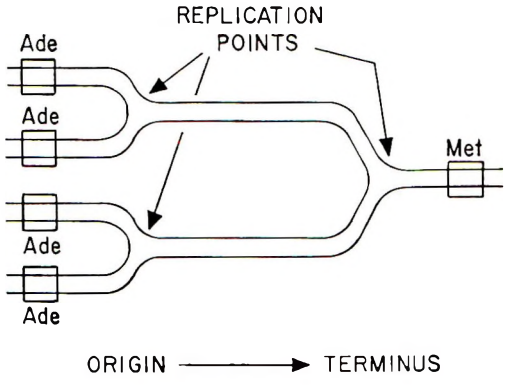


Fig. 3. Diagrammatic representation of duplication of *Bacillus subtilis* chromosome during rapid growth on enriched media showing initiation of second reproductive cycle before completion of first cycle.

the purified medium but present in the crude enriched broth. In a very short time Dr. Yoshikawa identified the factor as being adsorbable on charcoal and to be replaceable by the four basic components of ribonucleic acid: adenine, guanine, cytosine and uracil (table 2). Thymine would not replace uracil. Omission of any one of the four bases slowed down the duplication of

TABLE 2

Effect of various supplements to charcoal-treated enriched medium on rate of replication of DNA in Bacillus subtilis[1]

Additions to medium	Adenine: methionine gene ratio of recipient cells		
	Exp. 1	Exp. 2	Average
None	1.94	1.89	1.92
Casein hydrolysate	1.91	2.38	2.15
A+G+U+C	3.97	3.50	3.74
Casein hydrolysate +A+G+U+C	4.00	3.62	3.81
A+G+U	2.09	2.03	2.06
A+G+C	2.10	2.10	2.10
A+C+U	2.20	2.10	2.05
G+C+U	1.95	1.77	1.86

[1] A = adenine, G = guanine, C = cytosine, and U = uracil.

DNA to the rate observed on the purified medium. One possible inference is that the four bases cause a more rapid rate of RNA production and this in turn leads to the production of a messenger RNA that codes for

a protein which speeds up the DNA polymerase cycle, perhaps a so-called "initiator" protein or enzyme.

Let us now turn to a brief review of Professor Agnes Fay Morgan's research that formed a part of the great era of expansion in nutrition. Her basic work was concentrated in three main fields: First, the effect of heat upon the nutritive value of proteins; second, studies with calcium and phosphorus metabolism as affected by vitamin D, by the parathyroid hormone and by the composition of the diet; and third, the metabolic effects of B vitamins, ascorbic acid, vitamin A and carotene. I think that it was in the third of these fields that her contributions were most exciting. During 1939 to 1940 she broke new ground with observations on depigmentation and adrenal damage caused by deficiencies of the vitamin B complex. It was my privilege to supply her with concentrates used in these early tests. We were working in the dark but we were able to concentrate a new factor by means of biological assays. She found it essential for normal pigmentation and adrenal function. In 1939 we found at Davis that the essential substance was pantothenic acid (4). Dr. Morgan followed up her earlier studies with a substantial series of later publications that documented many of the earlier findings in terms of pure substances and a number of these articles are listed in the bibliography (5–11). Her finding of the relation of pantothenic acid to adrenal cortical function was made clearer by the subsequent finding that pantothenic acid is a component of coenzyme A, which is needed for the utilization of acetyl groups in the synthesis of steroids. Dr. Morgan also made major contributions in the field of vitamin A and carotene metabolism, tryptophan metabolism, riboflavin deficiency and the relation of the thyroid gland to the utilization of vitamin A.

In addition to her fundamental research, she has published many articles relating to the more practical or medical aspects of nutrition. These include studies on the supplement feeding of underweight children, experiments on the vitamin value of foods as changed by drying, cooking or storage and investigations of nutritional status in geriatrics patients. These latter studies have particularly occupied her in more recent years.

The list of Dr. Morgan's collaborators, co-authors and students is far too voluminous even to summarize. I am most happy to note that many of them have journeyed here on this most auspicious occasion.

LITERATURE CITED

1. Yoshikawa, H., and N. Sueoka 1963 Sequential replication of *Bacillus subtilis* chromosome. I. Comparison of marker frequencies in exponential and stationary growth phases. Proc. Nat. Acad. Sci., *49:* 559.
2. Spizizen, J. 1958 Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate. Proc. Nat. Acad. Sci., *44:* 1072.
3. Yoshikawa, H., A. O'Sullivan and N. Sueoka 1964 Sequential replication of the *Bacillus subtilis* chromosome. III. Regulation of initiation. Proc. Nat. Acad. Sci., *52:* 973.
4. Jukes, T. H. 1939 Pantothenic acid and the filtrate (chick anti-dermatitis) factor. J. Amer. Chem. Soc., *61:* 975.
5. Morgan, A. F., and H. D. Simms 1939 Adrenal atrophy and senescence produced by a vitamin deficiency. Science, *89:* 565.
6. Morgan, A. F., and H. D. Simms 1940 Greying of fur and other disturbances in several species due to a vitamin deficiency. J. Nutr., *19:* 233.
7. Morgan, A. F., and H. D. Simms 1940 Antigrey hair vitamin deficiency in the silver fox. J. Nutr., *20:* 627.
8. Becks, H., and A. F. Morgan 1942 The effect of deficiencies of the "filtrate fraction" of the vitamin B complex and of nicotinic acid on teeth and oral structure. J. Periodontol., *13:* 18.
9. Hurley, L. S., and A. F. Morgan 1952 Carbohydrate metabolism and adrenal cortical function in the pantothenic acid-deficient rat. J. Biol. Chem., *195:* 583.
10. Guehring, R. R., L. S. Hurley and A. F. Morgan 1952 Cholesterol metabolism in pantothenic acid deficiency. J. Biol. Chem., *197:* 485.
11. Butler, L. C., and A. F. Morgan 1955 The content of adrenocorticotrophic hormone in the pantothenic acid-deficient rat. Endocrinology, *56:* 322.

Nutritional Status, U.S.A.

GLADYS A. EMERSON
Professor of Nutrition, School of Public Health, University of California, Los Angeles, California

"Nutritional Status U.S.A.," (1) published in 1959 was compiled and edited by our Honoree of 50 years. Her milestone in nutrition reporting is the first reference in the bibliography of Recommended Dietary Allowances (2) revised in 1963. The same place is held in Dr. Ruth Leverton's review on "Nutritional well being in the U.S.A.," *Nutrition Reviews,* November, 1964 (3) and Dr. Grace Goldsmith's "Clinical problems in the U.S. today," *Nutrition Reviews,* January, 1965 (4).

The United States was a healthy place to live in 1959 and so it is today. Health, in Dr. Morgan's compilation, was measured by choice of foods. Her study represented a combination and interpretation of regional surveys of 12,000 children, adolescents and adults who were 50 to 80 years of age from 39 of the then 48 states (Alaska and Hawaii were not included). The nutritional status was found to be good. This general statement resulted from an appraisal of 7-day records of food intakes, physical examinations, biochemical analyses of blood and urine, and in some cases, dental examinations and x-rays of bones and teeth. The nutrition of boys and girls to the age of 12 was adequate, "even deluxe," except that the calcium intake was slightly low in the girls. Boys 13 to 20 years of age received adequate intakes of all nutrients except ascorbic acid. Girls in this group had adequate or high intakes of only three nutrients: vitamin A, riboflavin and niacin. They were seriously low in calcium, iron, vitamin B_1 and vitamin C, and borderline for calories and protein. Adult men had average or high intakes of all nutrients except calories and thiamine. The women had good average intakes of all nutrients except calories, vitamin B_1, calcium and riboflavin. The nutrients most likely to be low in children and adults were vitamins A and C, calcium and iron. Dr. Morgan pointed out that the low calorie intakes of women could not be cited as a deficit as the subjects were by no means on the average underweight. The evaluations were based on the 1953 and 1958 Recommended Dietary Allowances. Calorie allowances were reduced in the current revision. The Dietary Allowances of the Food and Nutrition Board, which include a margin of safety, should not be construed as requirements.

Dr. Leverton (3) stated that sufficient kinds and amounts of food are available in the U.S.A. to provide the total of nutrients and energy to meet the Recommended Dietary Allowances. The nutritive value of the food supply cannot be used alone in assessing the nutritional status of the individual although without an adequate supply there would be little likelihood of well being. This is evidenced by the widespread malnutrition in technically underdeveloped areas. The body's requirements are for nutrients rather than for individual foods.

W. N. Pearson (5) has given a biochemical appraisal of the vitamin nutritional status in man. He has suggested the use of blood levels of ascorbic acid and vitamin A and urinary excretion of the B vitamins as indices of vitamin nutritional status. This guide has been employed for use in population groups by the International Committee on Nutrition for National Defense (6). Grace A. Goldsmith (4) has listed the most important nutritional problems in 1965 as obesity, iron deficiency

anemia and malnutrition as secondary to disease states not primarily nutritional in origin. Nutritional deficiencies due solely to an inadequate diet have almost disappeared. Nutrition problems in Dr. Goldsmith's "today" and Dr. Morgan's "1959" are about the same.

The change in the American diet from 1879 to 1959 has been subject to review by Bennett and Peirce (7). Food and calorie consumption based on food at the retail level declined during this period. An increase was noted in the purchase of foods of low calorie content and a steady decrease occurred in the "consumption" of starchy foods such as wheat flour, corn meal and potatoes. Only slight differences were reported in the purchase of "meaty" foods, although within the group, pork consumption declined but was balanced by increased purchases of poultry. The simple carbohydrates increased in volume until a plateau was reached in 1930. The purchases of fat increased somewhat from 1920 to 1940 and thence plateaued. Butter and lard purchases decreased and vegetable oils gained in public favor decade by decade. The overall consumption of milk and milk products increased with condensed, evaporated and skim milk and cheese gaining in popularity. Technological advances in the processing of foods such as canning and freezing and in refrigeration led to increases in the purchases of certain fruits and vegetables listed in the "watery" class. The frozen food industry had just started at the beginning of World War II. The sales of frozen fruits and vegetables, as well as canned goods, greatly increased after 1949 with a corresponding decrease in the purchase of some fresh fruits and vegetables. Bennett and Peirce projected that the major food groups most likely to increase in per capita consumption appeared to be milk and meat. A slight decline in fat intake may occur due to continued nutritional warnings against the high intake of fats in general, and animal fats in particular. The watery fruits and vegetables may continue to increase their proportional importance and the drift toward canned, and especially frozen items, may well continue. Had this forecast been made in 1965 dehydrated products would have been listed. The change in food patterns was dealt with in some detail by Antar, Ohlson and Hodges in 1964 (8) who analyzed changes in retail market food supplies in the last 70 years in relation to the incidence of coronary heart disease with special reference to dietary carbohydrates and essential fatty acids. Heart diseases accounted for the deaths of 659,410 persons in 1960. When adjustments were made for increased life span, crude statistics, etc., it was widely accepted that arteriosclerotic heart disease had increased. Heart disease due to infection declined during the same period. The increase in age-adjusted death rates from coronary artery disease occurred mainly among men. From 1940 to 1954 the rise in incidence among white men amounted to about 40% and with white women the corresponding increase was only 16%. The major changes in the diet described by these authors were essentially the same as given by Bennett and Peirce (7). Antar *et al.* (8) stressed the overall dramatic increase in supplies of simple sugars and syrups with a decrease of complex carbohydrates especially in the first part of this century. The ratio of polyunsaturated to saturated fatty acids had increased 37% since 1909. These data do not fit the hypothesis that low ratios of polyunsaturated to saturated fatty acids in the food supply contribute to the incidence of coronary heart disease in the United States. The changes in dietary carbohydrates were considered as a possible factor. Yudkin (9), as early as 1957, pointed out that the incidence of ischemic heart disease in different countries showed a better relationship to the intake of sugar than any other major foodstuff. Yudkin, in 1965, in a review in *Food and Nutrition News* pointed out that the incidence of heart disease was low in Yeminite Jews until they moved to Israel and changed their dietary habits, which included the ingestion of large quantities of sugar.

Evidence is increasing for the need of additional iron by women and children. A greater allowance was provided in the current revision of Recommended Dietary Allowances. According to C. W. Woodruff

(cited by Goldsmith (4)) the most common dietary deficiency in North America is that of iron, expressed as hypochromic anemia in infants. Shaw and Robertson (10) reported that 24.7% of the patients 6 to 24 months of age admitted to the Columbus (Ohio) Children's Hospital had a hemoglobin concentration of less than 10 g per 100 ml. Most of the cases of anemia with documented diagnoses were iron-deficient in origin. Filer and Martinez (11) stated that adequate iron nutrition is difficult to achieve. The mean iron intake of infants is considerably below that recommended by the Committee on Nutrition of the American Academy of Pediatrics. Marsh and coworkers (12) reported that supplementary iron provided in a milk formula was absorbed by both full-term and premature infants. Gorten and Cross (13) found that iron-fortified formulas offered to premature infants from the newborn period, afforded effective prophylaxis against iron deficiency. Hematological observations from the tenth week of life were indistinguishable from those of healthy term infants receiving optimal nutritional care. The majority of premature infants, who derive iron solely from iron-fortified and iron-containing solid foods, even when these foods are fed from an early age, developed true iron deficiency anemias. Premature infants have less body iron than normal-term infants as the rates of acquisition of body mass and of iron are greatest as term approaches.

Haughton (14) reported that in an underprivileged preschool population (90% under 3 years of age) of New York City, 18.9% had hemoglobin levels below 10 g per 100 ml.

The adolescent's food patterns are frequently those of his parents. According to Beal (15) girls 12 to 14 years old developed seasonal food habits having better diets when attending school in the winter than in the summer. Dr. Morgan reported that teenage girls had the poorest diets of any population group in the late 1950's. As mentioned, their intakes of vitamins A and C, calcium and iron were below the recommended allowances. The increased incidence of teenage pregnancies is of paramount concern. In 1962 14% of all babies born and 38% of first born were to mothers 19 years old or younger. Premature births, toxemia and anemia are prevalent in this group. Lund (16) in 1951 reported that one-half of over 4,000 women at the prenatal clinic of the Charity Hospital in New Orleans were anemic at the time of their first visit.

Obesity remains a problem with some of the teenage population. Unglaub in 1964 (reported by Goldsmith (4)), observed a 29% incidence of obesity in 1,000 children 10 to 16 years of age in five rural areas in southern Louisiana. This finding was associated, at least in part, with hypothyroidism due to low intakes of iodine. Huenemann (17) has been concerned with obesity in teenagers in Berkeley. In her study of 11,000 teenagers the prevalence of obesity increased with each succeeding year. For both boys and girls the increment was from 11% in the ninth grade to 14% in the twelfth grade. Although some teenagers are growing huskier, many are becoming taller than their forebears who were their age a generation ago. This trend of increased stature has been reported from Ohio and Iowa to Guatemala, Japan and Lebanon.

Middle-aged women and, to a lesser extent, men are prone to overweight. The Recommended Dietary Allowances provide for a decrease in calorie intake with progressing age. Downward adjustments of 5, 8 and 10% respectively, from the reference individual of 25 years are recommended for age periods 35 to 55 years, 55 to 75 years and 75 years and beyond. Alastair Cooke (18) castigated the Food and Nutrition Board for taking a negative approach to the problem of obesity. His dim picture of the group was an unappetizing one bringing to mind Mencken's definition of a Puritan as a man who is afflicted with "the haunting fear that someone, somewhere may be happy." An editorial in *Lancet* on "The American and his diet" (19) suggested a positive solution to weight control in recommending more activity. The editors further stated that the evidence is mounting that physical exercise is beneficial in that it delays the onset of degenerative disease.

Tuttle and co-workers (20) found that the total amount of essential amino acid nitrogen required for maintaining nitrogen balance is greater in men over 50 years of age than in young adults. The quantities of methionine and lysine needed to maintain nitrogen balance appeared to be greater in older men than in younger men.

Osteoporosis occurs frequently in older people, particularly in post-menopausal women. Calcium absorption may be defective in patients with this disorder (21).

The overall nutritional picture in 1965 is good. Iron deficiency in youth and pregnant women and obesity and calcium deficiency in older individuals are of the most concern. The Council on Foods and Nutrition of the American Medical Association stated in 1962 that "despite the promise of diet in therapy, there is not sufficient information available at the present time to warrant a change in the American diet aimed at preventing heart disease in the general population" (22). Watkin (23) has suggested that new developments in molecular biology may provide means of retarding or halting the aging process. The substitution of young functioning RNA for aged RNA may ensure enzyme synthesis and thereby cell life.

LITERATURE CITED

1. Morgan, A. F. 1959 Nutritional Status, U.S.A. California Agricultural Experiment Station Bull. 769, Berkeley.
2. National Research Council, Food and Nutrition Board 1964 Recommended Dietary Allowances, publ. 1164. National Academy of Sciences—National Research Council, Washington, D. C.
3. Leverton, R. M. 1964 Nutritional well-being in the U.S.A. Nutr. Rev., *22:* 321.
4. Goldsmith, G. A. 1965 Clinical nutritional problems in the United States today. Nutr. Rev., *23:* 1.
5. Pearson, W. N. 1960 Interdepartmental Committee on Nutrition for National Defense. Suggested guide for interpreting dietary and biochemical data. Public Health Rep., *75:* 687.
6. Manual for Nutritional Surveys, ed. 2. 1963 Interdepartmental Committee on Nutrition for National Defense, National Institutes of Health, Bethesda, Maryland.
7. Bennett, M. K., and R. H. Peirce 1961 Food Res. Inst. Stud., *2:* 95.
8. Antar, M. A., M. A. Ohlson and R. E. Hodges 1964 Changes in retail market food supplies in the United States in the last seventy years in relation to the incidence of coronary disease, with special reference to dietary carbohydrates and essential fatty acids. Amer. J. Clin. Nutr., *14:* 169.
9. Yudkin, J. 1957 Diet and coronary thrombosis, hypothesis and fact. Lancet, *2:* 155.
10. Shaw, R., and W. O. Robertson 1964 Anemia among hospitalized infants. Ohio Med. J., *60:* 45.
11. Filer, L. J., Jr., and G. A. Martinez 1964 Intake of selected nutrients by infants in the United States: an evaluation of 4,000 representative six-months-olds. Clin. Pediat., 3: 633.
12. Marsh, A., H. Long and E. Stierwalt 1959 Comparative hematologic response to iron fortification of a milk formula for infants. Pediatrics, *24:* 404.
13. Gorten, M. K., and E. R. Cross 1964 Iron metabolism in premature infants. II. Prevention of iron deficiency. J. Pediat., *64:* 509.
14. Haughton, J. G. 1963 Nutritional anemia of infancy and childhood. Amer. J. Public Health, *53:* 1121.
15. Beal, V. A. 1961 Dietary intake of individuals followed through infancy and childhood. Amer. J. Public Health, *51:* 1107.
16. Lund, C. J. 1951 Studies on the iron deficiency anemia of pregnancy, including plasma volume, total hemoglobin, erythrocyte protoporphyrin in treated and untreated normal and anemic patients. Amer. J. Obstet. Gynecol., *62:* 947.
17. Huenemann, R. L. Unpublished observations. (See also Amer. J. Clin. Nutr., *18:* 325, 1966.)
18. Cooke, A. 1964 The return to calories. Listener, *71:* 867.
19. Editorial 1964 The American and his diet. Lancet *1:* 1373.
20. Tuttle, S. G., S. H. Bassett, W. H. Griffith, D. B. Mulcare and M. E. Swendseid 1965 Further observations on the amino acid requirements of older men. II. Methionine and lysine. Amer. J. Clin. Nutr., *16:* 229.
21. Spencer, H., J. Menczel, I. Lewin and J. Samachson 1964 Absorption of calcium in osteoporosis. Amer. J. Med., *37:* 223.
22. American Medical Association, Council on Foods and Nutrition 1962 The regulation of dietary fat, a report of the Council. J. Amer. Med. Ass., *181:* 411.
23. Watkin, D. M. 1965 New findings in nutrition of older people. Amer. J. Public Health, *55:* 548.

Some of the Developments in Food Production and Their Impact on Nutrition

EMIL M. MRAK
Chancellor, University of California, Davis, California

In developing my chain of thought with respect to what this paper should be, I found myself in a quandary as a result of the varying meanings of the word "production." I have asked several people; some answers referred to agricultural production, others referred to manufacturing. Even Mr. Webster leaves one somewhat confused for he indicates that production is the act or process of producing, bringing forth, or exhibiting to view the "production" of commodities.

Food production, therefore, can refer to anything from the farm to the factory. I have chosen, therefore, to dwell mostly on the farm production and to a lesser degree on manufacturing, as related to nutrition.

I think it would be well to look at the history of food production and the problems which have faced man from the very beginning. If one brings together the existing information or speculation on this subject, it appears to have had its beginning in the middle Paleolithic or Stone Age; the total population of the world could hardly have been more than a few hundred thousand, or perhaps one million at best. Without doubt, life was miserable and the wretches called man existed in what we might today term as poverty. Ways of exploiting nature brought meager returns, and, I suppose paradoxically, we could say that the planet was then overpopulated. The food crises that exist in some nations of the world today, have existed since the earliest times of man.

Early man lived where food was available, but after the discovery of agriculture, 10 to 15 thousand years ago in the Neolithic Age, the easily tillable land was the focus of effort. The expenditure of labor, without doubt, rose to meet food production, and as food became more available, population increased. There has been, therefore, the interplay of the very strong forces of food, labor and population and their effects on nutrition.

The development of agriculture did not come all at once. As in the case of most great developments, it progressed very slowly from the stick to wooden plow to the modern techniques. Technical agriculture brought enormous changes. It has enabled man to change from the Nomadic type of life to the more fixed type and introduced the use of products in urban areas and the storage of products for use during the non-productive period. This caused a change in food habits and, of course, still greater increases in population.

So the race went on and still goes on. And in most of the world the population is still ahead of food production and we are even using other means of exaggerating the problem in addition to that caused by agricultural production. The introduction of antibiotics, drugs and health education by the World Health Organization (WHO) has decreased the death rate, especially among the young, and increased the life span, and the birth rate continues at a roaring pace. Hence, our population increases are streaming ahead of the advances in food production. The technical know-how that has done so much for some of the new world countries and Western Europe has not found its way into many other areas of the world.

According to the Food and Agriculture Organization (FAO), only one-sixth of the

world population is well fed. Even the Royal Bank of Canada in its monthly letter has stated "of all our problems, none is more important than that of hunger." It is commonplace for others to tell us that men's social skills have not kept up with their mechanical skills. Here is another area, seldom thought of, in which men have not kept up. For 99% of the time man has been on earth he was a food gatherer, and only during the remaining one per cent has he been a food producer. He has not yet learned the new technique effectively.

In order to understand the condition of people in the underdeveloped countries, we need to compare the consumption figures of livestock products in a few areas of the world. To obtain our needed proteins from meat, fowl, fish, eggs, milk, cheese, etc., the diet of people in North America includes about 25 per cent livestock products, and that in Europe about 17 per cent; but in Asia and other parts of the world where they do not obtain needed protein, the annual food intake is 3 per cent or less. This, indeed, is a tragic situation in what we consider a modern world.

Canada publishes a food guide and in 1961 the Canadian Council on Nutrition prescribed one serving of meat, fish or poultry every day with eggs, cheese, dried beans or peas as alternatives. Compare that with the typical diet of a rice-eating country such as India. The city dweller in Canada consumes nearly 5 pounds of food per day, as compared with that of the Indian who consumes about 1.25 pounds, of which 85% is rice—deficient in protein, fats, and vitamins.

The Canadian food guide specifies further that children up to 11 years of age should have two and one-half cups of milk a day; adolescents, four cups; adults, one and one-half; and expectant and nursing mothers, four cups. In Calcutta there are 6 million people who get no milk at all. Most of the world's children must pass directly from breast feeding to a diet composed of inadequate, starchy, nutritionally poor foods. India has 20 per cent of the world's dairy cattle, yet produces less than one-sixth of the yield in more advanced countries. I saw at first hand cows in Brazil producing but a liter per day as compared with over ten liters in this country. The lack of animal feed and the failure to control diseases are the reasons.

The fact is that extremely few, perhaps less than 1%, of the people who live in India will ever in all their lives, experience what the North American family considers a good daily meal.

Why do we find this tremendous difference—this difference between North America and Europe on the one hand, and many of the other nations of the world on the other? Many things are responsible, but perhaps it can be best summed up by saying that North America and Europe are taking advantage of the technological age—the technological advances that have been made in the production, preservation, and distribution of foods during very recent times.

Let's look at some of the developments that have taken place in our own country. Great strides have been made in the development of new varieties, the control of disease and pests, soil improvement, irrigation and mechanization. The results of all these efforts have been to increase the production of foods while lowering the cost to the consumer. The manpower requirements of agriculture have decreased dramatically since 1850, when one farm worker produced products for 4 people, as compared with 1959, when he produced enough for 25 people. Crop production per acre has increased by over 40% during the past 20 years and the output per unit of total input has increased at least 20%.

As one may expect, the consumer has benefited from these changes to such an extent that the food bill today takes a considerably smaller portion of the net income than it did in 1950. Recent data of the United States Department of Agriculture show that in 1950 the food bill took about 25% of the income, whereas in 1963 it took only 18%. Although, since 1950, the trend has been to decrease, it is difficult to say how long this will continue. Not only has the cost of food gone down, but the diversity and availability of quality have improved tremendously. We know a great deal more about factors influencing the retention of vitamins and other nutri-

ents so important to health, and we have certainly taken advantage of this knowledge in our country.

These great changes in the farm producing areas have had other influences. According to Dean Butz of the Indiana College of Agriculture, only 7% of our present day working force is in agriculture, and this is still decreasing. In fact, he has pointed out that we could get along on 3%, as compared with the Orient, where they use 90%, and in Russia, where 50% of the working force is in agriculture. Dean Butz has stressed these points because many people have been thrown on the labor market, a number of whom are probably suffering from malnutrition as a result of the economic situation that has confronted them.

It seems ironic that we are living in an era of increasing farm productivity, yet in a day when we talk about poverty in our own country. In a recent monograph concerned with agriculture and the public interest, Keyserling pointed out that during the period 1947–1955, as a base period, the yield per acre of wheat rose from 17.4 bushels in the base period to 26.2 in 1964; corn from 38.8 to 62.1; and for all crops, from 307 pounds to 524.

If we use the period 1957 to 1959 as a 100% of production and from this consider the comparative production in 1964, it would be 111.

Such changes have brought forth the concern with "surpluses" in this country. Keyserling believes the situation has been overstated and indicates that the appropriate measure of the magnitudes of the so-called farm surpluses is to measure the annual use in ratio to the annual production. The ratio of consumption to production of wheat, according to Keyserling, was 86% in 1960 and 115% in 1964. He has indicated similar situations for certain other crops. This evidently means an increasing population and a decreasing production of certain types of crops.

Keyserling has pointed out that a second appropriate measurement of the magnitude of the so-called farm surpluses is the ratio of annual use to stocks accumulated over the years. In the case of wheat, the ratio was 62% in 1960 and 92% in 1964. Thus, in 1960, it could have been said that the accumulated stocks were equal to the wheat reserve for somewhat more than one and a half years; and in 1964 to a reserve of only 13 months.

The point that Keyserling is striving to develop is that the farm surpluses are really not as great as they have been politically pictured. In fact, he states that the farm surpluses in this country are a tiny fraction of other surpluses in our economy. He indicates that in 1964 the percentage of farm input not currently consumed is but 0.6%, as compared with idle steel capacity of 21%, and unused power of 11.8%, and the true unemployment as 8.3% of the civilian labor force.

I have stressed this point of view because I have a fear that many of us may have a false sense of security insofar as food production and its ultimate effect on nutrition in this country are concerned.

The technological age has done other things. We have reduced the number of food producing farms until today there are about 600,000 in the United States. The investment on the farms is great indeed. Butz has pointed out that it amounts to about 230 billion dollars, which is equal to one-third of our national debt. He has cited as a specific example, the situation in Indiana where the farm investment amounts to about $87,000 per worker and he has compared this with industry in general, which is $20,000 per worker.

If we want high production in the developing countries so the people will have an ample supply of food and eventually good nutrition—more than just calories—then modern technological procedures must be used. This means technically trained people and eventually investments in farm equipment.

During the past 20 years we have had the longest famine-free period in history. Much of this is due to the fact that we have been able to produce more in this country than needed. If such a situation is to prevail in the future then more will have to be produced, not only in this country but throughout the world. I indicated above that our surpluses according to Key-

serling amount to very little—about 0.6%. With our rising population this really isn't very great.

Not only will the world need more food in the future, but in this country, too, we will need more food. In 1960 the United States Department of Agriculture (USDA) pointed out that it will be necessary for our farmers to produce a great deal more food than is produced today to meet the needs of our surging population. It was pointed out that since 1920, our crop production per acre has increased 47%, our output per animal breeding unit is up 47%, and the output per man is up about 249%. Furthermore, the efficiency of marketing agricultural products has increased. The volume of farm products marketed has increased about 25% during the 15 years to 1960, but the number of workers involved in marketing has increased only 10%. The hourly earnings of marketing employees increased 54%, yet labor cost per unit on the farm products was up only 31%.

The question is, of course, how much more improvement can we expect? As our population grows, we will have to produce about 42 billion pounds of red meat by 1975, which is about 14 billion more than produced in 1960. Fruits and vegetables needed by 1975 will be about 57 billion tons, roughly 17 more than in 1960. Milk production needed in 1975 will be 172 billion pounds or about 42 billion more than in 1960. In the case of eggs, the requirements will increase by about 14 billion or a total of 84 billion by 1975. These are just a few examples of what will confront the farmer and why a higher density type of agriculture is essential in order to meet the needs of our growing population.

The question arises as to where we will produce this additional food. There is no doubt that we can substantially increase production per acre on the existing land. We can also do much to prevent the losses from pests during storage. In 1954, the USDA estimated that to offset losses in agricultural production caused by pests, an additional 88 million acres of land would have to be cultivated and losses subsequent to harvest would amount to the production of about 33 million acres. Losses caused by pests are still with us and they are considerable. Dr. George Decker of the University of Illinois has pointed out that we are still sharecropping with insects and pests in a most generous way. He indicates that in many cases we are producing more pest protein per acre of feed than we are of edible animal protein. The awareness of this situation offers great challenges, providing the technologists are not hamstrung by the steady stream of social protest books, which seem in some cases to bring emotions to destructive and harmful peaks before they are forgotten. The May 14 (1965) issue of the *New York Times* pointed out that Rachel Carson's rousing *Silent Spring* was such a book—one in which the author does not trouble the reader by interrupting her crusading rhetoric with references to the sources of her quotations and statistics.

The question has been raised concerning unused land areas and availability of land in other areas of the world. In the 50 states, including Hawaii and Alaska, there are about 19 hundred million acres of land and roughly 30 million acres of water. It is apparent that as population increases, the land area per person will decrease. When the pilgrims arrived in our country the Indians had about 2,500 acres per person; today we have only 11 acres per person. In the future, of course, this will decrease further and perhaps even before the year 2000 the average per person may be half of what it is today. The picture, however, is not as good as it may appear on the basis of what I have just said. I think we can approximate that about a quarter of the 19 hundred million acres of land (less than 5 hundred million) may be considered good agricultural land now in use. If our requirements for agricultural production increase, it might be possible to add another 150 or 200 million acres by the introduction of irrigation, the use of drainage, deforestation, and the conversion of a great deal of pasture land to highly cultivated farms.

Our agricultural land is decreasing at the rate of several million acres a year as a result of the conversion of some of the finest soil into ribbons of highways, air-

ports, sprawling cities, and so on. In California over 100,000 acres per year are going out of production. Of course, if people change their ways and stop smoking so much, there is a considerable amount of tobacco land that could be used for the production of fiber and for use as pasture, but this remains to be seen.

The land picture in the world is such that there is little hope of bringing in vast new acreages. There may be opportunities in arid areas but we do need arid land research before this can be done.

One of the answers to the problem in this country, I believe, will be the development of high density agriculture. There may be some reduction in quality for whenever we mechanize or move toward high production and lower cost, we sacrifice some quality. In doing this, however, we will have animals and plants closer together and in some cases, can produce more crops per year. Under such conditions it will be necessary to use procedures that may very well be frowned on by certain vocal individuals.

The use of agricultural chemicals has been severely criticized in many circles. High density agriculture will certainly require the use of these agents to control insects, plant pathogens, nematodes, weeds, animal diseases, etc. Furthermore, it will necessitate the use of defoliating agents and growth-control chemicals. These must be used in a manner that will insure the maintenance of residues at low levels and within tolerances established by the Food and Drug Administration.

But what about the rest of the world? If the evolving countries are not ready for such advanced technology, the only answer is to train people to apply technology that is compatible with the development of the country, with the view of improving it at as rapid a rate as possible. Then too, there is much to learn about these lands: What crops will grow best? Where will be the greatest yields? What would be best from the standpoint of nutrition? Here, too, we must establish our views on what an evolving nutrition should be. Should we aim for calories first—volumes of food—or do we wish to develop a fully balanced diet immediately? Truly these matters need judgment and must be studied carefully. If we can teach people to give up the stick for the wooden plow, we can go to more complicated agriculture quickly. We should continue to orient them more and more to advanced technology until the time comes in the not-too-distant future when they may very well be able to handle the complicated procedures used in the more advanced countries today. But preservation and distribution must also be considered. In the case of preservation, at first simple methods should be used, moving eventually to more advanced procedures, such as canning. Indeed, the world food problem is complicated and must progress in an evolutionary way. I sincerely hope that it is a rapid evolution.

I had an opportunity to observe one of the Rockefeller operations termed ACAR in Brazil. In this operation the farmers are actually shown how to plant seeds, how to cultivate and how to develop small farms with the view of eventually developing larger, better and more productive ones. The ACAR loans small quantities of money if needed, and I must say that the loans have almost invariably been repaid.

In this country, we may reach the maximum production before too many years go by, but we may very well be confronted with other problems. A serious one is the possibility of changing food habits. We should understand what brings these about—some may result from the manner of living and others from nutrition and medical discoveries. We walk less, we exercise less, and so we need fewer calories. Accordingly, the consumption of cereals has been decreasing for a number of years, while that of fruits and vegetables has increased. Then, again, there are certain scientific observations related to health that have had a profound influence on agricultural production. As the momentum of medical discovery increases, nutritional discoveries increase, and we can expect more of this in the future.

The cholesterol problem has certainly influenced food habits insofar as those containing unsaturated fats are concerned. I can assure you that the producers of eggs

have felt the impact of publications relating to cholesterol. Studies in these areas are important, and understanding of the total problem is essential to agriculture and food production. Nutritional studies by such agencies as the Heart Institute and the National Institutes of Health may appear far removed from agricultural production, but this is hardly the case, for some of the observations made in those institutions may well influence food habits, which in turn, will influence farm planning and production.

Recently, Dr. Mark Hegsted of Harvard made some pertinent remarks with respect to nutrition and agriculture. He pointed out that 20 years ago the nutritionists could set up the basic seven; but it has become clear that those happy days are gone. Obesity, cholesterol and polyunsaturates have become household words. Dr. Hegsted remarked that the evidence continues to accumulate that the diet which nutritionists and agriculture promoted so vigorously in the past, may not be the ideal diet. He indicates further that though we must maintain the gains we have made in health in the past 30 or 40 years, we must not necessarily maintain the same diet. It is clear to me, therefore, that discoveries may change food habits, which, in turn, may very well change the food production.

Finally, I would say that the direct production of protein from field crops looks like a real possibility for the not-too-distant future. In order to obtain 3,000 calories per day, an individual must eat the equivalent of about 1.8 pounds of plant material consisting of 0.7 pounds of carbon per day. From the standpoint of food technology, it is conceivable that eventually we might be compelled to change our diet pattern and eliminate the middle man, or the animal. At present we grow a great deal of vegetable matter and feed it to animals, and animals in turn are used for human food. The efficiency is between 5 and 10% depending upon the animals and the conditions of growth. As an example of this, forage grains for steers yield about 43 pounds of protein per acre, silage for milk 77 pounds, and unprocessed soy beans about 450 pounds per acre. We might very well learn to obtain our protein directly from alfalfa. In areas that grow several crops per year, the yield could be as high as 1,500 pounds per acre—for example, in Imperial Valley. This, of course, is the extreme.

I believe, however, that the future will be more a matter of technology than geography. When speaking of technology, I am thinking in the broadest terms involving production on the farm, preservation, distribution, storage, etc. I am thinking in terms of producing foods that offer the individual the best in nutrition.

In the developing nations, we will be confronted with the necessity of continuing education in the areas in technology. In our country, I believe, there will be the necessity of continuing education, but of a different type. The farm producer of food is finding himself more and more in a cost-price squeeze in spite of increased efficiency and high production. Gross receipts to the farmer are decreasing, while production and other expenses are continuing to increase. The USDA points out that the gross farm income for 1964 is expected to drop slightly below the 41 billion of 1963. At the same time the USDA states that with an increase in farm expenses, the net increase for 1964 will likely be lower and, in fact, may even be as much as 5% below the twelve and one-half billion estimated for 1963. It is quite possible that this trend may continue for some time to come. Production inputs have risen since 1960, and the total farm expenses have increased above 45%. Such costs result from increases in taxes, wages, real estate, labor, farm machinery, fertilizers, and other materials used on the farm. The farm producer of food, therefore, finds himself more and more in the cost-price squeeze in spite of increased efficiency and high production per acre.

He must be able to minimize costs and especially to use the modern methods of technology. If we increase the cost in this country, and if we handicap his use of such materials as agricultural chemicals, then the impact will be great both in the cities and in the urban areas. When people realize the facts and become educated to

what confronts all of us from the standpoint of availability, cost and nutritive value of food, then there may be a difference. Today, the urbanite is impressed with what he has been told about surpluses—to him, the abundance and variety of nutritious foods. I fear the worm of abundance has really spoiled our people, but I also believe it won't be many years before this worm will be turning.

CLOSING REMARKS

AGNES FAY MORGAN

Dr. McCollum, Dr. Morgan and Dr. György at reception prior to the Agnes Fay Morgan Symposium, University of California, Berkeley, May 8, 1965.

Closing Remarks by Agnes Fay Morgan

When I came to the University of California in 1915 I first met President Wheeler as I was on my way to my laboratory and he was taking a horseback ride around the campus as he frequently did. He recognized me and stopped to chat. We discussed prospects of nutrition teaching and research, he on horseback, me on the ground. Of course there may still be men on horseback on the campus, but only figuratively.

There were only a few buildings then but the campus was far more beautiful than it is now. At that time the classicists ruled this and other universities but the scientists had already established a foothold and were destined to go on irresistibly, like the Conquistadors of Latin America. G. N. Lewis had just come here from Boston, Joel Hildebrand from Pennsylvania, H. M. Evans from Johns Hopkins, K. F. Meyer from Zurich. T. B. Robertson, the biochemist, had succeeded Jacques Loeb and was laying a solid foundation in his field. Andrew Lawson, the eminent geologist and rebel, was about ready to start the faculty revolution of 1919.

My first troubles were budgetary. A tight-fisted board of research gave us $600 one year for the research of the department and I considered this a remarkable windfall. However, I found later that they had given the Chemistry Department $13,000 for their research. This I considered obviously unfair. I think no one would agree with me then or now.

The support we later received from both President R. G. Sproul and Dean C. B. Hutchison was indeed heartening. There was never any wavering in their belief in the need for both teaching and research in food chemistry and nutrition and in the encouragement and support of women students and teachers of these subjects. Our faculty colleagues in chemistry, biochemistry and the biological sciences were likewise generous in cooperation and support of the newcomer, nutrition. I mention in particular David Greenberg, C. L. A. Schmidt, I. L. Chaikoff, H. M. Evans, W. V. Cruess, Sam Lepkovsky, M. A. Joslyn, G. Mackinney, Emil Mrak, and K. F. Meyer.

There wasn't much in the books on nutrition then. Graham Lusk brought out his remarkable *Science of Nutrition* in 1906, Sherman, his *Chemistry of Food and Nutrition* in 1911 and McCollum, the *Newer Knowledge of Nutrition* in 1918. This was about all there was in English. I found Carl von Noorden's three huge volumes on "Metabolism and Medicine" in German in the library and spent much time poring over them. I decided that there was too much unknown and that we should at once start several lines of research, so as to have something to teach.

We began with pedigreed rats and mice left us by T. B. Robertson and E. S. Sundstroem when they left Berkeley in 1918, then we graduated to rhesus monkeys and dogs in collaboration with K. F. Meyer and Hermann Becks. Pigeons came next in the study of the effect of sulfuring and drying on the thiamine of fruits. We maintained an inbred colony of cocker spaniels for some 16 years studying vitamin metabolism in 12 generations and about 1,200 dogs.

We had many distinguished summer residents in our department, all of whom brought us substantial contributions of ideas and enthusiasm. In 1920 Louise Stanley, who later became the first director of the Bureau of Home Economics in the United States Department of Agriculture (USDA), spent the summer in our department. Her illustrious successor Ruth Leverton, one of our speakers today, now directs that bureau, which has undergone a number of changes of name.

In 1922 Lafayette Mendel spent the summer session here welcomed by Icie Macy

Hoobler who was one of his distinguished students and a member of our department. In 1923 Dr. E. V. McCollum was here. I believe our real start in modern nutrition began with these visits. My son was born in May, 1923, and I was quite excited and pleased over that event. Dr. McCollum remarked that if I had just had my fifth as he had, I'd hardly notice the event! I didn't believe this then nor do I now. It is gratifying to welcome Dr. McCollum here 42 years later and to find that his dry humor is as delightful as ever.

I well remember my first meeting with Paul György in 1937. The vast extent of his researches in the B vitamin complex astonished and inspired us all. Those were exciting days, with Dr. György out in front on riboflavin, vitamin B_6, pantothenic acid, and biotin, leading the way for a large group of us to follow. Tom Jukes and Sam Lepkovsky were among the leaders too and I was indeed lucky to have their encouragement and cooperation.

The credit that I have been given so generously for the researches done in our department should be shared by the young women who had the ideas, carried out the experiments and wrote up the results. I encouraged them, criticized, added my interpretation of the data. They should be standing here beside me, about 100 able dedicated women. Quite a few of these former students have carved professional niches for themselves in the ranks of nutrition teaching and research. I'll mention a few who are here from near and far today. Laura Lee W. Smith from Cornell, Mary Rose Gram from the University of Tennessee, Lotte Arnrich from Iowa State University, Carey D. Miller from the University of Hawaii, Florence B. King from Vermont, Margaret Morehouse of the University of Southern California, Margaret Chaney of Connecticut, Vera Mrak of Davis, Gladys Emerson of the University of California, Los Angeles, Lucille Hurley of Davis, Hazel Murray of Davis, Lura M. Morse and Nobuko Mizuno of the University of Minnesota. Many others of the Berkeley department and local residents belong in this distinguished list. There were men students also of merit and distinction. Col. R. R. Guehring and Captain Kozad of the U. S. Army Quartermaster staff and Harold W. Carroll of the Naval Radiological Research Laboratory were among them.

But I am more interested in the future of nutrition than in its past. If we could find some way of adding the word "molecular" to our department name we might be able to command more prestige, funds and followers. The no-holds-barred struggle for that word by several departments lately has been most enlightening. However, whatever the name, many grave problems of nutrition remain unsolved. For example, the mode of action and functions of vitamins C, A and D are not yet known. Probably this is true of vitamin E as well, although I sometimes think this may be a nonspecific antioxidant reaction. George Briggs doesn't agree. He connects it with selenium and other substances. Harold Olcott has some different ideas about this also.

What further genetically controlled errors of metabolism remain to be uncovered such as the lately detected phenylpyruvic acid-galactose problems? What other birth defects such as hare lip and club foot, formerly thought to be hereditary or an act of God, may be shown to be due to nutritional failures during pregnancy? Lucille Hurley today described such errors in small animals as due to a lack of pantothenic acid, manganese or zinc. Other such failures remain to be detected. Tom Jukes has shown us that enriched nutrition may influence the genetic picture. This is a dazzling new direction for the future.

Emil Mrak has outlined what marvels of improvement in the lot of the ill-fed millions of the world can be achieved by increased production through enlightened agriculture and food technology. Collaboration with the nutritionists is indeed a must for this end.

What is the mechanism of serum cholesterol control? Is there a demonstrable relationship between dietary fats, serum cholesterol and coronary heart disease or stroke? So far we have statistical possibilities, commercial exploitation of "polyunsaturates," even in dog foods, but no acceptable scientific evidence. Does sugar

rather than fat raise the serum cholesterol and predispose to atherosclerosis? On this point we have studies of food habits, records of average food intakes over the years, a few clinical studies of serum levels but not solid experimental evidence. Dr. Griffith has pointed to the bewildering array of new information on the synthesis and catabolism of fat and has indicated the need for information on the multicellular as well as the intracellular metabolism of the fatty acids. His plea for consideration of the whole man in the study of fats, carbohydrates and atherosclerosis is indeed sound.

Is atherosclerosis inevitable with aging? What are the normal changes due to healthy aging? One of the most discouraging findings in aging people is the degeneration of muscle, with increases in fat and water content, loss of protein and loss of functioning cells. Is this necessary? Can it be prevented by exercise or change in some dietary constituent? I asked one of my friends who has been watching his weight, what success he was having in reduction. He's been studied lately by Captain Behnke, who weighs his subjects under water and makes a number of body measurements. He replied, "My weight hasn't changed but my composition is all shot to pieces." Further research on body composition under many conditions of age, diet, sex and exercise are needed and some are now under way in our department.

As Gladys Emerson told us today our own nation is, on the whole, well nourished, perhaps over-nourished but there are weak spots in the overall picture. Iron deficiency anemia, obesity, coronary disease, osteoporosis in older women, amino acid imbalance in older men are some of the conditions probably amenable to nutritional treatment.

These are only a few of the puzzles of nutrition. They have been known for years and much work has been done on each of them but no definitive solutions have yet been found. "Molecular" or not, the future of nutritional sciences is bright and attractive in its chance of serving science and humanity. I should like to eavesdrop on the Symposium of a Hundred Years of Nutrition Research in 2015.

ACKNOWLEDGMENTS

The Symposium was sponsored jointly by the Department of Nutritional Sciences, University of California, Berkeley, and the Dairy Council of California. Its organization was under the direction of Richard L. Lyman, Chairman, George M. Briggs, Rosemarie Ostwald, Mary Ann Williams, and E. L. R. Stokstad.