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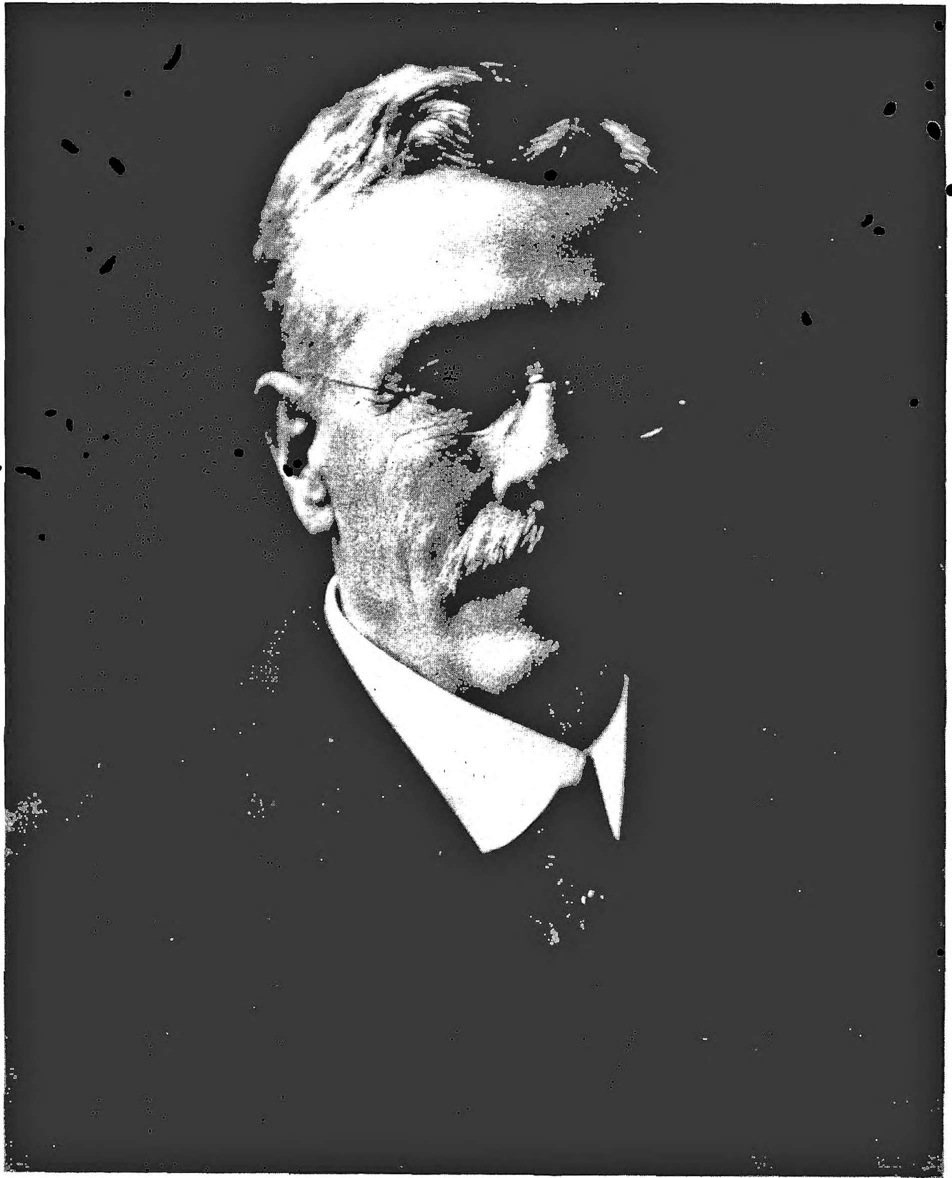
HENRY PRENTISS ARMSBY

(1853–1921)



HENRY PRENTISS ARMSBY

HENRY PRENTISS ARMSBY

(September 21, 1853 – October 19, 1921)

"He has left a monument such as few men can boast and his work will live on and continue to fruit in the lives of those who succeed him." These are the words of Dr. E. W. Allen in an editorial tribute to Armsby in the Experiment Station Record, Vol. 45, No. 7, p. 601, 1921.

Henry Prentiss Armsby was born in Northbridge, Massachusetts, September 21, 1853, the only child of Lewis and Mary A. (Prentiss) Armsby. Lewis Armsby, a skilled cabinet maker, moved with his family from Northbridge to Whitinsville and later to Millbury, Massachusetts while Henry was still a young boy. It was in these towns that Henry received his elementary school training. At the age of 15 he entered the Worcester County Free Institute of Industrial Science which later became the Worcester Polytechnic Institute. In 1871, in the first class to graduate from this Institute, he received the degree of Bachelor of Science. He remained at Worcester for one year as instructor in chemistry.

The next two years were spent in graduate study at the Sheffield Scientific School at Yale College where, in 1874, he received the degree of Bachelor of Philosophy. It was here that Armsby became acquainted with Samuel W. Johnson, Professor of Agricultural Chemistry and Director of the Connecticut Agricultural Experiment Station under whose inspirational leadership Armsby embarked upon his remarkable career of investigation. His first paper entitled, "The Decay of Nitrogenous Organic Compounds," was presented before the American Association for the Advancement of Science at Hartford in August, 1874.

After completing his studies at Yale, Armsby taught natural sciences in the High School at Fitchburg, Massachusetts, for

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one year. This was followed by a year of intense work and study in Leipzig, Germany, with his friend E. H. Jenkins of New Haven. During this sojourn abroad Armsby came into contact with Gustav Kühn, Director of the foremost Agricultural Experiment Station of Germany located at Möckern, near Leipzig. At this Experiment Station digestion and energy metabolism experiments with cattle were being conducted with the aid of a Pettenkofer respiration apparatus. This fruitful year made a lasting impression upon Armsby and without doubt determined the field of his future work. While at Leipzig he published (Jour. Pract. Chem., 13, 333, 1876), the results of his work there in a paper entitled, "Ueber die Einwirkung der Schwefelsäure auf Phosphorsauren Kalk" (The Influence of Sulfuric Acid upon Calcium Phosphate).

Upon his return to the United States he accepted a position at Rutgers College, New Brunswick, New Jersey, where he taught chemistry during the year 1876 to 1877. During this year at Rutgers he published a paper in Germany entitled, "Ueber das Absorptionsvermögen des Bodens für Basen" (The Power of the Soil to Absorb Bases).

From New Brunswick he was called back to New Haven at the recommendation of his former teacher, Director Samuel W. Johnson, to be chemist at the newly organized Connecticut Agricultural Experiment Station. The 4 years in this position were important and eventful for Armsby, crowded with advanced studies, experimentation, and writing. Filled with enthusiasm from his studies abroad and from contact with the great German scientists, he began the translation of Wolff's "Landwirtschaftliche Fütterungslehre," the outstanding text of this era on animal feeding. However, Armsby found so many changes and additions were needed to make the work apply to American conditions that in his hands it became a new book. This was published in 1880 under the title, "Manual of Cattle Feeding." It was the first book of its kind in the United States and was received with widespread favorable comment. In the preface Armsby makes

the following statement which characterizes his life-long philosophy and conviction that progress in agriculture depended upon the understanding of fundamental facts and principles. "In the writer's view, the highest usefulness of a work like the present does not consist simply in giving receipts which shall enable the farmer to feed his stock more economically, or to produce more milk or more or better beef, but in so elucidating our knowledge of the unchanging natural laws, chemical and physiological, of the nutrition of animals, that the attentive student shall be able to adapt his practice to the varying conditions in which he may be placed, and, more important still, shall be able to appropriate intelligently the results of new investigations and follow or take part in the advances of the science."

This book brought Doctor Armsby into prominence in connection with the subject of animal feeding and nutrition and foreshadowed his entire career. In it he presented together for the first time not only the results of investigations, but also an explanation of the processes by which results had been obtained. It passed through several editions and was for a long time used as a standard textbook.

Armsby's intense interest in animal feeding during this period is shown in a series of papers on the digestion, the composition and utilization of feeds by domestic animals. Closely allied subjects were included within the scope of his writing such as the chemistry of soil, milk and silage with scholarly analysis of problems dealing with the advancement of agricultural science. In these early days of his career he was frequently invited to appear before scientific organizations and various agricultural groups to discuss topics of mutual interest. He received the degree of Doctor of Philosophy from Yale University in 1879.

On October 15, 1878, he married Lucy A. Harding of Worcester, Massachusetts. In the years which followed, 5 sons were born to them: Charles Lewis, Ernest Harding, Sidney Prentiss, Henry Horton, and Edward McClellan. In the year 1881 Armsby accepted the position of Vice-Principal

and Professor of Agricultural Chemistry at the Storrs Agricultural School (now the University of Connecticut) where he remained for two years. During this period appeared the "Farmers Annual Handbook," by Armsby and Jenkins. Two years later Armsby was called to Wisconsin to become Professor of Agricultural Chemistry and Associate Director of the Agricultural Experiment Station. It was here that the first truly scientific digestion experiment in this country was planned and executed and which was published in 1885 in the American Journal of Science. The experiment in which two sheep were fed clover hay, malt sprouts and cottonseed meal, was so well planned that it would be difficult today to suggest improvement.

Even at the comparatively young age of 34 the name of Armsby was synonymous with leadership in agricultural science and in 1887 he was called to State College, Pennsylvania, to become Director of the Agricultural Experiment Station. The Hatch Act had been approved and signed by President Cleveland in March of that year and on June 30, the Trustees of The Pennsylvania State College took action accepting the provisions of the Act and establishing the Experiment Station at the Pennsylvania State College. Armsby's staff consisted of William Frear, Vice-Director and Chemist; William A. Buckhout, Botanist; George C. Butz, Horticulturist; William C. Patterson, Superintendent of Farm; and Harry G. Patterson, Laboratory Assistant. All these men with the exception of Harry G. Patterson, who later became Director of the Maryland Experiment Station, remained with the College until their deaths.

Armsby came to State College late in the year of 1887 in the vigor of early manhood with a wealth of training and experience which had come to no other young American chemist at that time. With his new staff he continued the work in Pennsylvania which had already been outlined and started largely by Doctor Jordan and Doctor Frear.

At this time there was considerable demand for "practical" investigations which would point the way for immediate in-

creased returns in agricultural practice. In an announcement which was issued as Bulletin No. 1 of the Pennsylvania State College Agricultural Experiment Station Armsby said, "It will be the aim of the Station to select for study such of these problems as appear to be of most immediate practical importance to the farmers, stock raisers, and fruit growers of the state, to bring all possible scientific appliances to bear on their solution, and by publishing the results of the investigations to make the knowledge thus obtained the common property of all." In discussing the question of abstract research versus practical experiments, he held that each had its place in the work of the experiment stations, that there was no innate antagonism between them and that, "no high ideal worthily followed ever interfered with faithfulness to the humblest duties." According to Armsby, "the station with the highest ideal of its functions will be the most efficient in the simpler and more prosaic duties which are a legitimate and proper and important part of its work."

Years later in his Annual Address as President of the Association of American Agricultural Colleges and Experiment Stations in 1899 Armsby said, "The function of the experiment station is not the impossible task of giving him (the farmer) recipes suited to every conceivable emergency. Its business is to enlarge his knowledge of the natural forces which drive his farm . . . and to teach him to control them instead of being controlled by them. It is not a device to save the farmer the trouble of thinking. On the contrary its consistent and insistent demand is that he think more. . . . The true field of work of the experiment station is the farmer's mind, not his acres."

Armsby had long been dissatisfied with the common methods of comparing feeds on the basis of their digestible matter alone. He realized the fundamental importance of the energy aspects of animal production and wrote extensively on this subject during his first 10 years at State College, pointing out these problems to the scientists of the United States Department of Agriculture. Their interest in the subject and

the confidence they had in Doctor Armsby led to a proposal made to the College that he undertake, in cooperation with the Bureau of Animal Industry, a series of investigations in the fundamental principles of animal nutrition.

In the spring of 1898 an agreement was reached between the Bureau of Animal Industry of the United States Department of Agriculture and the Pennsylvania State College Agricultural Experiment Station to conduct a highly scientific nutritional research program at State College under the Directorship of Doctor Armsby. This proposed line of work involved a number of new problems and measurements with farm animals that had never been undertaken. Armsby wanted to measure the complete intake and outgo of the experimental animal whether the items were gaseous, liquid or solid, and to account for the final disposition and distribution of the total feed intake.

After spending some time in Europe during the summer of 1898 studying various existing apparatus for respiration calorimetric research, Armsby decided to adopt the general principle of the respiration calorimeter at Middletown, Connecticut, which had been devised by Atwater and Rosa for experiments on man. The many technical problems encountered in the construction of such an apparatus for use with cattle were successfully solved by Armsby and his associate for many years, Professor Jons August Fries of the Pennsylvania Agricultural Experiment Station. Professor I. Thornton Osmond, Professor of Physics from 1879 to 1907 assisted in the design, construction and installation of this new Respiration Calorimeter. Exhaustive tests were made to verify the validity of results to be obtained with this new apparatus. The heat and carbon dioxide produced by the combustion of a known amount of ethyl alcohol in the respiration calorimeter was determined by precisely the same procedure that was to be followed during the experimentation with animals. The agreement between theoretical values of heat and of carbon dioxide with those actually obtained was virtually perfect.

Experimentation with the new respiration calorimeter which began in 1902 may be regarded as the realization of Armsby's life-long ambition to demonstrate the dependence of farm practice upon sound scientific principles of chemistry, physiology and energetics. By use of the respiration calorimeter he was able to make both direct and indirect calorimeter measurements of the heat produced by farm animals and to point out the relatively large amount of feed energy represented by this heat. This new apparatus also made it possible to measure quantitatively the methane produced by fermentation in the rumen, to compute the body gain of fat and protein and to obtain virtually perfect concordancy between the values for heat production as determined directly and indirectly. The years of work which followed attracted much attention in this country and abroad. Visitors from other countries interested in both animal and in human nutrition, including medical groups studying nutrition, came to Armsby's laboratory to see his unique apparatus and to benefit from his mastery of the many aspects of energy metabolism and animal nutrition. Many people were aware of the existence and location of the Pennsylvania State College only as the place where Armsby operated his respiration calorimeter.

When the work of the Pennsylvania State College was divided into schools in 1900, Doctor Armsby became Dean of the School of Agriculture. He retained this office until 1904 when at his request it was turned over to Doctor Buckhout in order that Armsby might give his entire time to the Experiment Station.

As Director of the Experiment Station for 20 years (1887–1907) much of Armsby's time and energy were required for practical investigations, administrative work and innumerable public demands. He held firm in his conviction that the future advancement of the nation's agriculture must rest on scientific research, but as the institution grew larger with demands from the public multiplying, the time for deep scholarly scientific research grew less and less. Consequently, Armsby

resigned from this position in 1907 to become Director of the Institute of Animal Nutrition which was then organized as a special division of the Pennsylvania State College. Professor J. A. Fries was made Vice-Director. The remaining 14 years of Armsby's life were spent in work involving his famous respiration calorimeter. Years later, in 1933, the Institute of Animal Nutrition was reorganized as a Department in the School of Agriculture of the Pennsylvania State College and of the Pennsylvania Agricultural Experiment Station.

Armsby was commissioned in 1905 by the Carnegie Institution of Washington to examine and report on the procedures at Wesleyan University, Middletown, Connecticut, preliminary to the establishment of a Nutritional Laboratory in Boston by the Carnegie Institution of Washington. Following the successful establishment of this laboratory he performed a similar service at the request of the Carnegie Institution of Washington in 1919 when a joint research project was initiated by the Carnegie Laboratory at Boston and the laboratory of Animal Nutrition at the New Hampshire Agricultural Experiment Station.

Of the 115 scientific publications by Armsby, about one-half this number appeared after he began his studies with the large respiration calorimeter at State College. These studies included meticulously controlled experimentation with cattle with complete accounting for the disposal of feed nitrogen and energy. His nutritive evaluation of feeds on an energy basis took the subject of animal nutrition out of the rule-of-thumb barn manual and laid before animal husbandmen the basic principles upon which any feeding practice must ultimately depend.

His writings and understanding are as authoritative today as when first elucidated more than 50 years ago. In very recent years the superior nutritive value of early-cut as compared with late-cut forage crops has been emphasized by numerous workers concerned with the nutritive value of roughages. In 1882 Armsby wrote as follows, "All these

results indicate that the richest fodder and the largest yield of digestible matters per acre may be obtained by cutting two or more crops of comparitively young grass in a season rather than one crop of over-ripe vegetation.''

His book, ''The Principles of Animal Nutrition,'' published in 1903 was the outstanding publication in agricultural science of that era and brought to light his deep scientific insight into the many problems in animal nutrition. The following is a passage taken from his discussion of the relationship between energy expenditure by the animal and the energy supplied in the feed.

''The metabolism of matter and energy in the body might be compared to the exchange of water in a mill-pond. The water in the pond may represent the materials of the body itself, while the water running in at the upper end represents the supply of matter and energy in the food, and that going down the flume to the millwheel the metabolism required for the production of physiological work as above defined. The water flowing into the pond does not immediately turn the wheel, but becomes part of the pond and loses its identity. Part of it may be drawn into the main current and enter the flume comparatively soon, while another part may remain in the pond for a long time. Pursuing the comparison still further, as but a small proportion of the energy liberated by the descent of the water in the flume takes the form of mechanical energy, most of it being converted into heat, so in the body but a small proportion of the energy expended in physiological work takes ultimately the form of mechanical energy.''

Another textbook, ''The Nutrition of Farm Animals,'' published in 1917 incorporated his clear understanding of the chemistry and physiology of nutrition with a vast amount of practical knowledge making this book an admirable basis for teaching and for research. His discussion of the significance of ''accessory ingredients,'' written during the period before the word ''Vitamins'' was in common usage, shows his scholarly appraisal of the role which these substances play

in nutrition. The vast amount of research and greatly increased knowledge concerning the identity and function of vitamins and the requirements for these substances which has come to pass since the time of Armsby has tended to create a warped perspective in the nutritive evaluation of foods. The following discourse is as pertinent and as sound today as when first formulated by Armsby.

"It is clear that the 'accessory ingredients' (using this simply as a convenient summary term for the various classes of substances indicated in the last paragraph) influence the nutritive value of a feeding stuff in an essentially different fashion than does the quantity of available ash, protein, and energy which it supplies. The latter limits the amount of production which the feeding stuff can support; the presence or absence of the former may determine the extent to which this potential value is actually realized.

"In some aspects of the matter, these 'accessory ingredients' might be crudely compared with the lubricants of a machine, which of themselves furnish neither power nor material, but which enable power derived from the consumption of fuel to be more efficiently used and therefore conduce to the production of a larger output.

"It is possible that in the future there must be added to the requirements already outlined for ash, protein (or amino acids) and energy for the various purposes of feeding, the requirements for the 'accessory substances' necessary to secure the most efficient functioning of the cells and organs of the body."

In the preface of this book Armsby subscribes wholeheartedly to the philosophy of his teacher and colleague Samuel W. Johnson who many years earlier had said, "Other qualifications being equal, the more advanced and complete the theory of which the farmer is the master, the more successful must be his farming. The more he knows, the more he can do. The more deeply, comprehensively, and clearly he can think, the more economically and advantageously can

he work. A true theory is the surest guide to a successful practice."

In the summer of 1908 representatives from 13 experiment stations and the Office of Experiment Stations of the United States Department of Agriculture met at Cornell University to discuss the desirability of forming a permanent organization of animal nutrition investigators. A committee was appointed headed by Doctor Armsby to survey the field of animal husbandry work and to prepare a report to include a proposed constitution for the new organization. This committee called the group together a few months later at Chicago. At this meeting in November, 1908, a constitution was adopted by 32 charter members. The name chosen for the new organization was The American Society of Animal Nutrition. "The objects of the society shall be to improve the quality of investigation in animal nutrition, to promote more systematic and better correlated study of feeding problems, and to facilitate personal intercourse between investigators in the field."

Armsby was elected President of the Society for the first three years of its existence. Under his capable leadership the new society prospered, and gradually became endowed with his spirit of research based on fundamental principles of physiology and nutrition. The first three annual meetings were held in November or December at Chicago. During the following 10 years the Society met each year in various midwestern states returning to Chicago for its 1920 meeting where it has continued to meet each year in November for a comprehensive program of scientific papers and discussions. The date of its meeting is quite appropriately planned to occur just prior to the annual International Livestock Show at Chicago. In 1912 the name of the organization was changed to "The American Society of Animal Production." The quantity and quality of the vast amount of scientific work which this Society has since accomplished is a monument to the enterprising zeal and faith of its founders. The Society now has 1,250 members from the United States and Canada

and publishes most of its highly technical and scientific work in its Journal of Animal Science.

The many honors which came to Armsby reflected the high esteem in which his work was held by his colleagues in this country and abroad. In 1904 the University of Wisconsin conferred upon him the Doctor of Laws, and from Yale University in 1920 and from Worcester Polytechnic Institute in 1921 he received the degree, Doctor of Science. He was elected a member of the Royal Society of Arts of Great Britain in 1911, and a foreign member of the Royal Academy of Agriculture of Sweden in 1912. His portrait hangs in the Saddle and Sirloin Club of Chicago, a symbol of the highest tribute from the American Society of Animal Production, of which Armsby had been a charter member.

He was called to Washington by the Commissioner of Agriculture in 1885 to a convention of delegates from the agricultural institutions out of which developed two years later, the Association of American Agricultural Colleges and Experiment Stations. During the ensuing years Armsby held several important positions in the Association, including that of secretary in 1890, president in 1899, a member of its executive committee and other committees of importance. He was chairman of the Association's committee on the experiment station exhibit at the World's Columbian Exposition at Chicago in 1903. He was chairman of the Committee on the Experiment Station Exhibit at the Chicago Exposition in 1893 and later held a similar position at the Exposition in Paris in 1900. At the Panama International Exposition held at San Francisco in 1915 he received the gold medal for the Institute of Animal Nutrition Exhibit — the model of the respiration calorimeter at State College. Armsby also served as president of the Society for the Promotion of Agricultural Science from 1905 to 1907 and on the editorial committee of the Journal of Agricultural Research for 5 years (1914–1919). With the formation of the National Research Council he became a member of the Committee on Food and Nutrition and saw in that organization a wonderful opportunity to

raise the plane of research of this country to a higher level of efficiency and usefulness. In the fall of 1918 he was sent to Europe by the United States Government as a member of the Inter-Allied Scientific Food Commission to survey the nutritional status of the war-torn peoples. Only a few months before his death which occurred October 19, 1921, he was consulted by the Czechoslovak Government through its legation in Washington with regard to the organization of a National Institute for Food Research in that country.

In a perusal of what has been recorded of Armsby in the writings of several of his contemporaries, it is significant to note that the following descriptive terms appear in all of them: modest; conscientious; meticulous in appearance, manners, and writing; a great thinker; a hard worker; cautious; accurate; patient; unassuming; and a very well balanced scholarly perspective with regard to the relation of one problem to another.

Armsby's work with the resipration calorimeter was far more than the development of a mere machine. With it he was able to measure precisely the economy of the animal mechanism in its use of protein and energy. He reduced the factors of feeding standards from three to two, based on protein and energy rather than on protein, carbohydrates and fats. He supplied a broader and more substantial basis for intelligent feeding practice and the foundation for a sound agricultural policy. He demonstrated the means of measuring animal feed on the basis of its energy supply and stated that "quantitatively the principal function of food is to supply energy," and that "a knowledge of the relative amounts of energy which can be recovered in various methods of utilization is a factor of prime importance in food conservation."

He studied the basic principles which underly and affect the transformation by the animal of products unavailable to man into human food. He demonstrated the great losses of energy which occur in the conversion of vegetable materials into animal products in feeding livestock. In this connection

he pointed out that animals receiving the proper amount of coarse fodder, may lose in one way or another, 29 to 86% of the energy of the grain fed with the roughage. A diversion to stock feeding of materials suitable for direct human consumption he therefore considered from this standpoint a wasteful procedure. Armsby demonstrated that the production of pork and milk was physiologically more economical than that of beef or mutton due to the extensive fermentation which occurs in the rumen of cattle and sheep and to the relatively large inedible portions of the carcass. He worked out the net available energy or production value of a considerable number of common feeds.

For 40 years Armsby was at the frontier of human knowledge. He and "Penn State" grew up together. "He was a proud possession." His classical work brought about the full realization by other workers in the field of nutrition that heat is a chief end-product of food.

In reviewing the outstanding accomplishments and qualifications of this great leader, it becomes increasingly clear that it was his breadth of vision, his orderly mental processes and analytical and well-balanced perspective that elevated him to the top position among his colleagues. Without such outstanding pioneers one cannot but wonder what would be the status of the world of science today. Perhaps the most fitting epitaph to characterize the life of Armsby is the one which he himself referred to while delivering a memorial address at the funeral of Dr. George W. Atherton, President of the Pennsylvania State College, who died in 1906. "Si monumentum quaeris, circumspice"—if thou seekest his monument, look about thee.

RAYMOND W. SWIFT

EFFECTS OF DIETARY CALCIUM AND PHOSPHORUS LEVELS UPON THE PHYSIOLOGICAL BEHAVIOR OF CALCIUM AND PHOSPHORUS IN THE RAT [1, 2]

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ONE FIGURE

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Previous reports from this laboratory by Hansard et al. ('50, '51a, '51b, '54b) have indicated that there are many nutritional factors that may affect the behavior of radiocalcium. These studies, however, have demonstrated that the rat readily adapts itself to dietary calcium changes by reciprocal absorption changes. In a recent review Nicolaysen et al. ('53) discussed the pertinent literature concerning the physiological behavior of calcium, especially in relation to absorption; and Duckworth and Hill ('53) reviewed reports concerned with the bone storage of calcium and phosphorus. Henry and Kon ('53) have recently described the relationship between calcium retention and body stores of calcium in the rat.

The studies here reported are concerned with basic investigations of the physiological behavior of calcium and phosphorus in growing rats being maintained on various dietary

[1] Published with the approval of the Director of the Tennessee Agricultural Experiment Station.

[2] Radioactive isotopes were obtained from the Oak Ridge National Laboratory on allocation from the U. S. Atomic Energy Commission.

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regimes. Radiochemical procedures were employed to follow the movement of labeled calcium and phosphorus in the bodies of animals maintained throughout the experimental period on rations that were identical except for variations in calcium and phosphorus content.

EXPERIMENTAL

Highly inbred Wistar rats of both sexes were used in these investigations. They were weaned at 30 days of age from mothers maintained on commercial rat pellets and placed on experimental rations as indicated. These rations were identical except for the variation in calcium and phosphorus content. All rats were given food and water ad libitum. Following the pre-experimental period of 55 days on these rations, each animal was given a single oral or intraperitoneal tracer dose of labeled calcium or phosphorus as described by Hansard and Comar ('53). They were then placed in metabolism cages equipped for the separate quantitative collection of urine and feces for complete 96-hour balance studies. At sacrifice samples of fresh tissue, feces and urine were taken for routine total calcium and phosphorus determinations and for the measurement of radioactivity by methods previously described by Comar et al. ('51). All radioisotope values, except those for the excreta, were calculated to an equivalent body weight basis and corrected to the percentage of *retained* radioactivity (Hansard, '53; Hansard et al., '53) thus permitting the measurement of the comparative behavior of that activity absorbed by the animal body.

RESULTS AND DISCUSSION

Effects of dietary calcium intake level

The first study of this series was designed to determine the effects of calcium status and dietary calcium intake upon the physiological behavior of radiocalcium. One hundred weanling albino rats were distributed among 4 equal groups and placed on the previously described cornstarch and casein

experimental ration, UT-AEC Ca-1 (Hansard et al., '51a) modified by the inclusion of calcium carbonate at the expense of cornstarch, to contain 0.013, 0.3, 0.5 and 1.0% calcium for lots 1, 2, 3 and 4, respectively. The phosphorus content of all diets was 0.4%. After a feeding period of 55 days the rats of lot 1, which received the low calcium ration, weighed an average of 62% of those in the high calcium groups and showed definite gross symptoms of low-calcium rickets. There were no significant weight differences between rats maintained on the other three diets. Following the conditioning periods, all animals were given a single dose of 10 to 20 μc of $Ca^{45}Cl$ and placed in individual metabolism cages. Food intake was measured, and periodic fecal and urinary collections were made during the 96-hour balance studies before the rats were sacrificed for the analysis of tissues.

Calcium absorption and endogenous excretion. The use of radiocalcium for the measurement of calcium partition in the gastrointestinal tract of cattle and rats has been previously discussed by Hansard ('53) and Hansard et al. ('50, '51c, '54a) and the effect of the level of dietary calcium upon the source of fecal calcium in cattle has been reported by Hansard et al. ('51c, '54b).

A modification of both the comparative balance procedure as discussed by Hansard et al. ('54a) and the isotope dilution method described by Visek et al. ('53) for cattle were used for estimation of the endogenous calcium in rats on the various rations. The first method consisted essentially of integrating the excretion data from animals dosed orally with that of similar animals receiving a single peritoneal injection of radiocalcium, and employing the total 96-hour balance figures to calculate the source of the fecal calcium as previously described (Hansard, '53). The isotope-dilution procedure gave results that closely paralleled those calculated by the comparative-balance method. It consisted of injecting selected animals on the various dietaries with a single tracer dose of labeled calcium and employing the 72-

to 96-hour balance and the blood plasma values at sacrifice after 96 hours to calculate the endogenous fecal calcium. Preliminary studies indicated that 72 hours after injection the secretion of Ca^{45} from the plasma to the feces had become fairly constant, and the ratios of the specific activities of the plasma and feces were used to estimate the per cent endogenous calcium as follows:

$$\frac{S.A._f}{S.A._p} \times 100 = E \text{ (per cent endogenous Ca), and then}$$

$$\frac{E \times \text{Ca intake (mg)}}{100} = \text{Fecal endogenous Ca (mg)}.$$

Neither of these methods gives information as to the pathway by which the endogenous calcium reaches the feces.

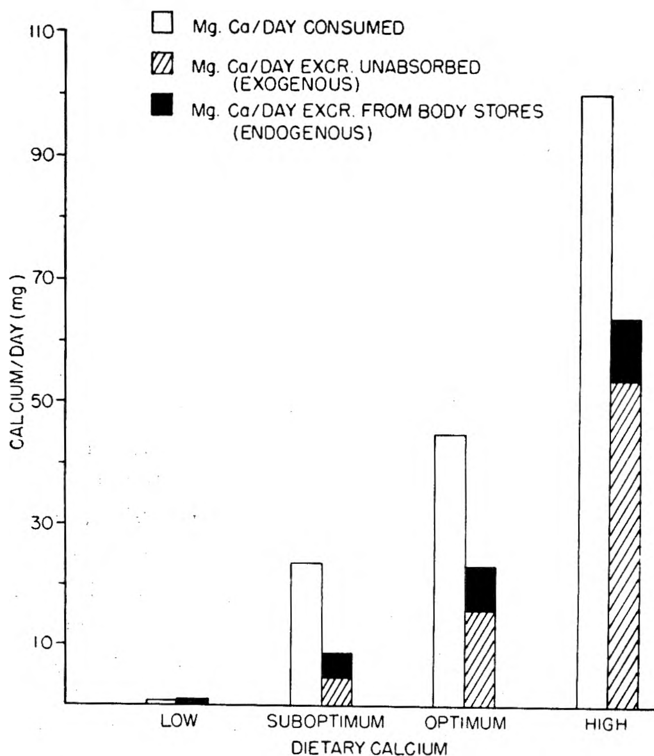

Fig. 1 The effects of dietary calcium levels upon fecal endogenous and unabsorbed calcium in rats.

However, both of them indicate an overall measure of the calcium loss from the body of rats on a normal dietary and permit a quantitative estimation of that fraction of dietary calcium passing through the gastrointestinal tract without being absorbed.

A summary of these values on the 4 levels of dietary calcium are graphically illustrated in figure 1. This indicates the magnitude of difference in daily endogenous and unabsorbed calcium and the total calcium excreted by rats maintained on identical diets, except for variation in calcium content. Animals on the lower calcium intake levels excreted a higher percentage of calcium from the body stores, but, as reported by Mitchell and Curzon ('39), the total calcium excreted from metabolic sources increased rapidly for those rats maintained on the higher calcium diets. This substantiates earlier reports and the findings by Hansard et al. ('51b, '53, '54b) indicating that body stores of the element materially affect the relative absorption of calcium from the tract and excretion into the tract. Hansard et al. ('51c) and Visek et al. ('53) have demonstrated that the addition of excess dietary calcium to rations for short periods of time, however, does not materially affect this body loss. It is apparent, therefore, that current intake is of less influence upon endogenous losses than is the calcium status of the animal at the time of measurement. These values illustrate further the ability of the growing rat to adjust its metabolic losses to the dietary calcium available from endogenous and exogenous sources, either by greater retention or by increased absorption of that calcium normally absorbed and re-excreted into the gastrointestinal tract (Hansard et al., '50, '51a).

The measurement of endogenous fecal calcium makes it possible to determine experimentally the *true* digestibility of the dietary calcium in these experimental rations. The values from the balance studies which are summarized in table 1 substantiate further the fact that there is decreased efficiency of utilization at the higher levels of calcium intake. Rats on low-calcium rations absorbed 98% of the dietary calcium,

whereas those animals maintained on high-calcium ration absorbed only 45%.

The losses of endogenous calcium per kilogram of body weight per day were 8, 23, 41 and 50 mg, respectively, for animals receiving daily 0.013, 0.3, 0.5 and 1.0% of dietary calcium. These values appear to be higher than those reviewed by Mitchell and Curzon ('39) but, except for the low-calcium group, represent animals on higher levels of dietary calcium at the time of measurement.

TABLE 1

Balance summary for rats maintained on different calcium intake levels

ITEM	UT-AEC RATIONS [1]			
	Ca-1	Ca-2	Ca-3	Ca-4
Weight at sacrifice, gm	103 ± 10 [2]	171 ± 15	171 ± 16	198 ± 20
Phosphorus content of ration, %	0.4	0.4	0.4	0.4
Calcium content of ration, %	0.013	0.3	0.5	1.0
Daily Ca intake, mg	0.8	24	45	102
Daily Ca excretion, mg	0.82	8.5	24	64
Apparent digestibility [3]	— 2	65	46	37
Ca-45 excretion, per cent of oral dose	3 ± 1	16 ± 8	40 ± 10	56 ± 10
Ca-45 excretion, per cent of I.P. dose	0.8 ± 1	2 ± 1	4 ± 1.5	7 ± 3
True digestibility of Ca [4]	98	86	63	47
Endogenous fecal Ca, per cent of total	98	47	29	16
Endogenous Ca, mg/kg body wt./day	8 ± 2	23 ± 5	41 ± 8	50 ± 8
Calculated Ca maintenance requirements, mg/rat/day [5]	0.82	5	11	21

[1] The initial age, and age at sacrifice was 30 and 85 days, respectively, for all animals.

[2] Mean ± standard deviation; 25 rats in each group.

[3] Apparent digestibility calculated as $100 - \left(\frac{\text{fecal calcium}}{\text{calcium intake}} \times 100\right)$.

[4] Calculated as $\frac{\text{Ca intake} - \text{fecal Ca} + \text{endogenous fecal Ca}}{\text{Ca intake}} \times 100$.

[5] Maintenance requirement calculated as $\frac{\text{mg of endogenous fecal Ca}}{\text{true digestibility}} \times 100$.

Dietary calcium intake and maintenance requirements. When the metabolic losses of calcium are known and the true digestibility of the dietary calcium is determined, it is possible to estimate the calcium maintenance requirements (Hansard et al. '54a) at the various levels of dietary intake. From the results in table 1, it is of interest to note that maintenance requirements for calcium varied with the nutritional status of the animal; and that this requirement was closely associated with the ability of the animal to adapt its metabolic losses to its mineral intake level. In the young growing rat receiving 24 mg of calcium per day the maintenance requirement was 5 mg, whereas when it had been receiving 100 mg of calcium per day, 21 mg were required to maintain it at this calcium status. This is in agreement with the reports of Hegsted et al. ('52) who suggested that a physiological adjustment to the calcium maintenance requirements necessitated that it be defined in terms of the nutritional status of the animal being maintained.

Calcium, phosphorus and ash content of tissue. Calcium, phosphorus and ash were determined for 15 selected tissues from the individual rats maintained from weaning to sacrifice on the 4 dietary calcium levels, but for brevity these values are not reported. It was of interest to note, however, that the variation in calcium level from 0.3 to 1.0% in lots 2, 3 and 4 made little difference in tissue analytical values. The bones from the low-calcium group showed evidence of near depletion for both calcium and phosphorus, with the exception that there was less change in the incisor and molar teeth. This was substantiated by the normal ash values, and would indicate that during the depletion period these latter tissues, as previously reported by Carlsson ('50), hold on tenaciously to the calcium and phosphorus, and apparently may be classed with the soft tissue for preferred deposition under conditions of stress. Depletion was more marked in areas of trabecular bone than those of cortical bone, indicating

relative availability from these sources for current use by the animal body.

Dietary calcium intake was not reflected in the analytical values for the soft tissues. This is in agreement with previous observations by Hansard et al. ('50, '51b) and Mitchell and Curzon ('39), that soft tissues are apparently maintained when necessary at the expense of bone minerals. The equilibrium existing between the bone, blood and soft tissue would

TABLE 2

The effect of dietary calcium intake level upon the distribution of retained calcium-45 in selected rat tissues [1]

TISSUE	UT-AEC RATION			
	Ca-1	Ca-2	Ca-3	Ca-4
Tibia shaft	13.3 ± 2	7.8 ± 2	7.44 ± 2	6.9 ± 2
Tibia epiphysis	15.0 ± 3	12.8 ± 5	12.6 ± 2	10.6 ± 6
Incisors	13.8 ± 5	18.5 ± 4	19.1 ± 4	17.7 ± 6
Molars	6.4 ± 3	7.0 ± 2	7.9 ± 3	7.6 ± 4
Mandible	18.5 ± 7	12.0 ± 3	11.0 ± 2	12.6 ± 4
Blood	.028 ± .01	.022 ± .002	.025 ± .003	.020 ± .002
G. muscle	.027 ± .002	.022 ± .003	.020 ± .003	.015 ± .002
Kidney	.025 ± .002	.033 ± .001	.020 ± .002	.025 ± .001
Liver	.013 ± .002	.018 ± .003	.008 ± .004	.007 ± .002
Spleen	.024 ± .005	.028 ± .003	.020 ± .002	.027 ± .002

[1] Calculated as per cent of *retained* calcium-45 per gram of fresh tissue. (Corrected to 100 gm body weight) (means and standard deviation).

probably require complete bone depletion before these tissues would be materially affected. In lieu of these findings and the fact that the balance data showed many of the low-calcium rats to be in positive calcium balance, it is indicated that adaptation to a limited calcium diet is a positive factor involving individual metabolic systems that must be given due consideration in the interpretation of the behavior of calcium in growing rats.

The behavior of retained radiocalcium. The concentration of *retained* radiocalcium as a function of the dietary calcium

intake level is presented for selected rat tissues in table 2. Calculations were corrected to 100 gm body weight, and for dose and absorption differences (table 1) to permit a measurement of the behavior in the body of that radiocalcium actually retained by the tissue itself. These data indicate little difference between groups in per cent calcium-45 *retained* by the various bones and soft tissues. It is pointed out, however, that a higher percentage of the calcium-45 dose was absorbed by those animals on the low-calcium diets, and that consideration is being here given to the behavior of 97 and 44% of the administered dose in tissues of the low- and high-calcium rats, respectively. The tibia and mandible of those rats on the low-calcium rations showed evidence of increased radiocalcium concentration. However, there was a wide variation between individuals, and even these values as corrected for absorption differences, did not appear to be highly significant. This would suggest body calcium status to be reflected directly by the fecal excretion values (Hansard et al., '50), and further emphasizes the importance of isotope absorption data for use in the interpretation of metabolic behavior (Hansard, '53).

The specific activity values for *retained* radiocalcium likewise demonstrate that the concentration of radiocalcium per unit of calcium present was not significantly affected by the dietary level, nor by the body stores of calcium, except for selected bones of the low-calcium rachitic group. The higher relative concentration of calcium-45 in the mandible and tibia in this low-calcium lot is a reflection of the magnitude of absorption differences and shows that there was an increased turnover rate and a tendency in the depleted bones to retain more tenaciously that calcium made available to them. As pointed out earlier, however, total calcium and Ca^{45} concentration differences in the soft tissue and in the bone of the groups on the higher levels of dietary calcium intake were not great. This indicates that the specific activity values generally paralleled the concentration values and for brevity they are omitted.

Influence of dietary calcium intake on phosphorus balance

The second phase of this study was concerned with the influence of current dietary intake and body stores of calcium upon phosphorus behavior. Fifty-eight weanling albino rats were divided into two groups and maintained on the previously described rations UT-AEC Ca-1 and Ca-3, containing

TABLE 3

Effect of dietary calcium intake level upon phosphorus absorption by rats

UT-AEC RATION	Ca-1	Ca-3
Number of rats	28	30
Average weight, gm [1]	120 ± .02	186 ± 9
Intake, mg/day		
Calcium	0.8 ± .02	45 ± 6
Phosphorus	22.0 ± 4	36 ± 10
Excretion, mg/day		
Feces:		
Calcium	0.8 ± .03	18.0 ± 3
Phosphorus	3.3 ± .8	8.8 ± 2
Phosphorus 32, per OS [2]	2.5 ± 1	4.4 ± 2
Phosphorus 32, I.P.[2]	1.4 ± .5	2.5 ± 0.8
Urine:		
Calcium	0.20	0.50
Phosphorus	28.5	17.1
Phosphorus 32, per OS [2]	24 ± 5	11 ± 2
Phosphorus 32, I.P.[2]	19 ± 1	12 ± 2

[1] Average weight (and standard deviation) at time of phosphorus-32 administration.

[2] Phosphorus-32 administered orally (OS) and intraperitoneally (I.P.) respectively, and reported as percentage of administered dose excreted per rat.

0.4% phosphorus and .013 and 0.5% calcium, respectively, for approximately 55 days. When gross deficiency symptoms were evidenced in the low-calcium group, all animals were dosed as indicated with a single tracer dose of P-32. They were then placed in individual metabolism units for 96-hour balance studies previous to sacrifice for tissue analyses.

The growth response of rats on the low- and normal-calcium diets, as noted in table 3, was similar to that observed for the

animals used in the first study. Total absorption of the orally administered P-32 for the low-calcium group averaged 73%, whereas the normal rats retained 85%. This showed that in the absence of adequate calcium, phosphorus absorption or retention, or both, were affected. Periodic urinary and fecal analyses indicated that the phosphorus was absorbed rapidly by the low-calcium rats, but was re-excreted into the urine at such a rapid rate that less was retained after 96 hours. Copp et al. ('51) reported a similar behavior in rats with low-phosphorus rickets. The observed high urinary excretion of P-32 (24 vs. 11% of the dose) in the low-calcium group indicates that a large part of the phosphorus was absorbed. However, results of the periodic balance studies showed that three-fourths of the current phosphorus intake was re-excreted by way of the kidney during the first 24 hours. Since there was no great loss of Ca-45 observed in this low-calcium group during the first study, it is evident that *absorbed* calcium and phosphorus do not behave alike, but are metabolized differently in the rat. Results of the conventional balance- and the intraperitoneal P-32-studies substantiate these findings. Whereas absorbed calcium is re-excreted into the tract and is subsequently re-absorbed, much of the dietary phosphorus absorbed from the tract is apparently re-excreted by way of the kidneys and is not retained. Therefore, a great part of the urinary phosphorus from rats on a diet adequate in phosphorus but low in calcium may actually be that which was absorbed from the food into the blood plasma and was subsequently re-excreted into the urine. This fact has been verified by comparative endogenous phosphorus values from this laboratory (Hansard, unpublished) which indicate a twofold increase in both fecal-endogenous and urinary phosphorus in rats maintained on low-calcium rations.

The results of the tissue distribution studies presented in table 4 show that the dietary calcium level had little effect upon the behavior of that phosphorus-32 retained by the tissue except that trabecular bone apparently does not retain for 96 hours the *absorbed* phosphorus-32 in the absence of

bone calcium. This is substantiated by the excretion values following both oral and intraperitoneal administration, and would indicate that the status of the body stores have more influence upon phosphorus retention than upon actual absorption from the tract itself. The tissue specific activity values reflect the magnitude of differences in the rate of phosphorus turnover attributed to the differences in calcium status of these animals, and indicate that there was a slightly

TABLE 4

Dietary calcium intake and the behavior of retained[1] *phosphorus-32 in the rat*

UT-AEC RATIONS	PER CENT DOSE PHOSPHORUS^{32}		SPECIFIC ACTIVITY[2]	
	Ca-1	Ca-3	Ca-1	Ca-3
Tibia shaft	6.3 ± 2.4[3]	5.1 ± 1.0	0.185	0.051
Tibia epiphysis	6.9 ± 3.5	15.9 ± 2.0	0.363	0.279
Incisors	9.1 ± 3.0	8.4 ± 3.0	0.091	0.060
Blood	0.31 ± .06	0.29 ± .08	0.910	1.120
G. muscle[4]	0.80 ± .30	1.0 ± .62	0.492	0.690
Kidney	1.90 ± .34	1.39 ± .41	0.662	0.468
Liver	2.01 ± .08	1.43 ± .43	0.688	0.530

[1] Tissue concentration values were corrected to *retained* phosphorus-32 per gram of fresh tissue. (See table 4.)

[2] Specific activity calculated as the per cent *retained* phosphorus-32 per gram of fresh tissue divided by the milligrams phosphorus per gram.

[3] Mean ± standard deviation.

[4] Gastrocnemius muscle.

greater retention of P-32 per unit of phosphorus in bones of the low-calcium rats.

Influence of dietary phosphorus intake on calcium balance

In a third experiment, a study was made of the effects of varying the dietary phosphorus level upon the behavior of Ca-45 when the dietary calcium was maintained at 0.5% of the ration. Fifty-eight weanling albino rats were grouped into 4 lots of 12 or more animals each, and placed on UT-AEC rations Ca-5, 6. 7 and 8. These rations were identical to

those described by Hansard et al. ('51a) except that cornstarch was replaced by calcium carbonate to give 0.5% calcium and by potassium diphosphate to give 0.18, 0.30, 0.5 and 1.0% phosphorus in the air dry rations. Following similar treatments previously described, all animals, at 85 days of age, were administered a single oral or intraperitoneal dose of Ca-45 and, following 96-hour individual balance studies, were sacrificed for tissue analyses.

During this period of rapid growth, weight changes were not affected by the variation in phosphorus intake or by the ratios of dietary calcium and phosphorus. Balance studies indicated no significant difference between either percentage of calcium or of calcium-45 excreted during the balance period. Calculation of endogenous calcium either by the comparative method or the isotope-dilution procedure showed no significant difference in the route of excretion or in the quantity of calcium lost from the body at these levels of phosphorus intake. It is pointed out, however, that with casein as the source of protein, it was not possible to lower the phosphorus intake sufficiently to create a stress, as was evidenced for calcium in rats on the low-calcium ration in the previous studies. The 0.18% level of dietary phosphorus appeared to be sufficient for growing rats at these levels of dietary calcium.

Distribution studies of radiocalcium at the various levels of phosphorus intake indicated that there was no significant difference in the deposition or turnover of *retained* Ca-45, total calcium, ash or phosphorus in selected soft tissues or bones under the conditions of this experiment.

SUMMARY

With the use of radiochemical procedures the behavior of labeled calcium and phosphorus was studied as a function of the dietary levels of these elements. Data obtained with more than 250 growing rats indicated that:

1. Current calcium intake was of less influence upon endogenous losses than was the calcium status of the animal at

the time of measurement. Endogenous fecal calcium increased from 1 to 10 mg per day for rats maintained on low- and high-calcium diets, respectively.

2. Utilization efficiency, as measured by *true* digestibility measurements, decreased with increased dietary calcium intake.

3. Total absorption and retention of radiocalcium were increased in rats with low calcium body stores, whereas radiophosphorus was absorbed but was subsequently re-excreted by way of the kidneys and less was retained.

4. The behavior of the Ca-45 retained by the tissues was practically independent of the dietary calcium and phosphorus levels studied, except that calcium-depleted animals demonstrated greater Ca-45 concentration and rate of turnover in all tissues.

5. Calcium maintenance requirements appeared to be a function of the animal's calcium status at the time of measurement. Animals reared to 85 days of age on rations furnished 24 and 100 mg of calcium per day required 5 and 21 mg of calcium per day, respectively, to maintain this calcium status.

6. Balance data appear to be basic for the interpretation of the behavior of Ca-45 and P-32 in the animal body. These simple procedures for estimates of endogenous calcium suggest application to studies of other factors involved in the physiological behavior of these minerals in animals.

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CONGENITAL MALFORMATIONS AS RELATED TO DEFICIENCIES OF RIBOFLAVIN AND VITAMIN B_{12}, SOURCE OF PROTEIN, CALCIUM TO PHOSPHORUS RATIO AND SKELETAL PHOSPHORUS METABOLISM [1, 2]

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Warkany and coworkers ('40, '42 and '44) were the first to show that a dietary deficiency of a B-vitamin caused congenital abnormalities in the rat. In their original work they used the Steenbock-Black rachitogenic diet supplemented with viosterol and about one-third of the offspring were congenitally malformed. The malformations were chiefly skeletal and included shortening of the long bones, fusion of the ribs and syndactylism. There were a few malformations of the soft tissues, including encephalocele and "open eye." All malformations were eliminated by riboflavin, and the percentage was decreased when the amount of calcium carbonate was lowered to 1%. More recently Giroud et al. ('50) and Gilman et al. ('52) have also studied riboflavin deficiencies in relation to fertility and congenital malformations.

Hydrocephalus occurred in about 2% of the offspring of dams that received a purified, casein-type diet (Richardson and Hogan, '46) and was prevented by the addition of folic

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[2] This is a portion of a thesis submitted to the Graduate School by R. B. Grainger in partial fulfillment of the requirements for the Ph.D. degree.

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acid (O'Dell et al., '48; Richardson, '51). Later, in an attempt to increase the number of hydrocephalic offspring, this purified diet was changed to include soybean oil meal as the source of protein. The incidence of hydrocephalus rose to about 20% (Hogan et al., '50) and was prevented by the addition of vitamin B_{12} (O'Dell et al., '51). The new diet contained no vitamin B_{12}, but it did contain a liberal amount of folic acid. We now know that the diet of Richardson and Hogan contained a significant amount of vitamin B_{12} but was practically devoid of folic acid. Thus, when the diet was deficient in folic acid the incidence of hydrocephalus was low, and when it was deficient in vitamin B_{12} the incidence was high. Numerous reports (Schultze, '49; Lepkovsky et al., '51; Bryden et al., '52) have shown the importance of vitamin B_{12} for the production of viable offspring, but there are few reports of congenital abnormalities which resulted from a vitamin B_{12} deficiency.

The corn-wheat gluten diet of Warkany and Nelson ('40) was entirely of vegetable origin and would be expected to be low in vitamin B_{12}. The occurrence of hydrocephalus was not reported, yet under the conditions in our laboratory one would expect a high incidence on such a diet. The observations reported here are concerned with the effects on congenital malformations of riboflavin and vitamin B_{12} deficiencies in diets in which the protein was supplied by corn and wheat gluten or by soybean protein. The source of dietary protein and the calcium to phosphorus ratio were also studied. Observations are reported on phosphorus metabolism in the skeleton of newborn rats as related to congenital anomalies.

EXPERIMENTAL

The experimental animals were albino rats originally of the Wistar strain. The females were housed in groups of 5 in wire-mesh cages until late in pregnancy when they were placed in individual cages on wood shavings. The offspring were sacrificed at birth and the dams returned to the breeding

cages immediately. After the newborn were examined for hydrocephalus (O'Dell et al., '48), eye defects, cleft palate and other gross abnormalities, they were cleared and stained according to the technique of Dawson ('26). The skeletal defects followed the general pattern described by Warkany ('41) except that there was a preponderance of sternal defects. The ocular defects were chiefly small or missing eyes as determined by gross examination. In most cases the dams were reared from weaning on their respective experimental diets, but in some preliminary experiments adult animals previously depleted of vitamin B_{12} were transferred to the corn-wheat gluten rations. As might be anticipated (O'Dell et al., '51) previous depletion increased the incidence of hydrocephalus on the vitamin B_{12} deficient diets. Since prior depletion had no effect on the incidence of other malformations the data from dams of all dietary histories were combined to simplify the presentation.

The alkaline phosphatase determinations were performed on the tibiae of newborn rats by a modification of the method of Bessey et al. ('46).[4] The rate of phosphorus turnover in the tibia was investigated with P^{32} using a modification of the method of Percival and Leblond ('48).[5] Total phosphorus was determined by the method of Fiske and Subbarow ('25) after wet ashing and the P^{32} activity was determined by use of a liquid counter (Tracerlab TGC-5). The Geiger-Mueller tube was coated with silicone and placed in 15 ml of solution contained in a tube so that about 7 cm of the counter were immersed.

Four different sources of protein were used in the basal rations, the compositions of which are shown in table 1.[6]

[4] The p-nitrophenyl phosphate was purchased from Sigma Chemical Co., St. Louis, Missouri.

[5] The P^{32} was obtained from the Oak Ridge National Laboratory, Oak Ridge, Tennessee, after allocation by the Isotope Division of the Atomic Energy Commission.

[6] The folic acid was supplied through the courtesy of Dr. T. H. Jukes, Lederle Laboratories, Pearl River, N. Y. The other crystalline vitamins were supplied through the courtesy of Dr. H. H. Draper, Merck and Co., Rahway, New Jersey.

The protein sources were: (1) corn and wheat gluten, (2) alpha protein,[7] a purified soybean protein, (3) water washed casein and (4) soybean oil meal. Ration 2732 is the modified Steenbock and Black diet used by Warkany and coworkers.

TABLE 1

Composition of basal rations

CONSTITUENTS	TYPE OF PROTEIN AND RATION NUMBER				
	Corn-wheat gluten 2732 [1]	Corn-wheat gluten 2705	Alpha protein 3071	Casein 2901	Soybean oil meal 2884
	%	%	%	%	%
Yellow corn	76	74			
Wheat gluten	20	20			
Alpha protein			30		
Casein (80)				30	
Soybean oil meal					70
Cerelose			51.7	52	22
Wood pulp			3	3	
Lard			8	8	2
$CaCO_3$	3	3			
NaCl	1	1			
Salts [2]			5	5	4
Methionine (DL)			0.3		
ADEK mix [3]		2	2	2	2
B-Vitamin mix [4]		+	+	+	+
Riboflavin [5]				+	+
Vitamin B_{12} [5]				+	+

[1] Animals on 2732 received 60 I.U. of Viosterol every 10 days.

[2] Richardson and Hogan ('46).

[3] The vitamin ADEK mix contained 2000 I.U. of A, 280 I.U. of D, 3 mg of alpha tocopherol and 1 mg of menadione in 2 gm of lard.

[4] The B-vitamin supplement supplied per 100 gm of ration: Thiamine HCl, 1.6; pyridoxine HCl, 1.6; Ca pantothenate, 4.0; biotin, 0.02; choline chloride, 100; and folic acid, 0.5 mg.

[5] 1.6 mg of riboflavin and 0.003 mg of vitamin B_{12} per 100 gm of ration.

Ration 2705 is a further modification that included the fat-soluble vitamins and thiamine, pyridoxine, pantothenic acid, folic acid and choline. The alpha protein diet (3071) contained the same vitamins as ration 2705 and the casein (2901)

[7] Alpha protein was purchased from the Glidden Co., Chicago, Illinois, and freed of sulfite by leaching with hot water (O'Dell et al., '52).

and soybean oil meal (2884) rations contained in addition riboflavin and vitamin B_{12}. The calcium and phosphorus contents of the rations were varied by adjusting the percentage of calcium carbonate and dicalcium phosphate in the salts mixture.

TABLE 2

Congenital malformations in offspring of females fed corn-wheat gluten type rations

GROUP NUMBER	BASAL RATION	VITAMIN SUPPLEMENT		OFFSPRING BORN	MALFORMATIONS		
		Riboflavin [1]	B_{12} [2]		Eye defects	Bone defects	Hydrocephalus
				no.	%	%	%
1	2732 [3]	—	—	101	14.9	26.8	23.7
2	2705 [4]	—	—	452	5.5	13.7	14.8
3	2705	—	+	194	0.5	16.5	0.0
4	2705	+	—	358	9.8	14.5	12.8
5	2705	+	+	188	0.5	8.0	0.0

[1] Supplement supplied 1.6 mg of riboflavin per 100 gm of ration.

[2] Supplement supplied 3 μg of vitamin B_{12} per 100 gm of ration.

[3] Ration 2732 contained no vitamin supplement, but the animals received 60 I.U. of viosterol orally every 10 days.

[4] Ration 2705 contained supplements of vitamins A, D, E and K as well as thiamine, pyridoxine, pantothenic acid, choline, biotin and folic acid (table 1).

RESULTS

Malformations on the corn-wheat gluten rations deficient in riboflavin and vitamin B_{12}

The original objective of this investigation was to determine whether or not the dams from our colony would produce offspring that had hydrocephalus and the malformations described by Warkany and coworkers when fed the Steenbock and Black rachitogenic diet supplemented with viosterol. The results obtained when this (2732) and similar diets were fed are shown in table 2. Of the offspring produced by dams fed ration no. 2732, 14.9% had eye defects, 26.8% had bone abnormalities and 23.7% were hydrocephalic. The incidence of skeletal malformations agrees well with the observations

of Warkany ('42). Supplementation of this diet with the vitamins other than riboflavin and vitamin B_{12} (2705) decreased the incidence of bone and eye abnormalities by about 50% (significant at the 1% level). The addition of vitamin B_{12} (group 3) to ration no. 2705 prevented hydrocephalus and virtually prevented eye abnormalities, but it did not affect the incidence of skeletal anomalies. The addition of riboflavin alone (group 4) did not decrease the incidence of any of the abnormalities.

When both riboflavin and vitamin B_{12} were added (group 5) there was a significant ($P < 0.02$) decrease in bone defects as compared to group 3 in which there was an uncomplicated riboflavin deficiency and as compared to group 4 ($P < 0.05$) in which there was an uncomplicated vitamin B_{12} deficiency. Stated in another manner, the addition of either riboflavin or vitamin B_{12} in the absence of the other one did not decrease skeletal anomalies but the removal of either one from the total supplement did increase the incidence. It is noteworthy that the vitamin supplement did not eliminate congenital malformations.

Malformations as related to riboflavin and vitamin B_{12} deficiencies in alpha protein rations

Since supplementation of the corn-wheat gluten rations with the known vitamins required by the rat did not completely prevent bone abnormalities and since the proteins of both corn and wheat gluten are of low biological value, it was deemed important to study the effects of the vitamin deficiencies in a diet which contained a more adequate protein. Alpha protein supplemented with methionine was chosen as a source of high quality protein that is low in both riboflavin and vitamin B_{12}.

A summary of the congenital abnormalities produced in the offspring of dams fed alpha protein rations is shown in table 3. The dams (group 6) that were fed the basal ration (3071) produced offspring that had 16.0% bone abnormalities,

but none had eye abnormalities or hydrocephalus. This incidence of bone abnormalities compares well with the incidence produced on the corn-wheat gluten ration (2705). The dams on this ration were depleted of riboflavin and reproduction ceased before they became depleted of vitamin B_{12}. Thus riboflavin was the first limiting factor for reproduction. In the absence of riboflavin the dams were not depleted of vitamin B_{12} and did not produce offspring with hydrocephalus or eye abnormalities.

TABLE 3

Congenital malformations in offspring of females fed alpha protein type rations (basal ration 3071)

GROUP NUMBER	VITAMIN SUPPLEMENT		OFFSPRING BORN	MALFORMATIONS		
	Riboflavin [1]	B_{12} [2]		Eye defects	Bone defects	Hydro-cephalus
			no.	%	%	%
6	—	—	94	0.0	16.0	0.0
7	+	—	375	6.7	14.4	11.7
8	—	+	112	2.7	24.1	0.0
9	+	+	399	0.5	1.8	0.0

[1] Supplement supplied 1.6 mg of riboflavin per 100 gm of ration.

[2] Supplement supplied 3 μg of vitamin B_{12} per 100 gm of ration.

The addition of riboflavin to the basal ration (group 7) produced an uncomplicated vitamin B_{12} deficiency and significantly increased the incidence of eye abnormalities (6.7%) and hydrocephalus (11.7%). As in the case of the corn-wheat gluten rations, the addition of riboflavin alone did not decrease the incidence of bone abnormalities (14.4%).

The addition of vitamin B_{12} alone (group 8) prevented hydrocephalus but significantly ($P < 0.05$) increased the incidence of skeletal malformations. This increase in malformations is no doubt due to the fact that a more severe riboflavin deficiency developed when vitamin B_{12} was present.

The ration that contained both riboflavin and vitamin B_{12} (group 9) prevented hydrocephalus, virtually prevented eye

defects and decreased the incidence of bone anomalies to 1.8% of the offspring born. When viewed from the standpoint of the ration containing both vitamins (group 9) it is clear that the omission of either vitamin B_{12} or riboflavin greatly increased the incidence of skeletal defects ($P < 0.01$). Omission of vitamin B_{12} resulted in hydrocephalus and eye defects but omission of riboflavin had little or no effect on these malformations.

TABLE 4

Incidence of congenital malformations as related to the source of dietary protein [1]

SOURCE OF PROTEIN	OFFSPRING BORN	EYE DEFECTS Incid.	EYE DEFECTS P values (vs stock)	BONE DEFECTS Incid.	BONE DEFECTS P values (vs stock)
	no.	%		%	
Casein	307	2.0	> 0.99	6.9	< 0.01
Corn and wheat gluten	188	0.5	> 0.99	8.0	< 0.01
Alpha protein	399	0.5	> 0.99	1.8	> 0.99
Soybean oil meal	213	0.9	> 0.99	1.4	> 0.99
Stock ration	261	0.8	...	1.5	...

[1] All diets contained the following B vitamins per 100 gm of ration: Thiamine hydrochloride 1.6, riboflavin 1.6, pyridoxine hydrochloride 1.6, calcium pantothenate 4.0, biotin 0.02, choline chloride 100, folic acid 0.5 and vitamin B_{12} 0.003 mg.

Malformations as related to the source of dietary protein

The alpha protein ration that contained all the known required vitamins produced a markedly lower incidence of skeletal anomalies (1.8%) than the corresponding corn-wheat gluten ration (8.0%). Whether the protective quality of the alpha protein ration is due to a more favorable amino acid composition or to an unrecognized nutrient is not known. In a search for other good sources of protein, another vegetable protein and an animal protein were tested for their protective value. The results are shown in table 4. Casein has been widely used in experimental diets and has been

found to be an adequate source of protein for the growing rat, but its protective action against embryonic damage is little if any greater than the corn and wheat gluten mixture. The incidence of bone abnormalities on the casein diet (6.9%) places it in the same category as the corn-wheat gluten diet, whereas the alpha-protein and soybean oil meal rations gave the same degree of protection afforded by the stock ration (1.5%) which was composed of natural foodstuffs.

Malformations as related to the calcium and phosphorus content of the diet

As shown in table 5 the high Ca: P ratio (4.4 to 1.0) in the corn-wheat gluten ration that contained all the known

TABLE 5

Incidence of congenital malformations as related to the calcium to phosphorus ratio in the diet [1]

SOURCE OF PROTEIN	HIGH Ca: P (4.4: 1)		NORMAL Ca: P (1.5: 1)		P VALUES
	Born	Bone defects	Born	Bone defects	High vs normal
	no.	%	*no.*	%	
Corn-wheat gluten [2]	88	6.8	100	9.0	> 0.70
Alpha protein [3]	200	5.0	195	2.1	< 0.01

[1] The rations contained the vitamin supplement listed in table 4.

[2] In the corn-wheat gluten rations the high Ca: P ratio was 1.23% Ca to 0.28% P; the normal ratio was 1.13% Ca to 0.77% P.

[3] In the alpha protein rations the high Ca: P ratio was 1.50% Ca to 0.3% P; the normal ratio was 0.9% Ca to 0.6% P.

required vitamins produced about the same percentage of skeletal defects as the normal ratio (1.5 to 1.0). As this ration was not entirely adequate in other respects the incidence of malformations was high in both cases. In order to test the effect of the Ca: P ratio in a more adequate ration, alpha protein was used as the protein source. With the alpha protein diet the incidence of skeletal anomalies was 5.0% on the high ratio compared to 2.1% on the normal ratio ($P <$

0.01). It is clear that a high Ca:P ratio increased the occurrence of malformations when the diet was not complicated by other deficiencies.

Alkaline phosphatase activity and P^{32} uptake in the tibiae of newborn rats

Skeletal defects were the most common abnormalities observed and the majority of these appeared to be the result of defective ossification, particularly in the sternum. To gain insight into the cause of the defective ossification the alkaline phosphatase activity and the rate of P^{32} uptake in the tibiae of newborn rats were determined.

TABLE 6

Alkaline phosphatase activity of tibiae from newborn rats

VITAMIN DEFICIENCY	NUMBER OF ANIMALS	ALKALINE PHOSPHATASE ACTIVITY, UNITS [1] PER GRAM FRESH TISSUE	P VALUES (vs none)
Riboflavin	3	0.83 ± 0.02 [2]	< 0.01
Vitamin B_{12}	19	1.63 ± 0.27	< 0.01
None [3]	10	2.05 ± 0.17	...

[1] 0.0001 (10^{-4}) unit of phosphatase activity liberated 0.05 μM of p-nitrophenol per tube (1.1 ml) in 30 minutes at 38°C. and pH 10.3.

[2] Standard deviation.

[3] Animals from ration 2884 and stock ration.

Table 6 summarizes the observations made on the alkaline phosphatase activity of the tibiae removed from riboflavin-deficient, vitamin B_{12}-deficient and control animals. A deficiency of riboflavin decreased the alkaline phosphatase activity to a value less than one-half that of the control animals. The activity of the tibiae from vitamin B_{12}-deficient newborn was about 80% of normal. These differences were highly significant statistically ($P < 0.01$).

The rate of phosphorus turnover in the tibiae of newborn rats from vitamin B_{12}-deficient dams and vitamin B_{12}-supplemented animals was determined by the specific activities of

the tissue after P^{32} administration. The results are shown in table 7. The radioactive phosphorus was concentrated in the bones in both cases. Although the deficient animals received a higher dose per gram of body weight, the recovery of P^{32} activity in the tibiae was decreased. The tibiae of the offspring from deficient dams were smaller but the amount of phosphorus per gram of tissue was higher than in the controls, a fact that may reflect faulty cartilage formation.

TABLE 7

P^{32} uptake in tibiae of newborn rats from vitamin B_{12}-deficient and B_{12}-supplemented dams [1]

VITAMIN DEFICIENCY	DOSE PER GM BODY WEIGHT [2]	RECOVERY PER GM TISSUE	P^{31} PER GM TISSUE	SPECIFIC ACTIVITY [3]
	cts/min. × 10^3	*cts/min.* × 10^3	*mg*	× 10^{-3}
Vitamin B_{12}	5420	30,760	21.9	0.26 ± 0.02
None	4330	33,340	15.8	0.49 ± 0.09

[1] The dams received ration 2884 with and without vitamin B_{12}. The values are averages of 6 offspring in each group.

[2] Within 7 hours after birth, about 10 μc of P^{32} as sodium phosphate in 0.1 ml of solution was administered subcutaneously and the animals sacrificed after 12 hours. Both tibia were removed, weighed and digested in 10 N H_2SO_4.

[3] Specific activity is defined as the counts per microgram of phosphorus divided by the dose per gram of body weight. The average, plus or minus the standard deviation, is recorded.

The specific activity in the case of the deficient animals was about one-half of the value obtained in the vitamin B_{12}-supplemented group. This difference was statistically significant at the 1% level.

DISCUSSION

The high incidence of hydrocephalus and eye defects observed when the animals consumed the unsupplemented corn-wheat gluten ration shows that the diet was grossly deficient in vitamin B_{12}. The fact that the addition of the known vitamins other than riboflavin and vitamin B_{12} decreased the incidence of skeletal abnormalities indicates that

the diet was deficient in at least one of these vitamins. A deficiency of folic acid may have caused the abnormalities, since according to microbiological assay the diet contained only about 17 μg of folic acid per 100 gm. In trials not reported here in which a casein-type ration was used, a folic acid deficiency has been found to increase bone malformations. Obviously, the diet is deficient in still other respects because supplementation with the known required vitamins still resulted in a relatively high incidence of skeletal defects. The protein of the diet is of low biological value and this may be the cause of the malformations. On the other hand, it may be deficient in an unrecognized nutrient.

Warkany and coworkers ('40, '42, and '44) did not report hydrocephalus or a high incidence of malformation in any soft tissue. This may have been due to the fact that their animals were not depleted of vitamin B_{12} or to the fact that they used a different strain. In most of their work, Warkany and coworkers used the Sprague-Dawley strain although they obtained comparable results with other strains. Normally the rats in this laboratory will be depleted of vitamin B_{12} when they have consumed an all-vegetable diet for a period of three months. However, the Sprague-Dawley strain seems to deplete less readily and to be less subject to congenital malformations when depleted of vitamin B_{12}. Out of 166 offspring produced in our laboratory by Sprague-Dawley dams fed a soybean oil meal ration deficient in vitamin B_{12} and mated with our Wistar strain males, there were only 7 cases (4.2%) of hydrocephalus and they were produced by two dams after being on the diet for about one year (unpublished data). Only a small number (74) of Sprague-Dawley offspring from vitamin B_{12}-depleted dams have been examined but none showed bone abnormalities. If the animals used by Warkany and coworkers were not depleted of vitamin B_{12} that would explain why riboflavin prevented skeletal malformations under their conditions but had no effect in this investigation unless vitamin B_{12} was included in the diet. Hartman et al. ('51) reported that high levels of riboflavin

promoted intestinal synthesis of vitamin B_{12} and tended to relieve a state of vitamin B_{12} deficiency. It is possible that the doses of riboflavin fed by Warkany and coworkers relieved any vitamin B_{12} deficiency that might have existed as well as the riboflavin deficiency. Under the conditions in this laboratory, vitamin B_{12} was required in addition to riboflavin.

The observations made on the alpha-protein rations agreed with those on the corn-wheat gluten rations relative to the effects of deficiencies of riboflavin and vitamin B_{12}. The alpha-protein ration was superior in that when supplemented with the required vitamins it gave more nearly complete protection of the developing fetus than the corn-wheat gluten ration or casein ration. A ration containing soybean oil meal also afforded protection.

In agreement with the observation of Warkany ('42) a high calcium to phosphorus ratio increased the incidence of bone abnormalities. This was more obvious when the diet was more nearly complete in other respects.

Deficiencies of riboflavin and of vitamin B_{12} in the maternal diet decreased the alkaline phosphatase activity of the tibiae of the newborn rats. This effect was more marked in the case of the riboflavin deficiency. The difference in alkaline phosphatase activities was correlated with more severe bone defects in the riboflavin-deficient offspring as observed grossly by the staining technique.

The effect of the low phosphatase activity in decreasing the rate of phosphorus incorporation in the skeleton was clearly shown by the P^{32} studies. The rate at which phosphorus was laid down, as measured by the specific activities, was markedly less in the vitamin B_{12}-deficient animals. Patrick and Schweitzer ('52) have also observed that vitamin B_{12} and other B-vitamins increased the rate at which P^{32} was laid down in the tibiae of chicks. The small tibiae of the deficient rats had more phosphorus per unit of weight

but the percentage of phosphorus incorporated in a given period of time was less. Apparently there was less organic matrix in the deficient animals and this was reflected in the lower phosphatase activity since the enzyme is associated with the cartilaginous tissue. Gross observation of the stained tibiae from deficient animals also indicated less cartilaginous tissue. Thus it appears that a deficiency of either vitamin B_{12} or riboflavin results in defective cartilage formation and a lower phosphatase activity. Consequently there is a decreased rate of ossification in the skeletal tissue.

SUMMARY

The corn-wheat gluten rachitogenic diet devised by Steenbock and Black is deficient in vitamin B_{12} and riboflavin, and possibly in folic acid. There was a high incidence of hydrocephalus, ocular defects and skeletal abnormalities among the offspring of dams fed this diet and the incidence of the malformations was greatly reduced by adding the known vitamins required by the rat. The addition of these vitamins to a diet in which the dietary protein was of soybean origin gave nearly complete protection. The omission of vitamin B_{12} resulted in hydrocephalus, eye defects and an increased incidence of bone defects. Omission of riboflavin had no effect on the incidence of hydrocephalus or eye defects but increased skeletal abnormalities. The omission of both gave a still higher incidence of skeletal abnormalities. A high Ca : P ratio also increased skeletal defects.

The alkaline phosphatase activity in the tibiae of riboflavin- and vitamin B_{12}-deficient newborn was lower than normal. The rate of phosphorus deposition in the tibiae of vitamin B_{12}-depleted offspring as measured by specific activities after P^{32} administration was also decreased. The latter facts give an indication of the cause of the high incidence of skeletal abnormalities in cases of riboflavin and of vitamin B_{12} deficiencies.

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EFFECT OF SOURCE OF DIETARY PROTEIN ON THE UNSATURATED FATTY ACIDS IN THE CARCASS FAT OF THE RAT [1,2]

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When animals are fed vegetable oils high in diethenoic or triethenoic acids, the depot fat generally reflects the level of these acids in the diet (Ellis and Hankins, '25; Ellis and Isbell, '26; Ellis, Rothwell and Pool, '31; Longenecker, '39). Cottonseed oil seems to be an exception in that when small amounts of this oil are fed to swine the lard produced is firmer than that obtained from control animals (Ellis and Isbell, '26; Ellis, Rothwell and Pool, '31). Hostetler, Halverson and Sherwood ('39) have shown that when up to 15% of cottonseed meal replaced corn in swine rations, the hardness of the lard produced increased proportionately to the level of cottonseed meal, but that with further increases in cottonseed meal, the hardness of the lard decreased. The high level of palmitic acid in cottonseed oil as compared to that in corn oil has been the generally accepted explanation of the hardening effects obtained when cottonseed meal replaced corn in animal rations. This explanation, however, would not account for the observed decrease in the hardness of the lard when the level of cottonseed meal exceeded 15% of the diet. The purpose

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of the study reported in this paper was to seek an explanation for this softening effect resulting from feeding diets containing high levels of cottonseed meal.

EXPERIMENTAL AND RESULTS

Weanling male rats of the Holtzman strain were used in 4 experiments. In each experiment the animals were ranked according to initial weight and divided into groups such that the mean weight for each group was about the same. In the first two experiments animals receiving the same diet were housed in the same cage, and in the last two experiments the rats were caged individually (in randomized blocks) in order to record food consumption.

The composition of the diets used in this study is presented in table 1. All diets, which contained nearly equal quantities of protein (N $\times$ 6.25), were fed ad libitum for 6 to 7 weeks. Normal weight gains of about 200 gm were obtained with the rats of all groups except the casein-fed (diet 8), in which the mean gain observed was only 138 gm.

At the end of the experimental period, the rats were decapitated, skinned and the entire gastro-intestinal tract removed. Each carcass was then minced with 250 ml of alcohol in a Waring Blendor. After boiling the minced carcass for 10 minutes, the alcohol was removed by filtration. The rat tissue was reextracted twice, using 100 ml of boiling alcohol; then the residue was washed with 200 ml of ether and dried in a vacuum oven at 50°C. The alcohol and the ether extracts were combined and brought to dryness under reduced pressure in an atmosphere of nitrogen. The remaining lipides were dissolved in ether, dried with anhydrous sodium sulfate and added to the previously dried rat tissue. This was extracted overnight, in a modified Pickel extractor, by anhydrous ether. The levels of the unsaturated fatty acids in the isolated depot fats were determined spectrophotometrically, after alkaline isomerization, according to the general procedure of Brice et al. ('52).

TABLE 1

Composition of the experimental diets[1]

INGREDIENT[2]	DIETS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
SM[3]	49.0	...	49.0	...	49.0	...	...	...	...	...	...	49.0	...	...	...	...	...
CM[3]	...	57.0	...	57.0	...	57.0	...	...	...	...	...	...	...	...	...	...	...
EA[4]	...	...	...	...	...	...	23.0	...	...	...	23.0	..	23.0	23.0	23.0	...	23.0
Casein[5]	...	...	...	...	...	...	...	23.0	...	...	...	...	...	...	...	...	...
Fibrin	...	...	...	...	...	...	...	...	23.0	...	...	...	...	...	...	...	...
SP[6]	...	...	...	...	...	..	...	...	...	23.0	...	...	...	...	...	...	...
MeOH ext'd SM	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	49.0	...
DL methionine	...	...	...	...	...	...	...	...	...	0.3	...	...	...	...	...	...	...
MeOH ext. of SM[7]	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	18.0
HCl ext.	...	...	...	...	...	...	...	...	...	...	...	...	...	0.75	...	...	...
Starch	45.0	37.0	45.0	37.0	50.0	42.0	68.0	68.0	68.0	67.7	68.0	45.0	73.0	72.25	72.0	45.0	50.0
CO	...	...	5.0	5.0	...	...	5.0	5.0	5.0	5.0	...	...	...	...	...	5.0	5.0
SO	5.0	5.0	...	...	...	...	...	...	...	...	...	...	...	...	1.0	...	...
Triolein[8]	...	...	...	...	...	...	...	...	...	...	5.0	5.0	...	...	...	...	...
Salts[9]	...	...	...	...	...	...	4.0	4.0	4.0	4.0	4.0	...	4.0	4.0	4.0	...	4.0
$CaCO_3$	0.5	0.5	0.5	0.5	0.5	0.5	...	...	...	...	...	0.5	...	...	...	0.5	...
NaCl	0.5	0.5	0.5	0.5	0.5	0.5	...	...	...	...	...	0.5	...	...	...	0.5	...

[1] The following vitamins were added per 100 gm diet: thiamine hydrochloride, 0.2 mg; calcium pantothenate, 2.0 mg; riboflavin, 0.4 mg; pyridoxine hydrochloride, 0.2 mg; folacin, 0.2 mg; nicotinic acid, 1.0 mg; vitamin B_{12}, 0.003 mg (1% in mannitol); and choline chloride, 100 mg. In addition oleum percomorphum providing 2000 I.U. of vitamin A and 420 I.U. of vitamin D was included per 100 gm diet.

[2] The following abbreviations are used: SM, soybean oil meal; CM, cottonsed meal; EA, egg albumin; SP, soybean protein; HCl ext., the ether soluble portion of acid hydrolyzed soybean meal; CO, cottonseed oil; and SO, soybean oil.

[3] Pre-press solvent-extracted cottonseed meal or solvent extracted soybean meal were used in one experiment in which diets 1 to 6 inclusive were fed. These meals, continuously extracted with ether for 24 hours, were used in all other meal diets.

[4] All rats fed egg albumin received 0.04 mg biotin every other day by intraperitoneal injection.

[5] Vitamin test; Nutritional Biochemicals Corporation.

[6] Alpha protein; Nutritional Biochemicals Corporation. Protein was washed until free of bisulfite.

[7] Methanol extract of soybean meal. One kilogram of starch was added to the 756 gm of methanol soluble material that was obtained from 5 kg of ether-extracted soybean meal.

[8] Emery 2230; Emery Industries Incorporated.

[9] Salts "W"; Nutritional Biochemicals Corporation.

The results of each experiment were subjected to statistical analysis. Since the means of the results from each diet that was used in more than one experiment were similar, the results were combined as presented in table 2. A probability level of 0.25 was chosen for significance of difference.

Analyses of cottonseed oil and soybean oil used in these experiments showed the following percentages of unsaturated fatty acids: for soybean oil, monoethenoid acids 33.5, diethenoid acids 47.1 and triethenoid acids 5.1; for cottonseed oil, monoethenoid acids 28.8 and diethenoid acids 45.1, There were no measurable amounts of triethenoid acids in the cottonseed oil and no tetraethenoid acids detectable in either of the two oils.

The addition of either 5% of soybean oil (diets 1 and 2) or 5% of cottonseed oil (diets 3 and 4) to diets containing either of the two meals resulted in greatly increased levels of diethenoid acids in carcass fat and a concomitant decrease in the monoethenoid acids when compared with the same basal diets with no added fat (diets 5 and 6). The addition of cottonseed oil effected no change in the triethenoid acid level in carcass fat, but the addition of soybean oil resulted in a 5-fold increase in triethenoid acid level. These results are as expected, in that the depot fat levels of the unsaturated fatty acids reflected the amounts of these acids in the oils fed. The carcass fat, however, of animals that received cottonseed meal and no added fat (diet 6) contained higher levels of diethenoic acids and lower levels of monoethenoic acids than their soybean-fed counterparts (diet 5).

In two of the three experiments in which direct comparisons were made, the levels of diethenoic acids in carcass fat were higher in rats fed cottonseed oil and cottonseed meal (diet 4) than in rats fed soybean meal and cottonseed oil (diet 3). In the third experiment, however, there was no significant difference between the diethenoic acid content of the carcass fat produced from these respective diets.

When the mean results of all experiments with diet 3 are contrasted with the mean for all the trials with diet 4 (table

TABLE 2

Mean unsaturated fatty acid content of rat carcass fat

DIET NO.	DIET [2]	NO. RATS	NO. OF EXP'TS.	UNSATURATED FATTY ACIDS [1]			
				Monoethenoid	Diethenoid	Triethenoid	Tetraethenoid
				%	%	%	%
1	SM + 5% SO	8	1	27.7 ± 1.14	29.5 ± .34	2.9 ± .10	2.6 ± .21
2	CM + 5% SO	8	1	27.5 ± 1.86	30.6 ± .64	2.4 ± .15	2.5 ± .07
3	SM + 5% CO	24	4	24.1 ± .53	27.9 ± .42	0.5 ± .03	2.8 ± .13
4	CM + 5% CO	21	3	23.0 ± .56	28.5 ± .51	0.4 ± .02	2.5 ± .12
5	SM + No Fat	8	1	52.3 ± .78	6.8 ± .28	0.6 ± .03	1.3 ± .15
6	CM + No Fat	8	1	43.9 ± 1.58	9.5 ± .11	0.3 ± .03	2.0 ± .33
7	EA + 5% CO	13	2	30.8 ± .72	20.6 ± .60	0.1 ± .04	1.7 ± .19
8	Casein + 5% CO	7	1	28.3 ± .90	21.3 ± .78	0.2 ± .04	3.3 ± .23
9	Fibrin + 5% CO	7	1	28.8 ± .90	20.8 ± .78	0.1 ± .04	1.7 ± .23
10	SP + 5% CO	7	1	26.7 ± .90	22.3 ± .78	0.1 ± .04	2.0 ± .23
11	EA + 5% Triolein	5	1	56.0 ± 1.11	6.7 ± .52	0.6 ± .07	1.4 ± .20
12	SM + 5% Triolein	3	1	55.1 ± 1.44	8.6 ± .68	0.9 ± .09	1.8 ± .26
13	EA + No Fat	6	1	54.4 ± 1.02	3.1 ± .47	0.4 ± .06	1.1 ± .18
14	EA + HCl Ext	6	1	53.6 ± 1.02	3.6 ± .47	0.3 ± .06	1.0 ± .18
15	EA + 1% SO	6	1	50.3 ± 1.02	7.3 ± .47	0.6 ± .06	1.5 ± .18
16	MeOH Ext'd SM + 5% CO	12	2	22.5 ± .88	27.4 ± .62	0.4 ± .04	2.4 ± .21
17	EA + MeOH Ext of SM + CO	6	1	27.6 ± 1.20	23.7 ± .94	0.4 ± .06	2.9 ± .03

[1] Each value accompanied by the standard deviation of the mean.

[2] See table 1, footnote 2, for key to abbreviations.

2), no significant differences are found with respect to the levels of any of the unsaturated fatty acids. No significant differences were found between the carcass fat of rats fed diets 1 and 2, as well as between those fed diets 3 and 4. It is doubtful therefore, if feeding soybean meal produces a carcass fat having an unsaturated fatty acid composition different from that resulting from feeding cottonseed meal when the diets contain either 5% cottonseed oil or 5% soybean oil.

Replacement of ether-extracted soybean meal (diet 3) or ether-extracted cottonseed meal (diet 4) by egg albumin (diet 7), casein (diet 8), fibrin (diet 9) or soybean-protein supplemented with methionine (diet 10) resulted in lower amounts of the diethenoid acids and higher levels of the monoethenoid acids in the carcass fat. Furthermore, with the exception of the casein-fed group in which growth was poor, the percentages of triethenoid and of tetraethenoid acids were lower when the purified proteins were fed than when either cottonseed meal or soybean meal was given. When triolein was used as the source of dietary fat, there was a tendency for the carcass fat produced by the egg albumin (diet 11) to contain smaller amounts of diethenoid acids than that produced by the soybean meal (diet 12).

It is well recognized that ether extraction alone does not remove all the ether-soluble substances from plant tissue. To be sure that the effects observed on the soybean and cottonseed meal regimes were not merely the result of residual unsaturated fatty acids in these feeds, 3 kg of ether-extracted soybean meal were hydrolyzed with hydrochloric acid and then extracted with a mixture of ether and petroleum ether according to the procedure of the Association of Official Agricultural Chemists ('50) for the determination of fats in cereal foods. The solvents were distilled off and the residual lipides, amounting to 45 gm, were added to starch and fed with an egg-albumin diet at a level equivalent to that in the original soybean meal.

The effects of this diet (diet 14) were compared with those from an unsupplemented egg albumin diet (diet 13) and with those from one to which 1% soybean oil was added (diet 15). Since it was presumed that any effect on rat carcass fat due to the residual oil of the ether-extracted soybean meal would be more pronounced on a low-fat diet than on a high-fat diet, cottonseed oil was omitted from these regimes. The feeding of the extracted residual soybean lipide did not alter the unsaturated fatty acid composition, but the addition of 1% soybean oil increased the diethenoid acid content of the rat fat by more than 100%. These results indicate that soybean and cottonseed meals contain an ether-insoluble factor that promotes the deposition of diethenoic acids in the carcass fat of rats.

An attempt was made to extract this factor with methanol by the following procedure: The ether-extracted soybean meal was treated with 10 volumes of 70% methanol for two hours, at room temperature, stirred occasionally and then filtered. This procedure was repeated twice and the resulting extracted meal was dried overnight at 50°C. in a forced air oven. The combined methanol extracts were concentrated in vacuo, dried on starch at 50°C., and ground. The methanol-soluble fraction was fed at a level equivalent to soybean meal.

Feeding the methanol-extracted meal (diet 16) resulted in essentially the same unsaturated fatty acid picture as that obtained with soybean meal that had not been treated with methanol (diet 3). The addition of the methanol extract of soybean meal to an egg albumin diet (diet 17) resulted in a higher percentage of diethenoid acids and a lower percentage of the monethenoid acids when compared with the results from the unsupplemented egg albumin diet (diet 7).

DISCUSSION

The results presented reaffirm the observations that the unsaturated fatty acids of the dietary fat exert a major effect on the fatty acid composition of the carcass fat of animals. In addition, there was a small but distinct increase in the

diethenoic acid level and a concomitant decrease in the monoethenoic acid level of the depot fat when ether-extracted meals, either soybean or cottonseed, replaced a purified source of dietary protein. It would appear that these meals contain substances that alter the metabolism of unsaturated fatty acids in the rat. The ether-soluble fraction of acid-hydrolyzed ether-extracted soybean meal fed at a level of 0.75% did not alter the unsaturated fatty acid composition of carcass fat. In contrast, the addition of 1% soybean oil resulted in a marked increase in the diethenoic-acid level. -Consequently, it is unlikely that the effects observed from feeding the meals are the result of residual diethenoic acids present in ether-extracted soybean meal or ether-extracted cottonseed meal. This hypothesis is supported further by the observation that when a 70% methanol extract of ether-extracted soybean meal is fed, the carcass fat has an unsaturated fatty acid composition approaching that obtained when the whole meal is fed.

From the standpoint of gross composition, the oil meal diets contained considerable quantities of carbohydrates other than starch. The presence in the meals of carbohydrate sources other than starch conceivably might influence the deposition or catabolism of unsaturated fatty acids. However, such reasoning would not seem a plausable explanation of the observed differences; inasmuch as the methanol extract of soybean meal produced the response. This would tend to indicate the presence of a substance (s) that exerts its effect in low concentration.

SUMMARY

1. The unsaturated fatty acid composition of the dietary fat is the primary factor affecting the fatty acid composition of the depot fat of the rat.

2. Replacement of the purified protein in the diet of rats with either ether-extracted soybean meal or ether-extracted cottonseed meal results in an increase in the level of diethenoid acids and a decrease in the level of monoethenoid acids in

the carcass fat. These effects apparently are not the result of residual plant oils in the meals.

3. Evidence presented indicates that soybean and cottonseed meals contain a factor (or factors?) capable of altering the metabolism of unsaturated fatty acids in the rat.

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PLACENTAL TRANSFER OF MO^{99} AND CA^{45} IN SWINE [1,2,3]

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In the study of factors influencing fetal development, the transfer of different mineral elements across the placenta has been found to vary in rate with the stage of pregnancy. Sodium is deposited most rapidly up to the middle of pregnancy, chloride during the early stages, and potassium, calcium and phosphorus most rapidly toward the end of the gestation period (Huggett, '41). Plumlee et al. ('52) made an extensive study of the placental deposition and turnover of Ca^{45} in cattle. The Pechers ('41) have reported on the placental transfer of radioactive calcium and strontium in mice. Wilde et al. ('46) demonstrated that P^{32} had a placental transfer coefficient in guinea pigs two to three times greater than that of labelled sodium; while Flexner and Gellhorn ('42) using labelled sodium, made a study of the placental transfer of this element in animals having placentas belonging to 4 different morphological types, one of which was the epitheliochorial placenta found in swine. The literature reveals no study of the occurrence of molybdenum in the fetuses of swine. The object of the present study was to evaluate the

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[3] The isotopes were obtained from Oak Ridge, Tennessee after allocation by the Atomic Energy Isotopes Commission.

deposition of this element in the fetus of swine using labelled molybdenum; concurrently a similar study was made using Ca^{45}.

EXPERIMENTAL

Four pregnant Duroc sows, no. 1 and no. 2 weighing 299 and 308 pounds, respectively, during their first pregnancies, and no. 3 and no. 4 weighing 335 and 300 pounds respectively, during their second pregnancies, were placed in metabolism racks and continued on a commercial stock ration. Approximately 6 days before farrowing, sows 1 and 2 were given 0.9 mc (millicurie) doses of Mo^{99} with Na_2MoO4 carrier and 0.3 mc of Ca^{45} as the chloride. Sows 3 and 4 received 3.16 mc of Mo^{99} and 0.87 mc of Ca^{45}. The isotopes were given by stomach tube in water solution. Carrier levels of 1.55, 3.10, 1.72 and 2.02 gm of Na_2MoO4 were given sows 1 to 4, respectively. The Ca^{45} as received contained 0.037 mc per mg of calcium in the form of $CaCl_2$, and was diluted to contain 0.1 mc per ml.

After isotope dosage, urinary and fecal collections were made manually until the sows were sacrificed 30 hours later. The sows were stunned by a blow on the head and the fetuses were obtained by abdominal hysterotomy. Blood samples were taken from the umbilical veins and the sow's heart. Samples of amnion, amniotic fluid, allantois, allantoic fluid, brain, spleen, pancreas, kidney, liver, heart, gastrocnemius muscles, vertebra and femur were taken from both the sows and the fetuses for Mo^{99} and Ca^{45} assays. The samples were digested with concentrated nitric acid, diluted to definite volume and aliquots taken for analysis. Mo^{99} determinations were made using dipping-type Geiger tubes, while calcium oxalate precipitates of the Ca^{45} (Shirley, Owens and Davis, '50) were prepared for dry Geiger tubes, using commercial scalers. The low intensity of the beta-particle emissions of the Ca^{45} compared to those of the Mo^{99} makes it possible to determine the Mo^{99} with relatively thick-walled dipping-type Geiger tubes in the presence of the Ca^{45}; the walls are penetrated by the Mo^{99} but not by the Ca^{45} emissions.

RESULTS AND DISCUSSION

Data are presented in table 1 for the concentration of Mo^{99} and Ca^{45} in the various tissues of the fetuses, expressed as percentage of dose per gram of tissue. The concentration of Mo^{99} in the fetuses of the sows of both first and second preg-

TABLE 1

Distribution of isotopes in tissues of fetuses expressed as percentage of dose per gram (10^{-4})

TISSUE	Mo^{99}		Ca^{45}	
	First pregnancy	Second pregnancy	First pregnancy	Second pregnancy
Amnion	—[1]	0.12	—	0.57
Amniotic fluid	0.08 ± 0.03[2]	0.05 ± 0.01[2]	0.29 ± 0.02[2]	0.76 ± 0.40[2]
Allantois	—	0.09	—	0.38 ± 0.38
Allantoic fluid	—	0.24 ± 0.11	—	1.00 ± 0.70
Placenta	0.11[3]	0.14 ± 0.04	0.37	0.10
Uterus	0.36 ± 0.19	—	0.51 ± 0.31	—
Blood serum	trace	0.03 ± 0.02[4]	0.36 ± 0.01	0.82 ± 0.40[4]
Blood cells	trace	—	0.01	—
Kidney	none	trace	0.25	0.64 ± 0.19
Brain	none	none	0.17	0.61 ± 0.26
Liver	0.07 ± 0.05	0.02 ± 0.01	0.13 ± 0.03	0.26 ± 0.06
Heart	trace	0.02	0.13 ± 0.06	0.03 ± 0.04
Gastrocnemius muscle	trace	trace	0.15 ± 0.01	0.42 ± 0.25
Femur	trace	trace	50.8 ± 1.0	105.0 ± 60.0
Vertebra	none	—	26.7 ± 3.0	—

[1] The dash indicates that no sample was taken. If the sample aliquot had less than 5 counts per minute of radioactivity above background it was assumed that none was present; if there were between 5 and 11 counts per minute only a trace was assumed to be present.

[2] Mean and standard deviation.

[3] Standard deviation was not sufficient to give values in first two decimal places.

[4] Whole blood.

nancies showed marked agreement. The Ca^{45} values for the fetal tissues of the second pregnancy in most instances were slightly higher than for the corresponding tissues of the first pregnancy. The differences were not significant.

The fluids and tissues that surround the fetuses had appreciably more Ca^{45} present than Mo^{99}. Only the blood serum,

liver and heart of the fetuses showed more than a trace of the molybdenum isotope. Considering the level of molybdate salt given with the isotope dose, it is apparent that very little molybdenum crosses the placenta to the developing fetus of swine. The quantity transferred would be comparable to trace element needs. Molybdenum has been demonstrated to be associated with xanthine oxidase (DeRenzo et al., '53).

TABLE 2

Distribution of isotopes in tissues of sows expressed as percentage of dose per gram (10^{-4})

TISSUE	Mo^{99}		Ca^{45}	
	First pregnancy	Second pregnancy	First pregnancy	Second pregnancy
Blood serum	0.20 [1]	—	0.40 [1]	—
Whole blood	—	0.23	—	0.40
Kidney	0.80 ± 0.60 [2]	0.28 ± 0.20 [2]	0.24	0.61 ± 0.18 [2]
Liver	0.70 ± 0.02	1.00 ± 0.10	0.14 ± 0.04	0.15 ± 0.21
Pancreas	0.07 ± 0.04	0.04 ± 0.01	0.63 ± 0.64	1.20 ± 0.40
Spleen	0.21	0.39 ± 0.05	0.12	0.13
Brain	0.05 ± 0.01	0.04 ± 0.02	0.17	0.69
Heart	0.65 ± 0.03	1.04 ± 0.03	0.03	0.11
Gastrocnemius muscle	none	trace	0.20	0.35
Small intestine	—	trace	—	1.30 ± 1.00
Large intestine	—	0.60 ± 0.30	—	5.20 ± 1.40
Femur	0.70 ± 0.30	0.40 ± 0.17	21.0	11.4
Vertebra	0.65 ± 0.40	—	26.0 ± 0.6	—

[1] Standard deviation was not sufficient to give values in first two decimal places.
[2] Mean and standard deviation.

Data for the concentration of Mo^{99} and Ca^{45} in the tissues of the sows, expressed as percentage of dose per gram of tissue are presented in table 2. Differences in concentration of Mo^{99} and Ca^{45} in the individual tissues probably are due to variations in the animals rather than to the effects of number of pregnancies. These data demonstrate a striking capacity of swine to absorb molybdenum, and distribute it throughout the body, although deposition of Mo^{99} in the bones was slight compared to that of calcium. Comparison of the data

in tables 1 and 2 shows that with so much Mo^{99} in the various tissues of the sow an effective barrier must exist to prevent transfer of the Mo^{99} to the fetuses. On the other hand the Ca^{45} isotope is as widely distributed in the fetal tissues as in those of the sow, with an appreciably higher concentration per unit of weight in the bones.

In table 3 data are presented for the fecal and urinary excretion of the Mo^{99} and Ca^{45} expressed as percentage of the dose. These data show that dietary molybdate salts are absorbed readily, and an average of 54.7% of the dose was excreted in the urine compared to an average of only 8.19% in the feces during the 30-hour evaluation period. The kidney is thus an important organ in the liberation of excess molybdenum salts from the body; yet it is very effective in retaining calcium. The feces are quite evidently the principal pathway for the excretion of calcium. By 30 hours after dosage only 3% of the Mo^{99} and 6% of the Ca^{45} remained in the contents of the alimentary tract. The wide variations in values between the sows could not be related definitely to number of times that the sows had been pregnant, their weight, or variation in the amounts of molybdate or calcium salts the sows received.

TABLE 3

Excretion of isotopes by sows expressed as percentage of dose

SOW NO.	PER CENT DOSE					
	Mo^{99}			Ca^{45}		
	Feces	Urine	G.I.[1] tract	Feces	Urine	G.I.[1] tract
1	6.63	58.0	1.8	47.8	0.18	7.9
2	18.90	36.9	1.2	54.2	0.26	4.7
3	0.23	50.1	6.4	...	0.27	..
4	7.02	74.1	3.7	39.1	0.29	..
Average	8.19	54.77	3.28	47.03	0.25	6.3

[1] Gastrointestinal tract.

SUMMARY

A study has been made to determine the extent to which molybdenum and calcium were transferred to the developing fetus in Duroc swine using the Mo^{99} and Ca^{45} isotopes. Mo^{99} was readily absorbed after oral administration to the sows and widely distributed in their tissues. Little or none was found in the various tissues of the fetuses, indicating a placental barrier to this element. Ca^{45} was readily transferred to the developing fetus and a greater percentage of it was found in the fetal bones per unit of weight than in the bones of the sows. No significant differences were observed in the uptake and distribution of the Mo^{99} and Ca^{45} isotopes between sows in their first and second pregnancies. Approximately 55% of the orally administered labelled molybdenum was excreted in the urine and 8% in the feces within 30 hours. Approximately half the intake of Ca^{45} was excreted in the feces and only a trace in the urine during the corresponding period.

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THE UTILIZATION OF CAROTENE

II. FROM SWEET POTATOES BY YOUNG HUMAN ADULTS [1]

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The importance of carotene as a source of vitamin A in the average diet in the United States is well recognized (Food and Nutrition Board, '48). Numerous absorption studies involving doses of purified carotene have been reported, but strangely enough, no report from an American laboratory has appeared in the literature on the utilization in the human of the carotene in any vegetable as estimated from fecal excretion.

Wilson, Das Gupta and Ahmad ('37) reported that about 10% of the carotene in raw carrots or cooked spinach was excreted in human feces, while van Eekelen, Pannevis and Engel ('38) found that two healthy male adults excreted 94 to 99% of the carotene in these two vegetables. Kreula and Virtanen ('39) studied the rate of carotene excretion of 4 persons aged 25 to 30 years. About 80% of the carotene in finely grated raw carrots was recovered after passage through the digestive tract, and 95% from coarsely grated raw or cooked carrots. Incubation of a mixture of carrots and feces at 37°C. for 24 hours resulted in no decrease in carotene. Virtanen and Kreula ('41) found, with a group of 8 subjects, that the fecal excretion of carotene amounted to 95 to 98% from well-chewed or cooked carrots, and 64 to 96% from

[1] Published with the approval of the Director of the Louisiana Agricultural Experiment Station.

finely grated material. Eriksen and Höygaard ('41) investigated the absorption of carotene from carrots and spinach during two 24-hour periods in 4 healthy men who had received a basal diet low in carotene. The fecal carotene from raw or cooked carrots amounted to 99 and 81% respectively; from spinach, the corresponding values were 55 and 42%. Leonhardi ('47) found, in an investigation based on 7 persons in 20 experiments, that the fecal excretion of carotene from fresh, dried and stored carrots, spinach, and dried sugar-beet leaves, averaged about 92% of the amount contained in the ingested vegetables. The excretion was less from fresh than from dried vegetables with a minimum excretion of about 85% from fresh carrots. Kreula ('47) studied the utilization of carotene in three normal subjects. On a fat-free diet, about 90% of the carotene was excreted when raw finely grated carrots were fed, but only 30 to 50% when the carrots were mixed with olive oil. In a later investigation Kreula ('50) found that subjects receiving a fat-free basal diet plus supplements of vitamin-free margarine and 0.8 to 8.0 mg carotene/day in various foods, after ingestion of the indicated sources of carotene, excreted amounts of carotene corresponding to the following percentages: crystalline β-carotene 100; dried carrot meal 100; carrot juice 87–90; olive oil ground with carrots in a meat grinder 43; tomatoes 43–82; spinach 100; and hay meal 93–100. Extensive experiments reported by Hume and Krebs ('49) indicated that the human fecal excretion of carotene varied from 44 to 76% of the amount ingested in canned carrots, 57 to 59% of that in canned spinach, and 59 to 73% of that in dehydrated cabbage.

Sweet potatoes are an important agricultural crop and a dietary staple, not only in Louisiana, but throughout the southern region of the United States. Increasingly, they are being included in the dietaries of other regions. With the world supply of animal sources of vitamin A comparatively limited and the plant sources of carotene relatively abundant, a study of the factors which affect the human utilization of

carotene in specific foods is of economic and physiological importance.

In the present experiment, the amounts of carotene excreted in the feces associated with basal diets essentially devoid of carotene and vitamin A but otherwise adequate, and the fecal carotene from these same diets supplemented with sweet potatoes, were measured over a period of two months using a group of 8 healthy young human adults as subjects.

EXPERIMENTAL

The subjects were student volunteers, usually seniors or graduate students majoring in chemistry. They were selected on the basis of good health, infrequent colds, absence of allergies, good scholastic standing, dependability, and cooperative personality. The acceptable subjects agreed to report on time for meals; to eat only the food served in the laboratory; to report at once any failure to collect specimens of excreta; to report any minor upsets such as colds or headaches; and to report medication of any kind. None of the subjects took part in strenuous athletic activities. The group consisted of 5 men and three women. In return for their cooperation the subjects received the diets free of charge. The age, sex, height, and weight of each subject are given in table 1.

The basal diet[2] was planned from the data in Tables of Food Composition (Bureau of Human Nutrition and Home Economics, '45) to supply as little carotene and vitamin A as possible but to be complete in every other way. The meals were prepared and served in the laboratory. All the breads, cakes, cookies, and biscuits were made with white unenriched

[2] The food served daily was selected from the following items to provide balanced meals: lean roast beef, ground lean beef, bacon, ham, pork coldmeats, pork sausage, Spam, white meat of chicken, beets, bleached celery, cauliflower, eggplant, Irish potatoes, white cabbage, white onions, white turnips, apple sauce, canned pineapple, canned pears, cranberry sauce, grapefruit, grapefruit juice, strawberries, rice, grits, farina, biscuit, toast, white bread, corn bread, unfortified margarine, jam, jelly, skimmilk (reconstituted from non-fat dry milk solids), white cake, chocolate cake, gingerbread, cookies, chocolate syrup, fondant, peppermint candy, sugar, coffee, tea, and Coca Cola.

flour or corn meal. The margarine was not fortified with vitamin A or carotene. The meats, fruits, and vegetables were consumed in the same amount by all subjects. All meats were divided before being cooked into weighed individual portions, while the fruits and vegetables were weighed after they were cooked. Dietitians' scales were always on the dining table. The subjects themselves weighed and recorded the amounts of the following items which they consumed ad libitum: breads, cakes, cookies, candy, rice, grits, farina, jam, jelly, sugar, and margarine. Throughout the experiment each subject ate 50 gm of raw apple with each meal because of the resulting beneficial effect in maintaining proper activity of the intestines.

Protein of animal origin was supplied by lean meat and non-fat dry milk solids. Each subject received daily the indicated amounts[3] of these two items which were varied periodically according to the following schedule. Subperiod 1, diet 1: 9 days; 150 gm meat and 12 gm milk solids. Subperiod 2, diet 2: 9 days; 150 gm meat and 120 gm milk solids. Subperiod 3, diet 3: 9 days; 300 gm meat and no milk solids. During the next 4 days the subjects again received diet 1.

Following this 31-day period of depletion of carotene and vitamin A, the subjects received during three 9-day periods (subperiods 4, 5, and 6) diets 1, 2, and 3 in sequence with a daily supplement of 60 gm of mashed sweet potatoes which supplied an average of 3500 μg carotene per subject per day according to the daily carotene analyses of the sweet potatoes by the method of Moore ('40, '42) and Moore and Ely ('41).

The subjects were weighed weekly. No significant changes in weight were observed throughout the experiment. The feces were quantitatively collected daily, weighed, frozen at 0°F., and composited[4] into individual three-day specimens before being analyzed for carotene by the technique discussed by van Eekelen, Engel and Bos ('42). Our application of their findings to routine use was as follows:

[3] The stated amounts of meat are the weights of the raw material.

[4] Feces which have been frozen and thawed are more easily mixed than feces which have not been frozen.

Ten grams of thawed feces was mixed with an equal weight of anhydrous Na_2SO_4. After standing 5 minutes this mixture was treated with 125 ml of 95% EtOH and boiled gently for 5 minutes; 0.5 gm of asbestos fiber was then added. When the suspended matter had settled, the supernatent liquid was decanted. Into an extraction tube[5], fitted with a cotton plug wet with 10 ml EtOH, and clamped in position for filtering into a 500-ml wide-mouthed Erlenmeyer flask containing 10 ml of 60% (wt/wt) aqueous KOH solution, the residue was transferred with the aid of the decanted liquid, all of which was filtered through the material in the extraction tube. The beakers were rinsed with 10 ml EtOH. The rinsings were added to the extraction tube which was then placed in the Erlenmeyer flask. The mixture was refluxed until the drainings from the cotton plug were colorless. The extraction tube was removed and the flask was cooled to room temperature. With shaking, 50 ml cold H_2O was added to the flask. The diluted extract was transferred to a separatory funnel. The flask was rinsed with three 25-ml portions of cold H_2O; these were added to the separatory funnel. The resulting solution was extracted with 4 30-ml portions of redistilled Skellysolve B, b.p. 68–69°C.; these portions were combined, washed with 4 250-ml portions of cold H_2O, and dried with anhydrous Na_2SO_4. The dry carotenoid solution was made up to a known volume with Skellysolve B. An aliquot of this solution was diluted with benzene to yield a 25% benzene-75% Skellysolve B solution (vol/vol), of which 50 ml was filtered through a 20 mm × 60 mm column of $Ca(OH)_2$[6]. The adsorbent was washed with 20 ml of a mixture of 25% benzene and 75% Skellysolve B, and the filtrate was made up to a known volume. The transmission of light through this carotene solution, as

[5] Extraction tubes were made from 30 mm i.d. × 195 mm Pyrex test tubes which were constricted to 12 mm i.d. at a point 110 mm from the mouth. A hole about 8 mm in diameter was blown in each tube on the lower shoulder of the constriction. The bottom of the tube was cut off diagonally at a point on the side of the 8-mm hole 40 mm below the hole, slanting to a point on the opposite side 25 mm below the hole.

[6] Fisher's analytical grade.

TABLE 1

Amounts of carotene excreted by the individual subjects

Subject no.	1	2	3	4	5	6	7	8
Sex	Female	Female	Female	Male	Male	Male	Male	Male
Age in years	21	27	18	22	19	21	28	28
Height, cm	157	173	174	173	169	183	167	170
Weight, kg	42	56	57	53	57	74	52	63
	Micrograms of carotene per three-day composite[1]							
Subperiod 1								
3rd composite	344 (2)	*	471 (2)	423 (2)	380 (2)	784 (2)	471 (2)	414 (2)
Subperiod 2								
3rd composite	492 (2)	536 (2)	687 (2)	824 (2)	373 (2)	457 (2)	778 (2)	1048 (2)
Subperiod 3								
3rd composite	251 (2)	761 (2)	349 (2)	651 (2)	444 (2)	762 (2)	417 (2)	539 (2)
Subperiod 4								
1st composite	1661 (6)	636 (4)	1489 (4)	2981 (9)	1794 (5)	955 (3)	1002 (4)	1660 (9)
2nd composite	4185 (23)	7376 (21)	7026 (19)	3298 (20)	5902 (21)	6242 (12)	4766 (25)	8405 (27)
3rd composite	4482 (35)	3771 (22)	4732 (17)	8164 (29)	5593 (19)	6770 (22)	5530 (36)	7456 (29)
Subperiod 5								
1st composite	8536 (30)	6861 (35)	7460 (24)	6912 (15)	10331 (23)	7786 (17)	12270 (28)	7434 (28)
2nd composite	6677 (22)	6085 (23)	7137 (21)	7540 (20)	6116 (25)	5691 (14)	4681 (22)	3883 (17)
3rd composite	4856 (25)	7247 (15)	7091 (19)	5009 (17)	3811 (17)	5885 (14)	7740 (23)	7302 (20)
Subperiod 6								
1st composite	4066 (22)	5208 (16)	7017 (19)	4090 (19)	924 (21)	6708 (16)	5545 (16)	5632 (25)
2nd composite	5098 (30)	5573 (22)	4147 (21)	*	6671 (28)	6372 (25)	6008 (36)	3935 (25)
3rd composite	5468 (20)	5944 (17)	4208 (14)	9709 (16)	2819 (12)	7102 (14)	5715 (28)	3676 (23)
24-hr. postperiod	1984 (15)	812 (18)	1992 (25)	*	*	1821 (12)	2325 (30)	4098 (27)
Subperiods 4, 5, 6								
Total	47013	49513	52299	47703	43961	55332	55582	53481

[1] Numbers within parentheses indicate micrograms carotene/gram feces in the composites analyzed.

* No feces voided.

compared with that through the mixture of 25% benzene and 75% Skellysolve B, was measured in an Evelyn colorimeter using a 440-mμ filter. The concentration of carotene was estimated by means of factors computed from measurements of similar solutions containing various known amounts of β-carotene.

The analytical results are given in table 1.

DISCUSSION OF RESULTS

During subperiods 1, 2, and 3 of the depletion period the fecal carotene averaged approximately 2 μg carotene/gm feces. During the repletion period, large variations in the fecal carotene values among the different subjects, and for the same subject from time to time, are shown by the data in table 1. During this 27-day period each subject ingested about 94.5 mg carotene in the sweet potato supplements. Expressed as percentages of this intake, the fecal carotene values for subjects 1–8 were respectively: 50, 52, 55, 51, 47, 59, 59, and 57; for the group of 8 subjects the overall average value was 53.8 ± 1.6 [7] %.

The average amounts of fecal carotene excreted by the group of 8 subjects during the last 6 days of subperiods 4, 5, and 6 were respectively 55.9 ± 3.6%, 57.6 ± 2.3%, and 48.9 ± 3.2% of the 6-day intake of carotene. The comparatively wide difference between the percentages associated with subperiods 5 and 6 possibly may indicate that differences between the proteins supplied by the milk solids and those supplied by the lean meat affect the excretion of carotene since it has been found in this laboratory (James and ElGindi, '53) that rats which received isocaloric, isonitrogenous, isophosphoric diets containing lactalbumin or gluten excreted 30 to 75% more carotene in the feces than did rats which received diets containing casein or zein, when the intakes of β-carotene were the same in all 4 diets.

[7] Standard error of the mean.

We have no complete and unequivocal explanation for the apparently smaller excretion of the carotene from sweet potatoes than of the carotene, as reported in the literature, from spinach, carrots, and of β-carotene itself. Undoubtedly, differences in analytical methods, experimental conditions and techniques, variations among the persons used as subjects, as well as differences in the compositions of the basal diets, would enter into a consideration of this question. In connection with their work with rats, Chou and Marlatt ('53) have pointed out that besides the source of fat in the diet, other factors which may affect the utilization of carotene from vegetables are possible isomerization of β-carotene to neo-β-carotene during processing, differences in absorption due to plant structure, and such characteristics of the diet as bulk, fat and tocopherol contents.

SUMMARY

The amounts of carotene excreted in feces associated with three basal diets essentially devoid of carotene and vitamin A but otherwise adequate, and the fecal carotene following the use of these same diets supplemented with sweet potatoes, were measured for two months using a group of three women and 5 men, 18–28 years of age. Protein of animal origin was furnished by lean meat and non-fat dry milk solids. The three diets supplied each subject daily with the following amounts of these two items: diet 1, 150 gm meat (raw wt.) and 12 gm milk solids; diet 2, 150 gm (raw wt.) and 120 gm milk solids; and diet 3, 300 gm (raw wt.) and no milk solids. The diets were fed 9 days each in sequence; then for 4 days, diet 1 was again in effect.

Following the 31-day depletion period, diets 1, 2, and 3 were fed, supplemented daily with 60 gm of sweet potatoes which provided 3500 μg carotene/subject/day, again in sequence for three 9-day periods. The feces were collected quantitatively daily, composited into three-day specimens, and analyzed for carotene. For the group of 8 subjects, the average amounts of fecal carotene associated with supplemented diets

1, 2, and 3 were respectively 55.9 ± 3.6, 57.6 ± 2.3, and 48.9 ± 3.2% of the carotene ingested in the sweet potatoes. The overall average value, which represented 216 subject-days, was 53.8 ± 1.6%.

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THE EFFECT OF HIGH LEVELS OF TERRAMYCIN AND STREPTOMYCIN ON LONGEVITY IN THE RAT[1]

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In contrast to the numerous published reports concerning the therapeutic and growth-stimulating effects of antibiotics with emphasis on practical application, relatively little information is available regarding their fundamental effects on life processes.

A vitamin– or protein–sparing action in sub-optimal diets has been observed by Lih and Baumann ('51), Daft and Schwarz ('52), Sauberlich ('52), Huang and McCay ('53), Machlin et al. ('52) and Pecora ('53). Black and Bratzler ('52) and Braude and Johnson ('53), however, found little improvement in energy and nitrogen utilization when either streptomycin or aureomycin was added to vitamin-supplemented diets. Reyniers and co-workers ('52) observed no improvement from antibiotic supplementation in germ-free growth studies. Gabuzda et al. ('52) and Faloon et al. ('53) reported loss in weight, negative nitrogen balance and increased riboflavin excretion in undernourished patients and in those with hepatic disease following aureomycin administration. Sarett ('52), however, found no increase in the urinary excretion of B-vitamins following oral administration of streptomycin. Some evidence indicating a favorable effect on reproduction and lactation has been obtained by Stern et

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al. ('50) and Uram ('53), but little is known about the effect of prolonged administration of antibiotics on life span.

The present investigation developed from an attempt to minimize by means of antibiotic prophylaxis the chronic pneumonia commonly observed in aged rats during longevity studies (French et al., '53).

EXPERIMENTAL

As described in detail in a preceding paper (French et al., '52), approximately 100 rats of each sex were obtained by repeated breeding of two closely related males with 8 closely related females (Wistar albino strain) and by using the second generation from these matings to insure maximum genetic uniformity. They were raised on a finely-ground standard rat diet,[2] and littermates were assigned to the control and the antibiotic supplemented groups when they reached 11 months of age. The animals in the control group continued on the same diet throughout the experiment while those in the supplemented group received during alternate months either streptomycin sulfate or terramycin hydrochloride incorporated at a level of 0.02% in the same diet. After 7 months of this regimen with no apparent improvement in the incidence or severity of chronic pneumonia, streptomycin was discontinued and terramycin was increased to a level of 0.04% of the diet. This supplied 20 to 25 mg of antibiotic per day per kilogram of body weight, or about half the amount recommended for oral therapeutic use in small animals. It should be noted that this amount is many times that commonly used to stimulate the growth of animals. Both groups were fed ad libitum, with weekly recording of food consumption throughout the life span of each animal. All rats were weighed at monthly intervals. The environmental temperature was maintained throughout the experiment at 27 ± 2°C., with few exceptions.

Approximately 10 rats of each sex from both groups were sacrificed for histologic study at ages ranging progressively

[2] Rockland Farms Rat Diet, complete.

from 12 to 20 months, and all animals were subjected to post-mortem examination at which time gross evidence of diseases and abnormalities was recorded. Symptoms of diseases and the time of appearance of tumors and other abnormalities were noted.

RESULTS AND DISCUSSION

Average food consumption and weight data of adult rats beyond the age of one year, as noted in a previous publication (French et al., '53), lose significance due to the variable occurrence of diseases, tumors and deaths. A rough comparison, however, of animals not obviously diseased in both the antibiotic-supplemented and control groups revealed little difference between either weight or food consumption; at the ages of one, one and one-half and two years the average weight of males in both groups ranged from 465 to 492 gm and from 295 to 321 gm for females, and the average daily food consumption ranged from 21 to 24 gm for males and from 15 to 18 gm for females.

TABLE 1

Summary of longevity data

DIET	SEX	NO. OF RATS	MEAN AGE AT DEATH [1]	RANGE IN LONGEVITY
			days	*days*
Antibiotic	Male	51	688 ± 21	385–1094
Supplement	Female	55	720 ± 27	333–1186
Control	Male	32	747 ± 27	354– 996
	Female	29	794 ± 31	437–1166

[1] Mean and standard error of the mean.

The average ages at death and ranges in longevity presented in table 1 summarize the essential longevity data. The decrease in length of life noted in both males and females that received the antibiotic supplement approaches significance at the 5% level when compared to the control group; and if compared to the carbohydrate-supplemented group of

an analagous study (French et al., '53), the decrease is highly significant and in the same order of magnitude noted for males that received a high fat diet, suggesting the possibility that a similar mechanism may be responsible, i.e., increased efficiency of food utilization. As in previous studies, a wide range in longevity was observed to reduce the statistical significance between groups to a minimum level.

TABLE 2

Summary of histopathology [1]

DIET	SEX	NO. OF RATS	BRONCHITIS, BRONCHO-PNEUMONIA	PASSIVE CONGESTION OF LIVER	NEPHRITIS	SPLENITIS
Antibiotic	Male	4	4	1	1	0
Supplement	Female	4	4	0	1	0
Control	Male	5	5	1	0	0
	Female	4	4	1	0	0

[1] Rats sacrificed at 12–20 months of age.

TABLE 3

Post-mortem frequency of diseases and abnormalities

DIET	SEX	NO. OF RATS	PNEUMONITIS	MIDDLE EAR DISEASE	GENERAL INFECTIONS	HEMATURIA	EDEMA	PARALYSIS	TUMORS	
									Mammary	Other
Antibiotic	Male	51	38	1	3	3	2	3	2	8
Supplement	Female	55	37	0	5	3	5	2	16	7
Control	Male	32	26	2	2	5	1	2	1	4
	Female	29	19	1	1	0	0	3	6	6

The minimum differences observed between the antibiotic-supplemented and the control groups in histopathological findings (table 2) and in the frequency of diseases and abnormalities as determined by post mortem examinations, (table 3) revealed no consistent reason for the decreased life span of the animals which received the antibiotic. All animals on both diets subjected to histologic examination were

affected with varying degrees of respiratory changes from chronic bronchitis to bronchopneumonia, generally of the non-suppurative type. Passive congestion of the liver was noted in one antibiotic-fed and in two of the control rats. Two antibiotic-fed rats exhibited kidney changes characterized in one by interstitial hemorrhage, tubular degeneration, and chronic glomerulonephritis and in the other by focal interstitial tissue proliferation and hemorrhage. The spleen of one antibiotic-fed animal showed reticular cell hyperplasia, fibrillary multinucleated giant cells and eosinophilia resembling Hodgkins disease. Certainly no beneficial effect on the most common disease of aged rats, chronic pneumonia with bronchiectasis (described by Ratcliffe, '42), resulted from the feeding of streptomycin and terramycin at levels of 0.02 and 0.04% of the diet, although the latter level is sufficient to produce a demonstrable blood concentration of the antibiotic (Welch, '50; Bliss et al., '50).

SUMMARY

In an attempt to minimize the chronic pneumonia commonly observed in aged rats by the continual administration of streptomycin and terramycin incorporated in the diet at high levels (0.02 and 0.04%) beginning at the age of 11 months, the average life span of albino rats of both sexes was decreased about 10%.

Neither beneficial nor deleterious effects of the antibiotic supplementation were revealed by gross and histologic examination.

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COLLAGEN FORMATION IN VITAMIN A-DEFICIENT RATS[1]

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Reports have appeared suggesting that rats and some other species, which synthesize enough ascorbic acid to be independent of an exogenous source, exhibit symptoms of scurvy when fed a vitamin A-deficient diet (Jonsson et al., '42; Mayer and Krehl, '48a). Mayer and Krehl ('48b) not only described lowered tissue ascorbic acid levels and scorbutic symptomatology in vitamin A-deficient rats, but the relief of these symptoms following intraperitoneal injection of ascorbic acid. No satisfactory criteria for determining scurvy in the rat exist, for the guinea pig has been the animal of choice in studies of experimental scurvy since Holst and Frölich ('07) demonstrated the latter's susceptibility to an ascorbic acid deficiency. The classic researches of Wolbach and Howe ('26), in which they demonstrated the need for ascorbic acid in wound repair and postulated its role in the formation of the intercellular matrix were accomplished with guinea pigs. More recently Robertson and Schwartz ('53) induced new fibrous tissue in guinea pigs by the subcutaneous injection of a suspension of Irish moss extractive and showed that the concentration of collagen in this repair tissue was a sensitive measure of the ascorbic acid status of the animal.

The present investigation was undertaken to see if this major biochemical function of ascorbic acid, namely collagen formation, was impaired in rats rendered "scorbutic" by deprivation of vitamin A.

[1] Performed in part under Contract N9onr82,800 with the Office of Naval Research.

METHODS

Sprague-Dawley rats suitable for vitamin A assay were obtained directly from the breeder. When received, the majority weighed between 40 and 60 gm; one shipment of 26 rats weighed between 95 and 115 gm. Diet A_1 of Mayer and Krehl ('48a), a "synthetic" diet deficient in vitamins A and C, was fed ad libitum throughout. Seventeen animals received supplemental vitamin A (2,500 I.U./rat/week); 25 were fed 25 mg of ascorbic acid from a pipet every other day or received diet A_1 containing 0.1% ascorbic acid; 107 received no supplementary vitamin A or C.

When regular weight gain had ceased in those rats not receiving vitamin A (after about three weeks) all rats were injected subcutaneously with 8 ml of a 1% suspension of Irish moss[2] extractive (Robertson and Schwartz, '53). The 107 previously unsupplemented rats were then placed into three groups. One group of 21 rats was fed 2,500 I.U. of vitamin A per rat per week, a second group of 38 was injected daily with 55 mg of sodium ascorbate and the third group of 48 received no additional vitamins. Rats which had been receiving either vitamin A or C prior to injection of Irish moss continued on the same regime.

Fourteen days after the Irish moss was injected, all surviving animals were killed and the new fibrous tissue was analyzed for collagen (Robertson, '50). Many animals died before the end of 14 days, but when new tissue was present, collagen was determined therein. These analyses were included in compiling the experimental data since analyses of induced fibrous tissue from a group of normal rats showed no significant difference in collagen concentration between the 10th and 14th day.

[2] We are indebted to Mr. L. Stoloff of Seaplant Chemical Corporation, New Bedford, Mass., for a purified preparation. Dr. M. P. Lamden of the Department of Biochemistry was kind enough to analyze this material and reported that it contains no more than 0.4 mg of *non-dialyzable* material assaying as ascorbic acid by the method of Roe and Oesterling ('44).

Representative teeth and ribs from each group were examined microscopically after histologic preparation.[3]

RESULTS

At no time during the experiment did the behavior or appearance of the animals suggest that ascorbic acid in any way ameliorated vitamin A deficiency. The following course is typical of those rats which did not receive vitamin A. Upon arrival the weight was 50 gm and weight gain continued for 14 days to a maximum of 100 gm. Irish moss was injected on the 17th day, at which time the weight had dropped to 98 gm. Slow weight loss continued, the general appearance became poorer and on the 24th day there was a distinct red rim around the eye suggesting xerophthalmia. When killed on the 31st day the weight was 86 gm. At autopsy there was evidence of hemorrhage into the gastrointestinal tract and the stomach was bloated.

The changes observed upon microscopic examination were similar in the teeth and costochondral junctions of all animals which did not receive vitamin A. These lesions were the same as those so beautifully described by Wolbach and Howe ('33) and as these investigators pointed out, differ from changes in comparable tissues of scorbutic guinea pigs.

Although 149 rats were used and injected with Irish moss, the mortality among rats not receiving vitamin A was so great that only 69 survived the 14 days usually allowed for formation of new fibrous tissue. In many animals the new tissue was so scant that complete analyses were not possible. By making use of analyses of tissue from all animals which survived at least 10 days following injection of Irish moss it was possible to accumulate 72 analyses for collagen concentration. In 51 of these samples there was sufficient material for analysis of water and fat permitting collagen assay on the basis of dry, fat-free tissue.

[3] It is a pleasure to thank Dr. F. W. Dunihue of the Department of Anatomy for invaluable assistance in the preparation and interpretation of histologic material.

The survival data and the results of collagen assay are presented in table 1. None of the data suggest that vitamin C affected either the mortality or collagen formation of vitamin A-deficient rats. The low collagen concentration in vitamin A-supplemented animals which is seen when the analyses are expressed on the basis of fresh tissue is mainly

TABLE 1

Collagen concentration in repair tissue and survival of vitamin A-deficient rats

GROUP	COLLAGEN [1]		SURVIVAL		
	% of fresh tissue	% of dry, fat-free tissue	Number injected with Irish moss	Number alive 14 days after injection	%
Vitamin A — throughout	1.21 (14) ± 0.12	9.0 (9) ± 0.7	17	16	94
Vitamin A since injection	1.11 (17) ± 0.06	8.7 (17) ± 0.5	21	21	100
Vitamin C — throughout	1.99 (12) ± 0.11	10.5 (3) ± 0.5	25	6	24
Vitamin C since injection	1.77 (13) ± 0.15	10.1 (9) ± 0.6	38	11	29
No supplement [2]	1.88 (21) ± 0.15	9.3 (14) ± 0.6	48	15	31

[1] The data are expressed as the mean ± its standard deviation. The numbers in parentheses are the number of tissue samples analyzed.

[2] The basic "synthetic" diet contained neither vitamin A nor vitamin C.

the result of the considerable admixture of fat with the new collagenous tissue. Collagen concentration in any of the 5 groups did not differ significantly when calculated on the basis of dry, fat-free tissue.

DISCUSSION

The above results are not readily reconciled with interpretations of investigations in which it is claimed that scurvy appears in vitamin A-deficient rats. The question arises whether ascorbic acid is necessary for collagen synthesis in

the rat or whether the criteria of scurvy used in these earlier studies, i.e., (1) some of the classic signs of scurvy such as hemorrhagic petechiae, swollen joints and gums and paralysis, (2) low tissue concentrations of ascorbic acid, or even (3) the beneficial effects of administered ascorbic acid, are specific for postulating an ascorbic acid deficiency.

There is at present no evidence available for definitely deciding either part of this question. However, it should be emphasized that the gross symptomatology outlined above is seen not only in avitaminosis A but also in hypervitaminosis A (Vedder and Rosenberg, '38; Moore and Wang, '45). It is not pathognomonic of ascorbic acid deficiency for not only may these stigmata arise from other causes but they are not necessarily seen in acute ascorbic acid deficiency in guinea pigs.[4] Low tissue levels of ascorbic acid, while inevitably accompanying a deficiency of the vitamin, are also seen in other conditions. Rodahl ('49) has reported that hypervitaminosis A, like hypovitaminosis A, decreased ascorbic acid concentrations in blood and liver.

A response to administered ascorbic acid might be considered the *sine qua non* for defining its deficiency. Nevertheless, we are all aware of the beneficial effects ascribed to this vitamin in the treatment of numerous conditions ranging from sterility to hay fever and of the equally numerous denials of such effects. The reported results in vitamin A deficiency in rats are similarly conflicting. Although Mayer and Krehl ('48a) found that ascorbic acid increased longevity, and decreased symptomatology, we found no beneficial effects. Jonsson et al. ('42), who, like Mayer and Krehl, believe that vitamin A-deficient rats have scurvy, found ascorbic acid without effect and suggested that exogenous ascorbic acid was not available to the organism kept in A-avitaminosis.

In view of these considerations and in view of the ability of the vitamin A-deficient rat to synthesize collagen, the suggestion that A-avitaminotic rats have scurvy should be critically re-examined.

[4] Unpublished data.

CONCLUSION

The growth of new repair tissue in rats has been stimulated by the subcutaneous injection of Irish moss extractive. The collagen concentration of this tissue was the same in normal rats, in vitamin A-deficient rats and in vitamin A-deficient rats given ascorbic acid. The results do not support the view that scurvy accompanies avitaminosis-A in the rat.

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SERUM INORGANIC SULFATE SULFUR AS A MEASURE OF THE SULFUR INTAKE OF SHEEP [1]

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FIVE FIGURES

(Received for publication May 24, 1954)

The importance of sulfur in ruminant nutrition has received considerable attention since the report by Loosli et al. ('49) of the microbial synthesis of methionine from inorganic sulfur and the demonstration by Block and Stekol ('50) of the presence of radioactive sulfur in milk protein following the feeding of radioactive sodium sulfate. Thomas et al. ('51) demonstrated that the addition of inorganic sulfate to a sulfur-deficient purified ration improved weight gains and the nitrogen and sulfur retention of sheep. Starks et al. ('53) found elemental sulfur effective in supplementing a low-sulfur ration for lambs. Recently Starks et al. ('54) have reported on the comparative quantitative requirements by lambs for elemental sulfur, sodium sulfate and DL-methionine.

In connection with feeding tests with sheep to study the effect of soil fertility on the nutritive value of the forage, inorganic serum sulfate levels were found to be rather closely correlated with the total sulfur content of the forage (Weir and Rendig, '52). The studies reported here were designed

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[2] Department of Animal Husbandry.

[3] Department of Soils.

[4] With technical assistance by T. S. Inouye.

to evaluate further this blood test as a means of assessing the sulfur intake of sheep.

EXPERIMENTAL

Yearling wethers weighing approximately 100 pounds were used for the three trials. Approximately 15 ml of blood was drawn from the jugular vein and inorganic sulfate determined in the serum by the colorimetric method of Letonoff and Reinhold ('36). This method is based upon the color developed by benzidine sulfate and sodium-β-naphthoquinone 4-sulfonate in aliquots of the protein-free uranium acetate filtrates of the serum. Sulfate so determined is referred to hereafter as "serum sulfate."

STUDIES WITH NATURAL ROUGHAGES

Trial I. Pellets made from a low-sulfur 5th-cutting alfalfa hay with 22% added cerelose [5] was used as the low-sulfur feed (sulfur content 0.165%). The control feed (sulfur content 0.262%) consisted of pellets made with alfalfa from the same source as above, cerelose, and in addition, methionine. Nine sheep were fed a maintenance ration of the methionine-supplemented feed for 8 days. The following 10 days they were fed at the same feed intake level and then given increasing amounts of the low-sulfur feed until they were eating all they would consume during the last 11 days of the trial. During the 8 days (period I) on the control ration the average daily feed intake ranged from 774 to 871 gm with an average daily sulfur intake of 2.0 to 2.3 gm (fig. 1 A). Blood samples taken each morning during period I contained an average of 3.4 to 3.9 mg of serum sulfate per 100 ml of serum. During the next 10 days (period II) when the lambs received the same amount of low-sulfur pellets, the average daily sulfur intake ranged from 1.3 to 1.5 gm. The serum sulfate values dropped to 2.2 on the third day, rose to 3.4 on the 4th day, and then steadily declined to a low of 0.7 mg per 100 ml

[5] Crystalline glucose, Corn Products Sales Co., Norfolk, Virginia.

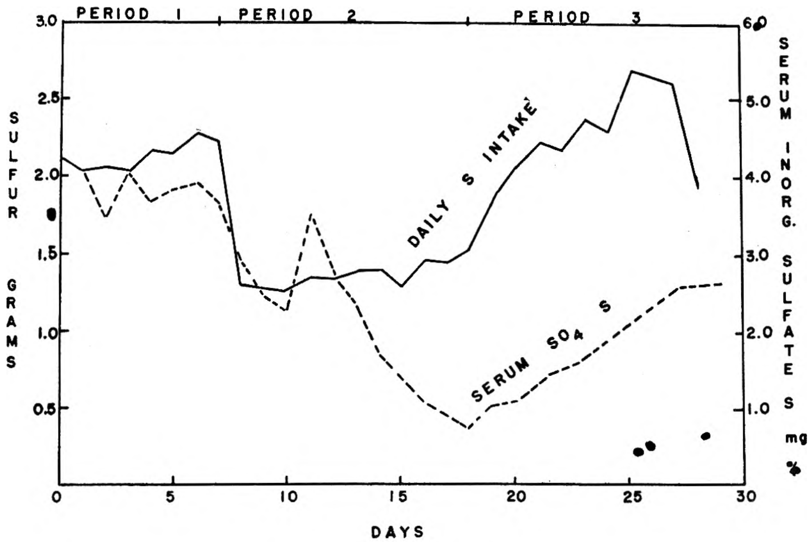

Figure 1 A

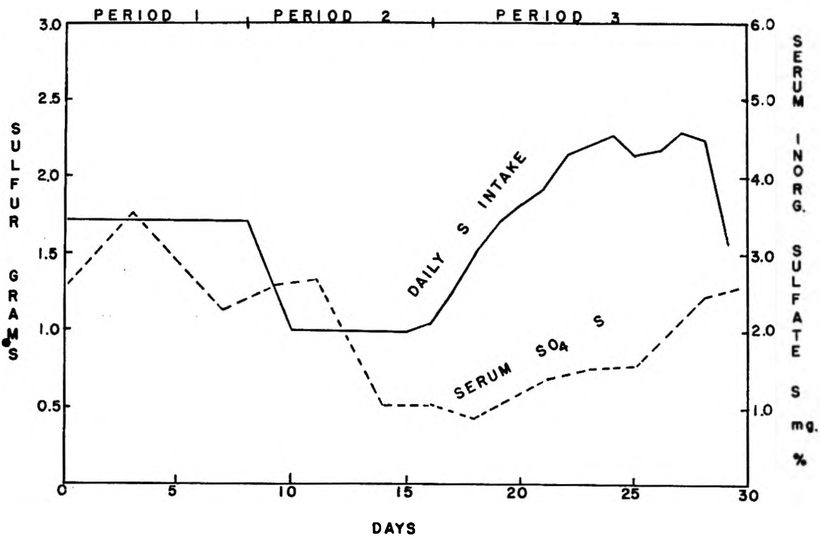

Figure 1 B

Fig. 1 Effect of sulfur intake on the serum sulfate level when lambs were fed on two low-sulfur hays.

A. Trial I. B. Trial II. Period I — lambs were given limited amount of low-sulfur hay with added methionine. Period — II lambs given same amount of low-sulfur hay. Period III — lambs given free access to low-sulfur hay.

of serum at the last bleeding of period II. In period III, with ad libitum feeding, the average daily intake increased from 944 gm on the first day to a high of 1,638 gm on the 8th day and then dropped to 1,192 gm on the last day. The corresponding sulfur intake was 1.6, 2.7, and 2.0 gm per day. With the increased sulfur intake the serum sulfate increased gradually, reaching 2.7 mg % at the end of the 11-day period.

Trial II. The design of trial II was the same as that of trial I, except that second cutting alfalfa hay was used in making up the pellets. The control pellets with methionine added contained 0.190% sulfur and the low-sulfur pellets contained 0.112% sulfur. The same sheep used in trial I were started on trial II at the close of trial I.

In period I the sheep ate 900 gm of the pellets daily with a sulfur intake of 1.7 gm (fig. 1 B). The serum sulfate values rose from an initial value of 2.6 to 3.6 on the third day and then dropped to 2.2 and 2.6 on the 7th and 8th day.

In period II on the same intake of feed but a daily intake of sulfur of 1.0 gm, the serum sulfate remained at 2.7 until the second day and then dropped to 1.0 mg % on the 5th and 7th days of the period.

On ad libitum feeding in period III, the sheep increased their feed intake to about 2,000 gm daily in the latter part of the period with a corresponding intake of 2.0 to 2.3 gm of sulfur per day. The serum sulfate continued to drop to a low of 0.9 mg % the second day of the period and then gradually increased to a value of 2.6 mg % at the end of the 14-day period.

STUDIES WITH PURIFIED RATIONS

To study further the effect of the sulfur level, 10 yearling wethers were placed on a purified ration (table 1) similar to that used by Starks et al. ('54). The sulfur content of this ration was only 0.02%.

Nine of the sheep ate satisfactorily but one consumed only 600 gm the first day, and thereafter refused to take more than 25 gm per day, and on some days completely refused

the feed. This sheep provided an interesting control as to the effect of starvation on the serum sulfate level. Starting with a value of 2.7 mg % the value dropped to 1.2 mg % by the 14th day and on the 21st day when it was withdrawn from the experiment, the serum sulfate value was 2.9 mg %.

The remaining 9 lambs were divided into three groups. Group I was left as a control on the purified ration. Group II was kept on the control ration for 14 days and then given

TABLE 1

Composition of semipurified ration

S content = 0.02%

	%
Cornstarch	35.4
Cerelose	22.5
Barley straw	15.0
Wood flock	15.0
Corn oil	3.5
Wheat germ oil	0.5
Urea	4.0
Choline chloride	0.1
Minerals (S free)[1]	4.0
Vitamins	
Vitamin D	750 U.S.P./lb.
Vitamin A	4000 U.S.P./lb.
Thiamine	1 mg/lb.
Riboflavin	1 mg/lb.

[1] Carbonate mixture of Thomas et al. ('51).

3 gm of elemental sulfur by capsule twice daily for the remainder of the trial. Group III was kept on the control ration for 20 days and then fed elemental sulfur added to the feed at the rate of 3 gm of sulfur per pound of feed.

The sheep in group I, on the control ration, consumed from 600 to 1,000 gm of feed daily with a sulfur intake of 0.12 to 0.20 gm per sheep (fig. 2 A). The serum sulfate dropped from an initial value of 3.0 mg % to 0.2 mg % on the 8th day and remained below 0.15 mg % for the duration of the trial.

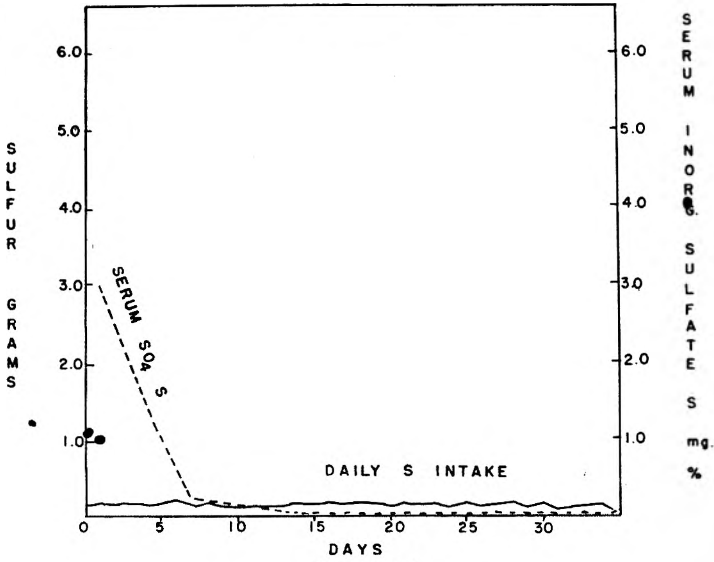

Figure 2 A

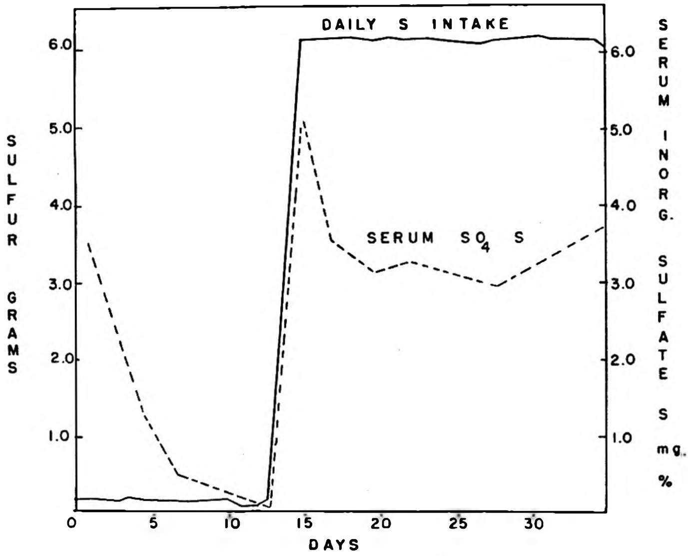

Figure 2 B

Fig. 2 Effect of a low sulfur intake on the serum sulfate level of lambs fed purified rations.

A. Purified ration only. B. Six grams of sulfur administered daily starting on the 14th day. C. Three grams of sulfur added per pound of feed on the 20th day.

The sheep in group II followed a similar pattern to those of group I during the 14 days they were on the control ration (fig. 2 B). The serum sulfate had dropped to 0.07 mg % at the last bleeding before the sulfur capsules were given. The 3-gm sulfur capsules were given first at the evening feeding of the 14th day. Two of the three lambs were given another capsule at the morning feeding. The three lambs were bled at 4 P.M. on the 15th day. The lamb which had

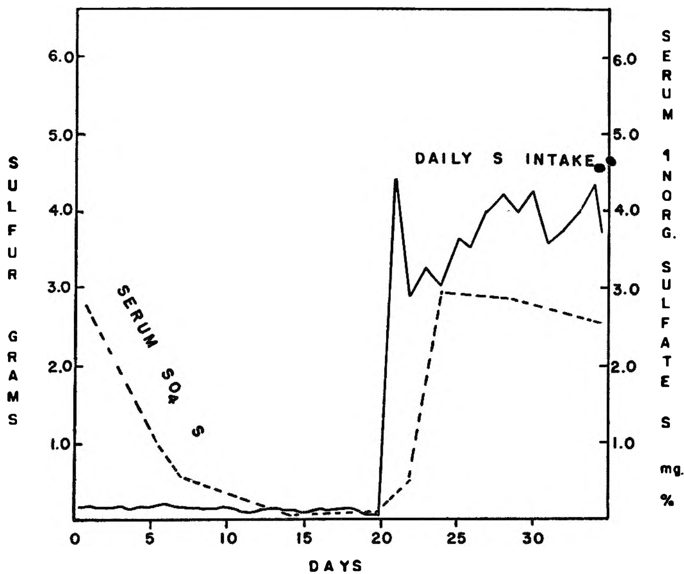

Figure 2 C

received only one capsule had a serum sulfate level of 2.5 mg %. The two lambs which had received two capsules each had serum sulfate values of 6.4 and 6.7 mg %. For the remainder of the trial each lamb received two 3-gm capsules of sulfur daily; the serum sulfate values ranged from 2.9 to 4.1 mg %.

The sheep in group III also reacted similarly to those of group I during the period they were on the control ration (fig. 2 C). Elemental sulfur was added to the feed at the rate of 3 gm per pound at the evening feeding of the 20th day. The sheep were bled the next morning and the serum

sulfate level had increased to 0.55 mg %. By the third day the serum sulfate was 2.9 mg % and remained at about this level until the close of the trial.

DISCUSSION

The results shown above clearly demonstrate that the serum inorganic sulfate sulfur level of the sheep can be changed by varying the sulfur intake. Trials I and II indicate that the serum sulfate is more nearly a reflection of the total sulfur intake than of the percentage of sulfur in the feed, but there is some indication that a low percentage of sulfur in the feed may have a depressing effect on the serum sulfate level. This effect was observed in period III in comparison with period I in both trials I and II when high sulfur intakes from large amounts of low-sulfur feed did not result in rapid increases in serum sulfate values.

The normal range for serum sulfate for sheep appears to be from 2.0 to 5.0 mg % of sulfate sulfur. This is the range obtained with sheep fed rations reasonably high in sulfur in other tests (Rendig and Weir, '54). These values are in agreement with those in the literature. Denis ('21) reports values of 2.5 to 4.0 mg % for sheep blood. Brown and Lewis ('41a) report the following values for inorganic sulfate sulfur in plasma ultrafiltrates of normal mammals: beef 3.44; hog 1.89; rat 1.9 to 2.4; rabbit 3.6 to 6.1; and man 0.82 to 1.17 mg %.

The values obtained for sheep in this study do not agree with those obtained by Whiting et al. ('54) who fed ewes on a basal ration containing only 0.09 sulfur. They report no depression in serum sulfate when the daily intake of sulfur was as low as 1.0 to 1.4 gm. The only apparent difference is that these authors determined serum sulfate by a different method. The method used here has been used extensively by other authors (Dziewiatowski, '54) and gave good results in recovery tests in our hands.

The metabolism of sulfur in the non-ruminant has been reviewed by Bach ('52). Sulfate is known to be one of the

end products of sulfur metabolism (Fromageot, '47). The excretion of urinary sulfate is known to be increased by the feeding of either methionine or cystine (Brown and Lewis, '41b). It is not surprising, therefore, that when methionine was added to the low-sulfur feed, there was an increase in serum sulfate.

Trial III demonstrated that the serum sulfate is reduced to very low levels by decreasing the sulfur supplied by the feed. The results obtained with the wether which refused feed indicated that this reduction is not a starvation effect. In this case, after an initial drop, the serum sulfate value rose to what is considered a normal level, presumably as a result of the breakdown of body tissue.

The marked response of the serum sulfate to the elemental sulfur given by capsule demonstrated that the sulfur was being taken into the body proper in some form. Denis and Reid ('27) observed a similar effect on the serum sulfate of a goat. These authors gave a goat 10 gm of powdered sulfur for 5 days and 20 gm for three days. Before feeding the sulfur, the inorganic blood sulfate was 6.5 mg %. On the 9th day, 18 hours after administration of the last dose of sulfur, the inorganic blood sulfate was 10.9 mg %.

It appears that this blood test is a useful tool in detecting a low sulfur intake in sheep. It might be used to determine whether the addition of sulfur to feed for ruminants is warranted.

SUMMARY

Serum inorganic sulfate sulfur in sheep is shown to vary with the sulfur intake of the animal. Two trials, using pelletted, low-sulfur alfalfa with added methionine, showed that the serum sulfate ranged from 2.0 to 4.0 mg % with a sulfur intake of 1.7 gm or more per sheep per day. When the same amount of the same feeds without the added methionine was fed, the serum sulfate dropped below 1.0 mg % on a sulfur intake of 1.0 to 1.5 gm per day. When fed on a low-sulfur purified ration (S = 0.02%), sheep were found to have very

low serum sulfate values. After 14 days on the purified ration all sheep showed values of less than 0.2 mg %. The administration of 3-gm capsules of elemental sulfur twice daily to each sheep fed the purified ration resulted in a rapid increase in serum sulfate. Addition of elemental sulfur to the purified ration resulted in a slower increase in serum sulfate.

The blood test appears useful in detecting a low-sulfur intake in sheep.

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EFFECT OF VARYING THE INTAKE OF CALCIUM PANTOTHENATE OF RATS DURING PREGNANCY

II. HISTOLOGICAL AND HISTOCHEMICAL STUDIES OF THE LIVER, ADRENAL, DUODENUM AND TIBIA OF THE YOUNG AT BIRTH [1, 2]

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FOUR FIGURES

(Received for publication March 1, 1954)

Numerous histological changes have been observed during advanced pantothenic acid deficiency in the mature rat (Follis, '46). Ashburn ('40) has described the adrenal in pantothenic acid deficiency as including: hemosiderin deposition, fibrosis, "congestion" hemorrhage, cellular atrophy, necrosis and scarring. Nelson and her associates ('50) observed changes in endochondal ossification of the tibia in young rats which were acutely deficient in pantothenic acid. Intestinal changes, particularly of the colon, have been described in some detail by Follis.

While certain chemical changes in newborn rats produced by females receiving different amounts of pantothenic acid were being investigated, the liver, adrenal, duodenum, and

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tibia were examined simultaneously (Everson, Northrop, Chung, Getty and Pudelkewicz, '54). In addition to investigating the structure of these tissues, the distribution and concentration of acid and alkaline phosphatase of each tissue were also measured. A limited number of studies have been completed in the past regarding the distribution of phosphatase in the organs and tissues of the rat, but as yet little is known of the significance of these findings and of how they are affected by various vitamin deficiencies.

EXPERIMENTAL

Female albino rats of the Wistar strain were used in the study and histological sections of the tissues of the young produced during second pregnancies were prepared and examined. The experimental females were maintained on the customary stock diet from the initiation of the study to the close of first pregnancies. At this time 35 females which had produced satisfactory first litters were transferred to purified rations or were maintained on the stock ration. The composition of the purified diet is given in table 1. The experimental animals were distributed into 4 groups: Group I, stock ration; Group II, purified diet (using vitamin mixture given in table 1); Group III, purified diet (same vitamin mixture plus 100 μg calcium pantothenate); Group IV, purified diet (same vitamin mixture plus 1 mg calcium pantothenate).

The newborn rats were killed with chloroform, and portions of the liver, duodenum, the entire adrenals, and the tibias from each animal were fixed. Tissues fixed in 10% neutral formalin were removed after 48 hours and were dehydrated in three changes of dioxane of one-half hour each. The tissues were then embedded in paraffin (Altman's mixture) after three changes in melted paraffin (53–56°C.) of one-half hour each. Sections were made at 6 μ thickness and the following stain techniques as described by Mallory were routinely used: Harris' hematoxylin and eosin, Heidenhain's hematoxylin, Weigert's elastic tissue stain, and Van Giesen's connective tissue stain.

For the histochemical demonstration of alkaline and acid phosphatase enzymes, the methods of Gomori ('39, '41) as modified by Deane and Dempsey ('45) were followed. Tissues of about 2 mm thickness were fixed in cold 80% alcohol (0°C.) for about 24 hours. These were then changed to 90%, 95% and finally absolute alcohol during the next 24 hours. The

TABLE 1

Composition of the purified ration

Vitamin-free casein	25%
Dextrose	55%
Swiftening	15%
Salts [1]	5%
Vitamin mixture fed daily per rat	
Choline chloride	10 mg
Inositol	5 mg
p-aminobenzoic acid	100 μg
2-methyl-naphthoquinone	250 μg
Thiamine hydrochloride	150 μg
Riboflavin	100 μg
Niacin	500 μg
Biotin	2.5 μg
Folic acid	6 μg
Pyridoxine hydrochloride	50 μg
Vitamin B_{12}	0.5 μg
Fat soluble vitamins	
Cod liver oil:	9 U.S.P. units vitamin D
	90 U.S.P. units vitamin A
Alpha tocopherol	750 μg

[1] Richardson, L. R., and A. G. Hogan — J. Nutrition, *32:* 459, 1946.

tissues were cleared in cedar oil for 24 hours and then in three changes of benzene of one hour each. These preparations were then embedded in melted paraffin as described for tissues fixed in general fixative. The paraffin blocks were sectioned at 6 μ thickness and mounted by use of Mayer's albumin-glycerin mixture. After removal of the paraffin, sections were incubated in buffered solutions containing sodium glycerophosphate and either lead or calcium ions. The solu-

tions were buffered with acetate or sodium barbital depending upon the pH desired. All solutions were examined immediately prior to incubation using a Beckman pH meter and were adjusted to pH 9.4 for alkaline phosphatase and pH 5.0 for acid phosphatase. The sections were incubated routinely for 6, 12, 24, 48 and 96 hours, together with control incubations. Following this treatment the sections were dehydrated, cleared and mounted in the usual manner. The sites of phosphatase activity were made microscopically visible by converting the precipitated phosphatase into visible brown sulfides as described by Gomori ('39). Control sections were prepared by omitting the substrate in the incubation solutions in order to reveal any possibility of preformed insoluble native phosphate. Chilled alcohol was found suitable as a fixative for both acid and alkaline phosphatase. The use of this solvent for the demonstration of both types of phosphatase has been recommended by Gomori ('45).

RESULTS

No structural differences were encountered for the 4 types of tissues examined which could be attributed to differences in dietary treatment of the females.

Histochemical data revealed that none of the tissues examined contained demonstrable acid phosphatase. Alkaline phosphatase concentrations were measured by applying arbitrary values of zero for negative, 1 for slight, 2 for moderate, 3 for intense, and 4 for very intense concentrations.

In the liver sections (fig. 1) all groups of young showed essentially the same distribution and concentrations of alkaline phosphatase as those of the stock young. The cell membrane and cytoplasm showed slight concentrations of alkaline phosphatase; the nuclear membrane and nucleoli were intensely stained. The general distribution from the portal veins and peripheral areas of the lobules to the central veins was about uniform.

Sections of the duodenum (fig. 2) likewise showed no important differences in the concentration or distribution of

phosphatase from those of the normal stock young. The cuticular borders of the villi were very intensely stained, but the crypts were less deeply stained than the upper half of the villi. The nuclear membrane and nucleoli were also very

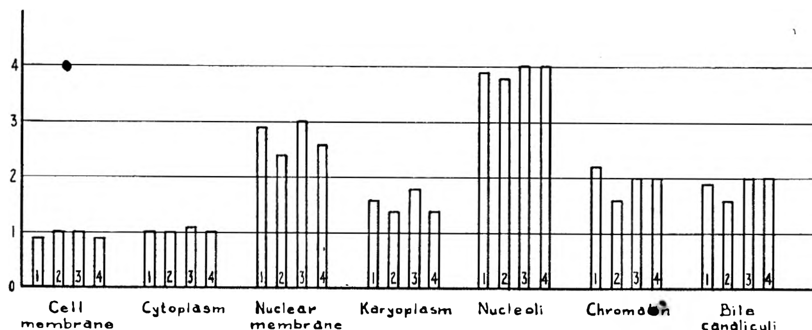

Fig. 1 Distribution and concentration of alkaline phosphatase in the liver.
Diets: 1. Stock, average of 50 samples.
2. No pantothenic acid, average of 14 samples.
3. 100 μg pantothenic acid, average of 36 samples.
4. 1 mg pantothenic acid, average of 42 samples.

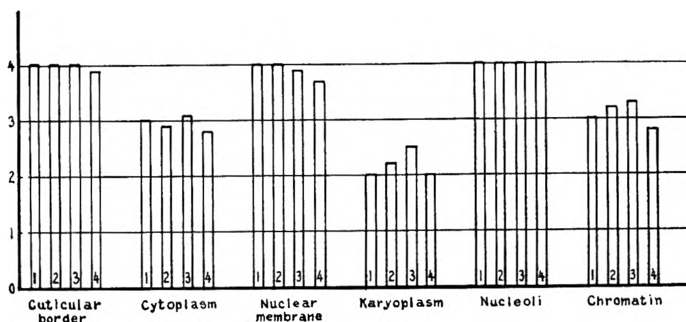

Fig. 2 Distribution and concentration of alkaline phosphatase in the duodenum.
Diets: 1. Stock, average of 49 samples.
2. No pantothenic acid, average of 13 samples.
3. 100 μg pantothenic acid, average of 35 samples.
4. 1 mg pantothenic acid, average of 42 samples.

intense. The cytoplasm was vividly stained as was the chromatin. The karoplasm ranged from moderate to intense.

In sections of the tibia (fig. 3) no real differences were observed in the concentration or distribution of alkaline phosphatase among the various groups. Very marked reactions

of alkaline phosphatase were noted in the perichondrium, periosteum, bone collar, interstitial spaces at the calcifying zones, and the osteoblasts, osteoclasts, and blood cells. The cell membranes of all the hypertrophic cartilage cells in the

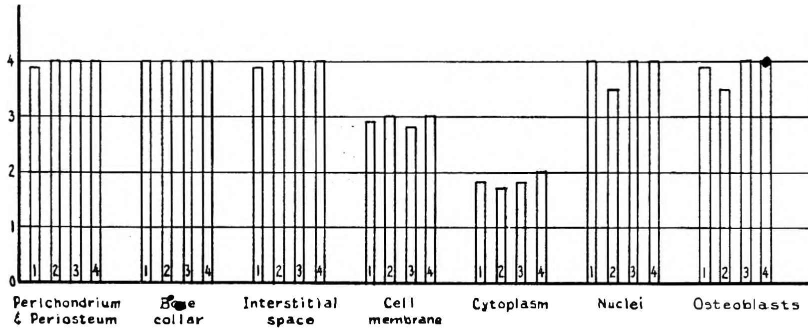

Fig. 3 Distribution and concentration of alkaline phosphatase in the tibia.
Diets: 1. Stock, average of 34 samples.
2. No pantothenic acid, average of 8 samples.
3. 100 μg pantothenic acid, average of 24 samples.
4. 1 mg pantothenic acid, average of 20 samples.

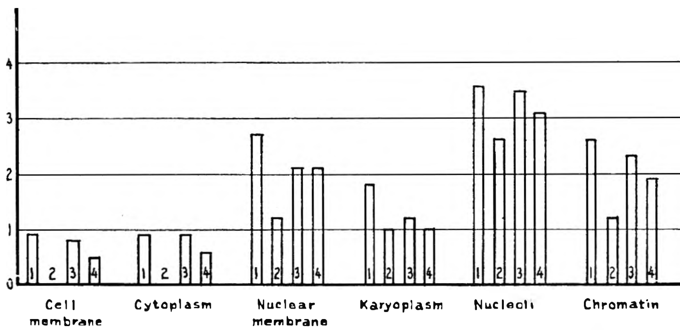

Fig. 4 Distribution and concentration of alkaline phosphatase in the adrenal.
Diets: 1. Stock, average of 49 samples.
2. No pantothenic acid, average of 13 samples.
3. 100 μg pantothenic acid, average of 36 samples.
4. 1 mg pantothenic acid, average of 42 samples.

cartilage cell columns were rich in this enzyme, whereas the cytoplasm showed only moderate amounts. The nuclei of these hypertrophied cells were very deeply stained. The hyaline cartilage cells at the epiphyses showed little or no difference among the 4 groups.

A significant difference (fig. 4) was encountered between the adrenal sections for young born to females receiving no pantothenic acid throughout pregnancy and those receiving the stock diet. Where the controls showed slight amounts of alkaline phosphatase in the cell membrane and cytoplasm, the deficient group showed none. Where the controls showed moderate phosphatase in the nuclear membrane, the deficient young showed only slight amounts. Likewise as the intensity was varied from slight to moderate in the karyoplasm for the controls, the young of animals receiving no pantothenic acid showed only slight activity. Where the controls showed intense reaction in the nucleoli and moderate in the chromatin, the young in the deficient group were correspondingly lower in activity.

Sections of the 4 tissues fixed with formalin showed little or no difference among the groups of young.

DISCUSSION

It will be observed from the histological findings that the demonstration of pantothenic acid dificiency in the rat at birth is difficult to accomplish. Even when the maternal diet contributed no pantothenic acid from approximately one week before conception to parturition, the young born exhibited no structural differences in the liver, adrenal, tibia and duodenum. Such young, however, were known to possess limited concentrations of pantothenic acid in the total body tissue and the pantothenic acid content of the blood was reduced (Everson, Northrop, Chung, Getty and Pudelkewicz, '54). Some rise in blood pyruvic acid concentration was also known to occur in these young. In spite of these changes, the development of the 4 tissues progressed normally histologically. When the distribution and concentration of acid and alkaline phosphatase were measured, it was found that the adrenals of the young with poorest pantothenic acid stores exhibited less demonstrable alkaline phosphatase. Young born of females receiving 100 μg or 1 mg quantities of calcium pantothenate daily throughout pregnancy, or the stock ration,

exhibited increased alkaline phosphatase activity. The fact that the litters of females deprived of pantothenic acid for some period of time were known to resorb in a high proportion of the cases suggests that more vital changes than those being investigated in the present study develop as a result of withdrawal of this vitamin from the maternal diet.

SUMMARY AND CONCLUSIONS

Histological and histochemical studies of the liver, adrenal, duodenum and tibia were made on newborn rats whose dams were fed several levels of pantothenic acid during reproduction. Tissues studied to date reveal little if any evidence of tissue change in the newborn rats except in the case of the adrenals which exhibited a decreased alkaline phosphatase reaction for the most deficient group of animals.

It appeared that the young of pantothenic acid-deficient females were normal at birth insofar as the structure of the 4 tissues studied was concerned. It would appear that whatever pathology may be produced in the fetal tissues by these dietary regimes is evidenced in the prenatal life of the fetuses thus contributing to early fetal resorptions.

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THE UTILIZATION OF CAROTENE BY HYPOTHYROID RATS

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The view that hypothyroidism decreases the ability of the organism to convert carotene to vitamin A was favored in the earlier literature as reviewed by Drill ('43). This postulation was based largely on observations of carotenemia and dark adaptation failures in clinical hypothyroidism. In more recent studies Johnson and Baumann ('47) reported that rats receiving thiouracil or thiourea stored very little vitamin A following carotene feeding as compared with control animals. Drill and Truant ('47) found that carotene failed to prevent xerophthalmia in vitamin A-deficient thyroidectomized rats, although preformed vitamin A was effective as a protective agent. On the other hand Remington, Harris and Smith ('42) reported that ocular symptoms in hypovitaminotic thyroidectomized rats were alleviated equally rapidly by giving either carotene or vitamin A. Employing a special bioassay technique Wiese, Deuel and Mehl ('48) tested the relative effectiveness of carotene in normal and hypothyroid rats for growth and found it to be the same. In another study these investigators (Wiese et al., '47) compared the vitamin A storage following the administration of a single large dose of carotene to vitamin A-deficient normal and hypothyroid rats and reported no significant difference between the averages of the two groups.

The criteria used to measure the effect of hypothyroidism in rats on carotene conversion in the present study included the determination of vitamin A in liver and kidney following

the administration of varying amounts of carotene, and following the depletion of stores built up from carotene, and of the residual amounts of carotene in the gastro-intestinal tract after the administration of a single dose of carotene.

PROCEDURE

Rats of the Long-Evans strain were employed in this study. When the young were two weeks of age the mothers were given a modified U.S.P. XII vitamin A depletion diet in which the casein had been increased to 35% at the expense of cornstarch. This procedure hastened the subsequent depletion of vitamin A stores in the young. These were weaned at 21 days of age to a diet of the following composition: vitamin test casein [1] 22, hydrogenated fat (primex) 5, salts [2] 4, yeast [3] 10, cornstarch 59 and mixed tocopherols [4] 125 mg %.

In the first series the appearance of a weight plateau was taken as a sign of vitamin A depletion. In the remaining experiments the animals were considered depleted three weeks following weaning, since repeated analyses of livers of animals treated in this fashion gave negative results as to residual vitamin A stores. Unless otherwise indicated the rats were allowed to eat ad libitum and were not fasted before autopsy.

Vitamin A was given orally as a concentrated distillate of fish liver oils diluted with cottonseed oil to the proper concentration.[5] A carotene concentrate from vegetable oils [6] was likewise diluted with cottonseed oil. The concentration of both supplements was checked at the beginning of each experiment and at frequent intervals by chemical analysis.

[1] Obtained from General Biochemicals, Inc.

[2] Hubbell, R. B., L. B. Mendel and A. J. Wakeman 1937. J. Nutrition, *14*: 273.

[3] Anheuser-Busch. Primary dried yeast, strain G.

[4] Concentrate of mixed tocopherols. Thirty-four per cent mixed tocopherols obtained from Distillation Products Industries, Rochester, New York.

[5] Distilled vitamin A concentrate. Natural ester form. 500,000 U.S.P. units per gram. Distillation Products, Inc.

[6] Carotene in oil. General Biochemicals, Inc.

Thiouracil was added to the diet at the level of 0.15%.[7] The thyroxine solution was made up in dilute sodium bicarbonate at a pH of 8.5 and 0.5 ml containing the daily dose was administered subcutaneously. The solution was refrigerated and prepared fresh twice weekly.

Determinations of basal metabolic rate were made in a multiple chamber apparatus designed by Benedict and MacLeod ('29).[8] As suggested by these authors calories/m^2/24 hours were calculated using an R.Q. of 0.72 and the formula $S = 9.1 \times W^{2/3}$.

Vitamin A in blood and organs was analyzed by a method described by Hebert and Morgan ('53). Carotene determinations were made either by direct colorimetric measurements of the supernatant petroleum ether extract or by chromatographic separation of the extract in a magnesium oxide-supercel column with benzene as the eluent. Determinations of maximum absorption curves were made in a Beckman spectrophotometer.

RESULTS

Series 1

Young male rats were depleted of their vitamin A stores as described above and were allowed to grow to early maturity on the same diet supplemented with a maintenance dose of 35 µg per day of carotene. When the animals weighed 200 gm they were divided into 4 groups, two serving as controls and two receiving thiouracil. After the establishment of hypothyroidism in two of the groups, liver vitamin A stores were built up in all animals over a 7-week period. One control and one hypothyroid group received an average daily dose of 86 µg of vitamin A, while two similar groups were given 440 µg per day of carotene. After a subsequent two-week depletion period the rats were autopsied.

[7] The thiouracil (Deracil) was furnished by the Lederle laboratories through the kindness of Dr. S. Hardy.

[8] Used with the kind permission of Dr. H. M. Evans, Institute of Experimental Biology, University of California, Berkeley.

Analysis of the livers showed that the vitamin A-fed thyroid-deficient animals stored about 33% more vitamin A than the controls, and that the carotene-fed thiouracil-supplemented rats had a greater than two-fold increase in concentration in comparison with their respective controls (table 1).

TABLE 1

The effect of thiouracil administration on the vitamin A content of the liver following supplementation with vitamin A and carotene
(Series 1)

GROUP	NO. OF RATS	SUPPLEMENT	BODY WT.	THYROID WT.	BASAL METABOLIC RATE		TOTAL VITAMIN A IN LIVER
		μg/day	*gm*	*mg*	*cal./m²/ 24 hrs.*	*% change*	*μg*
I. Control	9	Vit. A, 86	331 ± 7 [1]	32 ± 3.0	697 ± 19	..	370 ± 33
II. Thiouracil-fed	9	Vit. A, 86	270 ± 12	96 ± 7.3	529 ± 25	— 24	495 ± 27
III. Control	10	Carotene, 440	313 ± 10	29 ± 1.4	747 ± 18	..	106 ± 23
IV. Thiouracil-fed	9	Carotene, 440	274 ± 14	80 ± 9.6	573 ± 48	— 23	271 ± 40

[1] Standard error of the mean.

The drop in basal metabolic rate by 24 and 23% in both thyroid-deficient groups indicates that the drug was significantly effective. This finding was substantiated by an almost three-fold increase in the weight of the thyroid glands.

Series 2

The results of the foregoing experiment were confirmed in the next study, where young growing male and female rats were used. Two dosage levels were employed, adjusted to body weight so that the more slowly growing thyroid-

deficient rats received less carotene than the actively growing controls. At each dosage level the normal and thyroid-deficient rats were matched by a third group receiving both 0.15% thiouracil and 5 μg thyroxine daily, the latter given to neutralize the effect of the former.

Subsequent to an initial depletion and a 17-day adjustment period to thiouracil 6 groups were formed as indicated in table 2. Carotene dosage was carried on for 21 days and the rats were sacrificed 35 to 40 hours after the last dose.

The three groups on a low carotene intake of 56.5 μg/100 gm body weight deposited small amounts of vitamin A in their livers approximately the size of the daily dose (Hebert and Morgan, '53). The findings of the first series were confirmed nevertheless, for in spite of the smaller dose received by the small animals in the hypothyroid group in this series, both male and female rats stored approximately 80% more vitamin A than did their corresponding controls. The absolute level of liver vitamin A was higher in all the females (Booth '52). The difference between the sexes is especially significant in this series since the females received smaller amounts of carotene than their male counterparts.

The female rats in the group given thyroxine as well as thiouracil showed liver storage values identical with those of the controls. This may be taken as an indication that the effects observed in the thyroid-deficient group were due to the lowered metabolic rate and not to a drug effect. Evidently 5 μg/day of thyroxine was insufficient in the male rats to counteract the effects of thiouracil since the growth of these males was retarded in comparison with that of the normal animals. The vitamin A stores in the thiouracil- and thyroxine-treated group were correspondingly larger than those of the controls, although not as high as in the thyroid-deficient rats. The differences are however statistically insignificant.

A certain amount of thyroid hyperplasia was seen in both sexes in the daily-dosed group, a fact which would also indicate that the thiouracil was probably not completely counterbalanced by the thyroxine (table 2).

TABLE 2

Vitamin A levels in liver and kidney of normal, thiouracil-fed and thiouracil plus thyroxine-treated rats after varying doses of carotene

(Series 2)

GROUP	SEX	NO. OF RATS	CAROTENE	BODY WT.	THYROID WT.	VITAMIN A: Liver Total	VITAMIN A: Liver /100 gm B. wt.	VITAMIN A: Kidney Total	VITAMIN A: Kidney /100 gm B. wt.	TOTAL LIVER CAROTENE
			μg/day	gm	mg	μg	μg	μg	μg	μg
I. Control	M	10	96	205 ± 12 [1]	20 ± 0.5	29 ± 4.3	14 ± 2.0	25.3 ± 4.1	12.6 ± 2.2	5.5 ± 1.1
	F	9	76	150 ± 12	15 ± 0.6	45 ± 7.2	30 ± 4.4	2.5 ± 0.5	1.6 ± 0.3	7.2 ± 1.4
II. Thio-uracil-fed	M	9	86	168 ± 9	85 ± 7.8	52 ± 4.8	31 ± 3.0	1.9 ± 0.2	1.1 ± 0.1	4.7 ± 0.4
	F	7	70	127 ± 7	69 ± 5.1	80 ± 4.9	63 ± 3.3	1.9 ± 0.5	1.5 ± 0.5	8.3 ± 0.8
III. Thiouracil and thyroxine-treated	M	8	89	180 ± 10	36 ± 5.0	39 ± 7.0	22 ± 3.9	7.5 ± 1.4	4.2 ± 0.8	8.9 ± 1.2
	F	6	73	148 ± 8	36 ± 1.6	45 ± 6.7	31 ± 5.0	2.6 ± 0.6	1.8 ± 0.5	12.7 ± 3.2
IV. Control	M	10	2040	219 ± 6	23 ± 1.7	393 ± 33	180 ± 16	[illegible] ± 1.6	5.6 ± 0.7	19.0 ± 1.5
	F	8	1670	169 ± 3	17 ± 1.0	465 ± 43	273 ± 24	[illegible] ± 0.5	1.8 ± 0.3	34.0 ± 5.2
V. Thio-uracil-fed	M	9	1620	162 ± 9	82 ± 8	352 ± 33	220 ± 18	2.0 ± 0.3	1.3 ± 0.2	24.0 ± 3.6
	F	8	1480	135 ± 4	75 ± 7	402 ± 49	297 ± 35	2.2 ± 0.3	1.6 ± 0.2	27.0 ± 3.8
VI. Thiouracil and thyroxine-treated	M	8	1910	205 ± 11	41 ± 6	440 ± 40	211 ± 14	7.4 ± 1.1	3.7 ± 0.6	23.0 ± 1.1
	F	8	1500	158 ± 5	41 ± 6	454 ± 64	305 ± 44	3.9 ± 0.4	2.5 ± 0.2	37.0 ± 2.8

[1] Standard error of the mean.

In the groups which received 20 times as much carotene as those just described the difference in total liver vitamin A disappeared. But on a body weight basis, in proportion to the dose, the deficient animals stored slightly, though statistically insignificantly, more vitamin A than did the controls. Again all female rats stored more vitamin A than their male counterparts.

Rats receiving large amounts of carotene had appreciable quantities of the provitamin in their livers (table 2). Mattson and coworkers ('47) reported that the yellow pigment found in livers of animals receiving carotene had no characteristic absorption band at 450 mμ. This was not so for the material extracted here. It was separated on a magnesium-oxide supercel column and a maximum absorption was found at the wave length characteristic for carotene. Expressed in terms of the daily dose the amount of carotene found in the liver amounted to 0.93% in the control males on high dosage and to 1.26% in the females of the same group.

Kidney vitamin A deposits were also determined in the rats in series 2. Only the control males and the males on the double therapy showed appreciable amounts of vitamin A in this organ. The thiouracil-treated males and all females irrespective of dose and treatment had only insignificant amounts of vitamin A in the kidneys.

Series 3

Since liver vitamin A stores had so far reflected absorption, conversion and utilization factors, it was decided to study the effect of hypothyroidism on the latter alone. The problem herewith became one of vitamin A rather than carotene metabolism, since to our present knowledge carotene, once converted to vitamin A, follows the same pathway as the ingested preformed vitamin.

A group of normal rats, designated pair-weighed, were matched in their body weight gain with deficient animals by food intake restriction. Rats depleted of their vitamin A

stores as described for series 2 were given 10 successive doses of 1,890 μg of carotene with the aim of building up uniform liver stores. Twelve representative rats, 6 from each sex, were sacrificed so that the initial vitamin A liver stores for the group could be assessed. The remaining rats were grouped in sets of triplets of the same sex with similar body weight. Each set furnished one control animal, one hypothyroid and

TABLE 3

The effect of growth restriction and hypothyroidism on the utilization of existing vitamin A stores

(Series 3)

GROUP	SEX	NO. OF RATS	BODY WT.	TOTAL VITAMIN A		% OF INITIAL STORES IN LIVER
				Liver	Kidney	
			gm	*μg*	*μg*	
I.						
Initial	M	6	170	335 ± 36[1]	8.4 ± 1.1	100
control	F	6	136	513 ± 36	3.3 ± 0.5	100
Control	M	11	284	18 ± 3	11.9 ± 2.8	5
	F	6	208	47 ± 15	2.0 ± 0.3	9
Control	M	11	222	50 ± 10	3.4 ± 0.6	14
pair-weighed	F	10	170	95 ± 31	2.1 ± 0.3	18
Thiouracil-	M	12	223	57 ± 8	1.7 ± 0.2	16
treated	F	9	163	100 ± 18	1.7 ± 0.1	19

[1] Standard error of the mean.

one pair-weighed control. Carotene was thereafter withheld from the diet and the rats were sacrificed 10 weeks later. Results are given in table 3. The initial vitamin A stores of 355 μg for the males and 513 μg for the females had greatly decreased in all groups. However differences previously observed in the storage experiments were again evident in the depletion study where utilization was possibly the only active factor. The normal control males retained 5% of the initial amount after 10 weeks' depletion while the hypothyroid males

retained 16%. Corresponding values for females were 9% for controls and 19% for hypothyroid females. The calorie-restricted rats had retained practically the same amounts of the initial stores as did the thyroid-deficient animals. Again larger amounts of vitamin A were found in the kidneys of the control males than in any other group.

In spite of similar weights it was quite evident that there was a basic difference in body composition between hypothyroid and pair-weighed animals. On gross inspection at autopsy the deficient rats showed a good deal of body fat in the pelvic and abdominal regions. The pair-weighed animals were longer but quite lean. Food intake records showed that the normal pair-weighed males consumed about 10% more food than did the thyroid-deficient ones. The difference in the females over the total period was 20% on the same basis.

Series 4

Cama and Goodwin ('49) had reported increased carotene excretion following the administration of small amounts of carotene to thiouracil-treated rats. No difference could be detected between normal and hypothyroid animals on high levels of intake since large amounts were excreted by both groups. In the present study the absorption of a single dose of 35 μg of carotene was determined by analysis for the residual amounts of carotene in the walls and contents of the gastrointestinal tract two hours following the dose.

Mature normal and hypothyroid rats of both sexes, maintained on a vitamin A free diet, were used. The percentage of the dose recovered after two hours was 34 for control males and 39 for thyroid-deficient males. Corresponding figures for the females were 26 and 30%. The slight increase of the thiouracil-fed rats over their controls is statistically insignificant, so that, essentially, the hypothyroid condition did not effect the absorption of carotene from the gastrointestinal tract under these experimental conditions. The females tended towards a somewhat better removal rate than did

the males. This correlates well with the incidence of larger vitamin A liver stores found in the females.

DISCUSSION

The results obtained in the foregoing investigations indicate that under the experimental conditions employed thyroid-deficient rats can utilize carotene efficiently as the sole source of vitamin A for both maintenance and storage. In some instances the ability of normal animals to store vitamin A from carotene may even be exceeded by that of the hypothyroid rats. That this is more likely due to a decrease in the need for vitamin A because of lowered requirement for essential functions such as body growth and tissue maintenance rather than to an increased efficiency of carotene absorption and conversion is substantiated by the data of series 3 and 4 and by the vitamin A-fed group in series 1. The results of series 2, where two dosage levels were employed, can likewise be interpreted by similar reasoning. In the normal and hypothyroid groups in this series, in which the amount of carotene administered was not far above the maintenance dose, the more rapidly growing control rats utilized a proportionately greater amount of formed vitamin A for metabolic purposes and consequently the quantity available for storage was correspondingly smaller. With a 20-fold increase of the dose the amount of available vitamin A formed was so large that the fraction used to satisfy metabolic requirements was not great enough to produce a difference in the balance stored in the liver.

The results of the depletion experiment in series 3 bear this out further. Since the hypothyroid state was induced only after uniform liver stores had been built up from carotene, utilization of vitamin A alone could be studied independently of absorption and conversion of carotene. The more rapid removal of liver vitamin A stores observed in the normal fully fed rats may have been caused by a greater need in these animals as compared with the thyroid-deficient rats. Normal

caloric-restricted rats showed the same rate of utilization as their paired hypothyroid mates, indicating further that body weight might be of primary importance in determining the need for vitamin A rather than metabolic rate, in spite of differences in body composition.

The results obtained here support the experimental evidence obtained by Wiese and coworkers ('47, '48) and by Remington et al. ('42). Both laboratories using quite different methods failed to demonstrate significant differences in the utilization of carotene due to hypothyroidism. However our results are in part in contrast with those reported by Johnson and Baumann ('47) and by Drill and Truant ('47). Although the former group found that the utilization of existing vitamin A stores is retarded in the thyroid-deficient animal ('48) they could not demonstrate appreciable vitamin A liver stores following carotene administration in the thyroid-deficient rats. In part the disagreement found on the subject of carotene utilization in hypothyroidism is undoubtedly due to discrepancies in experimental procedures and in the criteria used to measure carotene utilization.

The amount of vitamin A in the kidney is to our present meagre knowledge not a function of the carotene-vitamin A conversion mechanism directly but is influenced by vitamin A metabolism in general. In the experiment cited here the normal male alone was capable of accumulating appreciable amounts of vitamin A in this organ. Females were unable to concentrate more than traces of the vitamin in the kidneys. Both hypothyroidism and caloric restriction prevented the accumulation of vitamin A in the male kidney. Johnson and Baumann ('48) concluded from similar observations that a rapid growth rate was essential for the shift from liver to kidney vitamin A deposits. Moore and Sharman's observations ('50) on the sex linked nature of kidney vitamin A stores are also confirmed here, since a comparison of individual male and female rats with similar weight gains showed greater liver stores and smaller kidney deposits for vitamin A in the female.

SUMMARY

The conversion of carotene to vitamin A and the distribution of vitamin A was studied in young growing or mature rats made hypothyroid with thiouracil.

On administration of small doses of carotene a relatively greater amount of vitamin A was recovered from the livers of the hypothyroid rats than from the normal controls. The difference disappeared with a 20-fold increase in dose.

A decrease in the utilization rate of vitamin A for metabolic functions by hypothyroid rats was demonstrated when hypothyroid and normal rats were depleted of similar preexisting stores since larger amounts were found in the deficient animals than in the controls. Growth retardation through caloric restriction in normal rats likewise resulted in larger residual vitamin A stores in quantities comparable with those found in hypothyroid rats.

The removal rate of a given dose of carotene in oil from the gastrointestinal tract was similar in normal and hypothyroid rats. This indicates a similarity of absorption rates in the two groups for the dose employed.

Normal male rats were capable of accumulating measurable amounts of vitamin A in their kidneys following carotene supplementation. Female rats whether normal or thyroid-deficient had only insignificant accumulation of vitamin A in their kidneys. Hypothyroidism and growth retardation due to caloric restriction were factors causing the disappearance of these vitamin A deposits in the male rat.

It is concluded that body weight and growth rather than basal metabolic rate govern the utilization of vitamin A and that neither absorption, transformation nor utilization of carotene is affected directly by thyroid activity.

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THE NUTRITIONAL STATUS OF PAPAGO INDIAN CHILDREN[1,2]

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This paper reports the results of a nutritional survey on Papago Indian children. This survey was part of a regional project in which selected population groups in the 11 Western States were studied. The Papago Indians were selected because they represent one of a few pure racial strains remaining in the United States; they exist on diets considered submarginal according to modern nutritional standards; and they give opportunity to determine the effects of an ample school lunch upon a population group.

EXPERIMENTAL

The survey was conducted during March and April, 1949 on the Papago Indian Reservation, with headquarters at Sells, Arizona. One hundred and fifteen Papago Indian children of both sexes were selected at random from schools supported by the United States Indian Service and from schools supported privately. These schools were located throughout the Reservation. One child was 11 years old, the remainder were 12 to 16 years old. Forty-eight of the children attended schools of the United States Indian Service, and 67 attended the pri-

[1] Arizona Agricultural Experiment Station Technical Paper number 335.

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vate schools. The children in both school systems were socially and economically comparable as far as their family backgrounds were concerned. Both school systems served a school lunch during the school year, from September through May.

Papago Indians are extremely proud of their heritage, and resent questioning of their personal habits. Therefore, members of the United States Indian Service advised that accurate dietary records would be impossible to obtain for Papago Indian children. General information relative to dietary habits was obtained from members of the clergy who associated closely with the Indians, from the managers of the several trading posts on the Reservation, and from members of the United States Indian Service.

The difficulties preventing satisfactory interviews concerning diet were even more pronounced for medical histories, so that no information as to previous medical history, care or treatment could be obtained. Clinical examinations were possible, and were made by a United States Public Health Service physician assisted by a registered nurse. Shoes and clothing were removed for this examination. The examination consisted of an assessment of nutritional status by physical signs (Sandstead and Anderson, '47), in addition to the usual procedures followed in a careful clinical examination. Symptoms widely acknowledged as associated with nutritional deficiency were included, as for example, gingivitis for ascorbic acid deficiency, increased vascularity and thickening of the bulbar conjunctiva, and hyperkeratosis of the elbows and knees for vitamin A deficiency, and asymptomatic stomatitis for vitamin B complex deficiency.

The children were brought to Sells for medical examinations and for collection of fasting blood samples. Blood assays were made in either a mobile laboratory loaned to the Western Region by the United States Public Health Service or in laboratories at the University of Arizona or at Colorado Agricultural and Mechanical College. Blood samples from each child were analyzed by current and well recognized microtechniques for the following: Vitamin A and carotene (Bes-

sey et al., '46); ascorbic acid (Lowry, Lopez and Bessey, '45); riboflavin (Burch, Bessey and Lowry, '48); total and free cholesterol (Shoenheimer and Sperry, '34; Sperry, '38); protein (Phillips et al., '34); glucose (Miller and Van Slyke, '36); hemoglobin (Wu, '22); hematocrit (Wintrobe and Landsberg, '35); sedimentation rate (Wintrobe, '42). For bone density determinations, the method of Mack, Brown, and Trapp ('49) was used. X-ray plates of the *os calcis* and hand were sent to Dr. Mack's laboratory for interpretation.

RESULTS AND DISCUSSION

Dietary intake. From the limited information obtainable, it is estimated that the Indians' intake of animal protein is low compared with average American standards. Beef cattle are raised on the reservation, but most of the beef is sold on the hoof to outside buyers and very little is consumed by the Indians themselves. Some wild game is eaten, but not enough is available generally to afford appreciable amounts of animal protein. Dairy products and poultry products are not generally a part of the diet. Fresh vegetables and fruits are used only sparingly. The main diet of the Indians is composed of beans, sugar and sirup, coffee, lard or fat pork, tortillas made of corn or wheat, and small amounts of native foods that grow in the desert. The native foods consist for the most part of mesquite beans, buds and fruit of cholla, and fruits of prickly pear and saguaro cactus. Some wild greens are available during certain parts of the year. A few families grow squash, corn, and other vegetables when weather conditions are favorable.

The noon lunch of the children attending the Indian Service school was carefully observed and found to be ample and well balanced. It furnished the following average daily nutrients as calculated (Watt, Merrill, '50) from a typical 5-day school week menu: calories 636, protein 22 gm, calcium 172 mg, iron 48 mg, vitamin A 2466 I.U., thiamin 0.42 mg, riboflavin 0.53 mg, niacin 3.8 mg, and ascorbic acid 57 mg. For example, one luncheon consisted of Spanish rice, green beans, carrot

sticks, bread and jam, milk, canned peaches; another consisted of roast beef, mashed potatoes, peas, cabbage salad, bread and honey, jello and fruit juice. Many of the foods in this lunch program came from government surplus. The noon lunch of the children attending private schools was limited and usually not well planned nutritionally. For example, lunch often consisted of bread, water and potatoes. Little coordination in lunch planning existed among the private schools studied.

Heights and weights. By analysis of variance (Snedecor, '46) the differences in height or weight between the Indian Service school children and the private school children were not significant. The mean weights for boys in the Indian Service schools were 128 ± 6.1 lb. and for those in private schools 119 ± 3.8 lb.; for Indian Service school girls 131 ± 6.3 lb. and for private school girls 129 ± 3.7 lb. The heights of boys in the Indian Service schools were 63.5 ± 0.61 and for those in private schools 63.6 ± 0.45 inches; for girls the measurements were 61.0 ± 0.41 and 60.9 ± 0.45 inches respectively. The mean ages for the Indian Service school boys were 13.9 years and for the private school boys 14.2 years; for the corresponding groups of girls, 13.8 years and 14.2 years respectively. It is the opinion of the authors that these values for heights and weights cannot be evaluated by comparison with the standard norms for white children. The Papago Indian has a body structure uniformly different from that of the white American.

Vitamin A and carotene. The data in table 1 show that the average vitamin A content of the blood of the children in both school groups was less than 21.2 µg per 100 ml, the standard of normality set by the Oxford Nutrition Survey (Sinclair, '47). There was no significant difference between the averages obtained in the two school systems. However, the carotene content of the blood from the Indian Service school children was definitely higher than that of blood from the private school children. These differences are highly significant ($t = 4.1$). According to the Oxford Survey, blood serum con-

TABLE 1

Analysis of blood from Papago Indian school children

DETERMINATION	INDIAN SCHOOLS — AMPLE LUNCH				BOYS AND GIRLS	PRIVATE SCHOOLS — LIMITED LUNCH				BOYS AND GIRLS
	Boys		Girls			Boys		Girls		
	No.	Content	No.	Content	Content	No.	Content	No.	Content	Content
Vitamin A μg/100 ml serum	29	21.2 ± 1.5 [1]	19	19.7 ± 1.5	20.6 ± 1.1	33	19.8 ± 1.2	34	19.5 ± 1.1	19.7 ± 0.81
Carotene μg/100 ml serum	29	91.5 ± 6.1	19	92.0 ± 6.4	91.7 ± 3.2	33	70.4 ± 5.4	34	72.8 ± 5.3	71.6 ± 3.8
Ascorbic acid mg/100 ml serum	29	0.871 ± 0.073	19	0.985 ± 0.094	0.916 ± 0.058	33	0.385 ± 0.040	34	0.585 ± 0.047	0.486 ± 0.034
Riboflavin μg/100 ml serum										
Free	27	0.97 ± 0.14	16	0.84 ± 0.16	0 92 ± 0.10	32	0.78 ± 0.11	27	0.97 ± 0.14	0.87 ± 0.091
Total	27	2.75 ± 0.16	16	2.85 ± 0.16	2.79 ± 0.12	32	2.65 ± 0.13	27	2.73 ± 0.15	2.68 ± 0.096
Cholesterol mg/100 ml serum										
Free	25	35.7 ± 0.51	16	38.9 ± 1.4	37.0 ± 0.69	33	35.1 ± 1.1	30	36.5 ± 1.1	35.8 ± 0.79
Total	25	137.6 ± 5.0	16	150.5 ± 5.7	142.7 ± 3.7	33	133.0 ± 4.0	30	139.0 ± 5.1	135.7 ± 2.8
Protein gm/100 ml serum	29	6.98 ± 0.050	19	7.03 ± 0.044	7.00 ± 0.034	33	6.79 ± 0.061	34	6.95 ± 0.047	6.87 ± 0.04
Glucose mg/100 ml serum	29	95.8 ± 4.8	19	103.1 ± 10.1	98.7 ± 5.2	33	99.7 ± 7.9	34	96.4 ± 8.6	98.0 ± 5.9
Hemoglobin gm/100 ml blood	29	13.6 ± 0.20	19	12.5 ± 0.37	13.2 ± 0.20	33	13.7 ± 0.19	34	12.5 ± 0.21	13.1 ± 0.15
Red Blood Count Millions/ml blood	29	4.90 ± 0.095	19	4.44 ± 0.105	4.72 ± 0.077	33	4.96 ± 0.100	34	4.41 ± 0.229	4.68 ± 0.068
White Blood Count Thousands/ml blood	29	9.34 ± 0.62	19	9.90 ± 0.53	9.56 ± 0.41	33	8.68 ± 0.600	34	9.15 ± 0.33	8.92 ± 0.215
Hematocrit % Volume	29	46.8 ± 0.59	19	44.3 ± 0.87	45.8 ± 0.51	33	46.7 ± 0.51	34	43.9 ± 0.44	45.3 ± 0.38
Sedimentation rate mm/hour	29	14.4 ± 1.5	19	21.2 ± 2.1	17.1 ± 1.3	33	17.0 ± 1.6	34	22.3 ± 1.7	19.7 ± 1.2

[1] Standard error of the mean.

taining less than 100 μg carotene per 100 ml is below normal, and serum containing less than 50 μg per 100 ml is extremely below normal. Sixty-five percent of the children in the Indian Service school assayed less than 100 μg per 100 ml blood serum and 4% contained less than 50 μg per 100 ml. Seventy-nine percent of the children in the private schools had blood which contained less than 100 μg and 31% had blood which contained less than 50 μg carotene per 100 ml blood serum (table 2).

According to the work of Merrow et al. ('52) the total amount of vitamin A activity in the blood more accurately reflects the nutrient intake of total vitamin A than either serum vitamin A or serum carotene alone. The total amount of vitamin A in International Units is readily calculated from the data in table 1 by multiplying the number of micrograms of vitamin A by 3.33 and the micrograms of carotene by 1.67, and adding the two. If the total level of vitamin A in the blood is used as a criterion of vitamin A status, the children in the Indian Service schools rate definitely better than the children in the private schools. The total vitamin A content of the blood of Indian Service school children is 221.3 ± 9.4 I.U. per 100 ml compared with 184.4 ± 5.8 for the private school children. These differences are highly significant ($t = 3.35$). The data in table 3 support the above chemical findings in that the incidence of clinical symptoms of vitamin A deficiency was greater in the private schools than in the Indian Service schools. Three to 4 times as many children in the private schools showed hyperkeratosis of the elbows and knees as in the Indian Service schools.

Ascorbic acid. The children from the Indian Service schools were considerably better nourished with respect to ascorbic acid than were the private school children. Tables 1 and 2 show a highly significant difference in the ascorbic acid content of the blood ($t = 6.52$) and table 3 shows a striking difference in the incidence of gingivitis. According to the Oxford Nutrition Standard of Normality (0.50 mg ascorbic acid per 100 ml), the serum concentration was below normal for only 15% of the Indian Service school children, as com-

TABLE 2

Distribution of Papago Indian children with respect to blood, vitamin A, carotene, and ascorbic acid

DETERMINATION	INDIAN SCHOOLS — AMPLE LUNCH						PRIVATE SCHOOLS — LIMITED LUNCH					
	Boys		Girls		Boys and girls		Boys		Girls		Boys and girls	
	No.	%	*No.*	%	*No.*	%	*No.*	%	*No.*	%	*No.*	%
Vitamin A/100 ml serum												
0–10 μg	3	10.3	1	5.26	4	8.33	2	6.06	2	5.88	4	5.97
10–15 μg	2	6.90	3	15.8	5	10.4	6	18.2	9	26.5	15	22.4
15–20 μg	10	34.5	7	36.8	17	35.4	9	27.3	8	23.5	17	25.4
Total below 20 μ	15	51.7	11	57.86	26	54.13	17	51.56	19	55.88	36	53.77
20–30 μg	9	31.0	6	31.6	15	31.2	14	42.4	12	35.3	26	38.8
30–40 μg	5	17.2	2	10.5	7	14.6	2	6.06	3	8.82	5	7.46
Total above 20 μg	14	48.2	8	42.1	22	45.8	16	48.46	15	44.12	31	46.26
Carotene/100 ml serum												
30–50 μg	1	3.45	1	5.26	2	4.17	11	33.3	10	29.4	21	31.3
50–75 μg	8	27.6	4	21.0	12	25.0	10	30.3	11	32.3	21	31.3
75–100 μg	10	34.5	7	36.8	17	35.4	5	15.1	6	17.6	11	16.4
Total below 100 μg	19	65.55	12	63.06	41	64.57	26	78.7	27	79.3	53	79.0
100–150 μg	8	27.6	7	36.7	15	31.2	7	21.2	6	17.6	13	19.4
150–200 μg	2	6.90	0	0	2	4.17	0	0	1	2.94	1	1.49
Total above 100 μg	10	34.5	7	36.7	17	35.37	7	21.2	7	20.54	14	20.89
Ascorbic Acid/100 ml serum												
0–0.3 mg	2	6.90	0	0	2	4.17	13	39.4	6	17.6	19	28.4
0.3–0.5 mg	3	10.3	2	10.5	5	10.4	16	48.5	8	23.5	24	35.8
Total below 0.5 mg	5	17.2	2	10.5	7	14.57	29	87.9	14	41.1	43	64.2
0.5–1.0 mg	13	44.8	9	47.4	22	45.8	3	9.09	18	52.9	21	31.3
1.0–1.5 mg	10	34.5	5	26.3	15	31.2	1	3.03	2	5.88	3	4.48
1.5–1.7 mg	1	3.45	3	15.8	4	8.33	0	0	0	0	0	0
Total above 0.5 mg	24	82.75	17	89.5	41	85.33	4	12.12	20	58.78	24	35.78

pared to 64% for the private school children (table 2). It is interesting that there were so many cases of gingivitis among the private school children whose blood serum averaged 0.486 mg of ascorbic acid per 100 ml, which is only slightly below the minimum set by the Oxford group.

The results in tables 1 and 2 show that the girls had a higher content of ascorbic acid in their blood serum than the boys. However, it is to be noted that in the Indian Service

TABLE 3

Physical signs often attributed to deficiencies in Papago Indian school children

DEFICIENCY SYMPTOMS	INDIAN SCHOOLS — AMPLE LUNCH				PRIVATE SCHOOLS — LIMITED LUNCH			
	Boys		Girls		Boys		Girls	
	No. examined	% with deficiency	No. examined	% with deficiency	No. examined	% with deficiency	No. examined	% with deficiency
Gingivitis	29	21	19	10	33	88	34	74
Bulbar Conjunctiva:								
(a) Increased Vascularity	29	79	19	79	33	94	34	94
(b) Thickening	29	59	19	68	33	94	34	76
Asymptomatic stomatitis	29	28	19	16	33	3	34	9
Hyperkeratosis:								
(a) Elbows	29	10	19	10	33	30	34	32
(b) Knees	29	14	19	10	33	48	34	32

schools, where the blood content of ascorbic acid was relatively high, the difference was not significant ($t = 0.95$) between girls and boys, but the difference was highly significant ($t = 4.17$) between the boys and girls in the private schools where the blood content of ascorbic acid was relatively low.

The main cause of the better ascorbic acid status of the Indian Service school children doubtless is due to the well-balanced school lunch which contained three glasses of fruit juice weekly as its principal source of ascorbic acid.

Riboflavin and niacin. There was no significant difference in the serum levels of free or total riboflavin between the Indian Service school children and the private school children (table 1). Both the average free and total riboflavin in both groups of children was within the approximate normal range (Sinclair, '47). There were some evidences of asymtomatic stomatitis (table 3) in both groups of children with the incidence somewhat higher in the Indian Service school than in the private school children. Only a few symptoms of niacin deficiency were noted.

Cholesterol, glucose, and protein. The average serum cholesterol was in the low normal range for both groups of children (Sunderman and Boerner, '49; Hawk, Oser and Summerson, '47). This is interesting since the Papago Indians eat large amounts of lard. Low serum cholesterol may be a racial characteristic.

The average serum content of glucose (Hawk, Oser and Summerson, '47), and protein (Sinclair, '47) were within the approximately normal range in both groups of children. There was no significant difference in either of these between the Indian Service school children and the private school children.

Hemoglobin, red blood cell count, white blood cell count, hematocrit, and sedimentation rate. There was no significant difference between the Indian Service school children and the private school children in hemoglobin, red blood cell count, white blood cell count, hematocrit and sedimentation rate. The hemoglobin was in the low normal range in both groups of children (Sinclair, '47). There was a significant difference between boys and girls in hemoglobin, red blood count, sedimentation rate, and hematocrit. This observation has been made many times and is standard textbook information.

Bone density evaluations. The bone density evaluations in table 4 show a favorable status in bone calcification for the Indian Service school girls (t values, *os calcis* = 2.12, phalanx — 5 — 2 center = 7.92, phalanx — 5 — 2 end = 2.54) but not

for the boys. There was a highly significant difference in bone density between boys and girls in the Indian Service school children for phalanx center and end (t values, center = 5.65, end = 3.33). This sex difference did not exist between the boys and girls in the private schools. It is apparent that the girls were more sensitive to lack of calcium and phosphorus than were the boys. This may be due to the fact that girls ordinarily reach puberty before boys.

TABLE 4

Bone density evaluations of Papago Indian children

	NO. OF INDIVIDUALS	OS CALCIS NOT CORRECTED FOR SOFT TISSUE	PHALANX – 5 – 2 – CORRECTED FOR SOFT TISSUE	
			Center	End
		B Index [1]	B Index [1]	B Index [1]
Indian school:				
(ample lunch)				
Boys	26	1.20 ± .034 [2]	1.35 ± .041	0.89 ± .024
Girls	15	1.31 ± .061	1.61 ± .021	1.02 ± .030
Private school:				
(limited lunch)				
Boys	33	1.17 ± .026	1.37 ± .039	0.92 ± .021
Girls	32	1.17 ± .024	1.42 ± .012	0.91 ± .030

[1] Number of X-ray equivalent grams of ivory per cubic centimeter of material.
[2] Standard error of the mean.

SUMMARY AND CONCLUSION

The data obtained from a nutritional survey on 115 Papago Indian children show that children receiving an ample school lunch are better nourished than those receiving a limited school lunch as judged by certain indices generally held to reflect nutritional status. The blood of the children receiving the limited lunch was lower in total vitamin A and ascorbic acid than the blood of the children receiving the ample lunch. Also, a higher percentage of the former showed symptoms usually associated with vitamin A and ascorbic acid deficiencies than did the latter group. Likewise, girls from the limited school lunch group showed poorer bone calcification than

those receiving the good lunch. No significant difference between the two groups of children was found for height or weight or age, values for hemoglobin, red cell count or white cell count, volume of packed cells, sedimentation rate or serum riboflavin, cholesterol, glucose or protein.

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VITAMIN B_1 ACTIVITY OF DISULFIDE FORMS OF THIAMINE

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TWO FIGURES

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The preparation of allithiamine from thiamine by treatment with allicin under mildly alkaline conditions has been reported by Matsukawa and Yurugi ('52). Fujiwara and Watanabe ('52) found that allithiamine could replace thiamine in the diet of rice birds and of the rat. These findings have recently been confirmed in the United States by Lilly et al. ('53).

It was considered of interest to extend the biological studies of these disulfide forms of thiamine in experiments with several vitamin B_1-requiring organisms. This paper presents growth data obtained with rats and chicks receiving these compounds in lieu of thiamine. The effect of the disulfide forms on thiamine stores of the rat and the anti-vitamin B_1 effect of oxythiamine have also been recorded. Finally, absorption and excretion studies were performed in man.

EXPERIMENTAL AND RESULTS

Vitamin B_1 deficiency was induced in the rat and chick by feeding a synthetic diet free of vitamin B_1. Both preventive and curative experiments were undertaken. Weanling rats and one-day-old New Hampshire chicks were used respectively. Allithiamine and propyl allithiamine (structures shown

below in fig. 1) were administered in 10% ethanol when injected. The biological activity of the disulfide forms was compared with thiamine mononitrate on a molar equivalent basis.

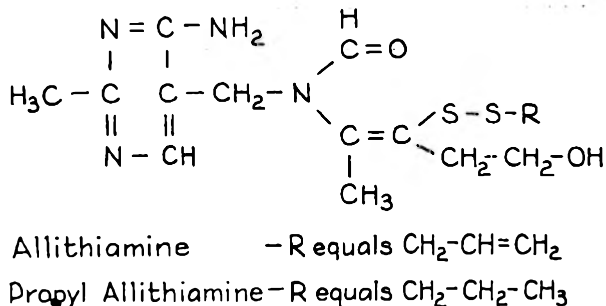

Fig. 1 Structure of vitamin B_1 disulfide forms.

TABLE 1

Response of vitamin B_1-deficient rats to equimolar doses of thiamine, allithiamine and propyl allithiamine

TREATMENT [1]	INITIAL	AV. WT. AND ( ) NO. ALIVE AT		
		1 wk.	2 wks.	4 wks.
3.07 μg Thiamine mononitrate	52 (18)	54 (17)	57	61 (17)
6.14 μg Thiamine mononitrate	53 (10)	65	77	103 (10)
9.21 μg Thiamine mononitrate	57 (5)	80	102	142 (5)
3.32 μg Allithiamine	52 (18)	54	56	58 (17)
6.64 μg Allithiamine	53 (10)	64	79	99 (10)
9.96 μg Allithiamine	56 (5)	80	104	139 (5)
3.32 μg Propyl allithiamine	52 (18)	55 (17)	59	62 (17)
6.64 μg Propyl allithiamine	52 (10)	65 (9)	84	109 (9)
9.96 μg Propyl allithiamine	56 (5)	78	105	145 (5)

[1] All injections by the subcutaneous route 5 times weekly.

Rat experiments

The data obtained in a curative experiment in rats are shown in table 1. Weanling rats were grown on a B_1-deficient diet of the following composition: Sucrose, 75; Labco casein, 18; Salts, 5; corn oil (fortified with adequate amounts of vitamins A, D, E and K), 2; B-vitamin supplements/kg diet:

riboflavin, 5 mg; pyridoxine · HCl, 5 mg; calcium pantothenate, 20 mg; nicotinamide, 10 mg; inositol, 50 mg; PABA, 10 mg; biotin, 50 µg; folic acid, 500 µg; and choline, 1 gm. At the end of three weeks their growth curves had plateaued and started to decline. Treatment was then begun as shown in table 1. The equivalence of the disulfide forms and thiamine was striking. Polyneuritic symptoms rapidly disappeared upon administration of any of the three compounds.

TABLE 2

Prevention of vitamin B_1 deficiency in rats by thiamine, allithiamine and propyl allithiamine

TREATMENT [1]	INITIAL	AV. WTS. AND NO. ALIVE ( ) AT			
		1 wk.	2 wks.	3 wks.	4 wks.
Basal diet (deficient)	39 (3)	61	63	48	all dead
Basal diet + 1 mg thiamine mononitrate	39 (5)	59	84	96	86 (5)
Basal diet + 1.1 mg allithiamine	39 (5)	62	92	113	129 (5)
Basal diet + 1.1 mg propyl allithiamine	39 (5)	58	89	109	131 (5)
Basal diet + 2 mg thiamine mononitrate	39 (5)	64	89	113	130 (5)
Basal diet + 2.2 mg allithiamine	39 (5)	64	95	118	151 (5)
Basal diet + 2.2 mg propyl allithiamine	39 (5)	60	92	117	149 (5)

[1] All additions expressed as mg/kg diet.

The results of a prophylactic experiment are shown in table 2. They again demonstrate the equivalence of the disulfide form and thiamine although there is some suggestion of a greater utilization of the open chain form when fed in the diet, instead of being administered by injection.

It was of interest to determine the deposition of thiamine (i.e. thiochrome forming material) in the liver of the rat after administration of thiamine or allithiamine. In this experiment a comparison was made of the effect of equimolar

doses of thiamine mononitrate and allithiamine on liver thiamine level. Weanling rats fed the basal deficient diet served as negative controls. Other groups received a dose

TABLE 3

Effect of thiamine mononitrate and allithiamine on rat liver thiamine levels

GROUP NO.	TREATMENT	THIAMINE [1] IN LIVER (μg/gram wet tissue)
1	Basal deficient diet	0.9
2	Basal + 1.42 mg allithiamine/kg	2.9
3	Basal + 1.31 mg thiamine mononitrate/kg	1.7
4	Basal + 0.1 ml 10% ethanol [2]	0.5
5	Basal + 8.85 allithiamine [3]	1.7
6	Basal + 8.15 thiamine [3] mononitrate	1.6

[1] Expressed as thiamine · HCl.
[2] Given intramuscularly 5 times a week.
[3] Dissolved in 10% ethanol and given intramuscularly 5 times a week.

TABLE 4

Inhibition index of oxythiamine in the rat against thiamine, allithiamine and propyl allithiamine

VITAMER ADMINISTERED	MOLAR RATIO (Antagonist/Vitamer)	NO. ALIVE/NO. TREATED [1]
Thiamine mononitrate	9	22/24
Thiamine mononitrate	18	9/22
Thiamine mononitrate	22	0/12
Thiamine mononitrate	23	2/ 9
Thiamine mononitrate	27	0/ 6
Allithiamine	9	21/24
Allithiamine	18	13/23
Allithiamine	22	3/13
Allithiamine	23	5/ 7
Allithiamine	27	0/ 6
Propyl allithiamine	9	12/16
Propyl allithiamine	18	9/16
Propyl allithiamine	22	4/13
Propyl allithiamine	23	2/ 8
Propyl allithiamine	27	0/ 6

[1] After 4 weeks of antimetabolite treatment.

of thiamine or allithiamine either in the diet or as in intra-muscular injection. At the end of three weeks all rats were killed, their livers extirpated, pooled for each group and assayed for total thiamine by the thiochrome procedure of Hennessey and Cerecedo ('41). The data obtained are shown in table 3. A partial depletion of the liver stores of rats on the deficient diet is seen in groups 1 and 4. The increase in thiamine livel after an oral or parenteral dose of thiamine mononitrate or allithiamine is also readily observed. Of particular interest is the possibly greater store of thiamine after oral ingestion of allithiamine (compare groups 2 and 3).

The effects of the disulfide forms against a thiamine antagonist, oxythiamine (Soodak and Cerecedo, '44), were also determined. Deficient rats were given a minimum dose of thiamine or allithiamine (3.07 μg and 3.32 μg respectively) with varying amounts of oxythiamine · HCl. Both inhibitor and vitamin were injected subcutaneously. The data shown in table 4 indicate that the inhibition index of oxythiamine in the rat is about 10 to 20 irrespective of the source of vitamin B_1, once again pointing to the identical vitamin B_1 activity of thiamine, allithiamine and propyl allithiamine.

Chick experiments

In the chick, preventive experiments were carried out. One-day-old New Hampshire chicks were given a synthetic B_1-deficient diet. Thiamine mononitrate or allithiamine were incorporated in the diet or injected intramuscularly. The results are shown in table 5. They again point to an equivalent activity of the disulfide forms and thiamine.

Human experiments

The experiments of the Japanese workers (Fujiwara and Watanabe, '52) indicated that a dose of allithiamine gave rise to a far greater excretion of thiamine than that observed after an equivalent dose of thiamine. It was considered important to repeat this experiment. Four healthy male

adults served as subjects. On the first day doses of 5, 10, 20 and 40 mg of thiamine mononitrate were administered in capsule form to each of the subjects respectively after the first morning urine was voided. Urine was then collected

TABLE 5

Vitamin B_1 activity of allithiamine in chicks

TREATMENT	AV. WTS. AND NO. ALIVE ( ) AT		
	0	16 days	26 days
Basal B_1 deficient diet	39 (33)	all dead	....
Basal + 1 mg thiamine mononitrate/kg	39 (26)	103 (23)	197 (17)
Basal + 3 mg thiamine mononitrate/kg	39 (26)	120 (25)	232 (25)
Basal + 1 mg allithiamine/kg	39 (33)	91 (12)	149 (9)
Basal + 3 mg allithiamine/kg	40 (33)	115 (33)	224 (31)
Basal + 10 μg thiamine mononitrate [1]	38 (10)	78 (10)	90 (9)
Basal + 30 μg thiamine mononitrate [1]	40 (10)	113 (10)	216 (10)
Basal + 10 μg allithiamine [1]	39 (10)	80 (10)	107 (10)
Basal + 30 μg allithiamine [1]	38 (10)	118 (10)	224 (9)

[1] Given intramuscularly each day.

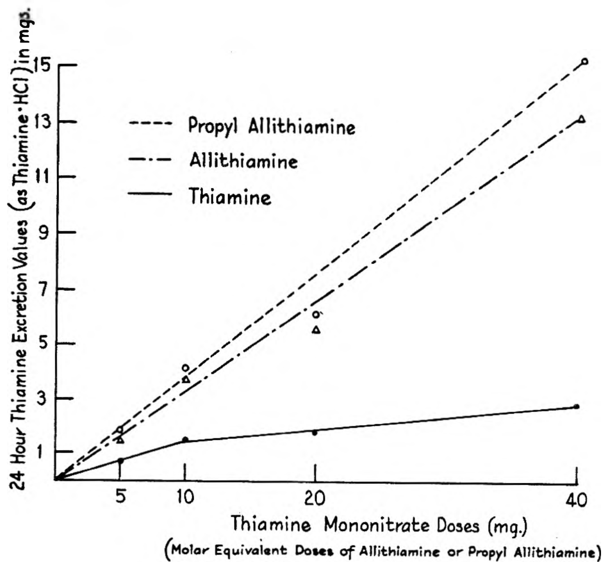

Fig. 2 Excretion of thiamine after oral administration of thiamine mononitrate, allithiamine and propyl allithiamine.

for the ensuing 24-hour period. No restrictions in diet were made except that abstention from alcoholic beverages was suggested. The urines were then assayed for total thiamine by the thiochrome procedure. Forty-eight hours later the same molar doses of allithiamine (viz. 5.4, 10.8, 21.6 and 43.2 mg) were administered to the same subjects, each individual receiving the molar dose corresponding to the dose of thiamine which he had received in the first test. The urines were then collected as described above and assayed for total thiamine. Three days later the experiment was again repeated with propyl allithiamine. The data obtained are shown in graphic form in figure 2. They clearly show that by increasing the oral dose of the disulfide forms of thiamine a far greater excretion value of thiamine was obtained than when an equivalent amount of thiamine was fed.

DISCUSSION

The data presented in this paper confirm the reports in the literature on the biological activity of disulfide forms of thiamine. There thus appears to be a quantitative conversion of these compounds to thiamine *in vivo*. Identical weight responses in vitamin B_1-deficient rats are observed when the disulfide forms are compared with thiamine in equimolar amounts (tables 1 and 5).

The data of table 2 suggest that a somewhat greater growth response is produced by the disulfide forms than by thiamine when the compounds are administered in the diet. Whether these data reflect an altered absorption of thiamine when the thiazole ring is opened and oxidized to a disulfide, or a greater stability in the intestinal tract of the disulfide forms, or both, cannot be answered. Another possible explanation lies in the difference of stability of the various forms in the experimental diet. Kandutsch and Baumann ('52) have recently reported on the stability of thiamine in synthetic diets.

The thiamine values in the liver of rats (table 3) which received a dietary supplement of thiamine or allithiamine lend support to the idea advanced above. It is noteworthy

that no differences in thiamine values were observed when the compounds were administered intramuscularly (table 3, groups 5 and 6).

Oxythiamine has been reported to be a potent anti-vitamin B_1 compound in the rat (Frohman and Day, '49). Under our conditions approximately the same inhibition index is observed with each of the vitamin B_1 compounds.

The urinary values observed in humans after an oral dose of each of the vitamins (fig. 2) are in agreement with the data of the Japanese workers (Fujiwara and Watanabe, '52). A better utilization of the disulfide forms is clearly suggested. Whether or not this reflects a greater absorption from or a greater stability in the intestinal tract again remains conjectural. In other human studies (Kirk and Chieffi, '51), it has been reported that one-half to three-fourths of an oral dose of 5 mg of thiamine is recovered in the stool. Large fecal thiamine levels have also been observed after oral thiamine administration in thiamine deficient patients (Youmans et al., '40). In spite of these large losses, the therapeutic efficacy of oral thiamine cannot be questioned. However, in view of the large fecal thiamine loss encountered during oral thiamine treatment, parenteral administration of thiamine has been suggested in those instances in which a rapid restoration of the blood thiamine level is desired (Kirk and Chieffi, '51).

SUMMARY AND CONCLUSIONS

Comparisons of the vitamin B_1 activity of thiamine, allithiamine and propyl allithiamine have been made.

When injected, the three compounds produce equivalent growth responses in rats and chicks.

There is some suggestion of a greater growth response observed with the disulfide forms than with an equivalent amount of thiamine-mononitrate when the compounds are included in the diet. This is also borne out by studies on liver thiamine levels after a dose of thiamine or allithiamine.

In rats, approximately the same inhibition index of oxythiamine is observed against thiamine, allithiamine and propyl allithiamine.

The oral administration of the disulfide forms to humans produces a greater urinary excretion of thiamine than is observed after the oral administration of an equivalent amount of thiamine.

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THE EFFECT OF METHOD OF ADMINISTRATION ON THE ABSORPTION AND STORAGE OF VITAMIN A BY DAIRY CALVES[1]

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TWO FIGURES

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Recent evidence has demonstrated that the method of administration has a marked effect upon the rate of absorption and efficiency of utilization of vitamin A. Thus, a knowledge of the procedure of vitamin A administration and of the effects of the various relevant factors is essential for accurate evaluation of the adequacy of dietary intake. It has been shown with rats (Popper and Volk, '48; Sobel et al., '48; Volk and Popper, '50; Lewis and Cohlan, '50) that vitamin A (both ester and alcohol forms) in aqueous dispersion is absorbed more rapidly from the intestinal tract and is utilized more efficiently than vitamin A in oil. Moreover, orally ingested vitamin A (alcohol) in aqueous dispersion is more efficiently transferred to the milk of the rat and is stored in greater quantities in the suckling than vitamin A in oily solution (Sobel and Rosenberg, '50). Chicks also have been found to utilize vitamin A ester more effectively when administered in the form of a water emulsion than when fed in an oil carrier (Halpern et al., '47). Humans have been observed to absorb aqueous dispersions of vitamin A (both alcohol and ester forms) more effectively than vitamin A in oil (Lewis et al., '47; Lewis et al., '50). Not all data support the above conclusions, however, since aqueous dispersion has been ob-

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served to decrease efficiency of utilization of vitamin A acetate in rats (Burns et al., '51) and vitamin A in alcohol form in chicks (March et al., '52), and to increase absorption and storage in rats only at high levels of supplementation (Ellingson et al., '51).

It has been demonstrated (Wise et al., '49) that in dairy calves a water dispersion of vitamin A (natural ester) is more effective than an oily solution in promoting rapid absorption. Also vitamin A is more rapidly absorbed when fed in milk by nipple feeder than when administered by capsule or in milk by stomach tube (Jacobson et al., '50). Inasmuch as rapid absorption in most instances has enhanced efficient utilization of vitamin A in other species, it seemed desirable to ascertain whether the factors which increase the rate of absorption of this vitamin from the gastrointestinal tract of the dairy calf also promote more efficient absorption and greater storage.

EXPERIMENTAL PROCEDURES

The experimental subjects, 24 calves from the Iowa State College dairy herd, received colostrum from their respective dams for three days following birth. During the period from 4 to 14 days of age, the calves were fed fresh whole milk twice daily at the rate of 10 pounds per day per 100 pounds of body weight. Subsequently the whole milk was replaced gradually, over a 4-day period, with reconstituted skimmilk. Thus, no whole milk was fed after 18 days. The skimmilk was limited to a maximum of 12 pounds daily per calf. All calves received, after 4 days of age, oat straw (approximately 18 months old) ad libitum and a low-carotene concentrate mixture (limited to 4 pounds per calf daily) composed of crushed oats, 50; wheat bran, 38; linseed oil meal, 10; steamed bone meal, 1; and iodized salt, 1%. Sufficient irradiated yeast was added to the concentrate mixture to provide a minimum of 300 I. U. of vitamin D daily per 100 pounds of body weight of calf. Water was available at all times subsequent to the whole milk feeding period. Each calf was weighed at the

beginning of the experiment and at weekly intervals thereafter.

The vitamin A depletion period, initiated for each calf at 18 days of age, was continued until blood plasma vitamin A values were reduced to an average of approximately 8 μg per 100 ml. In preliminary observations on calves not included in this report the plasma vitamin A levels were depleted to 4 μg. A high mortality rate indicated that this procedure was impractical under the conditions of this experiment for calves of this age: thus, the higher depletion level was selected.

At the end of the depletion period the calves were allotted randomly to 4 experimental groups, each composed of 5 Holsteins and one Brown Swiss. Subsequently vitamin A supplements were administered twice weekly. At each supplemental feeding, vitamin A was administered at the rate of 10,500 μg per 100 pounds of body weight. Each calf received per 100 pounds of average body weight a total of 126,000 μg of vitamin A, administered over a 6-week period.

The dispersion media and methods of administration of the vitamin A[2] were as follows: group I, corn oil in skimmilk given by nipple bottle; group II, Tween 80[3] in skimmilk given by nipple bottle; group III, corn oil given by capsule; and group IV, Tween 80 given by capsule. The vitamin A concentrate was a natural ester form distilled from fish liver oil and contained the equivalent of 57,300 μg of vitamin A alcohol per gram. The vitamin supplement fed to groups I and III was composed of one part of the vitamin A concentrate dissolved in 7 parts of corn oil, whereas that administered to groups II and IV was a similar amount of vitamin A dispersed in 7 parts of Tween 80.

Subsequent to the supplementation period, the calves received the same diet as that fed during the initial depletion period. This terminal depletion was continued for each calf

[2] Supplied by Distillation Products Inc., Rochester, N. Y. through the courtesy of Dr. P. L. Harris.

[3] Polyoxyethylene sorbitan monooleate, supplied by Atlas Powder Co., Wilmington, Delaware, through the courtesy of Dr. W. K. Abbott.

until the blood plasma vitamin A values for two successive weeks averaged approximately 5 μg per 100 ml.

Departures from the foregoing procedures occurred in the first two replicates (first two calves in each group). The total vitamin A administered was the same but because of slightly lower initial supplementation levels a period of 8 weeks, rather than 6 weeks, was employed. Also two animals from these replicates, one each from groups I and III, were sacrificed near the end of the terminal depletion period to determine liver and kidney storage of vitamin A.

On the first Friday after each calf was placed on experiment and at weekly intervals thereafter samples of venous blood were obtained and, following centrifugation, the plasma was analyzed for carotenoids and for vitamin A employing the saponification method of Allen et al. ('49). During the vitamin A supplementation period the blood samples were drawn approximately 60 hours after the semi-weekly vitamin A administration.

RESULTS

Criteria employed in comparing the effects of the method of administration on absorption and storage of vitamin A were blood plasma vitamin A levels at the end of the vitamin A supplementation period, length of the terminal depletion period and changes in body weight. The data were evaluated by analyses of variance and covariance (Snedecor, '46). To minimize interpolation between weekly plasma vitamin A values, a blood level of 5.6 μg vitamin A per 100 ml of plasma was chosen arbitrarily in the analysis of the data as the end point of the terminal depletion period.

The mean blood plasma vitamin A levels of all groups rose sharply during the first week of supplementation (fig. 1), the increase being greatest for group II (vitamin A emulsified in milk by Tween 80, fed by nipple bottle). Thereafter, the values remained rather constant except for those of group II, which increased at a lesser rate during the remainder of the supplementation period. The mean terminal depletion

time (fig. 2), following supplementation, was longest for group II followed in order by groups IV (vitamin A in oil plus Tween 80, fed by capsule), I (vitamin A in corn oil, fed in milk by via nipple bottle), and III (vitamin A in corn oil, fed by capsule).

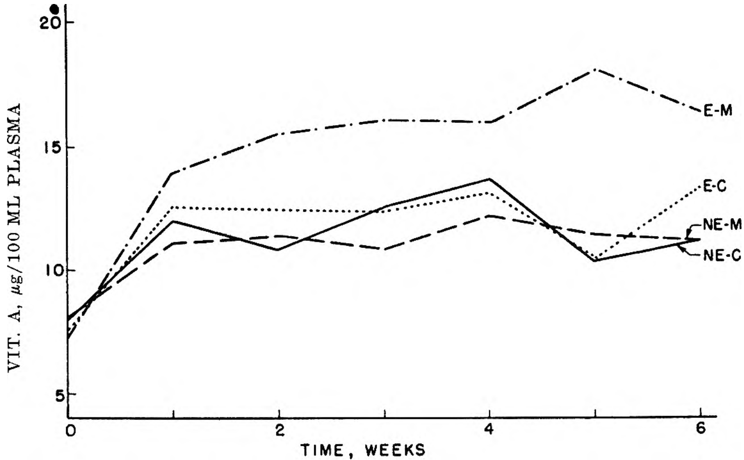

Fig. 1 Effect of dispersion medium and method of administration of vitamin A supplements on blood plasma vitamin A levels. (Four calves per group; vitamin A supplementation at rate of 10,500 µg twice weekly per 100 pounds body weight. E-M = emulsified, milk; E-C = emulsified, capsule; NE-M = non-emulsified, milk; NE-C = non-emulsified, capsule).

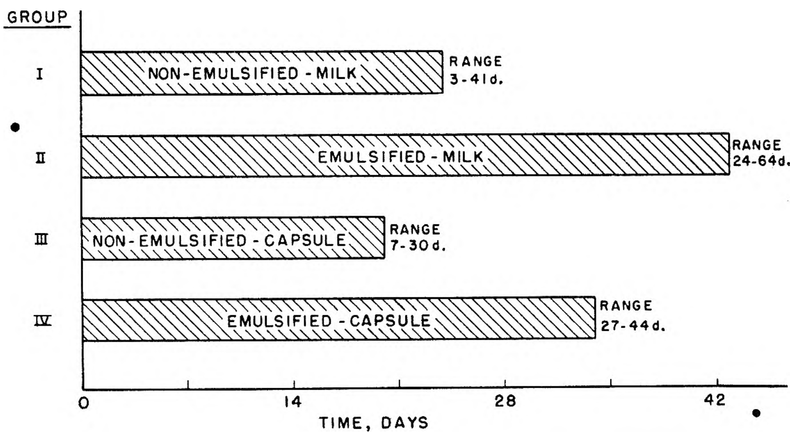

Fig. 2 Effect of dispersion medium and method of administration of vitamin A supplements on terminal depletion times of groups shown in figure 1.

The first two replicates, one composed of Holsteins and the other of Brown Swiss, except where otherwise indicated, were excluded from the analysis of the data because of differences in the length of the supplementation period and because some of these animals were sacrificed for liver and kidney analyses. The results from these two replicates, however, were similar to those of the last 4 (figs. 1 and 2) in which 4 Holsteins (two males and two females) were allotted to each group.

Inasmuch as the ages of the calves at the beginning of the supplementation period were dependent upon the age when the first blood samples were taken (since blood was collected on the Friday following entry on experiment) and upon the time required for initial depletion, slight variations were observed, ranging from an average of 30 days for calves in group III to an average of 36 days for those in group II. Since a significant correlation was found between blood plasma vitamin A levels at the end of the supplementation period and the age of the calves when the first blood samples were taken, an analysis of covariance was indicated. The adjusted means showed a significant difference at $P = 0.01$ between the blood plasma vitamin A levels resulting from feeding vitamin A in oil and those following administration of vitamin A with Tween 80. Conversely, differences between milk and capsule methods of administration were not significant at $P = 0.05$. Statistical analysis indicated further that plasma vitamin A levels and body weights at the beginning of the 6-week supplementation period, body weights at the end of the supplementation period and initial depletion time had no significant effect on plasma vitamin A values at the end of the supplementation period.

Plasma vitamin A levels and body weights at the beginning of the supplementation period, body weights at the end of supplementation and ages when the first blood samples were taken had no appreciable effects on terminal vitamin A depletion time. Since, however, a significant correlation was found between terminal depletion time and initial depletion time, an analysis of covariance was employed. This analysis

revealed a significant difference at P = 0.05 between the adjusted depletion times of calves fed vitamin A in oil and those of calves receiving this vitamin with Tween 80. The difference between milk and capsule methods of administration was not significant at P = 0.05. Inclusion of the data for the first two replicates, however, revealed a significant difference not only between oily and aqueous (Tween 80) dispersions but also between milk and capsule methods of administration, the difference being much greater in the former instance.

All blood plasma samples used for vitamin A determinations were analyzed for carotenoids to provide an index of carotenoid consumption. In all instances the plasma carotenoid values decreased rapidly during initial depletion and remained at levels near zero throughout the vitamin A supplementation and terminal depletion periods, thus indicating that no appreciable amount of carotenoids was present in the vitamin A depletion diet.

Mean weights in pounds at the beginning and at the end of the supplementation period for calves in the last 4 replicates of groups I, II, III, and IV, respectively, were 108 and 155; 91 and 137; 96 and 133; and 105 and 147. The differences in weight gains during the supplementation period were not significant. Moreover, no major differences in the incidence of diarrhea or lacrimation were observed. Calves in group II, however, possessed smooth haircoats and were the most vigorous, whereas those in group III had rough haircoats and were less vigorous than calves in the other groups.

Three calves, two from the first two replicates and another used in preliminary observations, with blood plasma vitamin A levels ranging from 5.5 to 9.0 μg per 100 ml were sacrificed to determine vitamin A storage. Total liver storage, which was correlated directly with blood plasma levels, averaged 2,206 μg per calf, and kidney storage was negligible.

DISCUSSION

The investigation reported herein has shown that vitamin A when administered to calves in the presence of an emulsify-

ing agent is absorbed and stored to a greater extent than when similar amounts of the vitamin are fed in oily solution. This occurred both when vitamin A was administered in milk by nipple feeder and when it was administered by capsule. Thus, the previously observed more rapid absorption of aqueous dispersions by calves (Wise et al., '49) apparently is indicative also of more complete absorption and storage.

The rate of absorption, however, is not a completely reliable criterion of efficiency of vitamin A utilization in the bovine, possibly due to the compound nature of the ruminant stomach. Previous observations (Jacobson et al., '50) have shown that the rate of absorption is much less rapid when vitamin A is homogenized in milk and fed by stomach tube (into the rumino-reticular cavity) than when a similar dispersion is fed by nipple feeder (into the abomasum), and that both of these methods of administration promote more rapid absorption than when the vitamin is fed by capsule. Moreover, other data (Wise et al., '50) have demonstrated that vitamin A in oil fed in milk by nipple feeder is absorbed much more rapidly than a similar quantity of the vitamin dispersed in milk by Tween 80 and administered by stomach tube. Thus, in the present investigation it might be expected that vitamin A in the oil fed in milk by nipple feeder would permit greater storage than a mixture of vitamin A and Tween 80 fed by capsule. It was observed, however, that calves fed the latter stored greater amounts of vitamin A as indicated by terminal depletion time. It appears, therefore, that the efficiency of utilization of vitamin A by the dairy calf is influenced to a greater degree by dispersion medium than by method of administration despite the earlier data indicating to the contrary (Jacobson et al., '50; Wise et al., '50).

The reason for the improvement in absorption resulting from aqueous dispersion is not entirely clear. It is possible that such dispersion results in absorption at a higher level of the intestine where vitamin A is absorbed more rapidly (Popper and Volk, '48). Furthermore, absorption at the

higher level of the intestine would allow less time for possible destruction of vitamin A in the gastrointestinal tract.

The comparatively slow rate of absorption of the vitamin A in a vitamin A-Tween 80 mixture delivered to the rumino-reticular cavity and the apparent efficient utilization suggest a relatively effective absorption despite a retarded passage of the vitamin through the gastrointestinal tract. Since this method of administration promoted somewhat less storage than delivery of the vitamin directly into the omaso-abomasal area, however, a reduction in vitamin A potency due to the greater time required to reach the point of absorption is suggested.

The marked rise during the first week of the supplementation period and the subsequent leveling off of the plasma vitamin A levels indicate that these values are inadequate as an indication of vitamin A storage (except in deficient animals). Similar conclusions have been reached by Sobel et al. ('48) in experiments with rats.

It has been demonstrated that an aqueous dispersion of vitamin A is utilized three times as efficiently by rats as a similar oily solution of this vitamin (Sobel et al., '48; Volk and Popper, '50). The present investigation indicates that the increased efficiency in dairy calves probably is not so great.

SUMMARY

A natural ester vitamin A concentrate was fed by various methods to young dairy calves, previously depleted of vitamin A reserves, to determine relative efficiency of absorption and storage of vitamin A. Vitamin A in oil and vitamin A in oil plus an emulsifying agent (Tween 80) were administered in milk by nipple feeder and by capsule.

Vitamin A absorption and storage, as measured by blood plasma vitamin A levels and by depletion time subsequent to vitamin A supplementation, were much greater from an aqueous dispersion than from an oily solution. There was an indication that administration of vitamin A in milk by

nipple feeder resulted in more efficient utilization than administration by capsule.

The point of deposition of a vitamin A supplement in the stomach of the dairy calf apparently has a greater effect upon the rate of absorption than upon efficiency of utilization of this vitamin.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Ruth M. Ward for technical assistance.

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EFFECT OF DIETARY AMINO ACID BALANCE ON FAT DEPOSITION IN THE LIVERS OF RATS FED LOW PROTEIN DIETS[1]

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INTRODUCTION

Both Singal and associates ('53) and Harper and associates ('53) have reported that threonine is effective in reducing the deposition of liver fat in rats fed low casein diets containing choline. The former workers also reported that lysine was required to maintain a normal level of fat in the livers of rats fed a purified diet containing crystalline amino acids. The observations that fat accumulated in the livers of rats fed a 9% casein diet only when the diet was supplemented with methionine and that threonine was less effective in reducing liver fat deposition when the diets contained less than 9% of casein (Harper et al., '54) suggested that the extent of fat deposition in the liver depended on the balance as well as on the absolute quantities of amino acids in the diet. Since the addition of large amounts of glycine and certain other related compounds to the 9% casein diet also reduced fat deposition, it appeared that certain non-essential dietary components also affected the deposition of liver fat. In order to extend these observations a study of fat deposition in the

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livers of rats fed proteins other than casein was undertaken on the assumption that differences in the amino acid composition of these proteins would be reflected by differences in the extent of fat deposition in the liver. The results of this study are reported below.

EXPERIMENTAL

Male weanling rats of the Sprague-Dawley strain weighing from 40 to 50 gm were used in all experiments. In each experiment rats were distributed equally among the various groups according to body weight. Each group was composed of 6 animals. The rats were kept in individual, screen-botton cages and were fed ad libitum. The individual animals were weighed weekly. At the end of two weeks, the rats were stunned by a blow on the head and decapitated. The livers were removed and stored at — 4°C. until the fat content was determined. This was done by ether extraction of the dried and ground liver. (Hawk and Elvehjem '53.) Nitrogen determinations of the proteins were made by the macro-Kjeldahl method using mercuric oxide as a catalyst.

The basal diet was prepared to contain the following: corn oil, 5; Salts 4 (Hegsted, Mills, Elvehjem and Hart, '41), 4; choline chloride, 0.15; DL-tryptophan, 0.1; DL-methionine, 0.1%; protein at the level indicated in the tables and the remainder sucrose. All supplements were included at the expense of sucrose. When fibrin was the dietary protein, tryptophan was omitted and 0.3% DL-methionine was added. When defatted and dried pork and beef were the dietary proteins, DL-tryptophan was included at the level of 0.1% and DL-methionine at 0.3% of the diet as was the case with casein. Vitamins were added to provide in milligrams per 100 gm ration: thiamine·HCl, 0.5; riboflavin, 0.5; niacin, 1.0; calcium pantothenate, 2.0; pyridoxine, 0.25; biotin, 0.01; folic acid, 0.02; vitamin B_{12}, 0.002; inositol, 10.0. Two drops of halibut oil fortified to furnish 1000 I.U. of vitamin A, 10 I.U. of vitamin D, 0.04 mg of 2-methyl-1,4-naphthoquinone and 0.8 mg of α-tocopherol were given weekly.

The egg albumin, fibrin, and gelatin used in the experiments were obtained commercially. The casein was an alcohol-extracted product prepared in the Department. The pork and beef proteins were prepared from market pork and beef. The meat was cooked, dried in a stream of warm air and freed of fat by extraction with petroleum ether.

RESULTS

Each experiment is reported separately because the liver fat values of control groups varied from experiment to experiment even though the relative differences between groups were consistent.

It is evident from the results presented in table 1 that fat accumulated in the livers of rats fed low levels of casein, pork, beef, or egg albumin and that as the level of the dietary protein was increased, the amount of fat deposited in the liver was reduced in each case. No accumulation of liver fat occurred in rats fed low-fibrin diets. The addition of threonine or glycine reduced fat deposition in every case and the coincident addition of both of these amino acids caused a further reduction. Threonine was more effective with the casein and beef diets than it was with the pork and albumin diets (groups 11 to 16, 21 and 22). Glycine was as effective as threonine in the pork diets (groups 18 and 19) and was considerably more effective than threonine in the albumin diets (groups 29 to 31). Threonine was more effective in the casein diets than in the albumin diets even when the proteins were fed on an equal nitrogen basis (groups 27 and 28). Since casein and egg albumin contain approximately the same amounts of threonine, groups 27 and 28 received approximately equal quantities of this amino acid.

The addition of 6% of gelatin to the albumin diets caused a greater reduction in liver fat than can be accounted for by the threonine content of the gelatin (groups 44 to 47 of table 2) although the growth response was about the same with each. The large amount of glycine provided by the gelatin probably accounts for the additional decrease in liver fat.

TABLE 1

Effect of level of dietary protein and of supplementary threonine and glycine on fat deposition in the livers of rats fed various low protein diets

EXPT. NO.	GROUP NO.	PROTEIN	SUPPLEMENT	GROWTH	LIVER FAT [1]	
				gm/wk.[1]	*% Dry wt.*	*% Wet wt.*
I	1	6% fibrin	None	3.2 ± 0.4	15.1 ± 2.1	4.5 ± 0.7
	2	7.5% fibrin	None	5.8 ± 0.6	15.2 ± 1.2	4.4 ± 0.4
	3	9% fibrin	None	12.8 ± 1.1	16.2 ± 0.9	4.8 ± 0.3
II	4	8% pork	None	12.2 ± 0.6	30.8 ± 2.5	10.5 ± 1.1
	5	10% pork	None	22.2 ± 1.8	18.6 ± 2.6	5.8 ± 0.3
	6	12% pork	None	28.4 ± 0.8	13.6 ± 0.9	4.0 ± 0.3
	7	8% beef	None	15.4 ± 1.0	27.2 ± 1.2	8.9 ± 0.5
	8	10% beef	None	21.5 ± 1.1	19.0 ± 1.2	5.8 ± 0.4
	9	12% beef	None	32.4 ± 1.2	11.7 ± 0.9	3.5 ± 0.3
	10	9% casein	None	17.6 ± 1.4	23.8 ± 1.0	7.6 ± 0.4
III	11	9% casein	None	16.3 ± 1.5	28.4 ± 2.2	9.6 ± 0.8
	12	9% casein	0.36% DL-threonine	20.2 ± 0.9	12.6 ± 0.9	3.7 ± 0.2
	13	8.1% beef	None	13.9 ± 1.3	26.4 ± 3.4	8.9 ± 1.3
	14	8.1% beef	0.36% DL-threonine	16.0 ± 1.4	16.4 ± 0.7	5.0 ± 0.2
	15	8.2% pork	None	10.8 ± 0.8	30.1 ± 2.7	10.0 ± 1.0
	16	8.2% pork	0.36% DL-threonine	15.7 ± 1.5	22.2 ± 2.0	7.0 ± 0.7
IV	17	8% pork	None	8.6 ± 0.8	43.8 ± 4.2	16.4 ± 2.2
	18	8% pork	0.36% DL-threonine	12.8 ± 0.9	22.0 ± 1.3	6.8 ± 0.5
	19	8% pork	1.5% glycine	7.6 ± 0.7	[illegible]	[illegible]

	23	11% egg albumin	None	12.7 ± 0.6	20.4 ± 1.0	5.8 ± 0.4
	24	11% egg albumin	0.36% DL-threonine	16.0 ± 0.8	16.4 ± 1.6	3.9 ± 0.6
	25	13% egg albumin	None	18.6 ± 0.6	16.5 ± 1.3	4.7 ± 0.4
	26	13% egg albumin	0.36% DL-threonine	20.4 ± 1.5	14.4 ± 0.5	4.0 ± 0.2
VI	27	9% casein	0.36% DL-threonine	22.2 ± 1.1	10.0 ± 0.3	2.9 ± 0.1
	28	10.5% egg albumin	0.36% DL-threonine	20.8 ± 0.8	15.3 ± 0.9	4.6 ± 0.3
VII	29	9% egg albumin	None	8.8 ± 0.7	33.6 ± 3.0	11.2 ± 1.3
	30	9% egg albumin	0.36% DL-threonine	12.2 ± 1.3	23.5 ± 3.2	7.8 ± 1.7
	31	9% egg albumin	1.5% glycine	7.5 ± 0.5	16.1 ± 1.1	4.4 ± 0.4
	32	9% egg albumin	1.5% glycine + 0.36% DL-threonine	11.9 ± 0.9	13.7 ± 0.8	3.7 ± 0.3
VIII	33	8% egg albumin	None	9.5 ± 0.5	25.9 ± 2.2	8.3 ± 0.9
	34	8% egg albumin	1.5% glycine	6.2 ± 0.7	16.8 ± 1.0	4.8 ± 0.3
	35	9% egg albumin	None	10.1 ± 0.8	21.6 ± 2.2	6.8 ± 0.8
	36	9% egg albumin	1.5% glycine	11.1 ± 1.3	13.1 ± 0.5	3.8 ± 0.2
	37	11% egg albumin	None	20.5 ± 0.8	14.6 ± 0.6	4.3 ± 0.2
	38	11% egg albumin	1.5% glycine	13.9 ± 1.5	12.0 ± 0.8	3.4 ± 0.2
IX	39	4.5% egg albumin + 4.5% casein	None	14.5 ± 1.1	20.0 ± 2.5	6.0 ± 1.0
	40	4.5% egg albumin + 4.5% casein	0.36% DL-threonine	21.0 ± 0.7	12.2 ± 0.4	3.6 ± 0.2
	41	4.5% egg albumin + 4.5% casein	1.5% glycine	13.5 ± 0.9	16.3 ± 0.6	4.8 ± 0.2
	42	9% casein	None	19.8 ± 1.6	23.4 ± 2.7	7.3 ± 1.0
	43	9% egg albumin	None	10.1 ± 0.8	21.6 ± 2.2	6.8 ± 0.8

[1] Values represent mean ± standard error of the mean for 6 animals.

TABLE 2

Effect of supplement of protein and amino acids on the deposition of fat in the livers of rats fed low albumin diets

EXPT. NO.	GROUP NO.	PROTEIN	SUPPLEMENT	GROWTH	LIVER FAT [1]	
				gm/wk.[1]	*% Dry wt.*	*% Wet wt.*
X	44	9% egg albumin	None	8.2 ± 1.0	28.1 ± 3.3	8.6 ± 1.4
	45	9% egg albumin	6% gelatin	13.5 ± 1.0	16.7 ± 1.8	4.7 ± 0.5
	46	9% egg albumin	1.5% glycine	7.6 ± 0.3	16.5 ± 1.4	4.6 ± 0.4
	47	9% egg albumin	0.18% DL-threonine	13.0 ± 1.3	20.8 ± 1.4	6.1 ± 0.5
	48	9% egg albumin	0.18% DL-threonine + 0.5% L-lysine	12.8 ± 0.8	22.6 ± 2.2	6.7 ± 0.7
XI	49	9% egg albumin	None	10.0 ± 1.1	28.1 ± 2.5	8.8 ± 1.0
	50	9% egg albumin	12% gelatin	18.5 ± 1.5	10.0 ± 1.1	3.0 ± 0.4
	51	9% egg albumin	1.5% glycine	7.4 ± 1.3	16.9 ± 1.3	4.7 ± 0.4
	52	9% egg albumin	0.36% DL-threonine	15.1 ± 0.9	22.7 ± 1.3	6.8 ± 0.5
	53	9% egg albumin	0.25% L-histidine·HCl	12.2 ± 0.9	22.9 ± 1.6	6.9 ± 0.5
	54	9% egg albumin	0.2% DL-valine	11.2 ± 1.1	32.5 ± 2.5	10.9 ± 1.3
	55	9% egg albumin	0.25% L-histidine·HCl + 0.2% DL-valine +			

	58	9% egg albumin	0.25% L-histidine·HCl	10.2 ⊥ 0.1	22.7 ± 1.4	9.1 ± 0.8
	59	9% egg albumin	0.25% L-histidine·HCl + 0.2% DL-valine + 0.36% DL-threonine	14.8 ± 0.9	22.7 ± 2.2	7.1 ± 0.8
	60	9% egg albumin	0.25% L-histidine·HCl + 0.2% DL-valine + 0.36% DL-threonine + 0.5% L-lysine	17.8 ± 0.6	13.7 ± 0.3	3.9 ± 0.1
	61	9% egg albumin	1% DL-leucine	6.8 ± 0.8	15.4 ± 1.0	4.5 ± 0.3
	62	9% egg albumin	1.5% DL-serine	8.2 ± 1.2	21.9 ± 2.9	5.9 ± 0.9
	63	9% egg albumin	2% fibrin	17.7 ± 0.8	15.7 ± 1.4	4.7 ± 0.5
	64	9% egg albumin	0.4% DL-serine + 0.3% DL-leucine + 0.36% DL-threonine	12.4 ± 0.8	13.7 ± 1.4	4.0 ± 0.5
XIII	65	8% egg albumin	None	8.6 ± 0.7	28.9 ± 2.8	9.8 ± 1.1
	66	8% egg albumin	0.25% L-histidine·HCl + 0.36% DL-threonine	15.3 ± 0.6	23.1 ± 2.9	7.2 ± 1.1
	67	8% egg albumin	0.25% L-histidine·HCl + 0.36% DL-threonine + 0.5% L-lysine	16.0 ± 0.8	18.1 ± 1.5	5.4 ± 0.5
	68	8% egg albumin	0.2% DL-valine + 0.36% DL-threonine	14.9 ± 0.7	22.5 ± 1.8	6.9 ± 0.6
	69	8% egg albumin	0.2% DL-valine + 0.36% DL-threonine + 0.5% L-lysine	9.7 ± 0.7	12.6 ± 0.7	3.7 ± 0.2
	70	8% egg albumin	0.25% L-histidine·HCl + 0.2% DL-valine + 0.36% DL-threonine + 0.5% L-lysine	14.2 ± 1.3	18.3 ± 1.6	5.6 ± 0.5
XIV	71	8% egg albumin	No tryptophan. No methionine	8.9 ± 0.9	26.4 ± 2.0	8.6 ± 0.8
	72	8% egg albumin	0.1% DL-tryptophan. No methionine	7.8 ± 0.7	32.8 ± 3.8	11.1 ± 1.5
	73	8% egg albumin	0.1% DL-methionine. No tryptophan	7.8 ± 0.9	32.7 ± 2.2	11.0 ± 1.0
	74	8% egg albumin	0.1% DL-tryptophan + 0.1% DL-methionine	8.6 ± 0.7	28.9 ± 2.8	9.8 ± 1.1

[1] Values represent mean ± standard error of the mean for 6 animals.

The addition of 12% of gelatin resulted in a growth stimulation greater than that obtained with 0.36% DL-threonine (groups 50 and 52).

Supplementation of the egg albumin diets with 2% of fibrin also caused a marked reduction in the amount of liver fat. Serine, leucine, and threonine in the amounts provided by the fibrin reduced the amount of fat in the livers as much as did fibrin (groups 63 and 64). It is interesting to note that leucine and serine alone are active only in relatively large amounts, the effect with leucine being accompanied by a considerable growth depression (groups 61 and 62), but the smaller levels of these amino acids when added with threonine were very effective in preventing the accumulation of liver fat. Unpublished results obtained in our laboratory, however, show that leucine also decreased liver fat deposition under conditions in which growth was not retarded.

The addition of lysine and threonine to 9% egg albumin diets caused no greater reduction in liver fat deposition than that obtained with threonine alone (groups 47 and 48), nor was a growth stimulation observed when the lysine was added. No significant effect on fat deposition occurred when either histidine or valine were added singly to the basal diet (groups 53, 54, and 58), nor was any change observed when histidine, valine, and lysine were added together (group 56). When histidine and valine were added with threonine, the reduction in liver fat was no more than that obtained with threonine alone (groups 52 and 55). However, when lysine was added in the presence of supplements of histidine, valine, and threonine, a marked decrease in the liver fat resulted (groups 59 and 60). Groups 67 and 70 show that valine need not be added to obtain this reduction in liver fat. However, when histidine was omitted, the addition of lysine with threonine and valine caused a considerable growth depression (groups 68 and 69) which could contribute to the low liver fat obtained.

The addition of tryptophan and methionine separately to the egg albumin basal diet (prepared without additional amounts of these amino acids) caused slight increases in the

liver lipids (groups 71 to 73), but when both were added together, no effect on the deposition of liver fat was noted (groups 71 and 74).

Supplementations of diets containing various levels of albumin with glycine resulted in a reduction of liver fat in each instance (groups 33 to 38). Marked growth depressions were observed at the 8 and 11% levels but 1.5% glycine did not retard growth when added to the 9% albumin diet. However, in other experiments, slight growth depressions with glycine were observed (groups 31, 46, and 51). While threonine was more active than glycine when added to low casein diets and the reverse was true for albumin diets, threonine was more effective than glycine in reducing liver fat when fed to rats receiving diets in which the protein was provided equally by casein and albumin (groups 39 to 43).

DISCUSSION

Amino acid balance and growth

The addition of threonine to low pork, beef, casein, and egg albumin diets which were supplemented with tryptophan and methionine caused a growth response in every case. Tryptophan and methionine, however, are not limiting amino acids in low albumin diets and their addition, either singly or together, did not improve growth in rats fed these diets.

Although histidine and lysine are calculated to be more limiting for growth than threonine in albumin diets, no growth response was obtained when the first two amino acids were added to the diet, while the addition of threonine alone resulted in an increase in growth in every case. It is possible that the threonine of albumin is not completely available to the rat. Pecora and Hundley ('51) found that although rice diets were presumably more deficient in lysine than in threonine, no growth response was obtained unless both threonine and lysine were added to the diets.

The addition of lysine to the albumin diet supplemented with valine and threonine caused an unfavorable amino acid

ratio for growth in the rat. A more severe histidine deficiency was probably produced since the addition of histidine to the diet brought the growth back to the level obtained when only valine and threonine were added to the diet.

Amino acid balance and deposition of liver fat

The liver fat results support the view that the ratio of the amino acids present in the diet affects markedly the deposition of fat in the livers of rats fed low-protein diets containing choline. The fact that threonine is more effective in reducing liver fat in rats fed certain proteins than it is when other proteins are fed, even when the actual amounts of threonine in the diets are the same, indicates that other amino acids are involved. Threonine is most effective in reducing liver fat deposition in rats fed casein, somewhat less effective when beef is fed, and still less active when pork and albumin are the dietary proteins.

The balance of amino acids in fibrin, supplemented with methionine, seems to be such that no fat accumulates in the livers of rats even when the protein is fed at as low a level as 6%. Although 9% of fibrin supplies the requirement of the rat for threonine, a threonine deficiency exists when lower levels are fed.

Since threonine is less effective in reducing the deposition of liver fat in rats fed 6% casein diets than in those fed 9% casein diets, it was suggested that amino acids other than threonine supplied in larger amounts by the higher level of casein are necessary for normal fat deposition (Harper et al., '54). Normal levels of liver fat are not obtained when threonine alone is added to 9% albumin diets but fat deposition is normal when lysine and histidine are added along with threonine. Lysine is not active at all unless the other amino acids are present. This further indicates that the actual amounts of certain essential amino acids in low protein diets is not the controlling factor in liver fat deposition under these conditions but that the accumulation of fat is also affected by the levels of other limiting essential amino acids.

The presence of certain non-essential amino acids in proteins may also be of importance as shown by the action of gelatin and glycine. The marked effect of glycine in albumin diets is not readily explained. Preliminary studies have indicated that its action is different from that of threonine but further investigation is required to clarify this point.

The suggestion of Singal et al. ('53) that an accumulation of liver fat may be a general sign of an amino acid deficiency is probably an over-simplification. Although in their experiments, in which they used diets markedly deficient in only a single amino acid, the accumulation of liver fat was apparently due directly to a deficiency of one amino acid, the results obtained in this and in a previous study (Harper et al., '54) indicate that the situation is actually much more complex. The results with the fibrin diets (table 1) and results with casein diets not supplemented with the sulfur amino acids (Harper et al., in press) indicate that the deposition of liver fat may be perfectly normal in animals receiving diets severely deficient in amino acids. Even the albumin diets supplemented with histidine, lysine, and threonine are still deficient in terms of their ability to support normal growth. However, the improvement in the balance of amino acids in the diet, even though the diet may still be deficient in amino acids, is sufficient to maintain normal fat deposition.

Flodin ('53) has suggested that there is an optimum amino acid balance for growth, that at any given concentration the rate of tissue synthesis is at a maximum when the proportions of amino acids absorbed are the same as those in which they are used in the "anabolic center." If they are not in the same ratio, the rate of tissue synthesis decreases. By analogy, it is possible that a specific amino acid ratio may be required for the maintenance of normal liver fat deposition.

SUMMARY

Fat which accumulates in the livers of rats fed low protein diets containing pork, beef, or egg albumin is reduced when the level of the dietary protein is increased. Supplementation

of these diets with gelatin or fibrin or with threonine and glycine lowered the amount of liver fat. Lysine in the presence of additional histidine and threonine further decreased the amount of fat in the livers of rats fed low protein diets containing egg albumin. Excessive deposition of liver fat was not observed when low protein diets containing fibrin were fed.

It is suggested that the maintenance of normal fat deposition in the livers of rats fed low protein diets containing choline depends upon the presence of a specific ratio of amino acids in the diet.

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PROCEEDINGS OF THE EIGHTEENTH ANNUAL MEETING OF THE AMERICAN INSTITUTE OF NUTRITION

CONVENTION HALL, ATLANTIC CITY, NEW JERSEY

APRIL 12–16, 1954

COUNCIL MEETINGS

Council meetings were held in the Ambassador Hotel on Sunday, April 11, and Monday, April 12. Formal actions of the Council are reported in the following minutes of the two business meetings.

SCIENTIFIC SESSIONS

The scientific program of the Institute consisted of 11 half-day sessions at which 114 papers were presented and two half-day sessions devoted to symposia. On Tuesday afternoon the symposium topic was "Basic and Applied Studies of the Newer B-Complex Factors" and on Wednesday afternoon "What can we do about Food Faddism." Both symposia were well-presented and the attendance was excellent. Ten papers were read by title.

BUSINESS MEETINGS

Two business meetings were held: one at 4:15 p. m. on Tuesday, April 13 and one at 4:00 p. m. Thursday, April 15. The following items were considered:

Tuesday, April 13. The meeting was called to order by President Conrad A. Elvehjem. The minutes of last year's business meetings, as published in the September 1953 issue of the *Journal of Nutrition,* were approved.

The President appointed Drs. B. S. Schweigert and N. B. Guerrant to serve as the Tellers' Committee for counting the

ballots for the annual election of officers. The Secretary transmitted the ballots to the Tellers' Committee.

The Council's recommendations on nominees for election to membership in the American Institute of Nutrition were presented. The recommendations were unanimously approved and the following were elected to membership:

John G. Bieri
Joseph H. Burchenal
Carey W. Carrick
Gerald F. Combs
Charles A. Denton
Ercel S. Eppright
Karl A. Folkers
Samuel M. Greenberg
Frederic W. Hill
Cloy B. Knodt
Carl H. Krieger
Albert L. Lehninger
Robert W. MacVicar
Barbara McLaren
Edwin T. Mertz
Edward H. Reisner, Jr.
Milton L. Scott
Betty F. Steele
Lester J. Teply
Calvin W. Woodruff

The report of the Treasurer, Dr. O. L. Kline, was submitted. The Auditing Committee, Drs. Joseph S. Butts and George Briggs, reported that receipts and disbursements were properly recorded in the Treasurer's books and that his report as of April 1, 1954, was substantiated by the records. The continuation of the $1.00 annual dues for next year, as recommended by the Treasurer and approved by the Council, was approved.

The Secretary, Dr. J. M. Orten, reported that as of April 1, 1954, the membership of the Institute included 372 active members and 34 retired members, totalling 406. Three members died during the past year:

James S. McLester, February 7, 1954
Howard B. Lewis, March 7, 1954
Walter C. Russell, March 10, 1954

The annual report of the Editor of the *Journal of Nutrition* was presented by Dr. George R. Cowgill. The report follows:

Annual Report of the Editor of the Journal of Nutrition

This annual report covers the volumes of the Journal of Nutrition published during 1953 as well as general matters

arising since the last annual meeting of the American Institute of Nutrition in Chicago, April 7, 1952.

Papers published and related data

Three volumes — numbers 49, 50 and 51 — were published during 1953. Each began with a biography. In addition, each volume contained 56, 40 and 50 articles, respectively, which, counted with the three biographies, give a total of 149 for the year compared with 142 for the previous year. The average number of pages per article proved to be 12.4; the corresponding figure for the previous year is 12.6 pages. During the year 184 manuscripts were received of which 20, or 10.9%, were rejected. The corresponding figures for the previous year are 197 submitted, and 37, or 20%, rejected.

Picture on front cover

In continuation of the policy adopted in 1949, the first issue of each volume carried a picture in the oval on the front cover. A larger picture was used as a frontispiece accompanied by a short biography. The pictures used in 1953 were those of Bunge, Lind and Hart.

Personnel of Editorial Board

At the last annual meeting three members retired after having served the usual term of four years. They were William J. Darby, D. M. Hegsted and Pearl P. Swanson. The new members elected to replace them were E. W. Crampton, Gladys A. Emerson and Ruben W. Engel.

Editorial problems

These continue to be much the same as those encountered in previous years. We have continued the policy of having a meeting of the editorial board sometime during the Federation meetings. At this time our editorial problems are reviewed and any matter relating to the welfare of the Journal

and its management is considered. This is mentioned here so that members of the American Institute of Nutrition will understand that the policies followed by the editor represent the considered opinion of the editorial board as a whole.

For the first time in my experience as editor the number of papers published in two successive monthly issues was unusually low, namely 8 in the July issue and 8 in that for August. Fortunately, this was offset by a greater number of papers being offered later, with the result that the total number published and number of pages printed for the entire year actually turned out to be greater than for the previous year. This should serve to illustrate one kind of problem which the editor of a scientific publication such as ours must face at times.

I wish to record my appreciation of the services rendered by the various members of the editorial board. The society is in debt to them for the time and effort they put into reading and evaluating manuscripts offered. Thanks should also go to the staff of the Wistar Institute which has the responsibility of getting the manuscript safely through the printing shop and finally published in our Journal. If all contributors were to read carefully the instructions which are printed on the inside back cover of each issue, the work of the editor's office and that of the printing shop would be greatly reduced. Greater cooperation to this end on the part of the members of the society would be deeply appreciated.

A motion expressing appreciation to Dr, Cowgill for his excellent administration of the Journal during the past year was unanimously passed.

The following reports from the Standing Committees were presented. Dr. L. A. Maynard, alternate to Dr. W. C. Russell, as representative to the Division of Biology and Agriculture, the Agricultural Research Institute, and the Food and Nutrition Board of the National Research Council reported on the actions of these groups during the past year.

Dr. Joseph H. Roe, representative to the 1953 meeting of the Agricultural Board and the Agricultural Research In-

stitute, reported on actions taken at this meeting. A summary of Dr. Roe's report is on file in the Secretary's office.

The representatives to the Joint Committee on Nomenclature with the Society of Biological Chemists, Drs. C. G. King and E. M. Nelson, reported that no problems on nomenclature had been referred to the Committee during the past year.

The Secretary read a report from Dr. Herbert Pollack, Chairman of the Committee on the Registry of Pathology of Nutritional Diseases, stating that arrangements have been completed with the East African Medical Survey and Filiariasis Research Commission for the receipt of complete biochemical data and liver biopsies on eight cases of kwashiorkor. Further work has been done on the histology of the starvation syndrome by the medical nutrition laboratory of the Office of the Surgeon General.

The representatives to the American Association for the Advancement of Science, Drs. J. H. Roe and F. J. Stare, reported that the Symposium "Comparative Nutrition Requirements of Animal Species" presented at the Boston meeting in December and cosponsored by the American Institute of Nutrition and Section N (Medical Sciences) of the AAAS was of outstanding interest and exceptionally well attended. Our representatives attended all Council meetings of the AAAS in Boston.

A summary of recent actions of the Federation Board was presented by the President, an important one of which is the probable purchase of a permanent home for the Federation in Bethesda, Maryland, near the National Institutes of Health. Slides of the building and grounds were shown later.

Recent actions of the Council were summarized by the Secretary. These included plans for next year's meetings with trial "conjoint" sessions with the other Societies of the Federation on three or four selected subjects, the continuing of a "time scheduled" program as was done this year, and the appointment of Standing Committees (with the exception of the Nominating Committee) by the incoming

rather than the retiring President as has been done by tradition in the past.

The meeting was adjourned at 5:10 p. m.

Thursday, April 15. The meeting was called to order at 4:00 p. m. by President Elvehjem.

The Tellers' Committee reported the results of their count of the ballots. A total of 230 ballots was cast. The following officers were declared elected for the year beginning July 1, 1954:

President: George R. Cowgill
Vice-President: W. Henry Sebrell, Jr.
Secretary: Ruben W. Engel
Councillor: James M. Hundley
Associate Editors (four-year term beginning May 1, 1954):
Grace A. Goldsmith
W. D. Salmon
L. D. Wright
C. R. Grau (to replace R. W. Engel) — for three-year term.

On the basis of suggestions given on the ballots President Elvehjem appointed the following Nominating Committee for 1954-55:

Wendell H. Griffith, Chairman
Paul L. Day
Joseph H. Roe
Douglas V. Frost
Icie Macy Hoobler

Under the heading of new business, Dr. Paul E. Howe suggested the topic "Translation of Nutritional requirements into Food" as a possible symposium topic for next year.

President Elvehjem announced that the Third International Congress of Nutrition is to be held in Amsterdam on September 13 to 17 inclusive, 1954. Dr. Paul Gyorgy was appointed to represent the American Institute of Nutrition.

The Third International Congress of Biochemistry is to be held in Brussels, August 1 to 6, 1955. Members of the American Institute of Nutrition are invited to participate. Circulars giving the necessary information will be mailed to members of the Institute sometime this summer.

The Secretary announced that both the Borden and Osborne and Mendel Awards will be available for presentation again next year.

The President announced that next year's meeting will be held in San Francisco, April 10 to 15, 1955.

The meeting was turned over to the new President, Dr. George R. Gowgill for a few comments and then was adjourned by President Elvehjem at 4:45 p. m.

ANNUAL DINNER AND PRESENTATION OF AWARDS

The Annual Dinner of the American Institute of Nutrition was held on Wednesday evening, April 14, in the Madison Hotel and was attended by a record group of 271 members and guests. The evening was a most pleasant one, climaxed by a program consisting of the introduction of new members, the presentation of Awards, and a few remarks by Dr. George R. Cowgill commemorating the Twenty-fifth anniversary of the publication of the *Journal of Nutrition.* Dr. Cowgill spoke in place of Dr. John R. Murlin, the first editor of the Journal, who was unavoidably absent because of an automobile accident.

The Borden Award in Nutrition was presented jointly to Dr. Agnes Fay Morgan of the University of California in Berkeley and Dr. Arthur H. Smith of Wayne University in Detroit, for their research contributions on the nutritional significance of the components of milk and particularly the effect of heat on the biological value of milk proteins. Dr. Morgan's achievements in nutrition were described by Dr. Thomas H. Jukes and Dr. Morgan responded with reminiscences of her work and with wise counsel to young investigators in the field. Dr. Harry J. Deuel, Jr. introduced Dr. Smith recounting their associations as graduate students at Yale and subsequently, following graduation. Dr. Smith responded urging nutritionists to remember not only their responsibilities to science but also their broader responsibilities to society as well.

The Osborne and Mendel Award was presented to Dr. Leonard A. Maynard of Cornell University in Ithaca "for his investigations on biochemical and nutritional aspects of metabolism and lactation and for his contributions as a teacher, administrator, and public servant in the field of nutrition." Dr. E. W. Crampton introduced the medalist with humorous and delightful comments on his background. Dr. Maynard responded with reminiscenses of his work in nutrition and of his associations with Professor Lafayette B. Mendel in whose honor the Award has been named.

COMMITTEES FOR 1954–55

The incoming President, Dr. George R. Cowgill, has appointed the following Standing Committees for the year beginning July 1, 1954:

Committee on Registry of Pathology of Nutritional Diseases

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

Consultants

P. R. Cannon
Paul Klemperer

Representatives to the Joint Committee on Nomenclature

C. G. King
E. M. Nelson

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 (for a term of three years)

Representatives to the American Association for the Advancement of Science

Joseph H. Roe, Section N (Medical Science) — one year
Vernon H. Cheldelin, Section C (Chemistry) — two years

Respectfully submitted,
JAMES M. ORTEN, *Secretary*
American Institute of Nutrition

BORDEN AWARD IN NUTRITION

Nominations are solicited for the 1955 Award and a gold medal made available by the Borden Company Foundation, Inc. The American Institute of Nutrition will make this award in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of the components of milk or of dairy products. 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 honor.

The formal presentation will be made at the annual meeting of the Institute in the spring of 1955. To be considered for the award, nominations must be in the hands of the Chairman of the Nominating Committee by January 1, 1955. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate consideration for the award.

Chairman, Nominating Committee:

W. D. SALMON
Animal Husbandry and Nutrition
Alabama Polytechnic Institute
Auburn, Alabama

OSBORNE AND MENDEL AWARD

Nominations are invited for the Osborne and Mendel Award of $1000.00 established by the Nutrition Foundation, Inc., for the recognition of outstanding accomplishments in the general field of exploratory research in the science of nutrition. It shall be given to the investigator who, in the opinion of a Jury of Award, has made the most significant published contribution in the year preceding the annual meeting of the Institute, or who has published a series of contemporary papers of outstanding significance.

The Award will be presented at the annual meeting of the American Institute of Nutrition.

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.

Nominations may be made by anyone. Nominations for the 1955 Award, accompanied by data relative to the accomplishments of the nominee, must be sent to the Chairman of the Nominating Committee before January 1, 1955.

Chairman, Nominating Committee:

DR. FLOYD S. DAFT
Institute of Arthritis and Metabolic Disease
National Institutes of Health
Bethesda, Maryland