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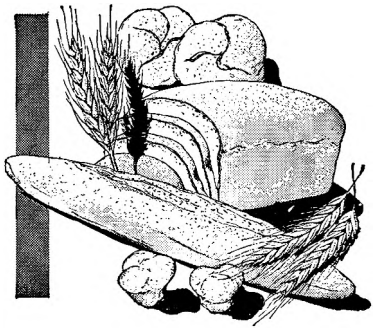

A Brief History of Milling and Baking by **SCIENCE WRITER**

CHAPTER XVI. *Improvements in Cleaning and Bolting*

Better Machines and Better Methods

From the time of Oliver Evans and his mechanized, water-powered mill in 1785 to the mid-1800s, when the Middle West was becoming America's grain empire, many improvements and refinements took place in milling methods.

Starting with the first process, preliminary cleaning of the wheat, it was found that a number of machines were needed —batteries of sieves to separate the wheat from dirt and foreign matter, and bolters to sift the flour after grinding.

Each of these processes was improved as time passed. Air was introduced, as blast or suction, in the cleaning process. Centrifugal force was used.

Let's examine each process in more detail.

They Made a Machine That Would Clean

The miller is faced with a difficult problem at the very beginning of his operation. He must clean the wheat that arrives at his mill. It must be rid of dirt, the beard removed. Sound grain kernels must be separated from diseased.

How was it done? Evans used a rolling screen of woven wire or perforated metal, but this took up much room in the mill and, more important, only a small fraction of the screen was in use at any time. So a more efficient system of flat sieves, set horizontally or slightly inclined, and oscillated by mill power, was substituted.

The sieves were so constructed that the wheatberry passed over them, but loose dirt, cockle weed, and other small particles passed through. Air was blown through the upper screen and across the top of the lower to remove dust, which was carried off through ducts to a room where it was allowed to settle.

A Success by Switching From Round to Flat

Having cleaned the grain, the miller channeled it to the grindstones and then to bolters. Bolting is a method of sifting through a fine screen or cloth. The bolters were cylinders or polygonal reels. Their purpose was to sift the meal, this dividing the flour into grades and removing as much of the bran as possible.

Again it was found that the cylinders or reels were much less efficient than flat sieves, which eventually replaced them except at certain special places in the mill.

Some Beneficial Results of Enrichment

With a long history of constant product and processing improvement it was only natural that millers bettered the nutritive value through enrichment of their family white flour with vitamin B_1, vitamin B_2, niacin, and iron when processing losses of these elements were fully understood. Bakers, too, followed suit by enriching their white bread and rolls with the vitamins and iron.

A half a generation has passed since enrichment became the general practice of millers and bakers in the United States. Think of the incalculable benefit to the health of millions of Americans that has resulted. Physicians, nutritionists, dietitions praise enrichment and continue to support it enthusiastically. In their professional work, they are seeing fewer and fewer cases of disease associated with deficiencies of these vitamins. Much credit must be given to white flour and bread enrichment.


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The next chapter titled: "An Analysis of the Wheatberry" will be published soon.

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LEROY SHELDON PALMER

(1887 – 1944)

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LEROY SHELDON PALMER

(March 23, 1887 – March 8, 1944)

Few scientists have such a breadth and variety of research interests as did Leroy Sheldon Palmer. Not only did he work effectively in several areas in the field of animal nutrition, but also he made outstanding contributions to dairy chemistry. Furthermore, his fundamental studies of the plant and animal pigments brought him recognition early in his active research career. Working in two agricultural experiment stations, he sought to unravel the fundamental biochemistry of the agricultural problems that he studied.

L. S. Palmer was born in Rushville, Illinois on March 23, 1887, one of 5 sons of a Presbyterian minister, Samuel C. Palmer and his wife, Annie Goodman Palmer. He was the twin brother of Robert C. Palmer who recently retired as vice president and research director after 41 years of service with Newport Industries, Pensacola, Florida. The two brothers were of strikingly similar physical appearance even in adulthood. For this reason the infrequent visits of Robert C. Palmer to the Department of Agricultural Biochemistry at Minnesota would sometimes cause confusion among the graduate students. One particular incident which comes to mind concerns a very detailed discussion between Robert C. Palmer and a certain graduate student on refinements of procedures for the isolation of β-lactoglobulin from milk. It was not until several days had passed that the student discovered that he had not been talking to L. S. Palmer.

The Palmer family moved to St. Louis, Missouri when the twins were very young, and they received their elementary and secondary education in the public schools of that city following which they enrolled in the University of Missouri in 1905. It

is interesting that Palmer's undergraduate training was not in a field particularly related to his future research work, but in chemical engineering in which he obtained the B.S. degree in 1909. His first published research work consisted of two papers on "Rapid Electrochemical Analysis: A Comparison of Several Methods" and "A New Electrolytic Method for the Preparation of Explosive Antimony" which were collaborative efforts of the twin brothers and were presented at the meetings of the American Electrochemical Society in May, 1909 and October, 1909 respectively.

L. S. Palmer was appointed Fellow in Chemistry at the University of Missouri for the year 1909–1910. There was at that time on the campus a Dairy Research Laboratory operated jointly by the University and the United States Department of Agriculture. This had been established a few years earlier through the efforts of Dr. C. H. Eckles, head of the Department of Dairy Husbandry. In October, 1909, Dr. R. H. Shaw, in charge of this laboratory, induced Palmer to resign his University fellowship and to become one of the chemists of the Laboratory. Shaw soon resigned and in 1911 Palmer was placed in charge of the laboratory. The research partnership thus formed between Palmer and Eckles was most productive; it persisted until the death of the latter in 1933. When a change in policy in Washington in 1913 caused withdrawal of Government support of the Dairy Research Laboratory, Eckles persuaded the University of Missouri to continue it and to appoint Palmer as assistant professor of dairy chemistry. Meanwhile Palmer was pursuing graduate work at the University, from which he received the degree of M.A. in 1911 and the Ph.D. in 1913.

In 1911 he married Gay Wilcox, a student of music at Christian College in Columbia and daughter of a newspaper editor of nearby Ashland, Missouri. The home thus established was an exceptionally happy one; Palmer was a most devoted husband and father and his wife's congenial and domestic ways helped to provide a refined and wholesome home life. They had three children — a daughter and two sons.

Doctor Eckles left Missouri in 1919 to accept the post of Chief of the Division of Dairy Husbandry at the University of Minnesota. There Ross Aiken Gortner had recently (1917) been appointed Chief of the Division of Agricultural Biochemistry which he was busily engaged in developing and expanding. Eckles and Gortner soon arranged the creation of an Associate Professorship in Agricultural Biochemistry for Palmer which he assumed in the summer of 1919. Initially he was in charge only of the work in Dairy Chemistry, but in 1922 he was promoted to Professor and placed in charge of the Animal Nutrition work as well. Upon Doctor Gortner's death in 1942 he became Chief of the Division and continued in that position until his own death early in 1944.

Palmer's doctoral thesis was a monumental study of "Carotin — the Principal Natural Yellow Pigment of Milk Fat." It developed out of a concern to discover the reasons for variation in the color of butter. Palmer attacked the problem in a very fundamental manner. He characterized the yellow pigments by the best methods at his disposal, including the chromatographic technique devised by Tswett a few years previously. Furthermore, he demonstrated that the carotinoid pigments in milk and in animal tissues originate in the plants consumed by the animal. This work was published in the form of 5 papers with Eckles in the Journal of Biological Chemistry in 1914. Thus Palmer initiated a comprehensive and important study of the carotinoids and other pigments which was culminated with the publication of his very well-known American Chemical Society Monograph in 1922 entitled "Carotinoids and Related Pigments." This book remained the standard text in the field for many years and is still referred to on many occasions. Palmer's prodigious capacity for work was amply demonstrated during these years, and fate was certainly fickle when he was denied the fame that would have been his had he discovered the important physiological relationship between the carotenes and vitamin A. However, in reading over his publications on this subject it is understandable why he steadfastly refused to accept the possibility of such a relationship.

As early as 1900 many biologists accepted the theory that all visible pigments of animals are essential products of animal metabolism. Their function was described in such terms as "protective coloration," "warning coloration," "sexual attraction," etc. Palmer's opinion of these theories is amply demonstrated by the following quotation from one of his publications: "Suffice it to say that the writer (Palmer) does not possess a biological viewpoint which is sufficiently developed along academic lines to appreciate 'function' as an abstract attribute of living organisms. Function, to be real according to his (again Palmer) conception, must be concrete or physiological. Thus, if such pigments possess a function they must be linked with some nutritional or metabolic process in the animal." Palmer dismissed the possibility of any relationship between carotinoids and vitamin A both on theoretical grounds and also as a result of many experiments. On a theoretical basis he could not understand that if the carotinoids possessed a physiological function why it was that certain species of animals were completely devoid of carotinoids in their tissues and secretions even when fed diets rich in carotinoid pigment. On an experimental basis he reported the results of an extensive investigation with poultry in which a carotinoid-free diet was fed. These animals grew normally and their subsequent egg production rate was also normal. This conclusively demonstrated for the first time that a species of animal which is normally pigmented with carotinoids did not require these pigments for growth or reproduction. In this he was entirely correct. However, his chick diet carried an ample supply of vitamin A in the form of fresh pork livers. Thus, one would, of course, not expect any nutritional value in supplemental carotinoid pigment. He believed that the animal body possessed the power to separate carotinoids from vitamin A. Palmer was never able to explain satisfactorily the results of a 1914 study in which he observed that the feeding of yellow corn to lactating cattle did not appreciably affect the color of the butterfat, but did cause rather large increases in the vitamin A content of the same fat.

Soon after going to Minnesota Palmer formed a close research association with Dr. Cornelia Kennedy that had a marked broadening effect on his research outlook and efforts. Miss Kennedy, at that time, had just obtained her Ph.D. degree in McCollum's laboratory at Johns Hopkins and she certainly had the background in the rapidly expanding field of nutrition to give Palmer valuable assistance. Their research association lasted for 24 years.

During this time the controversy over the relationships between vitamin A and certain of the carotinoid pigments continued. In reading over some of these publications it is very interesting to note that the research worker of that day was considerably less restrained in his criticism of divergent results reported by another worker than is the case today.

Palmer's last published work on the relationship of carotinoids and vitamin A was in 1921. In it he reported on a very extensive study using the albino rat. The diet used was carotinoid-free and he obtained normal growth and reproduction, but, again as in his work with calves and chicks, his diet contained a source of vitamin A in the form of carotinoid-free egg yolk. Thus, in his own mind, at least, there could be no possibility of a relationship between carotinoids and vitamin A. It was not until 1930 that the preplexing question as to why the yellow-red plant pigment carotene exhibits vitamin A activity although the familiar vitamin A of liver oils is essentially a colorless substance was answered when Paul Karrer definitely established the chemical basis for this relationship. It was indeed unfortunate that Palmer had not experimented with diets devoid not only of carotinoid pigment but also of vitamin A.

Palmer's nutrition research from the early 1920's until his death covered a very broad field. His work was not confined to the rat laboratory, but encompassed the experimental livestock barns as well. His experimental animals included the chick, pig, lamb, dairy cow and horse. His various accomplishments with these species certainly demonstrates the desirability of the application of biochemical knowledge and tech-

niques to agricultural research. It is interesting that in many of the nutrition studies with livestock he would carry out concurrent experiments with laboratory animals. For example, in his studies on the ascorbic acid needs of the calf, the diet fed to these animals was also fed to guinea pigs. The results showed that while the guinea pigs developed scurvy in 30 days the calves performed normally for a period of one year. Furthermore, he was able to demonstrate synthesis of vitamin C in calves by feeding the livers from some of the animals to guinea pigs on a scurvy-producing diet.

A similar procedure was used in his studies with Doctor Eckles of the B vitamin needs of the calf, published in 1926. Rations made up of natural feed ingredients were used. It was found that while the calves grew normally to maturity and also produced normal offspring, the rats died within from two to 5 weeks on the same diet, a condition which could be corrected by brewers' dried yeast. In discussing their results they concluded that the only rational explanation for their observations would be to assume B vitamin synthesis in the digestive tract.

Although Palmer was always much more interested in fundamental nutrition than in the applied, he could combine the two with practical results. This is well exemplified in his work with mineral (particularly phosphorus) nutrition in cattle.

In certain areas of western Minnesota farmers were experiencing great difficulty in raising cattle. The condition in the cattle was characterized by a severe loss of appetite and in some cases even a depraved appetite. In the latter case some of the animals were observed eating virtually anything to which they had access. Death losses were high in many herds. Preliminary observations indicated that the diet of these animals, which was composed principally of prairie grass or hay, was very low in phosphorus. Blood plasma phosphorus levels in these animals were found to be as low as 1 mg % as compared to a normal level of 4 mg %. The entire situation was confusing because in the same regions where the abnormal cattle were found it was observed that the waters were very

high in sulfates, particularly magnesium sulfate. There followed a very extensive series of studies of phosphorus nutrition of cattle and the possible effects of the injection of high levels of magnesium. Palmer soon found that the condition primarily was one of phosphorus deficiency and that magnesium did not play an important role. However, even though the answer to the problem was known, the fact that many of the farmers were not able to take advantage of it is clearly shown in Palmer's publication in *Science* for October 10, 1930. He wrote: "The economic condition of many of the farmers in the affected regions of Minnesota is pitiable. The seriousness of the situation becomes apparent when it is realized that these people have no surplus cash income with which to start alleviating their plight through the purchase of fertilizers or proper feed supplements rich in phosphorus." Fortunately, today one rarely encounters phosphorus deficiency in cattle due to the ever increasing use of phosphate fertilizers and perhaps of more importance, the use of supplemental phosphate in the ration.

It is a truly amazing commentary on Palmer's ability to carry on a prodigious amount of work in different fields, that at the same time that he was working with phosphorus in cattle, with dairy chemistry and carotinoids, he initiated a series of studies on "The Fundamental Food Requirements for the Growth of the Rat." The first of these papers appeared in the Journal of Biological Chemistry in 1927. While it is not known for certain what prompted these studies, it seems possible that he was in disagreement with the commonly-held view of that time. This view was that the essential food requirements for the rat for growth and prolonged well-being can be expressed in terms of energy, biologically active protein, mineral salts and the then known vitamins. To Palmer's critical mind one could not make this statement with any degree of assurance until each individual ingredient of the diet was supplied in its chemically pure state. While this was obviously impossible at the time, he made a particular effort to obtain as pure a source of protein as it was possible to make.

He chose casein as the protein, no doubt because he was familiar with it, and his product was as pure as any of the so-called vitamin-free caseins available today. Using this casein in his diet he was unable to obtain normal rat growth unless, of course, such natural supplements as dried yeast were used. It is rather puzzling that Palmer never recorded any attempts to purify further some of the essential factors in materials such as yeast other than by autoclaving to destroy thiamine and by the usual alcohol-ether separations. Be that as it may, Palmer soon recognized that the wide variations in growth rates he was obtaining between rats fed the same diet was not conducive to orderly interpretation. He then turned to Mitchell's paired-feeding method and found marked differences in efficiency of food utilization between the different pairs consuming the same kind and quantity of food during the same period of time. These results disturbed him very much and what followed gives one an insight into Palmer's way of thinking. He reasoned that if he had a strain of rats which was uniform in efficiency of food utilization, comparisons of the effects of different diets on growth would be of much greater significance. Furthermore, he believed that with a strain of animals of this type the paired-feeding method would attain its full usefulness. It is obvious from the foregoing that Palmer had little respect for statistical treatment of data even though he required all of his graduate students to at least expose themselves to courses in statistics. By proper selection and inbreeding he and Doctor Kennedy produced two strains of rats differing widely in their efficiency of food utilization. These were called the "high" and the "low" efficiency strains and were well established by 1933. He and his group then embarked on a research program which was still active at the time of his death. These studies were concerned with the physiological and biochemical differences which could be associated with the marked differences in efficiency of food utilization between the two strains of rats. While Palmer's original purpose was to develop a strain of rats with a uniform efficiency of food utilization to be used to study the then unknown vitamins,

he appeared to become more and more engrossed in studying the differences between the two strains. Any study of the factors involved in efficiency of food utilization is enormously complex even though it is very basic in animal production. Perhaps for this reason it has been largely ignored by the animal breeder.

While it is not the purpose of this paper to go into any detailed study of the results of Palmer's many years of study with the two strains of rats, it can be stated that a number of physiological differences were found. However, many more years of effort would have been necessary to define these differences adequately.

It should not be inferred from the preceding that all of Palmer's later activities were confined to studies with the "high" and "low" efficiency strains of rats. His interests still remained broad. At the time of his death, he was actively engaged in studies of the mineral nutrition of cattle and swine, the nutritive value of honey, and the vitamin E requirements of rats and cattle.

Doctor Palmer's work in Dairy Chemistry was originally concerned with studies of the milk pigments and of factors affecting the composition of milk and milk fat. His work on the carotinoid pigments has already been mentioned. He also dealt with lactochrome (riboflavin) in milk, defining variations in concentration and also characterizing it chemically to some extent.

The Dairy Research Laboraory at Missouri had been established primarily to study factors affecting milk composition and Palmer at first continued work already begun by his predecessors. Such factors as plane of nutrition of the cow, parturition, gestation, stage of lactation, age of the cow and the feeding of cottonseed products were investigated in a careful and classical series of studies. This series laid the foundation for much of the more recent work on the composition of milk. These studies as well as those on the milk pigments reveal the characteristic trait of both Palmer and Eckles to attack prob-

lems in a straightforward and fundamental way and to secure adequate data to permit valid conclusions.

Palmer began to manifest an interest in other aspects of dairy chemistry while still at Missouri. No doubt this interest developed in part out of problems that arose in the collection and analysis of samples in the study of milk composition. Three fields of interest which he thus opened up and later amplified in further studies at Minnesota were lipolysis in milk, deteriorative changes in dairy products and the colloidal chemistry of milk.

Palmer's name is inseparably linked with early studies of milk lipase largely because he erroneously concluded in the early twenties that lipase does not exist in normal milk. Actually all normal milk contains lipase but it is not active unless the milk is cooled or otherwise treated to "activate" it, perhaps by promoting its adsorption on the surface of the fat globules. Palmer's experimental procedures and techniques failed to reveal this elusive relationship. It was discovered by others in the thirties and forties and recently very neatly elucidated by one of Palmer's former students, N. P. Tarassuk. Although Palmer was in error with regard to lipase in normal milk, he did correctly assign the bitter defect of milk from cows late in lactation to lipase action. In this case the enzyme needs no activation treatment.

Palmer was always much interested in the chemistry of deteriorative processes in dairy products. He directed work in this field at intervals as graduate students interested in such problems came to him. Significant contributions were made to the understanding of the chemistry of oxidative defects of butter and dry whole milk, oxidized flavor in pasteurized milk and browning of evaporated milk.

An M.A. thesis completed in 1918 on the churning of cream and a short paper published in 1919 on the physicochemical state of the milk proteins reveal Palmer's developing interest in the colloidal chemistry of milk. At Minnesota this interest became his ruling passion in dairy chemistry — fostered, no doubt, by his association with Doctor Gortner who was one of the foremost champions of colloid chemistry at that time.

Immediately after arrival at Minnesota, Palmer outlined an experiment station project on the colloidal chemistry of milk, particularly the basic mechanism of the churning process. During the next few years he became intensely interested in the mechanism of the clotting of milk by rennin. As it turned out the vast majority of his contributions to dairy chemistry were made in these two areas. Seven of his 10 Ph.D.'s and three of his 4 M.S.'s in dairy chemistry at Minnesota studied some aspect of one or the other of these problems.

The work begun with the objective of elucidating the mechanism of the churning process soon developed into a study of the nature and properties of the so-called "membrane" materials which are adsorbed on the surface of the fat globules of milk. In 1924 Palmer and a visiting Swedish chemist, E. Samuelson, found that phospholipids constitute a significant portion of the "membrane" material. Phospholipids had been ignored by previous workers, who, having extracted their preparations with fat solvents and discarded the extracts, had concluded that the "membrane" consisted mainly of protein. Over a period of about 20 years Palmer and his students made a thorough study of the composition of both natural and artificial "membranes" and their disruption by the churning process. Appropriately enough, the last paper in dairy chemistry which Palmer prepared personally was a review (published posthumously) of the chemistry of the fat globule "membrane."

In the twenties Palmer and two of his students studied the mechanism of the gravity creaming process in milk. Their principal contribution in this area was the recognition that the rise of fat globules is promoted by a substance or substances in the milk plasma. This was later shown by other groups to be a protein(s) which is adsorbed on the surface of the fat globules when milk is cooled and which promotes clustering or clumping of the globules.

In studying the coagulation of milk by the enzyme rennin, Palmer and his students defined some of the physico-chemical

effects of the enzymatic action on casein, demonstrating that both the charge and the hydration of the caseinate particle are reduced by this action. They also defined the inhibitory action of heat treatment on the clotting of milk by rennin and showed that it involves an effect on the colloidal calcium caseinate-phosphate particles. Palmer's interests in the fat globule "membrane" and rennet coagulation merged in a study with Tarassuk which demonstrated that "membrane" materials liberated from the fat globules during churning inhibit rennet clotting. It was also found that certain fatty acids liberated from the fat by lipolytic action are very inhibitory to the clotting. Finally Palmer and his student Hankinson made great progress in the purification of the enzyme rennin itself; this phase culminated in Hankinson's crystallization of the enzyme shortly after he left the University of Minnesota.

Throughout his dairy chemical research Palmer laid great stress on the use of simplified systems of purified constituents to study reactions and processes in the most basic and uncomplicated fashion. He was especially ingenious in devising systems suitable for a particular problem.

Palmer's contributions to Dairy Chemistry were made on a "shoestring" so to speak. He never had more than two graduate students in the field at any one time and often only one. He was never satisfied with the support for his "first love" in science usually pointing out in his annual progress reports that additional areas could be explored with more adequate finances and assistance. Nevertheless, he was undoubtedly satisfied with the division of his efforts between dairy chemistry and nutrition because he refused offers of positions which would have allowed him to concentrate on one or the other. Certainly he accomplished a great deal in dairy chemistry with the resources available to him. His contributions were recognized in 1939 when he was named by the American Chemical Society as the first recipient of the Borden Award in Dairy Chemistry.

Palmer's research publications comprise a total of 185 journal articles and experiment station bulletins. His mono-

graph on the carotinoids has already been mentioned. In addition he contributed chapters to a number of books — notably the chapter on vitamins in R. A. Gortner's Outlines of Biochemistry, those on Milk Pigments and Rennet Coagulation in the American Chemical Society Monograph entitled "Fundamentals of Dairy Science" and that on Vitamin A in the 1939 American Medical Society Monograph "The Vitamins." His manual of "Laboratory Experiments in Dairy Chemistry" published in 1926 is well known.

Although Palmer was primarily interested in research, he contributed considerably to scientific development through his teaching activities. He expressed his attitude toward teaching when, in accepting the position at Minnesota he wrote Gortner, "With regard to the course . . . which you state that I would be expected to teach, I may say that I have no objections whatever to some teaching work; in fact I have always regarded it important that a man engaged almost wholly in research work should carry at the same time a few hours of teaching." He was not especially enthusiastic about teaching, but he recognized its necessity and its benefits to the researcher. He did not rise to great heights as an inspirational lecturer on account of some hesitancy in delivery and a tendency to somewhat involved and obscure phraseology. Nevertheless, his lectures were exceptionally well organized and presented a tremendous amount of information and critical evaluation. Without doubt his best course — and his favorite — was Dairy Chemistry. He organized this course when he came to Minnesota and gave it each year until his death. Intended primarily for upperclassmen and graduate students, it presented an outline and a critical evaluation of the chemistry of the constituents and properties of milk and of dairy processes. For sheer content and organization it was an invaluable orientation to anyone interested in serious research in the field. The laboratory phase of this course, designed to acquaint the student with techniques in the study of the properties of milk and its constituents, was exceptionally well developed. Such a laboratory course was unique at the time that the manual was published in 1926.

Palmer also taught an introductory course in Animal Biochemistry for undergraduates and an Advanced Animal Nutrition course for upperclassmen and graduates. He presented the latter in the form of a critical survey of such topics as Experimental Background for the Discovery of the Vitamins, Protein and Amino Acid Metabolism, Biological Value of Protein in Natural Foods, and Mineral Elements in Nutrition.

Palmer was undoubtedly at his best as a teacher in the graduate seminars which he conducted in Dairy Chemistry and Nutrition. The former is especially memorable to those who attended it. The participants included students from Dairy Bacteriology and Dairy Technology as well as Agricultural Biochemistry. They met for lunch following which one of the students or occasionally Palmer himself discussed the "topic of the day." Generally these seminars embraced a single unified theme throughout an academic quarter. Palmer's critical and analytical processes of thought were never better demonstrated than in these seminar sessions.

One of Palmer's greatest contributions to science was the graduate students whom he advised. At Missouri he guided three or 4 students to the Master's degree and at Minnesota he directed 42 to the Ph.D. and 19 to the M.S. He constantly sought to instill and develop the critical approach which was his own hallmark. He was always ready to help students with advice and counsel but he expected them to employ their best efforts to think out their problems for themselves. His questions and comments were designed to stimulate the student to forsake snap judgments and to consider all possble alternatives. He had no patience with laziness (either mental or physical), procrastination or bluffing, and he dealt with these defects directly and caustically.

He had a fine discriminating judgment of the relative importance of various activities, and he liked to concentrate his full attention on the problem at hand whether it was teaching a class, conducting an experiment, writing a research report or catching his limit of fish. There were those who thought that Palmer was rather stern and reserved around the labora-

tory. Indeed he was not given to passing the time of day with students and colleagues, but this was a reflection of his preoccupation with the task at hand. Furthermore, he suffered periodically from gastritis and arthritis, neither of which are conducive to geniality. Certainly he was most helpful to his students and his door was open — figuratively at least —to anyone sincerely wishing his counsel and advice. He enjoyed having friends, colleagues and graduate students in his home where he was always a friendly and genial host. The Palmers always entertained with true "Southern hospitality."

Doctor Palmer was a member of a number of the leading scientific societies and honorary fraternities in the country. He took an active part in the affairs of the American Chemical Society, serving as councillor in 1922 and as chairman of the Minnesota section for one term. He was president of the Minnesota Chapter of Sigma Xi in 1938–39. He was particularly active in the American Dairy Science Association. He attended many of the annual meetings at which he and his students presented a great many papers. The Association profitably employed his critical talents as Associate Editor of the Journal of Dairy Science from 1917 to 1927 and again from 1933 until his death. He was collaborator in the U. S. Pharmacopoeia Vitamin Standardization Committee in 1937 and in the U. S. Department of Agriculture Survey of the Vitamin A Potency of Butter in 1942–44. He was not a regular attendant at scientific meetings other than those of the American Dairy Science Association, feeling that his time could be better utilized in producing research rather than in talking about it. Nevertheless, he was a nationally recognized authority in his fields of interest.

He took great interest in campus affairs and found much pleasure, stimulation and comradeship with his colleagues of the agricultural staff in their Biological Club (popularly and affectionately referred to on the campus as the "Bug Club").

Palmer's life was not entirely given to study and research. He was keenly interested in sports and participated actively. Undoubtedly he found his most complete and refreshing re-

laxation in a day of fishing on one of Minnesota's many lakes. He especially looked forward to an annual fishing expedition with a group of colleagues from the Dairy Department. He forgot cares and worries completely on such trips, for when he fished, he *fished!* He immensely enjoyed a round of golf on a warm summer afternoon. He bowled regularly and for a number of years captained a departmental team in a campus league. One of the authors vividly recalls Palmer's intense exhilaration when he once bowled a score of 218 — his lifetime record!

In general outline Palmer's career appears similar to those of a great many men in agricultural colleges and experiment stations. It was distinguished by the intensity with which he worked and by the fundamental, basic and critical approaches that he brought to a number of agricultural problems. He always wished to get to the roots of any problem and he was never satisfied with anything but the complete picture. Thus he worked doggedly on certain projects for many years. He contributed much to our knowledge of animal nutrition and dairy chemistry and undoubtedly would have contributed much more had his body been of commensurate strength with his mind. Small in stature physically, he was a "giant" in his chosen fields of scientific endeavor.

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IRON ABSORPTION AND METABOLISM

I. INTERRELATIONSHIP OF ASCORBIC ACID AND VITAMIN E

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There is good evidence that ascorbic acid enhances iron absorption in man. Moore and Dubach ('51) and later, Steinkamp, Dubach and Moore ('55) used Fe^{59} to show that ascorbic acid and foods containing ascorbic acid enhance the absorption of food iron. Gorten and Bradley ('54) treated iron-deficient children with oral iron and ascorbic acid. They found that ascorbic acid exerts a favorable influence on iron absorption and postulated that the vitamin also improves iron utilization peripherally.

There are only a few reports in the literature in which vitamin E has been mentioned as having hematopoietic activity. Indovina ('51) noted that the administration of vitamin E to E-deficient rabbits caused an increase in the formation of hemoglobin. Scott et al. ('55) observed a microcytic anemia in conjunction with a low reticulocyte count in vitamin E-deficient chicks. These observations indicated to the authors that vitamin E may be concerned in erythropoiesis. Recently Day and Dinning ('56) observed anemia in vitamin E-deficient monkeys.

Dam et al. ('48) reported that ascorbic acid has an *in vivo* sparing action on tocopherol in the chick. Overman ('42) reported earlier that in the rat ascorbic acid increased the resistance of the animal's fat to the development of rancidity.

Preliminary studies of the effect of ascorbic acid on iron absorption in the rat led to the observation that an inter-

relationship seemed to exist between ascorbic acid and vitamin E in iron metabolism. Experimental evidence is presented here which shows that iron with a combination of ascorbic acid and vitamin E has more effect in the enhancement of hemoglobin synthesis and the maintenance of hemoglobin than does iron with either vitamin alone.

METHODS AND PROCEDURES

Three separate experiments on rats are reported here. Experimental animals were raised on a basal iron-deficient diet from the 12th day post partum. The rats were weaned on the 21st day after birth and were maintained on the iron-depletion diet until the hemoglobin concentrations became uniformly low (usually < 6.0 gm/100 ml of blood). In all experiments, when group assignments were made, the rats were balanced as regards litter mates and hemoglobin concentrations. Hemoglobins were determined on samples of tail blood by the method of Evelyn and Malloy ('38). All animals were maintained on the basal iron-deficient diet throughout the experiments. Supplements were given orally by dropper (vitamin E) or by stomach tube (iron and ascorbic acid).

All animals were maintained individually in hanging $\frac{1}{2}$-inch mesh wire-screen galvanized cages, and were fed the iron-low basal diet and given distilled water ad libitum. The basal diet was composed of, in grams per kilogram, powdered whole milk [1] 970, casein [2] 10, cellulose [3] 20. Micronutrients were incorporated into the milk diet to give the following in milligrams per kilogram: thiamine hydrochloride, riboflavin and pyridoxine, 5 each; calcium pantothenate, inositol and para-aminobenzoic acid, 50 each; niacin, 20; 2-methyl-1,4-naphthoquinone and folic acid, 1 each; biotin, 0.1; vitamin B_{12}, 0.02. Choline chloride was mixed with the casein to give 400 mg/kg

[1] Spray Dried whole milk, Albumina Supply Co., New York.
[2] Vitamin Test, General Biochemicals, Inc.
[3] Methocel, Dow Chemical Co.

of diet. Trace salts were incorporated into the milk diet to give in milligrams per kilogram: cupric sulfate 3.1, ammonium citrate 611.1, manganese sulfate 8.1, ammonium aluminum sulfate 3.7, potassium iodide 1.6, and sodium fluoride 20.3. No vitamin E was added to the diet. The only source of vitamin E was the whole powdered milk, which is estimated to contain 0.5 mg tocopherol per 100 gm (Lange, '50). A food consumption of 10 gm per day gives a vitamin E intake of approximately 0.05 mg.

The animal room was maintained at a constant temperature of 77° ± 1°F., and the humidity was kept at approximately 50%. Timed lighting gave 12 hours light and 12 hours darkness throughout the experiments.

Ferrous sulfate ($FeSO_4 \cdot 7H_2O$, A.R.) was used as the sole source of iron. All iron and ascorbic acid supplements were dissolved in 1% HCl and prepared fresh daily. Rats were intubated on a weight basis (5 ml/kg body weight). The volume of liquid given the rat remained constant per unit of body weight regardless of supplements administered. Animals were tubed 5 days weekly for the first two experiments. Details of experiment 3 will be given later.

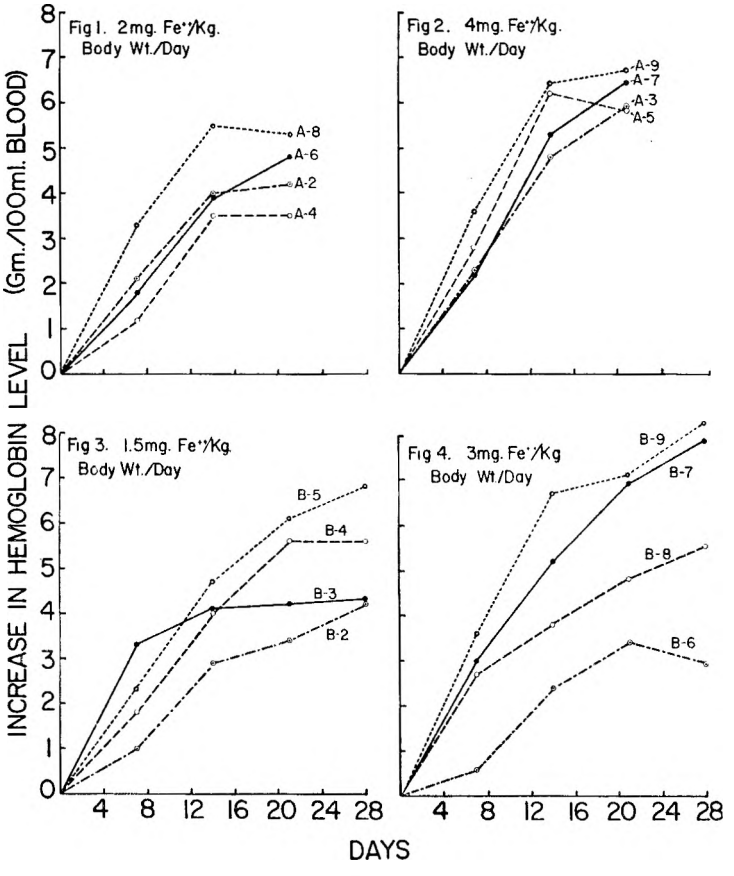
EXPERIMENTAL AND RESULTS

Experiment 1. Iron-deficient rats were assigned to 9 groups for supplementation with iron, ascorbic acid and vitamin E. Each group contained 5 rats except for the negative control group which contained two rats. The experimental period was 21 days. The average hemoglobin regeneration data above the baseline of 6.0 gm/100 ml blood are presented in figures 1 and 2. Hemoglobin values were obtained at 7-day intervals. The negative controls (group A-1; not shown on figures) gained an average of 1.6 gm of hemoglobin/100 ml of blood during the experimental period.

At both levels of iron (2 and 4 mg/kg of body weight) a greater stimulation of hemoglobin regeneration in the iron-deficient rats was observed when iron was administered with

both vitamin E [4] and ascorbic acid (group A-8, figure 1; and group A-9, figure 2) than with either vitamin alone (groups A-4 and A-6, figure 1; and groups A-5 and A-7, figure 2).

Experiment 2. The experimental procedures were the same as those used in experiment 1 except that the vitamin E was



Figs. 1–4 Average hemoglobin regeneration; figures 1–2, regeneration above baseline of 6.0 gm/100 ml blood; figures 3–4, regeneration above baseline of 5.0 gm/100 ml blood. Iron administered orally 5 times weekly. ⊙——⊙ iron alone; ○———○ iron plus vitamin E; ●——●——● iron plus ascorbic acid; ⊙ - - - - ⊙ iron plus vitamin E and ascorbic acid.

[4] Natural mixed tocopherol esters (34% solution, Distillation Products Industries) suspended in 95% ethyl alcohol and administered twice weekly at a level of approximately 3.5 mg/dose.

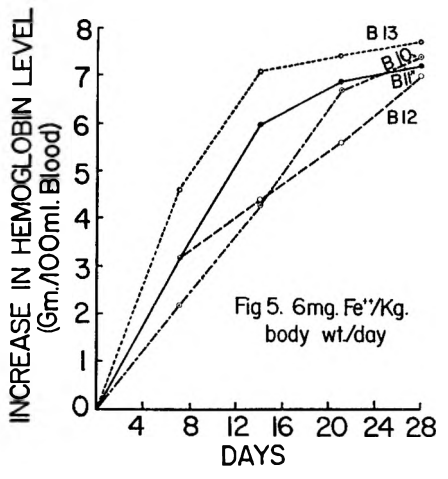


Fig. 5 Average hemoglobin regeneration above baseline of 3.5 gm/100 ml blood. Iron administered orally 5 times weekly. ⊙——⊙ iron alone; ○——○ iron plus vitamin E; ●——● iron plus ascorbic acid; ◘——◘ iron plus vitamin E and ascorbic acid.

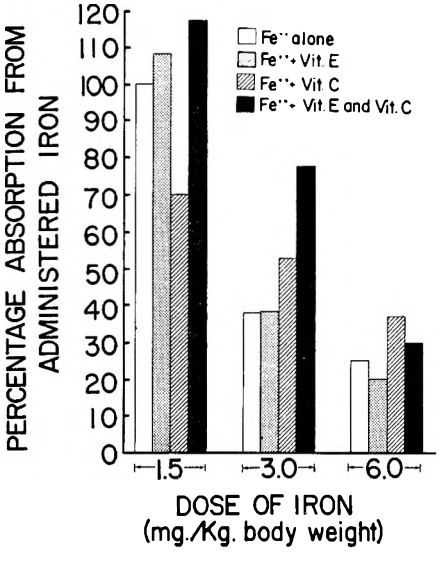


Fig. 6 Average percentage of iron absorption as measured by conversion into hemoglobin during the second week of experiment 2.

administered at a higher dosage level and without dilution with alcohol. One drop of vitamin E concentrate (same as used in experiment 1) was administered orally semiweekly so that each rat received a total of approximately 12 mg of the tocopherol esters per week.

Iron-deficient rats were assigned to 13 groups (4 rats per group) for supplementation with iron, ascorbic acid and vitamin E. The experimental period was 28 days.

The average hemoglobin regeneration data above the baseline of 5.0 gm/100 ml blood are presented in figures 3, 4 and 5. The negative controls (group B-1; not shown on figures) gave the following average gains in hemoglobin (gm/100 ml blood): −0.8, −0.8, −0.6 and −1.0 on the 7, 14, 21 and 28th days respectively.

The percentage of administered iron absorbed and converted to hemoglobin was calculated for the various groups of experimental rats for the second week. The data are presented in figure 6. The formula used for calculating the percentage absorption was:

$$\frac{\left[\left(\begin{matrix}\text{body wt.} \times \text{Hgb} \\ \text{14th day}\end{matrix}\right) \text{ minus } \left(\begin{matrix}\text{body wt.} \times \text{Hgb} \\ \text{7th day}\end{matrix}\right)\right]\left[\left(\begin{matrix}\text{blood vol.} \\ \text{of rat (\%)}\end{matrix}\right)\left(\text{mg Fe/gm Hgb}\right)\right]}{\text{Total mg Fe administered}} \times 100$$

The average blood volume was presumed to be 6.7% of the body weight (Cartland and Koch, '28); "Hgb" is the hemoglobin concentration in grams per 100 ml of blood and the value for "mg Fe/gm Hgb" was 3.35. It was assumed that the iron administered late in the second week and not yet converted into hemoglobin was counterbalanced by the iron given late in the first week and converted to hemoglobin during the second week.

Experiment 3. Iron-deficient rats were assigned to 5 groups of 8 rats each (5 males and three females) for supplementation with iron, ascorbic acid and vitamin E. In this experiment the rats were depleted of iron to an average of 5.3 gm hemoglobin per 100 ml blood by the method described in experiment 1. Three massive doses of iron were administered at

the start and on the 6th and 10th days of the experiment. Each dose contained 25 mg Fe(ous)/kg body weight and was administered by stomach tube. The groups administered ascorbic acid received their vitamin with the iron and at no other time. The vitamin E (same material as used in experiments 1 and 2) was administered at a dosage level of 12 mg on the same day as each of the three doses of iron. One

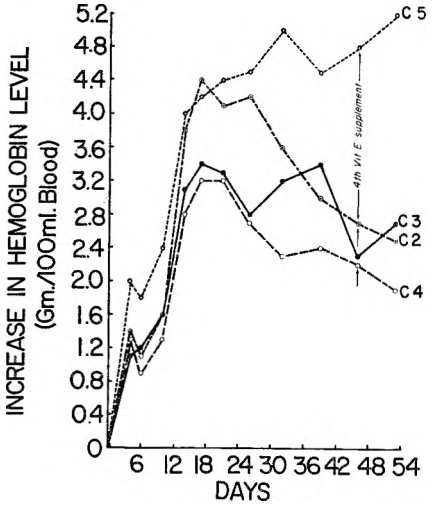


Fig. 7 Average hemoglobin regeneration above baseline of 5.3 gm/100 ml blood; 25 mg Fe/kg body weight administered at the start and on 6th and 10th days only. Vitamin E alone administered again on 46th day. ⊙———⊙ iron alone; ○———○ iron plus vitamin E; ●———● iron plus ascorbic acid; ●-----● iron plus vitamin E and ascorbic acid.

additional dose of vitamin E esters (approximately 25 mg) was given to all rats on the 46th day. The experimental period was 53 days.

The hemoglobin regeneration data are presented in figure 7. The negative control group is not presented; however, the average hemoglobin regeneration remained below 1.3 gm/100 ml blood throughout the experiment. Again as in the previous experiments the iron with both ascorbic acid and vitamin E caused the most rapid hemoglobin synthesis and ultimately

TABLE 1

Summary of hemoglobin data for 46th day and of hemoglobins, erythrocytes and hematocrits on 53rd day[1]

GROUP NO.	HEMOGLOBIN GAIN				ERYTHROCYTES $\times 10^6$		HEMATOCRIT	
	46th day	"P"	53rd day	"P"	53rd day	"P"	53rd day	"P"
	gm/100 ml		*gm/100 ml*		*per mm³*		%	
C-1 Controls	0.4 ± 0.5	0.01	1.3 ± 0.7	0.05	5.05 ± 0.04	N.S.	28 ± 1.1	N.S.
C-2 Fe(ous) alone	2.7 ± 0.5	0.10	2.5 ± 0.5	0.10	4.78 ± 0.49	N.S.	24 ± 2.9	0.05
C-3 Fe(ous) plus vitamin C	2.3 ± 1.2	0.05	2.7 ± 1.4	0.10	5.94 ± 0.34	N.S.	33 ± 2.9	N.S.
C-4 Fe(ous) plus vitamin E	2.2 ± 0.9	0.05	1.9 ± 0.9	0.05	4.80 ± 0.60	N.S.	25 ± 3.4	0.05
C-5 Fe(ous) plus vitamins C and E	4.8 ± 0.6	...	5.2 ± 0.9	...	5.67 ± 0.12	...	33 ± 1.9	...

[1] Includes standard error of the mean $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$ where "d" is deviation from the mean and "n" is number of observations. Probability of difference from group C-5 is given for other groups based on "t" test (Snedecor, '46).

the highest concentration of hemoglobin. After about the 17th day of the experiment (7 days after the last iron supplement) the mean hemoglobin concentration in groups C-2, C-3 and C-4 began to decline. After 45 days group C-5 differed significantly or approached a significant difference from each of the other groups with respect to hemoglobin concentration (table 1).

The weight gains of all groups of rats were similar except for group C-4 males (table 2).

TABLE 2

Weight gains (46 days) of rats on experiment 3

GROUP NO.	SUPPLEMENTS	WEIGHT GAINS [1]	
		Males	Females
		gm	*gm*
C-1	None	(3) 105 ± 15	(3) 54 ± 11
C-2	Fe(cus)	(5) 101 ± 10	(3) 73 ± 6
C-3	Fe(cus) plus vitamin C	(5) 100 ± 16	(3) 60 ± 7
C-4	Fe(cus) plus vitamin E	(5) 69 ± 12	(3) 81 ± 9
C-5	Fe(ous) plus vitamins C and E	(5) 124 ± 6	(3) 66 ± 4

[1] Includes S.E.M. (See footnote 1, table 1.) and number of rats alive (figures in parentheses).

The only weight gain that differs significantly from the weight gain of the C-5 group is that of the C-4 males.

A summary of all blood data obtained terminally on the rats on experiment 3 is presented in table 1. Calculations of factors relating hematologic findings to the degree of anemia are presented in table 3.

DISCUSSION

The experimental data indicate that in the iron-deficient rat on a milk diet, iron is converted to hemoglobin more efficiently when given with ascorbic acid and vitamin E than when given alone or with either vitamin separately. It is not clear from the data presented whether the improved hemoglobin picture of the groups receiving iron and both vitamins is due to an increased absorption of iron, an increased rate of utilization of iron, or both.

TABLE 3

Summary of calculations relating hematologic findings (table 1) to degrees of anemia (53rd day)[1]

GROUP NUMBER	MEAN CORPUSCULAR HEMOGLOBIN [2]	"P"	MEAN CORPUSCULAR VOLUME [3]	"P"	MEAN CORPUSCULAR HEMOGLOBIN CONC.[4]	"P"
	μμg		*μ*^{3}		*gm/100 ml packed cells*	
C-1 Controls	16.3 ± 0.7	0.10	54.9 ± 2.7	N.S.	29.7 ± 1.1	0.001
C-2 Fe(ous) alone	16.3 ± 1.9	0.10	50.4 ± 4.5	N.S.	32.2 ± 1.2	0.01
C-3 Fe(ous) plus vitamin C	18.4 ± 2.4	N.S.	55.5 ± 4.6	N.S.	32.9 ± 1.7	0.05
C-4 Fe(ous) plus vitamin E	15.9 ± 1.0	0.05	51.6 ± 1.9	N.S.	30.8 ± 1.7	0.01
C-5 Fe(ous) plus vitamins E and C	21.3 ± 1.9	...	57.2 ± 5.2	...	37.8 ± 0.5	...

[1] See footnote 1, table 1, for S.E.M. formula.

[2] $\frac{\text{Hemoglobin in grams per 100 ml blood}}{\text{Erythrocytes in millions per cubic millimeter}} \times 10 = \text{M.C.H.}$

[3] $\frac{\text{Volume of packed cells per 100 ml blood}}{\text{Erythrocytes in millions per cubic millimeter}} \times 10 = \text{M.C.V.}$

[4] $\frac{\text{Hemoglobin in grams per 100 ml blood}}{\text{Volume of packed cells per 100 ml blood}} \times 100 = \text{M.C.H.C.}$

The calculated percentage absorption of iron in experiment 2 indicates a superior effect for iron plus both vitamins at the two lower levels of iron. The absorption picture closely parallels the gross hemoglobin regeneration data in that generally greater effects from the vitamins were observed at lower levels of iron than at higher levels.

Experiment 3 shows that the group receiving three massive doses of iron plus both vitamins maintained a relatively high hemoglobin concentration throughout the experiment. The other groups failed to achieve or maintain a similar hemoglobin level. Apparently the rats on iron alone or on iron plus only one of the two vitamins failed to re-utilize efficiently the iron released by the normal turnover of red blood cells, or the various groups of animals differed in rates of excretion of iron. Stevens et al. ('53) have shown a constant loss of 0.5% per day of total body iron from both normal and "iron-heavy" mice. It would seem possible that a change in excretory loss of iron might be a factor contributory to the observed decreases in hemoglobin in three of the 4 test groups.

The similar weight gains of the rats, except for group C-4, during the experimental period make it improbable that differences in blood volumes could account for the differences observed in the regenerated hemoglobin. It would seem more likely that the animals that were supplied with iron plus ascorbic acid and tocopherol (group C-5, exp. 3) were better able to retain body iron and to re-utilize iron from the normal catabolism of the red cells. In experiment 3, in which vitamin E was given to all rats on the 46th day, including the negative controls for the first time, a boost in the average hemoglobin was noted in both groups that had received vitamin C earlier (C-3 and C-5, table 1). The negative controls also showed an increased level of hemoglobin during the week after the supplement of vitamin E (table 1).

A properly balanced redox system, especially in the bone marrow, would seem important in determining the rate of transfer of ferric iron of siderophilin or of ferritin to the ferrous form of hemoglobin. This same relationship may

be a factor in the absorption mechanism as well, since reduced iron appears to be transformed to the oxidized state in ferritin of the gut and then to the reduced state to cross the cell membrane into the blood.

It is hoped that additional work with antioxidants, reducing agents and radioactive iron will elucidate further the mechanisms related to the hematologic improvements seen in anemic rats receiving iron with ascorbic acid and vitamin E.

SUMMARY

The effects of supplements of iron with ascorbic acid and vitamin E on hemoglobin regeneration were studied in milk-fed anemic rats.

The rate of hemoglobin regeneration was consistently greater in rats supplemented with iron plus ascorbic acid and vitamin E than with iron alone or with iron plus either of the vitamins.

In a long-term study hemoglobin levels were better sustained after the cessation of iron supplements if the iron had been given concomitantly with ascorbic acid and vitamin E than if given alone or with either vitamin separately.

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IRON ABSORPTION AND METABOLISM

II. SUBSTITUTION OF N,N'-DIPHENYL-*P*-PHENYLENEDIAMINE (DPPD) FOR VITAMIN E

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In a previous report (Greenberg et al., '57), evidence was presented relating ascorbic acid and vitamin E to improved iron absorption and metabolism in the rat. Numerous reports of the effectiveness of diphenyl-*p*-phenylenediamine (DPPD) as a biological antioxidant and, in this respect, a substitute for vitamin E have appeared (Singsen et al., '55; Matterson et al., '55; Bunnell et al., '55).

DPPD [1] was substituted for vitamin E in this experiment in order to ascertain whether an antioxidant, not known to have other vitamin E-like biological activities, would stimulate hemoglobin regeneration as reported for vitamin E in the earlier experiments.

PROCEDURE

The general plan of study, the description of the method of iron-depletion of the rats, and the conditions under which they were housed and fed have been described (Greenberg et al., '57). The diet was modified by the dilution of the powdered whole milk with carbohydrates, by an increase in the choline chloride level and by the addition of vitamins A and D to the diet.

The diet as used in this experiment was as follows, in grams per 100 gm: powdered whole milk,[2] 72.5; cornstarch, 16.5;

[1] B. F. Goodrich Chemical Co.

dextrose, 2.0; sucrose, 6.0; cellulose,[3] 3.0. Micronutrients were included at the following levels (milligrams per kilogram): thiamine hydrochloride, riboflavin, pyridoxine, 5 each; niacin, 20; calcium pantothenate, *i*-inositol and *p*-aminobenzoic acid, 50 each; folic acid, 2 Me-1,4-naphthoquinone, 1 each; biotin, 0.1; vitamin B_{12}, 0.02; choline chloride, 450. Vitamin A,[4] 20,000 USP units and 2,000 USP units of vitamin D_2 [4] were added per kilogram of diet. The following trace elements were included at the following levels (milligrams per kilogram): cupric sulfate, 3.1; ammonium citrate, 611.1; manganese sulfate, 8.1; ammonium alum, 3.7; potassium iodide, 1.6; and sodium fluoride, 20.3.

The young iron-depleted rats were divided into 5 groups of 9 animals each. The groups were balanced as regards sex, littermates and hemoglobin levels. At the start of the experiment the animals were approximately 7 weeks old, and absolute hemoglobins averaged 4.0 gm/100 ml blood.

At the start of the experiment and on the 5th and 11th days massive doses of iron [25 mg of Fe(ous)/kg body weight] were administered by oral intubation to all rats except the negative controls. Ferrous sulfate ($FeSO_4 \cdot 7H_2O$, A.R.) was dissolved in 1% HCl, and all solutions were adjusted to a volume of 5 ml/kg. When ascorbic acid was administered (20 mg/kg body weight; groups 3 and 5), it was dissolved with the iron immediately before intubation. The ascorbic acid was administered with each dose of iron and twice weekly thereafter. All solutions were prepared fresh on each of the dosage days. Two of the groups (4 and 5) had DPPD incorporated into the diet at the level of 0.1% at the expense of sucrose from the first day iron was administered. These two groups continued on the DPPD-supplemented diet throughout the experiment.

The experiment was continued for 7 weeks, during which time hemoglobins were determined by the method of Evelyn

[2] Spray Dried Whole Milk, Albumina Supply Co., New York.

[3] Methocel, Dow Chemical Co.

[4] "Crystalets," 500,000 USP units vitamin A acetate and 50,000 USP units vitamin D_2/gram. Chas. Pfizer & Co., Inc.

and Malloy ('38) twice weekly during the first two weeks and weekly thereafter. Erythrocyte counts and hematocrits were determined on the 7th, 14th, 28th and 49th days. All hematologic studies were made on morning samples from the tail blood of the rats. At termination of the experiment blood volumes of rats from three of the 5 groups were determined by the Evan's Blue dilution method (Wang and Hegsted, '49). Two of the control groups (2 and 4) and the group with the

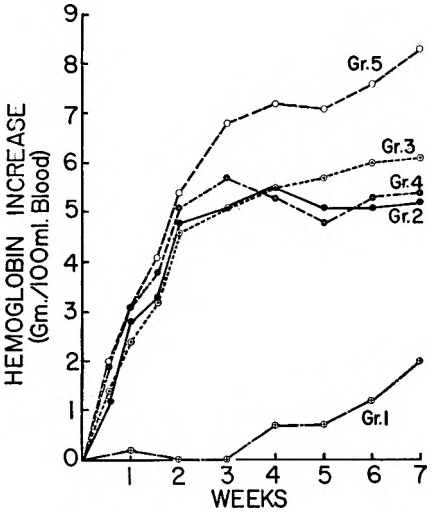

Fig. 1 Supplements administered at the start, 5th, and 11th days of the experiment. Vitamin C was administered to groups 3 and 5 twice weekly thereafter. Group 1, negative control; group 2, iron alone; group 3, iron plus ascorbic acid; group 4, iron plus DPPD; group 5, iron plus DPPD and ascorbic acid.

highest hemoglobin regeneration level (group 5) were chosen for these determinations.

RESULTS

The data on hemoglobin regeneration are presented in figure 1. The negative controls (group 1) showed very little hemoglobin regeneration through the 5th week; however, from the 5th to the 7th week (termination) the average hemoglobin of the negative control group increased by 1.3 gm/100 ml blood.

Hematocrit determinations and erythrocyte counts were made at the end of the first, second, 4th and 7th weeks. The results are presented in table 1. Calculations were made of relations of hematocrits, erythrocyte counts and hemoglobin levels for the groups studied (table 2). These calculations give the mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration, factors related to the degree and type of anemia.

TABLE 1

Hematocrit and erythrocyte counts on tail blood of rats at end of weeks indicated (5 rats per group)

GROUP NO.	SUPPLEMENTS	HEMATOCRITS (Per cent red cell volume)				ERYTHROCYTES (10^6) (Millions per mm^3)			
		1	2	4	7	1	2	4	7
1	None	18	21	26	28	3.7	3.4	4.5	5.9
2	Fe(ous)	27	41	38	36	4.5	6.0	6.3	7.6
3	Fe(ous) plus vit. C	28	41	39	42	4.9	5.1	7.1	7.5
4	Fe(ous) plus DPPD	28	39	37	37	4.0	4.8	6.3	6.1
5	Fe(ous) plus DPPD plus vit. C	31	47	45	45	4.4	5.2	6.8	6.6

Blood volumes were determined on the 49th day; the average blood volumes (ml/100 gm body wt.) including standard deviations were as follows:

Group 2 (Iron alone, 6 rats)	7.7 ± 2.0%
Group 4 (Iron plus DPPD, 5 rats)	8.0 ± 0.4%
Group 5 (Iron plus DPPD plus ascorbic acid, 7 rats)	8.0 ± 1.4%

DISCUSSION

These experimental data support our previous observations (Greenberg et al., '57) that the administration of both an antioxidant and ascorbic acid with iron promotes a greater hemoglobin regeneration response than does the same level of iron when given alone, with ascorbic acid or with the antioxidant. Although clear evidence exists that DPPD is an antioxidant and that it can replace or spare vitamin E for this activity, there is little positive evidence at present that this compound can replace vitamin E for all its known bio-

TABLE 2

Calculations of mean corpuscular volume [1] (M.C.V.), of mean corpuscular hemoglobin [2] (M.C.H.) and of mean corpuscular hemoglobin concentration [3] (M.C.H.C.) from analytical data on tail blood of rats. Results given at end of weeks indicated (5 rats per group)

GROUP NO.	SUPPLEMENTS	M.C.V.				M.C.H.				M.C.H.C.			
	WEEKS	1	2	4	7	1	2	4	7	1	2	4	7
		μ^3				$\mu\mu g$				*gm/100 ml packed cells*			
1	None	49	59	59	48	12.3	11.6	11.4	10.9	25	20	20	24
2	Fe(ous)	60	68	59	48	15.8	15.8	15.3	12.4	27	24	26	26
3	Fe(ous) plus vit. C	57	83	56	57	14.9	18.0	15.6	15.8	26	22	29	27
4	Fe(ous) plus DPPD	70	82	59	62	17.3	18.4	15.4	16.0	25	22	27	26
5	Fe(ous) plus DPPD plus vit. C	74	92	67	68	18.0	20.3	18.0	20.5	24	22	28	30

[1] $\frac{\text{Volume of packed cells per 100 ml blood}}{\text{Erythrocytes in millions per cubic millimeter}} \times 10 = \text{M.C.V.}$

[2] $\frac{\text{Hemoglobin in grams per 100 ml blood}}{\text{Erythrocytes in millions per cubic millimeter}} \times 10 = \text{M.C.H.}$

[3] $\frac{\text{Hemoglobin in grams per 100 ml blood}}{\text{Volume of packed cells per 100 ml blood}} \times 100 = \text{M.C.H.C.}$

logical activities. In regard to hemoglobin regeneration, positive vitamin E-like activity (either direct or through vitamin E-sparing effect) has been observed in this study.

No direct indication of the site of activity or the mechanism by which the improved hematopoiesis occurs when iron is administered to anemic animals along with both an antioxidant and ascorbic acid is revealed in this experiment. In the previous studies with vitamin E and in these data there are indications that more than enhanced intestinal absorption is responsible for the increased hematopoiesis observed in group 5 (iron plus DPPD plus ascorbic acid). In both experiment 3 of our former report (Greenberg et al., '57) and in this experiment the rate of hemoglobin regeneration appeared to be essentially equal for all groups during the period of iron administration. It was after this period that differences in hemoglobin levels were revealed.

The increase in absolute hemoglobin level observed in experiment 3 of our previous paper, where iron was administered with vitamin E and ascorbic acid and in this experiment where iron was administered with DPPD and ascorbic acid, theoretically could be related to an increase in the total iron absorbed. On the other hand this increase in hemoglobin level might have resulted in part from increased reutilization of iron from the normal catabolism of red blood cells or from diminution of iron excretion in the feces or urine. Further studies with radioactive iron may help to explain some of these observations.

The increased average hematocrit observed in group 5 (iron plus DPPD plus ascorbic acid) over the average hematocrit of any of the other groups at any of the test periods (table 1) is additional evidence supporting the observation of increased hemoglobins also observed in this group. The superiority of the supplements in group 5 over those in any other group is further supported by the results of the calculation of factors indicative of recovery from iron-deficiency anemia. In all cases mean corpuscular volume and mean corpuscular hemoglobins and in some cases mean corpuscular

hemoglobin concentrations were higher in group 5 than in any other group.

Blood volumes of the rats of three of the groups, including group 5, were essentially identical. These findings tend to eliminate the possibility that the observed increases in hemoglobin levels and in hematocrits in the group receiving iron with DPPD (vitamin E in the previous report) and ascorbic acid might have been due to hemoconcentration.

The results of this and of our previous experiments suggest that either vitamin E or a biologically active antioxidant and a synergistic substance (such as ascorbic acid) are interrelated and significantly improve iron metabolism in the iron-deficient rat.

SUMMARY

The effect of substituting an antioxidant [N,N′-diphenyl-*p*-phenylenediamine (DPPD)] for vitamin E was tested in hemoglobin regeneration studies on milk-fed anemic rats.

The average levels of hemoglobin regenerated and the average hematocrits were higher in the group of rats supplemented with iron plus ascorbic acid and the antioxidant than in groups receiving iron alone or iron plus either ascorbic acid or DPPD.

ADDENDUM

Since completing this work, it has been called to our attention that the U. S. Food and Drug Administration has banned the addition of DPPD to poultry feed (Anon., '56). This action was taken because of toxic effects in pregnant rats (Oser and Oser, '56).

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THE DIETARY COMPOSITION AND ADEQUACY OF THE FOOD CONSUMED BY YOUNG MEN ON AN AD LIBITUM REGIMEN [1]

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Estimates of the nutrient intake of a population are generally made for one or more of three purposes: first, to determine the adequacy of the nutrient intake by comparison with some standard recommended allowance; secondly, to compare the intakes of two or more population groups with similar occupation or activity patterns; and thirdly, to arrive at some estimate of the requirement for a particular nutrient (FAO, '49; Keys, '49–'50).

Many studies have been conducted within the past few years to measure and to assess the adequacy of the average dietary intake of the healthy male soldier in hot and temperate environments in various areas throughout the United States (Consolazio et al., '56; Howe and Berryman, '45; Johnson and Kark, '47; Schor and Swain, '49). These investigations were made among military personnel consuming packaged or garrison rations under the usual messing procedures. Information was not available, however, as to the composition

[1] The opinions expressed in this paper are those of the authors and do not necessarily represent those of any governmental agency.

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and apparent adequacy of the soldiers' dietary when exposed to an ad libitum intake of relatively acceptable foods.

The practice of self-feeding or self-selection of foods by farm animals, particularly for fattening purposes, is well known (Morrison, '45). Rats have demonstrated the ability to exhibit normal growth and reproduction when forced to select their own diet from the essential nutrients placed in separate containers (Mitchell and Mendel, '21; Osborne and Mendel, '18; Richter et al., '38). Comparable studies of self-selection, or ad libitum intakes as applied to man are practically non-existent, with the exception of the investigation of Davis ('28) with children. Davis allowed newly weaned infants to select their own foods ad libitum from a wide range of foods of animal and plant origin. The list contained only natural foods and no incomplete or canned foods. It was found that the infants were able to maintain themselves after weaning for as long as 12 months without any apparent deleterious effects. What can be expected from adult humans on an ad libitum regimen with their previously acquired tastes, idiosyncrasies, habits and preferences? The present study was designed to investigate this question by allowing soldiers unlimited quantities of available food items.

EXPERIMENTAL

This study was conducted at Fort Carson, Colorado, during a 4-week period in January and February, 1955. One hundred healthy male soldiers undergoing advanced military training served as subjects. The men ranged in age from 18 to 25 years (av. 21.5 $\pm$ 1.0); in height from 162 to 189 cm (av. 174.5 $\pm$ 6.4) and in weight from 52 to 98 kg (av. 70.4 $\pm$ 8.8).[4] The men were housed in two barracks buildings and were required to eat in an adjacent mess-hall building.

Weekly body weight measurements were obtained immedi-

[4] Detailed data on age, height and body weight for each subject are available (Konishi et al., '56a).

ately upon arising in the morning and after micturition. Body heights were obtained at the beginning of the study.

The subjects were allowed ad libitum quantities of foods available throughout the study. The diet was similar to the ordinary garrison ration except that extra quantities of all menu items were available. The foods were prepared under the supervision of a dietitian, with emphasis upon maximum acceptability. This included the purchase of superior cuts of meat and the limitation of canned foods to minimize the psychological aversion to canned foods of the "C" ration type which is frequently observed among soldiers.

To facilitate determination of the food consumed, each food was prepared as homogeneous as possible. This was accomplished by intentionally omitting from the menus heterogeneous food mixtures such as gravies, stews, mixed fruit salads, etc. The meats were trimmed of all visible fat prior to serving. The amount of each food taken by a subject was measured by placing the large container of food upon a direct reading scale and recording the weight before and after serving each man. The amount of a food item consumed by an individual was then obtained by simply subtracting the weight of the plate refusal from the total quantity taken. The subjects were also allowed to obtain additional food items at a designated post exchange facility where the item and the quantity purchased were recorded.

Representative samples of each food item served daily in the mess hall or purchased in the exchange facility during the 28-day period were obtained and analyzed for proteins (Keys, '40), fat, moisture, and ash (A.O.A.C., '50), and the carbohydrates were calculated by the usual method of weight-difference. The caloric intake was derived by applying the general Atwater factors of 4, 9 and 4 Cal. to the grams intake of protein, fat and carbohydrate, respectively (FAO, '47; Maynard, '44).

The subjects were maintained on a relatively moderate training program throughout the study. The most strenuous activity was a weekly 7-mile contest march.

RESULTS

During the course of the study, two of the subjects were hospitalized; therefore, this report is based upon the data for 98 men.

The mean daily nutrient intakes for all the subjects during each week of the study and the overall averages are shown in table 1. It will be noted that the intakes of calories, protein and carbohydrate were highest during the second week and decreased during the subsequent two weeks. The mean daily individual intakes of the nutrients ranged from 2882 to 4642

TABLE 1

Mean daily intake of calories, protein, fat and carbohydrate, by weeks[1]

WEEK	CALORIES[2]	PROTEIN	FAT	CARBOHYDRATE
	Av. ± S.D.[3]	Av. ± S.D.	Av. ± S.D.	Av. ± S.D.
	Cal.	*gm*	*gm*	*gm*
1	3412 ± 753	150.5 ± 38	150.6 ± 37	360.4 ± 93
2	3925 ± 661	161.4 ± 35	169.0 ± 34	433.1 ± 80
3	3833 ± 751	153.0 ± 31	175.4 ± 43	406.7 ± 85
4	3505 ± 700	138.0 ± 30	154.2 ± 36	386.2 ± 91
Mean	3669 ± 750	150.7 ± 35	162.3 ± 39	396.6 ± 91

[1] Daily nutrient intakes for each individual are available (Konishi et al., '56a).
[2] Includes calories from alcoholic beverages.
[3] Standard deviation.

Cal., 115 to 191 gm of protein, 111 to 207 gm of fat, and 279 to 534 gm of carbohydrate.

The average intake of calories from sources outside the mess hall was 190, 169, 131 and 118 during the first, second, third and 4th weeks, respectively. The mean daily intake for the 28-day period was 152 Cal. or 4.1% of the total calories consumed.

The modicum of calories consumed outside the mess reflects the high acceptability and the ad libitum availability of the foods offered. The 152 Cal. was less than the amount from such sources consumed by patients in various U. S. Army Hospitals (Konishi et al., '56b). These patients consumed

over 200 Cal. from sources outside the mess, representing 7.5% of their total calorie intake. Even more striking is the comparison with soldiers in ordinary U. S. Army training camps (Consolazio et al., '56), where the amount was approximately 18% of the total, or 700 Cal. from exchange facilities, vending machines, etc.

The average fat consumption was 162.3 gm per day or 39.8% of the total calories consumed. Figure 1 illustrates

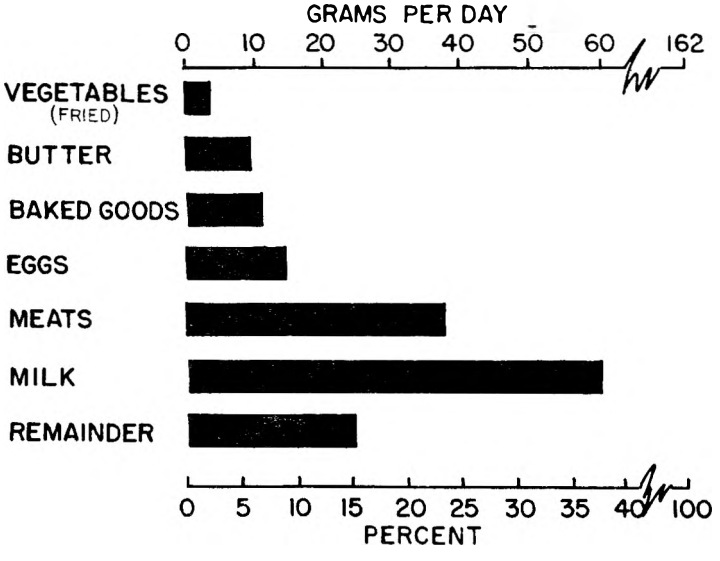

Fig. 1 Sources of average daily fat consumption from selected food groups.

graphically the quantity and percentages of fat contributed by 6 selected food groups. The fat from animal sources (i.e. milk, butter, eggs and meats) supplied 76% of the total fat intake per day. As noted, milk was the greatest single source of fat calories. This is not attributable to the percentage of fat in milk *per se*, or in milk equivalents, but to the quantity of fresh milk consumed (table 2). As a matter of interest, the highest quantity of milk consumed by an individual in one day was 3920 gm or 16 one-half-pint cartons. The volume of milk consumed by these soldiers was beyond all expecta-

tions. The average consumption increased steadily for three weeks before declining somewhat during the last week (Vawter and Konishi, '57).

The relatively high intake of protein, 151 gm per day, in this study is attributable primarily to the volume of fresh

TABLE 2

Average quantities of food consumed on an ad libitum regimen and in ordinary army messes

FOOD GROUPS	CONSUMED		
	Ad libitum [1]	1945 [2]	1941–1943 [3]
	gm/man/day	*gm/man/day*	*gm/man/day*
Meat, fish and poultry	249	379	413
Eggs	89	75	68
Milk and milk products (milk equivalents)	1757	572	461
(Milk)	(1537)		
Fats, butter and spreads	11 [4]	32	38
Sugar and syrups	18 [5]	127	119
Cereals and grain products	273	232	257
(Bread)	(117)		
Beans, other legumes (dry)	87	16	25
Vegetables, leafy green or yellow	58	137	163
Tomatoes	59	74	70
Citrus fruits	108	151	113
Potatoes	185	255	255
Vegetables, other	41	112	114
Fruits, other	123	96	163

[1] Complete individual daily intakes of all foods are available (Vawter and Konishi, '57).

[2] Average food consumption based on data of Schor and Swain ('49).

[3] Average food consumption based on data of Howe and Berryman ('45).

[4] Value for butter only.

[5] Value for sugar only.

milk consumed. The mean daily intake of 1537 gm of milk furnished approximately 50 gm of protein, or one-third of the total protein consumed. Whether the men were psychologically influenced by the presence of the nutrition team or whether the method of food preparation resulted in a relative "dryness" of the foods reflecting the higher fluid intake is unknown. Coffee and tea were available at all times, how-

ever, without any apparent influence upon the milk consumption.

The average quantities of the other food groups consumed are also shown in table 2. It is interesting to note that, in spite of the ad libitum availability, meats were consumed in such small quantities. The absence of gravies, particularly for roasts, was possibly a major factor in decreasing the intake of meat. The intake of vegetables was also significantly lower than that observed in regular army feeding.

TABLE 3

Mean calorie and protein intakes as a percentage of recommended allowances

NUTRIENT	REFERENCE	RECOMMENDED ALLOWANCE	INTAKE AS PER CENT OF RECOMMENDED ALLOWANCE	
			Av. ± S.D.	Range
			%	%
Calories	NRC [1] and FAO [2]	$152W^{0.73}$	107 ± 9.9	82–138
Calories	AR 40–564 [3]	3,000	122 ± 10.8	93–155
Protein	NRC	1 gm/kg body wt.	212 ± 26.7	159–276
Protein	AR 40–564	100 gm/day	151 ± 15.8	115–191

[1] National Research Council ('53).

[2] Food and Agriculture Organization of the United Nations ('50).

[3] Army Regulation 40–564 ('56).

An increase in body weight was observed in 95 of the 98 men. The average increase over the 28-day period was 2.03 kg with a range of minus 1.20 to plus 5.41 kg. These weight increases indicate that the calorie intake was in excess of the average daily requirement. This is further supported by the data in table 3 which show that the mean calorie and protein intakes were greater than present-day recommended allowances (NRC, '53; Army Regulation, '56). The calorie intake of 74 of the 98 men was greater than the recommended allowance as determined by applying individual body weights to the formula:

$$E = 152\ W^{0.73}$$

where E is the total calorie requirement and W the body weight in kilograms (FAO, '50; NRC, '53). The constant, 152,

was used on the basis of an assumed activity level of 3200 Cal. per day where the individual is neither sedentary nor engaged in hard physical labor. Adjustments were not made in the formula for the slight differences from the reference standards for age or in environmental temperature.

During the ages of 18, 19 and 20 years, obviously, a large percentage of the male population has not attained physical maturity, and additional intakes of food for growth purposes

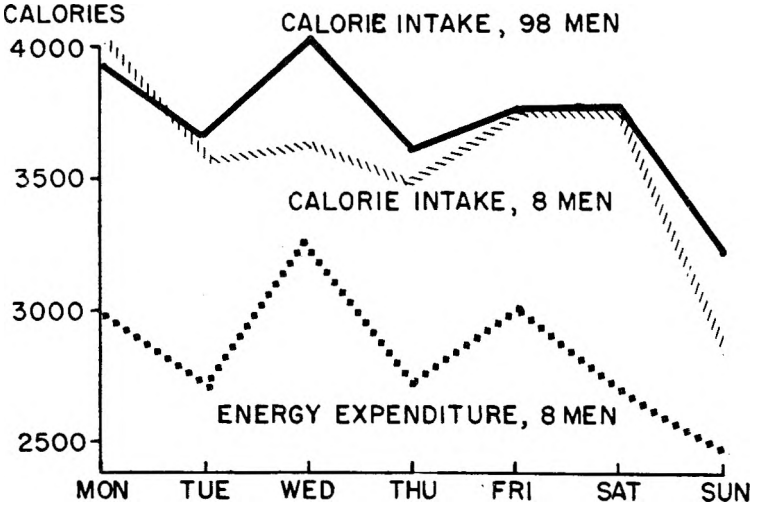

Fig. 2 Relation between caloric intake and energy expenditure for each day of the week (unadjusted for body weight change). The lower curve represents the average energy expended for each day of the week by 8 of the 98 men during a 14-day period; the middle curve represents the average calories consumed by the 8 men and the upper curve the average calorie intake for all 98 men during the same 14-day period.

are probably not uncommon. As a measure of this influence, the average calorie intake of each subject was compared according to age, height and weight. Age did not correlate significantly with calorie intake, nor with body weight and body weight change. The lack of correlation may reflect, in part, the narrow range of ages represented in this study. The correlation coefficient for height and calorie intake was 0.1837 which also was non-significant. The individual body

weight and calorie intake comparison resulted in a highly significant correlation coefficient of 0.2892 ($P < 0.01$). Partial correlation coefficients for body weight and calorie intake independent of age and height were 0.2932 ($P < 0.01$) and 0.2306 ($P < 0.05$), respectively.

Extensive daily energy expenditure studies were determined in 8 of the 98 men during a 14-day period.[5] The results of this study revealed a highly significant correlation between the energy expended and calories ingested during the same day. Inasmuch as the entire group of men followed the prescribed activity schedule, the mean energy expenditure of the 8 men may be assumed to represent that of the 98 men. In addition, the average calories consumed during each weekday by the 8 men did not differ significantly from that consumed by the 98 men during the same day. On the basis of this similarity, the relation between the average calorie intake for each day of the week and the energy expenditure of 8 and 98 men, on corresponding days, has been calculated as in figure 2. (The curves can be observed to move in a parallel manner for each day except Saturday.)

DISCUSSION

One of the reasons for conducting a nutrient intake study was to arrive at an estimate of the calorie requirements for a population under a particular situation. Many factors influence the calorie requirements of an individual. These include basal metabolic rate, body size, age, sex, energy expenditure and environment (FAO, '50; Orr and Leitch, '37–'38). The relation of intake to expenditure was illustrated in figure 2. The parallel relationship of the calorie intake and energy expended on the same day, with the exception of Saturday, is in contrast to the results obtained by Edholm et al. ('55). These authors found the best correlation between the dietary intake and the energy expended two days earlier. The high average intake on Wednesdays in the present study was ex-

[6] Brockett, Konishi, Brophy, Marcinek, Michalowicz, Grotheer and Kashin. Unpublished data.

pected, since the activity schedule for that day included a 7-mile contest march. The activity schedule for Monday included a three-minute step test which did not, in itself, involve a large expenditure. However, the decreased consumption of food on Sundays as a result of preacquired habits and tradition, would possibly influence the intake on the following day. Garry et al. ('55) also found that the intake of food on Sundays was significantly less than that for the other weekdays in studies of miners and clerks. A higher intake of calories on Monday, as a result of the decreased intake on Sundays was not apparent in their studies. The relatively high intake on Saturday cannot be explained on the basis of activity.

However, to assume that the optimal calorie requirement is synonymous with calorie requirement for energy balance excludes two major considerations. First, the energy expenditure reflects, within limits, the calorie intake (Keys, '49–'50). Experience has shown that humans have the ability to adapt themselves to limited intakes of calories (FAO, '50; Keys, '49–'50; Mitchell, '44). On a prolonged regimen restricted in calories, for example, the body conserves energy by decreasing basal metabolic activities and by decreasing the desire or ability to perform muscular work. The overfed organism, in turn, increases its mass and, as a consequence, the energy cost of certain activities is increased (Erickson et al., '45). Secondly, to consider the calorie requirement for energy balance as the calorie requirement, assumes that the existing body weight is ideal (Keys, '49–'50). Unfortunately, the "ideal" body weight for optimal health and physical performance is open to controversy. Nevertheless, it would be of interest to determine the level of calorie intake required for energy balance for this study in spite of the above limitations. Inasmuch as the majority of the subjects gained weight, it becomes necessary to adopt a procedure for estimating the calorie intake value of a unit of weight gain. Keys et al. ('55) recently investigated the composition of the weight gained by men overeating for 6 months on a constant activity regime and found it to be approximately 63% fat, 22% cells (of which

20% is protein) and 1% glycogen. Utilizing these values, the theoretical metabolizable energy equivalent of each gram of body weight gain was calculated as follows:

$$9.45 \times 0.63 = 5.95 \text{ fat Calories}$$
$$4.35 \times 0.20 \times 0.22 = 0.19 \text{ protein Calories}$$
$$4.22 \times 0.01 = 0.04 \text{ glycogen Calories}$$
$$\text{Total} = 6.18 \text{ Cal. per gram of body weight gain.}$$

This figure, however, does not represent the calories to be consumed in order to gain one gram of body weight. Additional calories are required to compensate for the loss through specific dynamic action, as well as the extra energy required to maintain and carry the increase in weight. As the effect of the latter factor probably would be small in this instance, only a 10% allowance for specific dynamic action need be included. Hence, the calorie intake equivalent for weight gain becomes approximately 6.8 Cal. for each gram of gain. If we assume that the composition of the weight gain in this study is similar to that observed by Keys, the subjects in this study, who gained an average of 73 gm per day, consumed 496 Cal. per day above the calorie balance level. Subtracting this value from the mean daily calorie intake (3669) would result in an apparent daily energy requirement of 3173 Cal. for weight maintenance.

The recommended allowance of calories for an activity level of 3200 Cal. is based on the equation $E = 152\ W^{0.73}$, suggested by the FAO Committee on Calorie Requirements (FAO, '50; NRC, '53). A corresponding equation was calculated from the data obtained in this study following adjustment for change in body weight. That is, the observed calorie intake of each individual was adjusted for body weight change by utilizing the 6.8 Cal. value for each gram of weight change. The adjusted intakes were then used to compute the following prediction equation:

$$E = 452.88\ W^{0.458}$$

where E is the estimated calorie requirement and W the body weight in kilograms. The two constants in the equation i.e., 452.88 and 0.458, were found to be highly significant when

tested by the t-test (Snedecor, '56). It was not possible to test the differences directly between these constants and the corresponding values in the FAO equation ('50) since the standard errors for the FAO constants are unknown. The exponential curves representing the two equations are shown in figure 3. It will be noted that the FAO equation would tend to overestimate the calorie requirements for those subjects in this study with the higher body weights.

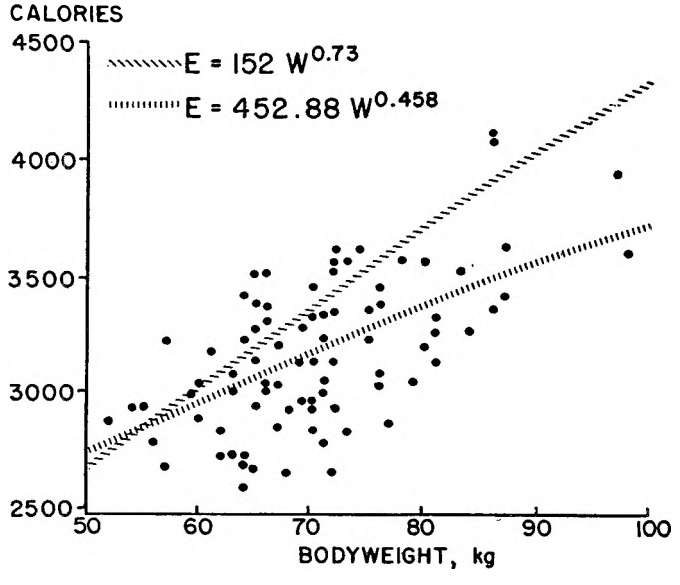

Fig. 3 Relation between body weight and caloric intake.

Table 4 presents the nutrient composition of the calories consumed in this study, that of Davis' infants, and those of soldiers in various training camps in temperate environments. The table shows that the percentage of calories from protein in this study was higher than that reported for soldiers, but equal to that reported for infants on an ad libitum intake. As previously mentioned, the subjects in the present study were not exposed to a true ad libitum intake in that choice of foods was limited to the foods offered. The menus, of

course, were planned to make available a balanced meal and this would tend to facilitate the ingestion of a nutritionally adequate diet. But evaluation of adequacy is difficult on the basis of available standards. While requirements for calories and protein have been defined, such is not the case with fat and carbohydrate.

The assessment of dietary adequacy on the basis of maintenance or change in weight also is difficult in the absence of fully defined standards. Standards of recommended weights are needed which will recognize occupation and activity level

TABLE 4

Nutrient composition of calories consumed by infants and soldiers

POPULATION	ENVIRONMENT	MEAN CALORIES PER DAY	CARORIES		
			Protein	Fat	Carbohydrate
			%	%	%
Infants [1,2]	Temperate	1211	17	36	47
Soldiers [3]	Temperate	3785	13	43	44
Soldiers [4]	Temperate	4265	13	43	44
Soldiers [5]	Temperate	3744	13	43	44
Soldiers [6]	Temperate	3669	17	40	43

[1] Davis ('28).
[2] Sargent et al. ('55).
[3] Howe and Berryman ('45).
[4] Consolazio et al. ('56).
[5] Schor and Swain ('49).
[6] Present study.

in addition to age, height and sex. It is obvious that clearly defined reference standards or criteria are particularly necessary for evaluating dietary adequacy when deficiency signs or symptoms are not apparent.

SUMMARY AND CONCLUSIONS

1. A food intake study of 98 soldiers was conducted under an ad libitum regimen during 28 days. Daily nutrient intakes were determined for each individual. The mean daily intakes of calories, protein, fat and carbohydrate from all sources were 3669 Cal., 150.7 gm, 162.3 gm, and 296.6 gm, respectively.

Ninety-five of the 98 men gained in body weight for an average gain of 2.03 kg over the 4-week period.

2. A parallel relationship was observed between calorie intake and energy expenditure for the same day. The intake was highest on Wednesday and lowest on Sunday, coincident with the highest and lowest activity levels, during the respective days.

3. By employing individual body weights and calorie intakes (adjusted for weight change) an equation was derived to estimate the calorie requirement for individuals of comparable age, sex and size as follows:

$$E = 452.88\ W^{0.458}$$

where E is the estimated calorie requirement and W the body weight in kilograms.

4. One of the most significant observations was the high intake of milk which averaged 1537 gm (one and one-half quarts) daily for each man.

5. The mean calorie and protein intakes were above the dietary allowances recommended by the National Research Council and above the minimum allowances specified by the Armed Forces for individuals under comparable activity levels.

ACKNOWLEDGMENTS

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DENTAL CARIES IN THE ALBINO RAT IN RELATION TO THE CHEMICAL COMPOSITION OF THE TEETH AND OF THE DIET

III. COMPOSITION OF INCISOR TEETH OF ANIMALS FED DIETS WITH DIFFERENT CA/P RATIOS

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On the basis of certain experimental observations there has been proposed the hypothesis (Sobel and Hanok, '48) that there is a relationship between the composition of the teeth and the fluid from which the tooth salts precipitate; and, that the composition of this fluid in turn is related to that of the blood serum, which may be modified by varying the Ca/P ratio of the diet. This relationship was first noted between the incisor teeth and blood serum of the albino rat fed diets with different Ca/P ratios (Sobel and Hanok, '48). Subsequently, a similar relationship was observed with respect to the molar teeth of the cotton rat (Sobel, '55). It had been suggested (Sobel, '52) that differences in susceptibility to dental caries might be related to the mineral composition of the teeth, which in turn might be related to the composition of the diet during the period in which the teeth are calcified.

In our previously reported experiments (Wynn, Haldi, Bentley and Law, '56) we found that the cariogenicity of the diet was affected by changes in the Ca/P ratio but that these

changes did not result in differences in the composition of the teeth. As we were primarily interested in the molar teeth and their susceptibility to caries, we did not deem it necessary to make chemical analyses of the incisors. It was later called to our attention that in Sobels's experiments the animals were placed on the experimental diets at 16 days of age whereas feeding was begun in our experiments when the animals were 21 days old, at which time calcification of the molars was much more nearly complete than in Sobel's experiments. This raised the question whether the different analytical results obtained in our experiments and in those of Sobel and his associates, might have been due (1) to the different Ca/P ratios or to different calcium and phosphorus levels of the diets or both, or (2) to the fact that in our experiments calcification of the molars had proceeded practically to completion at the initiation of the feeding period. To resolve this question it was decided to proceed further with our investigation and analyze the incisor teeth of the same animals. Fortunately these teeth had been preserved. The feeding period had extended over 70 days. As the rat's incisor replaces itself almost every 40 days all the tooth substance at the termination of the experiment would have been formed while the animal was on the experimental diet. It has been observed that the changes induced in the rat's incisors by dietary means are most pronounced at 70 days. (Gaunt, Irving and Thompson, '39). Analysis of the incisors should therefore show whether the composition of the tooth substance formed during the calcifying period was affected by the differences in the Ca/P ratios employed in these experiments.

EXPERIMENTAL PROCEDURE

Albino rats were selected in groups of 4 from the same litter at weaning and placed immediately on the experimental diets which were the same for each animal except for differences in the Ca/P ratio. There were 24 animals in each group. The diets, which had been found to be cariogenic, consisted of 64% sucrose, 20% casein, 8% fat, 4% yeast and liver extract and 4% salt mixture with vitamin supplements (Haldi and

Wynn, '52). All 4 diets contained 0.5% calcium, which provided an optimal calcium intake (Sherman and Booher, '31; Cox and Imboden, '36). The phosphorus content in diet 1, derived solely from the yeast and casein, was equal to one-half the calcium content giving a Ca/P ratio of 1:0.5. The amount of phosphorus in the other diets was varied by the addition of approximately neutral sodium phosphate [1] to give Ca/P ratios of 1:1, 1:2, and 1:3. These ratios and the calcium and phosphorus levels in the 4 diets have been shown to produce normal calcification of bone (Brown, Shohl, Chapman, Rose and Saurwein, '32). The calcium and phosphorus content of all the diets was verified by analysis.

The daily food intake of the 4 animals in each group was maintained fairly constant and exactly equalized at the end of each 10-day interval. All the animals were kept on the experimental diets for 70 days. At the end of the 70-day feeding period the animals were sacrificed and the incisors removed, cleaned and dried. They were then ground to pass through a 100-mesh sieve and dried in a vacuum. In order to obtain sufficient tooth substance for analysis the incisors of three animals on each diet were pooled. Enamel and dentin were separated by the flotation method of Manly and Hodge ('39) as modified by Gilda ('51).

The dried enamel and dentin were analyzed for nitrogen, calcium, phosphorus, magnesium and carbon dioxide by the following procedures: nitrogen, micro-Kjeldahl; calcium, Sobel, Rockenmacher and Kramer ('44); phosphorus, Fisk and Subbarow ('25); carbon dioxide, Van Slyke and Folch ('40), as modified by Sobel, Rochenmacker and Kramer ('44); magnesium, Young and Gill ('51). These methods are of sufficient precision to detect small changes in tooth composition. The agreements between results of replicate runs on the same samples were as follows: $N_2 \pm 0.02$; Ca $\pm$ 0.04; P $\pm$ 0.07; $CO_2 \pm 0.024$; Mg $\pm$ 0.005.

[1] Approximately neutral sodium phosphate was prepared by mixing 1 gm mol. of $Na\ H_2PO_4$ and 1 gm mol. of $Na_2\ HPO_4$. This mixture in solution had a pH of 6.5.

RESULTS

The composition of the enamel and dentin of the incisor teeth of the animals on the 4 diets with different Ca/P ratios is presented in table 1. It is apparent that varying the Ca/P ratio by changing the phosphorus content of the diet while maintaining the calcium content constant at the 0.5% level did not affect the precentage composition of either the incisor enamel or dentin in nitrogen (organic material), calcium, phosphorus, magnesium and carbon dioxde.

TABLE 1

Composition of incisor teeth of rats on diets with different Ca/P ratios[1]

Ca/P IN DIET	N	Mg	CO_2	Ca	P	Ca/P
	%	%	%	%	%	
			DRIED ENAMEL			
1:0.5	0.17±0.02	Trace	1.45±0.04	33.1±0.1	16.9±0.1	1.96±0.01
1:1	0.15±0.01	Trace	1.42±0.08	33.0±0.8	16.8±0.4	1.96±0.02
1:2	0.15±0.03	Trace	1.42±0.10	33.1±0.4	17.0±0.1	1.95±0.02
1:3	0.15±0.01	Trace	1.43±0.07	33.0±0.6	16.7±0.3	1.98±0.02
			DRIED DENTIN			
1:0.5	2.64±0.02	1.15±0.02	1.97±0.08	24.5±0.6	14.1±0.2	1.74±0.03
1:1	2.61±0.07	1.25±0.05	2.03±0.05	24.7±0.3	14.2±0.1	1.74±0.03
1:2	2.59±0.04	1.18±0.10	2.04±0.03	25.1±0.7	14.2±0.4	1.77±0.01
1:3	2.52±0.06	1.23±0.11	1.98±0.05	25.1±0.4	14.2±0.1	1.77±0.02

[1] Each value in the table is an average of 4 pooled samples. Each pooled sample was obtained from the teeth of three animals. In view of the small number of samples the ± values are given for the average deviations instead of the standard deviations.

The Ca/P ratio was lower in the dentin than in the enamel. These results are comparable with those reported by Hartles ('51) on the enamel and dentin of incisors of rats fed a high-sucrose diet and those of Sobel and Hanok ('48). These latter investigators found significantly lower Ca/P ratios in the dentin than in the enamel of incisor teeth of animals fed diets with different Ca/P ratios.

No magnesium could be detected in the enamel by microchemical analysis but traces were found upon spectrographic analysis. The dentin, on the other hand, was found to contain

more than 1% of magnesium. The organic portion of the dentin, represented by the nitrogen content was, as one would except, many times higher than in the enamel.

As it may be of interest to the reader to compare the composition of the molars and incisors, data on the enamel and dentin of the molars reported previously (Wynn, Haldi, Bentley and Law, '56) on the diet with a Ca/P ratio of 1:1 and those of the incisors on the same diet are presented in table 2. There was practically no difference between the composition of the molar and incisor enamel with two exceptions: the CO_2

TABLE 2

Comparison of the composition of molar and incisor enamel and dentin [1]

	N	Mg	CO_2	Ca	P	Ca/P
	%	%	%	%	%	
Molar enamel	0.19±0.01	0.14±0.03	2.44±0.03	33.2±0.2	16.7±0.2	1.99±0.02
Incisor enamel	0.15±0.03	Trace	1.42±0.10	33.0±0.8	16.8±0.4	1.96±0.02
Molar dentin	2.82±0.09	0.28±0.01	3.85±0.14	26.7±0.3	13.3±0.2	2.01±0.03
Incisor dentin	2.61±0.07	1.25±0.05	2.03±0.05	24.7±0.3	14.2±0.1	1.74±0.03

[1] Each value in the table is an average of 4 pooled samples. Each pooled sample was obtained from the teeth of three animals. In view of the small number of analyses the ± values are given for the average deviations instead of the standard deviations.

content was appreciably higher and the magnesium slightly higher in the molar than in the incisor enamel. The composition of the molar dentin, on the other hand was different from that of the incisor dentin in all the constituents analyzed and also in the Ca/P ratio. The differences with the exception of that of the nitrogen content were definitely statistically significant. The small difference in the nitrogen content of the incisor and molar dentin was of borderline significance. Final decision on its significance must await more work on the problem and more data. The most striking difference was in the magnesium content which was several times larger in the

incisors than in the molars. A similar difference in the magnesium in the incisor and molar dentin of the albino rat has been observed by Toverud ('23).

DISCUSSION

The present experiments, like those of Sobel and Hanok ('48), are concerned with the incisors, which grow continuously and are worn down by attrition. Under these circumstances calcification is a continuing process. In our experiments, unlike those of Sobel and Hanok, the composition of the incisors was not affected by changing the Ca/P ratio of the diet. How then can the different results in these two sets of experiments be reconciled? Most probably by the fact that the calcum and phosphorus content and the Ca/P ratios of our diets were within what might be called physiological limits, whereas in the experiments of Sobel and Hanok they were beyond these limits. The calcium in our experiments was maintained constant at 0.5% of the diet, which has been found to provide an adequate calcium intake. The phosphorus content was also adequate. The low-calcium-high-phosphorus diet of Sobel and Hanok contained only 0.028% of calcium. In the other two diets of Sobel and Hanok, the phosphorus content was low, namely, 0.124% as compared with 0.25% in our lowest phosphorus diet (Ca/P ratio 1:0.5). Our other three diets contained two, three and 4 times this amount of phosphorus. There was likewise a wide difference between the Ca/P ratios, namely, 1 : 27, 1 : 0.7 and 1 : 0.1 on a percentage basis in Sobel and Hanok's diets. It would therefore appear as if the hypothesis relating the inorganic composition of the incisor teeth to the calcium and phosphorus content and Ca/P ratio of the diet may be applicable only under certain conditions. In this connection it is of interest to recall that Gaunt and Irving ('40) found that with Ca/P ratios between 1:0.25 and 1:2, normal incisor teeth were formed in the rat only when the amount of these elements in the diet was at least 0.3%. When the intake of calcium and phosphorus was not adequate, the calcification of the teeth was more disturbed by diets with Ca/P ratios of

1:2 than by diets with Ca/P ratios of 1:1 or 1:0.25. The experiments of Toverud ('23) also have a bearing on this problem. Toverud found that changes in the composition of both growing incisors and the already-formed molars and also in the calcium content of the blood could be induced by feeding a diet deficient in calcium.

SUMMARY

Albino rats were fed 70 days from weaning cariogenic diets with 4 different Ca/P ratios, namely, 1:0.5, 1:1, 1:2 and 1:3.

The ratios were changed by maintaining the calcium content at the 0.5% level and varying the phosphorus content.

The nitrogen, calcium, phosphorus, magnesium and carbon dioxide content of the enamel and dentin of the incisor teeth were not affected by these dietary changes in the Ca/P ratio.

These results are discussed with reference to the hypothesis that changes in the Ca/P ratio of the diet may affect the composition of the blood which in turn may affect the composition of the developing tooth.

ACKNOWLEDGMENT

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DIET COMPOSITION AND MINERAL BALANCE IN GUINEA PIGS[1]

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The guinea pig is highly susceptible to soft tissue calcification. Wulzen and Bahrs ('41) first reported a stiffness syndrome which has been studied in detail and reviewed by van Wagtendonk and Wulzen ('50). The syndrome was characterized by stiff joints and by extensive soft tissue calcification and necrosis. A similar syndrome was reported by Hogan et al. ('50, '54) in guinea pigs that consumed excess phosphorus. Smith et al. ('49) also observed metastatic calcification when certain diets were fed for long periods.

Roine et al. ('49) pointed out that for maximum growth the guinea pig has a high requirement for potassium and magnesium. House and Hogan ('55) observed that high levels of these elements protect guinea pigs from soft tissue calcification. Magnesium deficiency has been implicated in metastatic calcification in other species including the calf (Moore et al., '38), the cotton rat (Constant and Phillips, '54) and the white rat (Tufts and Greenberg, '38). The rabbit has a high requirement for potassium (Hove and Herndon, '55), but part of the requirement can be met by other cations (Wooley and Michelson, '54). The high cation requirement of guinea pigs is partly due to their inability to conserve fixed bases by excreting ammonia in the urine (O'Dell et al., '56). Gum arabic has been reported to be superior to celluflour as a

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source of bulk (Booth et al., '49; House and Hogan, '55), possibly because of its mineral content.

The reluctance of investigators to use the guinea pig for balance studies may have resulted from the fact that the habits of the species are not readily adaptable to the necessary conditions. Nevertheless, it seemed that a study of mineral balances might help explain the superior nature of gum arabic, the injurious nature of high levels of phosphorus, and the ameliorative function of high levels of potassium and magnesium in the diet of the guinea pig. Balance studies of calcium, magnesium, potassium, sodium and phosphorus are reported here.

The balance studies show that a high phosphorus intake results in a negative balance of potassium and magnesium. Although the endogenous fecal excretion of the various minerals was not determined and hence true absorption cannot be calculated, apparent absorption values are reported because they strongly indicate that a high phosphorus level impairs magnesium absorption.

EXPERIMENTAL

Guinea pigs 14 weeks of age or older were used. In general, the animals had received a purified diet from weaning, and in all cases they consumed the respective experimental diets during a three-week preliminary conditioning period. During this period they became adjusted to a small individual cage, to a water bottle, and to a pelleted ration. An additional period of three or 4 days was required for the animals to adjust to the metabolism cage proper.

The metabolism cage was made of galvanized hardware cloth and was small enough to prevent the animals from turning around. The usual size was $9 \times 4 \times 3$ inches and it restricted their movements considerably. Restriction of movement was necessary to prevent coprophagy and to permit accurate collection. The animals were removed from the metabolism cage one hour each day and allowed to exercise in small cages with raised wire bottoms from which quanti-

tative collection could be made. The feces, urine, and refused food were collected daily and fresh food was weighed into the feeder. The dry weight of the food offered as well as that of the food refused was determined and weight of food consumed was determined by difference. Distilled water was supplied ad libitum. Collections were made for a period of 10 days and the daily samples were pooled and refrigerated. The pooled samples of feces were dried in vacuo, weighed, and homogenized before removal of an aliquot for analysis. Two milliliters of concentrated HCl were added to the urine collection bottle daily to serve as a preservative. The fecal receptacle and funnel were rinsed with dilute HCl and distilled water to remove dried urinary salts and the rinsings were combined with the urine sample. Each urine sample was filtered and the total volume determined before removal of an aliquot for analysis.

Phosphorus was determined by a modification of the method of Fiske and Subbarow ('25) after the sample was wet ashed with a mixture of sulfuric, nitric and perchloric acids. In the case of the urine samples it was necessary to remove the excess tin which arose from washing the cage with acid. This was done by treatment with cupferron before ashing. For the other elements, another aliquot was dry ashed at about 550°C. After removal of phosphate ion by use of an ion exchange resin, the calcium and magnesium were determined by the versene titration method of Cheng and Bray ('51). Potassium and sodium were determined by a commonly accepted flame photometric method.

The basal ration was the same as used by O'Dell et al. ('56) and contained acid-washed casein 30; sucrose 47; celluflour 15; soybean oil 4; salts [2] 4% and vitamin supplements. This diet contained about 0.9% of calcium, 0.4% of phosphorus, 0.5% of potassium, 0.1% of sodium and 0.1% of magnesium. The phosphorus content of the diet was increased by use of the salt mixture of Richardson and Hogan ('46) and by the

[2] The salt mixture of Hubbell et al. ('37) to which was added 2.5 gm of $MnSO_4 \cdot 4H_2O$ per 100 gm.

TABLE 1

Experimental design and performance of animals. Daily average for a 10-day period

RATION					AV. WT. OF ANIMALS		FOOD CONSUMED	DIGESTIBILITY OF DRY MATTER	MINERAL CONSUMPTION				
No.[1]	Approximate composition			Type of bulk [2]	Initial	Gain or loss			Ca	Mg	K	Na	P
	P	K	Mg										
	%	%	%		gm	gm		%	mg	mg	mg	mg	mg
3883	0.4	0.5	0.1	GA	513	1.9	21.4	95.0	214	12.9	116	25.7	94.5
3884	0.4	0.5	0.1	C	502	−0.5	22.4	80.2	190	6.7	130	29.1	103.0
3349	0.4	1.5	0.3	GA	538	3.3	21.3	94.8	217	72.3	345	21.3	93.7
3479	0.4	1.5	0.3	C	586	0.05	20.4	81.3	208	67.2	277	22.4	85.5
3514	0.9	0.5	0.1	GA	491	2.1	17.3	92.2	163	13.8	97.0	40.2	154.0
3480	0.9	0.5	0.1	C	457	0.4	18.9	79.5	179	14.2	86.6	47.6	174.0
3516	0.9	1.5	0.3	GA	570	1.4	23.0	93.2	258	78.1	395	59.7	210.0
3515	0.9	1.5	0.3	C	536	2.1	22.2	80.5	206	77.0	306	62.5	206.0
3476	1.8	0.5	0.1	GA	381	0.8	14.7	89.3	147	12.6	70.3	125.0	255.0
3475	1.8	0.5	0.1	C	475	−2.5	21.1	78.2	213	12.7	90.7	181.0	375.0
3478	1.8	1.5	0.3	GA	540	2.3	16.0	94.6	164	57.5	225	132.0	289.0
3477	1.8	1.5	0.3	C	417	2.5	19.6	78.5	200	70.8	276	170.0	351.0

[1] The same as our laboratory number. There were three or 4 animals per group.

[2] Celluflour (C) or Gum Arabic (GA).

addition of 3.6% $NaH_2PO_4 \cdot H_2O$. The potassium content was increased by the addition of 2.7% potassium acetate and the magnesium by addition of 0.5% magnesium oxide.

The experimental design and the approximate composition of the diets are shown in table 1. There were 4 diets of low (0.4%), 4 of medium (0.9%) and 4 of high (1.8%) phosphorus content. Within each group two diets had added potassium and magnesium and will be designated as high-potassium and magnesium diets. At each potassium and magnesium level one diet contained celluflour and the other gum arabic.

RESULTS

The average daily performance of the animals is shown in table 1. Most of the animals were near mature weight and gained slowly or not at all during the 10-day experimental period. The food consumption averaged about 20 gm per day except for the animals on ration 3476 and this was partly due to their smaller initial weight. The daily calcium consumption averaged about 200 mg. The intake of magnesium ranged from 7 to 78 mg, potassium from 70 to 395 mg, sodium from 21 to 181 mg and phosphorus from 86 to 375 mg, varying chiefly because of the composition of the diet. The consumption data were calculated from the average percentage composition determined by at least three analyses.

The average percentage digestibility of the dry matter of gum arabic diets was 93.5% (range, 89 to 95%) and of celluflour diets 79.8% (range, 78 to 81%). The difference between the average digestibility of the two is 13.5%, a number which is about equal to the percentage of bulk added. One may conclude that celluflour is poorly digested whereas gum arabic is almost completely digested.

The effect of dietary constituents on the absorption of minerals is summarized in table 2. For brevity of presentation the gum arabic diets were combined and compared with the combined celluflour diets and the high potassium and magnesium diets compared with the diets of lower content. The terms absorption or apparent absorption as used here

TABLE 2

Apparent absorption of minerals by the adult guinea pig

MINERAL	DIETARY VARIABLES COMPARED						
	Type of bulk		K and Mg level		Phosphorus level		
	C (22)[1]	GA (20)	Low (19)	High (23)	0.4% (14)	0.9% (15)	1.8% (13)
	%	%	%	%	%	%	%
Calcium	46 ± 4[2]	58 ± 4	48 ± 4	54 ± 4	70 ± 2**	45 ± 3	37 ± 4**
Magnesium	65 ± 14	76 ± 10	20 ± 71**	78 ± 3**	84 ± 10	77 ± 3	46 ± 23
Potassium	84 ± 2	95 ± 2	81 ± 2	92 ± 1	92 ± 2	90 ± 2	84 ± 3
Sodium	78 ± 3	91 ± 1	80 ± 3	86 ± 3	80 ± 5	83 ± 3	84 ± 3
Phosphorus	43 ± 2	49 ± 4	46 ± 2	45 ± 4	46 ± 4	42 ± 3	48 ± 5

[1] Number of trials averaged is shown within parentheses.

[2] Standard error of the mean.

** P value less than 0.01 when compared by the Fisher "t" test.

may be defined as 100 minus the percentage of the consumed nutrient recovered in the feces. The absorption of calcium, magnesium, potassium, and sodium was about 10 percentage units greater when gum arabic diets were fed than when celluflour diets were fed. Phosphorus absorption was essentially unaffected.

The higher levels of potassium and magnesium had no effect on the percentage absorption of phosphorus but increased that of calcium, magnesium, potassium and sodium. It seems unlikely that the difference in absorption of calcium and sodium is beyond the limit of experimental error. The percentages of potassium and magnesium absorbed were higher and the absolute amounts absorbed were markedly higher when the high-potassium and magnesium diets were fed. The difference in percentage absorption of magnesium was statistically significant at the 1% level. On the low-magnesium diets, some animals excreted more magnesium in the feces than they consumed.

The absorption of calcium and magnesium on the high level of phosphorus was about 50% of the value observed on the low level of phosphorus. The effect on sodium and phosphorus was of little or no significance. Although not shown in table 2, the average absorption of magnesium on the low-magnesium, high-phosphorus diets was —60% compared to + 66% on the medium-, and + 42% on the low-phosphorus diets. There was a trend toward lower absorption of potassium as the dietary phosphorus level was increased. Although the percentage difference was not great, the absolute difference was of considerable importance to animals that consumed a low-potassium diet.

The effect of dietary constituents on mineral balance is summarized in table 3. Animals that consumed gum arabic showed a higher retention of calcium and potassium than those on celluflour, but the differences were not statistically significant. High levels of dietary potassium and magnesium increased the retention of these cations and the effect on magnesium retention was significant at the 1% level. The

TABLE 3

Mineral balance of adult guinea pigs

Average balance expressed as milligrams per day per kilogram of body weight

MINERAL	DIETARY VARIABLES COMPARED						
	Type of bulk		K and Mg level		Phosphorus level		
	C (20)[1]	GA (19)	Low (19)	High (20)	0.4% (14)	0.8% (15)	1.8% (10)
Calcium	37 ± 9[2]	54 ± 12	44 ± 10	46 ± 11	65 ± 8	33 ± 13	37 ± 15
Magnesium	−1.5 ± 6	−3 ± 4	−11 ± 6**	6 ± 3**	0.2 ± 4*	9 ± 2*	−23 ± 8*
Potassium	20 ± 10	35 ± 12	20 ± 9	34 ± 13	48 ± 14	30 ± 13*	−6 ± 5*
Sodium	9 ± 6	−3 ± 6	−1 ± 6	7 ± 6	0.8 ± 3	−0.8 ± 6	12 ± 11
Phosphorus	41 ± 9	29 ± 11	37 ± 11	34 ± 9	29 ± 5	38 ± 11	42 ± 23

[1] The number of animals used is shown within parentheses.

[2] Standard error of the mean.

* P value less than 0.02.

** P value less than 0.01.

effect on the other elements was small and of little significance.

Sodium and phosphorus balances increased as the consumption of these elements increased, but the calcium balance was decreased by high levels of phosphorus. The most significant effect of the high-phosphorus level was to change the magnesium and potassium balance from positive to negative. A major consideration throughout this investigation was to determine when the animals were properly adjusted to the experimental diets. In these trials the animals on the medium-phosphorus diets were in exceptionally good condition and the animals on the low-phosphorus diets were slightly below

TABLE 4

Apparent absorption, pathway of excretion, and balance of minerals in adult guinea pigs fed purified diets [1]

MINERAL	APPARENT ABSORPTION	EXCRETED IN THE URINE [2]	BALANCE PER KG OF BODY WEIGHT PER DAY
	%	%	*mg*
Calcium	69	62	65
Magnesium	88	87	7
Potassium	94	93	53
Sodium	78	78	0.2
Phosphorus	44	34	24

[1] Rations 3349 and 3479, 4 animals each.

[2] Expressed as per cent of total urinary and fecal excretion.

normal in appearance. Consequently, the higher magnesium balance for the medium-phosphorus than for the low-phosphorus diets probably does not represent better nutrition.

From the standpoint of comparative physiology it is of interest to compare the guinea pig with other species as regards mineral metabolism. In table 4 are shown the average values of apparent absorption, pathway of excretion and balance of the various elements in animals fed the most nearly adequate purified diets used in this laboratory. The percentage of apparent absorption of calcium and magnesium is higher for the guinea pig than has been reported for most species. Since apparent absorption is not corrected for the

portion of the element excreted by way of the intestine, it is not possible to tell whether this difference lies in their ability to absorb the element more effectively or in differences in pathway of excretion. Regardless of this consideration, the adult guinea pig absorbs a high percentage of the calcium and magnesium in a purified diet and excretes a similar percentage of the excess elements by way of the urine. The percentage absorption of the other elements is comparable to that observed in other species.

The ratio of calcium to phosphorus retained should be about 1.5 to 1. The ratio observed in these animals was almost 3 to 1. This discrepancy probably arises due to analytical error and to the short duration of the balance study. The retention of potassium is greater than that of sodium as would be expected, but the high retention of potassium may reflect an accumulation of analytical errors.

DISCUSSION

The superiority of gum arabic over celluflour and other sources of bulk reported by Booth et al. ('49) and by House and Hogan ('55) is no doubt due in part to its ash content and in part to the fact that its high digestibility allows more complete absorption of cations. Since gum arabic is at least 90% digested by the guinea pigs, it seems unlikely that its value lies in providing "bulk" in the usual sense of the word. Larrivee and Elvehjem ('54) noted that chinchillas fed gum arabic diets produced far less feces than those fed collulose. This difference was attributed to constipation in the animals fed gum arabic. Guinea pigs fed gum arabic diets also produce small and scanty fecal pellets, and it seems possible that the explanation in both species is the high digestibility of gum arabic.

It is well recognized that a high calcium content of the diet increases the severity of magnesium deficiency and raises the magnesium requirement, but to the knowledge of the authors the detrimental effect of phosphorus on the absorption of magnesium has not been recognized. When the dietary

potassium and magnesium levels were low, the apparent absorption of magnesium was low and the absorption was decreased further by high levels of phosphorus. It is recognized that the endogenous excretion of magnesium would account for an appreciable amount of the fecal magnesium in the case of the low magnesium diets, but the important point is that high levels of phosphorus decreased the absorption regardless of the magnesium level.

Guinea pigs fed high-phosphorus diets that contain levels of potassium and magnesium commonly fed to other laboratory animals, such as rats and chickens, fail for at least two reasons; there may be other less obvious reasons. They go into a negative potassium balance, because of their inability to conserve fixed bases effectively (O'Dell et al., '56). They also show a negative balance of magnesium which is apparently due to the effect of phosphorus on the absorption of magnesium. There was no evidence for an increased urinary excretion of magnesium on the high-phosphorus diets.

Since a magnesium deficiency is known to cause calcinosis in other species, the soft tissue calcification observed in the guinea pig is probably due largely to a deficiency of this element. A high content of phosphorus in the diet contributes to the syndrome primarily by interfering with magnesium absorption and secondarily by maintaining a high blood phosphorus level (O'Dell et al., '56).

SUMMARY

Guinea pigs near maturity were used to study the effect of type of bulk, and of dietary levels of potassium, magnesium and phosphorus on apparent absorption and retention of calcium, magnesium, potassium, sodium and phosphorus.

The most significant observation was that high dietary levels of phosphorus caused a negative balance of magnesium in guinea pigs that received about 0.1% of magnesium in the diet. The low retention was primarily due to the decreased absorption of magnesium in the presence of excess phosphorus regardless of the magnesium level. A similar though less

marked effect was observed on the absorption and retention of potassium and calcium.

Gum arabic was found to be highly digestible whereas celluflour was essentially indigestible. The absorption of all cations was about 10 percentage points higher from diets that contained gum arabic than from those that contained celluflour.

Guinea pigs absorbed approximately 70% of the calcium and 90% of the magnesium consumed when they were fed a purified diet that contained 0.9% of calcium, 0.3% of magnesium and 0.4% of phosphorus. Large portions of the excreted calcium and magnesium, 62 and 87% respectively, were eliminated by way of the kidneys.

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THE EFFECT OF VITAMIN D-DEFICIENT DIETS CONTAINING VARIOUS CA:P RATIOS ON CATS [1,2]

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The few nutritional studies of cats available indicate that the nutritional requirements of cats may differ from those of other species in many details. Gershoff et al. ('57) have reported an inability of cats to convert carotene to vitamin A. They also found that the absorption of vitamin A by cats was related to the fat content of the diet. These workers and others (Krehl et al., '55; daSilva, '50) have reported that cats appear to require high-fat diets and they routinely feed cats purified diets containing 25 to 30% of fat. Most studies of experimental rickets have been conducted on rats, a species in which rickets cannot be produced unless the ratio of dietary calcium and phosphorus is altered. The desirability of studies of rickets in other species in addition to the peculiarities in the cat's need for fat and fat-soluble vitamins led to the

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[2] Preliminary results were presented at the 20th Annual Meeting of the American Institute of Nutrition, Atlantic City, New Jersey, 1956.

present study of the effect of feeding vitamin D-deficient rations and various Ca : P ratios to cats.

EXPERIMENTAL

Three- to 6-month-old kittens of mixed sexes were used in these experiments. Before being admitted to the laboratory, they were dusted with an insecticide, dewormed and vaccinated against feline distemper. The cats were housed individually in metal mesh cages and food and water were maintained ad libitum. Before being placed on experiment, the kittens were fed a high-fat, high-protein purified diet for two weeks so that those which would not accept purified diets could be identified and discarded. The remaining animals were divided into 4 groups and fed diets for periods up to 21 months with and without vitamin D which contained either 1% of calcium and 1% of phosphorus or 2% of calcium and 0.65% of phosphorus. These diets contained casein 32.1, corn oil 13, hydrogenated fat 13, Jones and Foster ('42) salt mix, minus its calcium and phosphorus salts, 1.8 and choline 0.3%. In addition the 1 : 1 Ca : P diet contained sucrose 36.4 and Ca_2HPO_4 3.4, the 2 : 0.65 Ca : P diet sucrose 34.3, Ca_2HPO_4 1.8 and $CaCO_3$ 3.7%. Vitamin supplements consisted of 4 mg thiamine, 8 mg riboflavin, 40 mg niacin, 20 mg Ca pantothenate, 4 mg pyridoxine, 1 mg folic acid, 0.2 mg biotin, 1 mg menadione, 25,000 I.U. vitamin A per kilo of ration. Control cats were given 250 I.U. of vitamin D_3 orally twice each week.

Periodically during the course of the experiment, x-rays and serum calcium, phosphorus, alkaline phosphatase and citric acid determinations were made. At death, fat-free femur ash was determined and histologic examination was made of the tissues.

RESULTS

Survival. The kittens did not accept the diets containing the 2 : 0.65 Ca : P ratio as well as they did those with the 1 : 1 ratio. At the end of 6 weeks, 7 of 17 kittens being fed the diet with the 2 : 0.65 ratio of calcium to phosphorus had died.

The poor performance on this diet appeared in part due to palatability factors since some of the kittens stopped eating on being fed this diet. However, other kittens ate well for a few weeks and then stopped eating. Twelve of the 13 animals fed the diet with the 1:1 ratio were alive after 6 weeks and all but one of them, a vitamin D-supplemented animal, were eating well and growing rapidly. These data are summarized in table 1. Most of the cats receiving vitamin D-deficient diets developed rickets in about 4 to 5 months. However, the rickets produced on the 1:1 ratio was more severe than that on the 2:0.65 ratio, possibly as a result of more rapid growth (table 1). By 11 months, three of the 6 cats

TABLE 1

Effect of vitamin D and Ca:P ratios on growth and survival of cats

Ca:P RATIO	NO. OF CATS	CATS ALIVE AT			WEIGHT GAIN IN 20 WEEKS
		6 weeks	20 weeks	21 months	
					gm
1:1 + vitamin D	6	6	5	4	1320 (710–2400)
1:1 no vitamin D	7	6	5	1	1295 (700–1720)
2:0.65 + vitamin D	8	4	3	1	1065 (700–1530)
2:0.65 no vitamin D	9	6	6	6	980 (620–1770)

fed the low-calcium (1:1) vitamin D-deficient diet had died, presumably because of anorexia caused by acute rickets. One cat died after three months with the additional finding of fatty nephrosis; and another, having survived the period of acute rickets, succumbed to a combination of acute sinus infection and cholangitis after 17 months on experiment. One cat in this group survived the entire experimental period, while none of the 6 cats fed the vitamin D-deficient, high-calcium (2:0.65) diet died. Three of the 4 cats fed the high-calcium diet with vitamin D which survived the 6-week initial period died after having been on experiment three, 10 and 16 months respectively. Fatty nephrosis was present in the first cat and cholangitis in the second. The cause of death of

the third cat was not determined, as a histological examination could not be made. The loss of these animals was unexpected, especially since the 6 vitamin D-deficieut cats for which they were controls survived the entire experiment. Four of the 6 cats fed the low-calcium diet with vitamin D lived through the experiment. These animals were healthy at all times and grew well. One of the two which died had a severe respiratory infection accompanied by a fatty nephrosis; the other, previously mentioned, did poorly during the entire period it was fed the experimental diet and a fatty nephrosis was the only lesion found by autopsy.

Serum studies. Serum analysis results (table 2) indicate that alkaline phosphatase determination is the most sensitive chemical means for evaluating the state of rickets in cats. Although these analyses were made throughout the experimental period, only selected data representing the results obtained are presented. There was a marked rise in serum alkaline phosphatase in most of the vitamin D-deficient cats in approximately the third month. The elevated phosphatase values were at a peak during the 5th to 7th months, then gradually decreased until the end of the experiment when all of the surviving cats showed approximately the same values whether or not they were receiving vitamin D. The serum alkaline phosphatase reached a higher level and decreased more slowly in the rachitic cats receiving the diet with a Ca: P ratio of 1:1 than in those receiving the 2:0.65 ratio. In each group of rachitic cats there was considerable variation in individual phosphatase values. These values appeared to be correlated with the degree of rickets as determined by x-ray examination of the animals. After having been on experiment for 7 months, the serum alkaline phosphatase of one of the rachitic cats fed the 1% calcium diet was 25.6 units. Inasmuch as alkaline phosphatase values are usually highest in growing animals and many enzyme systems have been shown to be sensitive to short fasting periods, food was withheld from this animal for 5 days. During this time the cat's weight dropped from 3060 to 2790 gm, but the serum

TABLE 2

Effect of vitamin D and Ca: P ratios on cat serum alkaline phosphatase, calcium, phosphorus and citric acid

Ca: P RATIO	NO. OF CATS	MONTHS ON EXPERIMENT	ALKALINE PHOSPHATASE [1]	Ca	P	CITRIC ACID
				mg %	*mg %*	*mg %*
1:1 + vitamin D	6	1	3.4 ± 0.9	...	...	...
	5	4.5	4.2 ± 0.2	9.6 ± 0.3	4.3 ± 0.5	...
	4	9	1.7 ± 0.6	10.8 ± 0.8	2.6 ± 0.6	5.9 ± 0.3
	4	21	1.0 ± 0.4	10.2 ± 0.5	3.4 ± 0.2	...
1:1 no vitamin D	6	1	4.3 ± 0.8	...	...	...
	5	4.5	12.1 ± 2.0	7.8 ± 0.5	4.2 ± 0.3	...
	5	9	7.9 ± 2.0	5.6 ± 1.0	1.7 ± 0.4	4.4 ± 0.1
	1	21	1.2	9.8	4.5	...
2:0.65 + vitamin D	4	1	3.8 ± 0.9	...	...	...
	3	4.5	4.0 ± 0.5	10.8 ± 0.2	3.9 ± 0.4	...
	3	9	1.4 ± 0.5	10.6 ± 0.4	3.1 ± 0.4	6.7 ± 0.4
	1	21	0.4	8.4	2.9	...
2:0.65 no vitamin D	6	1	3.1 ± 0.6	...	...	...
	6	4.5	9.0 ± 1.8	9.4 ± 0.3	2.8 ± 0.5	...
	6	9	3.7 ± 0.9	10.9 ± 0.7	1.8 ± 0.2	5.7 ± 0.4
	6	21	1.6 ± 0.2	9.6 ± 0.2	2.6 ± 0.3	...

[1] Alkaline phosphatase reported in units of Bessey et al. ('46). Figures include standard error of the mean.

alkaline phosphatase was not significantly affected, being 22.4 at the conclusion of the fasting period, a decrease which could be expected as a result of normal variation in phosphatase values.

All through the period of active rickets serum calcium values of rachitic cats fed the 1:1 Ca:P ratio were significantly lower than those of animals receiving the same diet with vitamin D. This was not true of the groups of cats receiving the 2:0.65 ratio. These two groups showed essentially the same serum calcium throughout the experiment.

Serum phosphorus was generally lower in the rachitic cats than their controls although not to a very great extent. In table 2 the serum phosphorus values at 4.5 months for both groups receiving the 1:1 Ca:P ratio are the same. This was the only set of determinations made during the period of active rickets in which this occurred. At 6.5, 7.5 and 9 months the serum phosphorus of the cats fed the 1:1 ration with vitamin D were 4.6, 5.3 and 2.6 mg %, respectively, and those of the rachitic cats fed the 1:1 ratio were 2.6, 3.1 and 1.7. Dickens ('41) has reported a low bone citric acid in a rachitic cat. Serum citric acid has also been reported depressed in rachitic animals of a number of species (Freeman and Chang, '50; Harrison and Harrison, '52; Steenbock and Bellin, '53). Serum citric acid values obtained when the cats had been on experiment 9 months indicated that this probably is true also for cats. The cats fed the 1:1 ratio without vitamin D had significantly less serum citrate than those in the other groups. The cats fed the 2:0.65 ratio without vitamin D also showed a trend toward having less serum citrate than their controls, but by this time their rickets was healing and probably their serum citrate had risen.

Urine studies. Studies of urinary calcium, phosphorus, nitrogen and citric acid initiated after the cats had been on experiment 8 to 14 months did not reveal any significant excretion differences which could be associated with the presence or absence of vitamin D. Urinary citric acid varied from 4 to 22 mg per cat per week and nitrogen from 6 to 13

gm per cat per week. Telfer ('24) has pointed out that calcium intake markedly influences phosphorus absorption, and Nicolaysen ('53) that the influence of phosphorus on calcium absorption is negligible. The results obtained in these experiments are in accord with these observations. Several cats from each group were placed on diets in which the calcium content was varied but the phosphorus held constant and

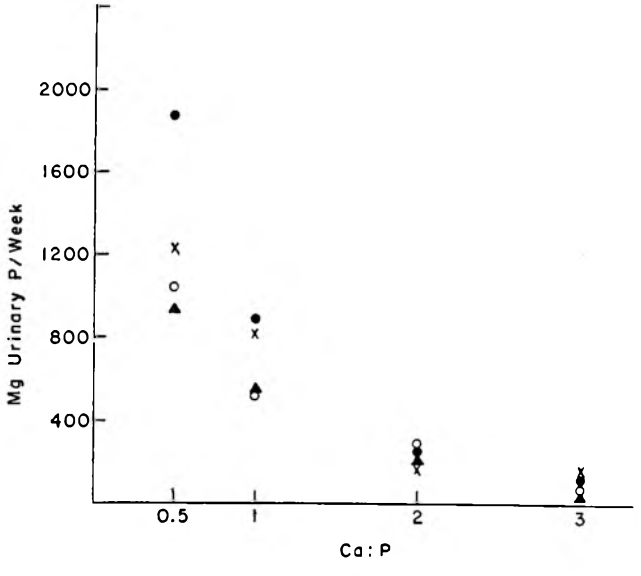

Fig. 1 The effect of dietary calcium on urinary phosphorus.
● = 1.01% P with vitamin D; X = 1.01% P without vitamin D
○ = 0.65% P with vitamin D; ▲ = 0.65% P without vitamin D

urinary phosphorus and calcium measured. The marked effect of dietary calcium on phosphorus excretion and therefore absorption is shown in figure 1 in which each point represents a minimum of 4 weekly collections obtained from two or more cats. On the 0.5:1, 1:1 and 2:1 Ca:P ratios urinary calcium was usually less than 10 mg per cat per week and slightly higher on the 3:1 ratio.

Bone ash studies. The ash content of the fat-free cat femurs was found to be over 50% for all cats which received vitamin

D as well as for those cats which did not receive vitamin D, but which survived the entire experiment. These values ranged up to 58% with the exception of one value of 62%. All of the cats which died during acute rickets showed considerably less bone ash with the lowest value being 36% in a cat which died after being on experiment 9 months.

Histologic studies. Autopsies were performed on all but two of the animals which survived the first 6 weeks of the experiment and hematoxylin- and eosin-stained sections of representative organs and decalcified bones were examined. The bones most frequently examined histologically were the ribs at the costo-chondral junctions, the distal end of the femur, the head of the tibia and a metatarsal. Parathyroids were seen in 11 cats representing each of the dietary groups.

Of the 5 animals examined which had been fed the vitamin D-supplemented 1:1 Ca:P diet for long periods, all had normal bones. Of the 5 animals studied histologically after a significant period of time on the vitamin D-deficient 1:1 Ca:P diet, three dying between the 9th and 17th month had severe rickets. The most marked changes were at the costo-chondral junctions producing the characteristic "rachitic rosary." Of the two remaining animals in this group, one which was killed after 21 months had normal bone with epiphyseal plates almost closed, and one which died during the third month had normal bone with epiphyseal plates present. The lack of rickets in this latter animal was probably due to the short period on the rachitogenic diet.

The histologic appearance of rickets as seen in ribs was characterized by a markedly irregular costo-chondral line, a thick irregular layer of mature cartilage, disorganization in the region of new bone formation, excessive osteoid, increased numbers of osteoblasts and a swollen costo-chondral junction. The rickets produced in these cats and that seen in man are the same morphologically.

Of the three animals examined on a 2:0.65 Ca:P vitamin D-supplemented diet, two had normal bones. The third, an animal which died after three months on the diet, had a slightly

irregular line of ossification at the epiphyseal plate of the tibial head without any other evidence of rickets. Of the 6 animals on the same diet without vitamin D, all except one, killed after 21 months, had some evidence of rickets or osteomalacia. The evidence of osteomalacia was an increased number of bone spicules in the metaphysis or adjacent to the costochondral junction, thick irregular bone spicules with crossed cement lines, slight osteoid excess and sometimes a residual irregular costo-chondral line. Some of these changes represented the "scars" of healed rickets and were not necessarily evidence of a true active osteomalacia.

Parathyroid size was estimated by the method of Luce ('23). The parathyroids varied from 0.76×0.39 mm to 3.23×2.65 mm. The two largest parathyroids, 3.23×2.65 mm and 3.21×2.51 mm, which were two of three glands found in a severely rachitic cat, contained some clear cells among the predominant chief cells. These two glands were the only two apparently increased in size. Castleman ('52), however, has pointed out the marked variability in size of the parathyroids in individuals with secondary hyperparathyroidism. Only one other gland was found to contain cells other than chief cells. Parathyroid hyperplasia has been produced by parenteral administration of phosphates (Drake et al., '37) or by marked calcium dietary deficiency (Luce, '23) and has been reported as being found occasionally secondary to rickets (Castleman, '52).

Of the incidental pathologic findings, the most interesting in addition to the pneumonia and purulent bronchitis which was observed in some animals was fatty nephrosis seen in 5 cats. This lesion appeared as a distinctive marked vacuolation in the luminal sides of the cytoplasm of cells of the proximal convoluted tubules and of Henle's loop. These vacuoles were observed in frozen sections stained with Sudan IV. Crystalloids were occasionally associated with them. The cytoplasm of involved cells was granular and refractile whenever the vacuole or vacuoles did not completely replace the cytoplasm. Although all 5 of the animals with this lesion died, the sig-

nificance of the lesion is not established. It has been found frequently in cats on other diets in this laboratory.

X-ray studies. At the onset of x-ray studies, 4.5 months after the beginning of the experiment, the average age of the cats was 7.5 to 10.5 months. As expected, no evidence of rachitic change was seen in cats which received vitamin D. Commonly seen in the initial film studies, especially of the cats which received vitamin D and the 2:0.65 Ca:P diet, was increased density in the metaphyseal regions (metaphyseal sclerosis). This usually disappeared within three months. Fusion of the radial epiphyses started when most of the cats were 15 to 16 months old.

The roentgen changes seen in cats which developed rickets are identical to those observed in man. The earliest diagnostic features are the rarefaction and irregular fraying of the epiphyseal plate. The clearly-defined epiphyseal plate fades into the soft tissue density of the adjacent epiphyseal cartilage. In later stages the roentgen changes are more marked. The ends of the long bones are enlarged and there is haziness and cupping of the diaphyseal ends of the bones. There is a wide radiolucent shadow between the epiphyseal ossification center and the end of the shaft. With healing, the recalcified epiphyseal plate casts a transverse linear shadow of increased density in the metaphysis beyond the end of the shaft at the level the epiphyseal plate would have reached had there been no rickets. The new epiphyseal plate thickens into a transversed band. The metaphyseal spongiosa is gradually recalcified and the radiolucent intermediate rachitic zone disappears as the shadow of the metaphyseal spongiosa fuses with that of the epiphyseal plate.

The degree of rickets in cats was graded using the widening of the radiolucent shadow between the epiphyseal center and the end of the shafts as a measure of comparison.

Five of the 11 cats deprived of vitamin D showed x-ray evidence of rickets on the initial films, and three cats had widened epiphyseal plates but no other roentgen criteria of rickets. All but one of the cats showed evidence of rickets

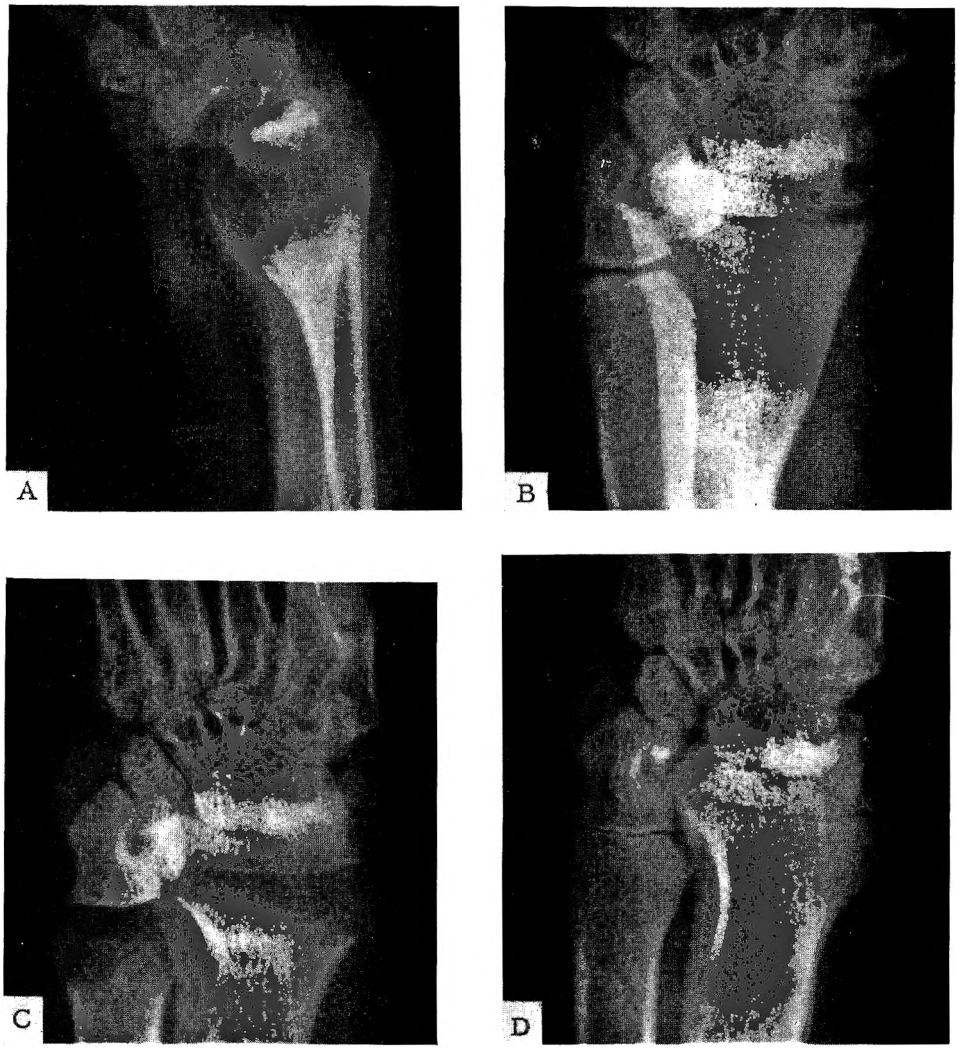

Fig. 2 Representative x-rays of cat 108 demonstrating spontaneous exacerbations and remission of rickets seen in cats receiving 2:0.65 Ca:P ratio without vitamin D.

A. 4.5 months on experiment. Moderate degree of active rickets.

B. 7.5 months. Rickets healed.

C. 10.5 months. Recurrence of moderate rickets.

D. 14.5 months. Rickets completely healed.

within 7.5 months of the onset of the experiment. One cat receiving the 2:0.65 Ca:P ratio without vitamin D showed no x-ray evidence of rickets at any time during the experiment.

In the cats receiving the 1:1 Ca:P ration, rickets generally became progressively severe, whereas a remarkable cycle of events characterized by spontaneous exacerbations and remissions of rickets was observed in cats receiving the 2:0.65 ratio. Ultimately, in both of these groups deprived of vitamin D, if the animals survived, the rickets was healed, although vitamin D was continually withheld.

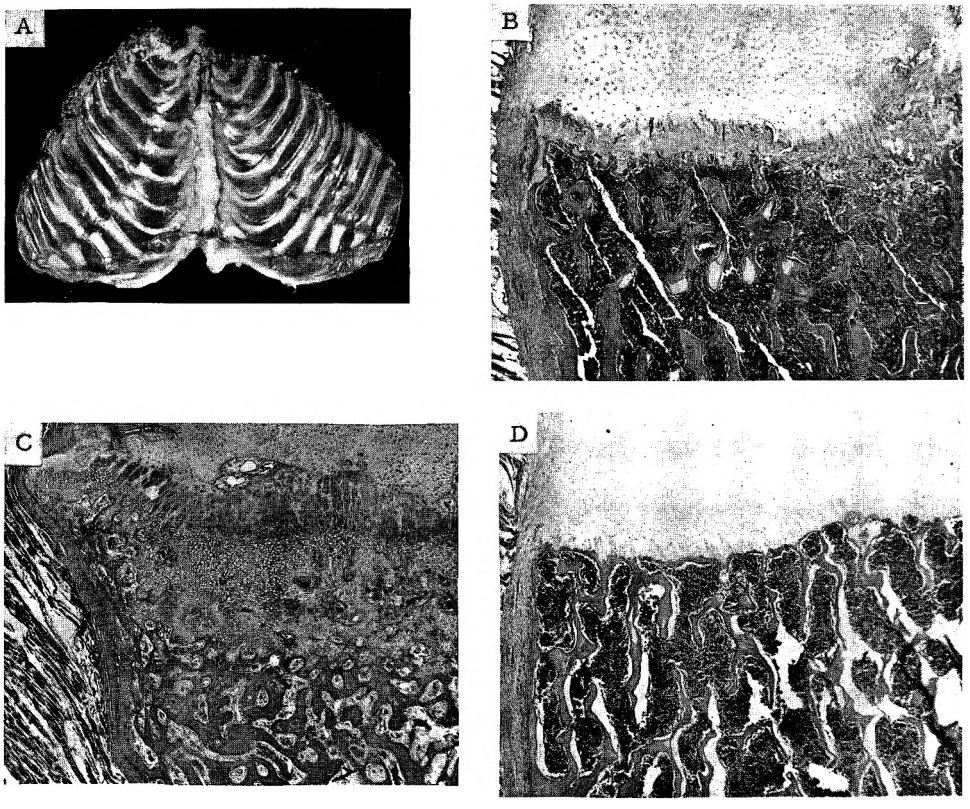

Fig. 3 A. Cat 97 — Rib cage with rachitic rosary.
B. Cat 108 — Costochondral junction with healed rickets and osteomalacia. × 40.
C. Cat 97 — Costochondral junction with active rickets. × 40.
D. Cat 87 — Normal costochondral junction. × 40.

DISCUSSION

Severe rickets can be produced in kittens on vitamin D-deficient diets without resorting to the use of unusual Ca : P ratios. In this respect the cat responds unlike the rat. Since it is generally accepted that the rachitogenicity of a diet increases as the Ca : P ratio deviates from 1 to 1.5 : 1, it was surprising that the rickets produced was more severe when 1% of calcium and 1% of phosphorus was fed than when 2% of calcium and 0.65% of phosphorus was fed. This may have been the result of what appeared to be the poorer growth of the cats fed the 2 : 0.65 Ca : P ratio.

The presence of a transverse band of increased density in the terminal segments of the radial shaft which has been referred to as metaphyseal sclerosis in this study is similar to the appearance produced by heavy metals in children, i.e., lead, bismuth, radium and phosphorus. However, heavy transverse bands may be found in apparently healthy children especially during the second to the 4th years of life.

In this study the presence of metaphyseal sclerosis is not considered significant. It is interesting that transverse bands of increased density in the metaphyseal areas were seen more commonly in the group receiving a diet of 2 : 0.65 Ca : P ratio with vitamin D. It may well be that the increased ratio of calcium to phosphorus results in local increased mineral deposition in the metaphyses. Thus the idiopathic metaphyseal density in asymptomatic children may also have its origin in an increased ratio of calcium to phosphorus dietary intake.

The spontaneous exacerbations and remissions of rickets observed in cats receiving the 2 : 0.65 ratio have not been observed in other species. These changes in degree of rickets could be determined by serum alkaline phosphatase determinations as well as x-ray criteria. Since those cats which survived the acute rickets of the first 12 months of the experiment, during which time their growth was greatest, showed a spontaneous healing of their rickets, it appears probable that the requirement of cats more than two years old for vitamin D is low.

Unfortunately, histologic examination was made of only one of the 8 kittens; 7 on the high ratio died during the first 6 weeks of the experiment. The reason for so many deaths is not apparent and, in the light of our experiences of the past 6 years in feeding hundreds of cats purified diets, is most unusual. The presence of fatty nephrosis in the one of these 8 kittens examined and in 4 other animals that died later is provocative but does not necessarily indicate that the renal lesion was important in causing death. Further work is necessary to determine the significance of this lesion in cats and its relation to the mineral content of the diet. There is no information in the literature concerning the mineral requirements of cats.

SUMMARY

Rickets has been produced in kittens fed purified diets lacking vitamin D. More severe rickets was produced by a diet containing 1% of calcium and 1% of phosphorus than by one containing 2% of calcium and 0.65% of phosphorus.

Cats which survive the acute rickets present during their rapid-growing period later develop a spontaneous healing of their rickets, indicating a low vitamin D requirement in young adult cats.

ACKNOWLEDGMENTS

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NUTRITION OF SALMONOID FISHES

V. CLASSIFICATION OF ESSENTIAL AMINO ACIDS FOR CHINOOK SALMON [1,2]

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INTRODUCTION

Although a large amount of experimental work has been published on the amino acid requirements of mammals and birds, the literature reveals no previous studies on classifying the indispensable amino acids for chinook salmon *(Oncorhynchus tshawytscha)*. Tunison et al. ('42) and Gerking ('52) reported experiments on measuring nitrogen balance in trout and sunfish, but exact control of the amino acid content of the diets was difficult with the ingredients used. As a prerequisite to amino acid studies, a satisfactory test diet containing a mixture of crystalline amino acids, vitamins, carbohydrates, fats, minerals and an inert binder was developed by Halver ('57b). Using this as the complete diet, we have dropped out, one at a time, all of the amino acids present in the test diet, and have compared the growth response of fingerling salmon on the complete and the deficient diets. The feeding experiments were carried out at the Salmon Nutrition Laboratory, U. S. Fish and Wildlife Service, Cook, Washington.

[1] Journal Paper no. 1086, Purdue Agricultural Experiment Station. The experimental data in this paper are taken from a thesis submitted by Donald C. DeLong in partial fulfillment of the requirements for the degree of Master of Science in Biochemistry, Purdue University, and served as the basis for his receipt of the 1956 Purdue Biological Society Graduate Student Award.

[2] Presented at the meetings of the American Institute of Nutrition, Atlantic City, New Jersey, April, 1956.

EXPERIMENTAL

The methods of diet preparation and general experimental feeding techniques were the same as those previously reported (Halver, '57a,b). In the deficient diets, α-cellulose flour replaced on an equal weight basis the amino acid dropped from the basal diet (table 1); the α-cellulose flour was considered an inert material. After preparation, the diets were stored in low-form fruit jars under refrigeration until fed.

TABLE 1

Ingredients of basal diet

CONSTITUENT	AMOUNT
	gm/200 gm diet
Mineral mix [1]	4.0
Amino acid mix [2]	70.0
Vitamin mix [1]	3.0
White dextrin	6.0
Corn oil	5.0
Cod liver oil	2.0
α-Cellulose flour	0.0
Water	100.0
Carboxymethylcellulose	10.0

[1] Mineral and vitamin mixtures the same as reported previously (Halver, '57b).
[2] Same as in diet C-G (Halver, '57b). This mixture supplies the following grams of amino acid per 200 gm of diet: L-arginine·HCl, 5; L-histidine·HCl·H_2O, 2.5; L-isoleucine, 4; L-leucine, 6; L-lysine·HCl, 5; L-methionine, 2; L-phenylalanine, 4; L-threonine, 2.5; L-tryptophan, 1; L-tyrosine, 4; L-valine, 4; glycine, 5; L-alanine, 3.5; L-aspartic acid, 5; L-cystine, 0.5; L-glutamic acid, 8; L-proline, 5; and L-serine, 3.

Feeding trials were conducted in screen-covered wood plank troughs that had been sealed with an inert plastic film. The spring water supply (3 gallons/minute/trough) was practically free of fish pathogens and remained at 47° ± 1°F. throughout the 10-week feeding period. Two trough sizes were available; 18 × 10 × 84 in. and 18 × 10 × 60 in. Approximately 5,000 actively feeding chinook salmon fingerlings were obtained from the Willard Fish Cultural Station, transferred to the experimental hatchery, and 20 lots of 200 fish each were hand counted into the troughs. Variation in total

weight of fish per trough was small. Throughout the remainder of the experiment, the entire population of each trough was weighed bi-weekly by one individual (DCD) by the weighing technique previously described (Halver, '57a).

Since the salmon had been actively feeding on a production diet in a hatchery raceway (8 × 80 ft.) the change to the experimental regime was great and the fish were given an adaptation period using a beef liver diet prior to the start of the feeding trial. After 5 days, the fish were feeding actively on the liver diet, on the 6th day the diet was altered to contain one-third complete amino acid test diet, on the 7th day to contain two-thirds test diet, and on the 8th and 9th days, to consist of the control diet alone. During the adaptation period, dead fish were replaced and at the end of the period an initial experimental weight for the fish in each trough was obtained (374 to 400 gm).

The fish were fed a slowly sinking diet expelled through a garlic press into the upper portion of the water. Diets were fed as long as the fish accepted them, and feeding ceased as soon as any portion of the diet reached the bottom of the trough. The fish would eat off the bottom but this was avoided as much as possible since leaching of nutrients from the diets must occur. Apparatus common to more than one trough was cleaned and disinfected between troughs to minimize inadvertent transfer of disease organisms or food particles. Fish were fed three times daily, 6 days weekly on a rigid schedule (8:00 A.M., 1:00 P.M., 4:00 P.M.). The fish seemed to consume more in the first two feedings each day. Troughs were cleaned partially daily without removing the fish, and were drained, cleaned, and disinfected during the biweekly weighing period.

RESULTS

Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine were found to be indispensable amino acids for normal growth of chinook salmon under the conditions of this experiment. No significant

growth was obtained on diets devoid of one of these amino acids, indicating that biosynthesis was negligible (table 2).

The feeding trials, with diets deficient in one of these amino acids, were initiated with 200 fish adapted to and actively feeding on the synthetic diet. Curbed intake of food was noted within 10 days in all cases. The fish in all 10 lots had a tend-

TABLE 2

Average data for chinook salmon on the various diets lacking in essential amino acids

DIETS	INITIAL WT.	AV. WT. 2ND WK.	AV. WT. 4TH WK.	AV. WT. 6TH WK.	AV. WT. 8TH WK.	AV. WT. 10TH WK.
	gm	*gm*	*gm*	*gm*	*gm*	*gm*
Arginine-deficient	1.88	1.84 (3)[1]	1.86 (0)	1.76 (0)	1.84 (2)	1.71 (4)
Basal				1.76	2.04 (1)	2.41 (0)
Histidine-deficient	1.88	1.99 (3)	2.03 (0)	2.01 (0)	1.91 (0)	1.80 (2)
Basal				2.01	2.35 (0)	2.74 (1)
Isoleucine-deficient	1.88	1.93 (0)	1.93 (1)	1.88 (1)	1.84 (1)	1.59 (2)
Basal				1.88	2.19 (1)	2.41 (2)
Leucine-deficient	1.91	1.86 (0)	1.90 (2)	1.68 (8)	1.63 (1)	1.42 (1)
Basal				1.68	1.86 (0)	2.11 (0)
Lysine-deficient	1.87	1.94 (0)	1.96 (0)	1.86 (1)	1.86 (0)	1.81 (1)
Basal				1.86	2.17 (0)	2.50 (1)
Methionine-deficient	1.88	2.00 (0)	2.05 (0)	1.99 (1)	1.83 (0)	1.82 (0)
Basal				1.99	2.20 (0)	2.47 (0)
Phenylalanine-deficient	1.96	2.11 (1)	2.09 (0)	2.07 (0)	1.96 (0)	1.87 (0)
Basal				2.07	2.38 (1)	2.71 (0)
Threonine-deficient	1.90	1.98 (0)	1.96 (0)	1.95 (1)	1.97 (2)	1.97 (1)
Basal				1.95	2.32 (1)	2.65 (0)
Tryptophan-deficient	1.92	1.93 (0)	1.96 (1)	1.94 (1)	1.86 (1)	1.77 (3)
Basal				1.94	2.36 (1)	2.49 (0)
Valine-deficient	1.93	2.00 (0)	2.05 (0)	1.97 (1)	1.94 (2)	1.78 (0)
Basal				1.97	2.15 (0)	2.38 (1)

[1] Numbers within parentheses indicate the mortality during the preceding two-week period.

ency to swim slowly to the surface, take a piece of food, chew it and then spit it out. They hovered near the surface when being fed, and ate very little.

At the end of the 6th week the deficient fish in each of the 10 lots were split into two subgroups by crowding them into one end of the trough and swirling them with a net. A second net was dipped into the swirling fish and approximately one-half

of the fish were removed. Both groups were hand counted and made equal. Subgroup 1 was continued on the same amino acid-deficient ration, and subgroup 2 was placed on the basal diet (table 1) containing all 18 amino acids (see recovery data, table 2).

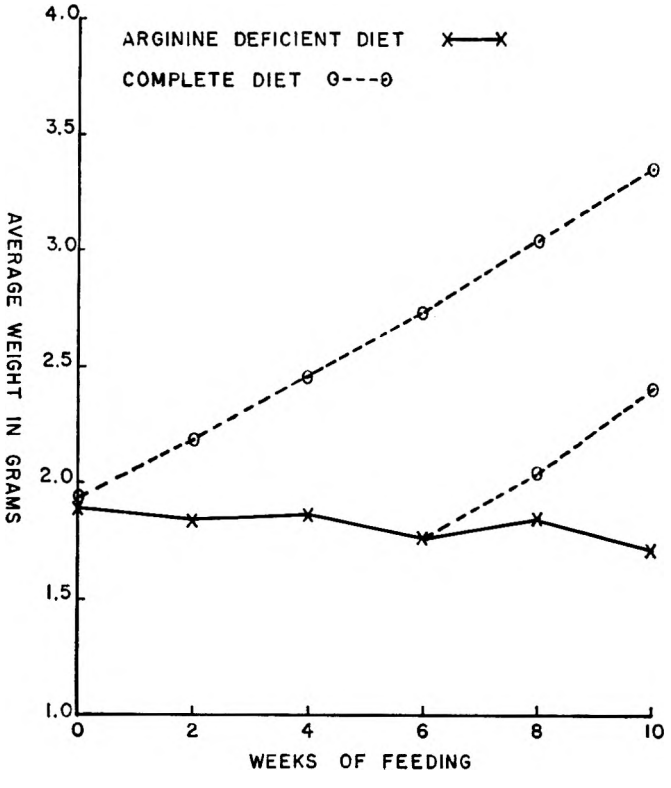

Fig. 1 Growth of arginine-deficient fish. The deficient group was divided after 6 weeks on the deficient diet and the missing amino acid was replaced in one of the two subgroups.

By the 7th day, fish in subgroup 2 were feeding actively, swimming rapidly to the surface, and even breaking the surface to obtain food. These fish showed an immediate and substantial growth response to the control diet. The fish in subgroup 1, on the deficient diet, continued to show a curbed intake of food and a relatively low degree of activity.

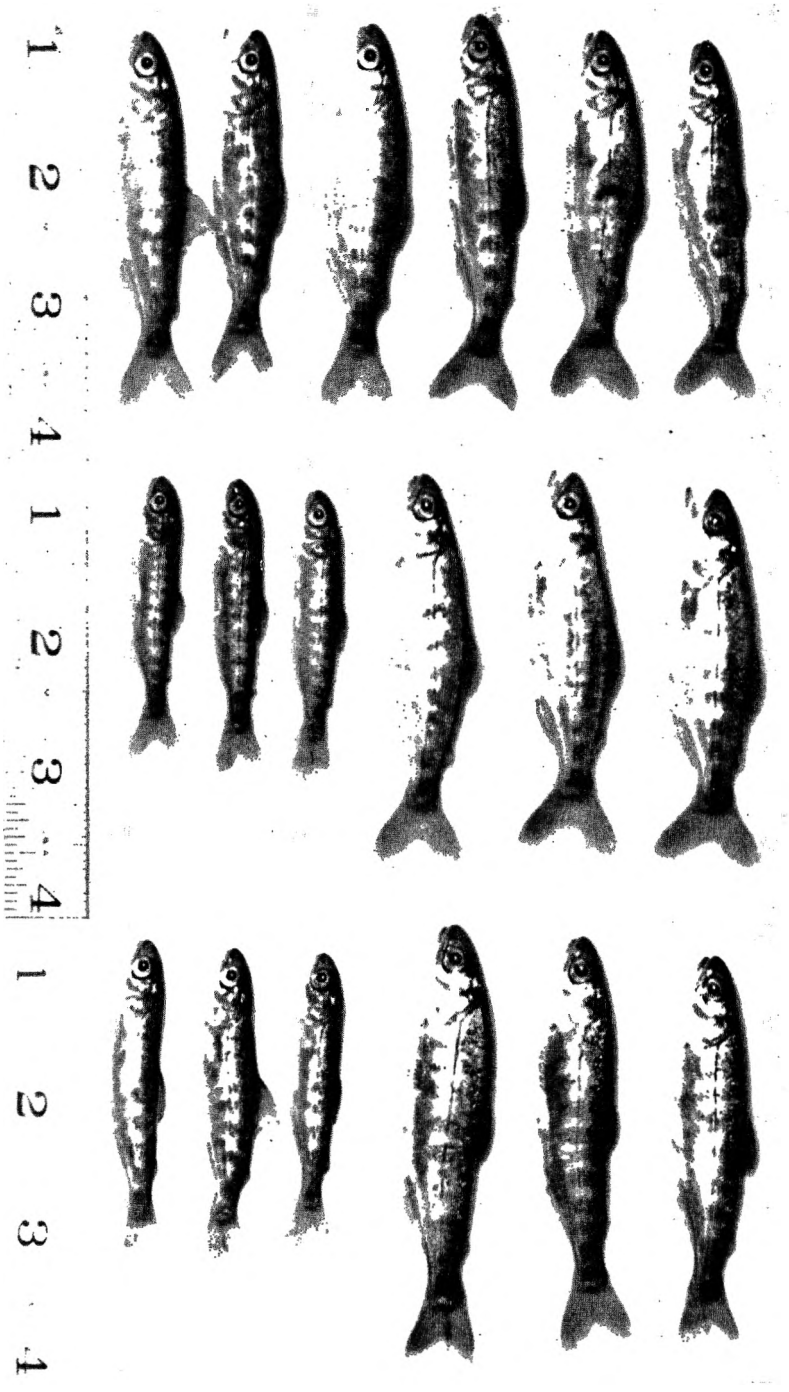

Fig. 2 Amino acid-deficient and control chinook salmon. Top row: glutamic acid-deficient (first three) and control fish. Center row: arginine-deficient (first three) and control fish. Bottom row: leucine-deficient (first three) and control fish.

The fish on the diets lacking in one essential amino acid were offered approxmately 600 gm of diet during the 10-week feeding period, but only a small percentage was actually eaten. The diet was fed very slowly to the fish, and was fed as long as any was being accepted by the fish. In contrast, fish in subgroup 2 fed actively and utilized the food with the same efficiency as that obtained with the two control lots.

The growth curves of fish receiving the diets deficient in essential amino acids, when compared with the average growth

TABLE 3

Average data for chinook salmon on basal diet and on diets lacking in dispensable amino acids

DIETS	INITIAL WT.	AV. WT. 2ND WK.	AV. WT. 4TH WK.	AV. WT. 6TH WK.	AV. WT. 8TH WK.	AV. WT. 10TH WK.
	gm	*gm*	*gm*	*gm*	*gm*	*gm*
Basal, lot 1	2.00	2.26 (0)[1]	2.48 (0)	2.78 (0)	3.10 (1)	3.42 (2)
Basal, lot 2	1.87	2.13 (0)	2.44 (1)	2.70 (0)	2.99 (1)	3.29 (2)
Alanine-deficient	1.90	2.13 (0)	2.40 (0)	2.65 (1)	2.97 (0)	3.23 (2)
Aspartic acid-deficient	1.92	2.16 (2)	2.47 (2)	2.76 (1)	3.07 (0)	3.46 (0)
Cystine-deficient	1.90	2.14 (1)	2.45 (0)	2.73 (0)	3.03 (2)	3.28 (1)
Glutamic acid-deficient	1.93	2.22 (1)	2.56 (1)	2.86 (0)	3.23 (1)	3.52 (1)
Glycine-deficient	1.89	2.11 (0)	2.47 (1)	2.83 (0)	3.11 (0)	3.33 (1)
Proline-deficient	1.91	2.11 (0)	2.39 (0)	2.76 (0)	3.01 (0)	3.29 (0)
Serine-deficient	1.94	2.18 (0)	2.49 (0)	2.83 (0)	3.13 (0)	3.45 (4)
Tyrosine-deficient	1.92	2.16 (0)	2.51 (1)	2.70 (1)	2.98 (1)	3.31 (0)

[1] Numbers within parentheses indicate the mortality during the preceding two-week period.

curve of the two control lots, were similar to that shown in figure 1 for arginine. Pictures comparing the appearance of typical fish selected from the arginine- and leucine-deficient lots with fish from the control lots are shown in figure 2 (center and bottom row).

Alanine, aspartic acid, cystine, glycine, glutamic acid, proline, serine and tyrosine were found to be dispensable amino acids for normal growth of chinook salmon under the conditions of this experiment. Fish on these diets continued to feed actively and to gain weight (table 3) throughout the

course of the feeding trial. The total food offered was approximately 1200 gm, which was utilized with approximately the same efficiency as that of the control groups. Mortality in each of the test groups was considered normal. Growth of the test groups closely paralleled that of the control groups, even

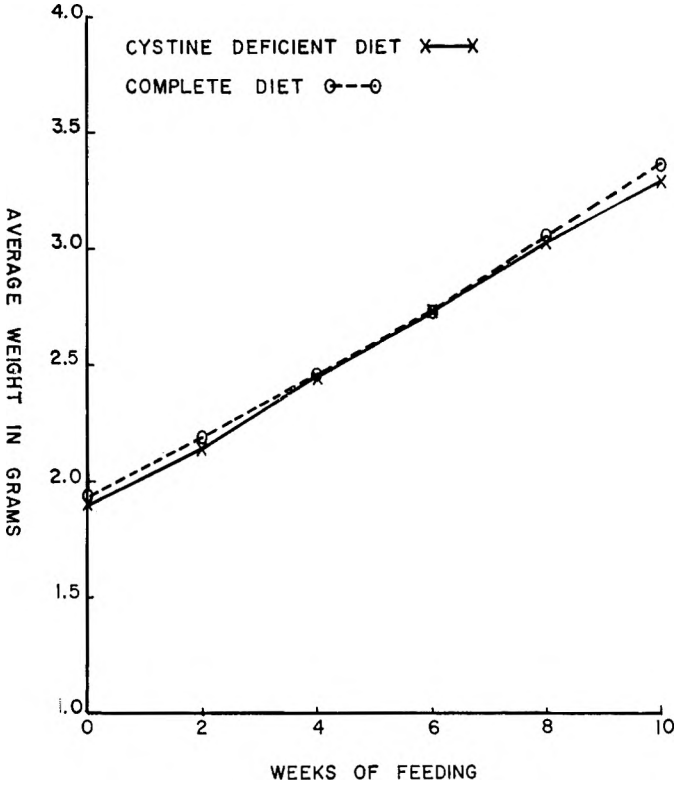

Fig. 3 Growth of cystine-deficient fish. No difference in growth was discernible between cystine-deficient and control lots of salmon.

though there was a reduction in the total protein content of the diet when each of the 8 amino acids was left out. Hydroxyproline was absent from all diets, including the control diet, and on the basis of the weight gains observed in all groups, can also be considered dispensable.

The growth curves of fish receiving the diets lacking in one of 8 dispensable amino acids tested, when compared with the average growth curve of the two control lots, were similar to that shown in figure 3 for cystine; the appearance of the fish in these lots was similar to that shown in figure 2 (top row) for glutamic acid.

DISCUSSION

Eighteen of the 19 amino acids were fed, each in its natural form, and all were considered to be present in excess of minimum requirements for good growth. The water temperature was low, and the growth rate was slower than that which can be obtained at higher water temperatures.

Food efficiency and the protein efficiency ratio could not be determined accurately. The fish on the diets deficient in a dispensable amino acid were actively feeding on the diet throughout the experiment. These fish consumed from 1100 to 1200 gm, which corresponds to a dry weight of 550 to 600 gm. This would indicate a food efficiency of 0.3 to 0.4 gm gained per gram of dry weight of diet. Considering the amount of the diet that was leached or not taken at all, the actual utilization was probably higher. The fish on the diets deficient in one indispensable amino acid were fed 500 to 600 gm of diet, or 250 to 300 gm of dry weight. These fish gained little or no weight, so a food efficiency index cannot be computed.

The mortality during each two-week period is recorded in tables 2 and 3. The mortality ranged from a high of 12 in the case of leucine to none in the case of proline, with three fish dying in one control group and 4 fish in the other. While the mortality rate was higher in some of the groups lacking a required amino acid, it is doubtful that the mortality could be attributed to the deficiency of the amino acid alone. Curbed intake could cause a deficiency in other essential nutrients, for Halver ('57a) has shown that chinook salmon of this size when deficient in pyridoxine have a 100% mortality in 6 weeks. In view of the variable individual characteristics of fish, the mor-

tality rate was considered of little diagnostic value, and probably normal.

The data indicate that the young chinook salmon, unlike the weanling rat (Borman et al., '46; Scull and Rose, '30) and pig (Mertz et al., '52) is unable to synthesize arginine; in this requirement the young salmon resembles the chick (Almquist and Grau, '44).

SUMMARY

Twenty lots of 200 chinook salmon were adapted to and fed a purified diet containing 18 L-amino acids as the only source of protein. Amino acid-deficient diets were formed by dropping one amino acid from the basal ration and replacing it with an equal weight of α-cellulose flour. Growth on these diets was compared with that obtained with the basal ration.

On the basis of the results obtained, the chinook salmon requires arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine for normal growth. No evidence was obtained for a partial biosynthesis of any of these componds. In contrast, alanine, aspartic acid, cystine, glycine, glutamic acid, proline, hydroxyproline, serine and tyrosine were not required for growth, and may be considered dispensable under the experimental conditions used.

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PROTEIN FACTORS AND EXPERIMENTAL RAT CARIES

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It is generally accepted that sugar and certain mineral components have essential roles in the cariogenicity of experimental animal diets (Sognnaes, '55). Recent studies in this laboratory suggest that dietary protein also may influence caries development. Thus McClure ('52) and McClure and Folk ('53, '55a) have implicated the effects of heat processing on the protein of certain cereal foods and particularly skimmilk powders as a dietary factor related to caries production in white rats. Their least cariogenic diet contained a lyophilized skimmilk powder, followed by increasing cariogenicity in diets containing a spray-dry and a roller-dry skimmilk powder. Autoclaving the spray- and roller-dry milk powders accentuated their cariogenic effect in a diet. Use of these diets has had an important advantage, namely, the development of extensive smooth-surface caries similar to that which is so frequently observed in human dentition (Losee and Nemes, '54). The diets also do not contain an excessive quantity of sugar.

In view of the well-established relation between heat treatment of certain protein foods and the unavailability of lysine (Griswold, '51), this amino acid became suspect as a dietary factor and it is of particular interest that supplementation of

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heat-processed cereal and milk powder diets with lysine was followed by a striking decrease in dental caries (McClure and Folk, '55b). Other factors are undoubtedly associated with the cariogenicity of these diets. Thus, it appears that a lysine supplement is not effective in reducing the cariogenic property of diets which contain an unautoclaved skimmilk powder and are adequate in lysine. The addition of minerals and fat reduced the cariogenic effect of this diet (McClure, Folk and Rust, '56).

The purpose of these present experiments was to attempt to resolve some of these complex dietary problems. In the heat treatment of natural protein foods a reducing sugar and perhaps certain salts are involved in the chemical changes occurring during the heat processing (Patton, '50). These experiments, therefore, also have studied the effects of autoclaving casein on caries production, using mixtures of casein, lactose and salts.

EXPERIMENTAL

Diets containing skimmilk powders. The composition of these diets is shown in table 1 and the caries results are presented graphically in figure 1. The basal diets of these experiments are the same as diets 635 and 636 (McClure and Folk, '53).

In experiment 216 (table 1) basal diet 635 was fortified with an amino acid mixture as well as by all the known B vitamins. In experiment 217, 11% casein replaced an equivalent amount of cornstarch. In both these experiments vitamins A, D and E were given orally.

As in all these studies in this laboratory, each control and experimental group contained from 35 to 40 rats [2] from the colony of the National Institutes of Health. They were started at weaning age weighing approximately 30 gm and litter mates were represented in control and test groups. There were two rats in each cage and the diets and distilled water were consumed ad libitum. After 56 to 60 days the animals

[2] Sprague-Dawley strain.

were sacrificed and the lower molar teeth examined for caries, as previously described (McClure and Folk, '53). The caries diagnosis is reported graphically in terms of incidence (percentage of rats having caries), number of carious lower teeth and an arbitrary score of severity (fig. 1).

As in previous studies (McClure and Folk, '53) with diet 635 unsupplemented, a high incidence of severe caries resulted

TABLE 1

Composition of modified skimmilk powder diets

	EXPERIMENT NO.				
	216 [1]	217	218	219	220
Roller-process skimmilk powder	35.0	35.0	...	...	...
Autoclaved roller-process skimmilk powder	...	...	35.0	35.0	35.0
Cerelose	18.0	18.0	18.0	18.0	18.0
Cornstarch	40.0	34.0	45.0	44.7	41.0
Liver powder	...	2.0	2.0	2.0	2.0
Casein	...	11.0	...	...	...
L-Lysine	...	...	...	0.4	...
Blood albumin	...	...	...	...	4.0
Vitamin mix 90 [2]	5.0	...	...	...	...

[1] Contained the following amounts of added amino acids per kilogram of diet: L-histidine·HCl, 2.5 gm; DL-isoleucine, 4.0 gm; L-lysine·HCl, 12.5 gm; DL-methionine, 2.0 gm; DL-phenylalanine, 6.0 gm; DL-tryptophan, 2.0 gm; DL-valine, 3.0 gm; L-cystine, 2.0 gm.

[2] Vitamin mix 90: thiamine·HCl, 20.0 mg; riboflavin, 20.0 mg; pyridoxine, 20.0 mg; calcium pantothenate, 50.0 mg; niacin, 50.0 mg; biotin, 2.0 mg; folic acid, 5.0 mg; inositol, 400.0 mg; *p*-aminobenzoic acid, 200.0 mg; vitamin B_{12}, 0.15 mg; menadione, 5.0 mg; liver powder, 2.0 gm; dextrin to 50.0 gm.

One drop of vitamin mix 60 (see table 2) was given orally every week.

in experiment 216 (fig. 1). This result thus indicates that neither essential vitamin nor amino acid deficiencies *per se* are critical factors in the cariogenicity of diet 635. The addition of 11% of casein at the expense of cornstach materially reduced the severity of caries, although it did not reduce the caries incidence.

In experiments 218, 219 and 220, which utilized basal diet 636 containing an autoclaved skimmilk powder, a supplement of 0.4% of L-lysine (exp. 219) was compared with equivalent

lysine added to the diet in the form of 4.0% blood albumin (experiment 220). The results of this study, shown in figure 1, were significant. A significant decrease in incidence and severity of caries followed the addition of both blood albumin and free L-lysine.

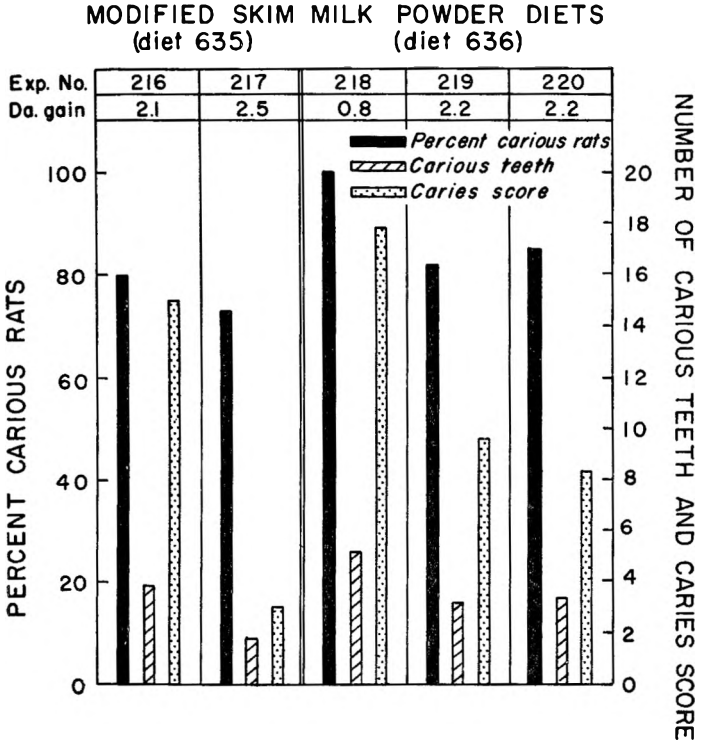

Fig. 1. Effect of vitamin, amino acid and casein supplements to diet 635 and of blood fibrin and L-lysine to diet 636. In experiment 216 the basal diet 635 was fortified with a mixture of essential amino acids plus all known B vitamins. In experiment 217, diet 635 was fortified with 11% casein. Diet 636 (autoclaved skim-milk powder) was used in experiment 218. This basal diet was supplemented by 0.4% L-lysine (experiment 219) and by 4.0% blood albumin (experiment 220).

Casein-base diets. The composition of these diets is given in table 2 and the caries data are presented in figure 2. An essential objective of these studies was to develop caries with a diet more refined than the milk powder diets, utilizing casein as a source of protein, and also to study the effects of

autoclaving casein in the presence of lactose and a mineral salt mixture. Casein was fed at levels of 13 and 24% and was either unautoclaved (diets 22 and 6), autoclaved with lactose (diets 23 and 20) or autoclaved in the presence of both lactose

TABLE 2

Composition of casein- and zein-base diets

	CASEIN-BASE DIETS		ZEIN-BASE DIETS	
	Diet number		Diet number	
	22, 23 [1] 24 [2]	6, 21 [1] 20 [2]	26, 10 9, 2	233 234
Casein	13.0	24.0	...	...
Zein	...	...	24.0	15.0
Cornstarch	...	...	...	25.8
Dextrin	41.6	30.4	...	...
Cerelose	18.0	18.0	51.3	18.0
Lactose	18.0	18.0	...	18.0
Salts [3]	2.5	2.5	4.0	2.5
DL-Methionine	0.1	0.2	..	..
L-Cystine	0.1	0.2	..	..
Choline chloride	0.2	0.2	0.2	0.2
Amino acid mix [4]	..	..	6.5	6.5
Vitamin mix 60 [5]	..	..	8.0	8.0
Vitamin mix 70 [6]	0.5	0.5	..	..
Vitamin mix 80 [7]	1.0	1.0	1.0	1.0
Vitamin mix 90 [8]	5.0	5.0	5.0	5.0

[1] Casein-lactose components autoclaved together at 15 lbs. for 15 minutes.

[2] Casein-lactose-salt components autoclaved together at 15 lbs. for 15 minutes.

[3] Hubbell, Mendel and Wakeman ('37).

[4] Same as used by Benton, Harper and Elvehjem ('55), except threonine was reduced from 1.2 to 0.6%.

[5] Vitamin mix 60: cottonseed oil, 80.0 ml; vitamin A, 5,500 units; vitamin D, 1,100 units and α-tocopherol, 200 mg per kilogram of diet.

[6] Vitamin mix 70: linoleic acid 5.00 ml in which was dissolved vitamin A, 5,500 units; vitamin D, 1,100 units; and α-tocopherol, 200 mg.

[7] Vitamin mix 80: ascorbic acid, 10.00 gm; sucrose, 490 gm.

[8] Vitamin mix 90: see table 1.

and the salt mixture (diets 21 and 24). Autoclaving consisted of placing the material mixed with 10% of water about 1 inch deep in a sealed pan and autoclaving for 15 minutes at 15 lbs. pressure. The diets containing 13% casein corresponded closely in quantity of sugar, protein and minerals, to the skim-milk powder diets (McClure and Folk, '55a).

The results of these studies appear in figure 2. A high incidence of relatively severe caries was produced by the 13% casein diets. By increasing casein to 24%, however, relatively few rats developed caries and it was present in a very mild form. Autoclaving the casein produced no apparent cariogenic

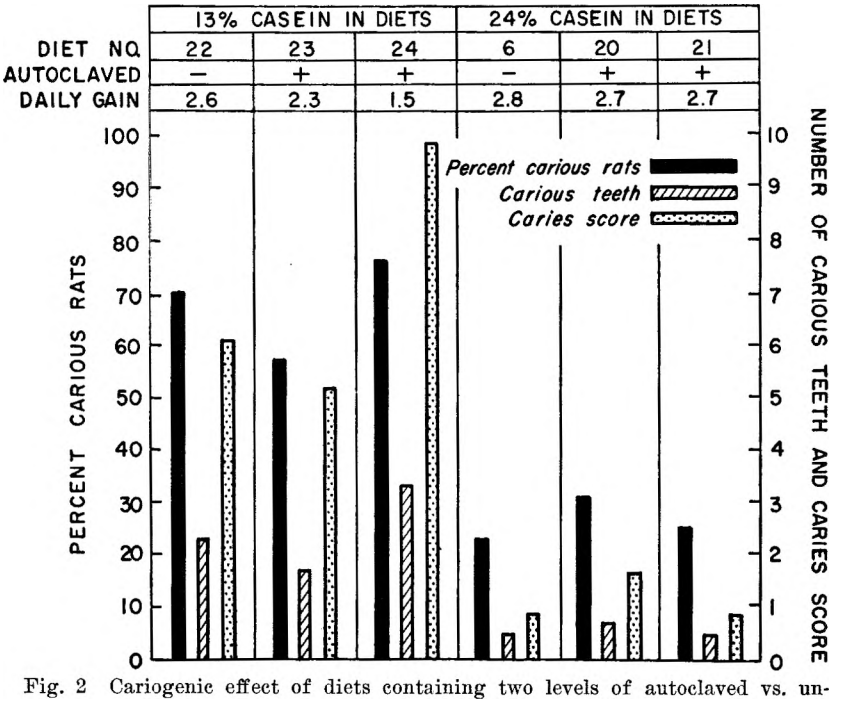

Fig. 2 Cariogenic effect of diets containing two levels of autoclaved vs. unautoclaved casein and at two different protein levels. Diets 22 and 6 were not autoclaved. The casein in diets 23 and 20 was autoclaved in the presence of lactose, while in diets 21 and 24 it was autoclaved in the presence of both lactose and salt components of the diet.

effect with 24% casein present in the diet but in the case of diet 24 (13% casein autoclaved with the lactose and minerals) the severity of caries became greater. The mean severity scores resulting from diets 22 and 24 were 6.3 ± 1.0 [3] and 10.0 ± 1.5 [3] respectively. The difference is significant by Fisher's "t" test at the 0.05 level.

[3] Standard error.

Zein-base diets. The composition of these diets is given in table 2. The amino acid supplement used is the same as amino acid mix no. 3 employed by Benton, Harper and Elvehjem ('55), except that the threonine level was reduced from 1.2% to 0.6% of the diet. Preliminary experiments indicated that growth was satisfactory on this reduced level of threonine.

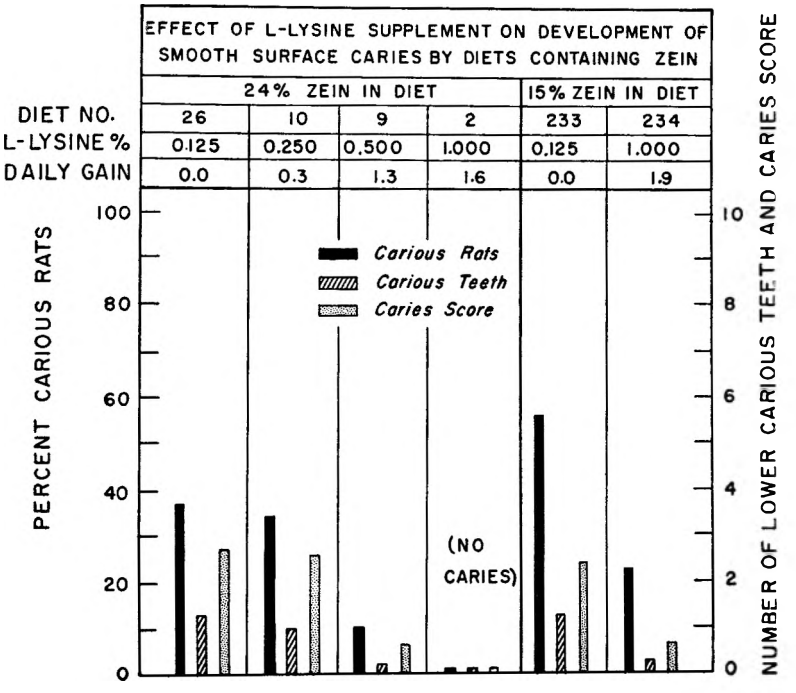

Fig. 3 Effect of L-lysine supplementation to lysine deficient diets on the development of smooth-surface caries in rats.

The lysine content was varied in order to provide diets containing the following L-lysine supplements: 1.0, 0.5, 0.25, and 0.125% (diets 26, 10, 9 and 2 respectively, fig. 3). A minimum 0.125% of L-lysine supplement was necessary to maintain the rats. The diets were made isonitrogeneous by the addition of glycine to compensate for the variations in lysine content. In another series of experiments the zein diet was modified as follows: reduction of zein from 24 to 15%, salts from 4.0 to

2.5% and cerelose from 52.0 to 18.0%. In addition, 18.0% of lactose and 26.3% of cornstarch were added in place of the cerelose. These changes made the diets more in keeping with the relative concentrations of these nutrients in the milk powder diets. These modified zein diets were studied at 0.125 and 1.0% levels of lysine supplementation (diets 233 and 234 respectively, fig. 3). The animals were fed the diets ad libitum for 90 days. As previously mentioned precautions were taken to ensure distribution of littermates among the experimental groups and 40 animals per group were used.

The results obtained with these diets, including daily weight gain, percentage of carious rats, carious teeth and caries score per rat, appear in figure 3. The daily weight increment of these experimental groups was directly proportional to the level of the lysine supplement. Caries incidence, carious teeth per rat, as well as the severity scores, also were directly related to the extent of the lysine deficiency. No rats on the 24% zein diets developed caries with 1.0% of L-lysine in the diet whereas 30 to 35% of the rats receiving only 0.125 or 0.250% of L-lysine developed caries with a severity score averaging approximately 2.5. Similarly when the pronounced deficiency of lysine in diet 233 was corrected in diet 234, by addition of 1.0% of L-lysine, there was a concomitant reduction in the incidence and severity of caries. The difference in caries severity is significant, probability < 0.05 by Fisher's "t" test.

DISCUSSION

The negative results following supplementation of diet 635 with essential B vitamins and amino acids contrast with the reduction in severity of caries due to the addition of 11% casein. With respect to protein, the amino acid mix and 11.0% casein were not equivalent on a nutrient basis, which may explain this caries difference. Total protein apparently can not be disregarded in this caries result, nor can the possibility of an imbalance of amino acid nutrients be ruled out as unimportant. The higher casein content of the diet might also

affect the oral environment and the oral spectrum of organisms. Disregarding the amino acid supplement in experiment 216, it does not appear that a deficiency of the B vitamins is involved in the caries potential of this diet.

The caries-inhibitory effect of blood albumin being comparable to that of free L-lysine suggests that systemic metabolic factors are involved in the cariostatic effect of L-lysine. This reasoning is based on the premise that little or none of the lysine in blood albumin would become available in the oral cavity. Additional data bearing on this hypothesis will be presented in a subsequent paper.

Experiments on the casein-base diets suggest that the notable reduction in caries may be the result of increasing the total dietary protein. Protein in highly cariogenic diets 635 and 636, containing milk powders, it may be noted, is at a level of approximately 13%. This may be a critical quantity of protein for such cariogenic effects. It is of particular interest that caries occurred at a high level in both incidence and severity in rats receiving the low-protein casein diets, particularly diet 22 which contained unautoclaved casein. The effects of autoclaving *per se* on the casein-lactose mixture apparently may be modified by the presence of minerals. The reduced growth obtained in experiment 24 suggests that a significant loss of lysine availability occurred under these particular autoclaving conditions.

Although a very significant difference in dental caries incidence resulted in rats receiving the 13% casein diets, in comparison with that observed when a 24% casein diet was used, there was no concomitant pronounced difference in the rate of growth. It thus appears that the growth requirements of the rat were equally satisfied, under the conditions of the present experiment, by both 13 and 24% casein in the diet. However, since the incidence of caries differed significantly in the rats fed the two diets, it appears that the dietary requirements related to cariogenicity may be distinct from those for optimum growth. In this connection it is of interest that Elvehjem

('56) has reported that levels of amino acids which may tend to produce optimum growth may not allow normal metabolic functions.

The inverse relationship observed between the caries incidence and the lysine content of the purified zein-base diets strengthens the previous observations on the caries inhibitory effect of L-lysine when added to cooked cereal diets and diets made lysine-deficient by the presence of autoclaved skimmilk powder. The increased caries resulting from the 15% zein diets, as compared with 24% zein, again may be an indication that there was a relationship between total protein and cariogenicity, although there were other differences in these diets.

SUMMARY

1. A cariogenic diet containing a roller-process skimmilk powder remained highly cariogenic after supplementation with known vitamins and essential amino acids. Caries severity was significantly reduced, however, by the addition of 11% of casein in place of cornstarch to this diet.

2. A supplement of blood albumin proved as effective as L-lysine in the reduction of caries produced by a lysine-deficient skimmilk powder diet.

3. Diets containing 13% of casein developed a high incidence of severe caries but caries was very limited with 24% casein in the diet. This striking caries difference was accompanied by only a slight difference in rate of growth.

4. An autoclaved mixture of casein, lactose, and Hubbell, Mendel and Wakeman salts compared with unautoclaved casein was associated with an increased incidence of caries when the casein content of the diet was 13% but not when it was 24%.

5. An inhibitory effect of a lysine supplement on caries was observed using purified diets containing zein as a source of protein. The result supports prior evidence that the cariogenicity of diets containing heat-processed skimmilk powders and deficient in lysine is due under some conditions to a critical deficiency of lysine.

6. The combined results of these studies suggest that the quantity of protein in the diet may be an important factor in the development of cariogenicity by experimental diets.

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OBSERVATIONS ON THE CHOLESTEROL, LINOLEIC AND LINOLENIC ACID CONTENT OF EGGS AS INFLUENCED BY DIETARY FATS [1]

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Over 20 years ago Cruickshank ('34) reviewed earlier work and reported further data on the influence of dietary fat on egg fat composition. The major objectives of such early studies, dating back to the turn of the century, were concerned with establishing general concepts about the degree of saturation in the depot fats of animals and of their products, such as milk and eggs. Because of these objectives, or because appropriate techniques in the science of nutrition were not available, these early workers either made no attempt, or were unable, to maintain hens in a normal physiological state on high-fat diets. Thus, when they tried to induce drastic changes in the depot and egg fat composition with a highly unsaturated fat, such as linseed oil, hens usually ceased laying within a short time.

Recent interest in the relationship between cholesterol metabolism, atherosclerosis and the dietary intake of saturated vs. the unsaturated essential fatty acids, namely, linoleic and linolenic acids (Ahrens et al., '54; Beveridge et al., '56; Holman, '56) has given new meaning and direction to the problem of altering the composition of egg fat. The present study was therefore undertaken (1) to study the feasibility of feeding

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large quantities of unsaturated fats to hens while maintaining normal egg production, and (2) to study the effect of feeding fats other than those previously studied (hempseed and linseed oils) on the cholesterol, linoleic and linolenic acid content of egg fat.

MATERIALS AND METHODS

Single-Comb White Leghorn hens were used in all trials. With the exception of the experiment represented in table 2, in which each of the three groups was composed of 24 birds,

TABLE 1

Diets

INGREDIENTS	BASAL RATION A	BASAL RATION B
	%	%
Corn meal	52.80	7.70
Soybean meal (50% protein)	20.00	30.00
Fiber [1]	....	10.00
Butyl fermentation solubles	2.00	2.00
Corn distillers solubles	2.00	2.00
Dried whey	2.00	....
Alfalfa meal (17% protein)	3.00	3.00
Dicalcium phosphate	4.00	4.00
Trace mineral mix [2]	3.00	3.00
Salt	0.50	0.50
Vitamin B_{12} supplement	0.50	0.50
DL-Methionine	0.10	0.20
Vitamins A and D_3 (10,000 A — 600 D_3)	0.10	0.10
Choline chloride	25 gm/100 lbs	25 gm/100 lbs
	90.00	63.00
Variables:		
Corn oil, safflower oil, tallow, corn meal	10.00	....
Tallow, soybean, linseed, and safflower oil, microcel [3]	....	20 or 30 5 or 7
Corn meal	100.00	to 100

[1] Solka-floc, manufactured by the Brown Company, Berlin, N. H.

[2] Mico concentrate, product of the Limestone Corp. of America, Newton, N. J.

[3] A calcium silicate preparation, product of Johns-Manville Products Corp., Manville, N. J.

5 birds were used for each treatment. The birds were housed in individual wire cages in a temperature-regulated room. Water and feed were allowed ad libitum, but in some instances feed consumption was measured daily. Egg production was recorded and eggs weighed and identified by hen and lot number. All eggs were stored in a refrigerator until broken out for analysis.

The rations used are given in table 1. Enough basal ration was usually mixed to last for some time, but the fat-containing final rations were freshly prepared every other day to diminish the danger of oxidation. When preparing the rations with absorbent and large amounts of fat, care had to be taken not to permit mixing for too long a time since continuous agitation eventually overcame the effectiveness of the absorbent and a wet feed again resulted. The oils were stored in dark bottles under refrigeration and fresh supplies were obtained frequently.

The yolks, from two eggs, selected randomly from each group of birds were pooled and an aliquot taken for extraction. Fat was extracted with a 2:1 chloroform-methanol mixture. After filtration, aliquots were taken for iodine number determinations by Hanus' method (Official Methods of Analyses — A.O.A.C., '56), and for cholesterol by the method of Zlatkis et al. ('53). Plasma cholesterol was determined directly by the same method.

For the determination of linoleic and linolenic acids, aliquots of the chloroform-methanol extract were evaporated to dryness at low temperature under carbon dioxide. The alkali-isomerization procedure of Brice and Swain ('45) as modified for large scale use by Collins and Sedgwick ('56) was then carried out. As recently pointed out by Mecchi et al. ('56) this method can not be expected to give absolutely exact values for linoleic and linolenic acids with chicken fat because of the presence of small percentages of tetra, penta, and hexaenoic acids; instead, results should be interpreted on a comparative rather than an absolute basis.

EXPERIMENTAL AND RESULTS

Eggs from hens receiving, respectively, no added dietary fat, 10% tallow or 10% corn oil were examined over a 7-month period. The iodine number and cholesterol content of the egg fat are given in table 2.

Inasmuch as the increase in unsaturation due to 10% corn oil was small, 10% safflower oil, which has a higher iodine number than corn oil (see table 5), was next tried with the same basal ration A. When, again, the iodine number of eggs collected from these hens did not rise above 85, it was decided to force-feed 15 gm of safflower oil twice a day to a group

TABLE 2

The influence of 10% dietary tallow or corn oil on the iodine number and cholesterol content of yolk fat over a 7-month feeding period [1]

FAT SUPPLEMENT TO BASAL RATION A [1]	IODINE NUMBER [2]					CHOLESTEROL [2]				
	2 mo.	5 mo.	6 mo.	7 mo.	Av.	2 mo.	5 mo.	6 mo.	7 mo.	Av.
						mg/gm yolk fat				
None	72	68	72	69	70	62	68	68	55	63
Tallow, 10%	71	72	67	63	68	64	69	53	62	62
Corn oil, 10%	79	84	78	79	80	69	62	65	67	66

[1] Twenty-four birds in each group.

[2] Over a 7-month period.

TABLE 3

The effect of force-feeding 30 gm safflower oil daily on egg production, feed consumption, iodine number and cholesterol content of egg fat

EFFECTS OF EXPERIMENTAL RATION [1]							
Hen no.	Egg production	Average daily feed consumption [2]	Body weight [2]		Iodine number and cholesterol content of yolk fat [3]		
			At start	At finish	Ration period	Iodine no.	Cholesterol content
	%	*gm*	*lbs.*	*lbs.*	*days*		*mg/gm fat*
1	70	92	4.7	3.3	1	73	54
2	58	59	5.1	4.2	5	84	54
3	45	92	4.1	3.8	12	79	71
4	82	87	3.8	3.6	24	85	58

[1] Daily force-feeding of 30 gm safflower oil.

[2] Over 40-day period on experimental ration.

[3] Determined on pooled eggs.

of 4 hens to see whether this procedure would yield egg fat with a greater unsaturation. This procedure was tried because the inclusion in the diet of levels of safflower oil exceeding 10% of the total diet always resulted in a finished feed which the hens would not eat because of its oily and clumping characteristics. The results of this trial, including egg production, feed consumption, and iodine number of eggs collected are shown in table 3. The inability to obtain more highly unsaturated egg fat by either including more oil in the diet (which proved physically unfeasible) or by force-feeding the oil separately, prompted the use of an absorbent when high levels of fat were to be included in the diet.

A calcium silicate preparation [2] with absorbent powers exceeding 4 times its own weight with either fat or water proved highly effective in overcoming the wetness of the feed with high levels of fat and thereby also made possible the successful production of eggs with a greater degree of unsaturation than had hitherto been possible. In table 4 are summarized the iodine values as a function of time on experiment for hens receiving, respectively, 30% safflower and 20% linseed oil. It can be seen that the degree of unsaturation in these eggs is greatly increased.

The major experiment of this series was designed to compare the effects of tallow, soybean, safflower, and linseed oils on the linoleic and linolenic acid content of the eggs. A summary of the composition of these fats is given in table 5. Five birds were assigned to each treatment group, and pertinent data, including egg production, body weight changes, plasma cholesterol levels, iodine number, linoleic and linolenic acid, as well as cholesterol content of egg fat, are summarized in tables 6 and 7. For comparison purposes values for normal eggs (from hens receiving no supplemental dietary fat) and for eggs obtained from hens on a completely synthetic amino acid diet (Fisher and Johnson, '56) containing 12% corn oil, are also included in table 7.

[2] Microcel, obtained through the courtesy of the Johns-Manville Products Corp., Manville, N. J.

Linseed oil produced a marked increase in the linolenic acid content of the egg fat whereas soybean oil, even though it contains appreciable amounts of linolenic acid, was essentially ineffective in this regard. Safflower oil produced the largest increase in egg fat linoleic acid although both linseed and soybean oil also doubled the linoleic acid content of egg fat. Tallow, in agreement with Cruickshank's findings, had no

TABLE 4

The effect of high dietary levels of safflower and linseed oil on the iodine number of egg fat in a diet to which has been added an absorbent to preserve the normal consistency of the fat-free ration

DIET PERIODS ON BASAL RATION B	IODINE NUMBER OF EGG FAT		CHOLESTEROL CONTENT OF YOLK FAT	
	30% S.[1,2]	20% L.[1,2]	30% S.[1,2]	20% L.[1,2]
days			*mg/gm fat*	
1	74	74	64	59
2	..	70	..	53
3	..	72	..	55
4	..	78	..	..
5	72	..	60	..
6	..	84	..	104
8	..	101	..	42
10	..	100	..	72
12	103	..	108	..
14	94	..	76	..
21	103	..	67	..

[1] Five birds on each treatment.

[2] Safflower oil, 30%; linseed oil, 20%.

TABLE 5

Composition of fats used

FAT	IODINE NO.	LINOLEIC ACID	LINOLENIC ACID
		%	%
Tallow	40 [1]	2.2 [1]	0.4 [1]
Corn oil	120	42.3 [1]	0 [1]
Safflower oil	135	61.9	trace
Soybean oil	125	43.9	7.8
Linseed oil	150	20.9	48.4

[1] These values are taken from table of Fat Composition, E. F. Drew Co., New York, N. Y. All other values were determined in this laboratory.

effect on the content of these two essential fatty acids. The eggs from the synthetic 12% corn oil-containing ration or the practical 10% corn oil-containing ration show only a slight increase in linoleic acid. Whether this is due to the lower level in the diet was not determined, although a separate trial not reported herein showed linseed oil to be equally effective at the 10 and 20% levels. The eggs produced on the synthetic diet show a slightly, perhaps significantly, lower level of linolenic acid which might be an indication that the hen cannot synthesize linolenic acid but can obtain it from the residual oils in the soybean meal portion of practical rations. The hens whose eggs were used in this instance had only been on the synthetic diet for one week and of course still had other fat reserves upon which to draw.

TABLE 6

Effect of various dietary fats on egg production, body weight changes, and plasma cholesterol levels during a 5-week experimental period

DIETARY FAT IN BASAL RATION B	EGG PRODUCTION	BODY WEIGHT		PLASMA CHOLESTEROL	
		At start	At finish [1]	At start	At finish [1]
	%	*lbs.*	*lbs.*	*mg %*	
Tallow, 20%	79	3.5	3.8	263	255
Linseed oil, 20%	75	3.6	3.9	283	338 (248)[2]
Safflower oil, 20%	71	3.7	3.8	280	249
Soybean oil, 20%	84	3.3	3.6	285	201

[1] After 5 weeks on experiment.

[2] One bird had an abnormally high level of cholesterol. Value within parentheses represents average of group excluding this hen.

DISCUSSION

The present study extends the observations of Cruickshank in several important ways: it has been shown here that hens can be maintained in proper egg production on diets containing high levels of such unsaturated fats as linseed oil. A marked increase in the linoleic acid without concomitant changes in the linolenic acid content of yolk fat can be achieved with fats rich in the former fatty acid but deficient or poor

TABLE 7

Changes in the egg fat composition as a result of feeding various dietary fats to hens[1]

DIETARY FAT	CHANGES IN EGG FAT COMPOSITION DURING VARIOUS TIME PERIODS															
	Iodine number					Cholesterol		Linoleic acid				Linolenic acid				
	Days	1	7	13	19	34	19	34	7	13	19	34	7	13	19	34
							mg/gm fat		%	%	%	%	%	%	%	%
None		69	..	..	..	69	55	55	..	..	..	8.9	..	..	..	3.8
Tallow, 20%		69	78	66	74	66	53	65	8.2	7.5	8.6	8.9	3.1	2.4	3.5	5.3
Linseed oil, 20%		63	84	..	99	97	80	90	16.2	20.4	13.4	18.4	14.5	15.4	16.0	18.4
Safflower oil, 20%		71	85	91	89	90	70	75	16.6	21.4	29.8	26.0	3.6	3.0	4.2	4.2
Soybean oil, 20%		66	78	85	92	83	64	74	9.0	23.7	23.2	17.8	3.6	3.7	4.0	4.8
Corn oil,[2] 12%		..	..	..	..	..	..	..	9.4	..	..	..	2.8	..	..	..
Corn oil,[3] 10%		..	..	..	..	..	..	..	..	..	..	9.9	..	..	..	3.6

[1] All analyses were carried out on duplicate samples.

[2] In a synthetic free-amino-acid-containing diet.

[3] These eggs are from the birds whose data are given in table 2 which had been on this diet for 7 months.

in linolenic acid. Earlier workers had used only fats which were reasonably high in both fatty acids. That soybean oil, which contains appreciable amounts of linolenic acid, exerts no appreciable effect on the linolenic acid content of eggs is interesting.

These results also confirm the observations of Cruickshank on the substitution of linoleic and linolenic acids for oleic rather than for the saturated acids. A calculation of the iodine numbers of linoleic and linolenic acids would indicate an even greater increase in the iodine number of the yolk fat, if the unsaturated acids had been substituted for saturated acids. Since this great rise in iodine number was not found, substitution must have been mainly for oleic acid.

In contrast to the observation of Cruickshank, the eggs from hens receiving linseed oil were not found to have any off-flavors. This may be due to the use of a more highly refined grade of oil.

The cholesterol content of egg fat shows little change with changes in fatty acid composition. More work needs to be done to ascertain if the values herein reported are true cholesterol values or represent other sterols absorbed from the vegetable oils.

The plasma cholesterol levels shown in table 6 deserve comment. The initial values are all higher than average, which may be explained in part on the basis that the hens had just started to lay (Leveille et al., '57) and in part, on their recent confinement in cages. Unpublished work in this laboratory has indicated a significant rise in plasma cholesterol levels for cage-confined vs. floor-housed birds, probably because of restriction in exercise. The vegetable oils produced a greater decrease in plasma cholesterol than did the tallow, but no conclusions can be drawn since the initial level for the tallow group was lower than the initial levels for the other oils. This phase of the study is being continued.

It is hoped that eggs with an altered fatty acid composition might be fed to human subjects to determine if the altered ratio of linoleic and linolenic acid exerts any influence on plasma

lipid levels as has been reported for certain pure unsaturated fats.

The effect of an altered fatty acid composition of eggs on embryonic development of the chicks is under investigation at the present time.

SUMMARY AND CONCLUSIONS

A practical means of incorporating large amounts of fat into a poultry ration without making it unpalatable because of oiliness, has been achieved through the addition of an absorbent to the diet. In this manner, the effects of tallow, corn, soybean, safflower and linseed oil have been studied in terms of the linoleic and linolenic acid composition of the egg fat. It was found that linseed oil produced a large increase in both the linoleic and linolenic acid content of egg fat, whereas soybean and safflower oil only increased the linoleic acid content, despite the fact that soybean oil contains 7 to 8% of linolenic acid. The cholesterol content of egg fat was essentially unchanged by alterations in the fatty acid composition.

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REVERSAL OF SULFAGUANIDINE TOXICITY IN THE RAT [1]

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Mackenzie, Mackenzie and McCollum and Mackenzie and Mackenzie ('41, '43) reported that sulfaguanidine and other sulfonamides were goitrogenic when fed to the rat at a level of 0.5 to 3.0% of the diet. This effect could be prevented by hypophysectomy, or by the administration of thyroxine, but not by iodine. Liver powder, fresh liver, *p*-aminobenzoic acid and certain other substances were also ineffective in preventing the goitrogenic effect of sulfaguanidine. Black, McKibbin and Elvehjem ('41) fed 0.5 to 2.0% sulfaguanidine to rats in a purified casein ration and observed that the growth depression of the drug at low levels could be prevented by feeding liver extract. At the higher level, many of the animals died. The feeding of liver extract decreased mortality but did not support optimum growth.

The feeding of sulfonamides to rats has been reported by several laboratories to produce deficiency symptoms that may be prevented or cured by the concommitant administration of vitamin K, niacin, biotin, pantothenic acid and folacin (Black et al., '42), (Daft et al., '42, Daft and Sebrell, '43), (Kornberg et al., '43), (Martin, '42), (Ransone and Elvehjem, '43), (Tepley et al., '47), (Welch and Wright, '43), (Wright and Welch, '44). Many of these investigators reported that liver was as effective as the pure vitamins but Ransone and

[1] Supported in part by a grant from the Research Corporation of America, New York, New York. Preliminary results were presented at the 20th annual meeting of the American Institute of Nutrition, Atlantic City, New Jersey. Fed. Proc. 15, Part 1, p. 738 (1956).

Elvehjem ('43) commented that two groups of rats grew better when fed sulfasuxidine, biotin and liver than when fed sulfasuxidine, biotin and a folic acid concentrate.

It is of interest that Axelrod et al. ('45) observed that the toxic effects of 0.5% sulfaguanidine in the diet of rats could be overcome by the feeding of norit eluate factor plus biotin or whole liver. When rats were fed 1.0% sulfaguanidine, these substances had only a transient effect on growth.

Tentori and Vivaldi ('50, '54a,) describe fatal neurological symptoms, torpor and paresis in rats fed 2% sulfaguanidine for 300 days or more. The addition of thiamine, folacin, vitamin B_{12} concentrates, liver extracts or brewers' yeast to the diet had no beneficial effect. The incorporation into the diet of 5% dried feces from rats fed a normal diet prevented the syndrome.

It had been observed in this laboratory that the feeding of 1% sulfaguanidine in a complete, purified diet to rats, resulted in growth cessation after 4 to 5 weeks, obesity, exophthalmia, dry, pale skin, and produced hyperplasia of the thyroid gland as reported by others (Mackenzie et al., '41; Mackenzie and Mackenzie, '43). When sulfaguanidine was incorporated into a stock ration, growth and the appearance of the animals were normal. It was found that meat meal, a component of the stock diet, resulted in normal animals, when fed at a level of 5% in a purified diet.

Whole liver or supplementation with large amounts of the known vitamins failed to restore growth. A comparison of meat meal with thyroid powder under various conditions indicated that the active principle in meat meal was not thyroxine.

EXPERIMENTAL

Weanling rats of the Holtzman strain, reared in our own colony or obtained from a commercial source, were used throughout this study. The animals were housed individually in raised wire screen cages and were fed ad libitum. A curative-type growth assay was used for testing various materials

for their growth-promoting effect on rats fed sulfaguanidine. Rats were prepared for assay by housing in large cages in groups of 6 to 8 and were fed diet A (table 1) until growth had declined to 5 gm per week or less (4 to 5 weeks). Samples to be assayed were added at a level of 5 or 10% at the expense of the whole diet. A comparable amount of peanut meal or soybean oil meal added to diet A provided a control diet. The animals

TABLE 1

Diets

INGREDIENT	A	B
	%	%
Ground wheat	55.0	
Alfalfa leaf meal	2.0	
Soybean oil meal	13.0	
Casein, crude	6.5	20.0
Sucrose [1]	8.9	64.4
Crisco	10.0	10.0
Salts 5 [2]	2.5	4.4
Vitamin A and D concentrate [3]	1.0	1.0
Choline chloride	0.0	0.2
Sulfaguanidine	1.1	

[1] Crystalline vitamins added with sucrose (mg/kg diet): Thiamine·HCl 4, riboflavin 4, niacin 20, Ca-pantothenate 10, pyridoxin·HCl 2, inositol 200, menadione 5, biotin 0.5, folacin 4.0, vitamin B_{12} 0.06. α-tocopherol 50 (in hexane solution).

[2] Salmon, W. D., J. Nutrition, *33:* 169, 1947.

[3] Contained 4,000 U.S.P. units vitamin A and 750 I. C. units vitamin D per gram; Viadex, Nopco Chemical Co., Harrison, N. J.

were fed the test diets for two weeks and an average gain of 5 gm or more per week was interpreted as a positive response. Whenever possible, equal numbers of both sexes of rats were used per group. Groups of three rats consisted of one male and two females. A more purified diet (diet B, table 1) comparable to that used by other workers, was also used to determine the effects of high vitamin supplementation. Sulfaguanidine, when added to diet B, was at the expense of sucrose.

RESULTS AND DISCUSSION

Table 2 depicts the results of a typical experiment to determine the component of a stock diet that was responsible for stimulating growth of sulfaguanidine-fed rats. Weanling rats (40 to 50 gm) were fed the indicated modifications of diet A and growth was measured through the seventh week. When it was apparent that meat meal stimulated growth, groups I and III were maintained through the 11th week and 10% meat meal was incorporated into the diet of group I. Group III was

TABLE 2

Effect of meat meal and skimmilk on the growth of rats fed a diet containing 1% of sulfaguanidine

GROUP (4 rats each)	DIETARY ADDITIONS [1]	AV. GAIN AT WEEK			
		2	5	7	11
		gm	*gm*	*gm*	*gm*
I	None	62	80	87 [2]	139
II	Meat meal,[3] 5%	66	177	236	
III	Skimmilk, 5%	61	84	94	103
IV	Sulfa omitted, Sucrose, 1%; casein, 5%	67	161	202	

[1] Diet A.

[2] Ten per cent of meat meal incorporated into the diet of group I after the 7th week.

[3] Meat meal obtained from Valleydale Packers, Salem, Virginia.

retained as a control. It is also evident that skimmilk is devoid of the active factor.

Effect of increased supplementation of vitamins. Results of experiments designed to evaluate the influence of increasing the protein or the known vitamins in the diet and to establish further the value of meat meal in stimulating growth of sulfaguanidine-fed rats is shown in table 3. Thyroid weights were obtained after the indicated feeding periods.

The growth response of meat meal was apparently not due to any of the known vitamins including biotin, folacin and vitamin B_{12}. Para-aminobenzoic acid and ascorbic acid had

also been tested previously. In one experiment, 4 rats which had been fed 1% *p*-aminobenzoic acid with 1% sulfaguanidine had an average weight of 123 gm after 17 weeks, as compared with 224 gm for control rats fed the same diet without sulfaguanidine. Lower levels of *p*-aminobenzoic acid (0.2%)

TABLE 3

Effect of vitamins and meat meal on growth of rats fed sulfaguanidine

EXP.	DIET	ADDITIONS	NO. RATS	AV. WT. AT WEEK			AV. THYROID WT.
				4	6	7	
				gm	*gm*	*gm*	*mg/100 gm body wt.*
I[1]	B	Sucrose, 1%	6	167	190	221	4.5
	B	Sulfaguanidine, 1% + vitamins, 2X	6	145	153	158	27.6
	B	Sulfaguanidine, 1% + vitamins, 2X + meat meal, 10%	6	142	180	197	23.2
II[2]	A	None	3	123	123		19.9
	A	Vitamins, 2X	3	122	119		14.2
	A	Meat meal, 5%	3	122	153		11.7
III[3]	B	Sulfaguanidine, 1%	4	146	159		...
	B	Sulfaguanidine, 1% + vitamins, 2X	7	151	156		...
	B	Sulfaguanidine, 1% + casein, 10%	4	143	157		...

[1] These diets were fed from weaning except that meat meal was not added until the 4th week. Vitamins, when added, were two times those shown in table 1.

[2] Diet A was fed to weanling rats for 4 weeks when growth had declined to 5 gm per week or less. Vitamins or meat meal were then added.

[3] Diet A was fed to weanling rats until growth was 5 gm per week or less (4 weeks). Diet B was then fed with the indicated modifications.

produced similar results. Also, 4 rats fed 200 mg of ascorbic acid per kilogram of diet and injected with 10 mg of ascorbic acid every other day weighed 148 gm (average) after the 11th week. Increasing the level of casein in the diet to 30% stimulated growth only slightly (experiment III).

Effect of various products. The growth effect of other substances added to diet A, after growth of sulfaguanidine-fed

rats had declined to 5 gm per week or less, is shown in table 4. The steak, pork shoulder, egg and liver samples were obtained from a local market. They were dried in a forced-air oven at 95° C. for 24 hours and then ground.

The fecal samples were obtained from animals fed a normal ration devoid of meat meal and were similarly dried and ground. It is evident that, with the exception of meat meal and thyroid powder, none of the products tested were able to overcome the growth inhibition resulting from sulfaguanidine feeding.

Tentori and Vivaldi ('50, '54a,) are of the opinion that some factor produced in the intestinal tract prevented sulfaguanidine toxicity. They were able to cure or prevent the appearance of deficiency symptoms by feeding dry feces from rats fed a normal diet. This has not been observed in this laboratory (table 4) although the conditions used here were not strictly comparable.

Three different sources of liver failed to stimulate growth of rats under the conditions of this study (table 4) although liver has been reported by numerous investigators to alleviate the toxic effects of various sulfonamides. The observations of Axelrod et al. ('45) suggest that the dietary level of the sulfonamide may be a critical factor. Ershoff ('54) observed that only two out of 7 samples of dried liver were effective in counteracting thiouracil toxicity. Another factor which may be of importance is the difference in potency of the various sulfonamides (Astwood et al., '43; Tabenkin et al., '53; Vanderlaan and Bissell, '46). Although liver was ineffective in this study, experiments to be reported indicate that hog stomach, hog pancreas and hog intestine were consistantly effective in preventing the toxic effects of sulfaguanidine. The exact composition of the meat meal used here is impossible to determine, but inquiry [2] discloses that meat meal is made of non-sellable portions of beef and hog carcasses, the intestinal tracts and occasionally an entire condemned animal.

[2] Valleydale Packers, Salem, Virginia

It is significant that, of several hundred rats prepared for assay, occasionally a rat has been observed to grow over an extended period when fed only the "deficient" diet. This phenomenon tends to occur more frequently in males than in females. It should be noted that animals fed the sulfaguani-

TABLE 4

Effect of various substances on growth of rats fed 1% sulfaguanidine

DIETARY ADDITIONS [1] (Diet A)	NO. RATS	AV. 2 WEEK GAIN
		gm
Peanut meal, 10%	10	4
Meat meal, 5%	10	45
Meat meal, 10%	10	31
Meat meal ash [2]	5	1
Cooked, dried whole egg, 10%	2	10
Dried egg yolk, 10%	3	2
Dried whole milk,[3] 10%	3	3
Dried calf liver, 10%	3	—1
Dried beef liver, 10%	3	3
Dried pork liver, 10%	3	5
Dried pork shoulder, 10%	3	5
Dried round steak, 10%	3	7
Dried rat feces, 5%	2	—4
Dried cow feces, 5%	2	2
Dried chicken feces, 5%	2	2
Dried distillers' solubles,[4] 10%	5	1
Soybean meal, 5% with 0.02% thyroid powder [5]	4	34

[1] The additions to the diet were made when weekly weight gains had declined to 4 to 5 gm.

[2] Ash equivalent to 5% meat meal (12 gm ash/kg diet). The meat meal was ashed at 600°C. for 12 hours.

[3] Borden.

[4] Kindly donated by Dr. L. E. Carpenter, Distillers Feed Research Council, Cincinnati, Ohio.

[5] Nutritional Biochemicals Corporation.

dine-containing diet and weighing more than 150 gm after the 5th week were never used for assay purposes. Experience has shown that these animals tend to continue growing and are useless for assay purposes. It has not been possible to determine whether synthesis in the intestinal tract may be the source of protective factors in such animals.

In early experiments, the details of which have not been presented here, a purified diet of methanol-extracted peanut meal was used. The symptoms under this dietary regime appeared to be more severe and rats of the Alabama Experiment Station and Sprague Dawley strains invariably died during the 12th to 14th week when the diet contained 1.0% of sulfaguanidine. Gross examination of the animals failed to disclose the cause of death. No deaths or severe symptoms have appeared when Holtzman rats were maintained for 360 days on the more natural type diet used in this study (diet A).

Stability of meat meal and thyroid powder. Table 5 summarizes the results of studies to determine the stability of the active principle in meat meal to acid and alkali and to compare its activity with that of thyroid powder.

It is clear that the activity of meat meal was destroyed after heating with either concentrated NH_4OH or 5% NaOH, but treatment with 6N HCl for 24 hours had little effect. Thyroid powder was unaffected by refluxing with concentrated NH_4OH or 5N HCl for 36 hours but the activity of meat meal was considerably decreased. However, treatment of meat meal with 5N HCl for shorter periods (13 hours) indicated that its activity was not decreased and that the active fraction was retained in the acid-insoluble residue (experiment II, table 5).

The growth factor in meat meal is stable to heat and acid hydrolysis for relatively short periods of time, but is all or partially destroyed by heating with NH_4OH or NaOH, suggesting that it is not thyroxine. As reported by Kendall ('29), thyroxine is stable under these conditions.

The possibility that thyroxine, if present in meat meal, was being destroyed or inactivated by some interaction with other components of meat meal has been ruled out since thyroid powder was as effective in promoting growth after treatment with NaOH in the presence of meat meal, as untreated thyroid powder (experiment III, table 5). A similar pattern of results was observed when the acid-insoluble residue of meat meal was

heated with and without thyroid powder in 2% NaOH for 48 hours.

It is significant that the administration of desiccated thyroid or thyroxine to rats fed 2% sulfaguanidine results in thyroid glands of normal weight (Mackenzie and Mackenzie, '43).

TABLE 5

Effect of heat, acid, and alkaline hydrolysis on the activity of meat meal and thyroid powder

EXP.	ADDITION AND TREATMENT [1]	NO. RATS	AV. 2 WK. GAIN
			gm
I	Meat meal, untreated, 100 gm	4	40
	Meat meal, dry heat, 95°, 24 hrs., 100 gm	3	32
	Meat meal, NH_4OH, 48 hrs., 100 gm	4	5
	Meat meal, 5% NaOH, 24 hrs., 100 gm	4	— 1
	Meat meal, 6N HCl, 24 hrs., 100 gm	4	32
II	Meat meal, untreated, 50 gm	4	40
	Meat meal, 5N HCl, 36 hrs., 50 gm	4	5
	Meat meal, NH_4OH, 36 hrs., 50 gm	4	19
	Thyroid powder, 5N HCl, 36 hrs.,[2] 0.2 gm	4	44
	Thyroid powder, NH_4OH, 36 hrs., 0.2 gm	4	43
	Meat meal residue, 5N HCl, 13 hrs.[3]	4	45
	Meat meal filtrate, 5N HCl, 13 hrs.[3]	4	— 3
III	Peanut meal, untreated, 50 gm	4	1
	Meat meal, untreated, 50 gm	4	40
	Meat meal, 5% NaOH, 24 hrs., 50 gm	4	19
	Thyroid powder, untreated, 0.2 gm	4	44
	Thyroid powder, 0.2 gm, meat meal, 50 gm, 5% NaOH, 24 hrs.[4]	4	43
	Meat meal residue, untreated, 10 gm	4	55
	Meat meal residue, 2% NaOH, 48 hrs., 10 gm	4	26
	Meat meal residue, 10 gm, thyroid powder, 0.2 gm, 2% NaOH, 48 hrs.[4]	4	53

[1] Samples were refluxed with 300 ml of HCl, NaOH or conc. NH_4OH for the time indicated, then neutralized and made up to 1 kg with diet A.

[2] Thyroid powder was mixed with 50 gm of soybean oil meal and treated as indicated.

[3] Fifty grams of meat meal, hydrolyzed with 5N HCl for 13 hrs., was divided into the acid-insoluble residue and the filtrate. The filtrate was evaporated to near dryness, neutralized, and made to 1 kg with diet A.

[4] Thyroid powder was mixed directly with meat meal or the acid-insoluble residue, and treated as indicated.

When meat meal was fed, thyroid weight was decreased slightly but was still two to three times larger than normal (table 3) suggesting that the growth effect was not due to thyroxin.

These experiments have compared the active factor in meat meal with the stability and behavior of thyroxin, whose characteristics have been well established (Kendall, '29). The possibility remains that the active principle in meat meal may be similar to growth-promoting factors of the thyroid gland other than thyroxin.

SUMMARY

Growth was inhibited when weanling rats were fed complete diets containing 1% sulfaguanidine. Addition of all known vitamins, including biotin, folacin and vitamin B_{12} failed to stimulate growth. Whole dry egg, whole milk powder, calf, beef and pork liver, pork shoulder, round steak, distillers, solubles at a dietary level of 10% also failed to stimulate growth. Dried rat, cow or chicken feces, at a level of 5%, were also without effect. Meat meal, of indeterminate composition, at a level of 5% of the diet stimulated growth of rats which had ceased to grow due to the ingestion of sulfaguanidine.

When meat meal (5%) was incorporated into the sulfaguanidine-containing diet of weanling rats, growth was as good or better than was observed in similar animals fed diets containing no sulfaguanidine or meat meal.

The growth-promoting factor of meat meal was stable to dry heat and refluxing with 6N HCl for 24 hours, but was destroyed or inactivated by refluxing with NH_4OH or NaOH. Activity of meat meal was retained in the acid-insoluble residue after refluxing with 5N HCl for 13 hours. Comparison of the stability of meat meal with thyroid powder indicates that the active principle in meat meal was not thyroxine.

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THE EFFECT OF RADIATION STERILIZATION ON THE NUTRITIVE VALUE OF FOODS

II. BIOLOGICAL VALUE OF PEA AND LIMA BEAN PROTEINS [1]

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We have previously reported the effect of irradiation sterilization on the nutritive value of some animal proteins, namely, the milk and beef proteins (Metta and Johnson, '56). In view of the fact that fresh legumes are commonly used in the American diet and the dry legumes form a good proportion of low-cost diets, experiments were undertaken to study nutritional changes due to irradiation sterilization of two commonly used legume seeds, peas and lima beans.

The purpose of this paper is to report the changes brought about in the nutritive value of the proteins of fresh frozen peas due to irradiation sterilization and conventional heat cooking (not sterilization) and the changes in the nutritive value of the proteins of fresh lima beans treated with increasing dosages of gamma irradiation, heat cooking, and a combination of irradiation sterilization and conventional cooking.

The data demonstrate that the digestibility of the pea protein is not changed due to cooking or irradiation. However, the biological value of the pea protein is reduced by 5% due to irradiation. In the case of the raw lima bean, irradiation either with 3 million r or 10 million r does not signifi-

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cantly alter the digestibility or the biological value of its protein, while heat cooking of beans irradiated at 3 million r improves the digestibility of nitrogen by 9%, and the biological value by 12%.

TABLE 1

Chemical analysis of the garden peas and the lima beans

	MOISTURE	CRUDE PROTEIN (N × 6.25)	ETHER EXTRACT	CRUDE FIBER	HEAT OF COMBUSTION, GROSS ENERGY
	%	%	%	%	*Cal.*
Garden peas					
Non-processed (fresh frozen)	80.7	5.44	0.16	1.99	88.2
Irradiation processed [1]	80.5	5.57	0.12	1.89	89.0
Heat cooked [2]	80.3	5.37	0.24	1.91	85.9
Lima beans					
Non-processed (fresh frozen)	65.8	7.59	0.17	2.14	150.0
Irradiation processed [1]	68.1	7.43	0.14	2.12	148.2
Irradiation processed [3]	66.2	7.45	0.18	2.17	150.2
Heat cooked [2]	66.7	7.45	0.24	2.15	151.1
Irradiation processed [1] and heat cooked	69.1	7.13	0.16	2.15	147.1

[1] Sterilized with 3 million r.

[2] Cooked in an autoclave for 4 minutes at 15 lbs. pressure.

[3] Sterilized with 10 million r.

EXPERIMENTAL

Frozen lima beans and green peas [2] were purchased from a local food store. All samples of peas and beans were repacked in no. 2 cans, frozen and preserved at — 20°F. Some of these cans were irradiation sterilized [3] with gamma rays. The samples remained frozen before and after irradiation.

[2] The lima beans (Phaseolus lunatus) were of the Agen brand and marked "Baby Lima Beans" and distributed by the Cascade Frozen Foods, Inc., Burlington, Washington. The garden peas (Pisum sativum) were of the "Green Giant Peas" brand and distributed by the Le Sueur Company.

[3] Irradiation was carried out at the Atomic Energy Commission's Material Testing Reactor (MTR) by the Phillips Petroleum Company.

Proximate analyses of the non-processed and heat- or irradiation-processed peas and beans did not reveal any significant differences for the nutrients analyzed (see table 1), no loss of nitrogen occurring as a result of irradiation.

The nutritive value of the legume proteins was measured on growing male albino rats of the Sprague-Dawley strain according to the biological value method of Mitchell ('24, '44). The samples of peas or beans, whether non-processed, heat-processed or irradiation-processed, were ground wet and incorporated wet into experimental diets to provide 10% protein (N $\times$ 6.25), on a moisture-free basis. The pea diets contained 25% cornstarch, 15% cerelose, 10% sucrose, 8% lard, 1.5% cod liver oil, 0.5% wheat germ oil, 5% minerals and 35% peas (on moisture-free basis). The composition of the lima bean diets was 14% cornstarch, 15% cerelose, 10% sucrose, 7% lard, 1.5% cod liver oil, 0.5% wheat germ oil, 4% minerals and 47% lima beans (on dry basis). Vitamins were incorporated per 100 gm (dry) diet as follows: calcium pantothenate, 2 mg; choline chloride, 200 mg; pyridoxine hydrochloride, riboflavin and thiamine hydrochloride, 0.25 mg each; nicotinic acid, 1 mg; *p*-aminobenzoic acid 5 mg; inositol, 10 mg; and 2-methyl-1,4-naphthoquinone, 1 mg. The methods used for preserving, for feeding of the diets, and for the chemical analyses of the nutrients, diets and excreta are described elsewhere (Metta and Johnson, '56). The proximate analyses of the experimental diets are given in table 2.

In each experiment there were three feeding periods. During the first and third periods test diets were fed according to the plan of the experiment, while during the second period all rats were fed a low-nitrogen diet, otherwise similar to the test diets. Each of the feeding periods was generally of 16 days of which the first 9 days were allowed for physiological adjustment to the diets and during the following 7 days urine and feces collections were made. The rats were on constant food intake during the last 5 days of the prefeeding periods and the subsequent 7 days of the collection periods.

TABLE 2

Chemical analysis of experimental diets

	PEA DIETS			BEAN DIETS				
	Fresh frozen peas	Irradiated peas (3 million r)	Heat-cooked peas	Fresh frozen lima beans	Irradiated lima beans (3 million r)	Irradiated lima beans (10 million r)	Heat-cooked lima beans	Irradiated plus heat-cooked lima beans (3 million r)
	%	%	%	%	%	%	%	%
On fresh basis								
Moisture	59.1	60.5	62.4	49.2	49.6	50.1	49.0	49.0
Crude protein	4.03	4.06	3.90	5.69	5.54	5.63	5.48	5.32
Ether extract	4.11	4.04	3.37	5.13	5.05	5.13	5.05	5.24
Crude fiber	1.37	1.46	1.61	1.57	1.59	1.60	1.56	1.56
Heat of combustion, Cal.	181	184	170	228	227	228	220	220
On moisture-free basis								
Crude protein	9.83	10.26	10.38	11.18	11.00	11.25	11.17	10.84
Ether extract	10.04	10.22	8.97	10.09	10.02	10.25	10.32	10.68
Crude fiber	3.35	3.69	4.29	3.10	3.15	3.21	3.05	3.50
Heat of combustion, Cal.	443	465	452	448	450	455	449	450

The effects of both irradiation sterilization and conventional heat cooking were tested on peas. The irradiation treatment used for the peas was 3 million r gamma-ray irradiation [4]; heat processing consisted of cooking the fresh peas in the autoclave for 4 minutes at 15 lbs. pressure. Six rats were used per treatment.

The plan of feeding the test diets was as follows:

PERIOD	GROUP 1	GROUP 2	GROUP 3
I	Non-processed peas diet	Cooked peas diet	Radiation-processed peas diet
II	4% whole egg diet	4% whole egg diet	4% whole egg diet
III	Radiation-processed peas diet	Radiation-processed peas diet	Non-processed peas diet

The presence of trypsin inhibitor in the lima beans (Borchers and Ackerson, '47) and the relative resistance of enzyme systems to irradiation (Proctor and Goldblith, '51) led us to investigate the effect of heat cooking and of increasing dosages of irradiation on the nutritive value of the lima beans. Since the resistance of the trypsin inhibitor to irradiation treatment masked the effect of the irradiation on the nutritive value of the lima bean protein, it was thought worth while to study a combined effect of irradiation and heat cooking on the same sample of the fresh lima beans. Five treatments were included in the plan of the experiment: treatment 1 consisted of the fresh frozen lima beans; treatment 2, lima beans irradiated with 3 million r; treatment 3, lima beans irradiated with 10 million r; treatment 4, heat-cooked lima beans; and treatment 5, heat-cooked lima beans which had been previously irradiated with 3 million r. A treatment of 10 million r was used to determine whether the trypsin inhibitor could be inactivated, as well as to study the changes in the nutritive value of the raw lima beans after the high irradiation dose. Five rats were used per treatment.

[4] r is roentgen.

The plan of feeding the test diets was as follows.

PERIOD	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
I	Raw lima beans diet	3 mr [1] lima beans diet	10 mr [1] lima beans diet	Cooked lima beans diet	3 mr [1] and cooked lima beans diet
II	4% egg diet	4% egg diet	4% egg diet	4% egg diet	4% egg diet
III	Cooked lima beans diet	3 mr [1] and cooked lima beans diet	Raw lima beans diet	10 mr [1] lima beans diet	3 mr [1] lima beans diet

[1] Million r.

Detailed statistical analysis of the nitrogen metabolism data was done according to the analysis of variance.

RESULTS AND DISCUSSION

The effect of heat cooking and of irradiation sterilization on garden peas. The irradiation-treated peas were not different in odor or appearance from the non-processed or heat-cooked peas. The rats were offered the diets containing non-processed, heat-cooked or irradiated peas in equal though restricted amounts and were found to consume the diets quite readily, no difference in the acceptability of the irradiated peas being noticed. There were no food refusals in any group. The food intake by each rat in each group was 5.5 gm/day during the first collection period and 6.8 gm/day during the third collection period. During both the periods the rats grew at the rate of approximately a gram a day and there was no difference between groups.

The digestibility and biological value of pea proteins. The digestibility and the biological value data are given in table 3. Since each animal was its own control, the individual differences between periods I and III were subjected to analysis of variance which showed clearly that there were no significant differences in the digestibility of the proteins of the raw and the processed (heat-cooked or irradiation sterilized) peas; however, there were differences among the biological values of the proteins of the variously processed peas. By the method of least squares it was found that the biological value of the

cooked pea protein (58%) did not differ significantly from the raw pea protein (raw minus heat processed, 0.52 ± 1.88), but that of the radiation sterilized pea protein was significantly ($P < 0.01$) lower than the raw pea protein (about 58%), the difference being 7.88 ± 0.94. The biological value of the protein of the irradiated peas (about 51%) was significantly ($P < 0.01$) lower than that of the heat-processed peas, the difference being 7.36 ± 1.64.

TABLE 3

Average coefficients of apparent and true digestibility and biological values of proteins for the various pea samples

EXPERIMENTAL PERIOD NO.	RAW PEAS	HEAT-COOKED PEAS	IRRADIATION-STERILIZED PEAS
		Apparent digestibility, %	
I	83.1	83.6	82.6
III	83.9	...	83.2
Mean	83.5	83.6	82.9
		True digestibility, %	
I	91.4	91.4	90.7
III	92.9	...	90.9
Mean	92.2	91.4	90.8
		Biological value, %	
I	59.0	57.8	51.9
III	57.6	...	49.1
Mean	58.3	57.8	50.5

Woods, Beeson and Bolin ('43) have reported that autoclaving (17 lbs. for 1.5 hrs.) of field peas decreased their value for growth of rats when compared with raw peas, and they concluded that moist heat destroys cystine in peas. Everson and Heckert ('44) have also reported that the efficiency of vine-ripened, raw pea protein decreases from 0.76 to 0.36 on autoclaving (15 lbs. for 45 minutes). Compared to the simple cooking of 4 minutes at 15 lbs. pressure, the autoclaving methods (comparable to commercial heat sterilization) used by Woods et al. may be considered rather severe and probably have destroyed cystine in their pea samples.

The effect of heat cooking and of irradiation sterilization on lima beans. The irradiation-treated lima beans were somewhat bleached and had a different odor in comparison with the raw or cooked beans.

Rats in all treatments consumed completely the diets offered during the first 9 days, but beginning the 10th day there were diet refusals in all the groups except the one receiving the diet containing the heat-cooked lima beans and the one receiving the diet containing the cooked irradiated lima beans. The daily intakes of diets containing the variously treated lima beans were as follows: raw, 3.99 gm; 3 million r irradiated, 4.47 gm; 10 million r irradiated, 4.25 gm; cooked lima beans, 6.37 gm; and 3 million r irradiated cooked, 6.32 gm.

In period III the experimental diets were interchanged as indicated in the plan. During this period also food refusals were found. The intakes of the lima bean diets (dry basis) per rat per day were as follows: raw, 5.03 gm; 3 million r irradiated, 7.73 gm; 10 million r irradiated, 6.70 gm; cooked lima beans, 7.75 gm; and 3 million r irradiated cooked, 7.73 gm. In both periods the intakes of diets containing either raw or 3 million r sterilized or 10 million r sterilized lima beans were significantly ($P < 0.01$) lower than those of the diets containing the heat-cooked lima beans whether or not the latter have been previously irradiation sterilized. Everson et al. ('44) reported that rats receiving vine-ripened raw lima beans lost weight, while those receiving autoclaved (15 lbs. for 45 minutes) lima beans grew reasonably. In our experiments, rats receiving the diets containing the raw or irradiated (but not cooked) beans hardly maintained their body weights, while those receiving the cooked beans, whether irradiated or not, grew at the rate of about 1 gm/gm protein consumed during the 38 days of feeding.

Borchers et al. ('47) have reported the presence of a trypsin inhibitor in raw lima beans and it appears that heat cooking (15 lbs. for 4 minutes) destroys this inhibitor and thus makes the beans acceptable. Since there was no signifi-

cant difference in the intake of the cooked compared to the cooked irradiated lima beans, it is evident that irradiation does not result in reduced acceptance of the lima beans by the growing rat.

The digestibility and biological value of the lima bean protein. The summary of the nitrogen metabolism data is given in table 4. Analysis of variance showed that there were no differences between rats treated alike. Hence, their 20 degrees of freedom were pooled with the 20 for rat times period

TABLE 4

Coefficients of apparent and true digestibility and biological values of proteins for the various lima beans

EXPERIMENTAL PERIOD NO.	FRESH FROZEN LIMA BEANS	3 MILLION R IRRADIATION-STERILIZED LIMA BEANS	HEAT-COOKED LIMA BEANS	3 MILLION R IRRADIATION-STERILIZED AND HEAT-COOKED LIMA BEANS	10 MILLION R IRRADIATION-STERILIZED LIMA BEANS
			Apparent digestibility, %		
I	59.9	61.2	67.4	71.5	63.2
III	62.2	61.3	68.1	70.9	62.8
Mean	61.1	61.3	67.8	71.2	63.0
			True digestibility, %		
I	68.2	69.0	76.9	81.3	71.9
II	67.9	71.1	76.4	77.3	72.0
Mean	68.1	70.0	76.6	79.3	72.0
			Biological value, %		
I	52.5	47.5	65.7	60.5	48.4
III	44.1	46.9	61.1	58.5	48.1
Mean	48.2	47.2	63.4	59.5	48.3

interaction. This gave an error mean square of 11.66 for true digestibility and 20.46 for biological value, with 40 degrees of freedom. In order to evaluate the treatment effects it was necessary to fit constants. This showed a favorable effect of heat amounting to about 9.32 percentage points for digestibility of nitrogen and 12.34 percentage points for biological value, both at 0.1% level. Also there was some indication of an effect of irradiation, digestibility of nitrogen increasing about 0.9 percentage points and biological value decreasing

about 1.3 percentage points for each million r, both significant only at the 10% level.

Proctor and Goldblith ('51) state "that in food materials a great deal more energy (by a factor of 10 or greater) is necessary for complete inactivation of enzymes than for complete destruction of bacteria." They found, for example, that a 10 million r cathode ray irradiation did not completely inactivate the peroxidase in milk, even though these workers have demonstrated that 2 million r is an effective dose for sterilization. Frankel-Conrat et al. ('52) have succeeded in isolating and characterizing a trypsin inhibitor from lima beans and have reported that this factor was relatively resistant to denaturation and not attacked by pepsin or papain. Our data are consistent with the presence of the trypsin inhibitor in the raw lima beans we used and indicate that irradiation even with 10 million r failed to inactivate this factor completely.

We found no difference in the digestibility or the biological value of the heat-cooked lima beans as compared with those of the irradiation sterilized plus heat-cooked lima beans. The digestibility of the nitrogen of the heat-cooked lima beans (77%) was not significantly different from that of the heat-cooked lima beans which had been irradiation sterilized (79%). The biological values of the heat-cooked lima bean and irradiation sterilized plus heat-cooked lima bean proteins were 63 and 60%, respectively, and these values were also not significantly different. Thus, irradiation sterilization with a 3 million r did not alter the nutritive value of the cooked lima bean protein.

SUMMARY AND CONCLUSIONS

The effect of irradiation sterilization and of heat cooking on the nutritive value of the protein in the fresh frozen garden pea and the lima bean was studied by the Thomas-Mitchell method. A 3 million r gamma irradiation was used to sterilize the frozen canned samples. No significant loss in nitrogen

of the peas or beans resulted due to irradiation. The diet containing irradiated peas was found acceptable to the rat. Irradiation of the lima beans did not improve the poor consumption of the raw lima bean diet.

Irradiation sterilization, like heat cooking, did not affect the digestibility of the raw pea protein (92%). While the biological value of the pea protein (58%) was also not affected by short-time cooking, irradiation sterilization with 3 million r dosage reduced it by 8%.

The rats consumed the raw lima bean diets reluctantly, and heat cooking improved markedly the acceptability of these diets. Cooking also increased the digestibility by 9% and the biological value by 12%. On the other hand, irradiation of the raw lima beans with 3 or even 10 million r did not significantly improve the digestibility or the biological value of their protein. These findings are apparently the result of the trypsin inhibitor present in raw lima beans which thus appears to be destroyed by heat and not by irradiation. No difference in the nutritive value of the lima bean protein was found when the heat-cooked beans were compared with the irradiated and subsequently cooked lima beans. This indicates no damage to the lima bean protein due to irradiation.

ACKNOWLEDGMENTS

The authors express their appreciation to Prof. A. I. Nelson and Dr. M. P. Steinberg of the Food Technology Department of the University of Illinois for allowing the use of their laboratory facilities for canning of the legume samples, and to Mr. John McGill for assisting with canning.

The authors also acklowledge the technical assistance of Mr. James R. Hollis and Mrs. Helen Sterrett.

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STUDIES OF PROTEIN RETENTION AND TURNOVER USING NITROGEN-15 AS A TAG

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Pasadena Foundation for Medical Research, Pasadena, California

(Received for publication April 5, 1957)

Methods employing isotopes have been used to investigate both protein metabolism and metabolic rates. Heavy water was used by Hevesy and Hofer ('34) to study turnover numbers for water in the human body. Iodinated casein tagged with I^{131} was used by Lavik et al. ('52) in the determination of protein absorption, as well as the measurement of fecal and urinary excretion of radioactive iodine. Methionine labeled with S^{35} has been used in the evaluation of certain phases of protein metabolism (Kinsell et al., '50), and P^{32} has been used to determine turnover of phospholipides in plasma (Cornatzer and Cayer, '50). Nitrogen-15 has been employed to study the rates of amino acid utilization and tissue protein turnover (Sprinson and Rittenberg, '49) and protein assimilation (Sharp et al., '56). A comparison of the uniformly labeled protein and labeled single amino acid techniques in protein utilization studies has indicated differences in the calculated rates of protein synthesis (White and Parson, '50).

In our investigation of protein metabolism, N^{15} was selected as the tag because it is the simplest specific protein indicator. The N^{15} technique is the most direct way in which the turnover of the protein pool may be estimated. Further, while fecal nitrogen can be identified as to food source by means of markers fed before and after protein ingestion, the same does not apply to urinary nitrogen excretion. Here N^{15} is a most useful means of identifying the excretion of nitrogen from a specific protein intake.

Studies from this laboratory have dealt with the absorption of N^{15} tagged yeast in normal, achlorhydric and hypochlorhydric subjects (Sharp et al., '56). The present investigation is concerned with the retention of absorbed N^{15} and the rate of protein turnover in the body in the same series of experiments.

PROCEDURE

Patients, feeding methods, N^{15} baseline determinations and all other procedures were those described in our earlier study. Urine specimens collected from each subject were pooled for that individual on an approximate calendar day basis for convenience.

The N_2 content of each pooled specimen was determined with a Kjeldahl procedure. The subsequent release of N_2 by a modification of the method of Rittenberg ('46) permitted N^{15} estimation on the mass spectrometer as before.

RESULTS

All pooled urine specimens collected for 5 days subsequent to tagged feeding were found to have an appreciable elevation of N^{15} concentration (table 1). The specimens with the peak concentration of N^{15} were found to be those of the second pooling for all subjects. In the final pooled specimens (the last 11 to 36 hours) of the 120-hour collection period the N^{15} concentrations were found to have declined to an atom percent excess of less than twice the baseline level.[1] These specimens contained less than 3% of the absorbed tracer (table 2).

Extension of the collection period for urine specimens over a longer time interval would have yielded additional information relative to the nitrogen metabolism rate of individuals. However, as may be seen from the individual average hourly excretion rate of the tag at the end of the 5-day collection period (table 2), the time interval used was sufficient for the average hourly rate of N^{15} excretion to fall to 0.06% of the absorbed tracer. Further collection of specimens would not

[1] The biologic baseline for our subjects was established in our previous study at 0.0066 atom per cent excess N^{15}.

significantly increase the amount of N^{15} excreted, and the values we have obtained permit reasonably accurate determination of the net retention of the tag and the relative protein utilization rates of the subjects studied.

The retention of absorbed N^{15} was determined for each individual (table 3). The average retention for the entire group was found to be 52.0%. For the young controls the average was 57.6%, but for the older subjects studied the average value dropped to only 49.1%. The reduced retention of protein

TABLE 1

The rise and fall of N^{15} concentration in urine specimens following tagged feeding

SUBJECT	INITIAL POOLED SPECIMEN		POOLED SPECIMEN WITH PEAK N^{15} CONCENTRATION		FINAL POOLED SPECIMEN	
	Hours after tagged feeding	Concentration of N^{15} above biologic baseline	Hours after tagged feeding	Concentration of N^{15} above biologic baseline	Hours after tagged feeding	Concentration of N^{15} above biologic baseline
		Atom % excess		*Atom % excess*		*Atom % excess*
L.G.	10	0.0873	10–34	0.1038	106–120	0.0084
G.S.	10	0.0668	10–36	0.0952	101–120	0.0063
A.E.	12	0.0970	12–33	0.1130	82–118	0.0097
S.B.	10	0.0878	10–34	0.0960	82–117	0.0094
B.S.	7	0.0395	7–36	0.1327	106–118	0.0027
N.H.	10	0.1019	10–35	0.1580	106–117	0.0128

TABLE 2

Per cent recovery of absorbed tracer in final pooled urine specimens

SUBJECT	HOURS AFTER TAGGED FEEDING	$\frac{N^{15} \text{ IN SPECIMEN}}{N^{15} \text{ ABSORBED}} \times 100$	$\frac{\text{AVERAGE } N^{15} \text{ EXCRETED/HOUR}}{N^{15} \text{ ABSORBED}} \times 100$
		%	%
L.G.	106–120	0.78	0.06
G.S.	101–120	0.72	0.04
A.E.	82–118	2.85	0.08
S.B.	82–117	2.99	0.09
B.S.	106–118	0.21	0.02
N.H.	106–117	0.82	0.07
			Mean
			0.06 ± 0.02

by the older group is probably due to lowered metabolic efficiency.

Using the ingenious format of Sprinson and Rittenberg, but basing our calculations on N^{15} absorbed rather than on the amount ingested, we derived protein synthesis rates and physiologic half-lives of N^{15} absorbed comparable to those which they obtained (table 4). We found as an average among our subjects the amount of nitrogen (including both fed and

TABLE 3

Retention of absorbed N^{15}

SUBJECT	N^{15} ABSORBED	N^{15} EXCRETED IN URINE	N^{15} RETAINED	$\frac{N^{15}\text{ RETAINED}}{N^{15}\text{ ABSORBED}} \times 100$	
	mg	*mg*	*mg*	%	
			Young controls		
L.G.	72.95	31.83	41.12	56.36	
					Mean
G.S.	77.62	31.92	45.70	58.89	57.6
			Older subjects		
A.E.	76.02	38.40	37.62	49.51	
S.B.	75.21	40.11	35.10	46.66	
					Mean
B.S.	75.43	35.34	40.09	53.13	
					49.1
N.H.	75.94	40.13	35.81	47.13	
				Mean	
				52.0 ± 4.2	

recycled) used for protein synthesis in the body to be 0.23 gm per kilogram per day, which agrees well with their value of 0.22 gm. Also, our finding of 75 days (average) for the half-life of N^{15} from yeast compares well with their value of 80 days from glycine.

We applied the same method of calculation to data published by White and Parson, and for one subject, A. W., for whom sufficient data were available, found a half-life of 91 days for N^{15} from tagged glycine, and 75 days for tagged yeast.

TABLE 4

Protein synthesis rate, size of nitrogen pool, and N^{15} "half-life" in human subjects

SUBJECT		AGE	WEIGHT	E	$\frac{\lambda_e}{\lambda_o}$		A	B	S	P	E_k	S_k	P_k	$t_{\frac{1}{2}}$	
		yrs.	*kg*	*gm*	*hrs.*	*hrs.*			*gm*	*gm*	*gm*	*gm*	*gm*	*days*	
					0–60	0–120									
Young controls	L.G.	24	58.1	13.4	0.378	0.436	0.447	0.77	16.6	39.0	0.231	0.286	0.671	60.6	Mean 61 days
					0–60	0–120									
	G.S.	24	72.7	14.3	0.385	0.411	0.412	1.06	20.4	32.7	0.197	0.281	0.450	61.7	
					0–59	0–118									
	A.E.	66	75.2	14.4	0.448	0.505	0.512	0.85	13.7	33.1	0.191	0.182	0.440	95.2	
					0–58	0–116									
Older subjects	S.B.	59	75.5	16.7	0.461	0.533	0.548	0.75	13.8	40.7	0.221	0.183	0.539	94.7	Mean 86 days
					0–59	0–118									
	B.S.	70	70.8	13.6	0.423	0.469	0.475	0.89	15.0	32.1	0.192	0.212	0.543	81.7	
					0–58	0–116									
	N.H.	70	43.3	12.3	0.459	0.529	0.542	0.75	10.4	30.3	0.284	0.240	0.700	72.2	
													Mean 0.231 → 75.0		

After Sprinson and Rittenberg ('49).

$\lambda_e = \lambda_o A(1-e^{-Bt})$

$A = \frac{E}{E+S}$

$B = \frac{E+S}{P}$

t = time in days

E Average urinary nitrogen excretion per 24 hours
λ_e Cumulative N^{15} excretion in urine to hours shown
λ_o N^{15} absorbed
A Numerical constant
B Numerical constant
S Nitrogen used for protein synthesis in the body per day
P Size of the nitrogen pool
$t_{\frac{1}{2}}$ "Half-life" in the body of N^{15}

E_k, S_k, P_k } Values as above per kg of body weight

We also found (table 4) that the young normal controls in our study (age 24 years) had a mean N^{15} half-life of 61 days, and the older subjects (average age 66 years) had a mean N^{15} half-life of 86 days. From these values we may deduce that the turnover of N^{15} (protein, nitrogen) in the young controls was approximately one and one half times that in the older subjects.

DISCUSSION

The nitrogen of any single amino acid may enter into its unique metabolic paths as well as entering nitrogen pools after deamination. Geiger ('47) has shown that all essential amino acids should be present simultaneously for the utilization of any of them. The protein and its constituent amino acids may enter all of the paths which each single amino acid may follow as well as entering the common nitrogen pools. From this point of view, it would be surprising should the half-life of N^{15} from tagged glycine, a non-essential amino acid, be comparable to a generally tagged yeast. From the relatively close agreement of half-lives, it would seem that the turnover number of much of the nitrogen of the human body is rather slow, as Sprinson and Rittenberg have already indicated.

The conditions of our experiment fulfill the requirement of no N^{15} intake prior to test feeding (as in classical endogenous nitrogen determinations) and permit us to disregard endogenous N^{15}. More prolonged experimental periods should allow the determination of endogenous nitrogen from N^{15} data. The use of absorbed N^{15} as a basis for calculation rather than N^{15} ingested is a further refinement of the tagged feeding technique.

For a 70-kg man we find a minimum nitrogen requirement from the pool [2] of 70×0.23 or 16 gm per day for tissue protein synthesis, which agrees closely with the value reported by Sprinson and Rittenberg.

From the turnover rates found for the young controls and the older subjects it would seem that physiologic aging is a

[2] The pool is defined by Sprinson and Rittenberg ('49) as containing both fed and recycled nitrogen.

result of a slowing down of the protein synthesis rates as well as of metabolic processes in general. In this study we have been able to determine rates of protein synthesis and compare them at different age levels.

SUMMARY

Nitrogen-15 from single tagged feeding has been used to measure nitrogen in urine from a given protein source. Comparative studies of retention of absorbed protein in young and older subjects have been presented. The average retention in the young subjects was found to be 57.6%, but only 49.1% in the older subjects. The half-life of N^{15} from yeast in the body was found to average 75 days for the entire group studied: for the young controls 61 days and for the older subjects 86 days. The rate of protein turnover in the young controls was found to be approximately one and one half times that in the older subjects. Increased physiologic age would seem a probable cause for this reduced utilization of protein. The average nitrogen requirement from the pool for protein synthesis in the body was found to be 0.23 gm per kilogram per day. The nitrogen requirement for maintenance for a 70-kg man was found to be 16 gm per day.

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

PALMER HOUSE, CHICAGO, ILLINOIS
APRIL 14–19, 1957

COUNCIL MEETING

Council meetings were held at the Palmer House, Chicago, on Sunday, April 14, Monday, April 15, and Tuesday, April 16. Formal actions of the Council are reported in the following minutes of the two business meetings of the society held on April 16 and 18 in the Palmer House.

SCIENTIFIC SESSIONS

One hundred and eleven papers were submitted by members. Seven of these were transferred to biochemistry, three to physiology, and 12 to intersociety sessions. Thus 89 papers submitted by members, together with 18 received from other member societies (8 from biochemistry, 7 from physiology, and three from pharmacology) constituted a program of 10 half-day sessions. In addition, one half-day symposium was arranged under the title "Nutrition and Food Technology."

BUSINESS MEETINGS

1. Minutes. The minutes of the 20th Annual Meeting, as published in the September 1956 issue of the *Journal of Nutrition,* and as corrected in the October issue of the Journal, were approved.

2. Election. The secretary transmitted the sealed ballots to the Tellers' Committee, Dr. Harry Spector and Dr. A. E.

Harper. The Committee reported election results on 230 ballots cast as follows:

President: R. R. Williams
Vice President: William J. Darby
Secretary: George M Briggs (3-year term)
Councillor: Paul Gyorgy
Associate Editors (4-year term beginning May 1, 1957)
R. W. Engel
P. L Harris
H. A. Schneider

By vote of 206 for to 5 against the constitutional amendment was adopted as follows:

Article V, section 4.

Filling of vacancies: *A.* Should a vacancy occur in any elective position, the Council, by majority vote, shall have the power to appoint a member of the society to fill the unexpired term until the following election.

(This section previously read as follows: The Nominating Committee shall fill all vacancies in elective positions except such as may occur at a meeting of the society.)

On the basis of the suggestion on the ballot returns, President Williams appointed the following Nominating Committee for 1957–1958:

D. V. Frost, Chairman
C. M. McCay O. L. Kline J.M. Orten B. L. Oser

3. Membership. The Secretary reported that as of April 1, 1957, there were 397 active members and 41 retired members, or a total of 438 members in the American Institute of Nutrition. The following members passed away during the year:

Dr. H. J. Deuel, Jr., April 17, 1956 (Past President)
Dr. Arthur D. Holmes, July 18, 1956
Dr. S. Brody, August 6, 1956
Dr. A. J. Carlson, September 2, 1956
Dr. H. R. Kraybill, September 30, 1956

Appropriate resolutions relative to the deceased were approved and a copy is on file in the Secretary's Office.

The following members resigned during the year:

Dr. E. E. Snell
Dr. J. W. Conn

4. Treasury. The Auditing Committee, Mary B. Patton and F. A. Hitchcock, reported that the Treasurer's financial statement was substantiated by the records of Treasurer J. B. Brown's Office. The Treasurer's and Auditors' reports were approved. Dues were approved at $2.00 per member for the coming year. A summary financial report follows:

Receipts		
Membership dues	$403.00	
Interest on bond	13.80	
Miscellaneous	4.00	
TOTAL		$420.80
Expenditures		
Guests, Annual Dinner	$ 20.00	
Secretary's Office:		
Secretarial assistance	150.00	
Postage	90.00	
Miscellaneous	8.36	
Treasurer's Office:		
Supplies	65.94	
Secretarial assistance	50.00	
National Academy of Science (aid in publication cost*)	50.00	
International Union of Nutritional Sciences	14.00	
Framing charter	6.30	
Miscellaneous	2.91	
TOTAL		$457.51

* Publication entitled *Challenge of the Life Sciences.*

5. Secretary's report of federal income tax-exempt status. During the year the financial records and other pertinent documents of the society were assembled and presented to the U. S. Treasury Department, Office of the Director of Internal Revenue, for an opinion relative to the income tax exemption status of our society. Based upon the evidence presented, the American Institute of Nutrition has been declared exempt from Federal income tax as an organization operating exclusively for educational and scientific purposes as described in section 501 (c) (3) of the Internal Revenue Code of 1954.

6. Journal of Nutrition. The Journal Editor, Dr. G. R. Cowgill, submitted an annual report, the summary of which follows:

	Nos.
Volumes of the Journal of Nutrition, 1956	58,59,60
Number of papers submitted	185
Number of papers rejected	33
Number of papers published	149
Number of pages per article	12.1
Number of supplements	2 (128 pages)
Number of biographies	3

Included in the report of the Editor was a provision that for the coming year each member be billed for the 4 volumes of the Journal (Vol. 61, 62, 63, and 64) to cover the period 1 May 1957 to 1 September 1958, total cost of the 4 volumes to be $8.50. The Wistar Institute further agreed to assume the extra postage costs of mailings of the Journal to members receiving the Journal in countries outside the United States. The Editor's report was approved with a vote of appreciation to Doctor Cowgill for the efficient handling of Journal affairs.

7. Reports of standing committees:

(a) Joint Committee on Nomenclature (C. G. King and O. L. Kline). The Committee has recommended discontinuance of the use of the term D-isoascorbic acid in the technical and lay literature because of the risk of confusion in regard to its relationship to the vitamin, ascorbic acid. Substitute names are under review. The report was approved.

(b) The Committee on the Registry of Pathology of Nutritional Diseases is coordinating its program of work with a similar committee activated by the American Society for Experimental Pathology. No formal report of Committee actions was received during the year.

(c) Representative to the Food and Nutrition Board and the Division of Biology and Agriculture, N.R.C. (N. R. Ellis). The report was approved as presented by Doctor Ellis and the written report is on file in the Secretary's Office. Doctor Ellis commented that the Ameri-

can Institute of Nutrition is well-represented in the affairs of the National Academy — N.R.C., since a number of its members hold important committee appointments in the Academy and Council. Doctor Ellis recalled the appreciation of the National Academy for the support from the American Institute of Nutrition in financing the publication, *The Challenge of the Life Sciences.*

(d) Representatives on the AAAS Council (P. B. Pearson and H. A. Schneider). This Committee was active in assisting with the co-sponsorship by our society of a symposium at the December 1956 AAAS meeting in New York City on the subject of "Grasslands in Our National Life." The Committee further reported actions of the AAAS Council of interest to our members. The journals, *Science* and *The Scientific Monthly,* will be merged at some future date, probably 1958. The proposal to assess affiliated societies $25.00 per year for travel expense of Council members was dropped. The report was approved.

(e) Representative to the Nutrition Division of F.A.O. (H. D. Kruse). The report dealt mainly with the new F.A.O. program of research and development, coordinated with WHO and UNICEF, which has as its major objective increasing the supply of high-protein foods for use in protein-deficient, under-developed areas of the world. The report was approved.

(f) Public Information Committee (Dr. Icie Macy Hoobler). A verbal report was presented and approved in which it was indicated that the Federation *ad hoc* Committee appointed to study means of expanding Federation public information activities should meet with the present Public Information Committee in the near future. Doctor Hoobler's resignation was accepted and a vote of thanks was extended for her continued interest and contribution to the Committee's activities.

8. Nominations for membership. The Council received 27 nominations for membership. The following were recommended by the Council and approved by the members present at the business meeting:

Lotte Arnrich	Norman H. Jolliffe
J. M. R. Beveridge	Joseph Kastelic
Myron Brin	F. A. Kummerow
Joseph Brozek	Gennard Matrone
Amber L. S. Cheng	William J. McGanity
Waldemar Dasler	D. W. Peterson
Bessie L. Davey	Ruth L. Pike
Leslie P. Dryden	Ray L. Shirley
H. D. Eaton	Sam C. Smith
Ralph T. Holman	Philip L. White

9. Actions of the Federation Board. The Secretary reported on the actions of the Federation Board in its Sunday meeting as follows:

(a) Proposal was approved that each member society of the Federation be allowed one room, rent-free, for use as office space at Beaumont House.

(b) A proposal submitted by a committee on revision of the Constitution proposed a constitutional change that would permit action on any proposal by a two-thirds vote of the membership of the Federation Board. It was agreed that the matter should be referred to society councils.

(The American Institute of Nutrition Council opposed the suggested constitutional change and proposed a substitute measure that would in effect result in increasing the efficiency of operation of the Federation Board. The substitute measure follows: A negative or contrary vote by a single society, either two or three of its votes on the Federation Board, on a particular measure would require that the same measure be placed on the agenda for the next meeting of the Federation Board and that interim committees be charged with a thorough study of the point of difference.)

The Secretary was instructed to transmit the substitute proposal to the Federation Board at its next meeting.

10. Organizing Committee Report. Grants totaling $50,000.00 have been confirmed for use in organizing an International Nutrition Congress in the United States in 1960. The Committee is hopeful that an additional equal sum, possibly more, can be raised to meet the needs of such a Congress. W.H. Sebrell, Jr., was appointed Chairman of the Program Committee for the International Congress, with William J. Darby, P. Gyorgy, and R. W. Engel as members.

Mrs. C. G. King has accepted the Chairmanship of the Women's Committee with Mrs. E. M. Nelson as Co-Chairman.

Delegates approved for the 1957 International Nutrition Congress in Paris, France, by the National Academy of Sciences, N.R.C., as recommended by the American Institute of Nutrition Council are as follows:

C. G. King, Voting Delegate
W. H. Sebrell, Jr., Voting Delegate
Grace Goldsmith
L. A. Maynard
R. R. Williams
P. Gyorgy
C. A. Elvehjem

Council approved the recommendation of the committees that M. O. Lee serve as Assistant Treasurer of the American Institute of Nutrition and that he be responsible for the disbursement of all funds raised in connection with the 1960 International Congress. Mr. Robert N. Harvey will be authorized to sign as disbursing officer in connection with funds committed by the Assistant Treasurer.

11. Miscellaneous items.

(a) Honoraria for secretarial assistance for the coming year were approved at $200.00 for the Secretary's Office and $100.00 for the Treasurer's Office. Council further proposed that up to $200.00 might be drawn upon to defray expenses of Council meetings, particularly if plans for International Congress in 1960 require more Council activity.

(b) Following a meeting of the Council with Representatives of the Borden Foundation, Inc., the following is a

revision of the Award statement as adopted in 1943 and revised in 1954. (Italics indicates language changed or added.)

BORDEN AWARD OF THE AMERICAN INSTITUTE OF NUTRITION

Adopted 1943; revised May and July, 1954
Revised April, 1957

(Italics indicates language added in 1957)

This 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 any of its components*. This award will be made primarily for the publication of specific papers during the previous calendar year, but the judges may recommend that it be given for important contributions over a more extended period not necessarily including the previous calendar year. The award may be divided between two or more collaborators in a given research, and may be omitted in any given year if in the opinion of the judges the work submitted does not warrant the award. Employees of the Borden Company are not eligible for this award.

The President of the American Institute of Nutrition shall appoint a Nominating Committee which shall solicit, initiate, and receive recommendations. *It shall be the duty of the Nominating Committee to canvas the field and, if necessary, initiate nominations to insure a sufficient number of well-qualified nominees.* Considerations of the nominations shall be the duty of a Jury of Award to be appointed annually by the President of the Institute. The recommendations of the Jury of Award shall be transmitted to the President of the Institute for consideration by the Council. Membership in the American Institute of Nutrition is not a prerequisite for nomination.

No individual who has received a Borden Award from another administering association shall be deemed eligible to receive the Borden Award of the American Institute of Nutrition unless it be for outstanding research on a different sub-

ject or for specific accomplishment subsequent to the first award.

The announcement of the recipient and the presentation of the award shall be made at the annual meeting of the Institute. At this time it is expected that the recipient of the award will review briefly the work upon which the award has been based.

(c) Since the society does not have a past president this year, R. W. Engel was appointed to serve on Council and to serve as Acting Past President on the Federation Board for 1957–1958.

(d) A Constitution (prepared in the Secretary's Office) for the U. S. National Committee of the International Union of Nutrition Sciences (IUNS) was adopted with certain corrections and changes. Final draft will be submitted to the International Relations Division, N.A.S. - N.R.C. The purpose of such a constitution and committee is to effect appropriate United States participation in the IUNS through the N.A.S. - N.R.C., which adheres to the IUNS on behalf of nutritional scientists of the United States.

(e) As a result of the actions of a screening committee appointed by the President to review applications for N.S.F. travel grants to the 1957 International Nutrition Congress, Paris, France, 6 travel grants have been awarded to young nutrition investigators who have not had previous opportunities to visit European educational and research institutions.

(f) The suggestion that a printed form be used next year for abstracts was agreed upon with the concurrent agreement that abstracts contain no more than 250 words in order to effect a further economy in the cost of publishing the abstract issue of *Federation Proceedings*.

(g) President R. R. Williams appointed a committee to review our present procedure for electing new members. The Committee consists of A. E. Harper, Chairman, A.

E. Schaefer, R. M. Leverton, C. M. Lyman, and F. J. Stare.

(h) Council recommended that the records of the Proceedings show appreciation of the American Institute of Nutrition for the valuable and efficient services of the retiring Secretary, who in turn wishes to record the faithful and efficient services of Mrs. Mary Cox, the Secretary's assistant.

(i) Council approved any funds accrued from the sale of dinner tickets should be applied to pay expenses of the Dinner Speaker, Dr. Eugene Campbell.

(j) Suggested symposia topics for future program planning.

1. Protein and amino acid requirements.
2. Mineral interrelationships in nutrition.
3. Nutrition in pregnancy.
4. Fats in human nutrition.

ANNUAL DINNER AND PRESENTATION OF THE AWARD

The annual dinner of the American Institute of Nutrition was held on Wednesday, April 17, in the Palmer House and was attended by 237 members and guests. The program high light was the presentation of the Osborne and Mendel Award to Dr. George R. Cowgill. The Award was given in recognition of his many pioneer and subsequent fundamental research contributions to our knowledge of the B vitamins and of protein nutrition; and for his numerous other broad contributions to the science of nutrition as a teacher, as editor of the *Journal of Nutrition,* and as an expert advisor in this field.

Doctor Joseph Roe reviewed the numerous contributions of Doctor Cowgill to our knowledge of nutrition and his able handling of Journal affairs. Doctor Eugene Campbell served as guest speaker and reviewed the program of the International Cooperative Administration as it relates to nutrition, particularly with respect to the program in under-developed areas of the world.

COMMITTEES FOR 1957–58

The following are the standing committees beginning July 1, 1957:

Committee on Registry of Pathology of Nutritional Diseases

Herbert Pollack, Chairman
O. A. Bessey
W. H. Sebrell, Jr.
C. L. Pirani, Secretary

Consultant, Paul Klemperer

Representatives to the Joint Committee on Nomenclature

O. L. Kline
Philip L. White

Representative to the Division of Biology and Agriculture, to the Agricultural Research Institute and to the Food and Nutrition Board, National Research Council

N. R. Ellis

Representatives to the American Association for the Advancement of Science

Howard A. Schneider, Section C (Chemistry)
Paul L. Day, Section N (Medical Science)

Representative to FAO

James M. Hundley

Representative on Federation Public Information Committee

R. W. Engel

Committee on Membership

A. E. Harper, Chairman
Ruth M. Leverton
C. M. Lyman
Arnold E. Schaefer

F. J. Stare

Respectfully submitted,
R. W. Engel, Secretary
American Institute of Nutrition

INVITATION FOR NOMINATIONS FOR 1958 AMERICAN INSTITUTE OF NUTRITION AWARDS

Nominations are invited for the 1958 annual awards administered by the American Institute of Nutrition. Nominations may be made by anyone, including all members of the Nominating Committees. The following information must be submitted: Name of the award for which the candidate is proposed and as convincing a statement as possible as to the basis for the nomination (this may include a pertinent bibliography but reprints are not required). *Five copies* of all documents, including seconding statements, must be sent to the Chairman of the appropriate nominating committee before January 1, 1958, to be considered for the 1958 award.

1958 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 any of 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. The award is usually given to one person, but if in their judgment circumstances and justice so dictate, the Jury of Award may recommend that it be divided between two or more collaborators in a given research. The Jury may also recommend that the award be omitted in any given year if in its opinion the work submitted does not warrant the award. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award. 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 — Harold H. Mitchell; 1946 — Philip C. Jeans and Genevieve Stearns; 1947 — Leonard A. Maynard; 1948 — Charles A. Cary; 1949 — Harry J. Deuel, Jr.; 1950 — Henry C. Sherman; 1951 — Paul György; 1952 — Max Kleiber; 1953 — Harold H. Williams; 1954 — Agnes Fay Morgan and Arthur H. Smith; 1955 — A. G. Hogan; 1956 — Frank M. Strong; 1957 — no award.

Chairman, Nominating Committee:
DR. W. C. ROSE
Department of Biochemistry
University of Illinois
Urbana, Illinois

1958 Osborne and Mendel Award

The Osborne and Mendel Award of $1,000 has been established by the Nutrition Foundation, Inc., for the recognition of outstanding basic research accomplishments 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 the year preceding the annual meeting of the Institute, or who has published recently a series of papers of outstanding significance.

The recipient will be chosen by a Jury of Award of the American Institute of Nutrition. As a general policy, the award will be made to one person. If, in the judgment of the Jury of Award, an injustice would otherwise be done, it may be divided among two or more persons. 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. Membership in the Institute of Nutrition is not a requirement for eligibility and there is no limitation as to age.

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

Chairman, Nominating Committee:
DR. ICIE MACY HOOBLER
Merrill-Palmer School
71 East Ferry Avenue
Detroit 2, Michigan

NOTICE TO CONTRIBUTORS

THE JOURNAL OF NUTRITION, a copyrighted periodical, appears monthly for the publication of original research bearing on the subject of nutrition and occasional reviews of the literature dealing with this subject.

THE JOURNAL OF NUTRITION is the official organ of the American Institute of Nutrition. The officers of the Institute for 1957–58 are: R. R. Williams, President; William J. Darby, Vice-President; George M. Briggs, Secretary; J. B. Brown, Treasurer; L. A. Maynard, E. W. McHenry, Paul Gyorgy, Councillors.

Preliminary notices, or papers already published or in press elsewhere, will not be accepted. Unusually long papers that would take a disproportionate part of a single issue can be considered only if published as a supplement, the entire cost of which is assumed by the author.

The paper must be accompanied by an author's abstract not to exceed 225 words, which will be published in The Wistar Institute Advance Abstract Card Service.

Manuscripts and drawings should be sent by express prepaid or by registered mail to the Editor, Dr. George R. Cowgill, Yale University Nutrition Laboratory, 333 Cedar Street, New Haven 11, Conn. If two complete copies of the paper are submitted, consideration by the Journal can be expedited.

Manuscripts and drawings should be submitted in complete and finished form with the author's complete address. All drawings should be marked with the author's name. The Wistar Institute reserves the right to return to the author for revision material which is not in proper form for the printer. When the amount of tabular or illustrative material or both is judged to be excessive, or unusually expensive, authors may be requested to pay the excess cost.

Manuscripts should be typed in double spacing on one side of bond or heavy-bodied paper 8½ × 11 inches and should be sent flat. Page 1 should include, in the following order: complete title, author's name, institution from which the paper came, with city and state, total number of figures, shortened form of title (not more than 35 letters and spaces), address to which the proof is to be sent. The text starts on page 2.

Tables, quotations (extracts of over 5 lines), and all other material usually set in type smaller than the text, should be typed each on a separate sheet. Footnotes to the text should be numbered consecutively (including those on page 1) and typed in order on a separate sheet. Explanations of figures should be treated in the same manner. Footnotes to a table should be made a part of the table, and should be typed directly beneath it. Citations of literature in the text should be made by author and by numerals to indicate the year of publication. Authors' names (followed by year, title of paper, etc.) should be arranged alphabetically in a list at the end of the text.

The original drawings, not photographs of drawings, should accompany the manuscript. When photographs are used for halftone reproduction, glossy prints should be sent. Authors should indicate on the manuscript the approximate position of text figures. If there are illustrations so large as to require mailing separately, reduced copies of them that can be mailed with the manuscript should be provided in order to expedite consideration of the paper.

Figures should be drawn for reproduction as line or halftone engravings, unless the author is prepared to defray the cost of a more expensive form of illustration. Letters and figures should be uniform and large enough so that no character will be less than 2 mm high after reduction to at least the width of the page (4¼ inches). Double plates will not be accepted. Figures should be numbered consecutively beginning with the text figures and continuing through the plates. The reduction desired should be clearly indicated on the margin of the drawing.

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Galley proofs and engraver's proofs of figures are sent to the author. All corrections should be clearly marked thereon. For further details important for the preparation of a satisfactory manuscript, ask The Wistar Institute for a copy of its leaflet entitled: **A GUIDE FOR AUTHORS.**

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