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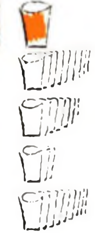

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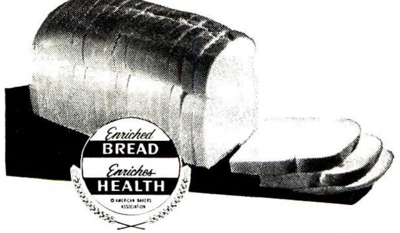

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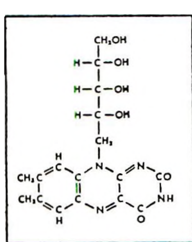
The Vital Story

A Quick History. Independent investigators, working separately to unlock several of nature's doors, sometimes open up unsuspected relationships. This happened with vitamin B_2.

Investigations. About 25 years ago, several groups, notably Warburg's, were investigating a "yellow enzyme" obtained from yeast. Almost simultaneously other investigators were studying a food factor that aided growth of laboratory animals.

What they found. Proceeding with chemical analysis of this growth factor, the team of Kuhn, György, and Wagner-Jauregg noted a relationship between the growth-producing agent and the "yellow enzyme." Their findings, and those of other researchers along similar lines, were published in 1933. Eventually, riboflavin and an essential part of the yellow enzyme were found to be identical and the unity of an essential nutrient and cellular metabolism was established.



Isolation of pure riboflavin was achieved by Kuhn and his co-workers, and by Ellinger and Koschara, in 1933.

Nomenclature. Known in the United States as riboflavin, this vitamin has also been called lactoflavin, ovoflavin, hepatoflavin, and vitamin G.

SYNTHESIS

By 1935, two eminent chemists, working separately, had synthesized riboflavin, practically in a dead heat. Prof. Paul Karrer of the University of Zurich, a collaborator of the Hoffmann-La Roche Laboratories, produced the first successful synthesis. Five weeks later Richard Kuhn of Germany announced his synthesis of the vitamin. Prof. Karrer subsequently shared the Nobel Prize in Chemistry for his work in vitamins and carotenoids.

The Karrer synthesis forms the basis for chemical processes in widespread use today by Hoffmann-La Roche and other leading manufacturers throughout the world. Riboflavin is also manufactured today by fermentation methods.

CHEMICAL AND PHYSICAL PROPERTIES

Riboflavin is yellow, slightly water-soluble with a greenish fluorescence and a bitter taste. Its empirical formula is $C_{17}H_{20}N_4O_6$. Vitamin B_2 produced by the Roche process is identical in every way with that occurring in nature.

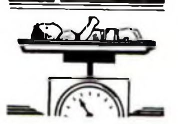
How does vitamin B_2 work? Riboflavin is a vital part of nature's chain of reactions for utilization of carbohydrate energy. It has been found to be a constituent of many enzyme systems and is thus intimately connected with life processes. It is probably required by the metabolic processes of every animal and bird as well as by many fishes, insects and lower forms of life. (In certain animals, however, the requirement may be synthesized by bacteria within the intestine.)

In the cells riboflavin goes to work attached to a phosphate group. This substance, known as riboflavin-5′-phosphate or flavin mononucleotide, may in turn be attached to still another essential substance, adenylic acid, forming flavin adenine dinucleotide. Either nucleotide then is attached to protein, thereby forming an enzyme, and takes its part in oxidation-reduction reactions.

Requirements in Human Nutrition. As we have seen, vitamin B_2 is essential to life. We have no special storage organs in our bodies for this vitamin, although a certain level is maintained in various tissues, with relatively large amounts found in the liver and kidneys.

MEASURING METHODS

In the beginning, riboflavin activity was described in "Bourquin-Sherman units" and requirements were thought to be very small. Subsequent research showed otherwise. Milligrams of weight became the unit and the Food & Drug Administration of the U. S. Dept. of Health, Education & Welfare established (July 1, 1958) a minimum daily requirement of 1.2 mg. of riboflavin for all persons 12 or more years old. For infants it is 0.6 mg. These requirements are designed to prevent the occurrence of symptoms of riboflavin deficiency disease. The minimum daily requirement for this vitamin for children from 1 to 12 years is 0.9 milligram.



Recommended allowances. The Food & Nutrition Board of the National Research Council has recommended the following daily dietary allowances of riboflavin, expressed as milligrams. These are designed to maintain good nutrition of healthy persons in the U. S. A.

Men	1.6	
Women	1.4	
" (3rd trimester of pregnancy)	2.0	
" (Lactating)	2.5	
Infants, 1-3 months	0.4	
" 4-9 "	0.7	
" 10-12 "	0.9	
Children, 1-3 years	1.0	
" 4-6 "	1.2	
" 7-9 "	1.5	
	Boys	Girls
Adolescents, 10-12 years	1.8	1.8
" 13-15 "	2.1	2.0
" 16-20 "	2.5	1.9

—of VITAMIN B_2 *by* Science Writer

(Riboflavin)

Deficiencies of vitamin B_2 appear in several ways in human beings. The eyes, the skin, the nerves, and the blood show the effects of too little riboflavin. Laboratory animals have demonstrated that a riboflavin-deficient diet can cause death of adults and can slow or stop growth in the young. Female animals, deprived of riboflavin in the diet, may produce offspring with congenital malformations.

Medical uses. To overcome and control deficiencies in human beings, physicians have pure riboflavin available for administration by injection or orally, by itself or with other "B" vitamins or multi-vitamin-mineral combinations.

How do we get our daily riboflavin? Vitamin B_2 has wide distribution throughout the entire animal and vegetable kingdoms. Good sources are milk and its products, eggs, meats, legumes, green leaves and buds. Whole-grain cereals have significant but not large amounts of riboflavin.

ADDITION TO FOODS

Cereal foods play a large part in our diet. To produce the white flour almost all of us want, millers are obliged to remove parts of the wheat that contain much of the grain's riboflavin and other nutrients. In addition, cereal grains are not rich sources of riboflavin. Millers meet this problem by *enriching* the grain foods for which federal standards exist with vitamins B_1, B_2, niacin and the mineral iron. In the case of vitamin B_2, however, they do more than *restore* the processed food to its natural riboflavin level; they *fortify* the food with enough of this essential vitamin to make it nutritionally more valuable than it was in nature.

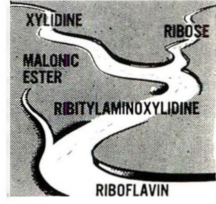
Acting to protect the good health of millions of Americans, bakers and millers adopted *enrichment* of white bread and white flour in 1941. Since that time, other foods, such as macaroni products, corn meal and grits, farina, pastina and breakfast cereals have had their food value increased by enrichment with pure riboflavin and other vitamins and minerals.

When enriching, fortifying or restoring, food manufacturers add the necessary quantity of riboflavin (and other vitamins and minerals) to the food during processing, so that the finished product meets federal, state, and territorial requirements or contributes to the consumer an amount of the vitamin that dietary experts believe significantly useful.

PRODUCTION

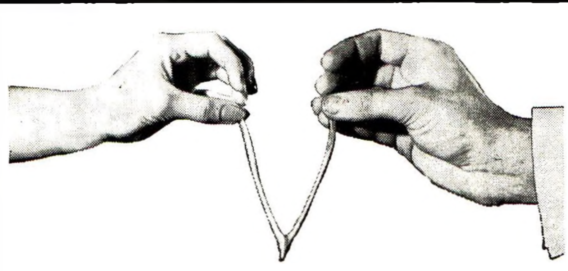
Prof. Karrer's synthesis of riboflavin was a laboratory success. Adapting the process to commercial production, however, demanded original thinking by chemists at Hoffmann-La Roche. The production of riboflavin by chemical synthesis requires the production of ribose, a rare sugar, at an early stage in the process. This special sugar must be made inexpensively if the synthesis is to be practical. Sugar chemistry is a difficult matter. In a brilliant piece of work, the Roche chemical experts developed a method to produce ribose on a commercial scale by an electrolytic process, thus overcoming a most troublesome problem. Subsequently, Roche chemists developed the first practical synthesis for riboflavin-5′-phosphate, identical with natural flavin mononucleotide.

Picture three streams joining to form a river and you have a simplified idea of the Roche process for synthesizing vitamin B_2. O-xylene and glucose are processed separately to form xylidine and ribose respectively. These are joined to form ribitylxylidine, which is then converted to ribitylaminoxylidine. Starting separately with malonic ester, which is processed through intermediate stages to alloxan, the third "stream" is then joined with ribitylaminoxylidine to form riboflavin. Purification occurs at each step of the synthesis. Riboflavin 'Roche' equals or exceeds U. S. P. standards.



By the tons. So efficient is the Roche process that pure riboflavin is produced *by the tons* for use in pharmaceutical products and processed foods. An interesting development by Roche is the production of riboflavin in different forms related to the method of end use. 'Roche' Regular riboflavin U. S. P. is especially useful in dry enrichment premixes, powdered dietary supplements, pharmaceutical tablets and soft gelatin capsules. 'Roche' Solutions type is preferred for the manufacture of solutions having low concentration. 'Roche' Riboflavin-5′-Phosphate Sodium is a highly and rapidly soluble riboflavin compound favored for all pharmaceutical liquid products and some tablets, lozenges, and capsules. It has a more pleasant taste than the bitter U. S. P. riboflavin.

This article is published in the interests of pharmaceutical manufacturers, and of food processors who make their good foods better using pure riboflavin 'Roche.' Reprints of this and others in the series will be supplied on request without charge. Also available without cost is a brochure describing the enrichment or fortification of cereal grain products with essential vitamins and minerals. These articles and the brochure have been found most helpful as sources of accurate information in brief form. Teachers especially find them useful in education. Regardless of your occupation, feel free to write for them. Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 1956 Bourdon St., St. Laurent, P. Q.



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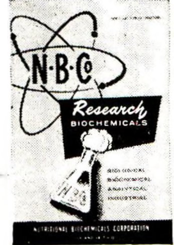

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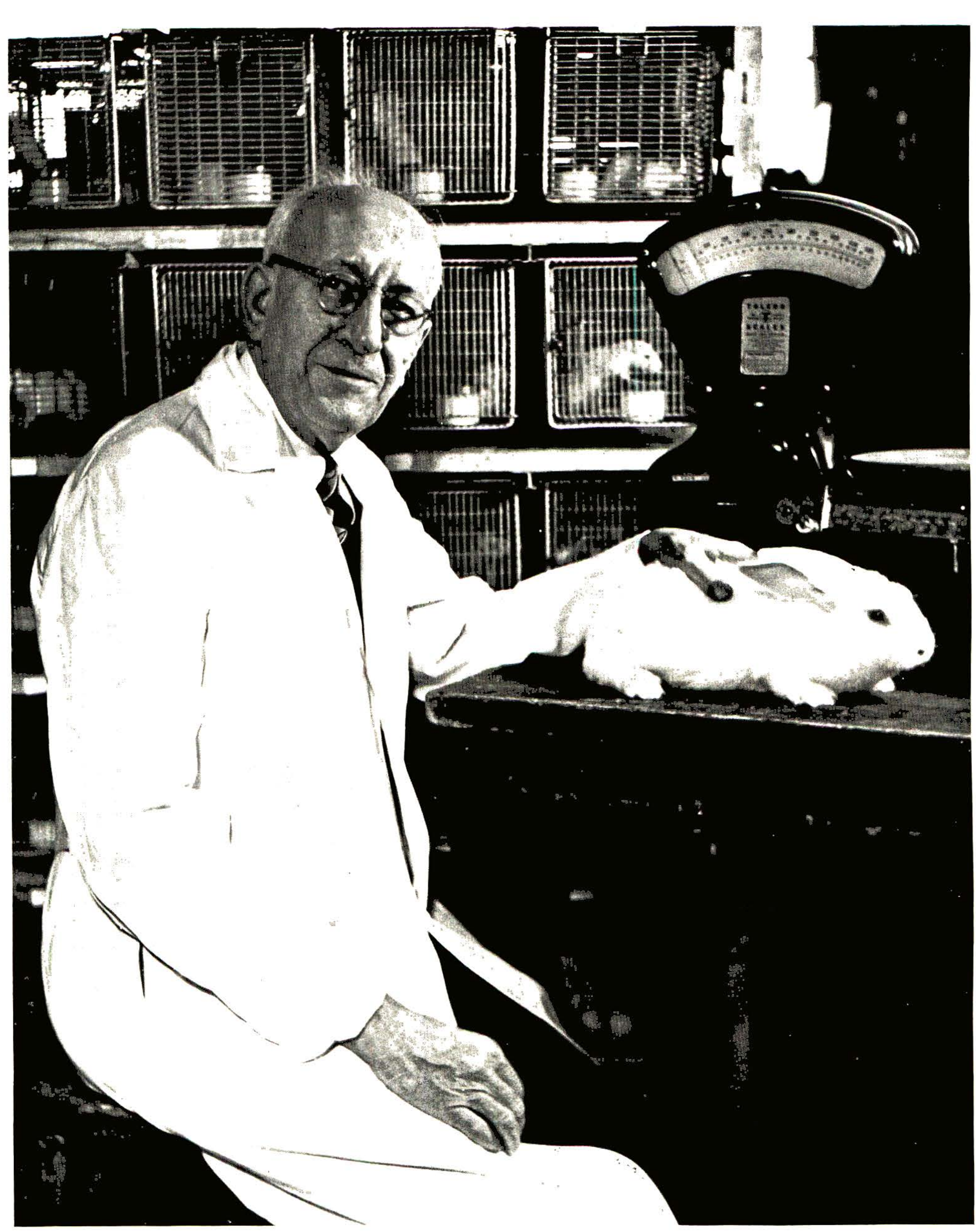
HENRY ALBRIGHT MATTILL

1883 – 1953



HENRY ALBRIGHT MATTILL

HENRY ALBRIGHT MATTILL

(November 28, 1883 — March 30, 1953)

In the biochemical teaching area of the new Medical Research Center at the State University of Iowa there has been placed a small bronze plaque, dedicated to the memory of Henry Albright Mattill, 1883-1953, Professor and Head of the Department of Biochemistry, 1927 to 1952.

Henry Mattill took no little pride in having had the opportunity at Iowa to work with some 2,000 medical students. To him, teaching was not an onerous obligation, but a privilege to be guarded jealously. His presentations were methodical and well-organized. To have overstressed his own field of research interest in teaching would have violated his sense of fair play. Beyond the introduction of subject matter, he strove to "cultivate unprejudiced objective thinking" and to emphasize "the human side of science and its intellectual and social implications." Through the classroom and the office he sought to mould his students as individuals into well-integrated personalities.

To many a graduate student he was a wise and helpful counselor, to others a Dutch uncle. In the days when financial aid was not so readily available as now, the gracious extension of a small loan or of assistance in securing a job eased many a crisis known only to the student involved. Staff and graduate students were members of a closely knit biochemical family, a feeling attributable in no small measure to the delightful hospitality extended several times each year through picnics or suppers at the Mattill home. The event often included the showing of exquisite Kodachrome slides depicting a vacation in the western mountains or in Europe, the reading of selections from the Pickwick papers or other gems of English literature or from the biography or writings of some famous scientist, or the rendition of a piano selection from the works of one of the master composers.

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Henry Mattill had grown up in a parsonage. His mother was a lover of music who had begun his piano training at the age of 7 and had instilled in her son a passion for beauty. There is little doubt that early exposure to strict religious discipline had also influenced profoundly his attitude toward life. He was born in Glasgow, Missouri, November 28, 1883, but during his early years his family had moved to Cleveland, Ohio where his father became joint manager of his denomination's publication house. His family was bilingual (German and English), its chief outside interests centering in the church. Upon his graduation from Cleveland Central High School, second in his class in scholarship, he entered Adelbert College, the undergraduate college for men at Western Reserve, with a student body of 250. In those days the curriculum provided little choice. Major emphasis was placed on the classics, other languages and literature, with the physical sciences second in importance. The faculty, which numbered about 25, included several noted scholars and others who were excellent teachers. One of these was Professor Edward W. Morley, head of the Chemistry Department. Professor Morley had begun his career as an ordained clergyman, but had later turned to science. Though largely self-taught, he had become a noted figure, remembered today for his accurate determination of the relative atomic weights of hydrogen and oxygen and for his participation in the famous Michelson-Morley experiment to determine the existence or non-existence of an ether drift. It was to his admiration for Dr. Morley as a man, a scientist and a teacher that Henry Mattill traced his motivation toward a career in chemistry. One of his prized possessions was a copy of the Smithsonian publication, "On the Densities of Oxygen and Hydrogen and the Ratios of their Atomic Weights," by Edward W. Morley, Ph.D. Above the desk in his office at the State University of Iowa there hung a framed photograph of Morley. Extracurricular activities at Adelbert centered primarily about the Glee Club, which he served as accompanist, business manager, and president, and the Y.M.C.A., which he represented in a number of

state and national meetings. In his sophomore year he was awarded a two-year honor in French. He graduated with an A.B. degree *magna cum laude* in 1906 and was elected to Phi Beta Kappa. In 1907, having completed the required work, he was granted the degree of Master of Arts in absentia by Western Reserve University.

Henry Mattill entered the University of Illinois at Urbana for graduate study in chemistry in the fall of 1906. He had wavered between chemistry and medicine as professions and it was undoubtedly in the hope of merging these interests that he transferred from agricultural chemistry to biochemistry when the division of physiological chemistry was instituted in 1907, with Philip B. Hawk as its first chairman.

Hawk had been an assistant in chemistry under Atwater, had studied under Chittenden and Gies, and had introduced Chittenden's course in physiological chemistry at the University of Pennsylvania in 1903. The period was one of transition from the old-fashioned medical chemistry to experimental physiological chemistry. Scurvy, beri-beri, and rickets were known, as were certain dietary measures which could be taken to prevent or cure them, but their etiology was obscure. Hopkins had just reported experimental evidence which indicated that no animal could live on a mixture of pure protein, fat and carbohydrate, even when the necessary inorganic material was supplied, but that "accessory factors" were also required. The use of the small experimental animal, so important in the development of our knowledge in this area, was as yet unique. It was not until Funk reported his concentration of the anti-beriberi substance from rice polishings in 1912 that attention was focused on the concept of "deficiency diseases" and "vitamins." The idea of protein quality was vaguely understood, but it took the brilliant studies which Osborne and Mendel first reported in 1912 to clarify the picture. The roles of the enzymes in digestion and fermentation had reached the stage where their classification as "organized" and "unorganized" ferments had been shown to be no longer tenable, but the dependence of their activity on pH

was not recognized until 1909 and but little evidence was yet available as to their probable chemical nature. The stage was not yet set for modern nutrition.

The title of Henry Mattill's doctoral dissertation was, "The influence of water-drinking with meals upon the digestion and utilization of proteins, fats, and carbohydrates." In those days the medical profession and the general public were convinced that water "should never be drunk at meals, and preferably not for at least one hour after the meal has been eaten. The effect of drinking water while eating is first to moisten the food, thus hindering the normal and healthful flow of saliva and the other digestive juices; secondly, to dilute the various juices to an abnormal extent; and thirdly, to wash the food elements through the stomach and into the intestines before they have had time to become thoroughly liquefied and digested. The effects of this upon the welfare of the whole organism can only be described as direful." The experiments involved two subjects who ingested three identical meals of graham (or oatmeal) crackers, peanut butter, butter, milk and water each day, with smaller quantities of water at stated intervals, for 11 to 28 days. Twenty-four-hour urine collections were analyzed for total nitrogen, ammonia, urea, creatinine, creatine, total and ethereal sulfates, and indican by the methods which then prevailed (Folin's newer procedures were not yet available). Analysis of the feces was made on each stool, in the fat series by saponifying, and estimating the fatty acids gravimetrically; in the protein series by determining the total fecal nitrogen and its partition into bacterial, 0.2% HCl soluble, acid-alcohol soluble, and residual nitrogen; and in the carbohydrate series by hydrolyzing with acid, rendering alkaline with sodium hydroxide, and determining the reducing power of the filtrate by the newly available Benedict method. Suffice it to say that the results of the study plainly indicated that "many desirable and no undesirable effects were obtained by the use of water with meals, and in general, the more water taken the more pronounced were the benefits." The data are incorporated in three publications in the Journal

of the American Chemical Society. The time-consuming nature of the studies outlined did not prevent other experimentation during these 4 years. Publications include a short report on "The diastatic enzyme of ripening meat," with A. W. Peters, and complete publication of "A method for the quantitative determination of fecal bacteria," with Hawk, and of three fasting studies with Paul E. Howe and Hawk: "Nitrogen partition of two men through seven-day fasts following the ingestion of a low-protein diet; supplemented by comparative data;" "Influence of an excessive water ingestion in a dog after a prolonged fast;" and "Distribution of nitrogen during a fast of 117 days" in which the same dog was involved. The Ph.D. degree was conferred in 1910.

Despite his research activity, Henry was able to augment funds furnished by assistantships and scholarships by serving as organist in the Methodist church in Urbana. He also found time for social life with a group of Gamma Alpha friends, including W. W. Cort in Parisitology, Warren Stifler in Physics, Alan Gleason and E. S. Reynolds in Botany, and Henry Rietz in Mathematics. It was at Illinois also that he met Helen Isham, Ph.D. Cornell, 1906, then a member of the Chemistry staff, who became his wife in 1912.

In the 10 years following his Illinois days, there was little opportunity for research. From 1910 to 1915 Henry Mattill served as assistant and associate professor at the University of Utah, where he taught biochemistry and physiology to the preclinical medical students and hygiene to all of the undergraduate men in the university. It is of interest to note that in the interval at Utah there appeared a paper with Mrs. Mattill on "Some metabolic influences of bathing in the Great Salt Lake." In 1915, disagreement between the staff and the administration led 20 of the faculty members to resign. Henry Mattill accepted a new position as Assistant Professor of Nutrition in the College of Agriculture at the University of California in Berkeley and was just becoming well-oriented when he was asked by Dr. John R. Murlin, then Lieutenant Colonel of the Sanitary Corps and director of the Division

of Food and Nutrition, Medical Department, U. S. Army, to take over the management of one of the nutrition teams whose business it was to improve the quality of the army's food and diminish its waste. Sharing in this effort in army camps in the south and with the expeditionary forces in France were Carlson, Shaffer, Woodyatt, Gephart, and many others.

Upon his discharge in July 1919, he accepted Dr. Murlin's invitation to join his staff as Professor of Biochemistry in the Department of Vital Economics at the University of Rochester. The medical school had not yet been established. Murlin's department was specially endowed, with little teaching responsibility and ample opportunity for research. By this time interest in "modern nutrition" was gaining momentum and Henry Mattill turned his attention to the nutritive properties of milk, with special reference to growth and reproduction in the albino rat. His initial study with Conklin ('20) suggested that the inability of rats to reproduce on a diet of milk alone might have been due to the lack in milk of substances necessary for successful adolescent growth and reproduction or to the presence in milk of substances inhibitory of growth in the adolescent and mature growth periods. The latter possibility had been inferred from a report by Osborne and Mendel of reproduction in rats fed whole milk powder, lard, and starch. It was investigated first (Mattill and Stone, '23) because of the partial reproductive success which had attended the supplementation of milk powder with starch and butter fat, possibly through dilution by these foodstuffs of an inhibitory factor in the milk. A footnote added to this second paper is of interest, "As this paper was going to press Evans and Bishop announced the existence of a substance, X, found especially in green lettuce leaves, whose presence in the diet is necessary to secure normal reproduction. Placental rather than ovarian function was improved by its addition. Several of our observations are confirmatory of theirs and the assumption of still another unknown dietary requisite would perhaps explain the reproductive failure we have thus far not been able to correct on milk rations." The work announced by

Evans and Bishop was published in full in the same month (March, 1923) that the paper of Mattill and Stone appeared. In less than a year, confirmation of these findings was had in the papers of Sure who recommended that substance X be called vitamin E. Evans' original interest had been in the mechanism of the estrus cycle which he found to be normal on his experimental diets. The rats would breed, ovulate, and conceive, yet fetal death invariably occurred. He subsequently comments (J. Am. Med. Assoc., *99,* 469 [1932]) "It is interesting that while experimenting with other food mixtures and engaged in other aims essentially the same conclusion and interpretation was quickly reached by Barnett Sure and H. A. Mattill, and the work of these investigators appeared so promptly that they must almost be reckoned with the original finders as promulgating the new conception and securing for it at once favorable attention."

However, Mattill had also called attention to sterility in the male. In his paper with Stone he stated "From the functional tests, from the weight of the testes, and from histological examination of sections it is concluded that male rats on milk rations suffered a gradual decline in reproductive function which becomes complete toward 200 days of age." A third paper with Carman and Clayton, published in 1924, presents an extensive description of the progressive testicular degeneration observed.

The variability of reproductive behavior with type and quantity of dietary fat led to the supposition that the vitamin E content of a foodstuff might depend not only on the amount originally present, but also upon its degree of destruction. This was confirmed by observations which showed a correlation between the degree of susceptibility to autoxidation of several fats with the reproductive behavior of rats reared on dietary mixtures in which these fats were incorporated. Oxidation could be accelerated by prooxygenic or delayed by antioxygenic substances, and there was some indication that vitamin E itself might possess antioxygenic activity.

In the fall of 1927, Dr. Mattill accepted a professorship at the State University of Iowa where he succeeded V. C. Myers as Head of Biochemistry, at that time recognized as a department in the College of Medicine, and as division of the Chemistry Department. The head of the Chemistry Department was Edward Bartow, whom Dr. Mattill had known at the University of Illinois and in the Sanitary Corps in France. Investigations begun at Rochester were continued at Iowa City. Concentration of the unsaponifiable fractions of lettuce and various vegetable oils yielded viscous residues. Acetylation of these destroyed their antioxygenic activity, but did not alter their vitamin E potency. Antioxygenic activity had been associated with the presence of free hydroxyl groups, probably phenolic. Further fractionation of the unsaponifiable residues with organic solvents effected the removal of the major portion of the antioxygenic activity and yielded a vitamin fraction with a potency estimated in retrospect to have represented a vitamin E content of at least 50%. Tests of such fractions indicated that vitamin E contained one or more readily esterifiable hydroxyl groups. The acetyl and benzoyl esters were effective in the rat, but the urethane and ether derivatives were not. Encouraged by McCollum's success in the preparation of crystalline allophanates of cholesterol and other sterols, reaction of the vitamin E concentrates with cyanic acid was tried in the hope that a crystalline vitamin E allophanate might eventually be obtained. Tests of the reaction mixture, however, showed that it was still very actively antioxygenic. Reasoning that such could not have been the case if the hydroxyl groups had undergone esterification, this course of procedure was not pursued further.[1] Some time later Evans and the Emersons succeeded in isolating the tocopherols by crystallization as the allophanates. Tests made by Olcott, in Mattill's laboratory and Emerson, in Evans', showed that the allophanates of the tocopherols and of α-naphthol were the

[1] I am indebted to Dr. Harold S. Olcott, who spent several years with Dr. Mattill in graduate and post-doctorate research, for this information.

only derivatives which retained their antioxidant activity after esterification of the hydroxyl group.

Isolation of the tocopherols made it possible to prove beyond doubt that pure vitamin E was antioxygenic, as had long been suspected. Considerable attention had already been given to the probable mechanisms of antioxygenic activity. This was now intensified. Primary antioxygenic action was found to be associated with ortho and para di- and polyphenolic compounds, or substances having similar electronic configurations. The effectiveness of this type of compound was prolonged synergistically by the addition of certain inorganic or organic acids that were inactive alone. Still other acids acted both as synergists and as stabilizers. From the physiological standpoint, alpha tocopherol was found to prevent the autoxidation of vitamin A in the intestine. Paralysis in the young of female rats on vitamin E-deficient diets was shown to result from a muscular, rather than from a nervous lesion. Unlike the normal weanling rat, the young rabbit cannot live without vitamin E. Within a few weeks muscle degeneration occurs and the animal becomes completely helpless. Creatinuria and loss of creatine from the muscle preceded the signs of paralysis or histological changes. The dystrophic muscle showed an abnormally rapid oxygen uptake which increased as the dystrophy progressed.

In such a brief sketch as this it is impossible to give a very complete survey of Dr. Mattill's research work or to correlate it adequately with the contributions of others with which it was intricately interwoven. During his 25 years at Iowa Dr. Mattill was working primarily in a teaching environment. His chief function at the research level was to train graduate students. The 120-odd publications of work directed by him include several studies of the biological value of the proteins of meat and cereals, a field in which he was also much interested. But the student who arrived with an established preference for work in an area quite apart from these fields was not commandeered or discouraged. The problem of how he might best attain his goal was carefully considered and he

was given the benefit of the best advice throughout his stay that could be afforded on the campus.

In his relations with his staff, Dr. Mattill showed a genuine and unselfish interest. Each member was encouraged to develop his own research program and, as additions to the staff were made, men were sought who could broaden the outlook with fresh points of view. His department was a congenial and cooperative one. Dr. Mattill was conscious of his academic responsibilities and ready as well to fulfill what he considered to be part of his obligations as a scientist. At the local level he had served the Iowa sections of the American Chemical Society and the Society for Experimental Biology and Medicine in various offices and Sigma Xi as its president. He had represented the medical research area of the university on the Council of the Graduate College and had been a member of the University Library Committee for several years. He was a Rotarian and a member of the Unitarian Church.

Dr. Mattill had also spent countless hours editing abstracts and manuscripts, managing editorial affairs, and planning scientific programs. He had served as associate editor of Biological Abstracts for 12 years, as a member of the Board of Editors of the Proceedings of the Society for Experimental Biology and Medicine for 7 years, and on the editorial boards of the Journal of Nutrition for 4 years and of Physiological Reviews for 5 years. He was secretary of the American Society of Biological Chemists from 1933 to 1938, a member of its council from 1938 to 1944, its vice president in 1951. He became its president in 1952. From 1944 to the time of his death he had served the society as a member of its editorial committee, the last 5 years as its chairman. He was a charter member of the American Institute of Nutrition.

In 1950, the Iowa Section of the American Chemical Society chose him as recipient of its third annual Iowa Award, in recognition of his research and his many years of devoted medical and graduate school teaching. In 1952, Western Reserve University conferred upon him the honorary degree of

Doctor of Science. He retired from his teaching duties at Iowa in July of that year.

Dr. and Mrs. Mattill had much in common. They had begun their home-making in Salt Lake City in the shadow of the mountains, where they loved to hike and camp. Several summers were spent in the Sierras. Into this environment they introduced their son John, who with his father became a miniature camera enthusiast of no mean ability. There were excursions to the national parks, in the Colorado Rockies, and several months before the second world war were spent abroad. In the winter months John and his father took up stamp collecting. The ties in the Mattill family were close, with each member strongly devoted to the others. By the time of Dr. Mattill's retirement, however, John had become director of publications at the Massachusetts Institute of Technology and had established a home of his own.

In the late spring of 1952, Dr. Mattill had submitted to surgery, but recovery had seemingly been complete. Extensive plans had been made for the years he and Mrs. Mattill had planned to spend together after his retirement, part of them in advancing nutrition in Central and South America. They had studied Spanish and the new program had been started at Havana, Cuba, where he became adviser to the Foundation for Medical Investigation in Nutrition. He was forced to return to Iowa City in December for further medical attention. His condition proved to be malignant and steadily worsened, but to the last he kept up his courage and interest, even dictating a message to be read at the dedication of the Cuban laboratories which his illness had forced him to miss. Death mercifully ended his suffering on March 30, 1953.

Scientific work is a matter of record which anyone may read. Qualities of character are not so easily conveyed. Dr. Mattill had a keen sense of principle. Though possessed of a quick temper, he had mastered too well the art of self-discipline to allow it often to get out of bounds. He was a champion of liberal education and social justice, ever alert to human factors and astute and constructive in his outlook.

He possessed a dignity which set him apart. Few knew him intimately, but many had felt the warmth of his personality. Possibly his noblest attribute was the humility with which he approached his work and his associates. Few men have been as completely unselfish and as genuinely interested in others. His warm friendliness, quiet humor, seasoned wisdom, and cordial scientific and personal companionship were sources of stimulus and of inspiration to all who knew him.

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ESTIMATED DIETARY INTAKE, URINARY EXCRETION AND BLOOD VITAMIN C IN WOMEN OF DIFFERENT AGES [1]

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From the Agricultural Experiment Stations in Ames, Iowa, Brookings, South Dakota, East Lansing, Michigan, Urbana, Illinois, and Lincoln, Nebraska

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INTRODUCTION

Gander and Niederberger ('36) reported that older people required larger amounts of vitamin C than younger people for tissue saturation as measured by urinary excretion. Older individuals needed 50 mg daily to maintain this state whereas younger individuals needed 25 to 32 mg. Rafsky and Newman ('41) observed that only two of 25 men and women, ranging from 66 to 83 years of age, showed continuous evidence of saturation; the others retained large amounts of ascorbic acid.

Kirchman (cited by Rafsky and Newman, '41) found that only one out of 50 patients, 50 to 87 years of age, had a plasma value as high as 1 mg of ascorbic acid per 100 ml. On the other hand, Kyhos et al. ('45) reported that the amounts of vitamin C needed to restore low blood concentrations to

[1] Contribution no. 11, Subproject 1 of the North Central Regional Cooperative Project NC-5, *Nutritional status and dietary needs of population groups.*

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normal values in 23 men, 20 to 75 years of age, appeared not to be affected by variation in their ages. Kirk and Chieffi ('53a, b) measured ascorbic acid in the whole blood of 61 men and 81 women ranging from middle to old age and concluded that for men ascorbic acid concentrations decreased with increasing age; for women no decrease with age occurred. Oral administration of 100 mg of the vitamin per day raised the values of vitamin C in the blood of 16 of these elderly patients. Three other subjects appeared to have impaired absorption of vitamin C because oral administration produced no changes but subcutaneous injections increased the concentration of the nutrient in the blood.

A cooperative regional project in the North Central States has been developed for the purpose of examining the nutritional status of women ranging in age from 20 to 90 years. This paper presents the findings with respect to ascorbic acid.

SOURCES OF DATA

Five hundred and sixty-nine women from 5 states participated in this study. After a period of at least one year, 68 of these women participated for a second time; the data obtained from them have been treated as if they were new subjects. Records of weighed, measured or recalled food intakes, and urinary excretion of vitamin C for 24 hours preceding and following a 400 mg load dose have been obtained. Vitamin C was determined in the serum (Lowry et al., '45; Bessey et al., '47), or whole blood (Roe and Kuether, '43) or plasma (Mindlin and Butler, '38). Iowa and South Dakota measured total ascorbic acid in urine (Roe and Kuether, '43), Michigan measured ascorbic acid (Evelyn et al., '38). Nebraska and Illinois did not assess urinary vitamin C. Table 1 is a summary of the kinds of data available from each of the cooperating state experiment stations.

The technique for obtaining subjects varied somewhat from state to state. However, the women were residents of the towns or cities in which the universities or colleges were located. Each woman was interested in the study and cooperated

TABLE 1

Sources of data in study of vitamin C status

STATE	STUDY NO.	NO. OF WOMEN	NO. AND KIND OF FOOD INTAKE RECORDS	FOOD TABLE USED FOR CALCULATION	NO. OF BLOOD DETERMINATIONS	NO. OF BASAL URINE EXCRETIONS	NO. OF LOAD TEST URINE EXCRETIONS
Illinois	I	114	114 (7-day measured)	USDA Handbook 8	114 (serum, total ascorbic acid)		
Iowa	I	49	49 (7-day weighed)	Bowes and Church	49 (whole blood, total ascorbic acid)	49	49
	II	47 (36 repeated from study I)	47 (7-day weighed)	Bowes and Church	47 (serum, total ascorbic acid)	47	47
Michigan	I	56	56 (1-day recall)	Donelson and Leichsenring	56 (serum, total ascorbic acid)	—	—
	II	17 (11 repeated from study I)	46 (2-day weighed)[1]	Donelson and Leichsenring	46 (plasma, ascorbic acid)[1]	46 [1]	46 [1]
Nebraska	I	49	49 (7-day weighed)		49 (plasma, ascorbic acid)	—	—
	II	224	224 (7-day measured)	Percentage counter (Leverton, '52)	224 (plasma, ascorbic acid)	—	—
South Dakota	I	56	56 (7-day weighed including 7, 7-day measured diets)	Bowes and Church and USDA #572	56 (plasma, ascorbic acid)	—	—
	II	25 (21 repeated from study I)	25 (7-day weighed)	Bowes and Church and USDA #572	25 (serum, total ascorbic acid)	25	25
Total		637 (68 repeated)	666 (97 repeated)		666 (97 repeated)	167 (65 repeated)	167 (65 repeated)

[1] Study II in Michigan included several observations for some of the 17 subjects.

TABLE 2

Mean intakes and mean blood concentrations of ascorbic acid of subjects in 5 North Central States

AGE IN YEARS	INTAKE (MG/DAY)				BLOOD CONCENTRATION (MG/100 ML)		
	No. subjects	Mean	Std. dev.	Range	Mean	Std. dev.	Range
Illinois							
Study I		7-day measured diets			Serum (total ascorbic acid)		
20–29	6	117	70.4	53–249	1.24	0.575	0.37–1.80
30–39	36	83	38.4	24–191	1.11	0.583	0.28–2.64
40–49	30	108	56.4	25–325	1.22	0.500	0.18–2.41
50–59	13	99	47.3	38–170	1.29	0.591	0.37–1.82
60–69	20	123	76.1	50–397	1.23	0.541	0.23–2.15
70–79	6	77	35.8	29–125	1.12	0.377	0.71–1.58
80–89	2	198	—	85–311	1.90	—	1.13–2.68
90–99	1	61	—	—	0.37	—	—
Total	114	102	58.5	24–397	1.20	0.553	0.18–2.68
Iowa							
Study I		7-day weighed diets			Whole blood (total ascorbic acid)		
30–39	12	77	37.9	30–161	0.92	0.422	0.2–1.5
40–49	15	89	31.6	37–167	0.84	0.416	0.3–1.3
50–59	14	83	25.5	35–123	0.84	0.363	0.3–1.4
60–69	3	146	—	109–172	1.07	—	0.8–1.4
70–79	5	50	3.6	45– 55	0.92	0.343	0.4–1.3
Total	49	84	35.0	30–172	0.88	0.380	0.2–1.5
Study II		7-day weighed diets			Serum (total ascorbic acid)		
30–39	8	81	32.9	40–127	1.01	0.712	0.2–2.0
40–49	13	115	46.8	64–228	1.45	0.486	0.6–2.0
50–59	10	81	32.5	35–148	0.85	0.438	0.1–1.4
60–69	8	133	101.3	26–362	1.20	0.499	0.5–1.9
70–79	7	83	70.5	23–237	1.29	0.599	0.3–2.0
80–89	1	60	—	—	0.6	—	—
Total	47	99	60.2	23–362	1.16	0.564	0.1–2.0
Michigan							
Study I		1-day recalled diets			Serum (total ascorbic acid)		
40–49	9	58	53.1	5–152	1.08	0.456	0.20–1.7
50–59	19	68	49.6	1–172	0.79	0.492	0.10–1.5
60–69	13	62	61.2	1–242	0.92	0.440	0.25–1.5
70–79	13	51	47.6	8–175	0.93	0.514	0.15–1.8
80–89	2	46	—	18– 74	0.62	—	0.60–0.65
Total	56	60	51.0	1–242	0.89	0.471	0.10–1.8

Study II		2-day weighed diets			Plasma (ascorbic acid)		
40–49	1 [1]	71	—	—	1.07	—	—
50–59	8 [1]	65	28.9	25–125	0.79	0.319	0.44–1.38
60–69	4 [1]	54	—	27– 79	0.98	—	0.68–1.38
70–79	4 [1]	54	—	28–110	0.60	—	0.15–1.04
Total	17	60	28.7	25–125	0.81	0.328	0.15–1.38
Nebraska							
Study I		7-day measured diets			Plasma (ascorbic acid)		
30–39	28	61	21.7	17–101	0.66	0.394	0.05–1.73
40–49	67	88	23.3	36–150	0.89	0.317	0.07–1.73
50–59	83	87	33.9	25–214	0.84	0.343	0.01–1.64
60–69	35	76	26.4	25–137	0.81	0.331	0.18–1.51
70–79	11	75	29.9	29–115	0.97	0.438	0.13–1.62
Total	224	82	29.1	17–214	0.84	0.350	0.01–1.73
Study II		7-day weighed diets			Plasma (ascorbic acid)		
30–39	14	80	53.4	23–211	0.77	0.392	0.21–1.32
40–49	14	118	40.7	67–213	0.92	0.322	0.50–1.67
50–59	10	140	58.5	24–252	0.86	0.323	0.09–1.35
60–69	8	92	45.4	35–175	0.88	0.345	0.30–1.32
70–79	3	65	—	29–137	0.50	—	0.27–0.89
Total	49	104	54.3	23–252	0.83	0.349	0.09–1.67
South Dakota							
Study I and II		7-day weighed diets [2]			Plasma (ascorbic acid) or serum (total ascorbic acid)		
30–39	11	57	11.8	40– 76	0.57	0.326	0.2–1.0
40–49	12	97	36.8	29–138	1.02	0.526	0.1–1.8
50–59	20	84	58.6	25–308	0.76	0.471	0.1–1.6
60–69	13	66	22.7	31–107	0.72	0.485	0.2–1.6
70–79	16	68	20.6	34–147	0.88	0.455	0.1–1.6
80–89	9	55	26.6	21– 98	0.51	0.330	0.1–1.1
Total	81	73	38.1	21–308	0.76	0.463	0.1–1.8

[1] Some subjects observed from one to three two-day periods.

[2] Includes 7, 7-day measured diets.

in such a way that results presented a typical picture of her dietary habits and nutritional state. Each woman was also in apparent good health, free from observable signs of disease, and capable of assuming responsibility as a member of a household or profession or both. These selective factors precluded securing samples which were unbiased representations of all women in their residential areas.

RESULTS AND DISCUSSION

Since the food intake records were obtained and analyzed in different ways, the data from each state have been handled separately and are summarized by age decades in table 2.

Mean intakes of ascorbic acid usually exceeded 70 mg, the amount recommended by the National Research Council for women, and generally varied little with age; beyond the age of 60 or 70 some decrease may have occurred. Women from 40 to 60 years of age often recorded somewhat larger mean intakes than either younger or older women. Their intakes averaged over 80 mg per day for women in each state with the exception of Michigan.

Blood levels, whether serum, plasma or whole blood, indicated that no major changes in ascorbic acid nutrition occurred with age, although lower average values were observed for some groups of subjects over 69 years of age. The ranges of values contributing to the means were large in all decades, hence, the standard deviations also were large.

The relationship of the blood concentration of vitamin C to the intake was examined graphically. Both the intake per day and the intake per kilogram of body weight per day were considered. Use of either of these intake measures produced a significant product-moment correlation with the corresponding blood values.

In figure 1, blood values have been plotted against the intake in milligrams per kilogram body weight per day. It was hoped that adjustment of the intake in terms of body weight might reduce the large variability observed for blood values corresponding to a given intake. The mean blood values for

class intervals of one-fourth unit (0.25–0.49, 0.50–0.74, etc.) have been computed for the observations along the abscissa and the plotted means connected by a solid line. The general course of the solid line for each graph supports the hypothesis

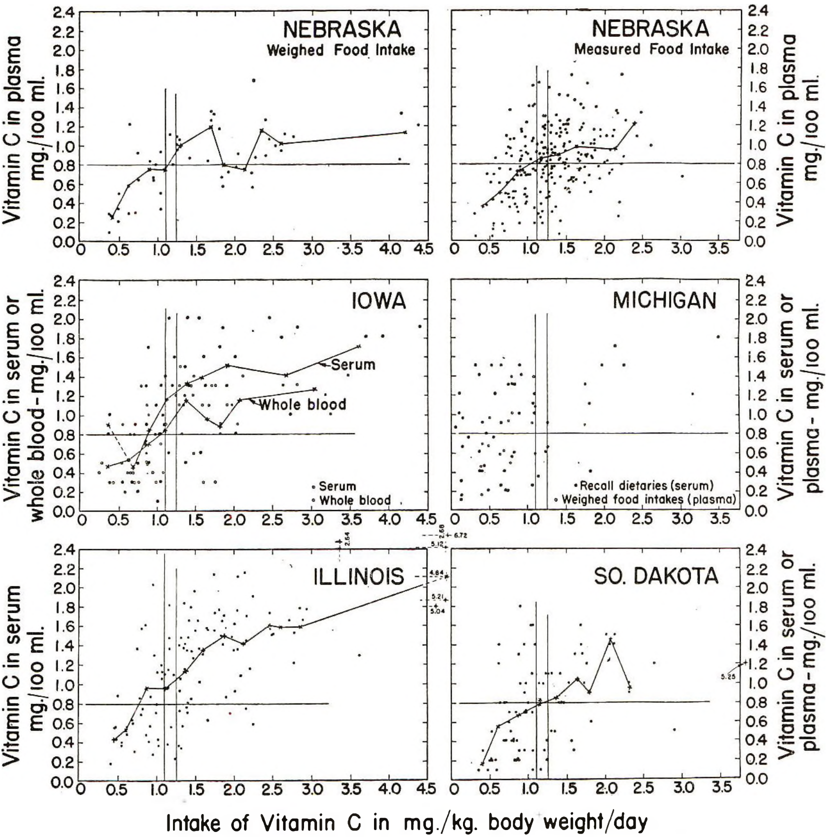

Fig. 1 Scattergrams of the vitamin C concentration in the blood (whole blood, serum or plasma) of women in 5 locations within the North Central States plotted against the intake calculated per kilogram body weight per day. A mean line has been superimposed and was obtained by averaging blood concentrations within 0.25 units on the abscissa.

that a curvilinear relationship rather than linear relationship exists between intake and blood concentration. However, the variation from the lines connecting the means is quite large.

Three states, Iowa, Michigan and South Dakota, studied urine excretions in addition to intake and blood concentrations

of vitamin C. Excretion of vitamin C after the load test and basal urine excretion were plotted against intake expressed as milligrams per kilogram body weight per day. Mean urinary excretions for class intervals of one-fourth unit were computed for the intake observations and the means connected by a solid line. The data for Iowa are shown graphically in figures 2 and 3. Basal excretions seemed to exhibit random variations at low intakes. When the average daily intake exceeded 1.1 mg per kilogram body weight per day, the relation of the basal excretion to the intake appeared to be nearly linear. Beyond intakes of 1.25 mg per kilogram body weight per day, the slope of the mean line of load dose excretion is less steep than at lower intakes.

Since the Iowa data were more extensive than the data from either Michigan or South Dakota, they were examined in more detail. Visual inspection of the three graphs presented here, indicated that intakes of less than 1.1 mg per kilogram of body weight per day were apt to produce blood values of less than 0.8 mg per 100 ml, and to result in basal urine excretions of less than 15 mg per day and load test excretions of less than 115 mg per day. Intakes greater than 1.1 mg per kilogram per day were apt to result in blood values above 0.8 mg % and in urinary excretions exceeding 15 mg per day or 115 mg following a load dose.

Roe, Kuether and Zimler ('46), studying the distribution of ascorbic acid in blood, suggested that in healthy subjects whole blood values below 0.6 mg per 100 ml indicated a negative balance between intake and bodily needs. Bessey and Lowry ('47) interpreted blood serum concentrations of 0.7 to 1.0 mg % as representing a good level of nutrition with respect to ascorbic acid. The value 0.8 mg %, chosen here, falls within the range of blood concentrations proposed by other workers as indicative of satisfactory nutrition. Harris and coworkers ('36) concluded from their studies that urinary excretion of 13 mg of vitamin C daily represented an intake which was borderline in adequacy. In the present study, ex-

cretions exceeding 15 mg per day were associated with blood levels considered satisfactory. Therefore the data concur with the interpretation of findings by Harris et al.

When division of the intake at 1.1 mg per kilogram body weight per day was used, 71% of the Iowa subjects could be classified into two distinct groups; in one, urine excretions and blood values were consistently above the expected figures, in the other, below. One-half of the remaining 29% of the subjects could not be classified into either of these groups because of the blood values.

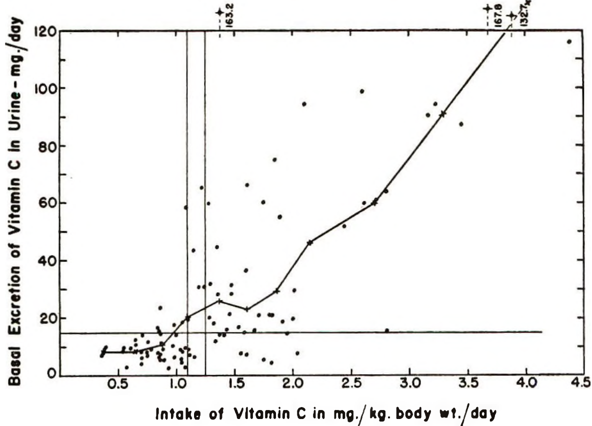

Fig. 2 Excretion of vitamin C in the urine of Iowa women during one day plotted against their mean daily intake of vitamin C calculated from a 7-day weighed food intake record.

When the Iowa data were ranked in order of vitamin C intake, 41 subjects had intakes which were less than 1.1 mg per kilogram per day. Of these subjects, 54% had blood values which were below 0.8 mg %, 85% excreted less than 15 mg per day when eating their regular diets, and 85% excreted less than 115 mg in the 24-hour period following the load dose. There were 55 subjects whose intakes of vitamin C were above 1.1 mg per kilogram per day. Of these, only 11% had blood levels below 0.8 mg %, 20% excreted less than 15 mg per day and 16% returned less than 115 mg after the load dose.

Of the 13 women over 70 years of age, 10 of them had intakes below 1.1 mg per kilogram per day. Only two had blood levels below 0.8 mg %, whereas 8 excreted less than 15 mg of vitamin C per day and less than 115 mg after the load dose.

Data from 25 cases in South Dakota and 17 cases in Michigan were examined in view of the relationships observed in the Iowa data. In both instances the percentage of subjects over 60 years of age was larger than in the Iowa sample. In the South Dakota sample, intakes of vitamin C greater than 1.25 mg per kilogram per day seemed necessary to produce

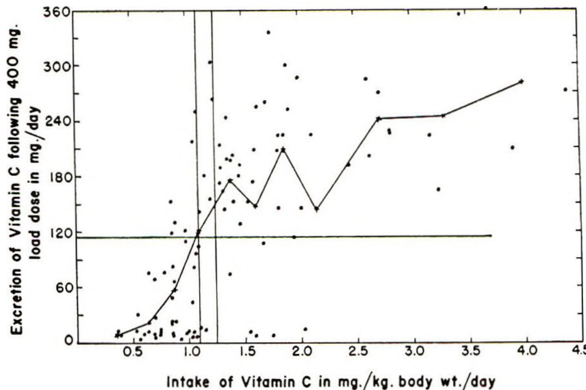

Fig. 3 Excretion of vitamin C in the urine of Iowa women during 24 hours following a load dose of 400 mg of ascorbic acid plotted against their mean daily intake of vitamin C calculated from a 7-day weighed food intake record.

basal urinary excretions exceeding 15 mg per day. Of 10 women consuming from 1.2 to 2.1 mg per kilogram per day, 6 excreted less than 15 mg per day and 7 returned less than 115 mg after the 400 mg load dose. On the other hand, only three had blood values below 0.8 mg %. Of 15 women having intakes less than 1.1 mg per kilogram per day, 8 had blood values below 0.8 mg % and all of them excreted less than 15 mg per day and less than 115 mg after the load dose.

The Michigan subjects had intakes ranging from 0.38 to 1.79 mg per kilogram per day but only three women exceeded 1.1 mg per kilogram per day. In one-half of the subjects,

low blood concentrations and low urinary excretions of vitamin C were concurrent with intakes below 1.1 mg per kilogram per day. In three cases the blood value was higher than 0.8 mg % while the urinary excretion was very low. Two women with low blood levels excreted relatively large amounts of vitamin C in the urine.

Frequently, the blood level appeared to indicate a somewhat better status than the urinary excretion of vitamin C, according to the criteria selected here. It is possible that the desirable concentration of vitamin C in serum, plasma, or whole blood is higher than 0.8 mg %. On the other hand blood levels may not respond as rapidly to changes in diet as the urinary excretion.

If the daily intake of vitamin C is irregular, lower correlation between the mean intake and either blood concentration or urinary excretion might result. Examination of dietary records indicated that irregular intakes occurred frequently. A preliminary study[2] of 49 Iowa women suggested that women with irregular vitamin C intakes had blood concentrations which deviated more from a mean line relating intake to blood values than those women with regular intakes.

High blood values occurred in many older individuals with low intakes and small amounts of vitamin C in the urine. This may be related to changes in kidney function with age since vitamin C is usually considered a threshold substance (Ralli et al., '38).

SUMMARY

The nutritional status of 569 women (68 of whom were studied twice) from 5 locations in the North Central states has been examined with respect to vitamin C. Whole blood, plasma or serum concentrations of vitamin C were related to the estimated dietary intake. Mean blood values for the state samples varied from 0.76 ± 0.463 to 1.20 ± 0.553 mg of vitamin C per 100 ml.

[2] Thesis submitted by Maxine Britton to the Graduate Faculty of Iowa State College for the degree of Master of Science.

Basal excretion of vitamin C and excretion after a 400 mg load dose were obtained from 135 of the subjects in Iowa, Michigan and South Dakota. At low intakes, basal urinary excretion of vitamin C exhibited random variation, but when the average daily intake exceeded 1.1 mg per kilogram of body weight, the relation of basal excretion to intake was nearly linear. Beyond intakes of 1.25 mg per kilogram of body weight, the slope of the mean line of load dose excretion was less steep than at lower intakes.

Age did not influence either the mean intake of vitamin C or its mean concentration in the blood of women participating in this study. Among Iowa women, vitamin C intakes of 1.1 mg or more per kilogram of body weight per day were associated with (1) blood values above 0.8 mg % in 89% of the cases, (2) basal urinary excretions of more than 15 mg per day in 80% of the cases, and (3) excretions of more than 115 mg per 24 hours after a load dose of 400 mg in 84% of the cases. Therefore, an ascorbic acid intake of 1.1 mg or more per kilogram of body weight from self-selected diets provides women with apparently satisfactory amounts of this vitamin. Individual intakes below 1.1 mg per kilogram of body weight occurred frequently in the populations examined.

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EFFECTS OF THIAMINE AND RIBOFLAVIN DEFICIENCY ON HISTIDINE METABOLISM [1,2]

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In studies of the effects of deficiencies of certain B vitamins on liver enzyme systems concerned with the straight deamination of histidine, elevated levels of histidase were found to accompany depletion of pyridoxal phosphate in the tissues of the rat (Baldridge and Tourtellotte, '57). This observation was interpreted as an adaptive increase in one route of degradation (via straight deamination) when a second (via transamination) was made less available.

Edlbacher and Becker ('40) have reported greater ammonia production following incubation of histidine with liver preparations from thiamine-deficient than with those from normal rats. Recently, thiamine has been implicated (Dalgliesh, '55) as a possible cofactor in tryptophan metabolism. As specific methods (Mehler and Tabor, '53) are now available for the assay of histidase and urocanase, it was of interest to investigate the effects of vitamin B_1 deficiency on these enzymes involved in the metabolism of histidine.

In studies of this type, even with pair-feeding techniques, comparison of results from deficient and control animals is difficult because of marked differences in body and organ

[1] Paper IV of a series on the metabolism of histidine.

[2] Supported in part by a grant from the National Institutes of Health, United States Public Health Service (Grant no. A-650-C3).

weights. In the present experiments, liver enzyme levels of thiamine-deficient rats can be compared with those of animals in a similarly poor nutritional state induced by deprivation of dietary riboflavin.

The results of these investigations provide further evidence for the concept of metabolic adaptation to a deficiency of essential cofactors by changes in tissue enzyme concentrations.

EXPERIMENTAL

Male weanling white rats were fed diets similar to those described previously (see Baldridge and Tourtellotte, '57, diet for pyridoxine-control animals) modified to contain 20% casein and 65.8% sucrose. Thiamine was omitted from the diet for one group and riboflavin from that for another. Corresponding control rats were pair-fed the complete diet. After 4 to 5 weeks the animals fed diets devoid of vitamin B_1 or B_2 exhibited the usual symptoms of the respective vitamin deficiencies (Jansen in Sebrell and Harris, '54; Horwitt, '54, ibid.). Data were obtained from 7 pairs in the thiamine-deficiency and from 8 pairs in the riboflavin-deficiency experiments.

The animals were killed during the 4th and 5th weeks of dietary control. After removal of portions of liver for determination of dry weight (overnight at 105°), homogenates were prepared with 1% KCl and aliquots were withdrawn for assay of rhodanese activity by the colorimetric procedure of Cosby and Sumner as adapted by Rosenthal et al. ('50) and further modified by Rosenthal and Vars, ('54). The homogenates were then centrifuged at 6000 *g* at 0°, and portions of the supernatant material were analyzed for histidase activity (formation of urocanic acid at pH 9.2) and urocanase activity (disappearance of urocanic acid at pH 7.4) as measured at 277 mμ with a recording spectrophotometer essentially as described by Mehler and Tabor ('53), (see also Baldridge and Tourtellotte, '57, and Baldridge, '58).

In table 1 are recorded the observed levels of liver histidase, urocanase and rhodanese. The levels of histidase were con-

siderably higher and those of urocanase were slightly higher in the livers of the thiamine-deficient than in the livers of control animals. In contrast, the activities of these enzymes were essentially the same in the livers of riboflavin-deficient and corresponding control rats. Liver rhodanese levels, util-

TABLE 1

Enzyme levels in livers of thiamine and riboflavin-deficient and pair-fed control rats [1]

ENZYME AND DIET	RANGE	MEAN	P [2]
	units/gm [3]	*units/gm*	
Histidase			
Thiamine-control	0.47–0.79	0.66	
Thiamine-deficient	0.80–1.07	0.92	< 0.01
Riboflavin-control	0.38–0.97	0.60	
Riboflavin-deficient	0.34–0.91	0.67	> 0.05
Urocanase			
Thiamine-control	0.68–0.94	0.83	
Thiamine-deficient	0.83–1.16	0.98	$< 0.05, > 0.01$
Riboflavin-control	0.66–0.98	0.80	
Riboflavin-deficient	0.51–0.88	0.73	> 0.05
Rhodanese			
Thiamine-control	1210–2020	1650	
Thiamine-deficient	1480–2120	1850	> 0.05
Riboflavin-control	1280–1960	1640	
Riboflavin-deficient	1090–1770	1500	> 0.05

[1] Seven pairs in the thiamine-deficiency and 8 pairs in the riboflavin-deficiency experiments.

[2] Probability level according to Fisher's "t" test (Youden, '51).

[3] Micromoles of product formed or substrate destroyed per minute per gram of dry liver tissue: histidase (formation of urocanic acid), pH 9.2, 30°; urocanase (destruction of urocanic acid), pH 7.4, 30°; rhodanese (formation of thiocyanate), pH 8.8, 20°. (See Baldridge and Tourtellotte, '57; Baldridge, '58).

ized as an index of general liver enzyme protein synthesis (Baldridge and Tourtellotte, '57), were similar in all groups.

The observed elevation in the levels of liver enzymes of the vitamin B_1-deficient compared with those of control rats cannot be ascribed to differences in body weight or in total

amount or percentage dry weight of liver tissue. As shown in table 2 similar manifestations of growth failure were observed in riboflavin-deficient rats, but there was no elevation in liver enzyme levels.

TABLE 2

Comparison of body weight, liver weight and dry weight of liver of thiamine-deficient, riboflavin-deficient and pair-fed control rats

DEFICIENCY AND DIET	BODY WEIGHT		LIVER WEIGHT		DRY WEIGHT OF LIVER	
	Range	Mean	Range	Mean	Range	Mean
	gm	*gm*	*gm*	*gm*	%	%
Thiamine						
Control	77–133	98	3.69–5.13	4.22	27.3–36.0	30.8
Deficient	47–69	55	2.15–2.94	2.40	28.5–33.5	31.0
Riboflavin						
Control	68–120	98	2.60–6.69	4.51	28.8–31.0	30.2
Deficient	33–57	47	1.24–3.68	2.57	28.4–32.7	30.6

[1] Seven pairs in the thiamine-deficiency and 8 pairs in the riboflavin-deficiency experiments.

DISCUSSION

The elevated rate of production of ammonia from histidine by liver preparations from thiamine-deficient rats reported by Edlbacher and Becker ('40) and referred to by them as increased "histidase" activity probably was due to enzymes in addition to the presently described histidase [which, as defined by Mehler and Tabor ('53), catalyzes the conversion of histidine to urocanic acid]. In any event, since such a marked increase in histidase activity has been found only during pyridoxine deficiency, it seems likely that the elevation observed in thiamine-deficient animals also represents a metabolic adaptation to denial of an alternate route for the degradation of histidine. If the reaction for which thiamine (or some derivative thereof) is required is similar to the vitamin B_1-dependent oxidation of the indole ring of tryptophan, an alternate metabolic route for histidine would involve oxidative cleavage of the carbon to carbon double bond in the imidazole ring of histidine prior to deamination of the alpha amino

group. Such a reaction involving histidine has not been demonstrated to date.

The present finding of unchanged histidase levels during riboflavin deficiency is similar to the results obtained with biotin and folic acid-deficient rats (Baldridge and Tourtellotte, '57; Baldridge, '58).

The slight increase in urocanase levels presently observed in thiamine-deficient rats might also be the result of metabolic adaptation to increased levels of substrate. However, no such elevation was noted during pyridoxine deficiency. Similarly, urocanase levels were unchanged in the livers of riboflavin-deficient animals as shown above, and in those of biotin-deficient rats.

SUMMARY

1. Levels of histidase were considerably greater (difference significant at a probability level of less than 1%) and of urocanase somewhat greater (difference significant at a probability level of between 5 and 1%) in livers of thiamine-deficient compared with those of pair-fed control rats. Liver rhodanese levels were the same in both groups.

2. Liver histidase, urocanase and rhodanese levels were not affected by lack of dietary riboflavin in the rat.

3. It is suggested that the observed increase in histidase activity during thiamine deficiency may represent a metabolic adaptation to denial of an alternate pathway for the degradation of histidine.

ACKNOWLEDGMENT

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THE ABSORPTION OF VITAMIN A IN DOGS FOLLOWING CHOLECYSTONEPHROSTOMY

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The ability to absorb vitamin A from the intestine has been used as an index of fat absorption, and the vitamin A absorption test, in which the serum levels of vitamin A are determined at intervals after the oral administration of the vitamin, has found its greatest clinical usefulness in the diagnosis of diseases of fat digestion and absorption such as pancreatic deficiency, sprue and celiac disease. The level of vitamin A in the plasma is lowered in parenchymal liver disease, and the rise observed after an oral test dose is also diminished in obstructive jaundice and in various types of liver damage, but the extent to which these findings are due to faulty absorption is uncertain. There have been some clinical evaluations of the effect of bile salts on the absorption of vitamin A, but there have been few experimental studies in animals.

Breese and McCoord ('40) determined the serum vitamin A levels following the oral administration of a fish liver oil concentrate to a group of children with catarrhal jaundice. Absorption was impaired, and there was no definite evidence of improvement when sodium taurocholate was given with the test dose of vitamin A. May, McCreary and Blackfan ('42) failed to note any beneficial effect of incorporating bile salts in the vehicle in which vitamin A was administered to a

patient with celiac disease. Bile salts given with the vitamin A or for several days before the test did not influence the absorption of vitamin A by the patients with liver disease studied by Popper and associates (Popper, Steigmann and Zevin, '43; Popper, Steigmann and Dyniewicz, '47) which was similar to the experience of Adlersberg et al. ('48) and of White and colleagues ('50).

Greaves and Schmidt ('35) reported experiments with a large series of rats with internal bile fistulas made by an anastomosis between the bile duct and the upper part of the descending colon by means of a small silver cannula. The ability to absorb various preparations of carotene and vitamin A was judged by the amounts required to restore the vaginal smears to normal in animals depleted of vitamin A. Vitamin A given as halibut liver oil was absorbed in sufficient amounts to correct the vaginal smear, but there appeared to be little or no absorption of carotene. However, a positive response was obtained when carotene was given with glycodeoxycholic or deoxycholic acid. In a review on the absorption and storage of vitamin A Clausen ('43) stated that dogs with long-standing biliary fistulas did not absorb vitamin A even when bile or bile salts were mixed with the vitamin, but that with continuous feeding of bile vitamin A absorption gradually improved. No details of his experiments are given. Bernhard and Ritzel ('53) observed the absorption of vitamin A in rats with thoracic duct fistulas. In the absence of bile, vitamin A absorption was decreased from 50 to 60% to about 1%, and the intraduodenal infusion of taurocholate or cholate increased absorption to about 5%.

Because of the limited experimental evidence available, we welcomed an opportunity to study the relationship between bile and vitamin A absorption in dogs following cholecystonephrostomy. In this operation the common bile duct is ligated, and an anastomosis is made between the gall bladder and the right renal pelvis, so that all of the bile is diverted from the intestinal tract and is excreted in the urine.

EXPERIMENTAL

The absorption of vitamin A was studied in 8 normal dogs and in 5 animals from two weeks to 18 months after cholecystonephrostomy had been performed. Dogs 1 and 3 were observed both before and after surgery. The dogs were maintained on commercial dog foods including both canned and dry preparations, but it was found necessary to supplement the diet of the operated animals with 50,000 units of vitamin A given intramuscularly about every two weeks in order to prevent symptoms of vitamin A deficiency. The operated dogs were being used for the assay of vitamin K, which was given or withheld as indicated by the plasma prothrombin levels. Supplements of vitamins D and E were not given, as no signs of deficiencies of these vitamins were observed.

Vitamin A absorption tests were done by determining the serum vitamin A levels in blood samples collected before and at intervals after the oral administration of a test dose of 3,000 units of vitamin A per kilogram of body weight. Most of the experiments were done with either a natural concentrate of vitamin A esters consisting chiefly of vitamin A palmitate with a potency of 500,000 units per gram or a preparation of vitamin A alcohol containing 2,300,000 units per gram.[1] Blood was drawn in the morning before the administration of the test dose and again three, 5 and 8 hours afterwards. No attempt was made to fast the animals, and usually at least a week elapsed between successive tests. Serum vitamin A was determined by a modification of the Carr-Price reaction as described by Kaser and Stekol ('43).

The normal dogs had a mean initial serum vitamin A level of 432 $\pm$ 50 units per 100 ml of serum and exhibited a definite increase in serum vitamin A during the 8 hours following the administration of the test dose. The data are shown in table 1.

[1] We are grateful to Dr. Philip L. Harris of Distillation Products Industries for the vitamin A ester and alcohol preparations.

Dogs differ from humans in the absence of carotene from their serum, the comparatively high initial values for serum vitamin A and the progressive increase in the serum levels throughout the 8-hour period. In the human the maximum serum vitamin A value during an absorption test usually occurs three to 5 hours after the test dose is given. In two

TABLE 1

Effect of vitamin A administration on serum vitamin A in normal dogs

DOG	FORM OF VITAMIN A GIVEN	INITIAL SERUM VITAMIN A	MAXIMUM SERUM VITAMIN A
		Units/100 ml	*Units/100 ml*
1	Ester	478	1092
	Alcohol	549	724
2	Ester	286	1052
	Alcohol	354	990
3	Ester	356	907
	Alcohol	613	1077
4	Ester	287	548
	Alcohol	370	672
	Ester	518	1018
			1217 [1]
5	Ester	437	1210
			1466 [1]
6	Ester	286	1068
	Alcohol	254	1125
7	Ester	284	322
	Ester	403	811
8	Alcohol	1014	1497

[1] Value 12 hours after administration of test dose.

animals the collection of blood samples was continued for 12 hours after a test dose of vitamin A ester, and still higher values were obtained at 12 hours than had been found at 8 hours. Vitamin A ester and vitamin A alcohol were absorbed apparently equally well. We have found this to be true of human subjects also, and the difference in the potency of the

preparations used seems to have been without effect, since there is no consistent difference between the values obtained for the two forms of vitamin A (P by the t test = 0.10 to 0.20). The first test with dog 7 shown in table 1 requires comment. During these experiments the dogs were allowed free access to food, and the vitamin A test dose was usually given with a canned commerical dog food containing meat. On this occasion the dog was given only bread and milk rather than a meat diet containing a relatively large amount of fat. The second curve with a much greater increase in serum vitamin A was obtained under the customary conditions.

The first two operated dogs to be studied had been subjected to surgery a year and a half earlier. They had been found to be deficient in vitamin K as indicated by prolonged prothrombin times and responded rapidly to the parenteral administration of very small doses of vitamin K (Quick and Collentine, '51). It seemed probable that they would also have impaired absorption of vitamin A. These two animals (9 and 10) were found to have serum vitamin A values of 139 and 201 units per 100 ml, respectively, which were lower than the levels found in any of the normal animals. Later they developed poor coats and infections of the urinary tract, at which time the serum of one of them contained only 52 units of vitamin A per 100 ml. In order to learn how rapidly the serum vitamin A might fall following cholecystonephrostomy, the level was followed in a three-month-old puppy. At the time of surgery the value was 596 units per 100 ml, but three weeks later it was only 48 units. At this time the administration of 50,000 units of vitamin A intramuscularly at weekly intervals was instituted, and the serum vitamin A gradually increased to 172 units per 100 ml 6 weeks later.

The data obtained on dogs after cholecystonephrostomy are given in table 2. The mean initial serum vitamin A value was 245 ± 17 units per 100 ml, which was significantly lower than in the normal animals (P by the t test < 0.01). When either vitamin A ester or vitamin A alcohol was given, little increase in serum vitamin A occurred. Comparisons of the

rises observed in normal and in operated animals by means of the *t* test showed that the probability of these results occurring by chance was < 0.01 for vitamin A ester and between 0.01 and 0.05 for the alcohol, with which fewer experiments were done.

TABLE 2

Effect of vitamin A administration on serum vitamin A in dogs following cholecystonephrostomy

DOG	FORM OF VITAMIN A GIVEN	BILE SUPPLEMENTATION	INITIAL SERUM VITAMIN A	MAXIMUM SERUM VITAMIN A
			Units/100 ml	*Units/100 ml*
1	Ester	None	320	454
	Alcohol	None	350	660
	Ester	1 gm desiccated bile with test dose	320	740
	Alcohol	1 gm desiccated bile with test dose	392	609
	Aqueous ester	None	344	605
3	Ester	1 gm desiccated bile with test dose	246	254
	Alcohol	1 gm desiccated bile with test dose	235	320
	Aqueous ester	None	272	462
9	Ester	None	129	170
	Alcohol	None	175	211
	Ester	1 gm desiccated bile with test dose	52	96
10	Ester	None	199	308
	Alcohol	None	214	246
	Ester	1 gm desiccated bile with test dose	106	200
	Ester	1 gm desiccated bile with test dose	192	265
	Ester	1 gm desiccated bile daily for 2 wks.	262	369
	Alcohol	1 gm desiccated bile daily for 2 wks.	331	435
	Aqueous ester	None	264	276
	Ester	100 ml fresh ox bile daily for 2 wks.	330	493
	Alcohol	100 ml fresh ox bile daily for 2 wks.	176	520
11	Ester	None	266	294
	Alcohol	None	295	332
	Ester	1 gm desiccated bile with test dose	170	287
	Alcohol	1 gm desiccated bile with test dose	302	342
	Aqueous ester	None	193	330

Attempts were made to improve vitamin A absorption by giving bile. No improvement was apparent when 1 gm of desiccated ox bile was given with either vitamin A ester or vitamin A alcohol, and the differences between the results with and without bile were statistically insignificant. With vitamin A ester as the test dose, a comparison of the maximum serum vitamin A levels in normal animals with those of operated dogs given 1 gm of desiccated bile with the test dose gave a P value of < 0.01. For a similar evaluation of the data for vitamin A alcohol P was 0.01 to 0.05. Possibly a slight increase in the response of the serum vitamin A to oral administration of vitamin A ester resulted when dog 10 was given 1 gm of desiccated bile daily for two weeks before an absorption test including the day of the test. It may also be significant that a gradual increase in the initial levels of serum vitamin A occurred during these experiments. The administration of 100 ml of fresh ox bile daily to one animal for 9 days including the day of the test seemed to improve the absorption of vitamin A, but the increase in serum level was still less than with most of the normal dogs.

Aqueous dispersions of vitamin A have been said to be more readily absorbed than oily preparations by normal human subjects and by patients with defects of fat digestion and absorption. Four operated dogs were given test doses of such a preparation [2] with approximately the same amounts of vitamin A as before, but there was no indication of any improvement in the absorption of vitamin A. These data are given in table 2.

DISCUSSION

Bile has long been believed to assist in the emulsification of fat in the intestine and indirectly in its digestion and absorption. According to the Pflüger-Verzár theory (Ver-

[2] U. S. Vitamin Corporation Aquasol Vitamin A Drops, a preparation of natural vitamin A made water-soluble by the use of sorethytan esters, 50,000 units per ml.

zár and McDougall, '36) this emulsification facilitates complete and rapid hydrolysis of triglycerides to glycerol and fatty acid, and the resulting fatty acids form water-soluble complexes with the bile acids. The more recent extensive investigations of Frazer ('40, '46, '50, '52a, b) have resulted in his partition hypothesis. It now appears that a large proportion of fat is absorbed in the upper portion of the small intestine as a fine emulsion following only partial hydrolysis. The necessary factors which favor emulsification of fat are an alkaline reaction, the presence of mono- and diglycerides arising from the incomplete hydrolysis of triglycerides by pancreatic lipase, and bile salts. Vitamin A is apparently absorbed in solution in this emulsified fat. In this connection it is of interest that one dog (no. 7 in table 1) had poor absorption of vitamin A when the customary food containing meat was not given with the test dose of vitamin A. Mendeloff ('54) has reported that poor absorption of vitamin A is found in fasting human subjects. However, the possibility that a factor other than dietary fat may be active is suggested by the fact that sham feeding was found to be effective in promoting the absorption of vitamin A in his experiments.

In our experiments the defect in the absorption of vitamin A was severe enough not only to give flat or low curves in vitamin A absorption tests, but to result in vitamin A deficiency after a period of time. The first dogs to be studied (nos. 9 and 10) were called to our attention because of the development of symptoms suggestive of vitamin A deficiency, and the condition of these animals improved with the parenteral administration of vitamin A. However, some vitamin A must have been absorbed or the animals' liver stores of vitamin A must been adequate, because vitamin A deficiency was not recognized until about 18 months after cholecystonephrostomy operations were performed. A young dog, which probably had limited stores of vitamin A and a high requirement, developed vitamin A deficiency soon after surgery. It is to be noted that all of the operated animals had lower

initial levels of sernm vitamin A in the absorption tests than did the normal dogs, and two of the vitamin-deficient dogs had values of approximately 50 units per 100 ml of serum on separate occasions when single determinations were done.

Our inability to correct the absorptive defect in animals with biliary fistulas by the administration of desiccated bile with the test dose of vitamin A was probably due to the failure to obtain complete restoration of optimum conditions for the emulsification of dietary fat with a single dose of desiccated bile. Because of the possibility that the absence of bile from the intestinal tract may have caused the development of a secondary absorptive defect, the daily administration of bile for one to two weeks was tried in an effort to reverse any such change. This, too, was unsuccessful.

SUMMARY

The absorption of vitamin A has been investigated in 8 normal dogs and in 5 animals subjected to cholecystonephrostomy. The animals with biliary fistulas had lower serum vitamin A levels than the normal controls, and two of them developed symptoms of vitamin A deficiency. Their ability to absorb both vitamin A esters and vitamin A alcohol was impaired as indicated by the failure to obtain a normal increase in the serum vitamin A levels following the administration of an oral test dose of vitamin A. Neither was there normal absorption of an aqueous dispersion of vitamin A. This absorptive defect was not corrected by the simultaneous administration of desiccated bile nor by giving desiccated bile or fresh bile daily for one to two weeks preceding the vitamin A absorption tests.

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THE ROLE OF BILE IN CALCIUM ABSORPTION[1]

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Wasserman and co-workers ('56, '57) have shown that certain food substances have the ability to stimulate the absorption of calcium from the digestive tract. Vitamin D, lactose, D- and L-lysine, and L-arginine were the most effective materials encountered. Little evidence was uncovered to indicate the mechanisms involved in the transport of calcium. One possibility offered was that these materials stimulated the flow of digestive juices which in turn produced the positive effect.

Of the intestinal secretions, bile has the greatest potential of being involved. The bile salts have long been recognized as being important in the prevention of gallstone formation and are known to undergo a markedly efficient resorption. Pavlov ('04) and Wisner and Whipple ('22) implicated bile salts in the metabolism of calcium after noting the osteoporosis that developed in biliary fistula dogs. von Beznak ('31) observed that oral administration of bile salts with milk to biliary fistula animals caused a rapid increase in the blood calcium and concluded that bile salts played a direct role in the absorption of the calcium. However, Greaves and

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Schmidt ('32) and Taylor et al. ('35) argued that the main effect of bile was to enhance the absorption of vitamin D and this then acted to increase calcium absorption.

With these older works in mind, it was decided to investigate the effect of biliary secretions upon the movement of calcium from the intestine into the body using the femur uptake of Ca^{45} as the criterion of absorption. To eliminate vitamin D status as a factor, only short-term experiments were planned. To insure a flow of bile, sodium taurocholate was given orally or intraperitoneally and when desired was coupled with bile duct ligations.

METHODS

University of Tennessee albino rats of Yale-Wistar origin, were used throughout this study and had been raised and maintained on a commercial pelleted dog food. Prior to use, the animals were fasted for 24 hours and then, under light ether anesthesia, were given 2 ml of a solution containing 4 μc of Ca^{45}, 0.26 mM of calcium (as $CaCl_2$), and 1 mM of glycine. At this time, either 1 ml of 0.9% NaCl or sodium taurocholate [4] was injected intraperitoneally. When bile duct ligations were made, the animals were allowed to recover for three hours before having the test solutions administered. L-Lysine was substituted for the glycine in the oral dosing solution when the ability of bile and bile-pancreatic duct ligation to alter the enhancing effect of lysine upon calcium absorption was tested. Other variations in the procedures will be noted in the text when pertinent.

After being dosed, the animals were maintained in the fasted state for 24 hours more and then were sacrificed. The femurs were removed, ashed, dissolved in 2 N HCl and then brought to 50-ml volume. To determine the amount of radioactivity in the bones, the calcium of a suitable aliquot of the ash solution was precipitated as the oxalate. As described by Comar ('55), the calcium oxalate was transferred to planchets, dried, and counted under a thin window GM tube. The results were expressed as the percentage of dose recovered in the two femurs.

[4] Nutritional Biochemicals.

RESULTS

The data of table 1 show that intraperitoneal administration of sodium taurocholate at levels of 0.037 and 0.074 mM per rat significantly increased the uptake of calcium from the intestine ($P < 0.01$). The effect appeared to be independent of age and sex, the bile salt increasing the Ca^{45} recovered in the femurs from 150 to 186% that of the control animals. In the male rats, the 0.074 mM level of bile salt failed to effect a further major increase in the uptake of Ca^{45} over that of a dose of 0.037 mM, indicating that nearly maximum response had been elicited by the smaller dose. Concurrent collections of the urine and feces of the young and adult female rats

TABLE 1

Effect of oral and intraperitoneal administration of sodium taurocholate on the Ca^{45} content of rat femurs [1]

Group	Route of Ca^{45} dose	Control	Millimoles of Na taurocholate orally		Millimoles of Na taurocholate I.P.	
			0.037	0.074	0.037	0.074
		% of dose in femurs	*% of dose in femurs*	*% of dose in femurs*	*% of dose in femurs*	*% of dose in femurs*
Young ♀	Oral	3.8 ± 0.3	4.1 ± 0.2		6.0 ± 0.2	
Young ♀	I.P.	7.6 ± 0.1	7.4 ± 0.6		7.3 ± 0.2	
Young ♂	Oral	4.2 ± 0.2		5.0 ± 0.3	6.4 ± 0.2	7.0 ± 0.2
Adult ♀	Oral	1.2 ± 0.1		1.1 ± 0.1		2.3 ± 0.6

[1] Values represent mean ± standard error of the mean; 6 animals per group; young rats ranged from 100 to 140 gm; adult rats weighed 250 to 350 gm.

gave results in substantial agreement with the femur isotope content. For the young females, 46.9, 46.1, and 13.0% of the Ca^{45} was found in the feces and digestive tracts of the control, oral taurocholate, and intraperitoneal taurocholate groups, respectively. In the same order, 1.3, 1.86, and 2.3% of the radioactivity was recovered in the urine. For the adult females, 82.3, 78.7, and 56.5% of the Ca^{45} was in the digestive tracts and feces, and 2.6, 4.5 and 1.96% in the urine for the control, oral taurocholate, and intraperitoneal taurocholate groups, respectively. When calculated on the basis of the percentage of the absorbed Ca^{45} appearing in the urine, these

data do not reveal that sodium taurocholate had any effect upon calcium once it was in the body proper. In agreement, neither oral nor intraperitoneal administration of the bile salt affected the distribution of the labeled calcium given intraperitoneally. To further pursue this point, embryonic chick bones were cultured for 7 days in media containing 0, 0.1, 1.0, and 10 mg % of desiccated ox bile. All groups showed the same uptake of Ca^{45}, reemphasizing the lack of effect of the bile salts on the handling of calcium inside the body.

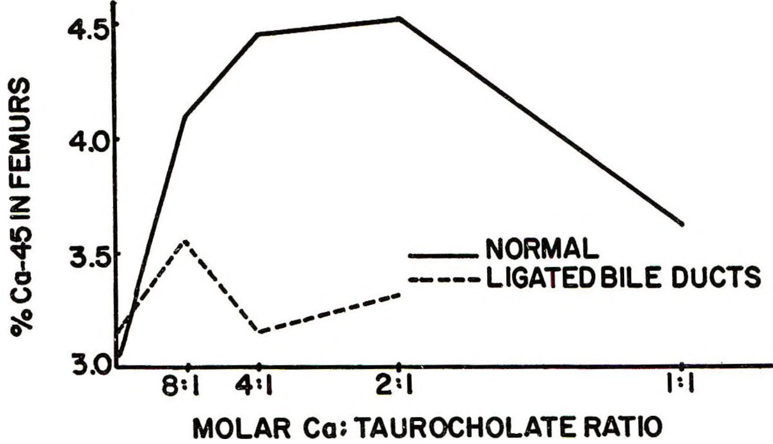

Fig. 1 The effect of various molar ratios of calcium to sodium taurocholate on the deposition of Ca^{45} in the femurs of normal and bile duct-ligated rats. For this study calcium was held constant while the amount of sodium taurocholate was varied.

In further experimentation using 14 male and 14 female rats of 100 to 130 gm, 0.009 mM of sodium taurocholate given intraperitoneally increased femur Ca^{45} to 124% that of the controls ($P < 0.05$). No difference in response due to sex was evident.

In table 1, the oral administration of sodium taurocholate did not affect the absorption of calcium to an extent that could be shown to be different from that of the controls. To investigate this in more detail, an experiment was performed in which calcium-sodium taurocholate ratios, on a

millimole basis, of 0, 8:1, 4:1, 2:1, and 1:1 were used. Each oral dose contained 0.26 mM of calcium, as $CaCl_2$, and the amount of sodium taurocholate was varied. Glycine was omitted from these solutions. Female rats of about 100 gm were used and normal rats were compared to rats with ligated bile ducts. Figure 1 shows that for the normal rats Ca^{45} deposition passed through a maximum between molar ratios of Ca: taurocholate of 4:1 and 2:1, indicating that oral administration of the bile salt was effective. A ratio of 4:1 increased the uptake to 146% that of the controls ($P < 0.1$) while a 2:1 ratio increased the uptake to 148% ($P < 0.05$). At ratios of 2:1 and 1:1, a marked diarrhea was evident. In rats with bile duct ligations, only the 8:1 ratio gave an apparent increase but this was not statistically significant. No diarrhea was seen and all of the rats of the 1:1 group died within 12 hours after dosing.

TABLE 2

The effect of bile duct ligation and intraperitoneal sodium taurocholate on the absorption of oral doses of labeled calcium [1]

Treatment	I.P. injection	No. of animals	Ca^{45} in femurs	Average increase in Ca^{45}
			% of dose	*% of control*
Sham operated	Saline	12	4.1 ± 0.3	100
Ligated duct	Saline	13	4.7 ± 0.2	114
Sham operated	Na Taurocholate	13	5.4 ± 0.3	131
Ligated duct	Na Taurocholate	8	4.2 ± 0.4	101

[1] Values represent mean ± standard error of the mean; 0.074 millimoles of sodium taurocholate or isotonic saline injected intraperitoneally into each rat; rats ranged in weight from 100 to 140 gm.

To pinpoint the functioning of the injected sodium taurocholate, rats with ligated ducts were contrasted to sham-operated rats and injections of 0.074 mM of the bile salt were compared to injections of isotonic saline. It can be seen in table 2 that intraperitoneal sodium taurocholate increased the femur content of Ca^{45} to 130% that of the controls ($P < 0.05$) and that ligations of the bile duct entirely removed this effect.

Ligation coupled with saline injection seemed to increase absorption but statistically this could not be shown to be significant. In subsequent studies this rise has not been observed.

In an experiment with adult female rats (250 to 300 gm), injections of 0.074 mM of sodium taurocholate intraperitoneally had no effect upon intestinal pH (table 3). In this trial control and test animals were sacrificed three and 10 hours after the last ingestion of food. This was also three hours after sodium taurocholate administration.

In a further study, 100 to 130 gm male rats were operated on so as to tie off only the bile duct, to tie off the bile-pancreatic duct, or to produce a sham-operated rat. After a three-hour recovery period these rats received 2 ml of the oral dosing solution in which glycine had been replaced by L-lysine. After 24 hours 8.4 ± 0.3, 8.1 ± 0.2, and $7.9 \pm 0.4\%$ of the Ca^{45} dose was found in the femurs of the control, bile duct ligated, and bile-pancreatic duct ligated groups, respectively. The differences were not statistically significant and thus the enhancing effect of lysine (Wasserman et al., '56) appears to be independent of biliary and pancreatic secretions.

DISCUSSION

Preliminary experiments indicated that the maximum enhancing effect of intraperitoneal sodium taurocholate was obtained when the bile salt was administered at the same time that the calcium test dose was given by gavage. A diminished effect was seen if the bile salt was given even one hour after the calcium dose. This indicated that the labeled calcium, present in a water solution, moved rapidly from the stomach to the intestinal tract. Natural foodstuffs would be expected to move slower giving more time for bile to be formed and mixed with the intestinal contents. Therefore, to portray adequately the normal function of bile in calcium absorption, it was deemed necessary to stimulate rapidly a copious flow of bile. To achieve this end, intraperitoneal injection of 0.037 mM of sodium taurocholate was used. It should be recognized that

TABLE 3

The effect of intraperitoneal injection of sodium taurocholate upon the pH of various segments of the gastrointestinal tract [1]

TREATMENT	NO. OF RATS	STOMACH	SMALL INTESTINE			CAECUM	LARGE INTESTINE
			1st third	2nd third	Last third		
		pH	*pH*	*pH*	*pH*	*pH*	*pH*
Killed 3 hours after eating							
Saline	5	4.3 ± 0.2	6.3 ± 0.1	6.7 ± 0.1	7.2 ± 0.2	6.7 ± 0.1	6.8 ± 0.1
Taurocholate	6	4.6 ± 0.2	6.4 ± 0.1	6.8 ± 0.1	7.4 ± 0.1	6.6 ± 0.1	6.7 ± 0.1
Killed 10 hours after eating							
Saline	4	2.8 ± 0.5	6.5 ± 0.1	6.7 ± 0.1	7.3 ± 0.2	6.5 ± 0.2	6.7 ± 0.1
Taurocholate	4	3.5 ± 0.3	6.6 ± 0.1	6.7 ± 0.1	7.2 ± 0.1	6.6 ± 0.1	7.3 ± 0.1

[1] Each figure represents the mean ± the standard error of the mean.

this is nearly three times the expected cholate content of a 100 gm rat (Lindstedt and Norman, '56). Nearly all of this injected bile salt would be put into the intestine via the bile duct; however, this output would extend over a period of about three hours (Lindstedt and Norman, '56). The data of Josephson ('41) with dogs and humans would suggest that only one-third of the injected material would appear within the first half-hour. This, plus the rapid movement of the test dose through the digestive tract, should have the effect of reducing the amount of bile mixing with the test dose to relatively normal levels. It should be kept in mind that even 0.009 mM of sodium taurocholate given intraperitoneally significantly increased the abosrption of calcium.

From the data developed in this paper it is evident that biliary secretions play a definite role in the movement of calcium from the intestine into the body. Stimulation of bile flow failed to produce significant changes in intestinal pH, thus removing this as a possibility. The necessity of maintaining the enterohepatic circulation intact, for either oral or intraperitoneal administration of sodium taurocholate, would indicate that the vehicle is contained within the bile itself. Though there is no direct evidence for it, the component involved in this transport is probably sodium taurocholate. From the data presented it can be seen that as the amount injected was increased a larger amount of calcium was deposited in the femurs. Since there was no internal effect detectable, this indicated an increase in absorption. The data of Klinke ('28) support this contention with the finding that solutions of cholic acid increased the solubility of $CaCO_3$, $Ca_3(PO_4)_2$, and Ca oleinate. Under his conditions it was noted that the ultrafilterable calcium fraction was increased. The failure to observe an increase in calcium absorption when the bile was given orally to rats with ligated bile ducts is probably an expression of the toxicity of the material rather than a contradiction of the above mechanism.

At present it is difficult to quantitate, in terms of a natural diet, the importance of bile in the absorption of calcium. No

doubt the composition of the diet, the calcium status of the animal, and the vitamin D status would confound such investigations. From the facts that absorption does occur in the absence of bile and increases when bile is added indicates that two mechanisms exist for calcium transport. That lysine could exert its effects independently of biliary secretions and at a much higher level than when $CaCl_2$ or $CaCl_2$ plus glycine is fed indicates yet a third mechanism. The relative importance and exact nature of these mechanisms is not yet understood.

SUMMARY

1. The influence of biliary secretion on calcium absorption from the intestine was investigated using intraperitoneal doses of sodium taurocholate to stimulate bile flow. The femur content of Ca^{45} 24 hours after administration of an oral dose of labeled calcium was used as criteria for absorption.

2. Sodium taurocholate injected intraperitoneally significantly increased the absorption of calcium from the intestine. The effect was produced in young and mature female rats, and young male rats.

3. Ligation of the bile duct removed the stimulatory effect of intraperitoneal sodium taurocholate upon calcium absorption.

4. Sodium taurocholate fed orally to young female rats significantly increased calcium absorption at a calcium: sodium taurocholate ratio of 2:1. Bile duct ligation prevented an increased absorption.

5. Sodium taurocholate given intraperitoneally had no influence upon the distribution of Ca^{45} injected intraperitoneally.

6. No significant changes in intestinal pH were seen when bile flow was stimulated.

7. The stimulatory effect of L-lysine upon calcium absorption could not be abolished by bile duct or bile-pancreatic duct ligation.

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EFFECTS OF SUPPLEMENTARY METHIONINE AND CHOLINE ON TISSUE LIPIDES AND ON THE VASCULAR STRUCTURE OF CHOLESTEROL-FED GROWING RATS [1,2]

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Studies of the influence of choline on cholesterol metabolism and on experimental atherosclerosis have led to contradictory reports. According to Hartroft et al. ('52), and Wilgram and associates ('54a,b, '55a,b), a choline deficiency (in the presence of adequate methionine) exerted a significant influence in producing vascular lesions in the rat; whereas other investigators have reported that a choline deficiency has no effect on the level of total blood cholesterol or on atherosclerosis, (Steiner, '38; Himsworth, '38; Baumann and Rusch, '38; Moses and Longabaugh, '50; Stamler et al., '50; Davidson et al., '51; Duff and Meissner, '54). On the other hand, Mann et al. ('53), Fillios and Mann ('54), Fillios et al. ('56) and Wissler and co-workers ('54, '55) reported a significant increase in the incidence of vascular lesions in animals fed a choline-rich diet. These authors further reported that choline-rich regimens produced a marked elevation in the

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serum lipide fractions. Results of more recent studies indicate that serum lipide fractions in monkeys, mice, and rats consuming choline-rich, methionine-deficient diets, may be altered and that the resulting changes lead to cardiovascular lesions. However, Broun et al. ('49), and Meeker and Kesten ('41), have failed to demonstrate such an effect.

In view of these contradictory reports, the relation of a choline deficiency, of a methionine deficiency, and of a combination of the two deficiencies to cholesterol metabolism and to experimental atherosclerosis seemed worthy of further investigation. The present report concerns some of the effects of high-cholesterol, low-choline diets and of high-cholesterol, low-methionine diets on the lipide fractions of certain tissues of the growing rat and on experimental atherosclerosis.

EXPERIMENTAL

Piebald rats of both sexes weighing from 40 to 57 gm and others ranging in weight from 100 to 144 gm were selected as experimental subjects. The rats were housed in individual all-metal cages provided with raised screen bottoms. With due consideration to distribution of sex, weight and litter, the rats were assigned to 11 experimental groups consisting of 10 to 13 animals per group. The respective experimental diets (compositions given in table 1) were fed ad libitum. Water was also furnished ad libitum. The rats were weighed at weekly intervals and the amount of food consumed recorded.

Fresh portions of the diets were prepared at biweekly intervals and stored at 3°C until used. The crystalline cholesterol was dissolved in warm corn oil before adding it to the other dietary constituents. A soy-bean protein, designated as C-1 protein by the manufacturer,[5] was used. This product was known to be deficient in sulfur-containing amino acids. Choline was incorporated in the respective diets in the form of the chloride in amounts 1.15 times the required weight of the base. In the methionine-supplemented diets, 0.6% of DL-methionine was used.

[5] The Drackett Products Company, Cincinnati, Ohio.

TABLE 1

Percentage composition of experimental diets

DIET NO.	1	2	3	4	5	6	7	8	9	10	11
Protein [1] (C-1)	19	19	19	19	19	19	19	19	19	19	19
Corn oil [2]	15	15	15	15	15	15	15	15	15	15	15
Cholesterol	5	5	5	5	5	5	5	5	5	5	5
DL-Methionine	—	—	—	0.6	0.6	—	—	—	0.6	0.6	0.6
Choline	0.10	0.05	0.025	0.10	0.05	0.2	0.05	0.01	0.2	0.05	—
Salt mixture (USP XIV) [3]	4	4	4	4	4	4	4	4	4	4	4
Vitamin mixture [4]	1	1	1	1	1	1	1	1	1	1	1
Sucrose	55.90	55.95	55.975	55.30	55.35	55.8	55.95	55.99	55.2	55.35	55.4

[1] A highly purified soybean protein obtained from the Drackett Products Company, Cincinnati, Ohio. The sulfur amino acid content of this protein as determined by the Wisconsin Alumni Research Foundation: methionine 1.0%, cystine 0.6%.

[2] Mazola.

[3] United States Pharmacopeia *XIV*, page 789.

[4] To each 100 gm of diet the following vitamins were added: thiamine HCl, 0.5 mg; riboflavin, 0.5 mg; pyridoxine HCl, 0.5 mg; Ca pantothenate, 5.0 mg; niacin, 8.0 mg; inositol, 100.0 mg; *p*-aminobenzoic acid, 100.0 mg; folic acid, 0.025 mg; biotin, 0.020 mg; vitamin B_{12}, 0.005 mg; ascorbic acid, 10.0 mg; menadione, 0.50 mg; α-tocopherol, 10.0 mg; vitamin A acetate, 1,250 I.U. and vitamin D_3, 180 I.U.

Care was taken to insure a uniform time interval between the removal of food from the cage and the securing of tissues from the rat. Blood for lipide analyses was obtained by cardiac puncture. Serum was separated after clotting and removed by means of a solvent pipet. Serum cholesterol was determined by the method of Zlatkis, Zak, and Boyle ('53). Serum phospholipides were determined by the combined methods of Youngburg and Youngburg ('30) and of Fiske and SubbaRow ('25). Total serum lipide was measured by the procedure of Sperry and Brand ('55). In the analyses of the liver, the whole organ was first homogenized and then the moisture content and the total lipide content determined by the methods of Sperry, '55. After the total lipides were determined, the desiccated residue was dissolved and made to a definite volume with the chloroform-methanol solvent (2:1) and aliquots of this solution were used in determining the various liver lipide components. The cholesterol content of an aliquot of the solution was determined by employing a modification of the Zlatkis, Zak and Boyle ('53) method for total cholesterol. Liver phospholipides were determined on another aliquot of the lipide extract by the method of Youngburg and Youngburg ('30) and Fiske and SubbaRow ('25). Adrenal cholesterol was determined by the method of Knobil et al. ('54).

The experiments were carried out in two phases (experiments 1 and 2). In experiment 1, the test animals, before being placed on the various dietary regimens, were maintained on a commercial stock ration[6] for 16 days in order to obtain basic tissue data. In this experiment, a study was made of the effects of feeding diets 1 to 5, inclusive (see table 1) to respective groups of growing rats (initial weight 100 to 144 gm) for a period of 12 weeks. At the end of the 12-week period, representative rats from group 3 were fed, for a period of two weeks, a diet containing 0.1% of added choline (diet 1) to determine the effect of further choline supplementation on the blood and tissue lipides.

[6] Wayne Stock Ration.

Experiment 2 was carried out in order to confirm some of the observations made during the first experiment and to determine whether the serum cholesterol concentration could be further influenced by increasing the choline content of the diet from 0.1 to 0.2% and, conversely, by lowering the choline content from 0.025 to 0.01% of the diet. In addition, a group of rats was fed a choline-free diet (diet 11) which contained 0.6% of added methionine. In experiment 2, groups of rats were fed diets 6 to 11 inclusive. At the end of the 12-week experimental period some of the rats receiving diet 6, on having manifested evidence of hypercholesterolemia, were given a dietary supplement of 0.6% of methionine (diet 9) for two additional weeks to determine whether dietary methionine affected the hypercholesterolemia.

RESULTS

Normal animals consuming commercial rations. The results of these studies are presented in condensed form in tables 2 and 3. Total cholesterol in the serum of young growing rats (12 weeks of age) consuming stock diets[7] in our laboratory varied from 60 to 82 mg%. The average for 29 males was 71 ± 11 mg% and for 29 female rats 69 ± 9 mg%. The total phospholipide in the sera of these rats varied from 75 to 105 mg%. The average adrenal cholesterol content for female rats was 5.7 ± 0.4% and for male rats 4.4 ± 0.3%. The average liver moisture content for these rats was 69 ± 2% and the averages of the various liver lipide components (expressed as percentage of wet weight) were as follows: total lipide, 3.7 ± 0.3%; total cholesterol, 0.2 ± 0.02%; total phospholipide, 2.7 ± 0.2%; and the ratio of cholesterol to phospholipide was 0.74. The mean values for the blood lipide and for the liver lipide of these normal animals did not vary as widely as did comparable data for the experimental animals.

Experiment 1 (with rats weighing 100 to 144 gm). At the start of experiment 1, 9 representative rats were anesthetized,

[7] Wayne, Purina or Rockland.

TABLE 2

Effect of composition of the diet of the rat on food intake, weight gain, and on the serum lipide content

(Average values are reported)

DIET AND GROUP NO.	WEEKS ON EXP. DIET	NUMBER OF RATS		INITIAL WEIGHT	FINAL WEIGHT	FOOD INTAKE	SERUM TOTAL LIPIDES	SERUM TOTAL CHOLESTEROL	SERUM PHOSPHO-LIPIDE	CHOLESTEROL/ PHOSPHO-LIPIDE RATIO
		M	*F*	*gm*	*gm*	*gm*	*mg %*	*mg %*	*mg %*	
Initial data	0	4	5	—	121	0	262	68	120	0.58
1	12	5	3	121	319	1042	994	290	149	1.95
2	12	6	2	121	315	1048	733	212	122	1.77
3	12	5	5	120	299	1011	657	181	102	1.82
3–1	12–2	3	2	306	322	176	884	252	129	1.97
4	12	5	3	120	371	1091	647	203	140	1.48
5	12	5	4	121	321	1017	580	203	117	1.74
6	12	5	5	49	272	973	1364	359	177	2.04
6–9	12–2	2	2	267	286	187	1098	236	183	1.29
7	12	3	7	51	245	913	741	217	143	1.56
8	12	4	7	53	264	970	631	194	130	1.50
9	12	4	5	48	298	1027	687	232	179	1.31
10	12	5	5	48	315	1064	544	207	135	1.54
11	12	3	5	48	296	989	516	217	139	1.63

TABLE 3

Effect of the composition of the diets on the lipide content of liver and adrenal tissues at the end of the 12- or 14-week experimental period

(Average values are reported)

DIET AND GROUP NO.	LIVER WEIGHT	RATIO OF LW/BW [1]	LIVER H_2O	LIVER TOTAL LIPIDE	LIVER TOTAL CHOLESTEROL	LIVER PHOSPHOLIPIDE	RATIO OF CHOLESTEROL/ PHOSPHOLIPIDE	LIVER TOTAL LIPIDE	LIVER TOTAL CHOLESTEROL	LIVER PHOSPHOLIPIDE	ADRENAL TOTAL CHOLESTEROL M	ADRENAL TOTAL CHOLESTEROL F
	gm		%	% *Wet wt.*	% *Wet wt.*	% *Wet wt.*	*Wet wt.*	% *Dry wt.*	% *Dry wt.*	% *Dry wt.*	% *Wet wt.*	% *Wet wt.*
Normal	4.9	0.041	68.6	3.8	0.2	2.59	0.08	12.2	0.7	8.2	3.60	4.72
1	11.7	0.036	56.3	24.2	9.0	2.62	3.45	55.4	20.7	6.0	6.16	7.20
2	11.9	0.038	54.6	26.9	11.0	2.49	4.50	59.3	24.4	5.5	6.23	8.52
3	11.4	0.039	51.6	31.4	14.3	2.21	6.49	65.0	29.7	4.6	7.14	8.65
3–1	11.5	0.036	55.9	25.2	11.2	1.95	5.86	57.0	25.6	4.6	5.91	7.40
4	13.1	0.035	57.6	22.7	8.9	2.39	3.76	53.1	20.8	5.7	5.98	8.24
5	12.1	0.037	56.4	24.1	10.7	2.47	4.43	55.2	24.5	5.7	6.94	8.54
6	9.9	0.036	57.0	23.5	8.2	2.56	3.34	54.2	18.9	6.0	5.54	6.98
6–9	10.7	0.036	57.9	22.6	6.9	2.94	2.35	53.7	16.4	7.0	5.97	6.96
7	9.7	0.039	54.8	25.5	9.9	2.52	3.96	56.2	21.8	5.6	6.00	7.13
8	11.0	0.042	53.8	27.8	11.1	2.44	4.59	60.2	24.0	5.3	5.99	7.54
9	11.6	0.037	57.3	22.3	8.4	2.72	3.12	52.1	19.5	6.4	6.46	7.24
10	12.3	0.038	56.9	22.9	9.5	2.71	3.61	52.9	22.0	6.4	7.15	7.27
11	10.9	0.036	57.4	22.9	9.7	2.57	3.81	53.7	22.7	6.1	6.51	7.73

[1] LW = liver weight, BW = body weight.

exsanguinated, and their livers and adrenals immediately removed for use in ascertaining pretreatment tissue lipide levels. These animals were selected from a group which had been fed a good breeding colony ration during the past 16 days in order to increase their body weight before being placed on experiment. A summary of the results obtained are presented in table 2.

The mean serum lipide components of the tissues of this control group of animals (expressed as mg%) were found to be as follows: total lipide 262 (range, 242 to 287), total cholesterol 68 (range, 64 to 72), and phospholipide 120 (range, 97 to 145). The cholesterol/phospholipide ratio was 0.58. The mean liver moisture content was 68.6% (range, 67.7 to 70.0) and the various lipide components of the livers (expressed as percentage of wet weight) were: total lipide 3.8 (range, 3.5 to 4.2), total cholesterol 0.21 (range, 0.18 to 0.22), and phospholipide 2.6 (range, 2.1 to 3.2). The cholesterol/phospholipide value was 0.081. The mean total adrenal cholesterol was 3.6% for males and 4.7% for the females.

The remaining rats were divided into 5 groups of 13 animals each. Individual rats were bled at intervals of three and 6 weeks. At the end of 12 weeks all of the animals were killed and their livers and adrenals immediately removed. Since some time may elapse before the effect of ingested fat has disappeared from the blood and preprandial values are obtainable, the blood and tissue samples used in these determinations were collected 12 hours after removal of food from the cages. When diets were fed which contained 19% of the sulfur amino acid-deficient protein but which were high in fat and in cholesterol, the concentration of blood cholesterol was related to the quantity of choline in the diet. The greater the amount of dietary choline the greater the hypercholesterolemia. Furthermore, the data indicate that the differences in concentration of serum cholesterol as observed in the blood of groups of rats receiving the various diets (diets 1, 2, 3) were not the result of differences in food in-

take, since the food intakes of the different groups were essentially the same. When 0.6% of DL-methionine was included in the diets (groups 4 and 5), the hypercholesterolemia was partially prevented. However, in the presence of adequate methionine, dietary choline exhibited no noticeable influence on the serum cholesterol levels. The animals comprising groups 4 and 5 consumed essentially the same amount of food as did the animals in groups 1, 2 and 3.

Experiment 2 (rats weighing 40 to 57 gm). In the previous experiment the hemorrhagic nephrotic syndrome frequently associated with a choline deficiency was not observed. In the second experiment younger rats were used in order to produce this choline-deficiency syndrome since the age of the rat is known to be important in this connection. A study was made also of the influence of increasing and decreasing dietary choline on the level of tissue lipides and on the vascular structure. An additional group of rats (group 11) also was included, the animals receiving a choline-free diet containing 0.6% of added methionine.

Of the 39 choline-deficient rats used in this experiment, 6 males and 5 females died from hemorrhagic kidney lesions occurring as a consequence of choline deficiency. The remaining 28 animals sustained a crisis of kidney damage and recuperated through the aid of intraperitoneal injections of choline. Deaths usually occurred on the 7th or 8th days of the experiment. This dietary imbalance resulted in the kidneys of these rats being transformed, on about the 6th day of feeding, from an apparently normal kidney to a greatly enlarged dark red, bloody structure. The mortality was highest on the 7th day. If the rat survived the acute stage, recovery was rapid, food consumption increased, and the animal gained weight. When diet 7 (0.05% of choline) was used, no deaths occurred among the female rats, but three male rats died from hemorrhagic kidney. On the other hand, when diet 8 (0.01% of choline) was fed to 9 male and 13 female rats, 3 males and 5 females succumbed to a condition resembling bilateral renal cortical necrosis. These observations indicated

that when a diet moderately deficient in choline (0.05%) is employed, female rats are more resistant to death from choline deficiency and when a diet highly deficient in choline (0.01%) is used both sexes are equally susceptible (33% of males and 39% of the female rats succumbed). All of the rats that received the high choline-supplemented diets appeared to remain healthy.

The data relating to variations in serum lipids are summarized in table 2. All cholesterol-enriched diets produced hypercholesterolemia, hyperlipemia, and hyperphospholipemia of varying degrees. In all rats, hypercholesterolemia was the principal alteration in the serum lipide pattern. Phospholipides increased but disproportionately less than did cholesterol, hence the ratio of the two increased significantly. The total serum lipide of all rats was elevated indicating that an increase in the neutral fat fraction had occurred.

The total lipide and the total cholesterol levels in the serum of the rats which had received diet 6 (no added methionine, 0.2% of choline) were increased 5-fold, whereas in rats receiving diet 9, which contained the same level of choline but was further supplemented with 0.6% of methionine, the serum total lipide was increased by only 2.6-fold and the total cholesterol increased by 3.4-fold. By lowering the choline content from 0.2% to 0.05% in the methionine-deficient diet the serum total lipide and total cholesterol were decreased 40%, and the lowering of dietary choline to 0.01% further lowered the lipide and cholesterol fractions. In the rats receiving the methionine-supplemented diets (diets 9, 10, 11) the hyperlipemia and hypercholesterolemia was partially prevented. Under these conditions supplementary choline had no effect on the hypercholesterolemia, whereas the lowering of the choline intake ameliorated the hyperlipemia.

The effect of dietary changes on established hypercholesterolemia. In experiment 1. At the end of the 12-week experimental period, 5 rats were selected at random from the low-choline, low methionine group (group 3) for further study. At this time they were bled by cardiac puncture to

obtain material for serum lipide determinations. They were then placed on a diet containing 0.1% of choline for two weeks (diet 1), after which they were sacrificed and examined in the usual manner. The influence of increased dietary choline on serum lipides at this stage of the experiment was striking. The serum total lipide, total cholesterol, and the phospholipide were found to have increased 25.7%, 28.2% and 21.0%, respectively. On the other hand the liver total lipide, the total cholesterol, and the phospholipide decreased 19.7%, 21.7% and 11.7%, respectively. The liver moisture increased 7.7% and the adrenal total cholesterol concentration in both female and male rats was significantly lowered.

In experiment 2. At the end of the 12-week experimental period 4 rats were selected from the group which had subsisted on the choline-supplemented, low-methionine diet (group 6) for further study. After cardiac puncture, these rats were placed on a diet supplemented with 0.6% of methionine (diet 9) and after two weeks were sacrificed and examined in the usual manner. The effect of methionine supplementation of low-methionine diet, in the presence of choline, on the blood lipides was dramatic. The serum total lipide and the total cholesterol decreased 19.5 and 24.3%, respectively. The cholesterol/phospholipide ratio decreased by 36.7% through a decrease in cholesterol concentration and an increase in the phospholipide fraction. The total liver lipide (expressed as percentage of wet weight) decreased from 23.5 to 22.6%, the cholesterol concentration from 8.2 to 6.9%, whereas the liver phospholipide increased from 2.6 to 2.9%. With this decrease in cholesterol concentration, the liver cholesterol-phospholipide ratio changed from 3.3 to 2.3.

Histological findings. For normal tissues in histological studies, healthy rats were selected from our breeding colony. These "normal" 4-month old male rats had an excellent record of food intake and weight gain, and exhibited optimal general appearance. Typical of the tissues taken from these rats is that of rat 5543. Microscopic examinations of the tissues from the experimental rats revealed that abnormalities

of the cardiovascular system were absent in all methionine-supplemented animals but were present in 9 of the 18 methionine-deficient choline-supplemented rats. The aortic lesions consisted of intimal thickening with fibrosis and hyalination. Microphotographs of three sections of tissues taken from typical rats (rats 50897, 50922 and 50960) are presented in plate 1. Rat 50897 (experiment 1) demonstrated a thickened intima. In this instance the smooth muscle cells of the underlying media were "pushed out" of their normal position indicating a hyperplastic intima. Rat 50922 (experiment 2) exhibited a mild lesion with fibrosis and intimal thickening, whereas rat 50960 had a aorta which contained a vast amount of hyaline tissue. In all of these animals both the serum lipide and cholesterol levels were greatly elevated. Rat 50960 had received a methionine supplement for two weeks prior to being sacrificed. However, this animal had previously consumed a diet lacking methionine for 12 weeks before being changed to the methionine-enriched diet. This lesion undoubtedly developed prior to methionine-supplementation. While it has been shown that atherosclerotic lesions are reversible, the process is apparently slow, requiring many days for the transformation back to normal in some animals. All of the vascular abnormalities recorded were observed in male rats, thus suggesting that estrogens offer some protection for the female.

DISCUSSION

No variation in concentrations of blood or liver lipides could be attributed to the sex of the test animal. On the other hand, the level of cholesterol in the adrenal gland was found to be closely linked with sex, the female rat exhibiting a higher adrenal cholesterol concentration than did the male. However, relationship between the serum cholesterol level and that in the adrenal gland could not be demonstrated.

The animals receiving supplementary methionine exhibited a faster rate of growth, indicating that the speed of protein synthesis is controlled by the intake of the indispensable amino acid. Rats of group 4, which received "optimal" cho-

line (0.01%), grew faster than did those of group 5 (0.05% choline), indicating that in the presence of adequate choline no methionine is required for lipotropism and indicating that there is a preferential utilization of methionine for growth.

A further study of the data indicates that choline is active in the formation of serum phospholipide. The greater the dietary choline intake the greater the serum phospholipide concentration. This relationship was found to hold true both in the rats receiving the methionine-deficient diets and in those receiving the methionine-supplemented diets. This increase in serum phospholipide with increasing dietary choline indicates that choline is a precursor in the formation of the choline-containing phospholipides of the serum. The rats consuming the methionine-deficient diets exhibited a rise in the serum cholesterol/phospholipide ratio when the diets were high in choline. In the rats receiving the methionine-supplemented diets, the ratio of serum cholesterol to serum phospholipide was found to be lowest in those receiving the higher choline supplement. This is believed to be due to an increase in serum phospholipide with increased dietary choline, whereas the serum cholesterol content remained essentially the same in both groups of rats.

Increases in the cholesterol/phospholipide ratio are interpreted by some investigators as predisposing the development of atherosclerosis. It seems rather generally believed that it is not the cholesterol level alone that is significant, but that the development of the disease depends on the serum cholesterol/pospholipide ratio. If this concept is correct, then diet 1 should have been the most atherogenic inasmuch as it produced the highest ratio of serum cholesterol/phospholipide.

In the present study it was observed that increasing the dietary choline resulted in an increase in the total lipide of the blood. On the other hand, methionine-deficient rats developed a severe lipemia while receiving the recommended level of choline, a condition which did not appear when lower levels of choline were fed. Rats which received the methio-

nine-supplemented diets developed a moderate lipemia and those receiving the diet with the higher content of choline developed a more severe lipemia. This hyperlipemia seemed to parallel the hypercholesterolemia observed in rats receiving the low-methionine diets. Rats which had received the methionine-supplemented diets exhibited lower levels of total lipide than did rats which had received the methionine-free diets and the total cholesterol values assumed an intermediate range. In evaluating the influence of methionine on total serum lipides it was observed that the serum lipide components varied in a similar manner when the choline intake remained unchanged and the methionine intake was varied. The serum lipide level of the rats comprising group 1 was 35% higher than that of rats of group 4, while the cholesterol and the phospholipide levels were 30 and 6% higher, respectively.

Data relating to liver size, liver moisture, and liver lipides are presented in table 3. For ease of comparison liver lipide values are expressed both as percentage of fresh and dry organ weight. However, expressing these data as percentage of dry organ weight did make a difference in the pattern of significance. Rats subsisting on the cholesterol-rich diets exhibited no liver lipide variations attributable to sex. The ratio of liver weight to body weight was increased by a decrease in the choline intake and decreased when adequate methionine was fed.

After 12 weeks of feeding all rats showed a similar pattern of tissue lipidosis varying only in severity (table 3). Cholesterol was the chief component of this hepatic lipidosis. The increase in cholesterol far exceeded the rise in phospholipide which was often insignificant. The total lipide concentration was also elevated, indicating a rise in the neutral fat fraction. Liver moisture increased with choline intake and also with increased methionine feeding. The ratio of liver cholesterol/liver phospholipide increased with a decrease in choline intake whereas methionine intake had little effect on this ratio.

Choline had a moderate lipotropic effect on the cholesterol-induced hepatic lipidosis in that it served to decrease the total lipide and total cholesterol fractions. Methionine, on the other hand, exhibited only a slight lipotropic effect on the liver lipide and on the total cholesterol fractions.

The pathological livers were greatly enlarged and engorged with fat. They were white in appearance, fragile in structure, and contained little blood. The actual amounts of water and fat increased in the fatty livers although the percentage of phospholipide remained fairly constant. Liver lipides normally comprise 3 to 4% of the total moist weight of the liver. However, the mean proportion of lipide in the livers of those rats having received diets containing 5% of cholesterol was 25.8% of the moist organ weight and the average water content was 55.4%. Individual livers were found to contain as much as 35.3% of lipides. These fatty livers developed rapidly and were detected as early as one week after the animals were placed on the experimental diets. The source of the accumulated lipide in these fatty livers was believed to be largely exogenous in origin. This was indicated by the fact that the cholesterol content of the lipide was extremely high.

The data presented in table 3 also indicate that the total adrenal cholesterol was markedly increased in rats receiving these diets and that adrenal lipidosis was increased by decreasing the choline intake of both male and female rats. On the other hand methionine had no effect on the deposition of fat in the adrenals.

Some investigators assume that the degree of cholesterolemia and the level of liver cholesterol found in cholesterol-fed animals are indices of cholesterol absorption. If this were the case the liver would tend to accumulate dietary cholesterol and thus act as a buffer to prevent increases in serum lipides. However, the consistent relationship between liver lipide and serum lipide suggests that choline has a mobilizing effect, *viz.* that it removes the lipides from the liver into the blood. Choline is known to stimulate the formation of phospholipides in the liver and thereby tends to move the fat from the liver to the fat depots.

The liver lipides and serum total lipide were further lowered when the diets were supplemented with racemic methionine, the methionine partially preventing the increased hypercholesterolemia. In this instance methionine may act directly on the metabolism of lipides or indirectly by inhibiting, in some manner, the absorption of cholesterol from the intestinal tract.

The data indicate that under certain conditions choline had an aggravating effect on hyperlipemia and in the case of the low-methionine diets had an aggravating effect on hypercholesterolemia.

It was noted that even moderate doses of choline were ineffective in maintaining liver cholesterol in the normal range of concentration when the cholesterol intake was excessive. The results from experiment 1 indicated that choline and methionine have a small but consistent lipotropic effect on the cholesterol-induced hepatic lipidosis. Here it appears that choline alone accelerated the removal of cholesterol and neutral fat from the liver inasmuch as both hepatic total lipide and total cholesterol decreased when dietary choline was increased. However, in the presence of supplementary methionine, the hepatic total lipide remained fairly constant and the hepatic cholesterol was lowered when the choline intake was increased, thus indicating that in the presence of methionine the primary effect of choline is on cholesterol and that the removal of neutral fat is a much slower process. In the absence of methionine the water content of the liver was found to increase with increased choline intake whereas in the presence of dietary methionine the hepatic moisture content remained fairly constant with changes in dietary choline. This indicated that the increase of lipotropic agents above some undetermined minimum had no effect on the water content of cholesterol-fatty livers. From the results of experiment 2, as with those of experiment 1, a consistent relationship between liver cholesterol and serum cholesterol was found to exist, as well as the other relationships already mentioned in connection with experiment 1. The relationship of

adrenal total cholesterol to diet composition was found to be similar to that already reported (experiment 1).

It appears obvious that choline has an aggravating effect on hyperlipemia, hypercholesteremia, and on hyperphospholipemia and that it exerts a strong lipotropic action when added to a choline-deficient diet. It would appear that the lipides are removed from the liver to the blood where they remain, thus causing the high serum lipide levels. The data also suggest that choline supplementation influences the absorption of the lipides from the intestinal tract and supports the general belief that choline prevented the accumulation of lipides in the liver. The data suggest that methionine exerted a limited lipotropic effect and brought about a lowering of the serum total lipide and the cholesterol levels. Unlike the results from the previous experiment, where choline supplements were employed, both liver and serum lipide concentrations were lowered except for that of the phospholipide fraction which was increased perhaps due to the increase in available methyl groups. This indicates that methionine acts directly on the metabolism in some manner to bring about the utilization of the excess lipide, withdrawing it from both liver and blood. On the other hand, methionine appears to act through limiting the absorption of cholesterol and fat from the intestinal tract. Methionine supplementation, in both preventative and curative studies, tended to maintain the lipides within the normal range.

Although the cholesterol content of a natural diet may be unimportant in reckoning the concentration of blood cholesterol, the composition of the diet may exercise a major influence through other constituents as is indicated by the data presented. It is recognized that the experimental work reported at this time has been done under extreme conditions in which excessive amounts of cholesterol (5%) were incorporated in the diets, amounts never ingested under normal conditions. However, high levels of cholesterol feeding have been found to be useful in the production and study of experimental hypercholesteremia and experimental atherosclerosis.

SUMMARY

The influence of choline deficiency, methionine deficiency and a combination of these deficiencies on lipide metabolism and on the development of atherosclerosis in the growing rat has been investigated. Mild aortic changes were produced in male rats by feeding diets high in cholesterol, adequate in choline, and deficient in sulfur-containing amino acids. The various serum lipide fractions were greatly increased in those rats consuming the choline-rich diets. Consequently, the elevation of these constituents appears to be a prerequisite to the production of certain cardiovascular disease. Under the conditions of these experiments the increased hyperlipemia, hypercholesterolemia, and hyperphospholipemia resulting from the ingestion of such diets was partially prevented by DL-methionine supplementation.

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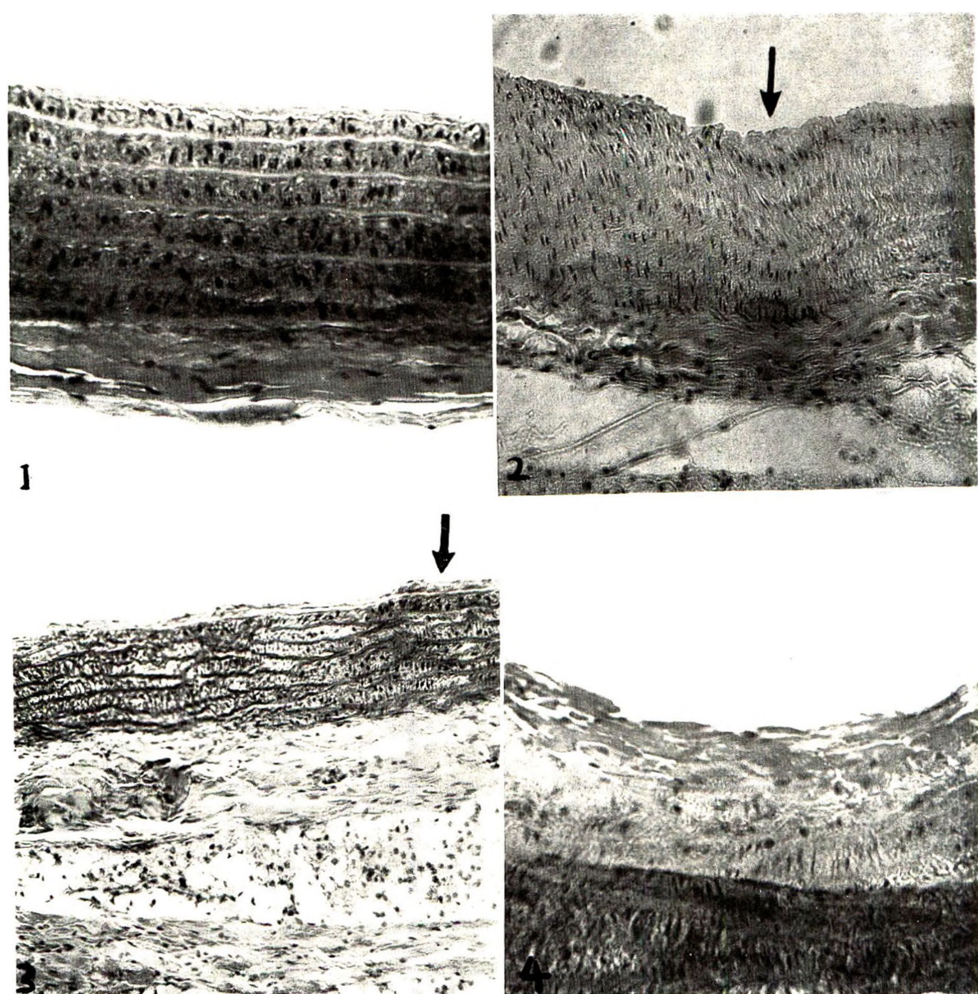

1 Rat 5543. Microscopic appearance of a stained section of the normal aorta of a control animal. × 80.

2 Rat 50897. Intimal thickening at arrow — note displacement of smooth muscle cells beneath the internal elastic membrane. × 160.

3 Rat 50922. Small lesion at arrow, with fibrosis and thickening of the intima. × 160.

4 Rat 50960. Hyalination (whole inner curvature) of the intima and inner surface of the media. × 160.

NUTRITIVE VALUE OF DIFFERENTLY PROCESSED GROUNDNUT[1] MEALS AND THE EFFECT OF SUPPLEMENTATION OF THE MEALS WITH AMINO ACIDS, ANTIBIOTICS AND VITAMIN B_{12}

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The high oil content of groundnut (45 to 50%) compared to soya bean (20%) and cottonseed (20%) makes the extraction of oil difficult in any single operation. For the best use of the flour from the point of view of its keeping quality, the oil content has to be reduced to a minimum (preferably $< 1\%$).

In the present investigations, groundnuts were processed under different conditions of heat and steam treatment for the efficient removal of oil and solvent. The amino acid composition of the differently processed meals was qualitatively compared with the amino acids of casein by paper chromatography and the thiamine content of the meals was assessed. Growth-tests (Osborne, Mendel and Ferry, '19) and nitrogen balance studies (Mitchell, '24; Mitchell and Carman, '26) on rats were carried out to evaluate the nutritive value of each of the meals.

The effects of supplementation of groundnut protein with amino acids, antibiotics and vitamin B_{12} were tested using the paired-feeding method of Mitchell and Beadles ('29), and some of the observations with supplementation were tested by the nitrogen balance method. A brief report of the

[1] Peanut.

work has already been published (Cama, Balasundaram and Malik, '55).

The subsequent two papers correlate the nutritional observations by electrophoretic changes in the different components of the protein (Cama, Malik and Nath, '58), and provide data on the effect of supplementation of groundnut protein with antibiotics and vitamin B_{12} on the liver reserves of vitamin A esters of the rat (Cama and Malik, '58).

MATERIALS AND METHODS

The groundnut meals were prepared under the following conditions from the same quality of seeds (Spanish variety):

Meal A: The cold solvent-extracted meal (raw) was prepared in the laboratory by passing pulverized groundnut grits through a 40-mesh sieve and as much as possible of the oil was removed by pressing in a small hydraulic press (pressure 4,000 to 5,000 lbs./sq. in.). The meal was shaken 5 to 6 times with low-boiling petroleum ether (40 to 60°) and finally washed on a filter with petroleum ether and dried at room temperature. The meal had a raw beany odour and was whitish in appearance. The percentage composition of the meal was: protein 45, oil 1.5 and moisture 9.2.

Meal B: was prepared using the common "expeller" process (screw-pressing) after slight cooking. This meal had a roasted odour and was brownish in appearance (protein 47, oil 10.6 and moisture 7.3%).

Meal C: was prepared from the "expeller" meal, by extraction with the non-flammable and non-denaturing solvent trichlorethylene in a pilot solvent-extraction plant. The extracted meal was steamed to remove residual oil and solvent under optimal conditions of open steam released from 25 lbs. compression for one-half hour (protein 52.3, oil 1.15 and moisture 4.3%).

Meal D: was the same as meal C but the residual solvent and oil were given an open steam treatment released from 100 lbs. compression for one hour. This meal received considerably more heat treatment and was dark brown in appearance (protein 52.3, oil 1.48 and moisture 5.3%).

Amino acids by paper chromatography: The amino-acid composition of the 4 differently processed meals was qualitatively compared with the amino acids of casein, after circular paper chromatographic separation of the amino acids from the protein hydrolysate. The technique and details were essentially the same as given by Giri and Rao ('52).

Samples (0.5 gm) of each of the 4 meals and caseinogen were mixed with 25.0 ml of 6N HCl and autoclaved at 15 lbs. pressure for 6 hours. After neutralization with sodium hydroxide and evaporation to dryness the residues were extracted with 10% *n*-propanol. Each of the solutions containing 75 μg of nitrogen in 75 μl was spotted on three different Whatman No. 1 filter papers and each paper was irrigated by any one of the following solvent mixtures —

(a) butanol : water : ethanol = 20 : 4.5 : 1
(b) butanol : water : acetic acid = 40 : 7 : 5 and
(c) butanol : water : acetic acid = 40 : 4 : 15 parts.

After irrigation, the papers were sprayed with 0.4% ninhydrin [2] in 95% acetone, dried and developed in an oven at 65°C for 15 minutes. The bands of the amino acids were qualitatively compared for colour intensity and photographs taken.

For tryptophan estimation 0.5 gm of the meal was mixed with 4.2 gm of anhydrous barium hydroxide and 10.0 ml of distilled water and autoclaved at 15 lbs. pressure for 8 hours. The solution was then neutralized with sulphuric acid and the same treatment as above was applied except that for irrigation only the butanol-water-acetic acid (40 : 7 : 5) mixture was used.

Thiamine estimation: Estimation of thiamine in the 4 meals was carried out by the modified thiochrome assay method of Hennessey and Cerecedo ('39).

Samples (2.0 gm) of each of the 4 meals were refluxed with 75.0 ml of 0.1 N H_2SO_4 for two hours and incubated overnight with 0.5 gm pepsin [3] at 37°C. The solution was

[2] Hoffmann-La Roche, Switzerland.
[3] Penick and Co., N. Y., U.S.A.

warmed to 100°C and on cooling it was mixed with 5.0 ml of 15 M sodium acetate, 0.5 gm of takadiastase [4] and again incubated overnight. The solution was filtered and aliquots were passed through a base-exchange zeolite [5] column. Thiamine was eluted with 25% hot potassium chloride in 0.1 N HCl. Each aliquot of the eluant was shaken with 3.0 ml of 1% potassium ferricyanide and 3.0 ml of 15% NaOH. The thiochrome formed was extracted with 10.0 ml of iso-butanol and fluorescence readings were taken.

Growth test: Four groups of albino rats, each with 4 females and three males and weighing between 25 and 30 gm were kept in individual cages. The average initial weights of each of the 4 groups were approximately equal. One of the 4 groundnut meals under comparison was given to each group, ad libitum, and the weight increases and the food intake were recorded (table 1).

The percentage composition of the diet with raw (A) groundnut meal was as follows: groundnut meal, 34.4; sucrose, 10; salt mixture,[6] 3.0; sodium chloride, 1.0; groundnut oil, 7.6; and maize starch, 44.0. The protein and oil content of the diet were 15 and 9% respectively. Each of the other three diets prepared with meals B, C and D also contained 15 and 9% of protein and oil respectively.

In addition, vitamin A was supplied as shark-liver oil,[7] mixed in groundnut oil at the rate of 300 I.U. of vitamin A per 10 gm of diet. The B-complex group of vitamins aneurin hydrochloride, 0.3; niacinamide, 2.0; riboflavin, 0.5; pyridoxin, 0.3 mg/kg and choline chloride, 2.0 gm/kg of diet were dissolved in water and added to the rations daily.

Paired-feeding test: Seven littermate pairs of albino rats, 4 females and three males in each group were pair-fed for 7 weeks, the diet of the littermate receiving the supplement being equalised with respect to that of the control rat. The composition of the diet, the levels of protein and oil and the

[4] British Drug Houses, U.K.
[5] Central Scientific Co., N. Y., U.S.A.
[6] Osborne and Mendel ('13).
[7] Fisheries Technological Laboratory, Bombay.

amounts of vitamin A and B vitamins added to the rations were the same as in the growth test.

The supplements of amino acids, antibiotics and vitamin B_{12} (table 2) were mixed daily in the food in aqueous solution. The following amino acids: L-lysine, DL-methionine, DL-isoleucine, DL-threonine, L-cystine, DL-tryptophan were each given at a level of 0.2% of the diet. Penicillin [8] and streptomycin [9] were given at the 0.02% level and aureomycin [10] was at the 0.03% level of the diet. Vitamin B_{12}[11] was given at the rate of 45 μg/kg of diet.

TABLE 1

Growth of rats in relation to protein intake of 4 differently processed groundnut meals

(All meals fed at the 15% protein level for periods of 7 weeks)

DIET	AVERAGE INCREASE IN WT. x	AVERAGE PROTEIN INTAKE y	PROTEIN EFFICIENCY RATIO x/y
	gm	*gm*	
Raw (A)	72.3	65.3	1.11 ± 0.041 [1]
Expeller (B)	79.0	55.0	1.43 ± 0.030
Control steamed (C)	72.6	50.4	1.44 ± 0.026
High steamed (D)	70.4	50.7	1.38 ± 0.038

[1] Standard error of the mean.

Nitrogen balance test: In each experiment, two litters of 4 female albino rats were used. They weighed about 50 to 60 gm at the beginning of each test and were between 40 and 45 days old. The cages used for the quantitative collection of urine and feces were as described by Cama and Morton ('51) and the Latin-square design of Macrae, Henry and Kon ('42) was followed.

The percentage composition of the standardising diet used at the beginning and end of each experiment to ascertain the amounts of endogenous urinary and fecal nitrogen was as

[8] Rhodia, France.

[9] Rhodia, France.

[10] Lederle Laboratories, Switzerland.

[11] "Macraban," Glaxo Laboratories, U.K.

TABLE 2

The effect of supplementation of groundnut protein with amino acids, antibiotics and vitamin B_{12} on the growth of rats in paired-feeding experiments
(Period: seven weeks)

DIET	AVERAGE INCREASE IN WT. x	AVERAGE PROTEIN INTAKE y	PROTEIN EFFICIENCY RATIO X/Y	MEAN DIFFERENCE	S.E.[1] DIFF.	P (PROBABILITY)[2]
	gm	gm				
Raw (A)	70.4	66.0	1.07	+ 0.04	± 0.0105	S***
Raw + lysine	73.5		1.11			
Raw	80.0	77.0	1.03	+ 0.08	± 0.0276	S**
Raw + methionine	86.0		1.11			
Raw	72.0	62.4	1.14	+ 0.09	± 0.0208	S**
Raw + isoleucine	76.8		1.23			
Raw	72.0	62.4	1.14	+ 0.09	± 0.0254	S**
Raw + threonine	76.8		1.23			
Raw	84.0	78.0	1.07	—	± 0.0212	N.S.
Raw + cystine	84.0		1.07			
Raw	70.0	66.0	1.07	— 0.07	± 0.0214	S**
Raw + tryptophan	66.0		1.00			
Raw	68.0	68.0	1.00	— 0.02	± 0.0207	N.S.
Raw + vitamin B_{12}	67.0		0.98			
Raw	95.0	88.0	1.07	+ 0.03	± 0.0119	S**
Raw + penicillin	98.0		1.10			
Raw	68.0	68.0	1.00	—	± 0.0394	N.S.
Raw + aureomycin	68.0		1.00			
Raw	95.0	88.0	1.07	—	± 0.0316	N.S.
Raw + streptomycin	95.0		1.07			
Expeller (B)	79.3	50.4	1.57	+ 0.17	± 0.0185	S***
Expeller + methionine	87.6		1.74			
Expeller	77.0	55.0	1.41	—	± 0.0562	N.S.
Expeller + vitamin B_{12}	77.0		1.41			
Expeller	77.0	55.0	1.40	+ 0.06	± 0.0263	S*
Expeller + aureomycin	80.0		1.46			
High steamed (D)	69.6	50.4	1.38	+ 0.21	± 0.0199	S***
High steamed + methionine	80.4		1.59			

[1] Standard error of the differences.
[2] S*** = Significant between 1 and 2% levels.
S** = Significant between 2 and 5% levels.
S* = Significant between 5 and 10% levels.
N.S. = Not significant.

follows: dry defatted whole egg, 5.4; sucrose, 10; salt mixture,[6] 3.0; sodium chloride, 1.0; groundnut oil, 9.0; cellulose powder,[12] 3.0; and maize starch, 68.6. The protein and oil contents were 4.0 and 9.0% respectively.

The diet with raw (A) groundnut meal contained the following percentage ingredients: groundnut meal, 20; sucrose, 10; salt mixture,[6] 3.0; sodium chloride, 1.0; groundnut oil, 7.5; and maize starch, 58.5. The protein and oil contents were 9% and the other diets containing meals B, C and D also contained 9% of oil and protein. In addition, vitamin A and the B-complex group of vitamins were supplied as described for the growth tests for the standardising as well as experimental diets.

Four nitrogen balance tests were carried out (table 3), using 10-day periods. The first three days of each period were for standardizing the rat for each change of diet and the last 7 days for the quantitative collection of urine and feces. The amount of nitrogen consumed during each 7-day period was noted. In test 1, raw (A), "expeller" (B), control-steamed (C) and high-steamed (D) meal diets were compared. Test 2 was carried out with supplementation of raw (A) groundnut meal diet with methionine, aureomycin and vitamin B_{12}. In test 3, the effect of supplementation of raw (A) groundnut meal diet with lysine, penicillin and streptomycin was examined and finally in test 4, "expeller" meal (B) diet was supplemented with methionine, aureomycin and vitamin B_{12}. The quantities of supplements added were the same as given during the paired-feeding test.

The significances of the differences between the differently treated groups in the paired-feeding test (table 2) as well as in the nitrogen balance experiments (tables 3 and 4) were statistically analysed by the t-test of significance as given by Davies ('49). The results were considered significant where P was between 5 and 10% or less than 5%, whereas those which had a value for P of more than 10% were not significant.

[6] See footnote 6, page 78.

[12] Carl Schleicher and Co., Germany.

TABLE 3

Mean data for biological values and true digestibilities of groundnut meals processed by different extraction procedures

DIET	PERIOD I	PERIOD II	PERIOD III	PERIOD IV	MEAN	STANDARD ERROR OF MEAN
Test 1			*Biological value*			
Raw (A)	41.0	53.1	54.6	56.9	51.4	± 2.62
Expeller (B)	53.2	59.0	62.3	66.5	60.2	± 1.89
Control steam (C)	63.5	65.3	67.4	59.6	63.9	± 1.68
High steam (D)	49.9	58.1	55.0	49.3	53.0	± 2.01
			True digestibility			
Raw (A)	89.8	91.7	92.5	90.4	91.1	± 0.53
Expeller (B)	90.2	94.0	94.6	93.8	93.1	± 0.83
Control steam (C)	85.8	87.9	92.6	94.5	90.2	± 1.73
High steam (D)	87.4	90.6	97.8	92.2	92.0	± 1.48
			Protein value [1]			
Raw (A)	36.8	48.6	50.5	51.4	46.8	± 2.54
Expeller (B)	47.9	55.5	59.0	62.4	56.2	± 2.05
Control steam (C)	54.4	57.4	62.5	56.5	57.7	± 2.06
High steam (D)	43.6	52.6	53.1	45.4	48.7	± 1.98
Test 2			*Biological value*			
Raw (A)	50.3	47.4	43.6	48.6	47.5	± 1.16
Raw + methionine	60.3	61.5	58.3	56.6	59.2	± 0.73
Raw + aureomycin	56.5	49.8	45.7	51.0	50.8	±11.50
Raw + vitamin B_{12}	55.1	51.1	51.6	51.6	52.3	± 0.85
			True digestibility			
Raw (A)	89.9	89.2	87.7	91.1	89.5	± 0.65
Raw + methionine	88.7	88.6	89.3	89.5	89.0	± 0.59
Raw + aureomycin	92.8	93.0	88.5	91.8	91.5	± 0.84
Raw + vitamin B_{12}	89.2	89.1	86.5	89.7	88.6	± 1.01
Test 3			*Biological value*			
Raw (A)	47.8	48.7	48.1	47.3	48.0	± 0.78
Raw + lysine	47.0	47.8	45.3	49.9	47.5	± 0.82
Raw + penicillin	52.3	51.7	49.2	52.9	51.6	± 0.95
Raw + streptomycin	49.6	52.9	46.6	47.8	49.2	± 1.02
			True digestibility			
Raw (A)	92.4	91.3	89.0	89.9	90.7	± 0.88
Raw + lysine	92.7	94.7	94.9	95.4	94.4	± 0.78
Raw + penicillin	94.4	94.0	94.4	92.8	93.9	± 0.84
Raw + streptomycin	93.7	90.4	92.3	92.3	92.1	± 0.82
Test 4			*Biological value*			
Expeller (B)	62.1	58.0	59.2	59.8	59.8	± 0.63
Expeller + methionine	66.9	65.8	65.4	67.1	66.3	± 0.96
Expeller + aureomycin	61.5	59.9	56.4	52.8	57.7	± 1.52
Expeller + vitamin B_{12}	63.6	62.8	57.2	53.9	59.4	± 1.78
			True digestibility			
Expeller (B)	93.7	94.3	92.2	93.9	93.5	± 0.68
Expeller + methionine	92.9	95.0	91.5	92.1	92.9	± 0.73
Expeller + aureomycin	95.4	97.3	96.5	97.3	96.6	± 0.41
Expeller + vitamin B_{12}	92.6	93.0	90.8	92.7	92.3	± 0.91

[1] Biological value × true digestibility.

TABLE 4

Statistical comparison of groundnut meals by the t-test

TEST 1	RAW			EXPELLER		CONTROL-STEAMED
	Expeller	Control-steamed	High-steamed	Control-steamed	High-steamed	High-steamed
Biological value						
Mean difference	+ 8.8	+ 12.5	+ 1.6	+ 3.7	— 7.2	— 10.9
Significance [1]	S**	S***	N.S.	S***	S***	S**
True digestibility						
Mean difference	+ 2.0	— 0.9	+ 0.9	— 2.9	— 1.1	+ 1.8
Significance	S**	N.S.	N.S.	N.S.	N.S.	N.S.
Protein value						
Mean difference	+ 9.4	+ 10.9	+ 1.9	+ 1.5	— 7.5	— 9.0
Significance	S***	S**	N.S.	N.S.	S**	S**

TEST 2	RAW		
	+ methionine	+ aureomycin	+ vitamin B_{12}
Biological value			
Mean difference	+ 11.7	+ 3.3	+ 4.8
Significance	S***	S*	S**
True digestibility			
Mean difference	— 0.5	+ 2.0	— 0.9
Significance	N.S.	S*	N.S.

TEST 3	RAW		
	+ lysine	+ penicillin	+ streptomycin
Biological value			
Mean difference	— 0.5	+ 3.6	+ 1.2
Significance	N.S.	S*	N.S.
True digestibility			
Mean difference	+ 3.7	+ 3.2	+ 1.4
Significance	S**	S**	N.S.

TEST 4	EXPELLER		
	+ methionine	+ vitamin B_{12}	+ aureomycin
Biological value			
Mean difference	+ 6.5	— 0.4	— 2.1
Significance	S***	N.S.	N.S.
True digestibility			
Mean difference	— 0.6	— 1.2	+ 3.1
Significance	N.S.	N.S.	S**

[1] S*** = Significant between 1 and 2% levels.
S** = Significant between 2 and 5% levels.
S* = Significant between 5 and 10% levels.
N.S. = Not significant.

RESULTS

Amino acids by paper chromatography: Qualitative examinations of amino acid bands by paper chromatography revealed that, in comparison with casein, the groundnut meals gave bands corresponding to methionine lysine, isoleucine and threonine which were very faint, whereas the colour intensity was stronger for cystine and tryptophan.

Thiamine content: The amounts of thiamine in raw (A), ''expeller'' (B), control-steamed (C) and high-steamed (D) meals were 6.6, 4.4, 4.7 and 1.9 µg/gm respectively.

Nutritive value of differently processed meals: The results of the growth test presented in table 1 indicate that the nutritive value as observed by the protein efficiency ratio is low for raw (A) as well as for high-steamed (D) meals. On the other hand, ''expeller'' (B) and control-steamed (C) meals have much higher protein efficiency ratios. The biological values of meals A and D as shown by nitrogen balance method (tables 3 and 4) are comparatively much lower than those of meals B and C. It is evident that the protein efficiency ratios and the biological values of the 4 meals vary in the same order.

When the figures for the biological values and the true digestibility coefficients are multiplied in the calculation of the ''protein value'' (Mitchell and Hamilton, '29), and the resultant figures are submitted to statistical analyses, it is observed that there is very good correlation between the biological values and the net protein value of the differently treated meals (tables 3 and 4).

Effect of supplementation with amino acids: As seen by the statistical significance of the paired-feeding values reported in table 2, lysine, methionine, isoleucine, and threonine had a good supplementary effect to the protein of raw (A) groundnut meal. Methionine supplementation to ''expeller'' (B) as well as high-steamed (D) meals, significantly enhanced the growth rate of rats whereas there was no effect on supplementation of raw (A) groundnut meal with cystine; tryptophan supplementation actually reduced the protein efficiency ratio.

Nitrogen balance experiments indicate that there is a substantial improvement in the biological values of raw (A) and "expeller" (B) meals when supplemented with methionine, but the true digestibility is not altered (tests 2 and 4, tables 3 and 4).

Effect of supplementation with antibiotics and vitamin B_{12}. Of the three antibiotics used in the present work as supplements to groundnut protein, only penicillin showed a supplementary effect as measured in the paired-feeding test (table 2) as well as by the biological values and the true digestibility coefficients (tables 3 and 4). Supplementation of meals with aureomycin and streptomycin had no effect in the growth test (table 2), whereas the true digestibilities were improved as seen by the nitrogen balance (tests 2 and 3, table 3). Aureomycin had a little beneficiary effect on the biological value of raw (A) meal.

Vitamin B_{12} supplementation to groundnut meal (A) had no effect in the growth test (table 2), but the biological value of the meal was improved (table 3). Supplementation of "expeller" (B) meal diet with vitamin B_{12} had no effect either in growth test (table 2) or on the biological value (table 3).

DISCUSSION

The conditions of solvent extraction of groundnut oil in the present studies are quite different from those employed earlier by Cama and Morton ('51). It is significant to note that even open steam released from 100 lbs. compression per hour to remove residual groundnut oil as well as the non-flammable solvent trichlorethylene compared to 2,000 lbs./hr. of open steam employed by Cama et al. ('51), very considerably affected the nutritive value of groundnut protein. The best nutritive value that could be obtained was by reducing exposure to heat and moisture to a minimum. The effect of heat treatment on the nutritive value of proteins was described earlier by Cama and Morton ('51).

The present work emphasizes that the different processing conditions employed affect the nutritive value of proteins as

determined by the protein efficiency ratios obtained by the growth test (table 1), the biological and the true digestibility values and the net protein values (tables 3 and 4). The heat-labile vitamin, thiamine, is also considerably affected by excessive steaming as seen from the results obtained with meal D. Although the thiamine values for "expeller" (B) as well as control-steamed (C) meals are lower than those of raw (A) meal, the loss in amount can well be sacrificed considering that the nutritive value of groundnut protein is significantly enhanced by gentle heat, which perhaps destroys the trypsin inhibitor believed to be present in unheated groundnut meals (Borchers and Ackerson, '47; Cama and Morton, '51). The low protein efficiency ratios (1.0 to 1.1) for meal (A) as seen from the data in tables 1 and 2 coupled with the low biological values (48.0 to 51.0), and the true digestibility coefficients (89.0 to 91.0) as seen from table 3, may be attributed to the presence of such an inhibitor, which affects the digestibility of the protein.

The protein efficiency ratios for "expeller" (B) and control-steamed (C) meals, vary between 1.40 and 1.57 (tables 1 and 2) and similar improvements in the biological values (60.0 to 64.0) and true digestibilities (90.0 to 93.0) table 3, are reproducible indicating the beneficiary effect of mild denaturation and consequent availability of essential sulphydryl groups for metabolism (Neurath, Greenstein, Putnam and Erickson, '44). However, in the case of high-steamed (D) meal, although there is not much deterioration as indicated by the growth test and the true digestibility, the biological value and the net protein value of the meal are considerably affected and the thiamine content drops appreciably. These nutritional observations on meals were closely related to the electrophoretic behaviour of proteins as reported in a subsequent paper (Cama, Malik and Nath, '58).

Supplementation of meals with amino acids (table 2) showed that groundnut protein is mainly deficient in methionine (Grau, '46). This is reflected in an increase in the protein efficiency ratio of meals B and D in the paired-feeding

test (table 2) as well as by an increase in the biological value of meal (B) when supplemented with methionine (table 3, test 4). Lysine, isoleucine and threonine improved the protein efficiency ratios but there was no improvement when the meals were supplemented with cystine. The addition of tryptophan significantly reduced the protein efficiency ratios which may well be due to an amino acid imbalance as reported by Ebisuzaki, Williams and Elvehjem ('52).

In comparison with casein, the amino acid bands of lysine, methionine, isoleucine and threonine of groundnut protein were much fainter whereas cystine and tryptophan bands were more intense. These observations are in agreement with the results of the paired-feeding studies on rats (table 2).

From the data in tables 2 and 3, it is evident that penicillin had the greatest effect on growth as well as on the biological value and the true digestibility values of groundnut protein. Supplementation of groundnut meal with aureomycin and streptomycin improved the digestibility of proteins (tables 3 and 4) which is in harmony with the concept that most antibiotics eliminate intestinal bacteria which absorb or destroy nutrients needed by the host (Stokstad, '54). However, the most striking evidence for a non-bacterial mechanism of the action of antibiotics is the recent work of Luckey, Gordon, Wagner and Reyniers ('56), who obtained significant growth responses to antibiotics in germ-free chicks and poults.

Supplementation of the groundnut meal (A) diet with vitamin B_{12} improved the biological value and the true digestibility coefficient (tables 3 and 4) but had no effect on the growth of rats in the paired-feeding test (table 2). There is now considerable evidence that the metabolic role of vitamin B_{12} is for methyl group synthesis and not for transmethylation reactions (Chang and Johnson, '55; Johnson, Firth and Mistry, '55). It is, therefore, likely that supplementation of groundnut meal, which is essentially deficient in methionine, with vitamin B_{12} meets the need for methyl group synthesis rather than for enhancing the growth rate of rats.

SUMMARY

Differently processed groundnut meals have been tested nutritionally by growth test as well as by nitrogen balance method on rats.

Unheated and overheated meals are nutritionally inferior to those extracted under gentle conditions of heat and steam treatment. The thiamine content of groundnut meal was much reduced by high-steam treatment.

Groundnut protein was observed to be deficient in methionine, lysine, isoleucine and threonine but not in cystine and tryptophan. The nutritional observations had good correlation with qualitative examination of amino acid bands on paper chromatograms and their comparison with the amino acids of casein.

Supplementation with penicillin at the 0.02% level of the diet improved the nutritional quality of groundnut proteins, but aureomycin at 0.03% and streptomycin at the 0.02% level of the diet were not effective.

Vitamin B_{12}, at 45 μg/kg of diet, improved the biological value of raw groundnut protein but had no effect on growth in the paired-feeding test.

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GASTRIC EMPTYING AND INTESTINAL ABSORPTION OF CARBOHYDRATE AND PROTEIN AS INFLUENCED BY THE NATURE OF THE TEST MEAL [1,2]

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The rate of glucose absorption appears to be governed by the rate at which it is emptied from the stomach (Fenton, '45; Reynell and Spray, '56). The rate of protein absorption has not been extensively investigated, partly because the mixing of dietary protein in the alimentary tract with endogenous protein of the digestive secretions makes interpretation of the data difficult. The purpose of the present investigation was to observe the disappearance of carbohydrate and protein from the alimentary tract as influenced by different carbohydrates and by high and low protein in the diet. There was a need to study separately the contents of the stomach and the small intestine and to extend previous results from this laboratory (Dreisbach and Nasset, '54).

METHODS

The composition of diets is given in table 1. Adult male albino rats, 175 to 260 gm, were adapted to the various diets 5 days before being used for absorption experiments. After

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[2] The data in this paper were taken from a thesis submitted by Spencer Rosenthal to the Graduate School of the University of Rochester in partial fulfillment of the requirements for the degree Master of Science, 1956.

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a fast of 48 hours 6.2 ml of a diet suspension, adjusted to pH 7.0 and containing approximately 1.5 gm of dry diet, were placed in the stomach through a #8 French catheter. Identical samples were delivered into beakers for chemical analysis. At intervals of 1, 2, 3, 4, 5 and 6 hours after feeding one rat was sacrificed by intraperitoneal administration of sodium pentobarbital. The pylorus was clamped off immediately and double ligatures placed at the cardia, pylorus and ileocecal junction. The stomach and small intestine were removed separately, rinsed quickly in 0.9% NaCl solution and blotted

TABLE 1

Composition of diets
(Grams of foods to produce one kilogram of moisture-free diet [1])

DIET NO.	DEXTRIN	BANANA (FRESH)	CASEIN	CORN OIL	SALTS [2]
1		2930	182	200	40
2	585		196	200	40
3			524	505	40
4		3840		200	40
5	768			200	40

[1] Vitamins added per kilogram: thiamine·HCl, 10 mg; riboflavin, 20 mg; niacin amide, 50 mg; folic acid, 1 mg; Ca pantothenate, 50 mg; pyridoxine·HCl, 250 mg; choline chloride, 2 gm; vitamin A, 6,500 I.U.; vitamin D, 1,500 I.U.; alpha-tocopherol, 50 mg; methyl-naphthoquinone, 15 mg.

[2] Wesson.

dry to remove any blood that adhered to the outside. The stomach and gut were opened and their contents promptly washed out with isotonic saline and diluted to 250 and 500 ml respectively. This material was homogenized, heated to 70 to 80°C to inactivate enzymes, and stored frozen in polyethylene bottles. Aliquot portions were analyzed for total carbohydrate, by the anthrone method (Morris, '48), and total nitrogen by the micro Kjeldahl method. This procedure was repeated 5 times for each diet, providing the results from 5 rats for each time interval.

RESULTS

Carbohydrate. Results of carbohydrate feeding are summarized in table 2. Columns for diets 1 and 4 are adjacent

TABLE 2

Recovery and computed absorption of carbohydrate from test meals containing different carbohydrate mixtures

ANIMALS SACRIFICED AT	1 HOUR				2 HOURS			
DIET [1]	1	4	2	5	1	4	2	5
Carbohydrate								
Fed at zero hr., mg	989	1154	802	1267	1073	1271	836	1267
Recovered from stomach, mg	723 ± 72 [2]	808 ± 153	399 ± 60	423 ± 72	490 ± 80	721 ± 77	258 ± 57	286 ± 57
Emptied from stomach, mg	266	346	403	844	583	550	578	981
Recovered from gut, mg	47 ± 11	44 ± 6	153 ± 22	378 ± 96	54 ± 12	74 ± 23	56 ± 21	203 ± 53
"Absorbed," mg	219	302	250	466	529	476	522	778
"Absorbed," % (of fed)	22	26	31	37	49	37	62	61

ANIMALS SACRIFICED AT	3 HOURS				4 HOURS			
DIET [1]	1	4	2	5	1	4	2	5
Carbohydrate								
Fed at zero hr., mg	974	1154	778	1267	987	1086	836	1267
Recovered from stomach, mg	344 ± 59	378 ± 95	73 ± 75	87 ± 36	240 ± 75	184 ± 58	8 ± 3	4 ± 1
Emptied from stomach, mg	630	776	705	1180	747	902	828	1263
Recovered from gut, mg	39 ± 7	59 ± 41	14 ± 9	91 ± 46	30 ± 4	46 ± 11	8 ± 4	10 ± 9
"Absorbed," mg	591	717	691	1089	717	856	820	1253
"Absorbed," % (of fed)	61	62	89	86	73	79	98	99

ANIMALS SACRIFICED AT	5 HOURS				6 HOURS			
DIET [1]	1	4	2	5	1	4	2	5
Carbohydrate								
Fed at zero hr., mg	984	1193	836	1267	981	1357	836	1267
Recovered from stomach, mg	100 ± 38	100 ± 27	+ [3]	25 ± 25	49 ± 18	95 ± 67	+	+
Emptied from stomach, mg	884	1093	836	1242	932	1262	836	1267
Recovered from gut, mg	21 ± 1	20 ± 4	+	12 ± 12	17 ± 2	23 ± 7	+	+
"Absorbed," mg	863	1073	836	1230	915	1239	836	1267
"Absorbed," % (of fed)	88	90	100	97	93	91	100	100

[1] See table 1 for composition of diets.
[2] Standard error of the mean.
[3] + = only traces of carbohydrate recovered.

because the source of carbohydrate was the same for both; columns for diets 2 and 5 are adjacent for the same reason.

Protein. Table 3 contains the mean results obtained by feeding diets 1, 2 and 3 which contained 16, 16, and 48% protein respectively. Table 4 summarizes the results of feeding diets 4 and 5. Since the glands and mucous membranes of the alimentary tract contribute to the chyme an appreciable quantity of endogenous protein during digestion, it is necessary to estimate its magnitude before computations can be made of the amounts of protein emptied from the stomach and absorbed from the gut. These computations are explained in the discussion. In this investigation the great preponderance of nitrogen presumably comes from protein and amino acids and hence the results have for convenience been reported as conventional protein ($N \times 6.25$). Small quantities of urea, ammonia, nucleic acid and other nitrogenous compounds are certainly included but it is unlikely that even large changes in their concentrations could alter the nature of the results or the conclusions derived therefrom.

DISCUSSION

Carbohydrate. Diets 1 and 4, containing fresh banana as the source of carbohydrate, were emptied from the stomach and also absorbed at nearly identical rates. Diets 2 and 5, containing dextrin as the source of carbohydrate, were emptied from the stomach and absorbed at approximately equal rates (table 2). The presence or absence of protein in these diets failed to make a significant difference in emptying the stomach. Diet 1 was emptied more slowly than diet 2 at all time intervals (Student's t test shows that at: 1 hr., $P < 0.012$; 2 hrs., $P = 0.21$; 3 hrs., $P < 0.003$; 4 hrs., $P < 0.005$; 5 hrs., $P < 0.05$; 6 hrs., $P = 0.13$). Similarly diet 4 was emptied more slowly than diet 5 ($P < 0.003$ for 1, 2, 3, 4 and 5 hrs.; 6 hrs., $P < 0.05$). The nature of the carbohydrate, therefore, seems to be important in determining the rate at which a meal is emptied from the stomach.

TABLE 3

Recovery and computed gastric emptying and absorption of protein from various test meals

ANIMALS SACRIFICED AT	1 HOUR			2 HOURS			3 HOURS		
DIET [1]	1	2	3	1	2	3	1	2	3
Protein									
Fed at zero hr., mg	295	283	342	320	311	342	289	290	342
Recovered from stomach, mg	237 ± 24 [2]	186 ± 35	114 ± 11	177 ± 24	132 ± 19	38 ± 5	133 ± 19	56 ± 27	43 ± 16
Emptied from stomach, mg	83	135	254	176	215	330	188	263	323
Recovered from gut, mg	109 ± 20	103 ± 7	151 ± 9	124 ± 14	121 ± 18	124 ± 14	135 ± 32	128 ± 26	104 ± 25
"Absorbed," % (of fed prot.)	28	48	74	53	69	97	65	91	94

ANIMALS SACRIFICED AT	4 HOURS			5 HOURS			6 HOURS		
DIET [1]	1	2	3	1	2	3	1	2	3
Protein									
Fed at zero hr., mg	294	311	342	293	311	342	292	311	342
Recovered from stomach, mg	78 ± 19	43 ± 11	17 ± 1	46 ± 17	42 ± 12	10 ± 2	33 ± 7	29 ± 12	9 ± 1
Emptied from stomach, mg	223	308	345	264	311	352	278	311	352
Recovered from gut, mg	129 ± 28	155 ± 23	153 ± 30	111 ± 19	124 ± 38	110 ± 29	141 ± 42	124 ± 44	97 ± 15
"Absorbed," % (of fed prot.)	76	99	101	90	100	103	95	100	103

[1] See table 1 for composition of diets.

[2] Standard error of the mean.

TABLE 4

Recovery and computed gastric emptying and absorption of protein from test meals low in protein

ANIMALS SACRIFICED AT	1 HOUR		2 HOURS		3 HOURS	
DIET [1]	4	5	4	5	4	5
Protein						
Fed at zero hr., mg	66	12	73	12	66	12
Recovered from stomach, mg	65 ± 10 [2]	29 ± 6	49 ± 4	32 ± 6	34 ± 5	23 ± 4
Emptied from stomach, mg	18	8	31	9	44	11
Recovered from gut, mg	146 ± 20	114 ± 24	122 ± 25	93 ± 17	106 ± 12	121 ± 37
"Absorbed," % (of fed prot.)	27	67	42	75	67	92

ANIMALS SACRIFICED AT	4 HOURS		5 HOURS		6 HOURS	
DIET [1]	4	5	4	5	4	5
Protein						
Fed at zero hr., mg	63	12	68	12	77	12
Recovered from stomach, mg	26 ± 2	16 ± 3	15 ± 1	13 ± 2	16 ± 5	18 ± 5
Emptied from stomach, mg	52	12	62	12	71	12
Recovered from gut, mg	113 ± 21	152 ± 33	77 ± 10	152 ± 21	87 ± 24	139 ± 34
"Absorbed," % (of fed prot.)	83	100	91	100	92	100

[1] See table 1 for composition of diets.

[2] Standard error of the mean.

The logarithm of the percentage of carbohydrate residue in the stomach is plotted as a function of time in figure 1. As indicated in the previous paragraph the differences between diets 4 and 5 are highly significant. The regression line for diet 4 reaches the logarithm of 100% residue 24 minutes after zero time which probably means that the stomach under these conditions did not begin immediately to empty in an exponential fashion. The regression line for diet V intersects the 100% line at —4 minutes which indicates that the stomach under these conditions began to empty at once and

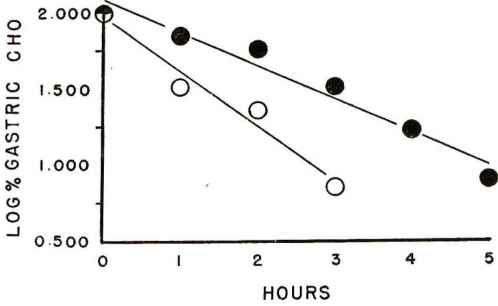

Fig. 1 Regression lines fitted by method of least squares. Ordinate is logarithm of gastric carbohydrate residue recovered at hourly intervals after feeding as indicated on abscissa.

$Y = 2.086 - 0.217\ X$ (diet 4, solid circles)

$Y = 1.976 - 0.362\ X$ (diet 5, open circles)

at a very high initial rate. The time required to empty 50% of the gastric carbohydrate, as predicted from the equations for the regression lines, is 107 minutes for diet 4 and 46 minutes for diet 5. To achieve 95% emptying 212 minutes is required for diet 4 and 383 minutes for diet 5.

The work of Fenton ('45) and of Reynell and Spray ('55) on the relationship of glucose absorption to emptying of the stomach was confirmed for total carbohydrate. In figure 2 the percentage absorption of carbohydrate is plotted as a function of the percentage emptied from the stomach for diets 4 and 5. Extrapolation of the regression lines to zero absorption gives intercepts at 6 and 47% emptying for the carbo-

hydrates of diets 4 and 5 respectively. This suggests that the initial rate of emptying of diet 5 was much faster than that of diet 4. The data in table 2 support this idea and show that the amount of carbohydrate recovered from the intestine continued to be greater for diet 5 than for diet 4 until the stomach was empty. The alimentary tract evidently responds quite differently to test meals which at first seem quite similar. Diets 4 and 5 were composed chiefly of carbohydrate and fat in about the same proportions. The obvious difference is in the source of carbohydrate. The carbohydrates of fresh banana formed a colloidal mass which at pH 7.0 and 24°C was

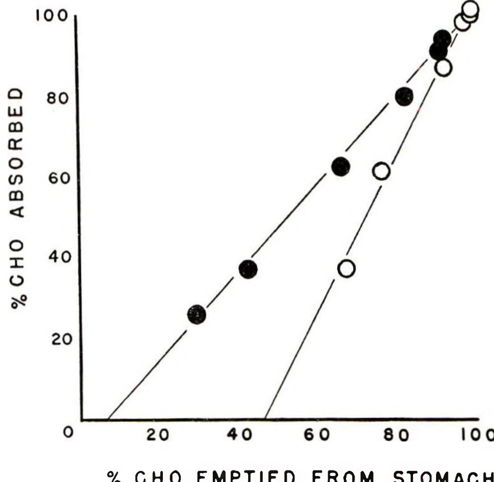

Fig. 2 Regression lines fitted by method of least squares.
$Y = -6.55 + 1.04\ X$ (diet 4, solid circles)
$Y = -88.8 + 1.89\ X$ (diet 5, open circles)

2.5 times as viscous as that which was formed from dextrin but it is impossible to decide on the basis of present data whether this difference was decisive. The chemical compositions of banana and dextrin carbohydrates are different and this may be a more important factor. Differences in alimentary responses to different foods may account in part for differences in their nutritive value. It was shown by Siliciano and Nasset ('53) that incorporation of banana in a diet low

in protein resulted in a significant increase in the retention of "absorbed" nitrogen.

Protein. The most striking feature of the data in tables 3 and 4 is the relatively constant load of protein ($N \times 6.25$) in the small intestine regardless of test meal or time after feeding. The average hourly recoveries of protein from the gut over a period of 6 hours were 125, 126, 125, 109, and 129 mg for diets 1, 2, 3, 4 and 5 respectively. The average protein recoveries for all diets were 125, 117, 119, 140, 115 and 118 mg at 1, 2, 3, 4, 5 and 6 hours respectively. The amount for a single group of animals ranged from 77 mg in the 5th hour on diet 4 to 155 mg in the 4th hour on diet 2. It is evident that the small intestine maintains a protein content in its lumen which is not greatly changed by the variety of test meals given in these experiments. This is consistent with the work of Lepkovsky et al. ('57) who reported that the water content and total dry weight of intestinal contents remained constant over a post-prandial period of 9 hours.

The quantity of gastric endogenous protein ($N \times 6.25$) was computed for each absorption period from the carbohydrate to protein ratio of the diet as established by chemical analysis. Test meals were homogenized and it was assumed that the stomach did not effect a physical separation of the carbohydrate and protein and that they left the stomach in the same proportions as they entered it. The excess protein in gastric contents was certainly endogenous and it was considered to be contributed chiefly by the stomach. The salivary and esophageal glands probably contribute small amounts. There was no systematic trend from the first to the 6th hour for any diet and there were no striking differences among the diets. The mean hourly values for endogenous gastric protein over the 6-hour period were 22, 36, 12, and 21 mg for diets 1, 2, 4 and 5 respectively. The overall mean of 23 mg of gastric endogenous protein was used in computing the results of feeding the non-carbohydrate diet (3). This may not be wholly justified but it probably represents a reasonable approximation.

The total protein recovered in gastric contents diminished exponentially with time until the level of endogenous gastric protein production was reached in 4 to 6 hours, depending upon the diet ingested. If plotted as in figure 1 the points fall very close to those given there for carbohydrate. The protein recovered from the small intestine, however, was essentially constant over the whole period of 6 hours. Since the dietary protein disappeared from the stomach while the intestinal protein remained constant, it follows that the gut must have absorbed an amount of protein equivalent to what was ingested. It seems evident from the present experiments that ingested protein must be considerably diluted with endogenous protein in the lumen of the intestine as previously suggested (Nasset et al., '55; Nasset, '57).

The classical determination of "digestibility" of protein involves subtraction of fecal protein ($N \times 6.25$) from food protein and expressing the remainder as a percentage of the protein fed. The basic assumption is that fecal protein is composed of undigested food protein. The fallacy of this assumption has been demonstrated repeatedly (Lusk, '28) but the term "digestibility" is still used in its literal sense. The present investigation adds more evidence that the digestive tract can contribute large quantities of protein to the chyme. In view of this it seems advisable to drop the term "digestibility" as applied to protein and fat. The digestive tract can and does contribute these nutrients from endogenous sources to the chyme during digestion. The amount of endogenous carbohydrate in chyme is normally very small. The classical concept of digestibility is strictly applicable only when the nutrient recovered from the feces can be identified as part of the material that was fed. The digestibility of cellulose in the cow or the beaver might be taken as examples.

The objection of Geiger et al. ('58) to the idea of a significant dilution in the digestive tract of dietary protein with endogenous protein is scarcely supported by their evidence. Their rats were smaller (140 to 220 gm) than those used in the present investigation (175 to 260 gm), dried skim milk

was the only protein-containing test meal fed, and a single absorption period of three hours was used. In one of their groups of animals they report recovery of 14.7 mg of N (92 mg protein) from the intestine which is within the lower range of values in tables 3 and 4. They report N recoveries from stomach plus intestine which are approximately three-fourths of the amount fed, implying at least that only one-fourth of the protein fed had been absorbed in three hours. In the present investigation 31 to 1400% of the ingested nitrogen was recovered from stomach and small intestine, depending upon the diet fed and the time between feeding and sacrifice of the animal. The lower the N intake, as in diet V, the greater the percentage recovery. Diet 3 (48% protein) consistently yielded the lowest percentage protein recoveries (tables 3 and 4), ranging from 77% in the first hour to 31% in the 6th hour. Diet 1 (16% protein) yielded protein recoveries in the alimentary tract which ranged from 117% in the first hour to 60% in the 6th hour.

Calculations were made regarding the probable contribution of nitrogenous compounds from various portions of the digestive system. Protein contributed by the gastric mucosa, as computed above, ranged from 12 to 36 mg. According to Junqueira et al. ('57) artificial stimulation of the pancreas in the anesthetized rat results in the secretion of approximately 2 mg of protein per hour. The pancreatic juice contained 3.4% of protein. According to Thomas ('50) the pancreatic juice of the dog may contain up to 10% of protein, depending on experimental conditions. It is not unlikely, therefore, that in the unanesthetized rat the value reported by Junqueira might be increased approximately three-fold. Salo ('37) found that a 15-cm denervated jejunal fistula in the dog produced 25 to 118 mg of protein per hour. Assuming this to represent 10% of the whole small intestine the output was 250 to 1180 mg/hour for the whole gut. On the basis of metabolic body size ($Kg^{3/4}$) the dogs used in Salo's investigation were approximately 30 times as large as the rats used in the present investigation. The estimate for endogenous

protein of intestinal origin is, therefore, approximately 8 to 39 mg. Adding the three estimates for gastric, pancreatic, and intestinal protein gives a minimum of 22 mg and a maximum of 94 mg. These estimates are probably low because the data for the intestine were obtained from fistulas which were separated from the gut proper and hence were never subjected to the normal chemical and mechanical stimuli of intestinal contents. Furthermore, bile, saliva and the cephalic phase of gastric secretion were not considered. This problem might be considerably clarified by feeding a labeled protein so that a direct determination could be made of its dilution with endogenous protein at various times after feeding.

SUMMARY

1. It was demonstrated that the stomach empties its contents in an exponential fashion. The rate of emptying appeared to be determined by the dietary sources of carbohydrate, which were fresh banana and dextrin.

2. The absorption of carbohydrate was a linear function of gastric emptying. The rate of absorption was greater for dextrin than for banana carbohydrate.

3. The recovery of protein from the small intestine tended to remain constant over a period of 6 hours, during which time all of the test meal protein had left the stomach. Reasons are given for the assumption that all of the test meal protein, or an equivalent mixture of dietary and endogenous protein, was absorbed in this period of time.

4. The results indicate that ingested protein is mixed in the lumen of the digestive tract with relatively large amounts of endogenous protein which originate in the digestive tract itself.

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DENTAL CARIES IN TWO STRAINS OF RATS FED DIETS OF TWO DEGREES OF FINENESS [1]

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INTRODUCTION

Hunt, Hoppert and Erwin ('44) and Stewart, Hoppert and Hunt ('53) have shown that different strains of rats vary in their susceptibility to dental caries and that coarse diets cause more severe caries than those finely ground.

Cox ('52) has criticized some work as using too few experimental animals. He recommends that a sufficiently large number be used to permit statistical evaluation of the results, and that complete details regarding the rats and diets should be reported.

Hartles, Lawton and Slack ('56) found that rats from the Harvard colony, when raised in Liverpool, England, were very much less susceptible to caries than those of the parent colony and emphasized "the dangers of comparing findings of different laboratories since small changes in the diet or a change in environment may influence the susceptibility to dental caries."

It seemed important to establish the reaction of a susceptible strain of rats when changed from the East coast of the United States to Hawaii and to compare it with a strain long inbred in Hawaii.

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[2] Captain, D. C., U.S.N. The opinions and assertions contained in this article are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

This paper presents results of the degree and extent of dental caries produced in two strains when a supplemented diet of natural foods was fed in two forms (1) all ingredients passed through a 28-mesh sieve, and (2) the same ingredients passed through a 48-mesh sieve. A simple system of scoring the teeth has been worked out by one of us (C.A.S.) that is particularly applicable to the type of caries found in these two strains of rats and which can readily be duplicated by two people. The number of carious teeth, the number of carious areas and the caries scores (extent) have been determined for the various groups of rats and the results evaluated by the analysis of variance.

EXPERIMENTAL MATERIALS AND PROCEDURE

Rats. The two strains of albino rats used are designated as the Hawaii and the Navy. The Hawaii strain was originally obtained by crossing female rats shipped to Hawaii from the Department of Physiological Chemistry at Yale University with males shipped from the Household Science Department at the University of California, Berkeley. The progeny have been inbred for more than 25 years.

The Navy strain was developed by one of us (C.A.S.) at the Navy Medical Research Institute, National Naval Medical Center, Bethesda, Md. Young males and females of the Navy strain shipped to Hawaii by air in October, 1951, have bred satisfactorily with a low mortality of the young.

Diets. Diet 15 (table 1) is the stock diet which has proved satisfactory, without supplementation, for reproduction and lactation for many years in Hawaii. The finely ground whole wheat flour was not sifted, as when tested only small bits of relatively soft bran were found to remain in the sieve. All other ingredients that were not in powdery form were ground fine enough to pass a 28-mesh sieve and combined in the proportions indicated. For diet 15A, all ingredients (except the "fine" whole-wheat flour) were ground separately in a ball mill until they would pass a 48-mesh sieve.

Procedure. Preliminary experiments indicated a marked difference in the response of the two strains of rats to coarse and fine diets. For the experiments reported in this paper, the two strains of rats were bred and fed at the same time of year under the same laboratory conditions.

Rats of both strains whose mothers had been fed diet 15 were weaned at 21 days and placed on diet 15 and diet 15A to achieve an approximately equal distribution of sexes from each litter. There were 20 to 22 rats in each group. They were

TABLE 1

Composition of stock diet 15

CONSTITUENT	AMOUNT	CONSTITUENT	AMOUNT
	gm		*gm*
Skim milk powder [1]	350	Yeast [2]	30
Whole wheat flour, "fine grind"	650	Wesson oil	46
Cornmeal (commercial)	150	Cod liver oil	4
White flour (enriched)	150	Salt mixture [3]	10
Brown rice flour	150	Iodized salt	10
Soybean flour	100	Total	1650

[1] Recently we have increased the protein content of the diet during pregnancy and lactation by the addition of fish meal.

[2] Strain G, Anheuser-Busch.

[3] Salt mixture: 90 gm calcium carbonate, 10 gm ferric citrate, 1 gm manganese sulfate.

housed in individual wire-mesh cages with raised screen bottoms. Cages, food and water cups were changed weekly. Tap water low in fluorine (0.03 to 0.05 p.p.m.) was used.

The rats were fed ad libitum for 100 days, and killed by gassing; the heads were removed and autoclaved for 15 minutes at 15 lbs. pressure; the jaws were cleaned, washed well in tap water, rinsed in alcohol and placed in ethyl ether for 5 days. The jaws, exposed to air to dry, were labeled before examining under a low power microscope.

Caries evaluation. The number of carious molars, number of carious areas, and the extent of each carious area were recorded using the diagram in figure 1 (as illustrated for the upper and lower left quadrants) and the criteria for the scores

listed in table 2. The results for the number of carious molars and carious areas, and the scores were tabulated and, after transformation, treated statistically.

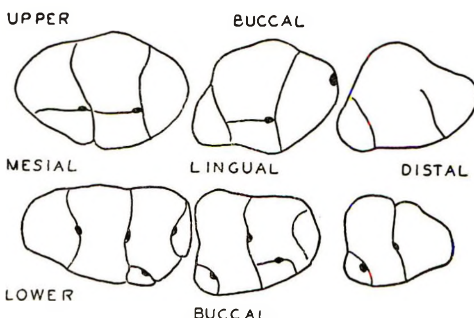

Fig. 1 Diagram of lower and upper left quadrants. Black spots indicate where carious areas are initiated.

TABLE 2

Criteria for scores for extent of caries

SCORE	DESCRIPTION OF LESIONS
1	a. Breakdown between cusps and/or b. Undermining of area on one or both sides of groove.
2	Cusps completely destroyed or thoroughly undermined on one side of groove.
3	All cusps surrounding groove destroyed or thoroughly undermined.

All carious areas are within the large or small grooves except the one found on the distal side of the upper second molar. Carious areas may extend between two carious grooves, in which case the score of both grooves must be given separately. When there was a question as to whether or not there was a cavity, it was given a zero score. A more precise but involved method of treating and rating the teeth was considered unwarranted in this work because of the ease with which the carious areas and their extent could be evaluated in these rodents.

RESULTS

The data for these experiments are summarized in table 3. It may be noted that the carious teeth, carious areas and the caries scores were always greater for the coarser diet for both the Hawaii and the Navy strains. It is also clear that the data indicate a greater susceptibility to caries of the Navy strain. The difference between the two strains was somewhat greater for the finely ground diet (diet 15A) than for the coarser diet.

TABLE 3

Summary of results of feeding two strains of rats diets of two degrees of fineness

CATEGORY OF INTEREST	HAWAII		NAVY	
Diet	15	15A	15	15A
No. of rats	21	20	22	20
Rats with caries	21	17	22	18
Rats with caries (%)	100	85	100	90
Carious teeth, total number	64	29	90	56
Carious teeth per rat	3.04	1.45	4.09	2.80
Carious areas (total)	70	29	134	60
Carious areas per rat	3.33	1.45	6.09	3.00
Carious scores (total)	96	36	163	63
Caries score per rat	4.57	1.80	7.40	3.15
Mean weight, males (gm)	468	400	403	359
Mean weight, females (gm)	273	255	252	241

Prior to subjecting the data to evaluation by the analysis of variance the original observations were, in all cases, subjected to transformation (Snedecor, pp. 314–321). The specific transformations employed being $(n + 3/8)^{1/2}$ for the numbers (n) of defective teeth and for the numbers of carious areas, and the $\log_{10}(s + 1)$ for the caries scores (s). In applying the analyses of variance to these transformed observations the methods appropriate to disproportionate subclass numbers were used whenever necessary (Snedecor, '56, pp. 379–382).

The differences in degree and extent of caries between the two strains were highly significant (tables 4 and 5). Likewise, the differences between diets were highly significant. The

Navy rats are more susceptible to dental caries than are the Hawaii rats and obviously retained a high degree of susceptibility when transferred from Bethesda, Md., and bred in Hawaii. There was no evidence of an interaction between strain and diet.

TABLE 4

Transformed (SR and L) means and mean differences, also rectified (N′ and S′) treatment means

STRAIN	DIET	TEETH		CARIOUS AREAS		SCORES	
		SR	N′	SR	N′	L	S′
Navy	15	2.073	3.92	2.468	5.72	0.856	6.18
	15A	1.690	2.48	1.735	2.64	0.548	2.53
Hawaii	15	1.790	2.83	1.850	3.05	0.686	3.85
	15A	1.306	1.33	1.306	1.33	0.401	1.52
Strain mean difference		0.332 [1]		0.527 [1]		0.159 [1]	
Diet mean difference		0.433 [1]		0.640 [1]		0.297 [1]	

[1] $P < 0.01$.

TABLE 5

Analyses of variance with reference to transformed (SR and L) data

SOURCE	d.f.	MEAN SQUARES FOR		
		Teeth (SR)	Carious areas (SR)	Score (L)
Between strains	1	2.2822 [1]	5.7588 [1]	0.5238 [1]
Between diets	1	3.8780 [1]	8.4781 [1]	1.8288 [1]
Strains × diets	1	0.0528	0.1850	0.0027
Error	79	0.2151	0.3025	0.0592

[1] $P < 0.01$.

The results indicate that using the Navy strain of rats, the proposed method of scoring, and diet 15A made up largely of natural foods, it should be possible to modify the diet and thus study some of the factors that tend to decrease or increase dental caries.

SUMMARY

Two inbred strains of rats have been compared for their susceptibility to dental caries when fed a diet of natural foods.

The Hawaii strain, inbred for more than 25 years, is very resistant to dental caries compared to the Navy strain developed at Bethesda, Md. The Navy strain retained its caries-susceptible characteristics when transported to and bred in Hawaii.

For rats of each strain it was found that the number of carious teeth, number of carious areas, and a score representing the extent of the carious areas, were all significantly smaller after feeding a diet ground to pass a 48-mesh sieve than when the same diet was passed through a 28-mesh sieve.

ACKNOWLEDGMENT

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THE DENTAL CARIES RESPONSE OF RATS FED CARIOGENIC AND NON-CARIOGENIC DIETS FOR DIFFERENT PERIODS OF TIME[1]

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A previous paper (Miller and Schlack, '58) reported the dental caries response of two strains of albino rats (Hawaii and Navy strains) to a diet composed largely of natural foods but containing small amounts of yeast, cod liver oil, and a salt mixture (diet 15A). A strain of rats developed at the Naval Medical Research laboratories at Bethesda, Md., was found to be susceptible to caries even on this diet which provides adequate nutrients for growth and reproduction and when all ingredients were finely ground to pass a 48-mesh sieve.

We found that the Hawaii strain showed little or no dental decay when fed the Harvard caries-producing diet (Sognnaes, '48), and that neither the Hawaii nor the Navy strain fed the cerelose diet of McClure ('51) showed the extent of caries that he reports.[2] It should be noted, however, that we used the Hubbell, Mendel and Wakeman ('37) salt mixture rather than the one recommended by Sognnaes. Our diet 15A causes mild caries, and when 17% of the whole wheat flour is replaced by sugar (diet 18A), causes moderate to severe caries. This caries-producing diet is similar to a diet simulating a typical American diet containing 17% sugar which was used by Zepplin et al. ('50). They reported that this diet caused as

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[2] Unpublished experiments.

severe carious lesions in the cotton rat as did a cariogenic diet with 67% sucrose.

The aim of the experiments reported here was to learn if feeding a good diet after weaning at 21 days, immediately after the appearance of the first and second molars, but not the third (Farris and Griffith ,'49), would protect the teeth when they were later subjected to a cariogenic diet. Also would changing from a diet that was certain to produce caries to one that produced little or no caries, result in more sound teeth at the end of the experimental period than if the caries-producing diet had been continued.

METHODS

Rats. Rats of the Navy strain known to be susceptible to caries (Miller and Schlack, '58) were used for both the preliminary and the final experiment. As 10 to 12 litters were needed for each experiment and since our small breeding colony does not produce this many litters at the same time, the young were started on the experimental diets as available over a period of about two months, but each litter was divided among the 4 groups. About a year and a half after the first experiment, rats of the same strain were used for experiment 2. The mothers of all young received diet 15 (same as 15A but all ingredients not ground to pass a 48-mesh sieve).

Diets. Two diets used were: diet 15A composed largely of natural foods but containing small amounts of yeast, cod liver oil, and a mineral mixture (Miller and Schlack, '58), and diet 18A in which sufficient of the whole wheat flour of diet 15A was replaced by powdered sugar to provide about 17% (16.97%) by weight and about 18% (17.73%) of the calories. All ingredients except the fine whole wheat flour were ground to pass a 48-mesh sieve. For both diets, skim milk powder (spray process) contributed 21.2% of the weight.

PROCEDURE

For experiment 1, 11 litters totaling 77 rats were divided and fed for 90 days as follows: Group 1 was given diet 15A

throughout the experiment; group 2, diet 15A for 42 days, followed by diet 18A for 48 days; group 3, diet 18A for 49 days and diet 15A for 41 days; and group 4, diet 18A for the entire period. The plan was to feed the cariogenic diet to groups 2 and 3 for the same number of days, but in one case at the beginning and the other at the end of the experiment.

For experiment 2, 10 litters of 80 rats were again divided to equalize the litters and sexes and fed the following diets for 100 days; group 1, diet 15A; group 2, diet 15A for 56 days followed by diet 18A for 44 days; group 3, diet 18A for 56 days followed by diet 15A for 44 days; and group 4, diet 18A. Here the rats in groups 2 and 3 were fed the same number of days at the beginning of the experiment before reversing the diets.

All rats were placed on the experimental diets at 21 days of age. They were housed in individual cages, fed the diets ad libitum and provided with local tap water which is low in total minerals and fluorine (0.3 to 0.05 p.p.m.).[3]

All jaws were prepared and examined using the method of caries evaluation previously described (Miller and Schlack, '58).

RESULTS AND DISCUSSION

The results of experiment 1 are summarized in tables 1, 2, and 3, and of experiment 2 in tables 4, 5, and 6.

Prior to subjecting the data to evaluation by the analysis of variance the original observations were, in all cases, subjected to transformation (Snedecor, '56, pp. 314–321). The specific transformations employed being $(n + 3/8)^{1/2}$ for the numbers (n) of defective teeth and for the numbers of cavities, and the $\log_{10}(s + 1)$ for the caries scores (s). In applying the analyses of variance to these transformed observations the methods appropriate to disproportionate subclass numbers were used whenever necessary (Snedecor, '56, pp. 379–382).

The analysis of variance measures the influence of diet as affected by the timing of the diet. The mean differences dis-

[3] Laboratory, Board of Water Supply, City and County of Honolulu.

played in the lower part of table 2 indicate that the effect of diet is more marked in the earlier stages than it is in the later stages. That the diets were directly additive in action is emphasized by the lack of significance of the constant

TABLE 1

Results of feeding 4 groups of rats diets with and without sugar for a period of 90 days

(Experiment 1)

CATEGORY OF INTEREST	GROUP 1	GROUP 2	GROUP 3	GROUP 4
Diets	15A (no sugar)	15A 42 days 18A 48 days	18A 49 days 15A 41 days	18A (17% sugar)
No. of rats	20	18	20	19
Rats with caries	15	17	19	19
Rats with caries (%)	75	94.4	95.0	100
Carious teeth (total number)	42	72	103	109
Carious teeth per rat	2.1	4.0	5.2	5.7
Carious areas (total)	52	93	148	160
Carious areas per rat	2.6	5.2	7.8	8.4
Carious scores (total)	62	121	199	238
Caries score per rat	3.1	6.7	10.5	12.5
Mean weight, males (gm)	334	369	338	349
Mean weight, females (gm)	226	220	226	227

TABLE 2

Transformed (SR and L) means and mean differences, also rectified (N′ and S′) treatment means

(Experiment 1)

GROUP	DIET (RELATIVE PERIODS FED)		TEETH		CARIOUS AREAS		SCORES	
	First half	Last half	SR	N′	SR	N′	L	S′
1	15A	15A	1.454	1.74	1.600	1.81	0.485	2.05
2	15A	18A	1.999	3.62	2.223	4.57	0.768	4.86
3	18A	15A	2.205	4.49	2.590	6.33	0.948	7.87
4	18A	18A	2.442	5.59	2.893	7.99	1.049	10.20
15 vs. 18 (first half)			0.603 [1]		0.836 [1]		0.376 [1]	
15 vs. 18 (last half)			0.389 [1]		0.461 [1]		0.191 [2]	

[1] $P < 0.05$.
[2] $P < 0.01$.

versus split comparison (table 3). This indicates that the teeth are protected to a considerable degree when diet 15A is fed for less than half the period at the beginning of the test followed by the cariogenic diet (18A) for the remainder of the experiment, contrasted with the cariogenic diet at the beginning, then changing to the non-cariogenic.

TABLE 3

Analyses of variance with reference to transformed (SR and L) data

(Experiment 1)

SOURCE OF VARIATION	d.f.	MEAN SQUARES FOR		
		Teeth (SR)	Carious areas (SR)	Score (L)
15 vs. 18 (first half)	1	6.9949 [2]	13.4511 [2]	2.7125 [2]
15 vs. 18 (last half)	1	2.9054 [2]	4.0795 [2]	0.6990 [1]
Constant vs. split diets	1	0.4556	0.4918	0.1591
Error	73	0.4130	0.5409	0.1088

[1] $P < 0.05$.
[2] $P < 0.01$.

For experiment 2 (tables 4, 5, and 6), the total period of feeding was extended from 90 to 100 days and, for groups 2 and 3, the initial feeding periods for the non-cariogenic or the cariogenic diets, respectively, were also extended; with resulting sharper contrasts in the effects of the two diets.

There is again a highly significant difference as a result of feeding diets 15A and 18A in the first part of the experimental period. The longer period of feeding the cariogenic or non-cariogenic diets at the beginning of the experiment exerted a correspondingly greater influence upon the condition of the teeth, as determined at the end of the experimental period.

The mean differences in table 6 emphasize, for all three criteria, that, as the initial feeding period was extended, subsequent changes of diet exerted no appreciable influence on tooth condition.

In other words, the rats fed diet 15A (non-cariogenic) for 56 days, followed by a cariogenic diet for 44 days had as good teeth as those which had diet 15A for the entire experimental period of 100 days. On the other hand, the rats in group 3

that had a cariogenic diet for 56 days followed by diet 15A for 44 days had as bad dental conditions as did the rats in group 4 that had the cariogenic diet for the entire 100 days.

Mellanby ('37) was among the first to emphasize the importance of nutrition to tooth structure and of good tooth structure to resistance to dental caries. Several investigators (Hodge, '43; Braunschneider, Hunt and Hoppert, '48) have found that the age at which rats are placed on cariogenic diets

TABLE 4

Results of feeding 4 groups of rats diets with and without sugar for a period of 100 days

(Experiment 2)

CATEGORY OF INTEREST	GROUP 1	GROUP 2	GROUP 3	GROUP 4
Diets	15A (no sugar)	15A 56 days 18A 44 days	18A 56 days 15A 44 days	18A (17% sugar)
No. of rats	20	20	20	20
Rats with caries	19	16	20	20
Rats with caries (%)	95	80	100	100
Carious teeth (total number)	43	42	117	121
Carious teeth per rat	2.2	2.1	5.8	6.0
Carious areas (total)	51	53	211	209
Carious areas per rat	2.6	2.6	10.6	10.4
Carious scores (total)	74	80	344	332
Caries score per rat	3.7	4.0	17.2	16.6
Mean weight, males (gm)	368	366	359	373
Mean weight, females (gm)	252	252	239	246

TABLE 5

Transformed (SR and L) means with corresponding rectified treatment means

(Experiment 2)

GROUP	(RELATIVE PERIODS FED)		TEETH		CARIOUS AREAS		SCORES	
	First half	Last half	SR	N′	SR	N′	L	S′
1	15A	15A	1.539	1.99	1.640	2.31	0.606	3.04
2	15A	18A	1.475	1.80	1.590	2.15	0.561	2.64
3	18A	15A	2.460	5.68	3.233	10.08	1.194	14.64
4	18A	18A	2.503	5.89	3.238	10.11	1.199	14.81

markedly influences the caries susceptibility, but both Hodges and the Michigan group were comparing rats placed on the caries-producing diets at 100 days, 150 days, and one year, contrasted with rats placed on the same diets after weaning. Sognnaes ('48) reported rats more susceptible to caries when the mother had a cariogenic diet than when the rats were placed on the same diet at weaning. He emphasized the effect of cariogenic diets in relation to the age of experimental animals and the maturation of the teeth.

The experiments reported here offer good statistical evidence that the diet of rats for the first 42 to 56 days after

TABLE 6

Mean differences and differences required for significance for 100-day experiment

(Experiment 2)

SOURCE OF VARIATION	MEAN DIFFERENCES FOR		
	Teeth (SR)	Carious areas (SR)	Score (L)
15 vs. 18 (first 56 days)	— 0.974 [1]	— 1.621 [1]	— 0.613 [1]
15 vs. 18 (last 44 days)	0.011	0.022	0.020
Straight vs. split diets	0.053	0.027	0.025
Difference required for significance ($P < 0.01$)	0.190	0.267	0.121

[1] $P < 0.01$.

weaning can markedly influence the condition of the teeth at the end of a 90-day or 100-day experimental period. If a diet that protects the teeth is first given for about half the period even when followed by a cariogenic diet for the remaining half, the teeth at the end of 100 days are in as good condition as when the non-cariogenic diet is furnished for the entire period. The reverse is also true, that when a cariogenic diet is fed for about half the period, and then followed by a non-cariogenic diet for the last half, the dental conditions are as bad as when the rats are given a caries-producing ration for the entire experimental period.

Although sound rat teeth may be more resistant to a cariogenic diet than are most human teeth, experiments such

as these help to explain some of the resistance to dental caries shown by sound human teeth when exposed for short or longer periods to caries-producing diets. They also suggest that it is to be expected that children who have a poor diet throughout their early years, especially during the pre-school period when the permanent teeth are being formed, may have a high rate of decay as young adults.

The dental condition of many of the young people in Hawaii show high DMF rates of 17 to 19 per person (Miller, Bickerton and Parke, '52, and Fanning, '52) even though as University students they report having relatively good diets.

SUMMARY

Matched litter mate rats were used in two sets of experiments employing two types of rations — diet 1 (15A), consisting of natural foods without sugar, produced little caries and diet 2 (18A), in which 18% of the calories in diet 1 were replaced with sugar, produced moderate to severe caries. When the non-cariogenic diet was fed for about half the experimental period of 100 days and the cariogenic diet the second half, the teeth were well protected; whereas if the cariogenic diet was fed the first half of the experimental period, the dental conditions were as bad as if the rats had the cariogenic diet for the entire period. Analogies with human dental conditions are drawn.

ACKNOWLEDGMENT

The author gratefully acknowledges the work of J. G. Darroch, Principal Statistician, Experiment Station, Hawaiian Sugar Planters' Association; Consultant, Pineapple Research Institute; and a member of the affiliated graduate faculty University of Hawaii, in reveiwing the results of the experiments and applying the proper statistical treatment.

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ELECTROPHORETIC ANALYSES OF GROUNDNUT [1] PROTEINS PREPARED UNDER DIFFERENT CONDITIONS OF EXTRACTION OF OIL

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Irving, Fontaine and Warner ('45) carried out solubility experiments to evaluate denaturation of groundnut proteins undergoing changes during processing. They observed that the meal prepared in the laboratory by solvent extraction was least affected, while the meal obtained from the mill-pressed cake was considerably denatured due to heat and steam treatment. These same authors (Fontaine, Irving and Warner, '45) reported moving boundary electrophoresis studies on the proteins of groundnut meal prepared in the laboratory. Two major and one or possibly two minor components at pH 9.26 were found to be present. Karon, Adams and Altschul ('50), making use of different buffers on the fractionation of groundnut protein and cottonseed protein by electrophoresis, observed that in the case of groundnut protein, identical protein patterns were obtained over a pH range of 8.3 to 10.2. Their results showed the presence of 4 components, two major and two minor. While Lord and Wakelam ('51) have studied the effect of modern mill-processing conditions on the solubility of various fractions of groundnut meal no work has so far been reported on the electrophoretic behaviour of differently-processed groundnut meals.

In a previous paper, nutritional investigations on groundnut proteins undergoing different conditions of extraction of oil were described (Balasundaram, Cama, Malik and Venkateshan, '58). The electrophoretic work reported in the

[1] Peanut.

present paper is on groundnut meal samples subjected to the same conditions of extraction.

MATERIALS AND METHODS

The preparation of groundnut meals A, B, C and D has been described by Balasundaram et al. ('58).

Extraction of meal

Water extract. For electrophoretic analysis the water extract of the protein was prepared as follows: 2.0 gm of the defatted meal was mixed with 20.0 ml of water and shaken for two hours at room temperature. The mixture was centrifuged at 2,000 r.p.m. for 15 minutes. The supernatant was then dialyzed against 200.0 ml of the veronal buffer of ionic strength 0.1, pH 8.6 for 12 hours at 4°C and after one change of buffer for 4 hours, with constant stirring at room temperature (25°C). The solution was next clarified by filtration through Whatman No. 42 filter paper.

Buffer extract: 2.0 gm of the defatted meal was extracted with 20.0 ml of the veronal buffer, ionic strength 0.1 and pH 8.6, for two hours, with constant shaking. The supernatant obtained after centrifugation was dialyzed as in the case of the water extract. After dialysis, the protein solution was filtered through Whatman No. 42 filter paper and analyzed electrophoretically.

Electrophoretic analysis of the extracts was carried out in Kern's micro-electrophoresis apparatus employing a voltage gradient of 3.4 V cm^{-1}. Ordinary dry cells were used as a source of current. The mobilities and area distribution were measured according to the method of Longsworth and MacInnes ('40) at room temperature and were reduced to 20°C by viscosity correction as given by Johnson and Shooter ('48).

The electrophoretic results of the water as well as the buffer extracts of the ascending (a) and descending (d) limbs are given in table 1.

TABLE 1

Electrophoretic analyses of differently-processed groundnut meals [1]

MEAL NO.	$-U \times 10^{-5} cm^2 volt^{-1} sec^{-1}$ (MOBILITY)							PERCENTAGE RELATIVE AREA						
	a_1	a_2	a_3	a_4	d_1	d_2	d_3	a_1	a_2	a_3	a_4	d_1	d_2	d_3
Water extract:														
A	7.10	11.80	15.90	19.34	10.86	16.52	20.86	14.5	34.3	46.2	5.0	46.7	46.0	7.3
B	6.23	11.25	16.14	19.14	10.53	16.01	19.11	7.0	33.0	56.0	4.0	34.5	60.5	5.0
C	—	11.69	15.95	19.04	10.87	16.0	29.11	—	16.9	78.9	4.2	17.3	76.2	6.5
D	—	11.13	16.03	19.66	9.76	15.70	21.52	—	19.8	79.6	9.6	15.4	78.9	5.7
Buffer extract:														
A	—	11.03	15.81	19.92	9.53	15.66	19.58	—	11.7	83.0	5.3	12.8	80.9	7.3
B	—	11.48	15.29	—	10.26	14.88	—	—	11.8	88.2	—	13.3	86.7	—
C	—	10.36	15.32	19.42	9.24	14.90	19.20	—	11.3	83.0	5.7	19.0	83.2	6.8
D	—	10.83	15.03	19.18	9.74	14.77	18.92	—	8.6	87.1	4.3	8.9	87.2	3.9

[1] At pH 8.6: u = 0.1; mobilities at 25°C.

RESULTS AND DISCUSSION

The physico-chemical characteristics of a protein are usually closely linked with its nutritional quality. From this point of view, an electrophoretic behaviour of a protein could very well reveal vital changes in protein make-up during treatment or processing which may be closely related to changes in the nutritive value of the protein.

Table 1 shows the mobilities of the ascending (a) and descending (d) limbs of the electrophoretic boundaries and the relative percentage area of the components observed. The results are given for water as well as buffer extracts of protein.

In the water extracts of raw (A) and "expeller" (B) meals, there are 4 components in the ascending limb (a values). The percentage of the first component, a_1, for the "expeller" (B) meal is only half that of the raw meal (A). This diminution in the first component is made up by an increase in the third component, a_3. On an average, the same amounts of components a_2 and a_4 are present in both the meals. It is interesting to record that in the control-steamed (C) and high-steamed (D) meals, the first component a_1, is completely absent and only about half the amount of the a_2 component is present as compared to raw (A) or "expeller" (B) meals. Simultaneously, there is a considerable increase in the a_3 component. The high-steamed (D) meal has a marked increase of the last component, a_4, as compared to that observed on the raw (A), "expeller" (B) or control-steamed (C) meals. Thus, the electrophoretic analyses of the water extracts of the 4 differently-processed meals reveal a striking change in the components with heat treatment.

In the descending limb (d values), three components are present and the picture is much the same. There is a progressive diminution in the first component, d_1, with increasing heat treatment accompanied by a corresponding increase of the d_2 component. The last component, d_3, shows relatively little change between the meals.

The differences amongst the meals in the electrophoretic patterns of the veronal-buffer-extracted protein are negligible. It may well be that, due to the alkalinity of the buffer, most of the components of groundnut protein are extracted to the same extent irrespective of their solubility differences. Consequently, during denaturation no significant differences could be observed in the buffer extract of the protein. There are three components in the ascending (a) and descending (d) limbs and not much difference is observed in the amounts of these components in the 4 meals. The middle components, a_3 and d_2, were predominant in all the meals. In the "expeller" (B) meal, however, there is complete absence of the last components, a_4 and d_3, in both the ascending (a) and the descending (d) limbs. Though this is only a minor component its absence in the buffer extract may perhaps be due to the very low concentration of the component which could not be detected in the electrophoretic pattern.

It is evident that the electrophoretic behaviour of differently processed meals has considerable relationship to the nutritional observations reported in a previous paper (Balasundaram et al., '58). The effect of progressive denaturation of the meals is strikingly revealed by the distribution of the different protein components in the unheated raw (A) meal compared to meal (D) undergoing heavy heat and steam treatment.

SUMMARY

Groundnut meals obtained by different conditions of modern mill-processing methods have been analyzed electrophoretically by the moving boundary electrophoretic method.

Different conditions of extraction significantly affect the relative percentage area of the protein pattern corresponding to each meal in the ascending as well as in the descending limb after extraction with water.

Mild denaturation of the protein, as observed under control-steamed (C) meal resulted in a complete absence of the a_1 component, a decrease in the a_2 component with a simultaneous increase of the a_3 component when compared to unheated

meal (A). Similar changes were also observed in the descending limb.

Extraction by veronal buffer did not indicate any differences amongst the meals.

ACKNOWLEDGMENT

We like to acknowledge our sincere thanks to Prof. K. V. Giri for his keen interest in the work.

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THE EFFECT OF PENICILLIN, AUREOMYCIN, STREPTOMYCIN AND VITAMIN B_{12} ON THE LIVER RESERVES OF VITAMIN A ESTERS OF THE RAT

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Lih and Baumann ('51) and Sauberlich ('52) showed that antibiotics exert a "sparing effect" on the requirements of the B-complex group of vitamins for the rat. The mode of action of the "sparing effect" has not yet been elucidated but it may well be due to either an increased synthesis of B-complex vitamins by intestinal microorganisms or to an increased efficiency of absorption of the vitamins from the gut walls.

The original observation of an increased storage of carotenoids in the serum and of vitamin A in the liver of chicks fed penicillin along with dietary carotene was made by Burgess, Gluck, Brisson and Laughland ('51). Similar observation of an increased storage of vitamin A in the livers of pigs fed an all-vegetable ration supplemented with penicillin and aureomycin was reported by Barber, Braude, Kon and Mitchell ('52). High and Wilson ('53) observed that vitamin B_{12} supplementation significantly increased vitamin A deposition in the liver from carotene-fed rats, but had no effect on the utilization of preformed vitamin A for storage. However, vitamin B_{12} in the presence of penicillin was ineffective towards the utilization of carotene for vitamin A storage. The results of Hartsook, Batchelor and Johnson ('53) also showed that antibiotics did not increase liver reserves of vitamin A when preformed vitamin A was supplied in the

rations. Furthermore, the avitaminosis syndromes were more severe and appeared much earlier in the aureomycin-treated group than in the control group. A little later, High ('55) reported that aureomycin did not affect the amount of vitamin A deposited in the liver and kidneys of either male or female rats when preformed vitamin A was administered.

In a previous paper, Balasundaram, Cama, Malik and Venkateshan ('58) reported the effect of supplementation of groundnut[1] protein with antibiotics and vitamin B_{12} on the growth and nitrogen balance requirements of the rat. The present investigation was taken up on the same group of rats after they were pair-fed for 7 weeks to examine the effect of supplementation with penicillin, aureomycin, streptomycin and vitamin B_{12} on liver storage of vitamin A esters.

EXPERIMENTAL

Paired-feeding test. The details of the paired-feeding test, composition of the diet and the amounts of supplements of antibiotics and vitamin B_{12} were described in a previous paper (Balasundaram et al., '58).

Determination of vitamin A esters in the liver. At the end of the 7-week period, the animals were anesthetised with chloroform and killed. The livers were separately ground with acid-washed sand and anhydrous sodium sulphate, and the tissue lipid was extracted 5 times with light petroleum. The combined extracts were reduced in volume at low pressure. Vitamin A esters were estimated spectrophotometrically by the three-point correction method of Cama, Collins and Morton ('51), using the absorption readings at 310, 325 and 335 mμ (light petroleum).

The results were considered significant when P was between 5 and 10% or less than 5%.

RESULTS AND DISCUSSION

In the present investigation, the rats were not depleted of their vitamin A reserves in the livers, but it was assumed

[1] Peanut.

that the liver vitamin A reserves of each litter-mate rat of the same sex were equal at the beginning of the paired-feeding test. Under the conditions of the stock colony diet used in our laboratory, the liver vitamin A reserves of a weanling rat were very small and usually varied between 30 and 40 I.U.

It is evident from table 1 that supplementation of groundnut meal with antibiotics and vitamin B_{12} significantly reduced liver storage of vitamin A esters. These observations emphasize that, whereas antibiotics and vitamin B_{12} have a "sparing effect" on the B-complex group of vitamins and also increase liver storage of vitamin A from carotene-fed rats as reported by previous investigators, the effect of antibiotics and vitamin B_{12} on feeding preformed vitamin A considerably lowered the liver reserves of vitamin A esters. High and Wilson ('53), Hartsook et al. ('53) and High ('55) reported that antibiotics

TABLE 1

Increase in weights and in liver reserves of vitamin A esters of rats fed groundnut proteins supplemented with antibiotics and vitamin B_{12}

(Period: seven weeks)

DIET	AVERAGE INCREASE IN WT.	AVERAGE LIVER RESERVES OF VITAMIN A ESTERS	MEAN DIFFERENCE	S.E.[1] DIFF.	P (PROBABILITY)[2]
	gm	*I.U.*			
Raw (A)	95	1157	— 218	± 41.2	S***
Raw + penicillin	98	939			
Raw	68	1183	— 225	± 59.0	S***
Raw + aureomycin	68	958			
Raw	95	1157	— 204	± 45.5	S***
Raw + streptomycin	95	953			
Raw	68	1183	— 96	± 19.5	S***
Raw + vitamin B_{12}	67	1087			
Expeller (B)	77	1405	— 289	± 112.6	S**
Expeller + aureomycin	80	1116			
Expeller	77	1417	— 176	± 80.0	S*
Expeller + vitamin B_{12}	77	1241			

[1] Standard error of the differences.

[2] S*** = Significant between 1 and 2% levels.
S** = Significant between 2 and 5% levels.
S* = Significant between 5 and 10% levels.

and vitamin B_{12} had no effect on the utilisation and storage of preformed vitamin A, but such a significant decrease in the liver storage of vitamin A esters as observed under the present conditions of paired-feeding tests has not been reported earlier. If the effects on rats are reproducible in other species of animals, then these results are of significance to the nutrition of farm animals where antibiotics and vitamin B_{12} are usually used as supplements to vegetable diets.

The marked effects of antibiotics and vitamin B_{12} in increasing the utilization of carotene for tissue deposition of vitamin A as contrasted to its almost deleterious effect with respect to the deposition of preformed vitamin A suggest that there may be some effect on the provitamin prior to its conversion to vitamin A. Such an effect may well be due to the enzymatic conversion of carotene to vitamin A or the absorption of the provitamin from the intestinal tract or alterations in the intestinal microflora prior to absorption.

SUMMARY

Supplementation of groundnut protein in paired-feeding tests with penicillin at 0.02%, aureomycin at 0.03%, streptomycin at 0.02% level of the diet and vitamin B_{12} at 45 μg/kg of diet significantly reduced the liver reserves of vitamin A esters in the rat when preformed vitamin A was included in the diet.

ACKNOWLEDGMENT

We are grateful to Prof. K. V. Giri for his keen interest in the work.

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INVITATIONS FOR NOMINATIONS FOR 1959 AMERICAN INSTITUTE OF NUTRITION AWARDS

Nominations are requested for the 1959 annual awards administered by the American Institute of Nutrition to be presented at the next annual meeting. Nominations may be made by anyone, including members of the Nominating Committees and non-members of the Institute.

The following information must be submitted: (1) Name of the award for which the candidate is proposed and (2) a statement as convincing as possible as to the basis for the nomination, stating the eligibility of the candidate (this may include the pertinent bibliography of the most appropriate and significant recent papers on which the nomination is based, but such bibliography is not necessary unless later requested by the Nominating Committee). Reprints are not required, nor are seconding statements. *Five copies of all documents* must be sent to the chairman of the appropriate Nominating Committee *before December 1, 1958,* to be considered for the 1959 awards.

General Regulations for A.I.N. Awards. An individual who has received one Institute award is ineligible to receive another Institute award unless it is for outstanding research subsequent to or not covered by the first award.[1] A Jury of Award composed of A.I.N. members, which makes final selection and remains anonymous, may recommend that an award be omitted in any given year if in its opinion the work of the candidates nominated does not warrant the award. An award is usually given to one person but, if circumstances and justice so dictate, a Jury of Award may recommend that any par-

[1] Including recipients of the former Mead-Johnson award. These are listed at the end of this notice.

ticular award be divided between two or more collaborators in a given research.

Membership in the American Institute of Nutrition is not a requirement for eligibility for an award and there is no limitation as to age. Presentation of awards will be made at the annual dinner at the annual meeting and time will be allotted on the scientific program for a brief review by the recipient of the work upon which the award has been based.

1959 Borden Award in Nutrition

The Borden Award in Nutrition, consisting of $1,000 and a gold medal, is made available by the Borden Company Foundation, Inc. The award is given in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of milk or its components. The award will be made primarily for the publication of specific papers during the previous calendar year, but the Jury of Award may recommend that it be given for important contributions made over a more extended period of time not necessarily including the previous calendar year. Employees of the Borden Company are not eligible for this award nor are individuals who have received a Borden Award from another administering association unless the new award be for outstanding research on a different subject or for specific accomplishment subsequent to the first award.

Former recipients of this award are: 1944 — E. V. McCollum; 1945 — H. H. Mitchell; 1946 — P. C. Jeans and Genevieve Stearns; 1947 — L. A. Maynard; 1948 — C. A. Cary; 1949 — H. J. Deuel, Jr.; 1950 — H. C. Sherman; 1951 — P. György; 1952 — M. Kleiber; 1953 — H. H. Williams; 1954 — Agnes Fay Morgan and A. H. Smith; 1955 — A. G. Hogan; 1956 — F. M. Strong; 1957 — no award; 1958 — L. D. Wright.

Nominating Committee:

H. H. WILLIAMS, *Chairman*
C. A. BAUMANN
G. V. MANN

Send nominations to:

DR. H. H. WILLIAMS
Cornell University, Savage Hall
Ithaca, New York

1959 Osborne and Mendel Award

The Osborne and Mendel Award of $1,000 and an inscribed scroll has been established by the Nutrition Foundation, Inc., for the recognition of outstanding recent basic research accomplishments in the general field of exploratory research in the science of nutrition. It shall be given to the investigator who, in the opinion of a Jury of Award, has made the most significant published contribution in approximately the calendar year preceding the annual meeting of the Institute, or who has published recently a series of papers of outstanding significance. Normally preference will be given to research workers in the United States and Canada, but investigators in other countries, especially those sojourning in the United States or Canada for a period of time, are not excluded from consideration.

Former recipients of this award are: 1949 — W. C. Rose; 1950 — C. A. Elvehjem; 1951 — E. E. Snell; 1952 — Icie Macy Hoobler; 1953 — V. du Vigneaud; 1954 — L. A. Maynard; 1955 — E. V. McCollum; 1956 — A. G. Hogan; 1957 — G. R. Cowgill; 1958 — P. György.

Nominating Committee:
ICIE MACY HOOBLER, *Chairman*
D. M. HEGSTED
J. S. DINNING

Send nominations to:
DR. ICIE MACY HOOBLER
Merrill-Palmer School
71 East Ferry Avenue
Detroit 2, Michigan

Former recipients of the Mead-Johnson Award presented by A.I.N. are: 1939 — C. A. Elvehjem; 1940 — W. H. Sebrell, Jr., J. C. Keresztesy, J. R. Stevens, S. A. Harris, E. T. Stiller, and K. Folkers; 1941 — R. J. Williams; 1942 — G. R. Cowgill; 1943 — V. du Vigneaud; 1944 — A. G. Hogan; 1945 — D. W. Woolley; 1946 — E. E. Snell; 1947 — W. J. Darby, P. L. Day, and E. L. R. Stokstad; 1948 — F. Lipmann; 1949 — Mary S. Shorb and K. A. Folkers; 1950 — W. B. Castle; 1951 — no award; 1952 — H. E. Sauberlich.

INVITATION FOR NOMINATIONS FOR FELLOWS

The Fellows Committee of the American Institute of Nutrition invites nominations for Fellows in the Society. Eligible candidates are active or retired members of the Society who have passed their sixty-fifth birthday (by the time of the annual meeting) and who have had distinguished careers in nutrition. Up to three Fellows will be chosen each year.

Nominations may be made to the Chairman of the Fellows Committee by any member of the Society.

Nominations (in 5 copies) are due by January 1. A supporting statement giving the reason for the nomination is desirable but not necessary.

Final selection will be made by the Fellows Committee and a suitable citation will be presented at the Annual Dinner in April. The following persons have been elected previously as Fellows of the Society:

Thorne M. Carpenter
George R. Cowgill
Eugene F. DuBois
Ernest B. Forbes
Casimir Funk
Elmer V. McCollum
Harold H. Mitchell
John R. Murlin
Harry Steenbock
Robert R. Williams

Fellows Committee:

T. H. JUKES, *Chairman*
PAUL GYÖRGY
COSMO MACKENZIE
W. D. SALMON
KARL FOLKERS

Send nominations to:

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American Cyanamid Company
Research Division
Pearl River, N. Y.

PROCEEDINGS
OF THE TWENTY-SECOND ANNUAL MEETING OF THE AMERICAN INSTITUTE OF NUTRITION

CONVENTION HALL, PHILADELPHIA, PENNSYLVANIA
APRIL 13–18, 1958

COUNCIL MEETINGS

Council meetings were held at Hotel Sheraton, Philadelphia, on Sunday, April 13, and Monday, April 14. Formal actions of the Council are reported in the following minutes of the two business meetings of the Society held on April 15 and 17 in Convention Hall.

SCIENTIFIC SESSIONS

There were a total of 125 papers submitted by members. Twenty-five of these were transferred to intersociety sessions or to the programs of other societies (three to physiology and 4 to biochemistry). With the 15 papers received from biochemistry, 7 from physiology, and three from pharmacology, a program of 11 half-day sessions was arranged. In addition, a half-day symposium on "Where are we headed in nutrition?" was arranged by Dr. King for Wednesday afternoon, April 16. Abstracts of all papers presented have been published in Federation Proceedings and the symposium talks will be published in the July issue of Federation Proceedings. Six intersociety sessions on "Atherosclerosis" (a total of 63 papers) were arranged under sponsorship of the Society. All sessions were well attended with the symposium drawing approximately 500 people.

BUSINESS MEETINGS

Held on April 15 with 90 present and on April 17 with 50 present — Dr. R. R. Williams, President, presiding:

1. Minutes. The minutes of the 21st Annual Meeting, as published in Journal of Nutrition *63:* 163, 1957 (September), were approved.

2. Election. The secretary transmitted the sealed ballots to the Tellers' Committee, Dr. Aaron Arnold and Dr. Chester D. Tolle. At the second business meeting the Committee reported election results on 257 ballots as follows:

To begin July 1, 1958:

President: William J. Darby
President Elect: D. Wayne Woolley
Councilor (replacing L. A. Maynard): Joseph H. Roe

To begin May 1, 1958:

Associate Editors (4-year term): O. Lee Kline
Edmund S. Nasset
Herbert Pollack

(See page 23 for list of all officers.)

3. Constitutional Amendments. By over two-thirds of all votes cast the following constitutional amendments were adopted (please refer to Federation Proceedings *16:* 904, 1957, for former wording of the Constitution and By-Laws):

A. *President-Elect.* By vote of 230 for to 5 against

1. Changed the wording "Vice-President" to "President-Elect" in
   a. Introduction, paragraph 3
   b. Article II, Section 2, line 3
2. Article III, Section 1, as changed, reads:

   Section 1. *Officers:* The officers, elected by members of the Society by majority vote, shall be a President, a President-Elect to be elected annually, and a Secretary and a Treasurer each of whom shall be elected to serve for a term of three years. Their terms of office shall commence on July 1 of the year in which they are elected. No person who has served as President is eligible for re-election as President-Elect.
3. Article V, Section 2, as changed, reads:

   Section 2. *Nomination of officials:* The Nominating Committee shall make at least one nomination for each of the offices to be filled, for each of the additional positions on the Council to be filled, and for each of the positions on the Editorial Board to be filled at the time of the annual meeting.

B. *Program.* By vote of 227 for to 6 against, a new section of Article VIII states:

Section 2. The Council shall, from time to time, set the rules on the number of papers which may be presented or sponsored by a member, as necessitated by changing circumstances but with regard to equality of privilege for all members.

C. *Auditing Committee.* By a vote of 235 for to 4 against, a new section of Article VI states:

Section 4. *Auditing Committee:* An Auditing Committee of two members, appointed for the coming year by the incoming President, shall be responsible for auditing the records of the Treasurer and for submitting a written report at the next annual meeting. One member shall be designated as Chairman and members are eligible for reappointment up to a total of three years.

D. *Fellows.* By a vote of 224 for to 14 against, a new section has been added to Article I which reads:

Section 5. *Fellows:* Active or retired members of the American Institute of Nutrition who have passed their sixty-fifth birthday and who have had a distinguished career in nutrition may be selected as Fellows of the Society. Such elections shall be made by a Fellows Committee of five members appointed by the incoming President. Nominations of candidates may be made to the Fellows Committee by any member of the Society. No more than three Fellows shall be selected each year (except that a maximum of ten may be selected the first year this provision is put into effect). A suitable citation shall accompany awarding of Fellowships at the annual meeting.

E. *Miscellaneous.* By a vote of 234 for to 1 against the following miscellaneous changes were made:

1. The word "proposed" is omitted in the first sentence of the introduction (in the sentence, "The name of the proposed Society . . .")
2. The last sentence in parentheses in Article III, Section 2, is deleted.
3. The title of Article V is changed to "Officers Nominating Committee and Elections."
4. The second and third sentences of Article VII, Section 2, part C, beginning with "In the event of a vacancy . . ." are deleted.

4. *Membership Status.* The Secretary reported that as of April 10, 1958, there were 408 active members and 45 retired members, or a total of 453 members in the American Institute of Nutrition (this is an increase of 15 members since last

year). Members present at the meeting stood for a moment of silence in memory of the following three members who passed away during the year:

J. H. Axtmayer, May 7, 1957
M. Bruger, November 26, 1957
S. M. Hauge, November 26, 1957

Appropriate resolutions which had been received relative to the deceased were approved and copies are on file in the Secretary's office.

The following members resigned during the year:

S. G. Smith
A. D. Welch

Dr. E. E. Snell, who was listed as having resigned in last year's minutes, has been reinstated.

5. *Treasurer's Report.* The report of Treasurer J. B. Brown was read and approved. A summary report from April 10, 1957, to April 1, 1958, is as follows:

Balance brought forward		$ 1,054.95
Receipts		
406 subscriptions to Journal of Nutrition	$ 3,440.50	
419 Federation dues	1,676.00	
416 Institute dues	832.00	
Contributions to Fifth International Congress on Nutrition—1960	23,850.00	
Nutrition Dinner—1957	93.17	
Smoker—1957	52.55	
Interest on bond	13.80	
Miscellaneous income	2.15	29,960.17
		$31,015.12
Expenditures		
Wistar Institute	3,440.50	
Federation Office	1,672.00	
Secretary's Office	390.00	
Treasurer's Office	142.60	
Federation Office for deposit in separate savings account for Fifth International Congress on Nutrition Fund in name of American Institute of Nutrition	23,850.00	
Miscellaneous	64.59	29,559.69
Balance on hand April 1, 1958		1,455.43
Bond (U. S. Savings)		500.00
TOTAL BALANCE		$ 1,955.43

The auditing Committee, Mary Brown Patton and Fred A. Hitchcock, reported that the Treasurer's accounts have been found in good order. Their report was approved. The original copies of the Treasurer's and Auditors' reports are on file in the Secretary's office.

At the second business meeting (April 17) dues of $2.00 for the coming year, plus a special assessment of $2.00 per year for the next three years for the 1960 International Congress on Nutrition, were approved. Provision will be made in the Treasurer's statements for additional contributions from members for the Congress.

6. *Journal of Nutrition.* The Editor, Dr. George R. Cowgill, submitted an annual report for 1957, a summary of which follows:

	Nos.
Volumes of Journal of Nutrition, 1957	61, 62, 63
Pages published	1842
Papers submitted	205
Papers withdrawn	1
Papers rejected	40
Papers published	151
Pages per paper	12.2
Supplements	0
Bibliographies (H. C. Sherman, Gerrit Grijns, and Leroy S. Palmer)	3

The Editor pointed out that the primary objective of 1800 pages per year in three volumes had again been attained. The 40 rejections for 1957 represent 19% compared with 12% in 1955 and 18% in 1956. The Editor also stated that two perennial problems still exist — many authors still fail to check their references carefully and fail to return their proofs promptly. The Editor announced that at the end of his 5-year term of appointment next year he plans to retire as Editor.

The Editor's report was approved with a vote of great appreciation to Dr. Cowgill for the efficient handling of Journal affairs. In view of Dr. Cowgill's decision to retire, it was announced that Dr. O. L. Kline has been appointed by the Editorial Board to receive suggestions for a new Editor not only from members of the Editorial Board but from all

interested members. Suggestions (in 12 copies) are welcome so that an Editor may be selected by the Editorial Board at the next annual meeting as in the past.

7. *New Members.* The Council received 55 nominations for membership. The following 52 candidates were declared eligible by the Council and were approved by members at the business meeting. (A brief guide of eligibility was sent to sponsors of new members this past year which was helpful in reducing the list of rejected candidates).

Erik Aaes-Jørgensen
Guillermo Arroyave
Joseph J. Barboriak
Orville G. Bentley
Finn W. Bernhart
John W. Bratzler
Ricardo Bressani
Edwin B. Bridgforth
Helen B. Burch
Lillian C. Butler
Meil-ling Wu Chang
Miriam F. Clarke
Hans Fisher
Katherine H. Fisher
M. R. Spivey Fox
Leo Friedman
Casimir Funk
Stanley N. Gershoff
Francisco Grande
John E. Halver
LaVell M. Henderson
John W. Hibbs
Charles H. Hill
Jacob A. Hoefer
Paul E. Johnson
Laurence W. Kinsell
Elmer J. Lease
Stanley Levey
Lewis E. Lloyd
Lawrence J. Machlin
Frank J. McClure
Russell F. Miller
Alexander B. Morrison
Elsie Z. Moyer
T. Keith Murray
Harold M. Nitowsky
William N. Pearson
William H. Pfander
Forrest W. Quackenbush
Hans R. Rosenberg
Padubidri S. Sarma
Klaus Schwarz
Florence I. Scoular
Edward L. Stephenson
Robert G. Tucker
Joseph J. Vitale
Harold D. Wallace
Robert H. Wasserman
Donald M. Watkin
Robert L. Wixom
Robert J. Young
Isadore Zipkin

The Secretary announced that new membership nomination forms have been approved by the Council and would be printed by next fall. These forms will give more information of value to sponsors and to the Council in determining qualifications of potential candidates. The Secretary also announced that there are still many eligible nutritionists who

are not members of the Society and that it was every member's duty to nominate such persons in the event they knew of them. Nomination blanks may be obtained from the Secretary after September 15.

8. *Reports of Committees and Representatives.*

A. Joint Committee on Nomenclature (O. L. Kline and P. L. White, with E. E. Snell, Chairman, and W. M. Sperry of American Society of Biological Chemists). The following is the complete report of this committee:

"The Committee has considered two items this year: (1) a proposal to substitute an appropriate trivial name for the compound now known as *d*-isoascorbic acid; and (2) a proposal to clarify scope of the term 'vitamin B_{12}.' Background for its deliberations and recommendations arrived at are considered below.

"1. *Isoascorbic acid.* The introduction of *d*-isoascorbic acid as an approved antioxidant for use by the food industry has introduced an objectionable situation. This compound, although it has no significant nutritive value or antiscorbutic activity, can readily be confused with the vitamin, ascorbic acid, which has both. Indeed, we understand that the Meat Inspection Service of the U. S. Department of Agriculture has allowed the name 'ascorbic acid' to be used for '*d*-isoascorbic acid' when the latter is added to cured meat for the purpose of inhibiting the fading of color. The situation is serious not only because it may misinform the public; there is also the real possibility of errors in chemical analysis for 'true' ascorbic acid. Because of this, a number of individuals in governmental, academic and industrial positions have suggested that use of the name, *d*-isoascorbic acid, be discontinued and a more appropriate name for use in technical and lay literature be selected to replace it.

"With this background, this committee several years ago initiated a study of possible alternative names. According to the revised rules for carbohydrate nomenclature published in *Chemical and Engineering News*, Vol. 31, page 1776, April 27, 1953, the correct chemical name for this compound is *D*-erythro-ascorbic acid. Several shortened names based upon this correct name have been considered (these included erythorbic, erythenolic, erythrorbic, erorbic, and eryorbic acids). The committee unanimously selected *erythorbic acid* as an appropriate trivial name for the substance.

"Dr. King attempted further to consult with the authors of the original paper in which the name *d*-isoascorbic acid was introduced. The first author, Dr. K. Maurer, apparently perished with his entire family at Jena during World War II. Dr. B. Sehied, his coauthor, could not be located and his name is not listed in any of the current directories of German

chemists. It is clear that no one is in a position to feel slighted in the least at not being consulted with reference to a proposed change in name.

"Accordingly, the Joint Committee on Nomenclature recommends that the Councils of the American Institute of Nutrition and of the American Society of Biological Chemists adopt the following resolutions:

(a) That the trivial name, *d*-isoascorbic acid, be abandoned as a trivial name for the antioxidant, *D*-erythroascorbic acid.

(b) That the trivial name *erythorbic acid* be adopted for use in the lay and technical literature as a synonym for *D*-erythroascorbic acid.

"2. *Vitamin B_{12}*. The first pure compound to be isolated with activity in controlling pernicious anemia in man and with 'animal protein factor' activity in rats was termed 'Vitamin B_{12}.' Subsequent research showed that a variety of slightly different substances with equivalent activity occurred naturally; 'cyanocobalamin' was recommended as a name for the form first isolated, and 'cobalamin' as a group name for the several naturally-occurring substances of similar therapeutic activity. The difficulty that arises is that most pharmacopaeias now employ vitamin B_{12} as synonymous with cyanocobalamin only, whereas general scientific usage has tended to make vitamin B_{12} a generic name that includes each of the closely related family of compounds (cyanocobalamin, hydroxocobalamin, etc.) with equivalent therapeutic activity. For example, Hawk, Oser and Summerson (Practical Physiological Chemistry, p. 1208) state: 'The term "vitamin B_{12}" refers chemically to cyanocobalamin but is commonly used to designate the cobalamin group with respect to their biological effect. For example, the vitamin B_{12} content of liver extract is commonly expressed in terms of a measurement of cyanocobalamin plus hydroxocobalamin.' This widespread usage is thus in conflict with legal requirements for labelling based upon U.S.P. specifications. To resolve this confusion the committee proposes:

(a) That 'vitamin B_{12}' be used as a generic term to refer to and include any or all compounds of this group that possess therapeutic potency or biologic activity in vertebrates.

(b) That 'cobalamin' be adopted as a chemical family name with the appropriate prefix designating a specific chemical substance (cyano-, hydroxo-, sulfato-, nitrito-, etc.).

These recommendations would be similar to that of past action of this committee in which the term vitamin B_6, originally synonymous with pyridoxine, was adopted as a group name to include pyridoxine, pyridoxamine and pyridoxal following discovery of these latter substances [see J. Nutrition *38*, 399 (1949) and *60*, 148 (1956)]."

At the first business meeting there was some question as to possible confusion which might arise from the word erythorbic acid. Also, questions were raised concerning vitamin B_{12} nomenclature. A motion to approve the report was defeated. At the second business meeting on April 17, at the direction

of the President, a motion to reconsider the report was approved. Dr. Kline then read the report in its entirety, which had not been done at the first meeting. Separate motions to accept the two parts of the report, with the provision that such acceptance might be subject to appeal to the Council through the Secretary by May 17, were approved with only one dissenting vote. (One letter was received by the Secretary within the time limit; this questioned the vitamin B_{12} part of the report. A copy of the letter was mailed to Council members who were polled for their decision as to necessary action. The Council voted unanimously to let the report stand as approved by the members.) As a matter of record, the report of the Joint Nomenclature Committee was also accepted by the American Society of Biological Chemists at its business meeting.

B. Committee on Registry of Pathology of Nutritional Diseases. No report.

C. Ad Hoc Public Information Committee (R. W. Engel, Chairman, A. E. Schaefer, and LeRoy Voris.) Because the report, presented by Dr. Engel, recommended the establishment of an important standing Public Information Committee of the Institute, it is given in detail as follows:

> "The American Institute of Nutrition is celebrating its Silver Anniversary. During the first 25 years of existence the society has not had a public information program. In the past few years a limited attempt has been made to satisfy requests from high school guidance teachers through the preparation of a single-page mimeographed statement describing career opportunities in the field of nutrition. Two to three hundred requests for such information have been received annually during the past several years.
>
> "The President's office and the Secretary's office of our society also receive inquiries frequently about a variety of matters relating to nutrition from such diverse areas as advertising agencies, high school students, college students, or other interested citizens, and from the feed and food industries.
>
> "The Institute should be able to respond to these inquiries and to furnish the public with factual statements that can be supported with sound nutrition research results, or to refer inquiries on such matters to

reliable information sources. Subjects of papers presented at our annual meetings deserve wider dissemination through the public press than has been the case in the past. Many newsworthy journal papers also might be publicized more widely by cooperating with reliable science writers. The Institute is obligated, through its constitutional objective, to further the extension of the knowledge of nutrition.

"In order for the society to assume a more responsible position in the dissemination of factual nutrition information the following recommendations are submitted for your consideration.

(a) That the American Institute of Nutrition establish a Public Information Committee composed of the Secretary of the Society and three other members;

(b) That the Public Information Committee should have representation from basic and applied areas of animal and human nutrition;

(c) That such a Committee should maintain a cooperative relationship with other agencies that are concerned with the dissemination of factual nutrition information, such as the Food and Nutrition Board, National Research Council, National Academy of Sciences, the American Medical Association, the American Dietetic Association, and the U. S. Public Health Service."

Dr. Engel also distributed copies of a new printing of a 4-page, two-color leaflet on "Career Opportunities in Nutrition" prepared in an interesting fashion by the Public Information Committee to answer requests from high school guidance programs and from individuals for such information. Additional copies may be obtained from the Secretary's office. This Committee also screened abstracts received for the program of the annual meeting for newsworthy findings to be forwarded to the Federation Public Information Committee. The report of the Committee was approved.

D. Representative to Federation Public Information Committee (R. W. Engel). Dr. Engel briefly reviewed the work of this committee in preparing publicity material for the scientific program and for the American Institute of Nutrition Symposium. As yet the Federated Societies have not found a common ground of understanding by which a full-time professional science writer can be made a part of the permanent staff.

E. Representative to the Food and Nutrition Board, the Agricultural Research Institute, and the Division of Biology and Agriculture, NAS-NRC (N. R. Ellis). The

report was approved as presented by Mr. Ellis and is on file in the Secretary's office. Mr. Ellis attended a number of meetings as the AIN liaison representative to the National Research Council. The report outlined the work and publications of the Food and Nutrition Board, the Agricultural Research Institute, the Food Protection Committee, and the Division of Biology and Agriculture. The report asked for the advice of nutritionists on the usefulness of the Handbook of Biological Data since revision is being considered. Dr. Olaf Mickelsen and Dr. Paul B. Pearson have been nominated as Society representatives to participate in a Symposium on Possible Uses of Earth Satellites in Relation to Life Sciences Experiments in Washington, D. C., on May 14 to 17, 1958.

F. Representatives to the AAAS Council (H. A. Schneider and P. L. Day). The representatives were active during the year and Dr. Schneider's brief verbal report of the year's activities was approved. It may be pointed out that members of AIN are automatically eligible as "Fellows" in AAAS. Applications may be made to the Secretary of AAAS.

G. Representative to the Nutrition Division of FAO (J. M. Hundley). Dr. Hundley gave a verbal report of the FAO program of research and development in the underdeveloped areas of the world. An FAO report on protein requirements will soon be available and other publications are in press. The report was approved.

H. Organizing Committee, Fifth International Congress on Nutrition, September 1–7, 1960, Washington, D. C. (Paul György, Chairman). Dr. György gave a verbal report of the many activities of the Organizing Committee. Several meetings of the entire committee and a number of subcommittee meetings have been held this past year. Plans are well underway for all activities. A budget of at least $250,000 is planned. It is expected that a number of travel grants will be avail-

able for younger foreign visitors. A total registration of 2500 to 3000 is expected. Interested nutritionists should write to Dr. M. O. Lee, 9650 Wisconsin Avenue, Washington 14, D. C. The report was approved. (A list of the entire committee may be found at the end of this report.)

I. U. S. National Committee, International Union of Nutritional Sciences (IUNS) (Paul György, Chairman). The final draft of the constitution, prepared by the Secretary's office, for the U. S. National Committee of IUNS was approved by the Council and by the NAS-NRC. The committee nominated by the Council of the American Institute of Nutrition has had one meeting (see list of committees for members and their terms of office). This committee will co-sponsor, with the American Institute of Nutrition, the 1960 International Congress on Nutrition and will effect appropriate United States participation in affairs of the IUNS through the NAS-NRC on behalf of nutritional scientists of the United States.

CONSTITUTION FOR THE U. S. NATIONAL COMMITTEE OF THE INTERNATIONAL UNION OF NUTRITIONAL SCIENCES (IUNS)*

1. *Purpose:* To effect appropriate United States participation in the International Union of Nutritional Sciences (IUNS) through the National Academy of Sciences-National Research Council (NAS-NRC) which adheres to the IUNS on behalf of the nutrition scientists of the United States.

2. *Function:*
   a. To advise the President of the National Academy of Sciences (NAS) on matters relating to United States participation in the IUNS.
   b. To nominate persons to serve as delegates at international meetings sponsored by the IUNS.
   c. To brief delegates to general assemblies and other meetings called by the IUNS.

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* Approved by the American Institute of Nutrition, September 1, 1957.
Approved by the Governing Board, NAS-NRC, October 13, 1957.

d. To arrange for scientific meetings in the United States in consonance with the objectives of the IUNS.
e. To direct particular attention to nutrition research that requires international cooperation.
f. To perform such duties as are required of national committees of adhering countries under the statutes of the IUNS.

3. *Membership:*
   a. The United States National Committee shall be composed of:
      (1) Appointed Members:
      Nine members nominated by the Council of the American Institute of Nutrition. Nominations shall be made in such a manner as (a) to insure representation from the broad field of human and animal nutrition, and (b) to provide representation from governmental and non-governmental organizations directly concerned with the field of nutrition.
      (2) Ex-officio members (voting):
         (a) President, American Institute of Nutrition.
         (b) Chairman of the Food and Nutrition Board, NAS-NRC.
      (3) Ex-officio members (non-voting):
         (a) Chairman of the Division of Biology and Agriculture, NAS-NRC.
         (b) Chairman of the Division of Medical Sciences, NAS-NRC.
         (c) Director of the Office of International Relations, NAS-NRC.
         (d) Officers of the IUNS resident in the United States.
   b. Nomination for membership shall be made annually and forwarded to the Chairman of the Division of Biology and Agriculture, NAS-NRC, by May 1 of each year. Appointment of the members shall be made by the President of the NAS upon recommendation of the Chairman of the Division of Biology and Agriculture, NAS-NRC, and shall extend from July 1 of the year of appointment through June 30 of the designated year of termination. Not less than five appointees shall be members of of the American Institute of Nutrition.
   c. Appointed committee members shall serve a three-year term, except that in the first year of operation under this Constitution nine members shall be appointed: three to serve for one year, three to serve for two years, and three to serve for three years. Therafter, three new members shall be appointed annually to serve for three consecutive years. Committee members may be reappointed, but continuous membership shall be limited to two consecutive three-year terms.
   d. Whenever an appointed member is unable to complete a three-year term, the Council of the American Institute of Nutrition shall nominate a new member to serve for the unexpired portion of the term of appointment.

4. *Subcommittees:* Whenever the need arises, the Committee may form *ad hoc* subcommittees to deal with matters relating to United States participation in the activities of the IUNS.

5. *Officers:* The officers of the Committee shall be a Chairman, a Vice-Chairman, and a Secretary-Treasurer, all of whom shall be members of the Committee

and citizens of the United States. The Chairman shall be nominated by the Committee for appointment by the President of the NAS. The Vice-Chairman and Secretary-Treasurer shall be elected by the Committee. The term of office shall be three years with permissive reelection.

6. *Meetings:* Meetings of the Committee shall be called by the Chairman at times and places designated by him. At least one such meeting shall be held each year. A majority of the voting membership of the Committee shall constitute a quorum.

7. *Finances:*
   a. The NAS-NRC shall, with the advice, cooperation, and active participation of the National Committee, undertake to solicit and obtain funds necessary for the payment of its annual contribution in consequence of its membership in the IUNS and for the activities and maintenance of the National Committee.
   b. To the extent possible the NAS-NRC shall cover expenses of the Committee, including travel and subsistence expenses of Committee members attending regularly scheduled meetings of the Committee.
   c. When expenses of the Committee exceed the amount of financial support available through the NAS-NRC, or when special projects require separate financing, the Committee may request authorization from the governing board of the NAS-NRC to solicit funds from outside sources.

8. *Amendments:* Proposals for amendments to this constitution may be initiated by an affirmative vote of two-thirds of the voting membership of the Committee. After such proposed amendments have been reviewed by the Council of the American Institute of Nutrition, they shall be submitted to the governing board of the NAS-NRC and shall become effective only upon approval by this board.

J. United States delegates to the Fourth International Congress on Nutrition, 1957, Paris, France, July 26–August 1 (C. G. King, Chairman, Paul György, L. A. Maynard, and W. H. Sebrell, Jr.). The complete report of Dr. King is on file in the Secretary's office. Dr. King reviewed the scientific program as well as the entertainment provided for members and guests. Attendance was slightly over 1000. Dr. D. P. Cuthbertson, Director of the Rowett Institute in Scotland, was elected chairman of IUNS succeeding Dr. E. J. Bigwood of Belgium. Prof. Leslie J. Harris of Cambridge University was re-elected Secretary General of the Union. The invitation of the U. S. delegates, on behalf of the American Institute of Nutrition and NAS-NRC, to hold

the Fifth International Congress in the United States in 1960 was accepted unanimously. The report was approved.

K. Committee on Membership Qualifications (A. E. Harper, Chairman, R. M. Leverton, C. M. Lyman, A. E. Schaefer, and F. J. Stare). Dr. Harper presented a progress report on the activities of this committee. The entire report, which was unanimously approved, is on file in the Secretary's office. In summary, the report stated that from discussions with other AIN members there appeared to be 4 general areas of agreement:

1. That membership in the American Institute of Nutrition should be restricted to those whose primary interest is in the science of nutrition; that is, primarily research workers.
2. That the requirements for membership are not clearly understood by the majority of members.
3. That the time restriction of five years as presently included in Article 1, section 1, of the By-Laws is not clearly understood by the majority of members.
4. That there should be included in the membership a class of honorary members.

The committee recommended that the present 5-year limitation in the By-Laws be removed to bring membership requirements more in line with those of other Federated Societies. It was also suggested that the Secretary, with the help of this committee, prepare an outline of requirements for eligibility as a guide to those who wish to nominate new members in an attempt to reduce the number of rejected candidates.

The committee also suggested that a change in the By-Laws be made to provide for Honorary Members in order to give recognition to distinguished and outstanding persons in the field of nutrition throughout the world who are not members of the Society (or may not be eligible for one reason or another).

The committee was instructed by the President to prepare the necessary proposed amendments for consideration by the members at the next annual meeting.

The committee encouraged the members of AIN to review the present list of membership (see Federation Proceedings for September 1958) and "to make every effort to nominate eligible candidates who have been bypassed for one reason or other" in any of the various fields of nutrition science (such as experimental animal nutrition, human nutrition, clinical nutrition, applied nutrition, agricultural chemistry, public health nutrition, food chemistry, nutritional biochemistry, etc.) and who, during their careers, have had experience in some basic phase of nutrition research.

L. AIN Representatives to Federation Board (R. R. Williams, R. W. Engel, and G. M. Briggs). Dr. Engel reported that the Federation Board met in Bethesda on January 22 and in Philadelphia on April 13 and April 16. A complete report is on file in the Secretary's office and will be published in Federation Proceedings.

Among the major actions of the Board, which is the managing body of the Federated Societies, were the following items:

1. The Bylaws shall be amended to provide that the Federation Board shall consist of three representatives designated by each of the constituent Societies for a period of three years, one to be replaced each year.

2. An appropriate Bylaw change shall be made by which the Federation shall establish an advisory panel composed of one representative from, and nominated by, each of the constituent Societies (except the Society of which the Chairman of the Board for that year is representative), from their representatives on the Board, for the purpose of consulting and advising the Chairman of the Board regarding urgent Federation problems requiring consideration between the regular meetings of the Board.

3. The Headquarters Committee shall be dissolved as of July 1, 1958, and its duties shall be assumed by the newly-established advisory panel.

4. The Finance Committee shall be abolished as of July 1, 1958, and its functions shall be taken over by the newly-established advisory panel.

5. The Past-Chairman of the Federation Board shall be invited to attend the Board meetings for the year following the term in which he served, and he shall also serve in an ex-officio capacity to the advisory panel.

It was recommended by the Council that the three AIN representatives to future Federation Boards consist of the President-Elect, the President, and the Past-President (or suitable alternates, if necessary, appointed by the Council) effective July 1, 1958. This will provide for maximum continuity of experience for Board members, each of whom will have a three-year term.

It was also recommended by the Council that the AIN representative to future Federation Advisory Panels be the immediate Past-President (or alternate designated by the Council) effective July 1, 1958.

Other actions of the Federation Board of interest to AIN members included:

1. Because of savings necessary in the printing of Federation Proceedings, this coming year abstracts will be printed in offset directly from the author's copy, with the stipulation that if re-typing is necessary a charge of $5.00 will be made to the author. Other savings will also be made, such as omitting the subject index from the March program issue and printing the directory issue in offset.
2. A general statement in support of the fluoridation of water supplies as a public health measure in the reduction of dental caries was approved.
3. Members who belong to two or more Federated Societies (and are thus billed twice or more for Federation dues) may request a subscription to Federation Proceedings for each membership. It is hoped that the duplicate set will be sent overseas or given to graduate students, rather than to libraries which would otherwise subscribe.

The entire report was approved.

9. Election of Fellows of the Society. By recommendation of the Council, and in conformity with the new constitutional amendment, at the business meeting on April 17 the following members, who have had distinguished careers in nutrition, were unanimously elected Fellows of the American Institute of Nutrition:

Thorne M. Carpenter
George R. Cowgill
Eugene F. DuBois
Ernest B. Forbes
Casimir Funk
Elmer V. McCollum
Harold H. Mitchell
John R. Murlin
Harry Steenbock
Robert R. Williams

(For the nomination of Dr. Williams, Dr. Darby temporarily assumed the chair.)

This is the first list of Fellows so chosen. In succeeding years up to three will be selected each year. Suggestions for names of candidates for this honor are welcomed by Dr. T. H. Jukes, chairman of the 1958–59 Fellows Committee. Candidates must be members of AIN and age 65 or over.

10. Appointment of Committee on Journal of Nutrition. Upon recommendation of the Council, a committee is to be appointed to evaluate the Journal of Nutrition and to look into its relationship with the American Institute of Nutrition and the Wistar Institute. The committee would also study possible improvements in the Journal, such as finding means of speeding up publication and publishing "News and Notes." Suggestions from members were urged and should be sent to Dr. D. M. Hegsted, Chairman, in the very near future. A motion made by Dr. McHenry requested that there be the utmost cooperation between the above committee and the Editorial Board. The motion was approved.

11. Report of Council Actions. Several other actions of the Council of interest to the membership were reported by the Secretary.

A. Awards. Several changes in the "Rules Governing AIN Awards" were approved in order to encourage nominations. These included a statement that the Nominating Committees shall make a preliminary screening of names received from sponsors or from members of the committee and may request bibliographic material of the highest ranking nominees (from associates or from sponsors). Approximately 5 names may then be sent to the appropriate Jury of Award with the necessary supporting material for final selection. Only bibliography of the most appropriate and significant papers on which the nomination is based is necessary. (In effect, this rule simplifies nomination and should increase the number of nominations to be considered.) A preliminary

nomination may now be made to the Nominating Committee without the usual seconding statements, pertinent bibliography, etc. However, this information may later be requested by the Nominating Committee.

The Council also approved that names of the highest ranking candidates would be held over each year for the new Nominating Committee.

The following new rule was also approved: "An individual who has received one Institute award shall be deemed ineligible to receive another Institute award unless it be for outstanding research subsequent to or not covered by the first award.[1]"

Members, or other interested persons, were encouraged to nominate qualified scientists for the AIN awards (see statement of rules and procedure for making nominations in the August, September and October issues of the Journal of Nutrition). The deadline has been moved up to December 1, 1958.

B. The Council expressed its interest in the possibility of permitting local groups of AIN members to form local sections composed of regular members and possibly local or associate members. This was left open for future proposals from local groups or for further discussion. No objections to this possibility were expressed.

C. The Council also expressed its interest in the possibility of a fall meeting of the Institute with a program of scientific papers submitted by members. This too was left open for further discussion and suggestions.

D. The Council asked for suggestions from members of a topic for the Nutrition Symposium at the next annual meeting.

E. The Secretary was instructed to send a congratulatory letter to Dr. C. A. Elvehjem on behalf of the American Institute of Nutrition on becoming President of the University of Wisconsin on July 1, 1958.

[1] Including recipients of the former Mead-Johnson award.

12. Society History. In connection with the 25th Anniversary of the Society a history is being written. Anyone with documents or other information on the early days of the Society or its founding is asked to notify the Secretary.

13. Acknowledgments.

A. A motion was unanimously approved that "members of the American Institute of Nutrition record their cordial and very hearty thanks for the courteous and very effective leadership of Dr. R. R. Williams over the past two years."

B. The Secretary wishes to acknowledge the excellent cooperation of Dr. R. W. Engel, former Secretary, and of Dr. R. R. Williams, without whose help and encouragement his job could not have been carried out. He also wishes to thank the Wistar Institute for kindly supplying the Society's printing needs this past year (stationery, ballots, award scroll, banquet programs, etc.).

14. The next annual meeting will be held in Atlantic City, New Jersey, on April 13–17, 1959.

ANNIVERSARY DINNER AND PRESENTATION OF AWARDS

A highlight of the entire meeting for many was the special 25th Anniversary Dinner Meeting on April 16 in Hotel Sheraton, Philadelphia. With Dr. R. R. Williams as toastmaster, a record total of 334 members and guests played host to the Award winners and to the original Founders of the American Institute of Nutrition in 1933 and first Editors of the Journal of Nutrition. A standing ovation was given to Dr. John R. Murlin (84 years of age), seated at the head table, the first Editor of the Journal and who, more than any other person, started the present American Institute of Nutrition. Standing ovations were also given to Dr. Ernest B. Forbes, and Dr. Elmer V. McCollum (original Founders) and to Dr. Icie Macy Hoobler and Dr. Paul E. Howe, members of the committee which prepared the first Constitution, all of whom were also

(Photo courtesy of Wyeth Intl. Ltd.)

Founders and Past Presidents of The American Institute of Nutrition attending the 25th Anniversary Meeting of the Society, Philadelphia, Pa., April 16, 1958.

Front row (left to right): Founders Icie Macy Hoobler, Ernest B. Forbes, John R. Murlin, E. V. McCollum, Paul E. Howe. Back row (left to right): Past-Presidents Paul L. Day, Arthur H. Smith, Albert G. Hogan, L. A. Maynard, Robert R. Williams, George R. Cowgill, Charles G. King, E. M. Nelson, W. H. Sebrell, Jr., C. A. Elvehjem. (W. H. Griffith, a Past-President, also attended the meeting but was unable to be present at the time the photograph was taken.)

seated at the head table. Messages were read from other Founders who could not be present.

Also in honor of the occasion the President asked all charter members who were present to stand and be acknowledged. Approximately 35 were present of the 98 remaining charter members who are still AIN members. Fourteen past-presidents were also present and were asked to stand and be acknowledged. Past secretaries, past treasurers, and past editors of the Journal of Nutrition were also asked to stand (and were nearly 100% in attendance starting with Dr. Murlin who was the first secretary-treasurer and editor). Mr. Ream of the Wistar Institute, which provided the souvenir dinner programs, was also introduced. (A few extra copies of the program are available from the Secretary until the supply runs out.) Special souvenir favors for each person present were kindly supplied by the Williams-Waterman Fund.

A photograph was taken of the Founders and Past-Presidents present at the dinner and is reproduced with these Proceedings.

The Borden Award of $1000 and a gold medal was presented to Dr. Lemuel D. Wright of Cornell University for his various outstanding studies in microbiological chemistry, including his studies on biocytin, on microbiological assay procedures, and for the discovery and synthesis of mevalonic acid, a component of cheese whey and an improtant intermediate in the biosynthesis of cholesterol. The presentation was made by Dr. Richard Barnes who reviewed the work on which the award was based.

The Osborne-Mendel Award of $1000 and a scroll was presented to Dr. Paul György for his discoveries concerning the prevention of experimental hepatic lesions in rats by antibiotics and antioxidants, for his studies with the *Lactobacillus bifidus* factor, and for his numerous other distinguished contributions to the science of nutrition. The presentation was made by Dr. Elmer V. McCollum who gave an interesting review of the studies on which the award was based.

Both recipients responded with a few interesting remarks of acknowledgment and thanks.

After the banquet the Biochemistry-Nutrition Smoker was held at the Hotel Sheraton with 788 present.

OFFICERS AND COMMITTEES—AMERICAN INSTITUTE OF NUTRITION

July 1, 1958–June 30, 1959

President: W. J. Darby, Vanderbilt University School of Medicine, Nashville, Tenn.

President-Elect: D. W. Woolley, Rockefeller Institute for Medical Research, New York, N. Y.

Past-President: R. R. Williams, 297 Summit Avenue, Summit, N. J.

Secretary: G. M. Briggs, National Institutes of Health, Bethesda, Maryland (1960)

Treasurer: J. B. Brown, Ohio State University, Columbus, Ohio (1959)

Councillors: E. W. McHenry (1959)
Paul György (1960)
J. H. Roe (1961)

Committees:

Nominating Committee: H. H. Williams (Chairman), Gladys Emerson, R. W. Engel, J. M. Orten, and O. L. Kline

Committee on Membership Qualifications (ad hoc): A. E. Harper (Chairman), R. M. Leverton, C. M. Lyman, A. E. Schaefer, and F. J. Stare

Committee on Nomenclature (joint with American Society of Biological Chemists): O. L. Kline (Chairman) (1959), P. L. White (1960)

Committee on Journal of Nutrition (ad hoc): D. M. Hegsted (Chairman), R. W. Engel, O. L. Kline, M. O. Lee, C. V. Moore, B. L. Oser, E. E. Snell, and Charlotte M. Young

Nominating Committee—Borden Award: H. H. Williams (Chairman) (1959), C. A. Baumann (1960), G. V. Mann (1961)

Nominating Committee—Osborne-Mendel Award: Icie Macy Hoobler (Chairman) (1959), D. M. Hegsted (1960), J. S. Dinning (1961)

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Also, ex-officio (voting) members are W. J. Darby (1959), Grace Goldsmith, C. G. King (1961); and ex-officio (non-voting) members are H. B. Steinbach, R. K. Cannan, W. W. Atwood, Jr.

Representatives to other organizations:

Federation Board: R. R. Williams (1959), W. J. Darby (1960), D. W. Woolley (1961)

Federation Advisory Committee: R. R. Williams (1959)

National Research Council Boards and Divisions: N. R. Ellis (1960)

American Association for the Advancement of Science Council:

P. L. Day (Section N—Medical) (1959)

H. A. Schneider (Section C—Chemistry) (1960)

Food and Agriculture Organization: J. M. Hundley (1960)

Federation Public Information Committee: R. W. Engel (1960)

Editorial Board of Federation Proceedings and Federation Secretaries Committee: G. M. Briggs (1960)

Officers and Committees of the Fifth International Congress on Nutrition, 1960

(Organized by American Institute of Nutrition and U.S. National Committee of IUNS)

E. V. McCollum, *Honorary President*

C. G. King, *President*

Organizing Committee:

Paul György, Chairman

M. O. Lee, General Secretary

G. M. Briggs, Recording Secretary

W. H. Sebrell, Jr., Chairman of Program Committee

E. L. Severinghaus, Chairman of Finance and Budget Committee

F. S. Daft, Chairman of Hospitality Committee (and Co-Chairman of Finance and Budget Committee)

W. J. Darby, Chairman of Lectureships and Travel Assistance Committee

Hazel Stiebeling, Chairman of Publications Committee

LeRoy Voris, Chairman of Public Information Committee

Other Members: C. H. Best, C. A. Elvehjem, Gladys Emerson, R. W. Engel, Grace Goldsmith, F. Gomez-S, J. M. Hundley, L. A. Maynard, E. M. Nelson, L. B. Pett, H. E. Robinson, F. J. Stare, R. R. Williams

Editorial Board—Journal of Nutrition: G. R. Cowgill, Editor (1959), J. S. Dinning (1959), J. M. Orten (1959), C. R. Treadwell (1959), C. P. Berg (1960), C. G. Mackenzie (1960), H. R. Bird (1960), R. W. Engel (1961), P. L. Harris (1961), H. A. Schneider (1961), O. L. Kline (1962), E. S. Nasset (1962), H. Pollack (1962)

Respectfully submitted,

GEORGE M. BRIGGS, Secretary,
American Institute of Nutrition

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