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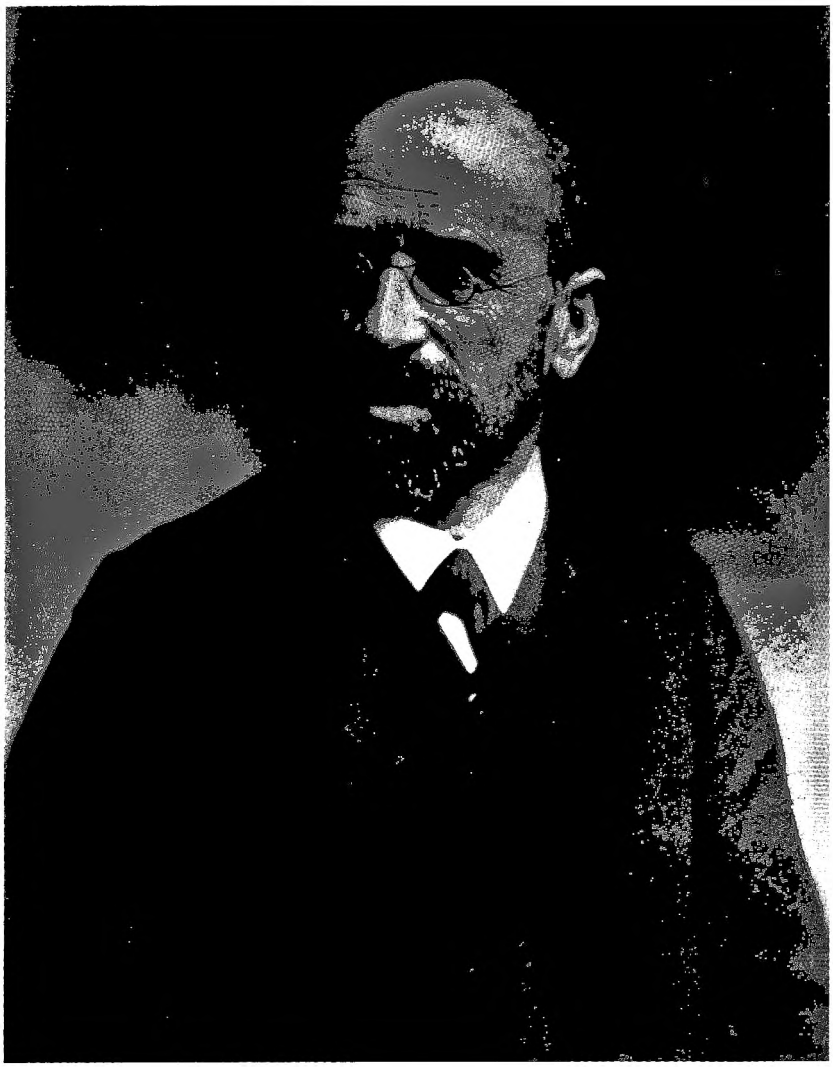
NATHAN ZUNTZ

(1847–1920)



NATHAN ZUNTZ

(October 7, 1847–March 23, 1920)

Nathan Zuntz was born on October 7, 1847 in Bonn, Germany, to Leopold and Julie Katzenstein Zuntz. His father was a merchant by trade and a profound and esteemed scholar of Hebrew history by avocation. Nathan, the eldest of 11 children, was early recognized as possessing a fine mind with a scientific inclination. Yet his first job, acquired at the insistence of relatives who hoped to interest him in business, was as an apprentice in a Bonn banking house. This phase of Nathan's career was short-lived as a result of his tipping a full bottle of ink on the ledgers! He was soon free to pursue his livelihood along lines more to his choosing.

As a boy, Zuntz was averse to convention and eagerly escaped his studies for the outdoors where he could indulge himself in his great love of natural phenomena. In spite of this he was an obedient and apt scholar, and it is reported that at the age of 4½ he was able to read the Bible in Hebrew, being coached in this enterprise by his father to whom learning and knowledge were almost religious duties.

The formal education of Zuntz was accomplished with dispatch. He finished at the gymnasium at 17, a year ahead of the usual, and immediately entered the study of medicine at the University of Bonn. He studied chemistry under Kekulè, physics under Clausius, and physiology under Pflüger. These three men, Pflüger in particular, greatly influenced his future interests. Zuntz used chemistry and physics effectively as tools to further his studies in his chosen field of physiology. Under Pflüger's direction he prepared his doctoral thesis, "Beiträge zur Physiologie des Blutes," in 1868. It concerned the binding of carbonic acid in the blood and the migration of carbonic acid between blood cells and plasma, and showed

that carboxy hemoglobin is a dissociable, not firmly bound, compound. After finishing his medical studies at 21, Zuntz spent some time as a rural physician in Oberpleiss in the Siebengebirge, but in 1869 went to Berlin for a semester to hear lectures by Frerichs, Virchow and Traube. In April 1870 he returned to Bonn, continuing his medical practice, and becoming assistant to Pflüger. At the same time he was appointed Privatdozent in Physiology at Bonn, and during the war of 1870–71 served as a civilian physician at the Bonn hospital.

During these years he worked 14 hours a day — a habit he continued through most of his working life. In 1873 he was elevated to assistant lecturer in physiology at the Landwirtschaftliche Akademie at Poppelsdorf and married Friederike Bing, who subsequently bore him three children. Also during this period Zuntz' parents died, leaving him as guardian of his 9 sisters. In 1874 he was named Extraordinary Professor of Anatomy at the Bonn Medical faculty. He remained at Bonn 6 more years, teaching and doing research in physiology and maintaining a private practice as a physician. In 1880 Nathan Zuntz made his final professional move when he went to Berlin to occupy the chair of teaching animal physiology at the newly established Landwirtschaftliche Hochschule. His appointment to this post was the result of the recommendation of Thiel, at the Poppelsdorf Akademie, who was impressed by the industriousness of the younger man. Upon leaving Bonn, Zuntz gave up his medical practice and in Berlin practiced medicine rarely and then only among his friends. The promise of greater freedom in research probably was an important factor in Zuntz' decision to join the Berlin agricultural faculty. Here was a unique opportunity to attack fundamental research problems on a variety of animal species under various conditions and to combine these fundamental studies with practical recommendations. At first the laboratory space was very small but this did not prevent extensive research. Later the laboratory was expanded to cover two floors rather than be

restricted to the original two rooms. In 1909 Zuntz drew up plans for a new laboratory designed specifically for his own needs. These plans called for divisions of pure chemistry, experimental physiology, metabolism research, bacteriological research and physical research as well as a dark room for optical work. Kurt Lehman was the first assistant Zuntz added to the staff. Soon Prof. Hagemann, who was already famous for his horse research at Poppelsdorf, joined the group. Other early members of the staff were Neuberg in chemistry, Caspari for physiology, Cronheim for fish research. The individual divisions made investigations of their own and also cooperated with one another in prosecution of larger problems. It was Zuntz' idea that there should be intermingling of the specialties in the attack on larger research problems.

Younger investigators were attracted to this laboratory not only from Germany but from other lands. They found Zuntz a man of keen understanding and wisdom and a helpful and kindly man who would listen to their ideas and give suggestions as to how these ideas might be tested in the laboratory. One of Zuntz' talents was devising methods and constructing apparatus, in view of which Loewy has called him, "Erfindungsreich."

Zuntz had an extraordinary knowledge of the literature of the day and a retentive memory. He had the habit of reading scientific reports with pen in hand, and made notes appraising the work on margins of the paper or on separate sheets. This habit and his exhaustive memory were invaluable for the numerous comprehensive and critical reviews which he produced on physiological questions. His aim was to establish the current state of a problem clearly and sharply.

He gave manifold helpful suggestions to his students in a spirit which wove a strong bond between Zuntz and these younger scholars and carried over into their later positions. One of his papers expresses his critical viewpoint and human quality in a concluding statement, acknowledging that he had contradicted many a highly esteemed investigator but

that he hoped the criticism would be taken as an effort to advance the cause and not as an expression of personal anger.

Zuntz spent the summer of 1908 in the United States, sharing the instruction in a course in biochemistry presented at the Third Session of the Graduate School of Agriculture of Cornell University at Ithaca, N.Y. Others participating in the instruction were: Dr. C. F. Langworthy, Dr. A. L. Winton, Dr. L. B. Mendel and Dr. H. P. Armsby. Zuntz presented 5 formal lectures and two seminars. He brought some of his respiration apparatus with him and demonstrated its use. It is reported that his lectures were of unusual interest and his visit of great value to scholars at the school because of the information and inspiration they provided.

Up to his 70th birthday he had 430 publications to his credit, most of which are listed in Landwirtschaftliche Jahrbucher, *51,* 329 (1917).

His major investigations were on the subjects of blood and blood gases, blood circulation, mechanics and chemistry of respiration, general metabolism and nutritional science, special metabolism of various foods, energy metabolism and heat production, digestion and absorption processes.

During his time at Bonn, in addition to his doctoral dissertation, he published with Pflüger a paper on the influence of acid on the content of O_2 in the blood. A second important research concerned the proof that the combination of carbon dioxide and hemoglobin is not firm, as was believed, but dissociable by oxygen. These findings formed the theoretical basis for the successful revival of carbon dioxide-poisoned persons by administration of oxygen by inhalation. A further important research during this era resulted in a publication with Rohrig, "Zur Theorie der Wärmeregulation" (Pflügers Arch. f.d. ges. Physiol., *4,* 1871). This showed that curarized rabbits lost the power of maintaining body temperature and that the metabolic rate decreased by half. Thus it was deduced that there was a reflex connection between skin and skeletal muscle and that one of the skin reactions to cold was to in-

crease muscle activity to increase metabolism. A further deduction was that maintenance of muscular tonus accounted for a large part of the total maintenance energy requirement. This is the basis of what was later called chemical heat regulation.

Joseph Barcroft gives us an insight into the disciplinary problems of research in those days with reference to Zuntz' incomplete studies of the effect of innervation on muscle metabolism. "Being struck with the fact that so important a research had been left by Zuntz in an obviously fragmentary condition, I asked him one night when we were in Teneriffe why he had never finished it. His reply, as nearly as I can remember it, was as follows: "When I commenced those experiments, I was assistant to Pflüger at Bonn. Pflüger came round one day and finding me at work said, 'What are you doing?' I replied, 'I am testing the effect of abolition of tone on muscular metabolism.' Pflüger said, 'Well, but you have not asked my permission to do this. Either you must stop these experiments or leave my laboratory.' I was not in a position to leave the laboratory, so I stopped the experiments.' "

Zuntz originated the concept of work of digestion as an explanation for increased metabolism following consumption of food. For example, V. Mehring and Zuntz, in a brief note (Pflügers Archiv. f.d. ges. Physiol., *15,* 634 [1877]) reported that of materials injected directly into the blood of a dog, lactic acid, glycerol or glucose did not markedly stimulate the metabolic rate, but peptone did so to a remarkable degree. On the other hand these 4 nutrients, as well as materials such as sodium sulfate, all increased oxygen consumption when fed. They concluded that the split products resulting from digestion of food protein account for the principal specific dynamic action (SDA) of the protein fed.

Prior to this they had concluded that the observed increases in metabolic rate following the feeding of nutrients, which indeed was greatest for protein and least for fat, was due to work of digestion.

By the time Zuntz went to Berlin he had, as we can see, accomplished much research. His ideas on nutrition at this time were clearly set forth in a review article published in Landwirtschaftliche Jahrbucher p. 65–117 for 1879. His fundamental viewpoints included the opinion that scientific investigation of animal nutrition had been impossible until the laws of chemistry and physics revealed the indestructibleness of matter and conservation of energy. He stated that, "Leben ist Stoffwechsel," and that oxygen rewinds the watch of life. He subscribed to Pflüger's unique theory of muscle contraction which stated that carbon and oxygen are loosely bound in muscle; stimulation increases their oscillation so they can join to form CO_2 and thus, by drawing closer, cause muscular contraction; CO_2 then comes off, the fibrils stretch again and new room for O_2 and other radicles is created.

On nitrogen metabolism, Zuntz recognized $N \times 6.25$ as an approximation and that proteins of equal nitrogen content could promote growth differently. He regarded non-protein nitrogen as useless for building protein in animals, so suggested that dialysis precede analysis of animal feeds for nitrogen.

He concluded that carbohydrate can form fat as well as produce energy, in contradiction to the teaching of Voit, and that a constant ratio for energy value of carbohydrate and fat in rations was not a realistic viewpoint due to the high work of digestion, caused by cellulose in the diet. He realized that fermentative bacteria were responsible for cellulose degradation in the rumen and that lactic and butyric acids, CH_4 and CO_2 were produced by this action and considered the acids to be of some use in the body as sources of energy.

Low environmental temperature would, he realized, increase metabolic rate by increasing metabolism of nitrogen-free substances. He visualized nitrogen metabolism as influenced only by the amount of nitrogen in the blood stream, while nitrogen-free metabolism was influenced by mechanical work, work of the digestive tract, sensuous impressions (wake vs. sleep) and temperature.

At this time he regarded the two major problems facing nutrition investigators as (1) evaluation of nitrogen content of feeds, and (2) evaluation of the different nitrogen-free constituents as influenced by type and presence of other constituents. He realized that this information could only be obtained through complete energy and nitrogen balance studies.

Thus we see the thinking of the young, yet well-established scientist at the time he went to Berlin.

It was one of Zuntz' basic premises that the manifestations of life could best be investigated through studies of oxygen consumption and the production of carbon dioxide and water by animals. In order to satisfy his desire for maximum accuracy and convenience, Zuntz modified the Pettenkofer respiration apparatus to make it possible not only to measure carbon dioxide production but also oxygen consumption. Actually Zuntz modified this apparatus several times, but best-known is the modification of Zuntz and Geppert which measured and took continuous aliquots of undiluted expired air and permitted analysis of the aliquots for CO_2 by absorption on KOH and then for O_2 by absorption on phosphorus. The original Zuntz-Geppert apparatus was made more portable by substituting for the relatively bulky wet-gas meter a much more compact dry-gas meter. This made possible for the first time investigations on respiratory metabolism far afield from the laboratory. In addition to its portability, the Zuntz-Geppert apparatus made possible the determination of respiratory exchange in relatively short periods by virtue of the greater concentration changes for the respiratory gases collected by mask than collected by inserting the whole animal in a chamber. Zuntz also made use of the Regnault-Reiset apparatus for many of his laboratory experiments, having constructed one large enough to contain a horse, and one for dogs in which tightness of the apparatus was made certain by immersing the entire chamber in water. One of his more unique pieces of apparatus was a respiration apparatus for fish.

Zuntz was of course interested in the subject of basal metabolism and made many measurements designed to study the influence of various factors on the basal rate of tissue oxidation. This led, ultimately, to his being credited by both Lusk and DuBois with first devising the proper technic of measurement of basal metabolism. Probably his most significant contribution to this technic was the observation that absolute muscular rest was necessary for accurate results. He was also interested in determining the factors which made up the basal metabolism and although he did not accomplish this in detail he did calculate that respiratory work and work of the heart accounted for 4.7 and 4.0%, respectively, of the basal metabolism of the horse. He carefully measured his own basal metabolism at intervals over a 29-year span and found a significant decrease during 1916–17, interpreting this as a result of undernutrition consequent to the food shortage created by the war.

In connection with his intense concern with respiratory metabolism, Zuntz made one of his most significant contributions by taking full advantage of the opportunities his unique apparatus afforded for accurately determining respiratory quotients. The table of Zuntz and Schumburg, showing caloric values of oxygen at different RQs, is still widely used in modified form.

One result of his studies of the respiratory quotient determined under a variety of conditions was to provide sound evidence as to the fuel for muscular exercise. He presented this evidence in a number of papers and reviews, and finally concluded that all three major nutrients may be used to supply the required energy but that ordinarily protein was not the preferred fuel. He did believe that vigorous exercise required protein for optimum performance but that for long-continued moderate labor, carbohydrate and fat would serve well. His opinions along this line were derived largely from his respiration experiments, in part from observations of food habits of laborers and athletes, and in part from studies which

showed increased urinary excretion of nitrogen to accompany hard muscular exercise.

Shortly after assuming his duties in Berlin, Zuntz published a discussion, "Bemerkungen über die Verdauung und den Nährwerth der Cellulose" (Arch. f. d. ges. Physiol. 49, 477–483 (1891), whose conclusion is widely quoted today as evidence that he was the first to suggest that the rumen flora played a role, not only in cellulose utilization (which was established experimentally by Tappeiner in 1884), but also in utilization of non-protein nitrogen by ruminants. He further advanced the hypothesis that amides such as asparagine might protect dietary protein from being fermented or assimilated by the flora in much the same way that soluble carbohydrates divert the microbiological action away from cellulose. At the same time he suggested that both starch and cellulose are attacked by the rumen flora since these substances were found to have equal energy value for ruminants; these materials he found of unequal value to the horse due to the "work of digestion" required by cellulose in this latter species. The falsity of the isodynamic replacement value of fat, starch and cellulose was interpreted by Zuntz as being largely due to fermentative action in the intestinal canal. Zuntz considered the work of digestion to result mainly from the effect of food in accelerating digestive secretions and muscular activity of mastication and of the intestinal tract itself, and realized that the energy thus spent would be useful to animals in a cold environment but that it was a waste in a warm environment and could not be converted to external work in any case. He made extensive investigations on the work of digestion in the horse, finding this to be about 9% of the resting metabolism 36 minutes after ingestion of a mixed ration of oats, straw and hay, with a further increase at the end of three hours. In further calculations he came to the remarkable conclusion that the work of digestion of crude fiber fed to a horse is greater than the metabolizable energy of the digested fiber. This calculation has been justly criticized by Armsby, yet it must be admitted

that the heat production increase following ingestion of high crude fiber ration by the horse is greater per unit of digested feed than if a low fiber ration is consumed.

Zuntz calculated the net energy value of feeds for horses, and although his methods were open to question he should be given credit for being the first to observe that the heat increment of feeding hay is greater than of grain and to point out that a correction should be made for this in metabolism studies.

The efficiency of mechanical work is a phase of energy metabolism which Zuntz investigated extensively in his work on the dog, man and particularly the horse. He found that unless the work load or speed was excessive, these three species could convert about one third of the potential energy of their food into mechanical work after making allowance for basal expenditures. In an article entitled, "Ueber den Stoffverbrauch des Hundes bei Muskelarbeit," appearing in Arch. f. d. ges. Physiol. 68, 191–211 (1897), he reports that about one third of the chemical energy in food can be used for external work, that the smaller the animal the greater the energy required for forward motion of equal mass through equal distance, and that the energy expended is nearly proportional to the surface area of the body.

In "Studien zu einer Physiologie des Marsches," Berlin 1901, Zuntz and Schumburg make a detailed report of studies dealing with metabolism in man as affected by training, type of work done, load carried, etc. Most of these investigations were carried out with 5 military students and included observations on effect of muscular work on heart, liver, condition of blood, capacity of lungs, elimination of nitrogen by skin, urine, feces and on the respiratory exchange. They found that training would definitely reduce the expenditure of energy for a specific job but did not influence the expenditure required for an unfamiliar variety of work.

In "Höhenklima und Bergwanderungen," Deutsches Verlagshaus Bong, 1906, Zuntz, Loewy, Muller and Caspari present a theoretical calculation and from it derive an astounding

conclusion. They determined by indirect calorimetry that the work of ascending a mountain required 2.353 calories for each kilogram lifted one meter, and stated that this energy is not converted into heat because it remains in the tissues as energy of position of the ascending body; it is, however, derived from muscle metabolism. Thus, they theorized, for each kilogram lifted one meter, 2.353 calories less heat are created than would correspond to the heat of combustion of the metabolized substance, and equally less than would be formed through metabolism of the same quantity of nutrients during horizontal walking. Moreover, during descent the energy of position which was stored through lifting the body will push the body downwards; the descent must be retarded by muscle tension and, just as in stretching a muscle through an attached weight, an amount of heat will be set free in the muscles, which does not come from chemical energy.

In "Untersuchungen über den Stoffwechsel des Pferdes bei Ruhe und Arbeit," (Landwirtschaftliche Jahrbucher 27, Ergänzungsband 3, 1898, 440 pp), Zuntz and Hagemann have summarized their long series of experiments on digestion and respiratory metabolism of the horse. These have been discussed in some detail by Armsby ("The Nutrition of Farm Animals," The Macmillan Company 1917, and "Principles of Animal Nutrition," John Wiley and Sons 1903). The major portion of the respiration studies (several hundred) were performed on a single horse. For these experiments on work production the animal was placed on a special "tread-power" which could be operated on the level or inclined; it could be driven by a steam engine, and in experiments on work of draft the animal pulled against a dynamometer. The experiments were individually of short duration and as many as 5 were made in one day under conditions of rest and varied types of work. The work of locomotion was found to vary with the speed so that in experiments on work and draft and of ascent or descent corrections were made for the speed of performance, there being an increase in expenditure of about 20% as the speed of walking increased from 78 to

98 meters per minute. These investigators found that whether work was performed at a walk or at a slow trot the net efficiency of work of ascent or of draft was about 35% unless the work became excessive, which caused efficiency to decrease.

Zuntz did not restrict his publications to research journals but also found time to popularize his findings in articles for the public and in practical recommendations to livestock raisers. Through his researches on metabolism in muscular work he came in close contact with sports and sought through numerous lectures and writings in sports journals to spread physiological knowledge in these circles. For this he was especially qualified, according to Loewy, by his ability to say complicated things in a simple manner.

In connection with his studies on human metabolism Zuntz organized and participated in three mountain expeditions and two balloon ascensions. On these expeditions as well as in the laboratory, he expressed his authority more as an informed fellow-worker than as an autocratic head; according to von der Heide, it was not so much a matter of working under Zuntz as of working with him.

The medical sciences also gained a good share of his attention, not only through his research reports but through chapters contributed by him to physiological and biochemical texts. A notable example of this is his chapter on nutrient and energy metabolism in the text, "Physiologie des Menschen," edited by Zuntz and Loewy, published in Leipzig 1913.

During the first world war, work in Zuntz' laboratories was turned to the matter of feeding the German people. His council was sought on how best to feed substitute materials to animals so as to keep for direct human use more of the grain products which normally were fed to animals. During the last war years, his heart began to fail, and in the fall of 1919 he was forced to give up teaching; he died in March 1920.

In spite of his long hours of work, Zuntz devoted much time to the affairs of the Berlin Physiological Society of which he

was an influential and revered member, and retained a wide breadth of interest beyond his profession, counting among his close friends men familiar with the fields of mathematics, poetry, philosophy and music. One of Zuntz' children, Leo, followed him into physiological research and published several papers with him. Apparently Leo Zuntz was also a physician since many of his papers are concerned with metabolism as affected by alterations in the reproductive cycle in the human.

The following quotation from an article (Exp. Sta. Rec. 7, 758–550, 1895–6) by Nathan Zuntz perhaps serves to explain the philosophy underlying his manifold and varied researches: "A scientific treatise on nutrition must have for a foundation the clearest possible explanation of the changes which the nutrients undergo in the organism and the function for which each is fitted."

ACKNOWLEDGEMENT

I am pleased to acknowledge the following assistance freely given me in the preparation of this sketch: Cambridge University Press, for permission to quote from Joseph Barcrofts' "Features in the Architecture of Physiological Function," Professor C. M. McCay of Cornell University, Paul Griminger, Marjorie Edman, and Alma White, University of Illinois, for bibliographic and translational aid. The two most complete accounts of Zuntz career are: "Dem Andenken an Nathan Zuntz," by A. Loewy. Arch. f. d. ges Physiol. 194, 1–19 (1922), and "Nathan Zuntz" by R. von der Heide, in Landwirtschaftliche Jahrbucher 51, 329–362 (1918).

R. M. Forbes

THE BIOLOGICAL VALUE OF OILS AND FATS

III. THE LONGEVITY OF RATS FED RAPESEED OIL- OR BUTTERFAT-CONTAINING DIETS

H. J. THOMASSON

Unilever Research Laboratory, Zwijndrecht, The Netherlands

THREE FIGURES

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INTRODUCTION

Previous work (Thomasson, '55a) has shown that the growth-promoting effect of various oils can differ considerably. Rapeseed oil, for example, causes a much slower growth than butterfat. The difference in growth between animals fed these two fats is associated with a different food intake; the rapeseed oil animals eat less than those fed the butterfat.

It is known that by a decrease in the food intake the life span of the animal can be lengthened. In some investigations that have been reported the animals received daily a limited amount of food (McCay et al., '35, '39a, b, '41; Riesen et al., '47; Ball et al., '47), or days of ad libitum feeding were followed by days of fasting (Carlson and Hoelzel, '46, '47).

With rapeseed oil animals a decreased food intake is obtained under ad libitum conditions. It is conceivable that the life span of rats receiving a moderate amount of this oil and showing a distinct decrease in the food intake, is also prolonged, as was observed with a regime of restricted feeding.

EXPERIMENTAL

For this investigation 96 male Wistar rats were used, which were caged individually when 21 days old. On the basis of

their body weights the animals were divided into two groups of 48 each (rapeseed oil and butter group).

The composition of the diets in parts by weight was as follows: ground rice, 33.6; casein, 20.9; dried brewers' yeast, 14.3; dried liver powder, 0.5; salts, 4.3; rapeseed oil or butterfat, 27.0. The butter was melted and the aqueous phase separated from the butterfat by decantation and filtration. The diets contained 23 Cal. % of protein and 50 Cal. % of fat, and were freshly prepared twice a week. Drinking water and food were available ad libitum. The fat-soluble vitamins were administered in the form of a prophylactic dose. At the beginning of the experiment and subsequently every 6 weeks the animals received 0.05 ml of a concentrate containing 2,000 I.U. of vitamin A, 12.5 μg of vitamin D_2, and 10 mg of tocopherol-acetate, dissolved in groundnut oil.

Each animal was weighed weekly. The quantity of food supplied was also determined weekly, but the amount of food actually consumed is not exactly known, since the wastage was not established.

The animals which had died were kept under formol for post-mortem examination. Most of these cadavers, however, were lost in the floods by which this country was afflicted in February 1953.

RESULTS

Longevity

It appeared that the rapeseed oil animals had a longer life than those of the butter group, the mean life spans being 669 and 545 days respectively. Application of Wilcoxon's test ('45) shows that the difference between these two means, 124 days or 23%, is highly significant.

The mortality curve (fig. 1), in which the experimental time is plotted against the percentage of mortality shows that:

1. At the moment that 50% of the butter animals had died (after 513 days), the mortality of the rapeseed oil group was only 17%.
2. After 645 days 77% of the butter animals and 50% of the rapeseed oil animals were dead.

3. The last butter animal died after 822 days, at which time 17% of the rapeseed oil group was still alive.

4. The last rapeseed oil animal lived 1021 days.

On the basis of these criteria the life of the rapeseed oil animals was 20 to 25% longer than that of the butter animals.

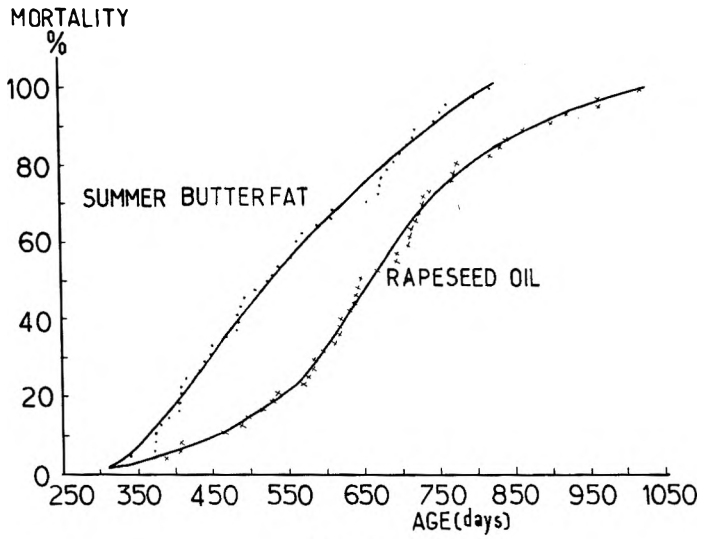

Figure 1

Body weight

The growth rate of the rats of the butter group was much greater than that of the rapeseed oil animals (fig. 2). The difference in weight between the two groups was noticeable on the first day, and steadily increased thereafter. After about 10 weeks the greatest difference, namely about 115 gm (40%), was observed; during the remainder of the experiment it gradually decreased. It is interesting to note (fig. 2) that the plateau (period of relatively unchanged body weight) on the curve for the rapeseed oil animals was maintained for roughly 25 weeks longer than for the rats in the other group. For the butter series this plateau was at a body weight of 400 to 420 gm; for the rapeseed oil group it was at 320 to 340 gm; the difference was 60 to 100 gm, or 15 to 30%.

At the time of death of the animals the mean body weight was 261 gm for those in the butter group, and 243 gm for the rapeseed oil group, considerably below the maximum for each group. The declines in weight were 140 to 160 gm and 80 to 100 gm respectively. Prior to death, therefore, the butter animals lost weight much more rapidly than the rapeseed oil animals. Expressed as a percentage, however, this decrease is approximately the same for the two groups, viz. 33 to 40% for the butter animals, and 33 to 44% for the rapeseed oil animals.

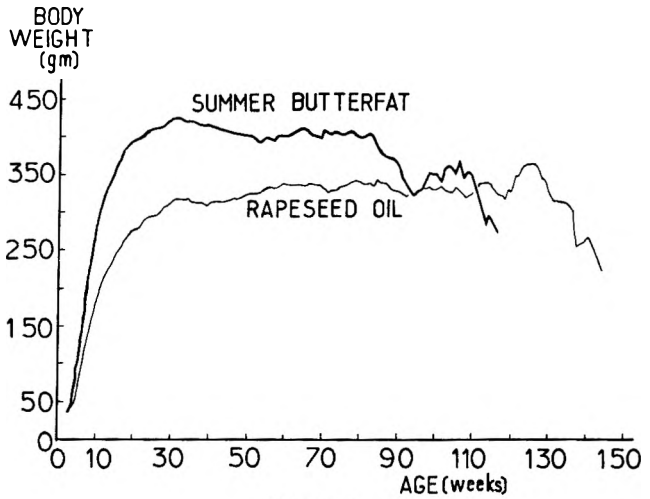

Figure 2

Food intake

Figure 3 records the amount of food consumed by the animals in the course of the experiment. The 10-week food-intake has been averaged per animal per week. As stated previously, the figures have only an orientating value, since they apply to the amount actually consumed plus the wastage. The graph shows that the butter animals consumed more food than the rapeseed oil animals. Over a period of 120 weeks the mean difference is 14%. This result is in accordance with the results of previous short-term experiments which extended

over 6 weeks (Thomasson, '55a). The total amount of food consumed (up to the time of death) amounts to 369,735 and 379,686 gm for the butter and the rapeseed oil group respectively, consequently 7,395 and 7,594 gm per animal. Thus, while the daily food intake is in favour of the butter group, the average consumption over the entire experimental period is nearly the same for both groups.

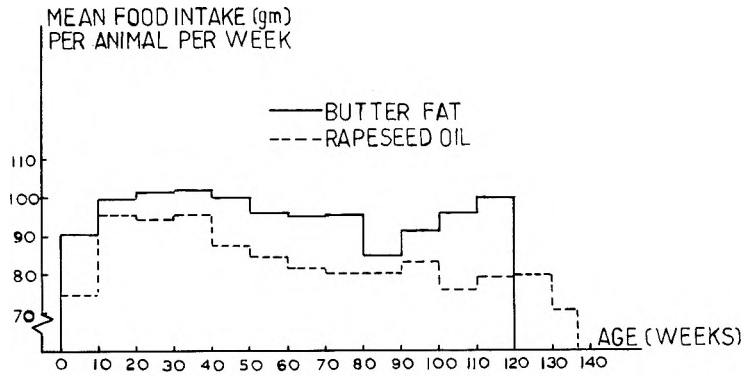

Figure 3

Pathological changes

In the course of the experiment the hind legs of the animals in each group showed cushion-like swellings of solid consistency located at the back of the sole. With the butter animals this change occurred significantly more often than with the rapeseed oil animals. Histological examination [1] showed that the leg-lesions consist of: (1) a defect of the epidermis and (2) a crust of necrotic connective tissue lying at the surface of the defect and separated from the sound tissue by a layer of chronically inflamed connective tissue.

Dr. Jansen further states: "Although the leg lesions were severer and occurred more frequently in the butter fat group than in the rapeseed oil group, the same phenomena were

[1] This investigation was carried out by Dr. M. T. Jansen, Chief Assistant of the Histological Laboratory, Utrecht, Director Prof. Dr. S. F. H. J. Berkelbach van de Sprenkel.

observed in both groups. It appears that the development of the lesion is not caused by failure of the epidermis, but that the epidermis is only secondarily destroyed. In the unaffected parts of the epithelium no differences between the animals of the groups could be detected.''

When found dead in the cage on the daily inspection, the animals were fixed in formol after opening the thorax and abdomen. The principal organs of a restricted number of these cadavers [2] have also been examined,[3] namely, thyroids, parathyroids, oesophagus, lungs, heart, aorta, spleen, liver, pancreas, kidneys, adrenals and testes. From this histological examination it appeared ''that in the livers of some of the rapeseed oil animals a slight indication was found of fat deposition and degeneration in the central parts of the lobes, while in the livers of the butterfat animals no abnormalities were observed. Besides it was found that in the kidneys of all the rats an increase of the connective tissue occurred, particularly between the blood vessels of the glomeruli. In a number of cases other parts of the nephron (tubuli contorti) had been destroyed or cystically widened. Although these changes were observed in animals of both groups, the rats of the butter group were more seriously affected than those of the rapeseed oil group.''

DISCUSSION

The present investigation demonstrates that male rats fed ad libitum with a diet containing 50 Cal. % of rapeseed oil live significantly longer than rats fed with a corresponding butter containing diet. The cause of the different longevity of butterfat and rapeseed oil animals is not quite clear. The pathological changes do not give distinct information regarding the cause of death. It is evident that the slight fatty liver of some of the rapeseed oil animals is insignificant in relation to the different life spans of the two groups. As regards

[2] As stated already the greater part of the cadavers was lost in the floods of February 1953.

[3] See footnote 1, page 21.

the kidney changes, however, this explanation is possible, for although these defects occured in both groups, the kidneys of the butter animals were more seriously affected than those of the rapeseed oil group. These kidney lesions, as well as the other changes of the butter animals resemble those found in the investigation by Kennedy ('51). This worker induced obesity in rats by destruction of the hypothalamus, and observed the following phenomena: hyperphagy, high fat content of the animals, shortening of the life span, trophic lesions of the skin ("pressure sores on the feet"), and kidney lesions. Kennedy expressed the opinion that the kidney defects caused the death of his animals.

In view of the relatively slight severity of the change, it is not very probable that the swellings of the hind-legs influenced the life span of the animals. It seems plausible to consider these disorders as a reaction to the irritation caused by the wire bottoms of the cages. In that case it is conceivable that the butter animals showed severer changes than the rapeseed oil animals, since by their greater body weight the irritation could be more intense. However, the analysis showed that there was no relation between the body weight and the severity of the swelling, for the degree of the swelling appeared to be approximately the same for the light and the heavy rapeseed oil animals, and the same applied to the butter animals. Consequently, the leg lesions of the light-weight butter animals were much more severe than those of the heavy rapeseed oil animals, although the body weights of these subgroups differed very little.

The absence of any such correlation is the reason for considering other factors than the higher body weight as responsible for the more serious leg lesions in the butter animals. The essential fatty acid content of butter is slight, corresponding to 1½% linoleic acid, as determined by means of the biological assay (Thomasson, '53), so that the requirements of the butter animals for essential fatty acids can hardly be met. Various research workers have described skin changes in animals deficient in essential fatty acids; special mention

is made here of the thickening of the epidermis, particularly of the stratum corneum, as observed by Williamson ('41), Hansen and Wiese ('51) and Ramalingaswami and Sinclair ('51). A tentative experiment in which three butter animals with pronounced lesions of the legs daily received an oral dose of sunflowerseed oil for 14 days did not support this view, since neither the growth nor the leg lesion responded to this treatment. A prophylactic treatment of butter fat and rapeseed oil animals as was applied in subsequent experiments (Thomasson, '55b) had no effect either. Consequently, formation of the leg lesions is not associated with a deficiency in essential fatty acids.

In all the experiments carried out hitherto in which it was possible to prolong the life span of the animals by dietary control, growth retardation occurred. The question, therefore, arises whether the retarded growth itself, consequently the small body weight, is the cause of a lengthened life span. From the analysis of the data it appeared that the correlation coefficients for the relation between the maximum body weight of each animal and its life span were 0.38 and 0.48 respectively for the butter and rapeseed oil group, and these values are highly significant. First the lightest butter animals die, then the heaviest butter animals, followed by the lightest rapeseed oil animals, while the heaviest rapeseed oil animals live longest. On the other hand a highly significant correlation exists between the number of days after which the maximum body weight was reached and the life span. For the butter and rapeseed oil groups the correlation coefficients were 0.71 and 0.79 respectively. These peculiarities demonstrate that the longer life span of the rapeseed oil animals cannot be explained simply by a slower growth or a smaller body weight.

Assuming that the different longevity of the butter fat and rapeseed oil animals is based on the decreased food intake of the latter group, the active substance would be known with fair probability, since the growth- and appetite-inhibiting substance in rapeseed oil was formerly identified as erucic acid (Thomasson and Boldingh, '55c).

Carroll ('51, '53) and Carroll and Noble ('52) ascertained that feeding of rapeseed oil or erucic acid causes an increase in size and cholesterol content of rat adrenals. Marked functional changes, however, were not found, and it appeared that such adrenals respond normally to stimulation. Carroll's view that the adrenal function is not changed on feeding rapeseed oil or erucic acid is supported by the present result, for it is hard to conceive that an altered adrenal function is attended with an increased life span.

The result obtained in the present experiment argues strongly against an unknown butter factor. This conclusion is in accordance with the investigations by von Euler et al. ('47) who observed no difference in life span between animals fed on butter and margarine respectively.

The prophylactic and therapeutic possibilities of growth-retarding fats and oils in combatting obesity has already been mentioned (Thomasson, '55a). Such a method parallels that of Pennington ('49), who prescribed a fat-rich diet to his obese patients, and obtained good results with it. In particular, no sensation of hunger occurred between meals, nor adverse phenomena, such as ketonuria, although 170 to 225 gm of fat were consumed per day. The nature of the fat has not been considered by Pennington, but it is probable that his method will yield better results if a special fat is used.

SUMMARY

In this investigation, male rats fed ad libitum with a diet containing 50 Cal. % rapeseed oil lived 20 to 25% longer than those fed a corresponding butterfat-containing diet.

Both the growth rate and the daily food intake of the rapeseed oil animals were lower than those of the butterfat animals.

The two groups showed pathological changes, namely lesions of the legs, and kidney degenerations, which were of a more serious nature in the butter group than in the rapeseed oil group. In the latter group a slight liver degeneration was observed, which did not occur in the butter group.

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LITERATURE CITED

BALL, Z. B., R. H. BARNES AND M. B. VISSCHER 1947 The effects of dietary caloric restriction on maturity and senescence, with particular reference to fertility and longevity. Am. J. Physiol., *150:* 511–519.

CARLSON, A. J., AND F. HOELZEL 1946 Apparent prolongation of the life span of rats by intermittent fasting, J. Nutrition, *31:* 363–375.

———— 1947 Growth and longevity of rats fed omnivorous and vegetarian diets. Ibid., *34:* 81–96.

CARROLL, K. K. 1951 Effects of dietary fats and oils on adrenal cholesterol. Endocrinology, *48:* 101–110.

———— 1953 Erucic acid as the factor in rape oil affecting adrenal cholesterol in the rat. J. Biol. Chem., *200:* 287–292.

CARROLL, K. K., AND R. L. NOBLE 1952 Effects of feeding rape oil on some endocrine functions of the rat. Endocrinology, *51:* 476–486.

EULER, B. v., H. v. EULER AND I. RÖNNESTAM 1947 Influence of fat nutrition upon the growth, the fertility and the longevity of rats. 2, 3. Arkiv f. Kemi, Mineral. Geol., *24:* 9–22.

HANSEN, A. E., AND H. F. WIESE 1951 Fat in the diet in relation to nutrition of the dog. Texas Rep. Biol. Med., *9:* 491–570.

KENNEDY, G. C. 1951 Experimental hypothalamic obesity. Proc. Roy. Soc. Med., *44:* 899–902.

MCCAY, C. M., M. F. CROMWELL AND L. A. MAYNARD 1935 The effect of retarded growth upon the lenght of life span and upon the ultimate body size. J. Nutrition, *10:* 63–79.

MCCAY, C. M., G. H. ELLIS, L. L. BARNES, C. A. H. SMITH AND G. SPERLING 1939b Chemical and pathological changes in aging and after retarded growth. Ibid., *18:* 15–25.

MCCAY, C. M., L. A. MAYNARD, G. SPERLING AND L. L. BARNES 1939a Retarded growth, life span, ultimate body size and age changes in the albino rat after feeding diets restricted in calories. Ibid., *18:* 1–13.

MCCAY, C. M., L. A. MAYNARD, G. SPERLING AND H. S. OSGOOD 1941 Nutritional requirements during the latter half of life. Ibid., *21:* 45–60.

PENNINGTON, A. W. 1949 Obesity in industry; the problem and its solution. Ind. Med. Surg., *18:* 259–260.

RAMALINGASWAMI, V., AND H. M. SINCLAIR 1951 The relation of deficiencies of vitamin A and of essential fatty acids to follicular hyperkeratosis in the rat. Brit. J. Nutrition, *5:* xi.

RIESEN, W. H., E. J. HERBST, C. WALLIKER AND C. A. ELVEHJEM 1947 The effect of restricted caloric intake on the longevity of rats. Am. J. Physiol., *148:* 614–617.

THOMASSON, H. J. 1953 Biological standardization of essential fatty acids (a new method). Int. Z. Vit. f., *25:* 62–68.

———— 1955a The biological value of oils and fats. I. Growth and food intake on feeding with natural oils and fats. J. Nutrition, In press.

———— 1955b Unpublished.

THOMASSON, H. J., AND J. BOLDINGH 1955c The biological value of oils and fats. II. The growth retarding substance of rapeseed oil. J. Nutrition. In press.

WILCOXON, F. 1945 Individual comparisons by ranking methods. Biometrics, *1:* 80–83.

WILLIAMSON, R. 1941 A note on the epidermis of the rat on a fat-free diet. Biochem. J., *35:* 1003–1005.

NUTRITIONAL STATUS OF SCHOOL CHILDREN 15 AND 16 YEARS OF AGE IN THREE IDAHO COMMUNITIES; BLOOD BIOCHEMICAL TESTS [1]

SHIRLEY V. BRING, KATHLEEN PORTER WARNICK [2] AND ELLA WOODS [3]

Department of Home Economics Research, University of Idaho, Moscow

ONE FIGURE

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This study was part of a cooperative project on the nutritional status of population groups in selected areas of the West. In the Idaho phase of this project the subjects were boys and girls, 15 or 16 years of age on their last birthday, who were reared in the three communities studied and who volunteered for the investigation. Two of the communities, Boise and Nampa, are neighboring cities in the southwestern part of Idaho; the third community, Coeur d'Alene, is in the northern part. Dental, dietary, physical and biochemical data were collected from January through May, 1951. The basis for the selection of the communities for study has been discussed in the reports of the dental and dietary phases (Porter and Woods, '54; Warnick et al., '55). This paper reports the findings of the biochemical tests on the blood. These data added to those from other studies of normal children may

[1] Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper no. 381. This study was part of the Western Regional Research Project on the Nutritional Status of Population Groups in Selected Areas of Idaho, with the cooperation of the Idaho Agricultural Experiment Station, the Bureau of Human Nutrition and Home Economics, and the Idaho State Department of Public Health. It was financed in part from funds appropriated under the Research and Marketing Act of 1946.

[2] Present address: 809 East 8th Street, Moscow, Idaho.

[3] Present address: 1416 North 13th Street, Boise, Idaho.

help to establish more definite ranges of normal for these blood constituents. In addition, these data should contribute to a more accurate evaluation of blood levels in terms of nutritional status.

METHODS

Fasting venous blood samples were obtained from 280 subjects during the forenoons. Hemoglobin, sedimentation rate, packed cell volume and serum ascorbic acid determinations were made immediately in the field clinic. Aliquots of the serums were refrigerated, packed in vacuum flasks containing dry ice and sent to the Home Economics Research laboratory at Utah State Agricultural College. There the serums of the Utah and Idaho phases of the regional study were analyzed for vitamin A, carotene, free and total riboflavin, free and total cholesterol, alkaline phosphatase, iron and cooper.

The microchemical methods of Bessey ('50) were used to determine vitamin A, carotene, free and total riboflavin, ascorbic acid and alkaline phosphatase. Free and total cholesterol were determined by the Sperry and Webb ('50) method, with the percent of light transmission read in a Model 6 Coleman Junior spectrophotometer at 625 mμ. Iron was determined by the method of Hamilton, Gubler, Cartwright and Wintrobe ('50) and copper by the method of Gubler, Lahey, Ashenbrucker, Cartwright and Wintrobe ('52). Hemoglobin, packed cell volume and sedimentation rate were determined by the Wintrobe ('51) methods. Hemoglobin was measured colorimetrically as alkaline hematin with a Fisher electrophotometer.

RESULTS AND DISCUSSION

Values of blood constituents

Mean values,[4] standard deviations and ranges for the blood constituents and the number of subjects of each sex for whom the determinations were made are presented in table 1. For

[4] Since there were unequal numbers of subjects in each group, the mean values by sex were adjusted for age and area differences.

BLOOD BIOCHEMICAL TESTS

TABLE 1

Means,[1] standard deviations and ranges of blood constituents of Idaho girls and boys 15 and 16 years of age

BIOCHEMICAL CONSTITUENT	SEX	NUMBER OF SUBJECTS	ADJUSTED MEAN [1]	STANDARD DEVIATION	RANGE
Serum					
Vitamin A	Girls	150	31.5	9.4	8.3–60.3
(μg/100 ml)	Boys	123	34.5	8.9	9.6–59.6
Carotene	Girls	150	122.1	41.0	13.5–286.0
(μg/100 ml)	Boys	123	125.5	43.5	47.0–290.5
Free riboflavin	Girls	153	0.75	0.64	0.13–5.82
(μg/100 ml)	Boys	124	0.63	0.39	0.06–2.78
Total riboflavin	Girls	154	2.40	0.84	1.03–8.21
(μg/100 ml)	Boys	124	2.39	0.80	0.93–6.55
Ascorbic acid	Girls	142	1.01	0.54	0.14–2.40
(mg/100 ml)	Boys	124	0.76	0.45	0.09–2.25
Free cholesterol	Girls	154	44.0	8.2	27.6–73.5
(mg/100 ml)	Boys	124	41.1	8.0	25.5–69.9
Total cholesterol	Girls	154	175.3	31.1	109.2–291.6
(mg/100 ml)	Boys	124	166.3	30.4	106.4–273.4
Alkaline phosphatase	Girls	150	2.05	0.88	0.57–8.49
(mmole units/100 ml)	Boys	121	4.14	1.81	0.95–10.79
Iron	Girls	141	104.8	43.3	31–361
(μg/100 ml)	Boys	115	113.0	33.2	33–209
Copper	Girls	151	119.7	22.8	54–194
(μg/100 ml)	Boys	122	112.1	20.6	64–170
Whole blood					
Hemoglobin	Girls	154	13.9	0.9	11.5–16.3
(gm/100 ml)	Boys	126	15.4	1.0	12.8–17.2
Packed cell volume	Girls	154	43.6	2.4	38.0–50.0
(% volume)	Boys	126	47.6	2.8	40.0–53.0
Sedimentation rate	Girls	152	14.1	8.0	1.0–36.0
(mm/hr.)	Boys	125	6.9	5.7	0.0–34.0

[1] Means adjusted for age and area differences in unequal number of subsamples.

uncontrollable reasons all of the determinations were not made for all of the subjects.

Figure 1 shows the distribution by sex of the individual values for the biochemical constituents studied. The cumulative charts readily show the percentage of values below any given level.

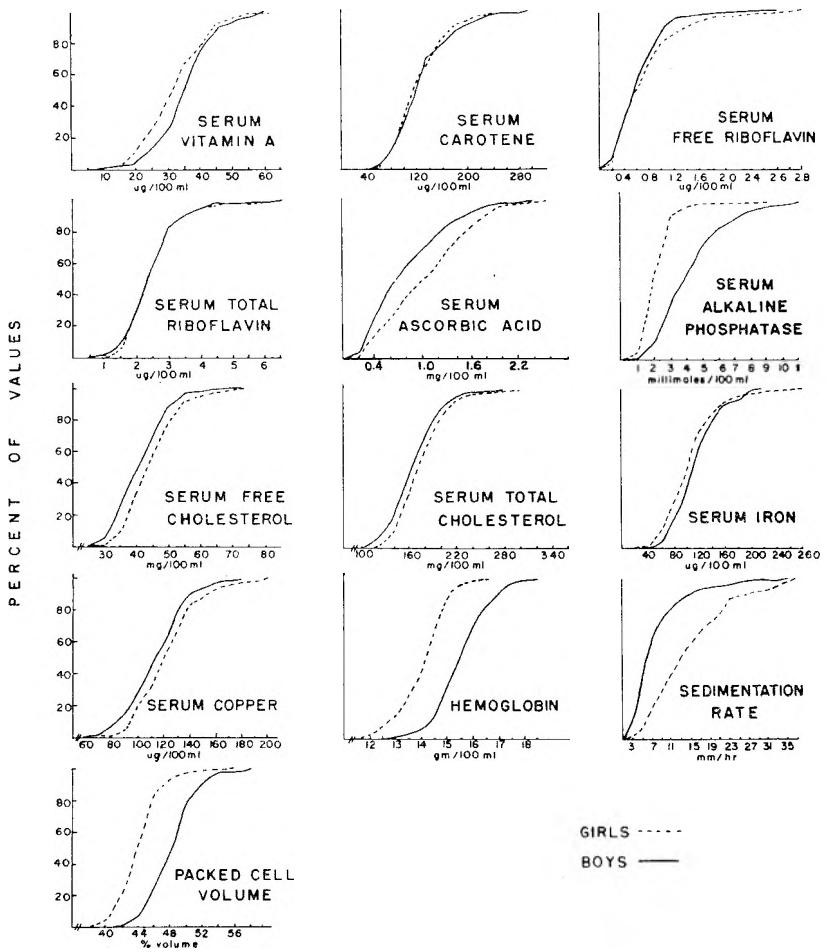

Fig. 1 Percentage of values below the specified levels for 13 blood constituents in the Idaho study. One value is not shown in the girls' graphs of serum free and total riboflavin and serum iron. These three values (5.82, 8.21 and 361 μg/100 ml) were omitted for reasons of expediency.

The normal range of values for most of the blood constituents has not been completely determined. There is need for more data before the lower and upper limits of normal for many of these blood constituents can be established.

Vitamin A and carotene. The mean concentrations of serum vitamin A for the subjects in this study were similar to those reported for adolescents in Utah (Wilcox et al., '54) but lower than the mean values reported for most of the student groups studied in the northeastern regional nutritional status study (Clayton et al., '53) and for the teen-age subjects in Oregon (Storvick et al., '51). In contrast, the mean serum carotene levels for the Idaho girls and boys were higher than those reported in Oregon (Storvick et al., '51) and for most of the groups of adolescents in the northeastern region (Clayton et al., '53). The Utah teen-age subjects (Wilcox et al., '54) had higher mean serum carotene values than the Idaho subjects.

Serum vitamin A values below 20 μg/100 ml were considered indicative of a "poor" level of nutrition by Bessey and Lowry ('47). Only 11% of the Idaho girls and 6% of the boys had values below this level. Bessey and Lowry ('47) classified serum carotene values below 75 and 125 μg/100 ml as "poor" and "fair," respectively. Clayton et al. ('53) found that many of the serum carotene values for the northeastern subjects fell below 125 μg/100 ml, so they classified values between 75 and 100 μg/100 ml as "fair." Nearly 60% of the Idaho subjects had serum carotene values below 125 μg/100 ml. Approximately one-third of the subjects had values below 100 μg/100 ml, but only 5% of the girls and 10% of the boys had values below 75 μg/100 ml.

Riboflavin. The mean serum total riboflavin values were similar to those found in fasting blood samples in comparable studies of normal subjects in Utah (Wilcox et al., '54) and Washington (Esselbaugh, '53).

Bessey and Lowry ('47) defined serum total riboflavin values below 2.5 μg/100 ml as indicative of a "poor" level of nutrition. Blood samples in that study were taken without

respect to meals eaten, which may account for the values being substantially higher than those found in fasting samples in the present study. Over 60% of the Idaho subjects had serum total riboflavin values below 2.5 μg/100 ml, although only 15% of the girls and 6% of the boys reported food intakes which supplied less than two-thirds of the National Research Council's recommended dietary allowances of riboflavin (Warnick et al., '55). Approximately one-half of the Idaho subjects had serum total riboflavin values below 2.3 μg/100 ml; approximately one-fourth had values below 1.9 μg/100 ml. Similar proportions of the subjects had serum free riboflavin values below 0.6 and 0.4 μg/100 ml.

Ascorbic acid. The mean serum ascorbic acid values were similar to or higher than those reported for the subjects studied in Oregon (Storvick et al., '51), New Mexico (Campbell et al., '53) and for most of the student groups studied in the northeastern region (Clayton et al., '53). The Utah boys (Wilcox et al., '54) had a higher mean ascorbic acid value than the Idaho boys.

There is evidence that the serum level of ascorbic acid reflects the recent level of intake (Ruffin et al., '44; Johnson et al., '45), but values below 0.4 mg/100 ml were considered by Bessey and Lowry ('47) as indicative of a "poor" level of nutrition. Sixteen percent of the Idaho girls and 25% of the boys had serum ascorbic acid values below 0.4 mg/100 ml.

Cholesterol. The average serum free and total cholesterol values were lower than those reported by Kornerup ('50) for 14- and 16-year-old children. Utah subjects 13 to 16 years of age had somewhat lower mean serum total cholesterol values but similar mean serum free cholesterol values (Wilcox et al., '54).

Muller ('45) reported normal values of plasma total cholesterol from 150 to 180 mg/100 ml. Values above 180 mg/100 ml were found for 39% of the Idaho girls and 31% of the boys; values above 200 mg/100 ml were found for 18% of the girls and 13% of the boys.

Alkaline phosphatase. The mean serum alkaline phosphatase value for the girls was less than one-half the value of the boys. The mean values in the present study were similar to those reported for Utah subjects (Wilcox et al., '54), but varied somewhat from results of other studies (Bessey and Lowry, '47; Harrison et al., '48; Clayton et al., '53).

Only 1% of the New York school children studied by Bessey and Lowry ('47) had serum alkaline phosphatase values above 8 mmole units/100 ml, which they classified as "unsatisfactory." The same percentage of Idaho girls, but 5% of the boys, had values above this criterion.

Iron. The mean serum iron values in this study were higher than the values reported by Schlaphoff et al. ('50) for samples drawn in the afternoon. Vahlquist ('41) and Dahl ('48) reported higher values for teen-age girls, but they did not report the time samples were taken.

Cartwright and Wintrobe ('52) suggested 44 to 215 μg/100 ml as the normal range of serum iron values. Only 2% of the Idaho subjects had serum iron values below 44 μg/100 ml.

Copper. The mean serum copper values for the Idaho adolescents were somewhat lower than Cartwright et al. ('45) reported for adults.

Cartwright and Wintrobe ('52) gave the normal range of serum copper values as 85 to 160 μg/100 ml. In the Idaho study, 3% of the girls and 13% of the boys had values below this range, 6% of the girls and 2% of the boys had values above.

Hemoglobin. The mean hemoglobin values were slightly higher than those reported for the Oregon subjects (Storvick et al., '51) and for most of the adolescent groups studied in the northeastern region (Clayton et al., '53).

Bessey and Lowry ('47) considered hemoglobin values between 11 and 13 gm/100 ml for girls and between 12 and 14 gm/100 ml for boys over 14 years of age indicative of a "fair" level of nutrition. Sixteen percent of the Idaho girls

and 6% of the boys had hemoglobin values in these ranges; none had lower values.

Volume of packed cells. The mean packed cell volumes found in this study are in good agreement with the values reported by Wintrobe ('51) for girls and boys of this age group. Only 5% of the girls and none of the boys had less than 40% volume of packed cells.

Sedimentation rate. Sedimentation rates above 20 mm/hr. for girls and above 10 mm/hr. for boys were designated as being above normal by Wintrobe ('51). Twenty-four percent of the girls and 18% of the boys in this study had sedimentation rates above these levels. Acute general infections, some chronic infections and some localized acute infections are accompanied by an increase in sedimentation rate (Wintrobe, '51). Nearly three-fourths of the Idaho subjects with high sedimentation rates were observed by the examining physicians to have colds, tonsilitis or a similar infection. Thirty-eight percent of the subjects with high sedmimentation rates were 10% or more above standard weight (Baldwin and Wood, '54); for 19% of the subjects with high sedimentation rates, overweight was the only abnormal finding in the phyical examination.

Sex, age and area differences

Mean values for each blood constituent grouped by sex, age and area are presented in table 2. Because it was impossible to secure data from equal numbers of subjects in each age and sex group in each area, the means were adjusted by the method of fitting constants by least squares to compensate for the different numbers of subjects in the groupings. For each of the blood constituents, the difference is given between the means for subjects 15 and 16 years of age and for each of the area comparisons. The smallest mean difference required for significance at the 5% level of probability is shown for each sex and age comparison and for the area comparison involving the smallest number of subjects (for the area comparisons involving larger numbers of subjects

TABLE 2

Means[1] *of blood constituents of Idaho subjects classified by sex, age and area with differences required for significance at the 5% level of probability*

COMPARISONS	SERUM									WHOLE BLOOD		
	Vitamin A	Carotene	Riboflavin		Ascorbic acid	Cholesterol		Alkaline phosphatase	Iron	Copper	Hemoglobin	Packed cell volume	Sedimentation rate
			Free	Total		Free	Total						
Sex	*µg/100 ml*	*µg/100 ml*	*µg/100 ml*		*mg/100 ml*	*mg/100 ml*		*mmole units/100 ml*	*µg/100 ml*	*µg/100 ml*	*gm/100 ml*	*%*	*mm/hr.*
Girls	31.5	122.1	0.75	2.40	1.01	44.0	175.3	2.05	104.8	119.7	13.9	43.6	14.1
Boys	34.5	125.5	0.63	2.39	0.76	41.1	166.3	4.14	113.0	112.1	15.4	47.6	6.9
Difference	— 3.0[2]	— 3.4	0.12	0.01	0.25[3]	2.9[3]	9.0[2]	— 2.09[3]	— 8.2	7.6[3]	— 1.5[3]	— 4.0[3]	7.2[3]
L.S.D.[4] (5%)	2.2	10.2	0.13	0.19	0.12	1.9	7.3	0.36	9.8	5.3	0.2	0.6	1.7
Age													
15 years	33.1	125.1	0.66	2.44	0.93	43.2	173.9	3.61	109.8	116.7	14.6	45.6	9.9
16 years	32.9	122.5	0.71	2.34	0.85	42.0	167.7	2.58	108.1	115.1	14.7	45.6	11.2
Difference	0.2	2.6	— 0.05	0.10	0.08	1.2	6.2	1.03[3]	1.7	1.6	— 0.1	0.0	— 1.3
L.S.D.[4] (5%)	2.2	10.1	0.13	0.19	0.12	1.9	7.2	0.36	9.8	5.3	0.2	0.6	1.7
Area													
Boise	33.5	128.3	0.71	2.35	0.81	43.5	171.5	2.89	117.8	121.9	14.9	46.6	10.1
Nampa	32.2	132.1	0.76	2.53	0.88	43.0	173.8	3.05	109.6	118.8	14.2	45.2	10.4
Coeur d'Alene	33.3	111.0	0.59	2.30	0.97	41.2	167.0	3.33	99.5	106.9	15.0	45.0	11.0
Differences:													
Boise:													
Nampa	1.3	3.8	0.05	0.18	0.07	0.5	2.3	0.16	8.2	3.1	0.7[3]	1.4[3]	0.3
Coeur d'Alene:													
Nampa	1.1	21.1[3]	0.17[2]	0.23	0.09	1.8	6.8	0.28	10.1	11.9[3]	0.8[3]	0.2	0.6
Coeur d'Alene:													
Boise	0.2	17.3[3]	0.12	0.05	0.16[2]	2.3	4.5	0.44[2]	18.3[3]	15.0[3]	0.1	1.6[3]	0.9
L.S.D.[5] (5%)	2.7	12.5	0.16	0.24	0.15	2.4	8.9	0.45	12.4	6.6	0.3	0.8	2.1

[1] Means adjusted for unequal subclass numbers.
[2] Difference between means significant at 5% level of probability.
[3] Difference between means significant at 1% level of probability.
[4] Least significant mean difference.
[5] L.S.D. between area comparisons based on smallest number of subjects.

a smaller mean difference would be significant). In the following discussion, all differences described as significant were statistically significant at the 1% level of probability unless it is specifically stated that the difference was significant at the 5% level of probability.

The mean values for the girls were significantly higher than those of the boys for sedimentation rate, ascorbic acid, free cholesterol and copper. The mean values for the boys were significantly higher than the values for the girls for hemoglobin, packed cell volume and alkaline phosphatase. Other workers have reported sex differences in the same direction as those found in Idaho for the mean serum values of one or more of these blood constituents (Mugrage and Andreson, '38; Cartwright et al., '45; Bessey and Lowry, '47; Harrison et al., '48; Clayton et al., '53).

The only statistically significant difference between age groups in this study was found in serum alkaline phosphatase values. This mean value was higher for the younger than for the older subjects. Similar age differences in serum alkaline phosphatase values have been reported by Bessey and Lowry ('47) and Harrison et al. ('48).

Area differences were noted in the values of several of the blood constituents. The Coeur d'Alene subjects had significantly lower mean values for serum carotene and serum copper than those studied in Boise and Nampa. The Coeur d'Alene subjects had a mean serum iron value significantly lower than the value for the Boise subjects. The Nampa group had a mean hemoglobin value significantly lower than the means for the other two groups. The Boise subjects had a mean packed cell volume significantly higher than the means for the other two groups. Of these area differences, only those in serum carotene and serum copper can be considered regional differences. Had this study been done in only one of the southwestern towns and in Coeur d'Alene, all differences between communities might have been attributed to geographic differences.

Correlations

Correlations were determined between nutrient intakes and the corresponding serum levels (Warnick et al., '55). Statistically significant correlations were found between the dietary intake and serum level of ascorbic acid and between the intake of vitamin A value and the serum carotene level. The correlation between the intake and the serum level of vitamin A was not significant. The intake of riboflavin was not significantly correlated with either serum free or total riboflavin.

In table 3 are the correlation coefficients among all comparisons of blood constituents for the girls and for the boys. Corrections were made for area differences in all correlations and for age differences in all correlations involving serum alkaline phosphatase. Highly significant positive correlations were found for both girls and boys between the following constituents of whole blood or serum: vitamin A and carotene; free and total riboflavin; free and total cholesterol; hemoglobin and packed cell volume; carotene and ascorbic acid; vitamin A and both free and total cholesterol; carotene and both free and total cholesterol; copper and sedimentation rate.

The first 4 of these correlations would be expected. The correlation between serum carotene and ascorbic acid might be explained by their common food sources — fruits and vegetables supply the major portion of these two vitamins. Highly significant positive correlations between serum vitamin A and carotene and between serum carotene and ascorbic acid have also been reported for the adolescents studied in the northeastern region (Babcock et al., '53) and in Oregon (Storvick et al., '51).

Vitamin A and cholesterol are fat-related compounds, so a metabolic relationship between them might be expected. Serum carotene also appears to be involved in this relationship. Collazo et al. ('34) reported that rats fed a vitamin A-deficient diet exhibited a considerable decrease in serum total cholesterol levels, while those given excess doses of vitamin A showed an increase in serum total cholesterol.

TABLE 3

Correlation coefficients[1] *among blood constituents of Idaho girls and boys 15 and 16 years of age*

BIOCHEMICAL CONSTITUENTS[2]	SEX	SERUM									WHOLE BLOOD		
		Carotene	Riboflavin		Ascorbic acid	Cholesterol		Alkaline phosphatase[3]	Iron	Copper	Hemoglobin	Packed cell volume	Sedimentation rate
			Free	Total		Free	Total						
Serum													
Vitamin A	F	0.27[5]	0.05	0.04	0.02	0.22[5]	0.26[5]	0.08	0.03	0.07	0.16	0.14	— 0.11
	M	0.29[5]	0.24[5]	0.31[5]	0.07	0.30[5]	0.30[5]	— 0.32[5]	0.34[5]	— 0.18[4]	0.15	0.12	0.02
Carotene	F		0.03	0.18[4]	0.39[5]	0.29[5]	0.29[5]	0.18	0.11	— 0.00	— 0.06	0.19[4]	— 0.04
	M		0.35[5]	0.25[5]	0.27[5]	0.38[5]	0.35[5]	— 0.07	0.18	— 0.10	0.15	0.18[4]	0.05
Riboflavin: free	F			0.75[5]	— 0.11	0.03	0.03	— 0.08	— 0.02	— 0.12	— 0.04	— 0.08	— 0.12
	M			0.58[5]	0.24[5]	— 0.01	0.01	— 0.15	0.18	— 0.14	0.02	— 0.02	0.00
total	F				— 0.09	0.19[4]	0.18[4]	0.04	0.06	— 0.12	— 0.03	— 0.01	— 0.07
	M				0.07	0.22[4]	0.22[4]	— 0.10	0.16	— 0.12	0.10	0.11	— 0.07
Ascorbic acid	F					0.08	— 0.10	0.14	0.01	0.04	— 0.18[4]	— 0.10	— 0.06
	M					— 0.00	— 0.03	— 0.09	0.27[5]	— 0.08	0.12	0.13	0.10
Cholesterol: free	F						0.97[5]	0.13	0.05	0.25[5]	0.13	0.18[4]	0.20[4]
	M						0.97[5]	— 0.08	0.26[5]	— 0.02	0.17	0.20[4]	— 0.02
total	F							0.11	0.01	0.21[4]	0.18[4]	0.18[4]	0.17[4]
	M							— 0.08	0.29[5]	— 0.04	0.21[4]	0.24[5]	— 0.03
Alkaline phosphatase	F								0.04	0.05	0.09	0.02	0.01
	M								— 0.21[4]	0.12	— 0.36[5]	— 0.35[5]	0.02
Iron	F									— 0.17[4]	0.16	0.10	— 0.19[4]
	M									— 0.09	0.39[5]	0.34[5]	— 0.09
Copper	F										0.05	0.08	0.40[5]
	M										— 0.02	0.01	0.49[5]
Whole blood													
Hemoglobin	F											0.49[5]	0.08
	M											0.81[5]	— 0.04
Packed cell volume	F												0.08
	M												— 0.08

[1] $r = \frac{\text{Covariance}}{\text{geometric mean of variances}}$; degrees of freedom for sums of products ranged from 111 to 123 for boys, 127 to 151 for girls; degrees of freedom for sums of squares ranged from 112 to 123 for boys, 138 to 151 for girls.

[2] All correlation coefficients were corrected for area differences.

[3] All correlation coefficients involving serum alkaline phosphatase were corrected for age differences.

[4] Significant at the 5% level of probability.

[5] Significant at the 1% level of probability.

In acute and chronic infectious diseases, elevated serum copper values have been observed by Valquist ('41), Cartwright et al. ('46) and Cartwright et al. ('48). Since sedimentation rate usually also increases in the presence of infection, this would explain the correlation between copper and sedimentation rate.

No significant correlation was found in the Idaho study between serum vitamin A and ascorbic acid. A positive correlation between these factors was reported in the studies in New York (Babcock et al., '53) and Oregon (Storvick et al., '51), and a negative correlation was reported in the West Virginia study (Babcock et al., '53). In the Oregon study (Storvick et al., '51) significant positive correlations were found between serum vitamin A and both hemoglobin and packed cell volume, but these were not found in the present study. In the studies reported by Storvick et al. ('51) and Babcock et al. ('53), however, the correlation coefficients were calculated using the combined data for both sexes.

In many of the comparisons among whole blood and serum constituents in the Idaho study, only the data of the boys showed highly significant correlations. This was true for the positive correlations between vitamin A and both free and total riboflavin, carotene and both free and total riboflavin, vitamin A and iron, free riboflavin and ascorbic acid, ascorbic acid and iron, iron and both free and total cholesterol, total cholesterol and packed cell volume, iron and hemoglobin, iron and packed cell volume, and for the negative correlations between alkaline phosphatase and each of the following: vitamin A, hemoglobin and packed cell volume. Only the correlation between free cholesterol and copper was significant for the girls alone.

In the light of present knowledge, there seems to be no reason for finding so many relationships between the blood constituents for the boys but not for the girls. Some may be chance relationships only. However, several findings in animal studies suggest riboflavin may be related to fat metabo-

lism (McHenry and Gavin, '38; Street and Cowgill, '39; Mannering et al., '41). If this is so, riboflavin may also be related to vitamin A. Kimble and Gordon ('39) reported that people with subnormal dark adaptation that does not respond to vitamin A therapy may be relieved by riboflavin therapy. Why there should be a significant correlation between these factors for the Idaho boys and not the girls is not evident, however. Further work is needed to clarify these findings of sex differences.

SUMMARY

Biochemical determinations were made on fasting venous blood samples from 280 school children 15 and 16 years of age in three Idaho communities.

The mean value for the girls was significantly higher than for the boys for sedimentation rate and for ascorbic acid, free cholesterol and copper of serum. The mean value for the boys was significantly higher than for the girls for serum alkaline phosphatase, hemoglobin and packed cell volume. The 15-year-old subjects had a mean serum alkaline phosphatase value significantly higher than the older children. The subjects in the northern community had mean serum carotene and copper values significantly lower than the subjects in the two southwestern communities.

The blood values of the girls and boys are compared to suggested limits of normal.

Highly significant positive correlations were found for both girls and boys between the following comparisons of whole blood and serum constituents: vitamin A and carotene; vitamin A and both free and total cholesterol; carotene and both free and total cholesterol; carotene and ascorbic acid; free and total riboflavin; free and total cholesterol; copper and sedimentation rate; hemoglobin and packed cell volume. In many of the other comparisons, only the data of the boys showed highly significant correlations. Further work is recommended to explain these sex differences.

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LITERATURE CITED

BABCOCK, M. J., M. M. CLAYTON, W. D. FOSTER, M. E. LOJKIN, R. E. TUCKER, A. H. VAN LANDINGHAM AND C. M. YOUNG 1953 Cooperative nutritional status studies in the northeast region. VI. Correlations. W. Va. Agr. Exp. Sta. Bull., 361T.

BALDWIN, B. T., AND T. D. WOOD 1954 Weight-height-age tables for boys and girls of school age. In: L. J. Bogert Nutrition and physical fitness: 636. Sixth edition. W. B. Saunders Co., Philadelphia.

BESSEY, O. A. 1950 Microchemical methods. In: Vitamin Methods, I: 303. Edited by P. Gyorgy. Academic Press, Inc., New York.

BESSEY, O. A., AND O. H. LOWRY 1947 Nutritional assay of 1200 New York state school children. In: Meals for Millions. New York State Joint Legislative Committee on Nutrition. Legislative Document 61.

CAMPBELL, A. M., R. J. GRASS, D. B. HACKER, M. HOTOPP AND E. M. LANTZ 1953 The relation of vitamin C to the condition of the gums of New Mexico school children in selected areas. N. Mex. Agr. Exp. Sta. Bull., 380.

CARTWRIGHT, G. E., C. M. HUGULEY, JR., H. ASHENBRUCKER, J. FAY AND M. M. WINTROBE 1948 Studies on free erythrocyte protoporphyrin, plasma iron and plasma copper in normal and anemic subjects. Blood, *3*, pt. 1: 501.

CARTWRIGHT, G. E., P. J. JONES AND M. M. WINTROBE 1945 Method for determination of copper in blood serum. J. Biol. Chem., *160:* 593.

CARTWRIGHT, G. E., M. LAURENTSEN, P. J. JONES, J. M. MERRILL AND M. M. WINTROBE 1946 The anemia of infection. I. Hypoferremia, hypercupremia and alterations in porphyrin metabolism in patients. J. Clin. Invest., *25:* 65.

CARTWRIGHT, G. E., AND M. M. WINTROBE 1952 The anemia of infection. XVII. A review. Advances in Internal Medicine: Vol. V. Edited by I. Snapper and W. Dock. The Year Book Publishers, Inc., Chicago.

CLAYTON, M. M., M. J. BABCOCK, W. D. FOSTER, S. STREGEVSKY, R. E. TUCKER, A. W. WERTZ AND H. H. WILLIAMS 1953 Cooperative nutritional status studies in the northeast region. V. Blood findings. Univ. Maine Agr. Exp. Sta. Bull., 516.

COLLAZO, J. A., I. TORRES AND SANCHEZ-RODRIGUEZ 1934 Das A-vitamin und der cholesterinstoffwechsel. Klin. Wochschr., *13:* 1678.

DAHL, S. 1948 Serum iron in normal women. Brit. Med. J., *I:* 731.

ESSELBAUGH, N. C. 1953 Personal communication.

GUBLER, C. J., M. E. LAHEY, H. ASHENBRUCKER, G. E. CARTWRIGHT AND M. M. WINTROBE 1952 Studies on copper metabolism. I. A method for the determination of copper in whole blood, red blood cells, and plasma. J. Biol. Chem., *196:* 209.

HAMILTON, L. D., C. J. GUBLER, G. E. CARTWRIGHT AND M. M. WINTROBE 1950 Diurnal variation in the plasma iron level of man. Proc. Soc. Exp. Biol. Med., *75:* 65.

HARRISON, A. P., C. RODERUCK, M. LESHER, M. KAUCHER, E. Z. MOYER, W. LAMECK AND E. F. BEACH 1948 Nutritional status of children. VIII. Blood serum alkaline phosphatase. J. Am. Dietet. Assn., *24:* 503.

JOHNSON, R. E., R. C. DARLING, F. SARGENT AND P. F. ROBINSON 1945 Effects of variations in dietary vitamin C on the physical well being of manual workers. J. Nutrition, *29:* 155.

KIMBLE, M. S., AND E. S. GORDON 1939 The importance of riboflavin and ascorbic acid in the utilization of vitamin A. J. Biol. Chem., *128:* lii.

KORNERUP, V. 1950 Concentrations of cholesterol, total fat and phospholipids in serum of normal man. Report of a study with special reference to sex, age and constitutional type. Arch. Int. Med., *85:* 398.

MANNERING, G. J., M. A. LIPTON AND C. A. ELVEHJEM 1941 Relation of dietary fat to riboflavin requirement of growing rats. Proc. Soc. Exp. Biol. Med., *46:* 100.

MCHENRY, E. W., AND G. GAVIN 1938 The B vitamins and fat metabolism. I. Effects of thiamine, riboflavin, and rice polish concentrate upon body fat. J. Biol. Chem., *125:* 653.

MUGRAGE, E. R., AND M. I. ANDRESEN 1938 Red blood cell values in adolescence. Am. J. Dis. Children, *56:* 997.

MULLER, G. L. 1945 The clinical significance of cholesterol. Am. J. Digest. Dis., *12:* 355.

PORTER, K. O., AND E. WOODS 1954 Dental caries prevalence in children 15 and 16 years of age in three Idaho communities. J. Dental Res., *33:* 542.

RUFFIN, J. M., D. CAYER AND W. A. PERLZWEIG 1944 The relationship between the clinical picture of a mild or early vitamin deficiency and laboratory determinations of vitamin levels. Gastroenterology, *3:* 340.

SCHLAPHOFF, D., F. A. JOHNSTON AND E. D. BOROUGHS 1950 Serum iron levels of adolescent girls and the diurnal variation of serum iron and hemoglobin. Arch. Biochem., *28:* 165.

SPERRY, W. M., AND M. WEBB 1950 A revision of the Schoenheimer-Sperry method for cholesterol determination. J. Biol. Chem., *187:* 97.

STORVICK, C. A., M. L. HATHAWAY AND R. M. NITCHALS 1951 Nutritional status of selected population groups in Oregon. II. Biochemical tests on the blood of native born and reared school children in two regions. Milbank Mem. Fund Quart., *29:* 255.

STREET, H. R., AND G. R. COWGILL 1939 Acute riboflavin deficiency in the dog. Am. J. Physiol., *125:* 323.

VAHLQUIST, B. C. 1941 Serum iron. A paediatric, clinical and experimental study. Acta Paediat., *28:* supp. 5: xii, 68, 374.

WARNICK, K. P., S. V. BRING AND E. WOODS 1955 Nutritional status of adolescent Idaho children. I. Evaluation of seven-day dietary records. J. Am. Dietet. Assn., *31:* 486.

WILCOX, E. B., L. S. GALLOWAY, P. WOOD AND F. L. MANGELSON 1954 Children with and without rheumatic fever. III. Blood serum vitamins and phosphatase data. Ibid., *30:* 1231.

WINTROBE, M. M. 1951 Clinical hematology: 95, 100, 289, 292, 303, 317. Third edition. Lea and Febiger, Philadelphia.

THE PANTOTHENIC ACID REQUIREMENT OF THE BABY PIG[1]

S. C. STOTHERS,[2] D. A. SCHMIDT, R. L. JOHNSTON, J. A. HOEFER AND R. W. LUECKE[3]

Michigan State College, East Lansing, Michigan

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Hughes and Ittner ('42), using a purified diet, were able to establish a pantothenic acid requirement for the weanling pig. Luecke et al. ('50), using natural feedstuffs, conducted a trial with weanling pigs which demonstrated the importance of proper B vitamin supplementation, especially pantothenic acid, to a low-protein corn-soybean meal ration. The recent development of sow's milk replacement diets has stimulated an increased interest in the vitamin requirements of the baby pigs. Weise et al. ('51) produced pantothenic acid-deficiency symptoms in the baby pig fed a synthetic milk diet.

No attempts, however, have been made to estimate the pantothenic acid requirement of the baby pig. This paper presents data obtained in experimental work designed to estimate this requirement and to study pantothenic acid-deficiency symptoms in baby pigs.

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[3] Departments of Animal Husbandry, Animal Pathology and Agricultural Chemistry, Michigan State College, East Lansing. This work was supported, in part, by a grant-in-aid from National Vitamin Foundation, Inc., New York, N. Y. The authors are indebted to Merck and Co., Inc., Rahway, New Jersey, and to Lederle Laboratories Division, Pearl River, N. Y., for the B vitamins used in this experiment.

EXPERIMENTAL

Baby pigs were removed from the sow after 72 to 96 hours depending on the vigor and size of the pigs at birth. The pigs were placed in metal cages with screened floors. Room temperature was maintained at approximately 70°F. Infra-red heat lamps supplied additional heat during the initial stages of the trial. No attempt was made to maintain a constant relative humidity.

Initially, all pigs were subjected to a 4-day depletion-adjustment period and fed a standard basal milk similar to that used by Miller et al. ('54). In this experiment however, the lard content was increased from 10 to 20% and the cerelose content was decreased accordingly. Other modifications were the inclusion of riboflavin at a level of 0.65 mg per kilogram of milk and the removal of calcium pantothenate from the solution of water-soluble vitamins. At the end of the depletion-adjustment period the pigs were lotted as fairly as possible, according to weight, sex and litter. All pigs were individually fed in order to obtain accurate food consumption records.

This experimental work was conducted with a basal lot receiving no calcium pantothenate, and 5 lots receiving calcium pantothenate at the levels of 5.0, 7.5, 10.0, 12.5 and 15.0 mg per kilogram of solids. The data presented represent the results of three experiments carried out at different periods of time. Analysis of variance showed no difference in replications so that the data are grouped and presented in one table. Some of the experimental levels studied were not duplicated in all three trials, resulting in an uneven number of pigs per level tested. All animals were fed regularly 6 times a day during the depletion-adjustment period, and 5 times a day during the experimental period. Feeding was essentially ad libitum.

Microbiological assays for pantothenic acid according to the method of Skeggs and Wright ('44) were carried out on the synthetic milk to verify the prepared concentration. The samples were digested using the enzyme mylase P as outlined by Buskirk et al. ('48).

The pigs were weighed every 4 days. Blood samples from an ear vein were taken regularly for all counts and hemoglobin determinations. All animals that died were necropsied shortly after death or in some cases were killed in extremis and blocks of tissues were taken and fixed in formal-saline. Hematoxylin-eosin was used on sections and Weil's ('45) stain for myelin sheaths. Nissl bodies were stained by the thionin technique described by Fletcher ('47). All tissues of deficient pigs were compared to those taken from control animals.

RESULTS AND DISCUSSION

The most important experimental results are given in table 1. Statistical treatment was applied to the results, using the method of Snedecor ('46) for analyzing mutliple classification variance.

The pigs in lot 1 receiving no calcium pantothenate developed a diarrhea in 14 to 28 days after being put on experiment. After 23 days one pig in this lot was used for recovery studies when it became very weak. Four intraperitoneal injections of a solution containing 100 mg of calcium pantothenate were given on successive days followed by 6 additional injections on alternate days. Within 24 hours the diarrhea decreased and the pig had an improved appetite. After three days the feces were quite firm and there was a marked improvement in the appearance and appetite of the pig. During calcium pantothenate therapy this pig gained 18.6 lb. within a period of 25 days as compared to 2.2 lb. gained during the first 23 days on trial. No severe locomotor incoordination was observed in the pigs receiving no calcium pantothenate (lot 1).

In lot 2, the 4 pigs tested developed diarrhea after 21 to 28 days, and "goose-stepping" in 32 to 39 days.

In the lot receiving 7.5 mg of calcium pantothenate per kilogram of solids (lot 3), most of the pigs developed diarrhea within 18 to 38 days. Weakness in the hindquarters was observed in all animals within 42 to 49 days. Three pigs in lot 4 (10.0 mg calcium pantothenate per kilogram solids) developed diarrhea in 21 to 28 days after being placed on trial and

TABLE 1

Response of baby pigs to synthetic milk diets containing different levels of calcium pantothenate

	LEVEL OF CALCIUM PANTOTHENATE IN MILLIGRAMS PER KILOGRAM OF SOLIDS					
	0	5.0	7.5	10.0	12.5	15.0
Lot number	1	2	3	4	5	6
Number of pigs	13	4	9	11 [1]	9	13
Days on test	.. [2]	.. [2]	40	40	40	40
Av. initial wt. (lb.)	4.23 ± 0.17 [3]	4.28 ± 0.32	4.20 ± 0.22	4.22 ± 0.18	4.10 ± 0.22	4.19 ± 0.18
Av. final wt. (lb.)	..	..	18.73 ± 0.88	20.42 ± 0.83	22.39 ± 0.88	21.91 ± 0.73
Av. daily gain [4] (lb.)	..	..	0.36 ± 0.02	0.40 ± 0.02	0.45 ± 0.02	0.44 ± 0.02
Solids per lb. gain (lb.)	..	..	1.22 ± 0.12	1.13 ± 0.11	1.05 ± 0.12	1.06 ± 0.10

[1] Two pigs in lot 4 died during the course of the experiment from causes unrelated to the level of calcium pantothenate fed, and consequently are not included in the calculations.

[2] Data on growth and feed consumption for lots 1 and 2 are not included since most of these pigs had died, been sacrificed in extremis or received calcium pantothenate injections before the end of the experimental feeding period. Pigs in any of the higher lots developing deficiency symptoms were kept for a period longer than 40 days for further observation and recovery studies.

[3] Standard error of the mean.

[4] Lots 5 and 6 gained significantly better ($P < 0.01$) than lot 3, and also significantly better ($P < 0.10$) than lot 4.

during the 7th week one of these three exhibited definite instability in its hind quarters. This apparent variation in pantothenic acid requirement of the pig has been observed and discussed by Luecke et al. ('53). That this variation is not confined to the pig alone is evidenced by the work of Hurley and Morgan ('52) and Hurley and Mackenzie ('54) who found highly variable growth responses in rats maintained on pantothenic acid-deficient diets. None of the pigs in lots 5 or 6 developed diarrhea or locomotor incoordination at any time during the trial.

Blood studies reveal that an abnormally high white blood cell count was associated with persistent diarrhea. Counts of 30,000 to 70,000 were found as compared to a normal count of 10,000 to 20,000. The high white cell counts were probably a result of the inflammation of the large intestine caused by a lack of pantothenic acid.

Pathological studies showed that the lesions present in the large intestine and nervous system of pantothenic acid-deficient pigs were essentially those described by Wintrobe ('40, '43), Luecke et al. ('49) and Sharma et al. ('52). Microscopically there was a marked decrease or complete disappearance of the goblet cells in the cecum, colon and rectum. There was an increase in the connective tissue of the submucosa and this feature was even more marked in the case of pigs which had diarrhea for a long period of time. The pantothenic acid-deficient pigs also showed loss of myelin, and degeneration of the dorsal root ganglion cells.

Microscopic pathology of deficient animals treated with calcium pantothenate revealed that myelin degeneration could still be found in the sciatic nerve. Also, fibrous tissue was still present in large amounts in the submucosa of the large intestine.

It was noted that the glomerular layer of the adrenal was decreased in thickness in almost all pantothenic acid-deficient pigs. However, in deficient animals treated with calcium pantothenate the layer showed a normal thickness.

A careful study of this experiment and the preliminary experiments showed that the 12.5 mg of calcium pantothenate per kilogram of solids was the lowest level fed in which no positive signs of pantothenic acid deficiency occurred. It thus appears to approximate the requirements of baby pigs, subjected to the experimental conditions used in this investigation. This requirement is similar to that reported by Moutsgaard ('53) for older pigs.

SUMMARY

After an initial depletion-adjustment period, 62 selected baby pigs were individually fed a "synthetic" milk for an experimental period of 48 days.

The results show that the pantothenic acid requirement of the baby pig for optimum growth and feed efficiency approximates 12.5 mg of calcium pantothenate per kilogram of solids.

The baby pigs receiving the milk devoid of calcium pantothenate developed a severe diarrhea usually within two to 4 weeks. Locomotor incoordination developed in an additional 7 to 10 days if the pigs did not become so weakened that they died prior to this time.

Almost all of the baby pigs receiving suboptimal amounts of calcium pantothenate developed a severe diarrhea usually within two to 4 weeks. Locomotor incoordination developed in most cases within 4 to 7 weeks.

Pathological studies showed that lesions present in the large intestine and nervous system of pantothenic acid-deficient pigs were essentially those described by previous workers. The increase in the connective tissue of the submucosa was more marked in the case of pigs which had diarrhea for a long period of time. This tissue was still present in large amounts in the submucosa of deficient animals treated with calcium pantothenate. Myelin degeneration was still found in the sciatic nerve of treated animals. The glomerular layer of the adrenals of almost all pantothenic acid-deficient pigs was decreased in thicknes.

LITERATURE CITED

BUSKIRK, H. H., A. M. BERGDAHL AND R. A. DELOR 1948 Enzymatic digestion of samples for microbiological assays of pantothenic acid. J. Biol. Chem., *172:* 671.

FLETCHER, D. E. 1947 Rapid staining procedures for paraffin section of formelin fixed tissue. J. Micropathology and Exp. Neurology, *7:* 299.

HUGHES, E. H., AND M. R. ITTNER 1942 The minimum requirement of pantothenic acid for the growing pig. J. Animal Sci., *1:* 116.

HURLEY, L. S., AND A. F. MORGAN 1952 Carbohydrate and adrenal cortical function in the pantothenic acid-deficient rat. J. Biol. Chem., *195:* 583.

HURLEY, L. S., AND J. B. MACKENZIE 1954 Adrenal function in the pantothenic acid-deficient rat. J. Nutrition, *54:* 403.

LUECKE, R. W., J. A. HOEFER AND F. THORP, JR. 1953 The supplementary effects of calcium pantothenate and aureomycin in a low protein ration for weanling pigs. J. Animal Sci., *12:* 605.

LUECKE, R. W., W. H. MCMILLAN AND F. THORP, JR. 1950 Further studies of pantothenic acid deficiency in weanling pigs. Ibid., *9:* 78.

LUECKE, R. W., F. THORP, JR., W. N. MCMILLAN AND H. W. DUNNE 1949 Pantothenic acid deficiency in pigs fed diets of natural feedstuffs. Ibid., *8:* 464.

MILLER, E. R., R. L. JOHNSTON, J. A. HOEFER AND R. W. LUECKE 1954 The riboflavin requirement of the baby pig. J. Nutrition, *52:* 405.

MOUTSGAARD, J. 1950 The vitamin B requirement of young pigs to ensure the best possible gain and food utilization. Reprint from the congress report of the VIth International Congress of Animal Husbandry.

SHARMA, G. L., R. L. JOHNSTON, R. W. LUECKE, J. A. HOEFER, M. L. GRAY AND F. TORP, JR. 1952 A study of the pathology of the intestine and other organs of weanling pigs when fed a ration of natural feedstuffs low in pantothenic acid. Am. J. Vet. Res., *13:* 298.

SKEGGS, H. R., AND D. L. WRIGHT 1944 The use of *Lactobacillus arabinosus* in the microbiological determination of pantothenic acid. J. Biol. Chem., *156:* 21.

SNEDECOR, G. W. 1946 Statistical Methods. Iowa State College Press, Ames.

WEIL, A. 1945 Textbook of Neuropathology. Second edition. Grune and Stratton, New York.

WEISE, A. C., W. P. LEHRER, JR., P. R. MOORE, O. F. PEHNISH AND W. V. HARTWELL 1951 Pantothenic acid deficiency in baby pigs. J. Animal Sci., *10:* 80.

WINTROBE, M. M., R. H. FOLLIS, JR., R. ALCAYAGA, M. PAULSON AND S. HUMPHREYS 1943 Pantothenic acid deficiency in swine. Bull. Johns Hopkins Hosp., *73:* 313.

WINTROBE, M. M., J. L. MILLER, JR., AND H. LISCO 1940 The relation of diet to the occurrence of ataxic and degeneration of the nervous system of pigs. Ibid., *67:* 377.

THE EFFECTS OF DIETARY MANGANESE AND THIAMINE LEVELS ON GROWTH RATE AND MANGANESE CONCENTRATION IN TISSUES OF RATS[1,2]

B. M. ANDERSON[3] AND H. E. PARKER

Department of Biochemistry, Purdue University Agricultural Experiment Station, West Lafayette, Indiana

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A metabolic interrelationship between manganese and vitamin B_1 has been reported by Perla and associates. Perla ('39) reported that the toxic effects of excess vitamin B_1 on lactation, maternal instinct and fertility could be prevented by adding manganese to the diet. A manganese deficiency had been previously shown by Orent and McCollum ('31) to produce the same adverse effects on maternal instinct and reproduction. Perla and Sandberg ('39) found that additional vitamin B_1 counteracted the toxic effects of excess manganese. Sandberg et al. ('39) reported that the depletion period of rats on a thiamine-deficient diet was consistently shortened when 1 mg of manganese was added daily to rats receiving a thiamine-deficient diet containing 6 mg of manganese per kilogram. By increasing the vitamin B_1 content of the diet these workers found a lowered retention of manganese in the animal body. Holtkamp and Hill ('50) were unable to find any antagonism between thiamine and manganese with respect to growth rate. In a more recent article Hill and

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[3] Present address: McCollum-Pratt Institute, Johns Hopkins University, Baltimore 18, Md.

Koltkamp ('54) reported that the storage of thiamine in the liver increased with supplementation of manganese until the dietary level of 10 mg of Mn per rat per day was reached and that thiamine supplementation of the diet from a level of 0.03 to 0.2 mg per rat per day increased (but not statistically significant) the storage of manganese in the liver.

EXPERIMENTAL

Forty-five male and 45 female weanling rats of the Wistar strain were divided equally into three groups and fed thiamine-deficient diets containing 0.8, 5.0 and 50.0 mg of manganese per kilogram, respectively. The percentage composition of the basal diet was as follows: ether and alcohol extracted casein, 20.0; cerelose, 70.0; fish liver oil,[4] 1.0; partially hydrogenated vegetable oil, 5.0; and minerals, 4.0. Vitamin additions in milligrams per 100 gm of diet were as follows: riboflavin, 1.0; niacin, 5.0; inositol, 30.0; pyridoxine hydrochloride, 1.0; choline, 200.0; biotin, 0.01; folic acid, 0.01; p-amino benzoic acid, 30.0; calcium pantothenate 5.0; 2-methyl-1,4-naphthoquinone, 0.50; and vitamin B_{12}, 0.002.

The animals were housed in individual wire cages and given distilled water to drink. Growth data obtained by daily weighings indicated that the rats in all groups were depleted of thiamine in 16 days. The rats in each group were then subdivided into three subgroups and given daily supplements of thiamine in doses of 2.5, 16 and 100 μg of thiamine per rat per day, respectively. Daily weighings were continued for two weeks after the thiamine supplements had started; thereafter the rats were weighed once a week. Four weeks after the thiamine supplements had started the animals were sacrificed by a blow on the head. Samples of the livers and hearts were excised and analyzed for their manganese content. The manganese content of the tisssues and samples of the diet were determined using the method of Gates and Ellis ('47).

[4] One per cent fish liver oil provided 436.67 U.S.P. units of vitamin A and 618.35 I.C. units of vitamin D per 100 gm of diet.

RESULTS

It was found that the time required to deplete the animals of thiamine was 16 days regardless of the concentration of manganese in the diet. The manganese level of the diet did not affect the growth rate of rats during the thiamine-depletion period. During thiamine supplementation the manganese level as well as the thiamine level affected the rate of gain of the rats. An analysis of variance of the average weekly gains of the rats showed that the differences due to thiamine levels, manganese levels and sex were all significant well beyond the 1% probability point and that there was no significant interaction between the effects of the manganese and thiamine levels on the growth rates. In other words, the growth rates varied directly with the levels of both thiamine and manganese, but the manganese level did not influence the response to the thiamine supplements and the level of thiamine supplementation did not affect the response to additional dietary manganese. The average weekly gains of rats receiving 2.5, 16.0 and 100.0 μg of thiamine daily were 9.4, 29.0 and 34.4 gm, respectively. The average weekly gain of all rats fed diets containing 0.8, 5.0 and 50.0 mg of manganese per kilogram were 22.0, 24.7 and 26.1 gm respectively.

Analysis of the heart and liver samples indicated that the different levels of thiamine did not affect the storage of manganese in these tissues (table 1). The storage of manganese in these tissues increased with the increased intake of manganese.

DISCUSSION

The observations on the time required to deplete rats of thiamine at different dietary manganese levels failed to confirm the report by Sandberg et al. ('39) that adding manganese to the diet shortened the depletion period. Many new growth factors have been discovered since the report by Sandberg et al. was published. Manganese may have exerted its effect on the depletion period through an interaction with some other limiting growth factor.

The growth rates of the animals failed to reveal any antagonism between thiamine and manganese. These observations are in agreement with the findings of Holtkamp and Hill ('50).

The liver has been reported to be the chief storage tissue for manganese in the animal body by Skinner et al. ('31) and Greenberg et al. ('43). Therefore, one would expect a metabolic interdependence between thiamine and manganese to be expressed by variations of the manganese concentration in the liver. However, the thiamine level did not affect the

TABLE 1

Effect of dietary manganese and thiamine on growth rate and manganese content of tissues

MANGANESE CONTENT OF DIET	DAILY THIAMINE SUPPLEMENT	AVERAGE WEEKLY GAIN	MANGANESE CONTENT OF DRY TISSUES	
			Liver	Heart
mg/kg	*μg*	*gm*	*mg/kg*	*mg/kg*
0.8	2.5	7.5	1.6	..
5.0	2.5	9.8	2.9	1.4
50.0	2.5	10.7	5.9	1.6
0.8	16.0	27.6	0.8	1.0
5.0	16.0	29.8	3.0	1.3
50.0	16.0	29.6	5.7	1.3
0.8	100.0	30.9	1.2	0.6
5.0	100.0	34.4	2.4	1.2
50.0	100.0	38.0	5.4	1.8

amount of manganese stored in this organ. The manganese content of the diet appeared to be the only significant factor influencing the amount of manganese stored by the liver and heart tissue.

SUMMARY

The time required to deplete rats of thiamine was not significantly altered by raising the manganese content of the diet.

The addition of either thiamine or manganese to diets deficient in both of these nutrients improved the growth rate of rats but there was no significant interaction between the effects of these two nutrients on the growth rate.

The amount of manganese present in heart and liver tissue was found to vary directly with the manganese level in the diet and was not significantly affected by the level of thiamine in the diet.

LITERATURE CITED

GATES, E. M., AND G. H. ELLIS 1947 Microcolorimetric method for the determination of Manganese in biological material with 4,4-tetramethyldiaminotriphenylmethane. J. Biol. Chem., *168:* 537.

GREENBERG, D. M., D. H. COPP AND E. M. CUTHBERTSON 1943 Studies in mineral metabolism with the aid of artificial radioisotopes. VII. The distribution and excretion, particularly by way of the bile of iron, cobolt, and manganese. J. Biol. Chem., *147:* 749.

HILL, R. M., AND D. E. HOLTKAMP 1954 Storage of dietary manganese and thiamine in the rat. J. Nutrition, *53:* 73.

HOLTKAMP, D. E., AND R. M. HILL 1950 The effect on growth of the level of manganese in the diet of rats, with some observations on manganese-thiamine relationships. Ibid., *41:* 307.

ORENT, E. R., AND E. V. MCCOLLUM 1931 Effects of deprivation of manganese in the rat. J. Biol. Chem., *92:* 651.

PERLA, D. 1939 The prevention of toxic manifestations of an excess of vitamin B_1 by supplements of manganese to the diet. Science, *89:* 132.

PERLA, D., AND M. SANDBERG 1939 Metabolic interdependence of vitamin B_1 and manganese. Reciprocal neutralization of their toxic effects. Proc. Soc. Exp. Biol. Med., *41:* 522.

SANDBERG, M., D. PERLA AND O. M. HOLLY 1939 Interdependence of vitamin B_1 and manganese. II. Manganese, copper, iron metabolism in B_1 deficient rats. Ibid., *42:* 368.

SKINNER, J. T., W. H. PETERSON AND H. STEENBOCK 1931 Manganese metabolism of the rat. J. Biol. Chem., *90:* 65.

RELATIVE EFFECTIVENESS OF ANTIBIOTICS IN RATS GIVEN LIMITING B VITAMINS BY MOUTH OR BY INJECTION [1]

J. D. JONES AND C. A. BAUMANN

Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

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It has been observed repeatedly that antibiotics in the diet stimulate the growth of rats fed diets suboptimal in an essential B vitamin, but that antibiotics are relatively ineffective when complete diets are fed (Linkswiler et al., '51; Lih and Baumann, '51; Sauberlich, '52; Guggenheim et al., '53). This points toward one of two mechanisms for their mode of action: an increased intestinal synthesis of the limiting vitamin, or a decrease in the portion absorbed or destroyed by intestinal microorganisms and thus withheld from the host.

The phenomenon of intestinal synthesis is well established, and increased amounts of riboflavin, pantothenic acid (Guggenheim et al., '53) and of folic acid (Monson et al., '54) have been found in the feces and the digestive tracts of rats or chicks receiving antibiotics in the diet. It has also been demonstrated that certain microorganisms such as the lactobacilli may absorb large amounts of pyridoxal from the medium (Snell and Rannefeld, '45), and a decrease in this phenomenon has been offered to explain the fact that the three forms of vitamin B_6 are essentially equal in rats fed aureomycin (Linkswiler et al., '51) although dietary pyri-

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doxine is superior to pyridoxal or pyridoxamine in the absence of antibiotics (Miller and Baumann, '45; Sarma et al., '46).

A distinction between the two postulated mechanisms might be made by feeding a diet devoid of an appropriate B vitamin, adding an antibiotic to the diet, and administering limiting amounts of the missing vitamin by injection. Under these circumstances a diversion of the limiting vitamin from the intestinal medium to the microorganisms would be unlikely. In the present study a comparison has been made of the effectiveness of penicillin, aureomycin, and streptomycin in rats given suboptimal amounts of thiamine, riboflavin or pantothenic acid either in the diet or by injection. Recently Schendel and Johnson ('54) have published results on two of these combinations, penicillin-thiamine and aureomycin-pantothenic acid. Studies on penicillin-thiamine have also been made on the chick (Waibel et al., '53).

EXPERIMENTAL

Series of male weanling rats of the Holtzman strain, 35–50 gm in weight, were divided into groups of 5 each. The rats were housed individually on screens and given food and water ad libitum. The basal diet (table 1) was fed as such or 25 mg/kg of penicillin, aureomycin or streptomycin were added. The vitamin under study was either omitted from the diet or it was added at a level that permitted only suboptimal growth in the absence of antibiotic. The animals on the deficient diets received subcutaneous daily injections of $\frac{1}{2}$ ml containing amounts of the missing vitamin that permitted growth similar to that in the control group receiving suboptimal amounts of vitamin in the diet. Some series contained additional groups in which antibiotics were added to a complete diet, and in the case of pantothenic acid, to a deficient diet. The penicillin derivatives, penicillamine and penicilloic acid [2] were tested in a low-thiamine diet. All series were repeated at least once.

[2] We are indebted to Dr. A. E. Schaefer of E. R. Squibb and Sons, New Brunswick, New Jersey, for these preparations.

The experiments were terminated after 5 weeks. The "percent effectiveness of the antibiotic" when the limiting vitamin was fed or injected was calculated from the expression $\frac{\text{weight gain with antibiotic} - \text{weight gain of control}}{\text{weight gain of control}}$, and a further comparison of these degrees of effectiveness made by calculating the "percent effectiveness of the injected dose," namely,

$$\frac{\%\text{ effectiveness of antibiotic when limiting nutrient is injected}}{\%\text{ effectiveness of antibiotic when limiting nutrient is fed}}.$$

TABLE 1

Composition of basal diet

INGREDIENTS	AMOUNT	VITAMINS ADDED	AMOUNT
	%		*mg/kg*
Sucrose	71.5	Riboflavin	3.0
Casein	18.0	Thiamine	6.0
Wesson salts	4.0	Pyridoxine	2.0
Corn oil	5.0	Nicotinic acid	25.0
L-Cystine	0.2	Calcium pantothenate	20.0
Choline chloride	0.1	Menadione	10.0
α-Tocopherol	0.003	Inositol	100.0
Glycerol	1.0	Biotin	0.1
		Folic	2.0
		Vitamins A and D were administered weekly by dropper	

RESULTS

Thiamine. Dietary penicillin and aureomycin consistently stimulated growth when the thiamine available to the rats was limiting; this was true whether the thiamine was given in the diet or by injection. In agreement with past observations (Lih and Baumann, '51; Guggenheim et al., '53) penicillin was more effective in thiamine deficiency than the other antibiotics tested. No effect was noted with these antibiotics when the basal diet contained adequate levels of thiamine (table 2 lines 23 to 25). Neither penicillamine nor penicilloic acid produced a consistent growth response when thiamine was limiting.

In several experiments with penicillin (table 2 lines 1, 4, 6, and 8) and aureomycin (lines 10 and 12) the apparent

TABLE 2

Response to dietary antibiotics of rats receiving suboptimal amounts of thiamine orally or by injection

LINE NO.	SERIES NO.	ANTIBIOTIC	THIAMINE		FINAL WEIGHT [1]		GAIN DUE TO ANTIBIOTIC	EFFECTIVENESS OF INJECTED DOSE
			Fed	Injected	No antibiotic	Plus antibiotic		
		25 mg/kg	*μg/gm*	*μg/day*	*gm*	*gm*	*%*	*%*
1.	I	Penicillin G	0.5		61 ± 7	118 ± 16	356.0	
2.				3.0	87 ± 11	128 ± 21	97.7	27.4
3.	II	Penicillin G	0.5–0.75		70 ± 14	185 ± 39	442	
4.				3.0–3.6	104 ± 13	147 ± 18	71.7	16.2
5.	IV	Procaine	0.75		164 ± 25	237 ± 29	37.6	
6.		penicillin		3.6	108 ± 9	152 ± 15	69.5	185.0
7.	VIII	Penicillin G	0.65		112 ± 16	196 ± 25	129.5	
8.				3.3–3.6	106 ± 13	146 ± 20	67.9	52.4
9.	II	Aureomycin	0.5–0.75		70 ± 14	140 ± 31	269.0	
10.		HCl		3.0–3.6	104 ± 13	127 ± 19	38.3	14.2
11.	VIII	Aureomycin	0.65		112 ± 16	142 ± 18	46.2	
12.		HCl		3.3–3.6	106 ± 13	116 ± 8	17.0	36.8
13.	IV	Streptomycin	0.75		164 ± 25	173 ± 30	4.6	
14.		sulfate		3.6	108 ± 9	138 ± 11	47.3	1019.0
15.	VIII	Streptomycin	0.65		112 ± 16	124 ± 33	18.5	
16.		sulfate		3.3–3.6	106 ± 13	117 ± 17	18.7	101.1
17.	IV	Penicillamine	0.75		164 ± 25	174 ± 22	9.2	
18.	VIII	HCl	0.65		112 ± 16	152 ± 35	61.6	
19.	IV			3.6	108 ± 9	105 ± 5	— 4.7	
20.	IV	Penicilloic	0.75		164 ± 25	132 ± 22	— 22.8	
21.	VIII	acid	0.65		112 ± 16	84 ± 20	— 43.0	
22.	IV			3.6	108 ± 9	116 ± 13	12.6	
23.	IV	Procaine penicillin	3.0		236 ± 16	245 ± 13	4.7	
24.	IV	Streptomycin sulfate	3.0		236 ± 16	211 ± 14	— 18.3	
25.	IV	Terramycin	3.0		236 ± 16	236 ± 20	...	

[1] The starting weights of the rats were: series I, 45 gm; series II, 44 gm; series IV, 44.6 gm; and series VIII, 47 gm.

effectiveness of the antibiotics was greatest when growth in the control group was relatively low. For this reason a comparison between the results of oral and parenteral experiments appeared to be valid only when growth in the appropriate control groups was similar, as in table 2 series VIII, lines 7 and 8, 11 and 12. Under these conditions penicillin was only 53.7% as effective in increasing growth when the vitamin was injected as when it was fed; and for aureomycin the corresponding figure was 37%. In other words, although the antibiotics stimulated growth even when there was no thiamine in the small intestine to be diverted to the microorganisms, the antibiotics were less effective than when some thiamine was present in the digestive tract from the diet.

Riboflavin. As in the case of thiamine, animals receiving limiting levels of riboflavin grew better when penicillin or aureomycin was included in the diet, and this was true whether the vitamin was fed or injected. The degree of stimulation due to the antibiotics varied somewhat (table 3 column 8) but in general the stimulation due to aureomycin was greater when the limiting amounts of riboflavin were in the diet than when they were injected. When growth in the control groups was equal (table 3 lines 11 and 16), aureomycin was 55% as effective when the riboflavin was injected as when it was included in the diet. For penicillin, however, the mode of administration of riboflavin did not appear to influence the effectiveness of the antibiotic. The standard deviation within groups of injected animals, as in the experiments with thiamine, was considerably less than that of those fed limiting amounts of riboflavin.

Pantothenic acid. Rats on a limiting dietary level of pantothenic acid, 3.0 mg/kg, showed consistent growth responses when penicillin or aureomycin was added to the diet. A similar but lesser increase in growth resulted when the vitamin was injected (table 4, columns 8 and 9). Some effect of the antibiotic was also noted in diets completely lacking in this vitamin (table 4 lines 19 to 21).

In general the results with pantothenic acid were more consistent than those with riboflavin or thiamine. When pantothenic acid was injected, penicillin was 70 to 90% as effective as when the limiting vitamin was in the diet; aureomycin was only 37 to 64% as effective for the parenteral as for the oral

TABLE 3

Response to dietary antibiotics of rats receiving suboptimal amounts of riboflavin orally or by injection

LINE NO.	SERIES NO.	ANTIBIOTIC	RIBOFLAVIN		FINAL WEIGHT [1]		GAIN DUE TO ANTIBIOTIC	EFFECTIVENESS OF INJECTED DOSE
			Fed	Injected	No antibiotic	Plus antibiotic		
		25 mg/kg	*μg/gm*	*μg/day*	*gm*	*gm*	*%*	*%*
1.	III	Penicillin G	0.75		174 ± 32	187 ± 13	10.4	
2.				3.6–12	130 ± 7	151 ± 18	25.7	247
3.	V	Penicillin G	0.75		117 ± 24	166 ± 16	67.0	
4.				6.0	141 ± 3	166 ± 10	24.7	36.9
5.	X	Procaine	0.75		124 ± 24	161 ± 18	48.6	
6.		penicillin		4.0	128 ± 16	164 ± 14	44.9	92.3
7.	III	Aureomycin	0.75		174 ± 32	156 ± 33	— 14.0	a
8.		HCl		3.6–12	130 ± 7	138 ± 11	9.8	
9.	V	Aureomycin	0.75		117 ± 24	144 ± 22	36.9	
10.		HCl		6.0	141 ± 3	154 ± 12	13.4	36.3
11.	X	Aureomycin	0.75		124 ± 24	148 ± 25	31.5	
12.		HCl		4.0	128 ± 16	142 ± 16	17.5	55.5
13.	X	Streptomycin	0.75		124 ± 24	126 ± 9	2.6	
14.		sulfate		4.0	128 ± 16	148 ± 28	25.0	961
15.	X	Chloromycetin	0.75		124 ± 24	133 ± 11	11.8	
16.				4.0	128 ± 16	159 ± 14	38.6	327
17.	III	Penicillin G	3.0		240 ± 31	257 ± 12	8.9	
18.	III	Aureomycin	3.0		240 ± 31	256 ± 25	8.4	

[1] The starting weights of the rats were: series III, 48.4 gm; series V, 43.8 gm; and series X, 47.8 gm.

TABLE 4

Response to dietary antibiotics of rats[1] receiving suboptimal amounts of calcium pantothenate orally or by injection

LINE NO.	SERIES NO.	ANTIBIOTIC	CALCIUM PANTOTHENATE		FINAL WEIGHT[2]		GAIN DUE TO ANTIBIOTIC	EFFECTIVENESS OF INJECTED DOSE
			Fed	Injected	No antibiotic	Plus antibiotic		
		25 mg/kg	*μg/gm*	*μg/day*	*gm*	*gm*	*%*	*%*
1.	IX	Procaine	3.0		158 ± 39	209 ± 35	46.2	
2.		penicillin		18	149 ± 14	194 ± 14	44.3	95.9
3.	VII ♀	Penicillin G	3.0		139 ± 21	172 ± 17	35.4	
4.				20	147 ± 9	172 ± 6	24.9	70.3
5.	VI	Penicillin G	3.0		150 ± 20	187 ± 33	34.9	
6.				24	170 ± 19	204 ± 20	27.0	77.3
7.	IX	Aureomycin	3.0		158 ± 39	224 ± 18	61.5	
8.		HCl		18	149 ± 14	188 ± 44	38.4	64.3
9.	VII ♀	Aureomycin	3.0		139 ± 21	182 ± 16	46.1	
10.		HCl		20	147 ± 9	164 ± 9	16.8	37.3
11.	VI	Aureomycin	3.0		150 ± 20	164 ± 16	13.2	
12.		HCl		24	170 ± 19	177 ± 5	5.6	42.1
13.	IX	Streptomycin	3.0		158 ± 39	178 ± 23	18.1	
14.		sulfate		18	149 ± 14	170 ± 16	20.7	114.4
15.	VII ♀	Streptomycin	3.0		139 ± 21	139 ± 13	...	
16.		sulfate		20	147 ± 9	161 ± 15	13.8	a
17.	VI	Streptomycin	3.0		150 ± 20	180 ± 11	28.2	
18.		sulfate		24	170 ± 19	192 ± 26	17.5	62.0
19.	VI	Penicillin G			121 ± 7	148 ± 25	35.1	
20.	VI	Aureomycin HCl			121 ± 7	125 ± 13	5.2	
21.	VI	Streptomycin sulfate			121 ±7	138 ± 16	22.1	

[1] Series VII consisted of female rats; all others were males.

[2] The starting weights of the rats were: series VI, 44 gm; series VII, 45.6 gm; and series IX, 47.5 gm.

pantothenate. Results with females (series VII) were very similar to those obtained with males.

Streptomycin. Others (Lih and Baumann, '51; Guggenheim et al., '53) have observed streptomycin to be less effective than aureomycin or penicillin in increasing the growth of rats fed diets limiting in thiamine or riboflavin and this has been confirmed in the present study. The observed percentage increases in growth due to the streptomycin were 4.6 and 18 in two trials with thiamine (table 2 lines 13 and 15), 2.6 with riboflavin (table 3 line 13), and 18, 0, and 28 with pantothenic acid (table 4 lines 13, 15, and 17). Unexpectedly, however, substantial increases in growth frequently resulted when streptomycin was fed to rats receiving a limiting vitamin by injection (table 2 line 14; table 3 line 14; table 4 line 14); in other words, streptomycin tended to be most effective when one of the essential vitamins was missing from the diet.

DISCUSSION

In spite of variations in the growth responses observed, penicillin and aureomycin consistently stimulated the growth of rats receiving limiting amounts of vitamins by subcutaneous injection. This was true for 5 different vitamin-antibiotic combinations, two of which have also been tested by Schendel and Johnson ('54). These results with rats differ from those observed with thiamine and penicillin in chicks (Waibel et al., '54). Since the levels of vitamins injected into the rats were not high, one would expect most of the injected dose to be distributed throughout the body, and only a minor fraction to reach the intestinal lumen where microorganisms could divert it for their own purposes. Under these circumstances the normal diversion of vitamins from the host to the intestinal microorganisms would not be sufficiently important so that a change in organisms could affect the growth of the rat by diminishing diversion. On the other hand an increase in the amounts of the vitamins synthesized by the intestinal microorganisms would be beneficial whether the limiting vitamin were administered in the diet or by injection. Hence it

would appear that antibiotics stimulate growth via the mechanism of intestinal synthesis at least to the extent that growth increases when the dosage of limiting vitamin is administered parenterally.

This latter stimulation, however, was usually much less than that observed when the dose of limiting vitamin was administered in the diet, and for the 18 comparisons made in the present study the relative effectiveness of penicillin and aureomycin in the parenteral experiments was approximately one-half that in the oral experiments. The data of Schendel and Johnson ('54) indicate aureomycin to have been 68% as effective when pantothenic acid was injected as when it was fed.[3]

One possible explanation for two observed levels of effectiveness for any given antibiotic is that both the mechanism of vitamin synthesis and that of diminished vitamin diversion may be operative when the antibiotic and the limiting vitamin are present in the digestive tract together, whereas the administration of limiting vitamin by injection permits the antibiotic to operate only by the mechanism of vitamin synthesis. According to this concept the discrepancy between the results of the two types of procedure might be a measure of the alteration in vitamin diversion that normally takes place when antibiotic is added to the diet.

Quite another possibility is that the effectiveness of an antibiotic upon sensitive organisms may vary with the completeness of the medium in which that organism is growing (Lih, '52). When the limiting vitamin is present in the diet, the medium (intestinal contents) is more nearly optimal for the organism than when a B vitamin is omitted from the diet. This latter situation may explain why streptomycin was frequently more effective when a vitamin was missing from

[3] Schendel and Johnson ('54) also reported an experiment on penicillin and thiamine, with data very similar to those in lines 1–4, table 2 of the present study. Unfortunately a low rate of growth in the control group often implies growth for only part of the experimental period, and under these conditions the apparent effectiveness of an antibiotic may be exaggerated.

the digestive tract (injection experiment) than when it was present in an amount suboptimal for the rat.

SUMMARY

Limiting amounts of thiamine, riboflavin, and pantothenic acid were administered to rats either by inclusion in the diet or by subcutaneous injection, and the effectiveness of dietary antibiotics on growth determined. Penicillin and aureomycin consistently increased growth under both conditions, but the magnitude of the increase was usually greater when the limiting B vitamin was in the diet than when it was injected. This was true in 5 of 6 experiments with thiamine, 6 of 6 with pantothenic acid, and three of 5 with riboflavin.

The results suggest that the antibiotics stimulate an increased intestinal synthesis of these vitamins, but leave the question open whether this is the only mechanism involved.

Streptomycin was relatively ineffective in diets containing limiting amounts of these vitamins, but it seemed to improve the effectiveness of the injected vitamins.

LITERATURE CITED

GUGGENHEIM, K., S. HALEVY, I. HARTMANN AND R. ZAMIR 1953 The effect of antibiotics on the metabolism of certain B vitamins. J. Nutrition, *50:* 245–254.

LIH, H., AND C. A. BAUMANN 1951 Effects of certain antibiotics on the growth of rats fed diets limiting in thiamine, riboflavin, or pantothenic acid. Ibid., *45:* 143–152.

LIH, H. 1952 Certain effects of antibiotics on rats and on *Leuconostoc citrovorum*. Ph.D. thesis. University of Wisconsin, Madison.

LINKSWILER, H., C. A. BAUMANN AND E. E. SNELL 1951 Effect of aureomycin on the response of rats to various forms of vitamin B_6. J. Nutrition, *43:* 565–574.

MILLER, E. C., AND C. A. BAUMANN 1945 Further studies on factors that affect xanthurenic acid excretion. J. Biol. Chem., *159:* 173–183.

MONSON, W. J., A. E. HARPER, M. E. WINJE, C. A. ELVEHJEM, R. A. RHODES AND W. B. SARLES 1954 A mechanism of the vitamin-sparing effect of antibiotics. J. Nutrition, *52:* 627–636.

SARMA, P. S., E. E. SNELL AND C. A. ELVEHJEM 1946 The vitamin B_6 group VIII. Biological assay of pyridoxal, pyridoxamine, and pyridoxine. J. Biol. Chem., *165:* 55–63.

SAUBERLICH, H. E. 1952 Effect of aureomycin and penicillin upon the vitamin requirement of the rat. J. Nutrition, *46:* 99–108.

SCHENDEL, H. E., AND B. C. JOHNSON 1954 Studies of antibiotics in weanling rats administered suboptimal levels of certain B vitamins orally and parenterally. Ibid., *54:* 461–468.

SNELL, E. E., AND A. N. RANNEFELD 1945 The vitamin B_6 group III. The vitamin activity of pyridoxal and pyridoxamine for various organisms. J. Biol. Chem., *157:* 475–489.

WAIBEL, P. E., W. W. CRAVENS AND C. A. BAUMANN 1953 Effect of dietary antibiotics on the growth of chicks receiving suboptimal amounts of thiamine by mouth or by injection. J. Nutrition, *50:* 441–450.

NITROGEN UTILIZATION DURING CALORIC RESTRICTION

III. THE EFFECT OF PRECEDING DIET [1]

DORIS HOWES CALLOWAY AND HARRY SPECTOR
Quartermaster Food and Container Institute for the Armed Forces
Chicago, Illinois

THREE FIGURES

(Received for publication February 14, 1955)

It has been shown by other workers that the composition of the diet fed preceding a period of fasting alters the carbohydrate (Samuels et al., '42; Roberts and Samuels, '43, '46; Hansen et al., '51), fat (MacKay et al., '41; Roberts et al., '44; Tidwell and Treadwell, '46), and protein metabolism (Voit, 1866; Roberts and Samuels, '49) during subsequent fasting. Variation in the protein-efficiency ratio in the mouse due to pre-test treatment (Bosshardt et al., '46) and in the nitrogen balance index in the dog (Allison et al., '46), related to prior state of protein nutrition, have been reported.

Evidence has been obtained in this laboratory that both the character of the diet and the plane of protein feeding before a period of caloric restriction affect ensuing nitrogen metabolism (Calloway and Spector, '53). This paper presents an extension of the work, including additional protein sources and levels.

[1] "This paper reports research undertaken at the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 530 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense."

PROCEDURE

Male albino rats of the Sprague-Dawley strain, weighing approximately 300 gm, were used in all experiments. Feeding, metabolic collections and chemical analyses were performed by methods previously described (Calloway and Spector, '55a). Complete vitamin supplements were given daily by dropper. Data to be presented in detail were drawn from three experiments.

In experiment I, one of 4 standardizing diets was fed for two weeks: (a) commercial stock diet [2]; (b) commercial stock diet diluted with sucrose to reduce the protein content to that of an 18% casein diet; (c) casein (28%) diet with the same composition as the commercial stock diet or (d) a purified diet containing 18% casein. Diets a and c supplied approximately 450 mg of nitrogen per day and diets b and d, 270 mg. During the following 12-day period of 50% caloric restriction, all animals were fed an egg albumin diet providing 160 mg of nitrogen. Representative animals, 8 per group, were sacrificed at the end of standardization and after 4, 8 and 12 days of caloric restriction. Analyses performed were: urinary and fecal nitrogen excretions; moisture, nitrogen and fat in liver and carcass; and plasma protein.

During the standardization period of experiment II, 6 animals per group were fed diets supplying 160, 275, or 455 mg of nitrogen from two sources, egg albumin or soy globulin. One group received a casein diet at the 160 mg nitrogen level and another, a protein-free diet. For 4 days of 50% caloric restriction the egg albumin diet, which supplied 160 mg of nitrogen, was fed to all groups. Liver composition was determined on receipt of animals from the supplier, after standardization and afer restriction. Urinary and fecal nitrogen excretions were measured during standardization and restriction.

In the third experiment, animals were fed the commercial stock diet for 10 days after receipt from the supplier to

[2] Purina Laboratory Chow.

minimize the effects of shipping weight loss and food restriction. The groups of 6 animals each then were maintained for two weeks on 4 types of diet, providing approximately 300 mg of nitrogen: (a) diluted commercial stock diet, (b) methionine supplemented Army ration (Ration, Individual, Combat [3]), (c) casein diet, or (d) egg albumin diet. The diets fed during

TABLE 1

Body weight losses after 4 days of 50% caloric restriction

PRE-STANDARDIZATION TREATMENT	STANDARDIZATION DIET			BODY WEIGHT LOSS ON RESTRICTION DIET: 50% CALORIES, 160 MG NITROGEN		
	Type	Nitrogen supplied	Calories supplied	Egg albumin	Casein + lactalbumin	Casein
		mg/day	*per day*	%	%	%
Experiment I:						
None	Commercial diet	462	46	10.4		
	Diluted comm. diet	275	46	8.5		
	Casein	445	46	6.6		
	Casein	270	46	6.9		
Experiment II:						
None	Protein-free	0	[1]	3.1		
	Egg albumin	168	48	7.6		
	Egg albumin	275	47	7.4		
	Egg albumin	455	48	7.2		
	Soy globulin	161	47	6.0		
	Soy globulin	278	48	7.1		
	Soy globulin	462	47	8.2		
	Casein	168	48	4.7		
Experiment III:						
Commercial diet for 10 days	Diluted comm. diet	341	54	7.1	7.5	
	Army ration	315	54	8.1	7.7	
	Casein	297	54	5.4	5.4	5.4
	Egg albumin	344	54	7.0	6.7	

[1] Fed ad libitum, average intake 44 Cal./day.

[3] Ration similar in composition to typical American diet but containing only processed foods. Growth studies indicate that 0.15% methionine supplementation is required for the rat although the ration is adequate for man.

a 4-day restriction period contained either egg albumin or a mixture of casein and lactalbumin [4] to yield 160 mg of nitrogen at half the caloric requirement. In addition, a group of casein pre-fed animals received casein during restriction. Liver measurements were made at the beginning and end of the standardization period and after restriction. Nitrogen balance was determined during restriction.

A summary of the diet plans is given in table 1.

RESULTS

Body weight relationships

Standardization. In experiments I and II, 46 to 48 Cal. were required to maintain body weight essentially constant during standardization. In experiment III, the requirement was 54 Cal. This same phenomenon of higher caloric requirement following commercial diet pre-feeding was reported in the preceding papers (Calloway and Spector, '53, '55b). The consistency of the finding and the uniformity of the observed caloric decrement suggest that the efficiency of food utilization is modified to the extent of 10 to 15%.

In experiment II, differences in body weight maintenance attributable to protein were observed. Protein-free feeding resulted in 14% body weight loss, although food consumption averaged 44 Cal. per day, only 4% less than the amount required to maintain body weight with adequate protein diets. The animals given the soy globulin diet, supplying 161 mg of nitrogen, lost 6.5% of their initial weight during the first 5 days of standardization but maintained constant body weight thereafter. Analyses of soy globulin obtained in this laboratory reveal that this protein is low in 7 of the 10 amino acids essential for rat growth with methionine the limiting one.

Restriction. The majority of comparisons presented are for the first 4 days of restriction as the degree of body weight loss attained was comparable to that anticipated in humans under military survival conditions. As previously reported

[4] Cas-al, precipitated whole milk proteins, Crest Foods Co.

(Calloway and Spector, '55a), variation in weight response occurred only in the first two days of restriction after which the rate of loss was the same for all experimental groups fed 50% of the caloric requirement. In continued restriction three to 3.5% weight losses were incurred over each two-day period.

As shown in table 1, weight losses were approximately 7 to 8% at the end of 4 days of 50% caloric restriction. The group standardized on the commercial diet lost a significantly greater amount, 10.4%, but this greater loss was offset when the diet was modified by the addition of sucrose and corn oil, being 8.5, 7.1, and 7.5%, respectively. Within each experiment, animals previously fed casein diets lost significantly less weight than those fed any other protein, but the source of protein fed during restriction was without effect. Body weight loss in the protein-depleted group was only 3.1%.

Nitrogen excretion and balance

Standardization. In the first experiment, all groups were in positive nitrogen balance during standardization. There was no significant difference between the two dietary nitrogen levels in this regard but the casein-fed groups exhibited greater retention than did those fed the commercial diet. (table 2). Two factors affected this balance: (1) the nitrogen of the commercial diet was 73% digestible (apparent) while that of casein was 90% and (2) of the nitrogen absorbed, less of the nitrogen in the commercial diet was retained — 4 and 8% as opposed to 10 and 13%.

During the period of standardization in experiment II, all groups fed protein were in positive nitrogen balance. Differences between proteins were apparent only when the intake was 160 mg of nitrogen per day, where the order of rank was egg albumin, 58 mg; casein, 35 mg; and soy globulin, 18 mg of nitrogen retained daily. There were no significant differences in protein digestibility. At higher intake levels, the balances were essentially the same. The fact that these animals continued to retain protein over a two-week period

in spite of caloric limitation sufficient to prevent weight increase, indicates that body composition may have undergone modification with protein replacing some other constituent. The group fed a protein-free diet lost nitrogen at the rate of 58 mg per day.

TABLE 2

Nitrogen balance during standardization and during 4 days of 50% caloric restriction

TYPE OF DIET	STANDARDIZATION		RESTRICTION		
	Nitrogen intake	Nitrogen balance [1]	Nitrogen balance days 1–4		
			Egg albumin	Casein	Casein + lactalbumin
	mg/day	*mg/day*	*mg*	*mg*	*mg*
Experiment I:					
Commercial diet	462	+ 13	— 367		
Diluted comm. diet	275	+ 16	— 236		
Casein	445	+ 38	— 288		
	270	+ 30	— 208		
Experiment II:					
Protein-free	0	— 58	+ 104		
Egg albumin	168	+ 58	— 272		
	275	+ 51	— 292		
	455	+ 54	— 381		
Soy globulin	161	+ 18	— 119		
	278	+ 56	— 273		
	462	+ 85	— 442		
Casein	168	+ 35	— 132		
Experiment III:					
Diluted comm. diet	341		— 176		— 175
Army ration	315		— 204		— 173
Casein	297		— 153	— 176	— 177
Egg albumin	344		— 234		— 235

[1] Mean, last two days.

Restriction. When fed 161 mg of egg albumin nitrogen per day, the protein-depleted animals retained nitrogen in spite of caloric restriction. All other groups were in negative nitrogen balance and the degree of negativity was directly proportional to the amount of nitrogen previously fed as

shown in figure 1. These data plotted from experiments I and II represent the first two and 4 days of restriction when the carry-over effect of the previous diet was most marked. The same trend was observed over days three and 4 as in one and two but the differences were of smaller magnitude; by the 5th and 6th days, previous diet effects were eliminated.

This carry-over effect was greatest where egg albumin was the prior protein source and least in the case of casein. It is apparent that differences among protein sources were

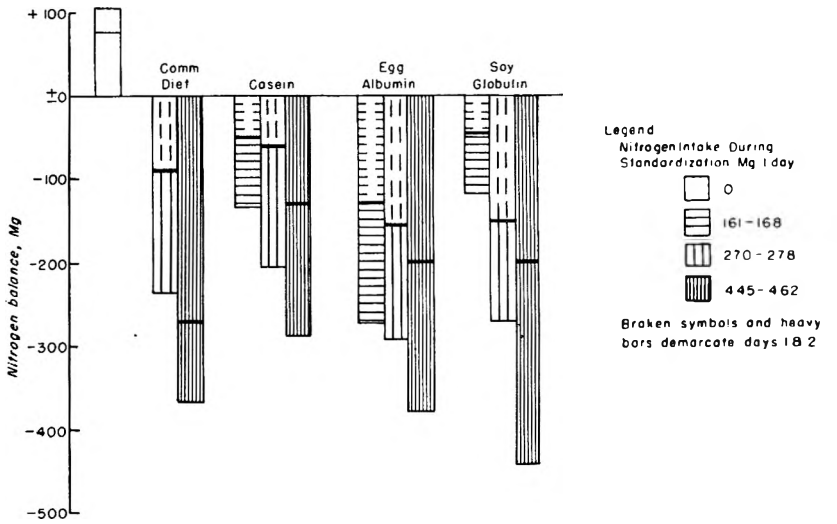

Fig. 1 Relationship between standardization diet and nitrogen balance during 4 days of 50% caloric restriction on an egg albumin diet supplying 160 mg N/day.

greatest where the intake was low, i.e., 160 mg of nitrogen. In this case, the difference attributable to previous protein source was over 200% greater with egg albumin than with either casein or soy globulin. At the highest intake, 445 to 460 mg, while variation was still found, the differences were no longer significant.

The balance observed following the regime in which 168 mg of nitrogen from egg albumin was supplied was comparable to that following several levels of other test proteins (fig. 1). Since the same retention followed ingestion of greater amounts

of nitrogen from this and other sources during standardization it is apparent that protein catabolism and nitrogen excretion increased. Presumably tissue regulatory mechanisms, specifically enzyme systems, were modified in response to a higher exchange of nitrogenous products. The result was a carry-over effect more related to previous intake level than to tissue retention.

To provide a well-nourished standard animal, it is suggested that a diet supplying approximately 160 mg of nitrogen daily from egg albumin would be the diet of choice as this diet promoted maximum nitrogen retention during standardization without excessive intake. If the animal is to be well-nourished, diets of lower nitrogen content or containing proteins of poorer biological value should be eliminated even though the carry-over effect is less marked.

Evidence from these experiments indicates that the frequently used allowance of two days to eliminate the effects of adjustment to change in diet is insufficient for restriction studies and that at least 4 days are required for this stabilization. A correlation coefficient was determined for the 4 days of restriction against previous nitrogen intake in 15 comparable groups [5] from this series and those reported in the previous papers (Calloway and Spector, '55a, b). A highly significant ($p = 0.001$) r of -0.75 was obtained and the regression equation described was $Y = -0.57 \times -73.33$, where $Y =$ 4-day nitrogen balance at 50% caloric restriction with 160 mg of egg albumin nitrogen and $X =$ previous daily nitrogen intake in milligrams.

In the third experiment, a smaller nitrogen deficit was incurred in all groups but the groups pre-fed egg albumin again exhibited greater losses than those given other sources of protein. Although nitrogen balance studies were not made during the standardization period of this experiment, the higher requirement for maintenance of body weight and the

[5] The protein-depleted group was eliminated in this computation as the response would vary with duration of depletion, but inclusion of those data results in a higher correlation, $r = -0.82$.

liver composition data (see below) suggest that the balance was negative for at least part of this period, resulting in partial depletion and, by inference, diminished output during restriction.

Variation in source of protein fed in the restriction phase was without significant effect. This would imply that when body protein was being catabolized in addition to dietary protein, the amino acid composition of the dietary protein was relatively less important in the common pool. In effect, the tissue protein might supplement dietary protein for needs other than energy production.

Liver composition

Standardization. In experiment I, livers weighed about 9 gm at the end of standardization and contained 310 to 342 mg of nitrogen and 269 to 317 mg of fat (table 3). The trend toward augmented liver nitrogen in the groups pre-fed the higher levels of nitrogen is not statistically significant.

The livers of animals sacrificed in experiment II, on receipt from the supplier, weighed an average of 10 gm and contained 358 mg of nitrogen, 11.8% of the total solids. At the end of the standardization period, all animals fed protein showed a statistically significant increase in liver nitrogen content and only extreme values were significantly different from one another. Liver weight also increased as would be expected on the basis that the animals are received in a semi-starved state and, secondly, because some growth potential exists. In all animals fed the minimum maintenance protein requirement, increases in hepatic weight and total nitrogen were parallel. When the protein allowance was liberal (275 or 450 mg N/day), increases in total liver nitrogen were greater than increases in liver weight; that is, diets of high protein content resulted in increased concentration of nitrogen per gram of tissue with relatively smaller increases in total tissue.

Differences in the biological value of the proteins fed during standardization were not reflected in the content of liver nitrogen and the lowest intake of nitrogen was equally

TABLE 3

Liver composition before and after standardization and after 4 days of 50% caloric restriction

EXPERIMENT NO.	INITIAL					AFTER STANDARDIZATION							AFTER 4 DAYS RESTRICTION					
	Wet wt.	Nitrogen		Fat		Diet		Wet wt.	Nitrogen		Fat		Diet supplying 160 mg N	Wet wt.	Nitrogen		Fat	
		Dry	Total	Dry	Total	Type	Nitrogen		Dry	Total	Dry	Total			Dry	Total	Dry	Total
	gm	%	*mg*	%	*mg*		*mg/day*	*gm*	%	*mg*	%	*mg*		*gm*	%	*mg*	%	*mg*
I						Commercial diet	462	9.3	11.4	342	10.6	317	Egg albumin	7.6	11.2	289	10.0	258
						Diluted comm. diet	275	8.7	11.6	310	10.1	269		8.1	11.0	305	10.8	298
						Casein	445	8.7	12.0	335	10.5	293		8.6	11.0	319	11.4	333
						Casein	275	8.6	11.4	319	10.6	296		7.7	11.6	298	10.0	254
II	10.0	11.8	358	9.7	296	Protein-free		9.8	10.5	298			Egg albumin	9.5	11.5	312		
						Egg albumin	168	12.3	11.6	436				10.1	12.2	346		
							275	11.6	12.3	466				10.3	12.4	351		
							455	11.1	12.6	456				9.9	12.4	343		
						Soy globulin	161	11.1	11.2	399				10.0	12.1	340		
							278	11.0	12.5	436				10.2	12.4	341		
							462	10.8	12.7	444				9.8	12.6	338		
						Casein	168	12.2	11.6	420				11.6	11.7	380		
III	15.2	11.8	482	8.1	332	Diluted comm. diet	341	12.9	11.4	415	9.5	341	Egg albumin	10.8	11.8	357	9.4	284
													Cas. + lact.	11.7	11.5	376	9.3	304
						Army ration	315	13.8	10.8	421	10.0	389	Egg albumin	11.0	12.0	368	8.3	257
													Cas. + lact.	11.6	11.8	374	9.6	306
						Casein	297	14.5	12.0	482	8.7	350	Egg albumin	12.6	11.7	407	7.7	268
													Cas. + lact.	11.5	11.2	366	9.1	292
													Casein	11.4	11.4	366	9.0	288
						Egg albumin	344	14.3	11.7	454	9.0	352	Egg albumin	11.9	11.8	388	7.8	258
													Cas. + lact.	12.1	11.9	393	7.2	238

as effective in production of liver nitrogen as the higher levels of each protein (table 3). The protein-depleted animals lost 17% of total liver nitrogen, due to a decrease in the percentage of nitrogen with essentially no change in the weight of the organ. No significant changes in moisture or ash content, approximately 68 and 4.4% respectively, occurred.

After initial pre-feeding with commercial stock diet, the liver weight of experiment III controls was 15 gm and nitrogen content, 482 mg. The standardization procedure resulted in diminished liver weight, greatest in the case of groups fed commercial diet or supplemented Army ration. In those two groups fed mixed proteins, the loss of liver nitrogen was 13 to 14%; where animal protein was fed exclusively, the losses were minimum in the case of egg albumin and absent in the casein group. There was essentially no change in total liver fat content, except in the group fed Army ration where the increase was 17%. The percentage of moisture and ash did not change significantly and, therefore, total changes paralleled changes in liver weight.

Restriction. At the end of 4 days of restriction, all groups previously fed protein exhibited losses of liver weight and nitrogen. In the first experiment, the animals fed the commercial diet incurred the only significant 4-day deficit. When restriction was continued to 8 days, the liver nitrogen content of the groups pre-fed high nitrogen diets was 258 to 262 mg and further restriction to 12 days produced no additional loss with totals of 259 to 261 mg. The rate of loss in the moderate level groups was more gradual, content being 281 to 282 mg at 8 days and 253 to 256 mg at 12.

Following restriction, the total liver nitrogen content of 338 to 351 mg was not significantly different among groups pre-fed egg albumin or soy globulin. Changes in these groups were of greater magnitude than in those of experiment I (table 3, 3rd column from right). Comparable losses were encountered in experiment III, where again only differences between extreme groups were significant. That is, the animals in the group which was pre-fed the commercial diet followed

by egg albumin lost significantly more; and those fed casein followed by egg albumin, egg albumin followed by egg albumin or by casein + lactalbumin showed losses that were significantly less when compared with each other. Adjacent mean values did not differ.

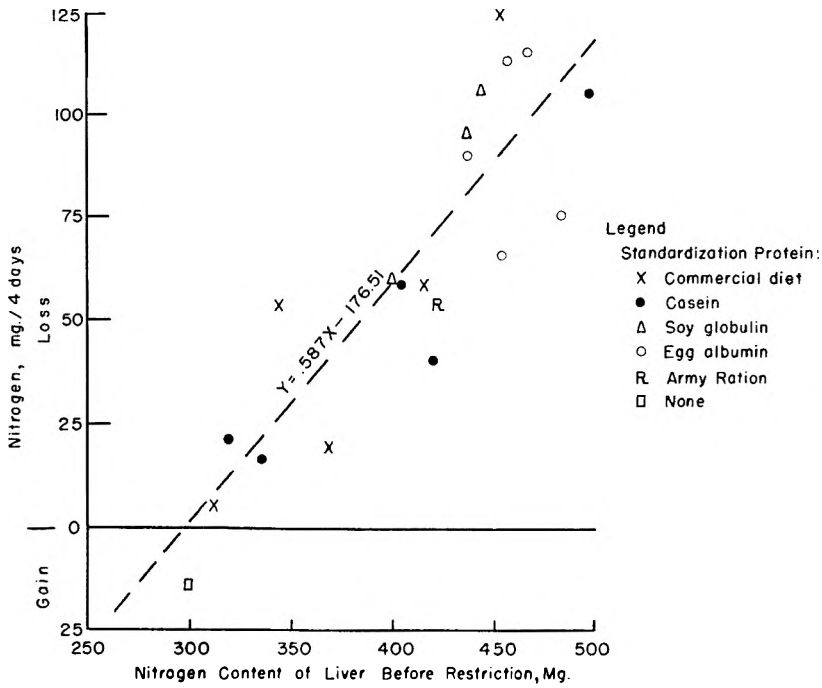

Fig. 2 Relationship between the content of liver nitrogen at the end of standardization and liver nitrogen loss after 4 days of 50% caloric restriction on an egg albumin diet supplying 160 mg N/day.

In protein-depleted animals there was increased nitrogen from 10.5 to 11.5% but no significant change in total content.

Reviewing the entire group of experiments, it appears that the loss of liver nitrogen was related to the initial content of the organ which was, in turn, chiefly related to organ weight. That this was true is apparent from figure 2, the 4-day loss being directly related to initial content ($r = +0.87$, $p, < 0.001$) in 20 groups from the complete series. The regression

equation was $Y = 0.587X - 176.51$ where $Y = 4$-day nitrogen loss in milligrams and $X =$ milligrams of initial liver nitrogen.

To determine the relationship between liver losses and overall nitrogen deficit the data were plotted as shown in figure 3. The complete absence of correlation is obvious ($r = -0.36$). Liver nitrogen changes were not quantitative criteria for the assessment of nitrogen metabolism and the only factor

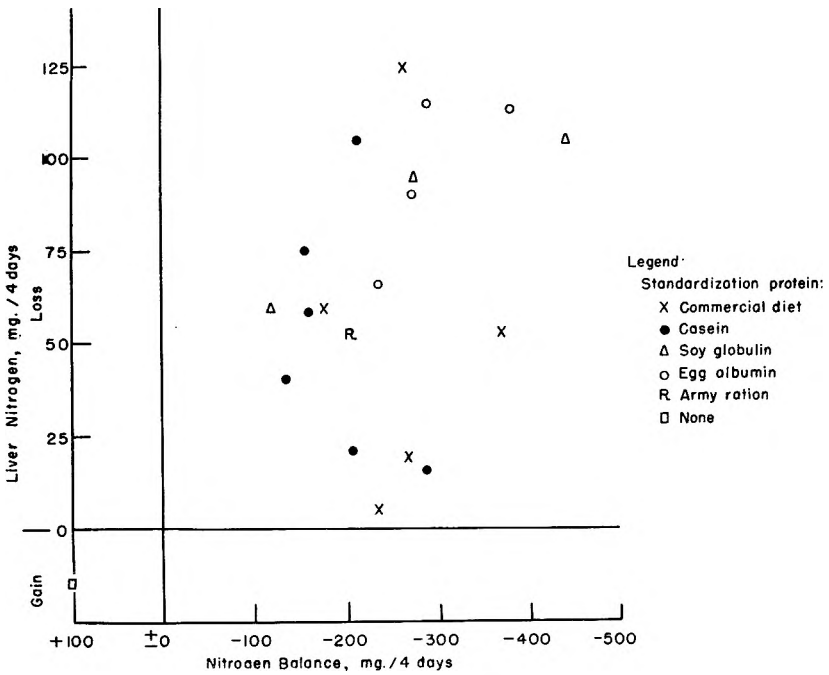

Fig. 3 Absence of relationship between nitrogen balance and liver nitrogen loss during caloric restriction.

which appeared valid was that changes were in the same direction, i.e., in no case was negative total balance concomitant with increased liver nitrogen. As a corollary, there is no evidence to indicate that expenditure of large liver nitrogen reserves resulted in significantly reduced losses from other tissues.

The factor or factors involved in the variation in liver weight have not been delineated as no controlled variable in

experiments to date can be designated as affecting the ratio of liver weight to body weight.

Other tissues

As shown in table 4, the nitrogen content of the carcass rose slightly, from 3.4 to 3.8%, as the body fat content diminished so that the loss of tissue protein was smaller than that of body weight. Total nitrogen loss as measured in

TABLE 4

Average carcass nitrogen and plasma protein after standardization and 4, 8 and 12 days of 50% caloric restriction

MEASURE	STANDARDIZATION DIET		STANDARDIZATION	RESTRICTION		
	Type	Nitrogen		4 days	8 days	12 days
		mg/day				
Carcass nitrogen, % (wet basis)	Comm. diet	462	3.43	3.58	3.75	3.86
	Comm. diet	275	3.48	3.44	3.79	3.74
	Casein	445	3.43	3.52	3.76	3.79
	Casein	270	3.37	3.61	3.90	3.83
Plasma protein, %	Comm. diet	462	6.48	6.72	6.34	6.21
	Comm. diet	275	6.73	6.54	6.54	6.15
	Casein	445	6.87	6.72	6.23	6.11
	Casein	270	6.22	6.60	6.02	6.00

the carcass represented about 1 gm or 9% over a 12-day period. The rate of loss was gradual and uniform, roughly 0.3 gm over each 4-day period, and was in good agreement with nitrogen balance data. Plasma protein levels declined slightly in all groups when restriction was continued for 8 to 12 days but all values are well within the normal range.

There were no significant differences among groups in carcass fat or moisture content. Initial fat content was 6% on a wet weight basis and decreased to 3% at the end of 12 days of restriction; moisture content was 68% throughout.

SUMMARY AND CONCLUSIONS

Young adult rats were fed a protein-free diet or diets with varying amounts of protein for a two-week standardization period either on receipt from the supplier or after a prefeeding period. Thus, 160 to 460 mg of nitrogen per day were supplied from a commercial stock diet, casein, egg albumin, soy globulin or Army ration. The animals were then subjected to 4 days of 50% caloric restriction on a constant intake of 160 mg of nitrogen. In one series, restriction was continued through 8 and 12 days. Nitrogen balance and liver composition were measured in all groups and, in addition, plasma protein and carcass composition were determined in the prolonged-restriction study.

If standardization was begun on receipt, 46 to 48 Cal. per day were required to maintain constant body weight; after refeeding with a commercial stock diet, the requirement was 54 Cal. During restriction, body weight loss was significantly greater in animals fed the unmodified commercial stock diet than in those fed from any other source.

During caloric restriction, the amount of negative nitrogen balance was directly proportional to the amount of nitrogen fed during standardization. Although this carry-over effect was greatest where egg albumin was the prior protein source, the variation due to level was smallest. Nitrogen balance was not significantly affected by protein source during restriction.

Liver nitrogen losses were correlated with the initial nitrogen content of the organ which was chiefly related to tissue weight. There was no correlation between liver nitrogen loss and nitrogen balance, but carcass nitrogen changes were in good agreement with balance data.

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LITERATURE CITED

ALLISON, J. B., J. A. ANDERSON AND R. D. SEELEY 1946 The determination of the nitrogen balance index in normal and hypoproteinemic dogs. Ann. N. Y. Acad. Sci., *47:* 245.

BOSSHARDT, D. K., L. C. YDSE, M. M. AYRES AND R. H. BARNES 1946 The use of mice for the measurement of the growth promoting quality of proteins. J. Nutrition, *31:* 23.

CALLOWAY, D. H., AND H. SPECTOR 1953 Influence of standardizing diet on nitrogen metabolism during caloric restriction. Fed. Proc., *12:* 410.

———— 1955a Nitrogen utilization during caloric restriction. I. The effect of dietary fat content. J. Nutrition, *56:* 533–544.

———— 1955b Nitrogen utilization during caloric restriction. II. The effect of variation in nitrogen intake. Ibid., *56:* 545–554.

HANSEN, R. G., W. J. RUTTER AND L. T. SAMUELS 1951 The effect of previous diet on the metabolism of glucose by rat diaphragm. J. Biol. Chem., *192:* 243.

MAC KAY, E. M., H. O. CARNE, A. N. WICK AND F. E. VISCHER 1941 The relation of fasting ketosis in the rat to the preceding diet and the liver fat. Ibid., *141:* 889.

ROBERTS, S., AND L. T. SAMUELS 1943 Influence of previous diet on insulin tolerance. Proc. Soc. Exp. Biol. Med., *53:* 207.

———— 1946 Previous diet and the role of the kidney in the metabolism of the eviscerated rat. Am. J. Physiol., *146:* 358.

———— 1949 Influence of previous diet on metabolism during fasting. Ibid., *158:* 57.

ROBERTS, S., L. T. SAMUELS AND R. M. REINECKE 1944 Previous diet and the apparent utilization of fat in the absence of the liver. Ibid., *140:* 639.

SAMUELS, L. T., R. M. REINECKE AND H. A. BALL 1942 Liver fats and glycogen of hypophysectomized rats on high carbohydrate and high fat diets. Proc. Soc. Exp. Biol. Med., *49:* 456.

TIDWELL, H. C., AND C. R. TREADWELL 1946 The effect of the preceding diet upon fasting ketonemia. J. Biol. Chem., *163:* 155.

VOIT, C. 1866 Ueber die Verschiedenheiten der Eiweissersetzung beim Hungern. Zeitschrift für Biol., *2:* 307.

THE EFFECT OF AUREOMYCIN ON THE APPARENT UTILIZATION OF VITAMIN A BY THE OVARIECTOMIZED RAT [1]

T. K. MURRAY AND J. A. CAMPBELL
Food and Drug Laboratories, Department of National Health and Welfare, Ottawa, Canada

ONE FIGURE

(Received for publication March 17, 1955)

The observation that antibiotics may influence the utilization of vitamin A was first made by Burgess et al. ('51), who noted an increase in liver stores of vitamin A, and in blood carotenoids, of chicks receiving penicillin. Coates ('53) reported similar results but pointed out that those chicks which did not respond to the antibiotic with increased growth, did not store more vitamin A than did the control groups. These authors attributed the growth response to the effect of penicillin in controlling a non-specific "infection," which otherwise reduced growth rate; the increased stores of vitamin A were considered to be a result of the increased growth. On the other hand, Hartsook, Batchelor and Johnson ('53) reported that aureomycin did not increase the utilization of vitamin A by the male rat, as judged by liver and kidney storage, growth or survival time. They suggested that aureomycin actually may have decreased the survival time of rats. Swick, Lih and Baumann ('51) found that penicillin did not increase the growth of rats on diets marginal in vitamin A.

In view of the relatively small volume of published work

[1] Some of these data were presented before the Canadian Physiological Society in Toronto, October 1954, and before the American Institute of Nutrition in San Francisco, April 1955.

on the effects of antibiotics on the metabolism of the fat soluble vitamins, and the lack of agreement among the various workers on the subject, it was decided to study the matter further in this laboratory. The experiments were based on the criterion of the effect of vitamin A on the cellular contents of the vagina of the ovariectomized rat. This technique has advantages in respect to specificity and precision over other criteria on which the assay of vitamin A may be based.

METHODS

The method used was the vaginal smear assay of Pugsley, Wills and Crandall ('44) with the exception that the diets contained raw corn-starch in place of dextrinized rice starch. Young, ovariectomized rats were fed the vitamin A maintenance diet until they were depleted of their vitamin A stores. Depletion was judged by the appearance of large numbers of cornified epithelial cells in the vagina. Depleted rats were distributed among groups according to weight, housed in individual cages and were fed the "vitamin A-free" diet. Aureomycin was incorporated in the diet by gradual dilution with casein.

In most experiments, three groups of 8 to 10 rats which received aureomycin, were compared with three similar groups which did not receive aureomycin. Each comparison thus involved 48 to 60 rats. The doses were dilutions in corn oil of the vitamin A acetate reference standard and were administered orally by means of a blunted needle on a tuberculin syringe. Four portions, each of 0.1 ml, were given over a period of two days. The total dose for each of the three levels amounted to 25, 50 or 100 I.U. Except where otherwise stated, the aureomycin was added to the "vitamin A-free" diet two days prior to dosing and thereafter throughout the test. After dosing, the cellular contents of the vagina were examined daily, and the number of days from the beginning of dosing to the reappearance of cornified epithelial cells was recorded. The response data were calculated on the basis of the log dose-response relationship. The effect of aureomycin

was assessed by calculating the "apparent potency" of the standard + treatment in terms of the standard alone, by usual bioassay procedures as outlined by Bliss ('51). The results of a typical three-dose assay are plotted in figure 1.

Since it was possible to use the rats in several successive assays, it was found useful to feed animals between tests on the vitamin A maintenance diet to which vitamin A had been added at the rate of 20 to 40 I.U./100 gm of feed. This diet kept the animals healthy but did not permit storage of vitamin A.

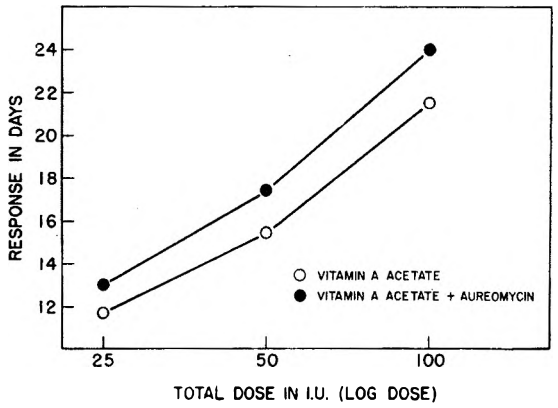

Fig. 1 Typical response lines showing the effect of aureomycin.

RESULTS

The effect of various levels of aureomycin on the utilization of vitamin A was studied in several assays in which the amount was varied from 33 to 330 mg per kilogram of diet. The results of 5 tests are summarized in table 1. Since a consistently significant effect was obtained at a level of 66 mg per kilogram, it was not established whether it was possible to obtain a significant response by the use of smaller amounts. The level of 33 mg per kilogram did not yield a significant response in the single test. It will be noted from the results of experiment 3, in which the groups of experiment 2 were reversed, that the aureomycin had no lasting effect on the utilization of vitamin A. It was therefore deemed permissible

to use the same rats for successive experiments. Rats which had received aureomycin were distributed randomly through the groups of subsequent assays.

In experiment 5, rats which had been fed aureomycin continuously from weaning age were compared in a series of assays with rats which had not received aureomycin. The effect of aureomycin appeared to increase to a certain extent with age or with the length of time that the rats had received aureomycin.

TABLE 1

The effect of aureomycin on the apparent utilization of vitamin A as measured by the vaginal smear assay

EXPERIMENT	AUREOMYCIN IN DIET	APPARENT INCREASE IN POTENCY	CONFIDENCE LIMITS (P = 0.05)
	mg/kg	%	%
1	33	9.8	± 13.0
2	66	35.0	± 11.2
3 [1]	66	40.4	± 16.5
4	330	21.1	± 16.8
5 [2]	66	18.5	± 16.9
	66	27.2	± 14.8
	66	50.5	± 17.3
	66	30.6	± 16.9

[1] Treatment and control groups of experiment 2 reversed 10 days before test.
[2] Successive assays where treatment was continued from weaning of rats.

In an attempt to determine whether the aureomycin exerted its effect only when it was present in the gut with the vitamin A, the aureomycin was fed at different stages of the assay as indicated in table 2. Experiments 1 and 3 were carried out at one time, while experiment 2 was completed in two parts. In every case, three groups which received aureomycin were compared with three other groups which did not receive it. The data indicate that aureomycin, when fed only during the dosing period, had no effect on the assay. When aureomycin was included in the diet from the day succeeding dosing with vitamin A, its effect in increasing potency bordered on significance at $P = 0.05$. If inclusion of aureomycin in the

diet was delayed for a further 24 hours there was no effect on potency.

It was suspected that the presence of small amounts of vitamin A in the "vitamin A-free" diet might explain the fact that aureomycin did not affect the assay unless it was fed throughout the assay. Lowe, Morton and Harrison ('53) had found it necessary to extract a diet similar to the one used in these experiments in order to obtain rapid vitamin A depletion of rats. Accordingly, the assays were repeated

TABLE 2

The effect of aureomycin, fed at various stages of the assay, on the apparent increase in vitamin A potency

AUREOMYCIN IN FEED	EXPERIMENT NO.	INCREASE IN POTENCY	CONFIDENCE LIMITS (P = 0.05)
66 mg/kg		%	%
Throughout test	1	13.9	± 20.6
Two days before dosing until one day after	1	— 1.7	± 20.5
	2	— 5.4	± 17.7
	3	+ 7.8	± 15.5
One day after dosing until end of test	1	15.5	± 16.2
	2	16.5	± 16.8
	3	11.8	± 15.4
Two days after dosing until end of test	2	5.8	± 18.8

using diets containing "vitamin A-free casein" which had been extracted for 6 hours with boiling ethanol and brewers' yeast which had been extracted for 24 hours with petrol ether. The results from two assays based on these diets showed that aureomycin significantly increased the response to vitamin A. It is unlikely, therefore, that the presence of small amounts of vitamin A in the diets was responsible for the observed effects of aureomycin.

Hartsook et al. ('53) added tocopherol to their vitamin A doses at the level of 5 mg per dose. It was of interest, there-

fore, to determine whether added tocopherols had any influence on the aureomycin effect which we had observed. Consequently, tocopherol was added to the vitamin A doses so that each total dose contained 5 mg and the effect of aureomycin under these conditions compared against its effect when the doses contained no added tocopherol. In each case, groups receiving aureomycin and the same doses of vitamin A were compared with those which did not receive aureomycin. The results of three such comparisons, shown in table 3, indicate

TABLE 3

The effect of aureomycin on the response of rats to vitamin A in the presence and absence of tocopherol

TOCOPHEROL[1]	AUREOMYCIN	EXPERIMENT NO.	INCREASE IN POTENCY	CONFIDENCE LIMITS (P = 0.05)
mg/total dose	*mg/kg diet*		%	%
none	66	1	25.0	± 10.4
		2	23.3	± 16.9
		3	16.4	± 19.7
4	none	1	— 6.1	± 16.0
		2	— 0.7	± 18.0
		3	— 4.6	± 17.6
4	66	1	21.5	± 17.5
		2	3.5	± 13.8
		3	25.8	± 23.1

[1] In experiment 1 d-α-tocopherol was used and in experiment 2 and 3 dl-α-tocopherol was used.

that added tocopherol had no effect on the utilization of vitamin A whether or not aureomycin was present.

It has been shown by Peterson, Dick and Johansson ('53) that aureomycin increased the intestinal synthesis of vitamin B_{12}. It was felt that this, perhaps, was the mechanism by which aureomycin affected the vitamin A utilization. High and Wilson ('53) reported that vitamin B_{12} increased the utilization of carotene as measured by liver and kidney stores. They were unable to show a significant effect on the utilization of vitamin A, but it was felt that a smaller effect might be

measurable in the present experiments with the more sensitive vaginal smear assay. Three treatments involving aureomycin and B_{12}, fed alone and together, were compared with groups which received neither aureomycin nor B_{12}. Each treatment involved 30 rats and the comparisons were made in the usual manner. The results shown in table 4 indicate that that vitamin B_{12} did not exert an "aureomycin effect" itself nor did it eliminate the effect of aureomycin. While the effect of aureomycin, when fed with vitamin B_{12}, was not quite significant as compared with the control groups, its effect was significant when compared with the groups which received vitamin B_{12} but no aureomycin.

TABLE 4

The effect of aureomycin on the response of rats to vitamin A in the presence and absence of vitamin B_{12}

VITAMIN B_{12}	AUREOMYCIN	INCREASE IN POTENCY	CONFIDENCE LIMITS (P = 0.05)
mg/kg diet	*mg/kg diet*	%	%
None	66	27.9	± 16.1
2.5	none	— 2.0	± 15.8
2.5	66	11.1	± 15.7

DISCUSSION

There appears to be no doubt that aureomycin increases the apparent utilization of vitamin A in the ovariectomized rat, as measured by the vaginal smear assay. The increase varied from 3.5 to 50.5%. The fact that aureomycin was found (Murray and Campbell, '55) to increase the growth rate of rats which received no, or suboptimal amounts of vitamin A but had no effect when adequate amounts of vitamin A were fed, suggests that the effect is, in some manner, actually concerned with vitamin A. The theory of Coates ('53) that the increase in vitamin A stores brought about by penicillin is a natural consequence of increased growth, does not seem to apply to the work reported here. Many

of the rats used in these experiments were essentially full grown.

An increase in the intestinal absorption of the vitamin A dose is perhaps the most obvious mechanism by which aureomycin exerts its effect. This theory does not account for the fact that a substantial response was consistently obtained when the feeding of the aureomycin was delayed until 24 hours after the last dose. This increase in response was found to be significant when the results of the three assays were combined. When the feeding of the aureomycin was delayed for a further 24 hours in one experiment, a very small increase in potency resulted. It should be remembered that when the feeding of the aureomycin was delayed until 48 hours after the last dose, rats receiving the low dose did not have many days to benefit from the antibiotic. When aureomycin was fed for two days before, during the two-day dosing period and for one day after, it had no effect. It seems unlikely, therefore, that aureomycin exerts its effect through an increase in absorption of the dose. It should also be pointed out in this connection that aureomycin by itself did not alter the vaginal smear of a vitamin A-deficient rat. It did, however, increase the time required to deplete young rats which were fed a vitamin A-deficient diet from weaning (Murray and Campbell, '55).

The above experiments suggested that the absorption of trace amounts of vitamin A in the diet might be involved. However, it was possible to obtain a highly significant effect of aureomycin even when both the yeast and casein had been subjected to exhaustive extraction.

When aureomycin was fed continuously from weaning, the effect of the antibiotic appeared to increase over successive assays. This may have been related to the length of time that the rats received aureomycin rather than the age or size of the rats, since in experiments where the antibiotic was not fed continuously, size and age did not seem to influence the results.

Vitamin E had no effect on the vitamin A assay in either the presence or absence of aureomycin. The antioxidant action of vitamin E is well known, but such effects were not in evidence in these experiments, perhaps because there was already sufficient vitamin E in the diluent oil and the diet. Vitamin B_{12} was also without effect on the assay, which confirms the observation of High and Wilson ('53) that vitamin B_{12} had no effect on liver stores after an oral dose of vitamin A. It also shows that aureomycin does not exert its effect on the vitamin A assay through increased synthesis of vitamin B_{12}.

The average positive effect of aureomycin in 14 assays in which no substantial change was made in the procedure was 24.9%. This means that when the Canadian Reference Standard Vitamin A Acetate (10,000 I.U./gm) was assayed against itself in the presence of aureomycin, its potency appeared to be 12,490 I.U. per gram. Since aureomycin apparently does not effect absorption of the vitamin A dose, the effect must be exerted during the period in which the rat is approaching a deficient state. Under these conditions any vitamin A-active substances that were made available would be used most efficiently and a very few units of vitamin A would have been sufficient to delay the appearance of cornified epithelia in the vagina for the two or three days required to cause the observed differences. Thus it would not be expected that aureomycin would cause a significant increase in liver stores and, in fact, no such increase has been found (Hartsook, Batchelor and Johnson, '53; Murray and Campbell, '55).

No clear explanation for the results observed has been found. It is possible that aureomycin increased the efficiency with which vitamin A was utilized after absorption. This possibility is weakened, but not eliminated, by the fact that aureomycin did not change the rate of disappearance of vitamin A from the liver, kidney and plasma of rats during depletion (Murray and Campbell, '55). Such an effect might occur only during the latter stages of depletion and involve quantities of vitamin A undetectable by the chemical method

used. A second possibility is that intestinal synthesis of vitamin A has taken place in the altered flora of the aureomycin-treated rats. Such a theory has been widely held for the B vitamins but seems less likely in the case of vitamin A activity.

It has been assumed in this paper that all vitamin A has been absorbed within 24 hours after dosing. This assumption, while apparently valid, remains to be proved.

SUMMARY

The effect of aureomycin on the vaginal smear assay for vitamin A has been studied. Aureomycin was added to the diet at the rate of 66 mg per kilogram and the response to doses of vitamin A acetate was compared with the response obtained in the absence of the antibiotic. Standard bioassay procedures were used.

Aureomycin increased significantly the response to vitamin A. This effect was relatively transient. Experiments in which aureomycin supplementation was begun 24 hours after the last vitamin A dose suggested that the effect was not a result of increased absorption of the dose. The addition of vitamin B_{12} to the diet did not mask the effect of aureomycin on the vitamin A assay. Likewise, the addition of tocopherol to the vitamin A doses had no influence on the vitamin A assay in either the presence or absence of aureomycin. Aureomycin increased the mean response to vitamin A by 25%.

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LITERATURE CITED

BLISS, C. I., AND P. GYORGY 1951 Vitamin Methods. Vol. II. Academic Press, Inc., New York.

BURGESS, R. C., M. GLUCK, G. BRISSON AND D. H. LAUGHLAND 1951 The effect of dietary penicillin on liver vitamin A and serum carotenoids in the chick. Arch. Biochem. Biophys., *33*: 339.

COATES, M. E. 1953 The mode of action of antibiotics in animal nutrition. Chemistry & Industry, *50:* 1333.

HARTSOOK, E. W., E. BATCHELOR AND B. C. JOHNSON 1953 Effect of aureomycin on utilization of vitamin A in the growing male albino rat. Proc. Soc. Exp. Biol. Med., *83:* 43.

HIGH, E. G., AND S. S. WILSON 1953 Effects of vitamin B_{12} on the utilization of carotene and vitamin A by the rat. J. Nutrition, *50:* 203.

LOWE, J. S., R. A. MORTON AND R. G. HARRISON 1953 Aspects of vitamin A deficiency in rats. Nature, *172:* 716.

MURRAY, T. K., AND J. A. CAMPBELL 1955 Effect of aureomycin on liver storage of vitamin A and on growth, depletion and survival time of rats. J. Nutrition, *51:* 101–110.

PETERSON, G. E., E. C. DICK AND K. R. JOHANSSON 1953 Influence of dietary aureomycin and carbohydrate on growth, intestinal microflora and vitamin B_{12} synthesis of the rat. Ibid., *51:* 171.

PUGSLEY, L. I., G. WILLS AND W. A. CRANDALL 1946 The biological assay of vitamin A by means of its influence on the cellular contents of the vagina of rats. Ibid., *28:* 365.

SWICK, R. W., H. LIH AND C. A. BAUMANN 1951 Contrasting effects of antibiotics in diets low in vitamin A or in members of the vitamin B complex. Federation Proc., *10:* 395.

EFFECT OF AUREOMYCIN ON LIVER STORAGE OF VITAMIN A, AND ON GROWTH, DEPLETION AND SURVIVAL TIME OF RATS [1]

T. K. MURRAY AND J. A. CAMPBELL
Food and Drug Laboratories, Department of National Health and Welfare, Ottawa, Canada

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It has been shown (Murray and Campbell, '55) that aureomycin influenced favourably the utilization of vitamin A in the ovariectomized rat as judged by the vaginal smear assay. It was therefore of interest to measure the effect of aureomycin on liver stores, depletion and survival time of rats in this laboratory.

METHODS

Rats were either dosed orally with corn oil dilutions of vitamin A acetate or obtained their vitamin A in the form of carotene and fish liver oil from fox starter mash.[2] Aureomycin was added at the rate of 66 mg/kg by gradual dilution with "vitamin-free" casein. Vitamin A was determined in liver, kidney and plasma by the antimony trichloride reaction after saponification and extraction with ether.

RESULTS

Effect of aureomycin on liver storage

A preliminary experiment indicated that aureomycin did not increase liver storage of vitamin A when the dosing period

[1] Some of these data were presented before the American Institute of Nutrition in San Francisco, April, 1955.

[2] Toronto Elevators.

was that generally used in this assay, namely two days. It was therefore decided to determine the effect of aureomycin on long-term storage trials. Two such experiments were conducted. In the first, 20 male and 20 female rats were divided into two groups at random. One group received fox starter mash, the other, the same diet plus 66 mg of aureomycin/kg. These diets were fed for 4 weeks and at the end of this period the rats were killed by decapitation and their livers analyzed for vitamin A. The results, shown in table 1, indicate a significant decrease in the liver weights and a highly signifi-

TABLE 1

Effect of aureomycin on vitamin A liver stores, body and liver weights of rats fed starter mash

DIET	STARTER MASH		STARTER MASH + AUREOMYCIN	
	Males	Females	Males	Females
Number	9	10	10	10
Duration, days	28	28	28	28
Body wt., gm	216.1	153.9	217.8	156.0
Liver wt., gm	9.9359 [1]	7.1869	9.0689	6.9082
Vitamin A/liver, I.U.	1412	1590	1643 [2]	1514
Vitamin A/gm liver, I.U.	143	223	182 [2]	221
Vitamin A stores/gm food	3.015	3.968	3.244	3.787

[1] Significant at $P = 0.05$.
[2] Significant at $P = 0.01$.

cant increase in vitamin A liver stores of males receiving aureomycin. The significance of this increase in vitamin A stores disappeared when it was considered on the basis of vitamin stored per gram of food eaten. No such differences occurred in the female group. It should be noted that the females stored as much vitamin A as the males although their food intake was smaller.

In the second experiment, 20 weanling rats, of each sex, were divided into two groups and were fed either the "vitamin A-free" diet or the same diet to which aureomycin had been added at the rate of 66 mg/kg. All rats were dosed orally, three times per week, with a corn oil dilution of vitamin

A acetate. The total weekly dose amounted to 150 I.U. and dosing was continued for 4 weeks. At the end of this period the rats were killed and their livers analyzed as in experiment 1. The data from this experiment shown in table 2 indicate that aureomycin did not increase significantly liver stores although rats of both sexes stored slightly more vitamin A when they received aureomycin. The livers of the female rats receiving aureomycin were significantly smaller, which contributed to the fact that the concentration of vitamin A in the livers of the aureomycin group was greater than in the controls. There was no difference between the liver weights of males, contrary to the results of experiment 1. It should be noted that in neither of these experiments did aureomycin affect the weight gain.

TABLE 2

Effect of aureomycin on vitamin A liver stores, body and liver weights of rats dosed orally with vitamin A

DIET	A-FREE		A-FREE + AUREOMYCIN	
	Males	Females	Males	Females
Number	10	10	10	10
Duration, days	28	28	28	28
Body wt., gm	156	132	159	128
Liver wt., gm	6.0113	4.7573 [1]	5.9462	4.2395
Vitamin A/liver, I.U.	346	524	370	581
Vitamin A/gm liver, I.U.	58.9	113.1	64.2	143.4 [1]

[1] Significant at $P = 0.05$.

Effect of aureomycin on liver, kidney and plasma vitamin A during depletion

Since aureomycin did not significantly affect the amount of vitamin A stored by rats from a given dose or a given food intake, it was felt that it may have exerted its influence through a more efficient use of the absorbed vitamin. An experiment was conducted in which liver, kidney and plasma vitamin A were measured at intervals during depletion. Male and female weanling rats were fed fox starter mash for 10 days to build up vitamin A stores. They were then divided

into two groups and were fed either the "vitamin A-free" diet or the same diet plus 66 mg of aureomycin/kg. At intervals, 4 male and 4 female rats from each treatment were killed and their livers, kidneys and plasma analyzed for vitamin A. The results are shown in table 3. Aureomycin did not influence the rates of depletion as measured by liver, kidney or plasma vitamin A. There were no consistent differences in body, liver or kidney weights between the groups. It was noted that females retained their kidney stores longer than did males.

Effect of aureomycin on depletion time, weight at depletion and survival time

The rate of vitamin A depletion can also be measured from observations of vaginal smears, weight at depletion and survival time.

In depletion and survival tests only female rats were used. At weaning age these rats were fed the maintenance diet previously described (Murray and Campbell, '55) either with or without aureomycin. As the rats became sexually mature their ovaries were removed, the same number from each treatment being operated on each day. After the operation, the rats were fed the "vitamin A-free" diet either with or without aureomycin. The cellular contents of the vagina were examined daily and the number of days to the appearance of cornified epithelia was recorded, as was also the weight at depletion. Depletion time, as judged by a plateau in weight gain for at least two days, was also calculated. The rats were maintained on these diets until death, and the age at death was recorded. Table 4 contains the results of three such experiments. Both the age and weight at depletion were significantly increased by aureomycin in two of the three experiments. Age at death was significantly increased by aureomycin in one of the three experiments. As was expected, the vaginal smear test reflected a lesser degree of deficiency than did the plateau of growth.

TABLE 3

The effect of aureomycin on serum, liver and kidney vitamin A during the depletion of rats

DIET	A-FREE		A-FREE + AUREOMYCIN		A-FREE		A-FREE + AUREOMYCIN		A-FREE		A-FREE + AUREOMYCIN		A-FREE		A-FREE + AUREOMYCIN	
SEX	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Days depleted	10	10	10	10	24	24	24	24	34	34	34	34	42	42	42	42
Wt. gain, gm	25.0	21.5	24.3	20.5	70	56	76	59	96	60	109	76	129.3	93.5	116.8	87.3
Liver wt., gm	3.15	2.40	3.23	2.42	5.79	3.97	6.07	4.46	6.36	4.56	7.91	5.09	7.30	5.34	6.48	4.93
Vitamin A/ liver, I.U.	176	124	166	128	42.6	19.3	52.6	13.2	N.D.[1]	N.D.	N.D.	N.D.	...	...	...	...
Plasma vitamin A, I.U./100 ml	203	150	177	150	N.D.[1]	N.D.	N.D.	N.D.	...	...	...	...	...	...	...	...
Kidney wt., gm	...	...	...	...	1.10	0.78	1.20	0.93	1.25	0.97	1.39	1.07	1.39	1.28	1.33	1.27
Vitamin A/ kidney, I.U.	...	...	...	...	21.8	11.5	20.8	10.4	5.6	13.4	5.2	9.3	0.6	3.3	0.56	2.3

[1] None detectable.

The effect of aureomycin on the survival time of male and intact female rats was also measured. Females which received aureomycin lived longer than did their controls but there was no difference between the groups of males.

TABLE 4

Effect of aureomycin on depletion time, weight at depletion and survival time of ovariectomized rats

TEST	DIET	NO. OF RATS	AGE AT DEPLETION		WEIGHT AT DEPLETION	AGE AT DEATH
			By vaginal smear	By weight		
			days	*days*	*gm*	*days*
	A-free	42	64.5	...	113.9	95.8
1	A-free + aureomycin	48	66.0 [1]	...	109.9	88.1
	A-free	16	54.2	61.2	110.9	72.2
2	A-free + aureomycin	14	57.7 [2]	72.6	126.1 [2]	93.0 [2]
	A-free	20	54.3	76.3	98.9	91.8
3	A-free + aureomycin	20	55.5	79.8	110.9 [2]	96.0

[1] Significant at $P = 0.05$.
[2] Significant at $P = 0.01$.

Effect of aureomycin on the growth of rats receiving very low doses of vitamin A

Weanling rats were maintained on the "vitamin A-free" diet until their weight levelled off. They were then divided into groups, half of which received the "vitamin A-free" diet, the remainder the same diet plus 66 mg of aureomycin/kg. All rats were dosed orally three times weekly with a corn oil dilution of vitamin A acetate. The calculated daily intake of vitamin A was either 1 or 2 I.U. Dosing was continued for 4 weeks and all animals were weighed at weekly intervals. Average weekly weight gains were calculated only for those animals which showed a weight gain at every weighing. The data are recorded in table 5. Aureomycin influenced favourably the weight gain of both male and female rats

when these rats received 2 I.U. of vitamin A per day. At the 1 I.U. per day level it had no effect. It will be recalled that aureomycin was without effect on growth rate (tables 1 and 2) when the dietary level of vitamin A was adequate.

TABLE 5

Effect of aureomycin on the growth of rats receiving very low doses of vitamin A

DOSE/DAY	2 I.U.				1 I.U.			
AUREOMYCIN	0		66		0		66	
SEX	M	F	M	F	M	F	M	F
mg/kg								
No. of animals	13	16	15	20	9	14	16	15
Mean weekly wt. gain	7.48	5.68	9.33 [1]	7.19 [1]	6.49	5.41	6.60	5.34

[1] Significant at $P = 0.05$.

DISCUSSION

Aureomycin did not increase the liver stores of vitamin A in rats which received the antibiotic for 6 days. When the dosing and treatment period was extended to 4 weeks both males and females stored more vitamin A when aureomycin was added to their food, but the differences were not significant however. In a 4-week experiment in which all the vitamin A was derived from the food, males which received aureomycin stored more vitamin A than did their controls. This difference was largely, but not entirely, the result of increased food consumption. Our results suggest that aureomycin may increase liver stores slightly but under the conditions of the present experiments this increase was not significant.

The time required to deplete young rats of their vitamin A stores was significantly increased by aureomycin whether depletion was judged by the appearance of cornified epithelia in the vagina or by a cessation of weight gain. Weight at depletion was also increased by aureomycin. However, when kidney and liver stores and plasma levels of vitamin A were measured during depletion, no differences could be found between rats receiving aureomycin and their controls.

Hartsook et al. ('53) found that aureomycin shortened the survival time of male rats which were fed a diet deficient in vitamin A. While our results on this phase of the work were quite variable, they suggested that aureomycin tended to increase the survival time of vitamin A-deficient rats. The effect seems to be more marked with females than with males which may explain why Hartsook et al. ('53) who used only males, found, in one test, that aureomycin shortened survival time.

Rats which were dosed with 2 I.U. of vitamin A per day grew significantly more when they were fed aureomycin. This was not true of rats in the long term liver storage tests which were given more than adequate amounts of vitamin A. This suggests that whatever the mechanism by which aureomycin exerts its effect, it is mediated through some factor with vitamin A activity. Aureomycin failed to increase the growth of rats receiving 1 I.U. per day. This level was not sufficient to promote regular weight gain in all rats and it may be significant that fewer animals were rejected from the aureomycin groups for failure to gain weight. Thus the animals remaining in the group which did not receive aureomycin may have been those with inherently better growth rates.

Considering the results as a whole, it would appear that aureomycin affects favourably all functions of vitamin A that have been tested with the possible exception of liver storage. This suggests that the quantities of vitamin A involved may be too small to be measurable as increased liver stores. In fact, the magnitude of the observed changes in depletion and survival time or in weight gain, make it obvious that very small amounts of vitamin A are involved. A similar conclusion has been reached by Murray and Campbell ('55) from a consideration of the results of vaginal smear assays and the possible mechanisms involved were discussed in that paper.

Some points of more general interest arise from this work. Aureomycin significantly decreased the liver weights of males in one experiment while in the next experiment females, but

not males, were similarly affected. Burgess et al. ('53) reported that penicillin reduced the liver weight per 100 gm of body weight in chicks. The differences found here were absolute, as there were no differences in body weight.

As has been reported by Brenner, Brookes and Roberts ('42) and by Booth ('50, '52), females stored more of a given dose of vitamin A than did males. However, when all the vitamin A was derived from the food, males stored as much vitamin A as did females, due to their greater food intake.

Moore and Sharman ('50) and Booth ('50, '52) reported that male rats stored more vitamin A in their kidneys than did females. This has been confirmed by the results reported in this paper. Booth ('52) also noted that males retained kidney stores longer, during depletion, than did females and that there was no difference between the sexes with regard to survival time. We found that females not only retained their kidney stores of vitamin A longer but also lived longer on a vitamin A free diet than did males.

SUMMARY

The effects of aureomycin on storage of vitamin A and on growth, depletion rate and survival of rats, have been studied. The increase in liver stores when aureomycin was fed for 4 weeks was not significant. Liver and kidney stores were unaffected during depletion. Rate of depletion was decreased and weight at depletion increased by aureomycin. Survival time of rats on a "vitamin A-free" diet was variable but seemed to be increased by aureomycin in females. Growth rates of rats on low vitamin A intakes were increased by aureomycin but were not affected when adequate amounts of vitamin A were fed. Females stored vitamin A in their livers more efficiently than did males, lived longer on a "vitamin A-free" diet and maintained kidney stores longer than did males.

It is concluded that aureomycin makes available amounts of vitamin A which effect survival time and depletion time

but which are not large enough to influence significantly the liver stores.

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LITERATURE CITED

BOOTH, V. H. 1950 The influence of sex on the storage of vitamin A. Biochem. J., *47:* XI iii.

———— 1952 Liver storage of vitamin A by male and female rats. J. Nutrition, *48:* 13.

BRENNER, S., M. C. H. BROOKES AND L. J. ROBERTS 1942 The relation of liver stores to the occurrence of early signs of vitamin A deficiency in the white rat. Ibid., *23:* 459.

BURGESS, R. C., M. GLUCK AND D. H. LAUGHLAND 1953 Biochemical changes in the penicillin-fed chick. Poultry Sci., *32:* 444.

HARTSOOK, E. W., E. BATCHELOR AND B. C. JOHNSON 1953 Effect of aureomycin on utilization of vitamin A in the growing male albino rat. Proc. Soc. Exp. Biol. Med., *83:* 43.

MOORE, T., AND I. M. SHARMAN 1950 Vitamin A in the kidneys of male and female rats. Biochem. J., *47:* XI iii.

MURRAY, T. K., AND J. A. CAMPBELL 1955 The effect of aureomycin on the apparent utilization of vitamin A by the ovariectomized rat. J. Nutrition, *57:* 89–100.

HEART FAILURE IN MICE RELATED TO MINERALS IN THE DIET [1]

JOSEPH T. KING, Y. CHIUNG PUH LEE AND M. B. VISSCHER
Department of Physiology, University of Minnesota, Minneapolis

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It was reported briefly that C_3H male mice fed on a certain semi-purified diet showed a high incidence of hydrothorax associated with cardiac hypertrophy, the maximum incidence occurring well beyond a year of age. Controls fed a commercial Fox Chow did not develop the syndrome (King, Lee, Carr and Visscher, '51).

Later we reported a study on the influence of this particular diet on the reproductive capacity of strains A and C_3H mice in which we found that while the diet was adequate for the A mouse in respect to preventing abortions and resorptions, it was inadequate for the C_3H strain (Lee, King and Visscher, '53). It was found that adding more wheat germ oil (or equivalent tocopherol) greatly reduced the number of interrupted pregancies and raised the average number of young per pregnancy to approximately the Fox Chow control value. The same improvement was noted when the diet, without additional tocopherol, was made with a more simple salt formula. It was clear that the mineral content of the diet in some way determined the amount of tocopherol required to maintain full-term pregnancy in the C_3H strain.

The present report concerns further studies of this diet, and certain modifications, as these influence the incidence and age of onset of the heart failure syndrome in C_3H male mice.

[1] This investigation was supported in part by a research grant from the National Heart Institute, of the National Institutes of Health, Public Health Service.

MATERIALS AND METHODS

Six groups of 20 C_3H male mice each were housed 20 per cage; one group of 20 was singly caged. Diets indicated were fed ad libitum. Tap water was continuously available. The air conditioned room was kept at 78 $\pm$ 2°F. Cages were made of sheet metal and wire screen. Mice were fed and weighed and the cages changed weekly. Autopsy was done on all mice found dead and on those sacrificed with ether when moribund. Apparently abnormal tissues were preserved for microscopic study. Mice were studied over the whole life span and careful records made of their clinical condition by experienced observers.

The basal diet used, in parts by weight, consisted of: glucose, 33; casein, 28; lard, 17; dried brewers' yeast, 8; alfalfa leaf, 4; salts (modified Jones-Foster [2] or modified Hubbell, Mendel and Wakeman), 4; cod liver oil, 2; wheat germ oil, 1.

RESULTS

The incidence of heart failure and some of the related data are shown in table I. The first two groups showed the contrast between the result obtained with a natural complex food and the basal diet made with the modified Jones-Foster salts. The latter diet caused a high incidence of heart failure, the absolute heart weights at autopsy being the highest observed in this series of diets. The same basal diet made with the modified Hubbell, Mendel and Wakeman salt formula gave results comparable to Fox Chow in respect to heart failure (group IIII). The average absolute heart weight was somewhat higher but not higher in relation to body weight, the ratio being the same as that seen in the Fox Chow group.

[2] Jones and Foster ('42) modified by the substitution of iodized table salt instead of sodium chloride to reduce the liberation of iodine. Hubbell, Mendel and Wakeman ('37) modified as follows: $CaCO_3$, 544; $MgCO_3$, 25; $MgSO_4$, 16; iodized table salt, 69; KCl, 112; KH_2PO_4, 212; "ferric phosphate soluble," N.F.VIII, powder, 21; and without the trace elements manganese, fluorine, aluminum and copper of the original mixture.

TABLE 1

Incidence of heart failure in C_3H male mice on various diets; heart weight/body weight ratios for mice with heart failure and for those dying of other causes

DIET GROUP	SALTS	MICE WITH HEART FAILURE				MICE DYING OF OTHER CAUSES			AV. AGE (RANGE) AT HEART FAILURE
		Incidence	Av. wt.[1]	Heart wt.	H.W.[2]/B.W.	Av. wt.[1]	Heart wt.	H.W.[2]/B.W.	
			gm	*mg*		*gm*	*mg*		*days*
I Fox Chow		0/20				31.9	136.4	4.30	
II Basal	JF	7/10	40.86	218.2	5.34	38.3	140.0	3.67	576.4 (471–594)
III Basal	HMW(m)[3]	0/10				35.1	150.1	4.30	
IV Basal	JF(m)[4]	9/20	35.3	208.9	5.89	33.5	145.6	4.35	625.8 (561–660)
V Crisco 17%	JF	0/20				33.2	147.9	4.50	
VI Crisco + lard[5]	JF	2/20	36.5	203.9	5.60	37.8	149.2	3.95	680 (587–774)
VII Basal without wheat germ oil	JF(10 ×)[6]	17/20	33.3	171.0	5.13	29.5	134.5	4.56	321 (274–375)

[1] One month before death.

[2] Heart weight in milligrams/body weight in grams.

[3] Modified Hubbell, Mendel and Wakeman salts.

[4] Modified Jones and Foster formula without the trace elements (cobalt, zinc, copper, manganese and iodine except that in the iodized table salt).

[5] Three per cent crisco + 14% lard.

[6] Modified Jones and Foster formula with 10 times normal trace elements.

Group IV shows the results when the trace elements were removed from the Jones and Foster formula. It is clear that the unphysiological properties of this formula cannot be attributed entirely to these constituents. However, the data do not permit the conclusion that the trace elements exerted no effect. The first case of heart failure occurred three months later than in the group on the diet containing the trace elements. The average age at failure was also higher.

Groups V and VI show the results of replacing lard, completely or in part, with an hydrogenated oil.[3] In each case improvement was evident, being more marked where lard was completely replaced. At the lower level of hydrogenated oil the average absolute heart weight was not abnormally high. However, in the two cases of heart failure noted the hearts were large, weighing 182 and 225.8 mg and they were typical in all essential respects as described below.

Group VII mice were fed a diet containing no wheat germ oil and 10 times the usual amount of trace elements in the salt mixture. Three mice died early of convulsions (238, 290 and 267 days — heart weights 125, 128.4 and 150 mg). All other mice showed typical cases of heart failure. This diet produced the highest percentage involvement at the earliest age.

In a typical case of heart failure of the type under consideration the mouse remained in apparent good health until about three weeks before death at which time the coat became rough and the mouse became less active. Careful observations revealed periods of a slow, deep type of respiration which became evident with time. Exophthalamus was present in some cases. Occasionally localized edema was seen around the head or hind limbs. More rarely generalized edema was seen.

Gross autopsy findings: The heart was enlarged in all diameters. A special finding was the extreme enlargement of the left auricle. The auricle frequently had yellowish white streaks on the surface. The blood-free heart was heavier than normal and was soft to touch. It lacked normal "tone" and

[3] Crisco.

placed on a flat surface and observed from the side it failed to show the normal plump outline.

Pleural effusion was present from a trace to 2 ml. Usually the fluid was clear. The liver was enlarged and more firm than normal. Ascites was not common in the C_3H mouse. The viscera frequently appeared congested.

Microscopic findings: The heart showed extensive fibrosis in the ventricles. Muscle fibers of the walls of the ventricles were found in all stages of deterioration. This was true less frequently in the septum and papillary muscles. The fibrosis had an irregular distribution. There was remarkably little infiltration of the acute inflammatory type in any stage thus far observed. There was little involvement of the endocardium. The coronary arteries appeared to be normal. In the auricles deterioration of muscle fibers was even more extensive than in the ventricles.

Of special interest was the presence and distribution of a yellow or brownish yellow pigment. In routine hemotoxylin eosin stain this material could easily be overlooked since it does not stain prominently especially when present in small granules or globules. Macrophages containing large amounts of the pigment were prominent even at low power. The pigment was also present in the cardiac muscle fibers where it was seen as small granules in various locations in the fibers. In various areas the total amount of pigment was proportional to fiber degeneration. On examination of a section with low power the areas showing fibrosis were easily seen and in such areas the pigment-laden macrophages were present. Elsewhere one could find with low power isolated or small groups of the dark phagocytic cells. Examination with higher power revealed fiber damage in such locations, usually in an early stage. Msucle fibers could be found showing very little histological change which, nevertheless, contained small amounts of this pigment.

The liver cords showed atrophy with widening of the sinusoids around the central vein in many cases. The general picture was that of chronic passive congestion.

DISCUSSION

Mice survive, without apparent ill effect, to and beyond a year of age on a diet which ultimately causes heart failure in most of those not dying earlier of other causes. In relation to the life span of the mouse this is a disorder which develops very slowly. That the disorder is due to a dietary defect is evident from the fact that it does not occur when the mice are fed a commercial Fox Chow. That it is a defect determined by mineral content of the diet is clear since it occurs regularly when the basal diet is made with the modified Jones and Foster salt formula and does not occur when the diet contains the modified Hubbell, Mendel and Wakeman salts.

The salt mixture which causes infertility of the C_3H female mouse by reason of fetal resorption and also causes heart failure later in life was found by Jones and Foster ('42) to yield superior results in short-term studies on bone growth in rats. This formula contains cobalt, copper, zinc, manganese and relatively large amounts of iodine. It is tempting to suppose that these elements, not present in the modification of the Hubbell, Mendel and Wakeman formula used in these experiments, account for the differences in the two mixtures noted above. However, the results in group IV (trace elements removed from the formula) offered inadequate support for this assumption although the onset of the disease entity was definitely postponed by such trace element removal.

While the available data do not permit a conclusion as to why the two salt mixtures produce such different effects, some of the facts are suggestive. As noted above the dietary defect which caused interrupted pregnancies in the C_3H mouse when the diet contained the modified Jones and Foster formula was corrected by addition of more wheat germ oil or by adding tocopherol as such. This raises the question whether the heart lesion which we observe when this salt mixture is used can be regarded as probably due to a relative tocopherol lack.

It is now generally recognized that the integrity of skeletal and cardiac muscle is dependent on adequate tocopherol supply (Mason and Emmel, '45; Follis, '48; Mackenzie, '53). Mason and Emmel ('45) reported a detailed study of vitamin E deficiency in the rat in which special attention was given to the origin, distribution and significance of a peculiar pigment arising in muscle fibers and perhaps elsewhere. They believed that the pigment represents "an abnormal intermediary product of cell metabolism or a metabolite having but a transitory existence in the E sufficient organism." Concerning the specificity of the pigment as a sign of E deficiency they state that the "accumulation of acid-fast pigment in the musculature and elsewhere appears to be a reliable and useful criterion of E deficiency with possibly greater specificity and practical significance than the biochemical criteria — which, unfortunately, have been established only for dystrophic skeletal muscle." In addition to the results on the abnormal pigment, this paper provides an excellent description of the histological changes in heart muscle in th E-deficient rat.

More recently Tobin ('50) has reported similar lesions in the hearts of mice fed a tocopherol-deficient diet containing 10% of cod liver oil. He also reports the presence of the pigment described by Mason and Emmel.

The lesions present in the hearts of our mice exhibiting heart failure are similar in all essential respects to those described above for vitamin E-deficient rats and mice. We believe the presence of the abnormal pigment to be particularly significant since we do not observe it in the hearts of mice dying of other causes. Even with low power examination the pigment-laden macrophages are easily seen and high power examination of the adjacent muscle fibers invariably reveals damage of some degree. It is possible to find muscle fibers containing some pigment without finding the pigment-containing macrophages in the same area but these are fibers showing minimal or no histological evidence of damage.

Because we have already found our basal diet made with the modified Jones and Foster salt to be inadequate to maintain uninterrupted pregnancy in the C_3H mouse unless supplemented with tocopherol and because the same diet produces a heart lesion indistinguishable from those produced in rats and mice by diets known to be deficient in vitamin E we tentatively assume that the observed heart failure is due to a tocopherol deficiency determined by this salt mixture. This assumption is consistent with preliminary results in other experiments in progress in the laboratory.

Even if we assume that the salt mixture produced a relative tocopherol lack it remains to be determined how this is brought about. It could be due to destruction of tocopherol before the diet is fed or destruction in the gut. Furthermore it is possible that the animal's requirement for vitamin E is raised by the salt formula.

Waddell and Steenbock ('28, '31) showed that certain constituents of the diet may determine the activity of vitamin E. Their use of ferric chloride to prevent vitamin E activity as determined by fertility of the rat is well known. Their results with ether and aqueous solutions of ferric chloride led them to conclude that the inhibitory effect was more evident as the contact between iron and the lipoid substances became more intimate. Mattill's work on the auto-oxidation of unsaturated oils and fats led to the conclusion that there is an oxidative destruction of some of the fat-soluble vitamin associated with such materials. In the paper by Cummings and Mattill ('31) they state "the efficiency of a given source of vitamin E depends in part upon the auto-oxidizable materials and the antioxidants associated with it." Salts of metals such as iron and copper are known to influence such oxidative processes and perhaps for this reason cause inactivation of vitamin E. In our recently reported study (King, Lee and Visscher, '55) on the role of minerals causing mouse "paralysis" we found that substituting ferrous sulfate for the usual ferric phosphate in a modified Hubbell, Mendel and Wakeman salt mixture had

the same effect as removing the wheat germ oil from the diet in respect to the incidence and age of onset of the "paralysis" syndrome.

It is also known that certain salt mixtures have a destructive effect on some B vitamins. Waibel, Bird and Baumann ('54) found that thiamine was more stable in a salt-free diet than in a diet containing salts and that a finely milled salt mixture was more destructive than a course mixture. Rombouts ('53) found that the Phillips and Hart ('35) mixture was more destructive to B vitamins than a U.S.P. mixture. Lyman and Elvehjem ('51) reported that much of the thiamine in certain purified rations containing the Phillips-Hart mixture could be destroyed in two weeks.

CONCLUSIONS

C_3H mice regularly develop heart failure late in life when fed a basal diet made with a modified Jones and Foster salt mixture. The disorder does not occur when the diet is made with a modified Hubbell, Mendel and Wakeman mixture nor does it occur when mice are fed commercial Fox Chow.

LITERATURE CITED

CUMMINGS, M. J., AND H. A. MATTILL 1931 The auto-oxidation of fats with reference to their destructive effect on vitamin E. J. Nutrition, *3:* 421.

FOLLIS, R. H. 1948 The pathology of nutritional disease. Charles C Thomas, Publisher.

JONES, J. H., AND C. FOSTER 1942 A salt mixture for use with basal diets either low or high in phosphorus. J. Nutrition, *24:* 245.

HUBBELL, R. B., L. B. MENDEL AND A. J. WAKEMAN 1937 A new salt mixture for use in experimental diets. J. Nutrition, *37:* 273.

KING, J. T., Y. C. P. LEE, C. C. CARR AND M. B. VISSCHER 1951 Spontaneous hydrothorax in mice related to strain and diet. Fed. Proc., *10:* 73.

KING, J. T., Y. C. P. LEE AND M. B. VISSCHER 1955 Relationship between vitamin E and dietary minerals in mouse "paralysis." Proc. Soc. Exp. Biol. Med., *88:* 406.

LEE, Y. C. P., J. T. KING AND M. B. VISSCHER 1953 Strain difference in vitamin E and B_{12} and certain mineral trace-element requirements for reproduction in A and Z mice. Am. J. Physiol., *173:* 456.

LYMAN, R. L., AND C. A. ELVEHJEM 1951 The lability of thiamine in certain purified rations. Proc. Soc. Exp. Biol. Med., *77:* 813.

MACKENZIE, C. G. 1953 Experimental muscular dystrophy. Symposium on Nutrition, Edited by Roger M. Herriot. The Johns Hopkins Press.

MASON, K. E., AND A. F. EMMEL 1945 Vitamin E and muscle pigment in the rat. Anat. Record, *92:* 33.

PHILLIPS, P. H., AND E. B. HART 1935 The effect of organic dietary constituents upon chronic fluorine toxicosis in the rat. J. Biol. Chem., *109:* 657.

ROMBOUTS, J. E. 1953 The destruction of B-vitamins by certain salt mixtures in purified rations. J. Nutrition, *50:* 255.

TOBIN, C. E. 1950 Effects of vitamin E deficiency and cod liver oil on myopathy in mice. Arch. Path., *50:* 385.

WADDELL, J., AND H. STEENBOCK 1928 The destruction of vitamin E in a ration composed of natural and varied foodstuffs. J. Biol. Chem., *80:* 431.

———— 1931 Vitamin E in iron treated dry rations. J. Nutrition, *4:* 79.

WAIBEL, P. E., H. R. BIRD AND C. A. BAUMANN 1954 Effects of salts on the instability of thiamine in purified chick diets. Ibid., *52:* 273.

PANTOTHENATE AND DIETARY CHOLESTEROL IN THE MAINTENANCE OF BLOOD AND TISSUE CHOLESTEROL ESTERS [1]

LEON SWELL, T. A. BOITER, HENRY FIELD, JR.
AND C. R. TREADWELL

General Medical Research, Veterans Administration Center, Martinsburg, West Virginia, and the Department of Biochemistry, George Washington University, School of Medicine, Washington, D. C.

ONE FIGURE

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It has been established that acetate serves as a primary precursor in the biosynthesis of fatty acids (Rittenberg and Bloch, '44) and cholesterol (Bloch and Rittenberg, '42). The synthesis of fatty acids and other types of acetylations are catalyzed by enzyme systems which require coenzyme A (Novelli, '53). Coenzyme A has also been shown to function in the synthesis of cholesterol from acetate (Klein and Lipmann, '53; Rabinowitz and Gurin, '54). Its role at higher levels is not known at the present time (Bloch et al., '54).

Pantothenic acid deficiency is associated with a decreased tissue content of coenzyme A and reduced capacity for acetylation (Olson and Stare, '51). If coenzyme A and acetylation are critical to the biosynthesis of cholesterol, it would be anticipated that the process would be inhibited in pantothenic acid-deficient animals. However, the available data concerning this point are somewhat contradictory. Guggenheim and Olson ('52) found that the cholesterol contents of

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liver, heart, adrenals and serum of rats were similar in pantothenic acid- and pair-fed control animals. Also there was no indication of a decreased rate of incorporation of carboxyl-labeled acetate into cholesterol in the deficient animals. Others (Guehring et al., '52; Morgan and Lewis, '53; Dumm et al., '53; Hurley and Mackenzie, '54) have reported lowered levels of cholesterol in adrenals, liver and serum and decreased cholesterol synthesis in liver slices of deficient rats (Klein and Lipmann, '53). Some of the data also suggested derangements in fat metabolism in these animals. Recently Boyd ('53) reported that if rats are maintained on a fat-free diet, the liver ester cholesterol concentration and the plasma ester concentration appear to be dependent on dietary pantothenate and that the presence of neutral fat in the diet could annul the decrease in liver and plasma cholesterol ester. The explanation was offered that while coenzyme A may be involved in cholesterol synthesis through utilization of acetate to form intermediates containing even numbers of carbon atoms, its role would be of less importance if similar intermediates became available through the catabolism of fat.

Earlier studies from our laboratories have shown that hypercholesterolemia can be produced in rats by feeding a fat-free diet containing cholesterol and bile salts (Swell et al., '55). The addition of fat to this diet merely accentuated the hypercholesterolemia. The incorporation of cholesterol or bile salts alone with or without fat in the diet did not have a pronounced effect on the blood cholesterol level. It was inferred that fat need not be present in the diet for cholesterol absorption to occur if sufficient bile salts are present and that the fat necessary for cholesterol esterification in the intestine can come from endogenous sources. In view of these findings, it appeared possible that the observed decline in liver and serum cholesterol esters reported by Boyd ('53) might be partially due to a relative deficiency of fatty acids. From this it would follow that the feeding of a fat-free diet containing cholesterol and bile salts to pantothenate-deficient

rats should not elevate the blood cholesterol, while it should in normal animals. Thus, a study was undertaken in which rats were fed complete and pantothenate-deficient diets containing cholesterol, fat and bile salts in various combinations. The levels of cholesterol were determined in the adrenals, liver and serum.

METHODS AND MATERIALS

Male and female rats from our stock colony and weighing 60 to 70 gm, were used. The animals were housed in individual

TABLE 1

Feeding regimen and weight gain on experimental diets

GROUP [1]	WEIGHT GAIN [2]	ADDITIONS TO DIET			
		Cholesterol	Sodium taurocholate	Olive oil	Pantothenate
	gm	%	%	%	
P-1	65 ± 8	..	..	..	+
P-2	57 ± 3	1	0.5	..	+
P-3	90 ± 30	..	..	25	+
P-4	75 ± 14	1	0.5	25	+
P-5	33 ± 11	..	..	..	—
P-6	30 ± 11	1	0.5	..	—
P-7	32 ± 20	..	..	25	—
P-8	39 ± 21	1	0.5	25	—

[1] Five to 7 rats per group; pair-fed, average food intake, 9.0 gm/day.
[2] Length of the feeding period 35 days.

cages. The blood cholesterol fractions were determined during a control period on pellet chow and then the animals were divided into 8 groups. Each group received a basal synthetic diet to which was added, in various combinations, 25% of olive oil, 1% of cholesterol and 0.5% of sodium taurocholate. Half of the groups were fed these diets without pantothenate. The feeding regimen is shown in table 1. The basal diet had the following percentage composition: vitamin-free casein 20, starch 24, glucose 24, Hubbell, Mendel, and Wakeman salt mixture 5, roughage [2] 2, and adequate amounts of crystalline

[2] Ruffex.

vitamins (except pantothenic acid). The remaining 25% of the diet was either fat, or in those diets containing no fat, the difference was made up by adding 25% of carbohydrate. The cholesterol and bile salts were substituted at the expense of the starch and glucose. The diets were pair-fed according to the following technique: after the first day all rats in all groups received the amount of food ingested the previous day by the rat having the lowest food intake. All animals received the diets for 35 days. At the end of three weeks, the animals on the pantothenate-deficient diet showed external symptoms of the deficiency such as poor growth, thinning of the hair and porphrin-stained whiskers. At death their adrenals were hemorrhagic.

The rats were bled from the tail vein and the blood samples collected directly into 0.2-ml pipettes and placed in 1:1 acetone-alcohol mixture for cholesterol analysis. Free and total cholesterol were determined on the whole blood by the method of Sperry and Webb ('50). At the end of 35 days, the animals were sacrificed by a sharp blow on the head and the liver and adrenals were removed. The liver was blotted free of blood, weighed and homogenized with saline in a Potter-Elvehjem apparatus. Total lipids were determined by extraction of an aliquot of the homogenates with ether-alcohol, drying with sodium sulfate, evaporating the solvent, and drying to constant weight. Free and total liver cholesterol were determined on the homogenates in the same manner as in the blood cholesterol determination. The pooled adrenals of each group were weighed and dried overnight at 70°C. The dried material was extracted in a soxhlet extractor with ether-alcohol for 24 hours. The solvent was evaporated off and the residue made to a definite volume with 1:1 acetone-alcohol. Free and total cholesterol were determined on the solution in the same manner as in the blood cholesterol determination.

RESULTS

The weight gain on the different diets is shown in table 1. The normal animals gained approximately twice as much

as did the deficient animals on comparable diets. The addition of cholesterol to the diet had no effect on the weight of the normal or deficient animals, while the addition of fat was followed by an increase in body weight of the normal rats. The blood cholesterol levels at zero, 10, 20, and 35 days during the feeding period in the different groups with and

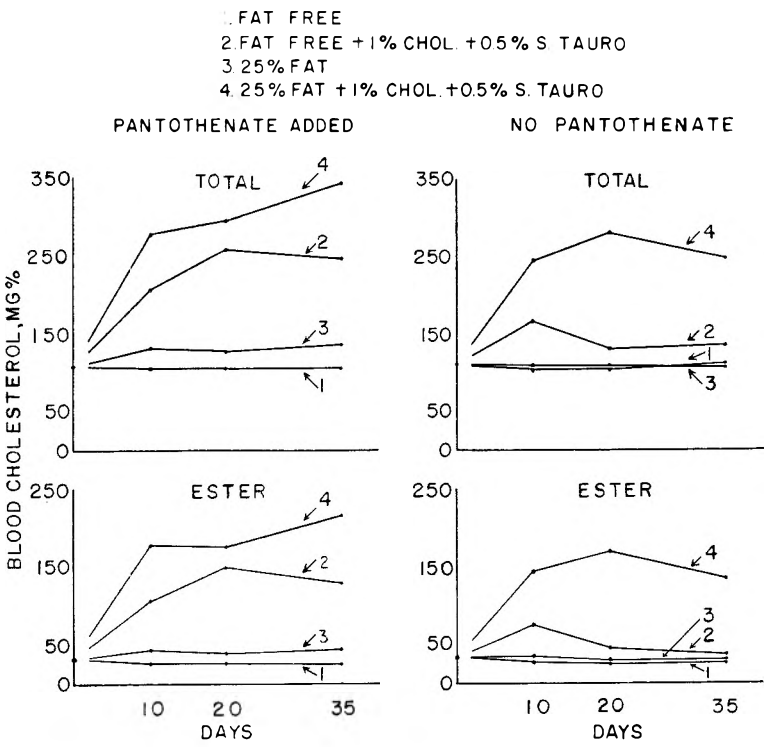

Fig. 1 The total and ester whole blood cholesterol of normal and pantothenate-deficient rats fed diets containing cholesterol, bile salts and fat in various combinations.

without pantothenate are shown in figure 1. Curves 1 (groups P-1 and P-5) show the effect of the fat- and cholesterol-free diet with and without pantothenate added. The total and ester cholesterol levels remained virtually unchanged during the feeding period in both the normal and deficient animals. Curves 2 represent the cholesterol levels of the animals re-

ceiving the fat-free diets containing cholesterol and bile salts. The total cholesterol increased markedly in the pantothenate group (P-2); principally in the ester fraction. The pantothenate-deficient animals (P-6) exhibited a small increase in total and ester cholesterol at the end of 10 days, but as the deficiency developed, the cholesterol levels declined and approached the control level. Curves 3 (groups P-3 and P-7) show the effects of the basal diet with added fat. Here the blood cholesterol level increased to a slight extent in the pantothenate group, but there was no change in the level in the deficient animals. The blood cholesterol levels of the animals on the diets (P-4 and P-5) containing cholesterol, fat, and bile salts are represented by curves 4. The ester and total cholesterol increased markedly in both the normal and deficient animals. At the end of 20 days the total blood cholesterol was 280 mg% in both groups. During the remainder of the feeding period the cholesterol level remained elevated in both groups, but it was slightly lower in the deficient group at the end of 35 days.

The liver lipid content of the rats on the different diets is shown in table 2. The weight of the livers of the pantothenate animals fed the diet (P-4) containing cholesterol, fat and bile salts was greatly increased. Their livers were fatty and contained large amounts of cholesterol (16.8%), mostly as the ester. The pantothenate group which received cholesterol and bile salts in a fat-free diet (P-2) also showed an increase in liver cholesterol, but of a smaller magnitude (1.7%). As can be seen with the addition of fat to the cholesterol and bile-salt diet, the deposition of cholesterol in the liver greatly increased. Those with fat alone added to the diet (P-3) without cholesterol and bile salt had only slight increases in the liver cholesterol. The non-cholesterol liver lipids of the normal animals were increased slightly when fat was added to the diet (groups P-3 and P-4). The livers of the pantothenate-deficient groups weighed considerably less than the normal groups, with the exception of group (P-8) which was fed a diet containing cholesterol, fat, and bile salts. The

TABLE 2

Effect of normal and pantothenic acid-deficient diets on liver lipids

GROUP	WT. OF LIVER	LIVER CHOLESTEROL	LIVER CHOLESTEROL (wet weight)			NON-CHOLESTEROL LIVER LIPIDS (wet weight)
			Free	Ester [1]	Total	
	gm	*mg/liver*	%	%	%	%
P-1	5.8 ± 0.7	15.6 ± 0.7	0.20	0.11	0.31 ± 0.06	6.4 ± 0.9
P-2	6.2 ± 0.3	68.3 ± 11.0	0.17	1.55	1.72 ± 0.20	6.9 ± 0.4
P-3	6.6 ± 1.1	38.8 ± 23.0	0.22	0.62	0.84 ± 0.29	7.7 ± 0.4
P-4	8.5 ± 1.2	867.8 ± 103.5	0.56	16.23	16.79 ± 1.78	9.2 ± 0.2
P-5	3.5 ± 0.9	9.6 ± 2.1	0.21	0.11	0.32 ± 0.05	8.9 ± 1.9
P-6	4.9 ± 0.2	16.8 ± 1.3	0.24	0.17	0.41 ± 0.09	6.6 ± 1.1
P-7	3.9 ± 0.3	10.4 ± 2.5	0.18	0.15	0.33 ± 0.07	6.7 ± 0.7
P-8	5.6 ± 1.4	125.1 ± 46.5	0.25	3.33	3.58 ± 0.42	7.6 ± 1.0

[1] Expressed as ester in terms of cholesterol oleate.

livers of this group were fatty and showed a large increase in total cholesterol (3.6%) which was almost all in the ester form. The pantothenate-deficient group (P-6) fed the fat-free diet with cholesterol and bile salts showed only a slight increase in total liver cholesterol when compared to the fat-free group with no additions (P-5). Also, the group fed the basal diet with fat (P-7) did not show an increase in cholesterol. The non-cholesterol liver lipids of the deficient animals did not change.

TABLE 3

Effect of normal and pantothenic acid-deficient diets on adrenal cholesterol

GROUP [1]	WT. OF ADRENALS	ADRENAL CHOLESTEROL	ADRENAL CHOLESTEROL (wet weight)		
			Free	Ester	Total
	mg	*mg/adrenals*	%	%	%
P-1	18.0	0.92	0.60	4.49	5.09
P-2	28.4	1.80	0.63	5.70	6.33
P-3	28.0	1.32	0.69	4.04	4.73
P-4	31.2	1.79	0.78	4.96	5.74
P-5	22.8	0.75	0.55	2.74	3.30
P-6	24.0	0.48	0.12	1.88	2.00
P-7	25.8	0.56	0.39	1.78	2.17
P-8	27.0	1.34	0.44	4.57	5.01

[1] The adrenals of each group were pooled, weighed and analyzed.

The following points are of interest when the effects of the different diets on the liver cholesterol and fat are compared in the normal and pantothenate-deficient groups. There was no difference in the total liver cholesterol, expressed as percentage, between the groups receiving the fat-free diet without dietary additions (P-1 and P-5), but there was a decline in the cholesterol content on an absolute basis in the pantothenate-deficient group. The normal animals showed a considerably greater increase in liver cholesterol than the pantothenate-deficient rats when they were fed a fat-free diet containing cholesterol and bile salts. Addition of fat to the latter diet produced an increase in the liver cholesterol of both normal and deficient groups, although the normal group showed a larger increase.

The adrenal cholesterol content of normal and deficient rats is shown in table 3. The percentage of total adrenal cholesterol in the normal groups receiving cholesterol and bile salts with and without fat in their diet (P-2 and P-4) increased, 20% and 13% respectively over the control group (P-1). There was a decline in the percentage of total adrenal cholesterol of all the pantothenate-deficient groups, except P-8 which received a diet containing cholesterol, fat, and bile salts. Those groups (P-5, P-6, P-7) showed decreases of up to 50% in the percentage of total adrenal cholesterol when compared to the normal animals receiving comparable diets.

DISCUSSION

The present study provides an alternative explanation for the previously reported (Boyd, '53) decline in liver and blood cholesterol ester of rats fed a diet devoid of fat and pantothenate and the reversal of this change by the addition of fat to a pantothenate-deficient diet. The increase observed, in the present study, in the blood cholesterol level of normal rats fed a fat-free diet containing cholesterol and bile salts was mainly in the ester fraction; the fatty acid utilized in cholesterol esterification must have come from endogenous sources. The pantothenate-deficient animals did not exhibit a comparable increase in blood cholesterol when a similar diet was fed, probably because of a lack of coenzyme A which is necessary for the synthesis of endogenous fat. When fat was added to the diet of the pantothenate-deficient animals, the blood cholesterol (ester) increased as in the normal animals. It would therefore appear that in the pantothenate-deficient animals there was a relative deficiency of fatty acids and that the previously observed changes in serum cholesterol ester may be partially explained on this basis.

The liver cholesterol contents followed much the same pattern as the blood cholesterol, although the pantothenate-deficient animals fed the cholesterol, fat, and bile salt diet did not have as much cholesterol in the liver as the normal animals fed a similar diet. However, the livers of those

animals contained excessive amounts of cholesterol. These findings are in agreement with those of Guehring et al. ('52). The latter authors suggest that the deficient animals may utilize exogenous cholesterol efficiently enough to prevent a rise in blood cholesterol and an accumulation of cholesterol in the liver. A decline in liver and blood cholesterol ester was not observed when rats were maintained on a pantothenate-deficient fat-free diet. The animals in the present study may not have been sufficiently depleted to demonstrate such changes. Winters et al., ('52) and Guehring et al. ('52) have also failed to observe a decline in the blood cholesterol of pantothenate-deficient animals.

The lowered adrenal cholesterol in the pantothenate-deficient groups, excepting the one fed a diet containing cholesterol, fat and bile salts, may be due to a decreased synthesis of adrenal cholesterol, and increased production of adrenal hormones due to stress or a combination of both. These findings are in agreement with those of Winters et al. ('52). The deficient group fed a diet containing cholesterol, fat and bile salts had an adrenal cholesterol higher than the other deficient groups. These animals also had a high blood cholesterol and it is possible that they were able to withdraw sufficient cholesterol from their blood to maintain a normal adrenal cholesterol level, or cholesterol was deposited by some other mechanism.

Boyd ('53) showed that adding fat to a cholesterol-free diet could prevent the decline of liver and serum cholesterol ester in pantothenate-deficient animals. The present data have demonstrated that adding fat to a high-cholesterol diet will produce hypercholesterolemia in deficient animals. Thus, it would appear that these effects are similar and are not due to decreased synthesis of cholesterol. However, it should be emphasized that the present data do not eliminate the possibility that there is a decreased synthesis of cholesterol in the pantothenate-deficient rat. There are two aspects of

the data which require further study for adequate explanations. First, the pantothenate-deficient animals had lower absolute amounts of cholesterol in the liver than the normals on comparable diets and with the same cholesterol intake. This may have been due to decreased absorption or increased utilization of cholesterol in the deficient animals. Secondly, in both types of animals with the same cholesterol intake the addition of fat to the diet produced a marked increase in the liver cholesterol (P-2, P-4, and P-6, P-8) esters. This may have been caused by increased absorption or decreased utilization of the dietary cholesterol.

SUMMARY

1. Following the feeding of a fat-free diet containing cholesterol and bile salts to normal animals, the blood cholesterol increased, while in pantothenate-deficient animals this diet had no appreciable effect on the blood cholesterol. Addition of fat to this diet accentuated the hypercholesterolemia in normal animals and produced hypercholesterolemia in the deficient rats.

2. No change in the blood cholesterol was noted when a cholesterol-free diet without fat was fed to normal and pantothenate-deficient animals.

3. The liver cholesterol was increased in both normal and pantothenate-deficient animals with hypercholesterolemia. However, the increase was greater in the rats receiving pantothenate.

4. The adrenal cholesterol declined in the pantothenate-deficient groups, with the exception of the group with hypercholesterolemia fed a diet containing cholesterol, fat and bile salts.

5. It is suggested that the previously observed decline in liver and serum cholesterol ester was partially due to a relative fatty acid deficiency whereby fatty acids were not readily available for cholesterol esterification in the intestine.

LITERATURE CITED

BLOCH, K., L. C. CLARK AND I. HARARY 1954 Utilization of branched chain acids in cholesterol synthesis. J. Biol. Chem., *211:* 687.

BLOCH, K., AND D. RITTENBERG 1942 On utilization of acetic acid for cholesterol formation. Ibid., *145:* 625.

BOYD, G. S. 1953 The possible role of coenzyme A in the biosynthesis of cholesterol in the rat. Biochem. J., *55:* 892.

DUMM, M. E., H. GERSHBERG, E. M. BECK AND E. P. RALLI 1953 Effect of pantothenate deficiency on synthesis of adrenal cholesterol following stress. Proc. Soc. Exp. Biol. Med., *82:* 659.

GUEHRING, R. R., L. S. HURLEY AND A. F. MORGAN 1952 Cholesterol metabolism in pantothenic acid deficiency. J. Biol. Chem., *197:* 485.

GUGGENHEIM, K., AND R. E. OLSON 1952 Lipogenesis in certain B-vitamin deficiencies. J. Nutrition, *48:* 345.

HUBBELL, R. B., L. B. MENDEL AND A. J. WAKEMAN 1937 A new salt mixture for use in experimental diets. Ibid., *14:* 273.

HURLEY, L. S., AND J. B. MACKENSIE 1954 Adrenal function in the pantothenic acid-deficient rat. Ibid., *54:* 403.

KLEIN, H. P., AND F. LIPMANN 1953 The relationship of coenzyme A to lipide synthesis. II. Experiments with rat liver. J. Biol. Chem., *203:* 101.

MORGAN, A. F., AND E. M. LEWIS 1953 The modification of choline deficiency by simultaneous pantothenic acid deficiency. Ibid, *200:* 839.

NOVELLI, G. D. 1953 Metabolic functions of pantothenic acid. Physiol. Rev., *33:* 525.

OLSON, R. E., AND F. J. STARE 1951 The metabolism *in vitro* of cardiac muscle in pantothenic acid deficiency. J. Biol. Chem., *190:* 149.

RITTENBERG, D., AND K. BLOCH 1944 Utilization of acetic acid for fatty acid synthesis. Ibid., *154:* 311.

RABINOWITZ, J. L., AND S. GURIN 1954 Biosynthesis of cholesterol and β-hydroxy-β-methyl-glutaric acid by extracts of liver. Ibid., *208:* 307.

SPERRY, W. M., AND M. WEBB 1950 A revision of the Schoeheimer-Sperry method for cholesterol determination. Ibid., *187:* 97.

SWELL, L., D. F. FLICK, H. FIELD, JR. AND C. R. TREADWELL 1955 The role of fat and fatty acid in the absorption of dietary cholesterol. Am. J. Physiol., *180:* 124.

WINTERS, R. W., R. B. SCHULTZ AND W. A. KREHL 1952 Studies on adrenal cortex of pantothenic acid-deficient rat. IV. Adrenal and serum cholesterol levels. Proc. Soc. Exp. Biol. Med., *79:* 695.

WATER RESTRICTION IN NUTRITION STUDIES

I. LEVEL OF FAT AND PROTEIN UTILIZATION [1]

MANUEL SCHREIBER AND CONRAD ARNOLD ELVEHJEM
Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

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INTRODUCTION

In a previous investigation (Schreiber and Elvehjem, '54) water restriction was used as a stress technique to study factors affecting the nasal excretion of a red pigment. In this paper we wish to present data obtained when water restriction was combined with variations in dietary composition. Special consideration will be given to the sparing action of fat on the utilization of dietary protein by the young rat.

EXPERIMENTAL PROCEDURE

Male weanling albino Sprague-Dawley strain rats weighing 40 to 45 gm were given water ad libitum but denied food for the first 24-hour pre-experimental period. The animals were then weighed, divided uniformly into groups with the same starting weight and individually housed in raised screen-bottom cages. All experiments were of three weeks duration.

The diets used are given in tables 1 and 2. Four per cent of Salts IV [2] was incorporated into the diets fed to groups 1 to 11 (table 1). Groups 12 to 23 received the same level of Salts IV/calorie. The amount of water-soluble vitamins in all diets

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[2] Salts IV (Hegsted, Mills, Elvehjem and Hart, '41).

TABLE 1

Experiment 1: The effects of water restriction on the growth and organ to body-weight ratios of weanling rats fed diets varying in nutrient composition. Experimental conditions: av. temp. 23°C.; av. relative humidity 33%

GROUP NO.	DIETS [1]			PROTEIN/ CALORIE	WEIGHT GAIN 3 WEEKS			WATER INTAKE (A GROUPS)		ORGAN TO BODY-WEIGHT RATIOS								
	Corn oil	Casein [2]	Sucrose		A	B	C			Thymus × 1000			Kidneys × 100			Liver × 100		
										A	B	C	A	B	C	A	B	C
	%	%	%	mg	gm	gm	gm	ml/day/ rat	ml/day/ dm^2 B.S.A.[3]									
1	5	50	41	122	102	2	.. [4]	22.3	9.8	4.3	0.2	..	1.4	1.5	..	5.7	4.5	..
2	5	25	66	61	127	25	— 9	17.6	7.1	5.1	3.3	0.0	1.2	1.3	1.8	6.6	4.6	5.0
3	5	18	73	44	126	30	— 1	16.3	6.6	5.0	3.2	0.2	1.2	1.3	1.6	6.2	4.6	4.8
4	5	18	73 [5]	44	129	35	— 2	17.4	7.0	5.1	3.6	0.2	1.0	1.2	1.5	5.7	3.9	4.3
5	5	10	81	24	77	32	6	11.8	5.7	3.2	3.7	0.3	1.0	1.2	1.3	7.4	4.9	4.9
6	5	10	81 [5]	24	105	29	1	17.6	7.6	4.0	3.1	0.2	0.8	1.2	1.4	4.9	4.0	4.1
7	25	50	21	98	104	2	.. [6]	20.2	8.8	4.0	0.1	..	1.4	1.7	..	6.8	4.8	..
8	25	25	46	49	135	19	— 8 [7]	18.2	7.1	4.9	2.7	0.0	1.2	1.4	2.0	6.5	5.1	5.7
9	25	18	53	35	125	25	— 4	17.3	7.0	4.0	3.2	0.0	1.0	1.2	1.7	6.8	4.1	5.0
10	50 [8]	25	21	39	117	17	.. [9]	17.2	7.1	4.8	2.2	..	1.1	1.4	..	6.4	4.1	..
11	50 [8]	18	28	28	101	25	— 5 [10]	18.0	7.9	4.2	2.9	0.0	0.9	1.2	1.7	6.1	4.6	5.4

Each group contained three animals. A = water ad libitum; B = 4 ml water/rat/day; C = 2 ml water/rat/day.

[1] All diets contained a "high level" of water- and fat-soluble vitamins. Salts IV was incorporated into all diets at a level of 4%.

[2] DL-Methionine was incorporated into all diets at a level of 2% of the casein content.

[3] Milliliters of water/rat/day/dm^2 of body surface area (B.S.A.) at the point of half maximum weight gain. B.S.A. = 0.1136 $W^{2/3}$ with B.S.A. in dm^2 and W the weight in grams (Carman and Mitchell, '26).

[4] All animals in the group died; av. survival time: 14 days.

[5] These diets contained dextrin instead of sucrose.

[6] All animals in the group died; av. survival time: 10 days.

[7] One animal of the group died on the 19th day.

[8] Corn oil, 25% + 25% lard.

[9] All animals in the group died; av. survival time: 17 days.

[10] One animal of the group died on the 17th day.

(in milligrams per kilogram of diet) were: thiamine·HCl 8, riboflavin 6, pyridoxine·HCl 4, niacin 50, Ca pantothenate 40, inositol 200, biotin 0.2, folic acid 4, vitamin B_{12} 0.04, and choline chloride 2000. All groups received the following amounts of fat-soluble vitamins/rat/week: vitamin A 1600 I.U., vitamin D_2 140 I.U., α-tocopherol 9.6 mg and menadione 1.2 mg. DL-Methionine was incorporated at a level of 2% of the casein in each diet. All diets were fed ad libitum. Water consumption records were kept in each experiment.

In experiment 1 water was fed at three levels: ad libitum (water bottle), 4 ml/rat/day (water cup) and 2 ml/rat/day (water cup). Because of the large number of groups in the first and subsequent experiments there were only three animals per water treatment per dietary group. The results of experiment 1 are seen in table 1.

RESULTS

Experiment 1

Groups given water ad libitum ("A" groups). With water ad libitum, the best growth was obtained in group 8A (table 1) fed a 25% protein diet containing 49 mg of protein/calorie. Poorest growth occurred in group 5A given a 10% protein diet with 24 mg of protein/calorie. The weight gains of groups 1A and 7A, fed 50% protein diets were inferior to those of groups 2A and 8A, fed 25% protein diets.

Although diets 11A, 6A and 5A had similar ratios of protein to energy, diet 11A, with fat as its main energy source, and diet 6A, with dextrin as its main energy source, promoted better growth than diet 5A. The main energy source of diet 5A was sucrose (see Harper and Katayama, '53 for a study and references on the difference between sucrose and dextrin or cornstarch when incorporated into low-casein diets fed to rats).

Since Richter and Brailey ('29) found close correlation between water consumption and body surface area (with rats of all sizes), the average daily water consumption was calcu-

lated on the basis of body surface area using the method of Carman and Mitchell ('26). Groups 1A and 7A, fed the highest protein diets, consumed the most water. Considering diet 3A as a conventional (synthetic) diet, groups 4A and 6A, fed dextrin in place of sucrose, and groups 8A through 11A fed higher percentages of fat, consumed more water than group 3A on both the per rat/day and the per square decimeter body surface area/day bases.

Groups given 4 ml of water/rat/day ("B" groups). Restricting water to 4 ml/rat/day produced growth results which differed from those obtained by feeding water ad libitum. Best growth occurred in groups fed 10 or 18% protein diets with 5% fat (groups 3B through 6B). Higher protein diets at all levels of fat depressed growth; poorest growth occurred in groups 1B and 7B fed 50% protein diets.

In diets 2B, 9B and 11B the protein level/calorie was progressively lowered, and the ratio of fat calories to total calories was progressively increased with no effect on the comparative growth rates of the animals of these groups. Fat, as well as protein, contributes *less* metabolic water *per calorie* than does carbohydrate. Therefore, fat at higher levels, like protein, reduces growth in rats fed water-restricted diets.

Groups given 2 ml of water/rat/day ("C" groups). The use of high-protein or high-fat levels or both in severely water-restricted diets induced fatalities (groups 1C, 7C and 10C). Weight losses were greater in most instances with animals receiving the higher-fat diets, while best growth was obtained with group 5C fed a low-protein, high-sucrose diet.

Organ to body-weight ratios ("A," "B" and "C" groups). The ratios of thymus to body weight for the "A" groups were with one exception (group 5A) similar. Restriction of water to 4 ml/rat/day produced very low ratios in groups fed high-protein diets and somewhat reduced values with high-fat diets. With severe water restriction the ratios approached zero. Progressive reductions in ratios accompanied intensification of water restriction with all diets.

In direct contrast to the thymus ratios, kidney ratios increased with all diets when water allotments were reduced; Schreiber and Elvehjem ('54) made similar observations in an earlier study. The high-protein diets produced the largest ratios of kidney to body weight at each water treatment.

Liver ratio values did not correlate well with water or protein intake but were largest with all diets when water was given ad libitum.

Experiments 2 and 3 (table 2)

The primary purpose of experiment 2 and its repetition, experiment 3, was to determine whether high-fat diets possessed the ability to spare dietary protein or increase its availability, since indications of this activity of fat were observed in experiment 1 (from the growth data of groups 11A, 6A and 5A).

Three levels of protein/calorie were used. At each protein level 4 percentages of fat were tested. Food and water (by bottle) were fed ad libitum to all groups and complete records of consumption were kept. The composition of the diets and the composite results of the two experiments are found in table 2.

Within the lowest and intermediate levels of protein, 12 mg of protein/calorie fed to groups 12, 13, 14 and 15, and 24 mg of protein/calorie fed to groups 16, 17, 18 and 19, the consumption of calories and the weight gains progressively increased as the amount of fat substituted for sucrose was progressively raised. The efficiency of protein utilization also increased with the higher-fat diets within the 12 or 24 mg of protein/calorie levels. Total body specific gravity values indirectly suggested that higher dietary fat did not apparently increase body fat content. An analysis by Scheer et al. ('47) of the carcasses of rats which had received diets containing widely varying amounts of fat (0 to 40%) with severely restricted and ad libitum caloric intakes indicated that no direct relation occurred between the fat intake and the extent of

TABLE 2

Experiments 2 and 3: The effects of diets fed to weanling rats in which fat was progressively substituted for carbohydrate (sucrose) at each of three protein/calorie levels. Effects on growth, water consumption, caloric and food intake, protein utilization, liver-fat content and organ to body-weight ratios. Experimental conditions: av. temp. 24°C.; av. relative humidity 48%

GROUP NO.	DIETS [1,2]			PROTEIN/ CALORIE	WT. GAIN 3 WKS.	WATER INTAKE		CALORIC INTAKE/ RAT/DAY	FOOD INTAKE/ RAT/DAY	PROTEIN EFFI-CIENCY [3]	LIVER FAT [4] (wet wt.)	ORGAN TO BODY-WEIGHT RATIOS		
	Casein	Fat	Sucrose									Thymus × 1000	Kidneys × 100	Liver × 100
	%	%	%	*mg*	*gm*	*ml/day/ rat*	*ml/day/ dm² B.S.A.*		*gm*		%			
12	4.8	1.0 (C.O.)[6]	90.4	12	12	5.0	3.5	20	5.1	2.32	13.6	2.2	1.2	7.5
13	5.0	5.0 (C.O.)	86.0	12	12	5.2	3.7	20	5.0	2.31	10.7	2.2	1.2	6.8
14	6.7	33.3 (Mixed)[7]	54.7	12	17	6.3	4.3	25	4.6	2.66	13.2	2.5	1.2	7.2
14-A [8]	6.7	33.3 (Mixed)[7]	54.7 [9]	12	38	10.2	6.1	38	7.0	3.91	12.6	2.4	1.0	6.6
14-B [8]	6.7	33.3 (Mixed)[7] antibiotics	54.7 [9]	12	36	9.8	5.9	33	6.1	4.24	12.7	2.8	1.0	5.9
15	9.6	82.7 (Lard)	0	12	35	10.9	6.6	37	4.7	3.72	15.8	2.4	1.1	6.8
15-A [8]	9.6	82.7 (Butterfat)	0	12	21	8.4	4.9	32	4.1	2.56	15.5	2.5	1.1	7.4
15-B [8]	9.6	82.7 (Veg. oil)[11]	0	12	25	11.8	7.6	33	4.2	3.00	8.8	2.0	1.2	6.1
16	9.5	1.0 (C.O.)	85.7	24	72	9.4	4.7	38	9.8	3.68	8.5	3.3	0.9	7.1
17	10.0	5.0 (C.O.)	81.0	24	74	10.5	5.2	40	9.9	3.60	6.8	3.7	0.9	6.8
18	13.3	33.3 (Mixed)[7]	48.1	24	87	13.1	6.1	45	8.3	3.77	5.2	3.7	0.9	6.5
19	18.2	74.5 (Lard)	0	24	105	15.2	6.6	53	7.1	3.88	8.1	3.9	0.9	5.7
20	19.0	1.0 (C.O.)	76.2	49	112	13.1	5.6	44	11.4	2.46	3.0	3.8	1.0	6.7
21	20.0	5.0 (C.O.)	71.0	49	133	14.3	5.7	49	12.0	2.65	3.2	4.4	1.1	6.4
22	26.7	33.3 (Mixed)[7]	34.7	49	121	14.8	6.1	48	8.7	2.48	4.1	4.2	1.1	6.4
23	33.0	60.4 (Lard)	0	49	117	15.0	6.3	48	7.1	2.37	5.7	4.4	1.1	6.6

[1] Salts IV was added to bring the dietary constituents up to 100%.
[2] Calories/gram of diets: 1% fat = 3.9; 5% fat = 4.1; 33.3% fat = 5.4; 60.4% fat = 6.8; 74.5% fat = 7.4 and 82.7% fat = 7.8.
[3] Protein efficiency = grams weight gain/grams protein consumed.
[4] Liver fat was determined by the method of Bixby et al. ('54).
[5] DL-Methionine was incorporated into all diets at a level of 2% of the casein content.
[6] Corn oil.
[7] Mixture of 16.65% corn oil and 16.65% lard.
[8] All groups but these had a total of 6 animals (three in each experiment); these groups contained three animals each (in but one experimental trial).
[9] These diets contained dextrin instead of sucrose.
[10] Antibiotics 0.04% penicillin + 0.04% aureomycin.
[11] Partially hydrogenated vegetable oil.

deposition of lipids in the tissue. Their results demonstrated that the superior growth of rats on high-fat diets represented an actual tissue growth and not simply the deposition of added fat. Rathbun and Pace ('45) have performed simultaneous analyses relating body fat content of guinea pigs to their body specific gravity. Therefore, the superior weight gains observed in our study with high dietary fat were most likely attributable to greater production of body proteins.

In but one experimental trial the protein-sparing action of 33% fat at the lowest protein/calorie level was enhanced with dextrin (groups 14 and 14A). Penicillin plus aureomycin did not improve growth any further with the moderately high-fat plus dextrin diet, but did increase the efficiency of protein utilization (groups 14A and 14B).

When the protein level was 49 mg/calorie the growth pattern was unlike those at the lower protein/calorie levels; best growth and highest efficiency of protein utilization occurred in the group receiving 5% fat (group 21). Increasing the percentage of fat above 5% at the highest protein level progressively depressed growth and protein efficiency in groups 21, 22 and 23 even though they consumed nearly identical quantities of protein and calories. This observation supplements the above finding of a protein-sparing action of high dietary fat at suboptimum protein levels. The use of high-protein diets in experiment 1 also reduced the growth rate of rats over that of those fed lower protein diets (groups 1A, 7A and 2A, 8A, table 1). Therefore, increasing the availability of a high dietary level of protein with high fat would subject the animals to the stress of excessive protein (casein + methionine) and prevent maximum growth. Earlier evidence for the growth-suppressing effect of high-casein diets has been reported by Drummond et al. ('22), Reader and Drummond ('25), Leatham ('47), Fenton and Carr ('51), Beckner ('53) and others.

At the lowest level of protein/calorie only that group given the highest fat diet (group 15), outside of the dextrin-fed animals, was able to match group 16 in caloric intake and protein

efficiency. These diets, 15 and 16, contained almost identical percentages of protein and similarly interchanged percentages of fat and carbohydrate, but differed by factors of two in protein level/calorie, in inducing animals to eat, e.g., grams of food, and in promoting weight gains.

Diets 19 and 20, containing similar percentages of protein and interchanged percentages of fat and carbohydrate, produced nearly comparable growth results, but in doing so animals of group 19, receiving the high-fat diet with 24 mg of protein/calorie, utilized the dietary protein much more efficiently than those of group 20 fed the high-sucrose diet with 49 mg of protein/calorie.

Water consumption data (based on body surface area) revealed that dextrin and high fat increased dietary water needs within each protein/calorie level not only because of their protein-sparing actions but also because of their inherent inferiority to sucrose, calorie for calorie, in contributing to the metabolic water pool.

Elevation of the protein level reduced the liver-fat content. Of the three diets containing 82.7% fat (in but one experimental trial) partially hydrogenated vegetable oil produced the lowest liver-fat value and increased water consumption the most; on the other hand, lard was superior to butterfat and partially hydrogenated vegetable oil in promoting growth and increasing the efficiency of protein utilization (groups 15, 15A and 15B). These growth differences with identical amounts of edible fats are in some respects similar to the observations of Hoagland and Snider ('40), Deuel et al. ('47), Barki et al. ('50) and others.

Analysis of organ ratios (table 2) indicated in most instances better correlation between different protein/calorie levels than within a given level. Thymus ratios correlated directly with protein levels. Spleen ratios showed similar but poorer correlation. Kidney ratios were lowest at the intermediate (24 mg) protein value, whereas liver ratios displayed no correlation with protein levels.

DISCUSSION

While studying the effects of water restriction on young rats fed diets varying widely in nutrient composition (experiment 1) we obtained suggestive evidence from the growth data of groups 11A, 6A and 5A that high-fat diets might be capable of sparing protein. This led to the observation in experiments 2 and 3 that caloric intake, protein efficiency and growth could definitely be improved in weanling rats fed suboptimum protein diets ad libitum when fat was progressively substituted for carbohydrate (sucrose) while the ratio of protein to energy was kept constant.

Deuel ('50) reviewed the non-caloric functions of fat in the diet. Munro ('51) extensively reviewed the efficacies of fat and carbohydrate as protein sparers under a variety of experimental conditions, and stated that carbohydrate has a specific role in the utilization of dietary protein, for which energy in the form of fat is not a substitute. The maximum growth and protein efficiency results we obtained within the 12 or 24 mg of protein/calorie levels with high-fat, carbohydrate(sucrose)-deficient diets 15 and 19 (table 2) do not follow this concept. There are several possible explanations for the above observations.

The presence of fat in a diet has long been known to modify the motility of the stomach and lead to the delay in gastric emptying. McSwiney and Spurrell ('35) found that the degree of gastric delay produced by fats increased with their concentration in the diet. They also observed that fat in the duodenum could produce gastric delay but that the extent of this effect was governed by intragastric conditions.

We have on several occasions fed the following diets containing equal amounts of carmine to young stock rats initially fasted for periods of approximately 18 hours and have obtained the following results: with the stock diet the first carmine-colored feces appeared in $3\frac{3}{4}$ hours; with a mixture of 75% of stock diet and 25% of lard, the time was $4\frac{3}{4}$ hours. Similarly, increases in the amount of lard to 50 and 75% resulted in further delays in the appearance of the carmine,

namely to 6½ and 10¼ hours respectively. The increasing delay in gastro-intestinal evacuation due to increased dietary fat might favor more complete digestion, absorption and ultimate utilization of the protein component of the diet.

Forbes et al. ('46a) found with growing rats that the gains in live weight and the digestibility and retention of nitrogen of diets containing the same amount of protein and energy improved in the order of increasing fat content (2 to 30%). In a later similar study with growing rats, Forbes and coworkers ('46b) observed a slightly but not significantly greater nitrogen retention on the low-fat diet when the supply of certain vitamins in their diets was greatly augmented.

The results obtained by these investigators are not surprising, inasmuch as they were feeding diets containing an optimum level of protein (22% and higher). With the highest level (49 mg) of protein/calorie we have also found that raising the fat above 5% reduced the rate of growth and the protein efficiency (see results of groups 21, 22 and 23, table 2). Our conditions very nearly parallel those of the Pennsylvania State College Group's isocaloric feeding experiments because the ad libitum caloric and protein intake of each of these three groups was almost identical.

In an earlier study by Forbes and Swift ('44) the two nutrient combinations which led to the minimum heat increments were those composed of protein and fat, and carbohydrate and fat. Next in increasing order of dynamic effects was the combination of protein, carbohydrate and fat. The least efficient combination as indicated by the maximum heat increment was the one containing no fat, e.g., carbohydrate and protein. The implication, therefore, was that fat in its combination with protein or carbohydrate or both conferred economy of utilization of food energy.

French, Black and Swift ('48) observed that decreasing the protein intake from 22 to 7% of the diet did not alter their previously reported superior energy utilization of high-fat diets by the growing rat. These investigators found that increased weight, increased body gains of fat and energy, and

decreased heat production were associated with the high-fat diet.

One possible explanation for the maximum caloric intakes by rats fed carbohydrate-deficient diets 15 and 19 might be found in the glucostatic theory of the regulation of food intake described by Mayer, Vitale and Bates ('51), which sees food intake conditioned by the variation in the blood glucose level.

The absence of sufficient dietary bulk in these very high fat diets as compared to equicaloric portions of the lower fat diets within the respective protein/calorie levels may also have stimulated the desire to eat. Whether the very high fat diets stimulated the secretion of abnormal amounts of bile with resulting increased emulsification and surface activation processes functioning as aids in the digestion and absorption of nutrients remains a possibility.

Finally it appears that the protein to calorie ratio is an important factor in nutrition studies especially if deviations from the typical diet are significant.

SUMMARY

1. The use of high-fat or high-protein diets or both under conditions of water restriction in almost all instances was responsible for larger weight loses and a greater frequency of fatalities of young rats than occurred with high carbohydrate diets. These results are in part attributed to the little recognized fact that fat, as well as protein, contributes *less* metabolic water *per calorie* than carbohydrate.

2. Progressive substitution of fat for carbohydrate (sucrose) in suboptimum protein diets fed ad libitum to weanling rats resulted in (a) increased caloric intake, (b) increased efficiency of protein utilization, (c) increased growth and (d) no change in total body specific gravity, although the ratio of protein to energy was kept constant.

3. At an optimum level of protein, progressive substitution of fat (above 5%) for carbohydrate (sucrose) resulted in (a) decreased efficiency of protein utilization, (b) reduced growth

and (c) no change in total body specific gravity, with the ratio of protein to energy maintained constant.

4. A number of possible explanations for the protein-sparing action of high-fat diets are discussed.

5. A study of organ to body-weight ratios showed (a) reduced thymus ratios and increased kidney ratios with intensification of water-restrictive measures and (b) larger thymus ratios concomitant with increased amounts of dietary protein. Liver ratio values were little affected by changes in water or protein levels of the diet. Spleen ratios were somewhat similar to those of the thymus.

6. The growth-promoting potential of an adequate diet cannot be based solely on the percentage of (high quality) protein. The level of protein/calorie must also be considered.

ACKNOWLEDGMENTS

We wish to acknowledge the help of the following workers and thank them for their co-operation: Dr. John N. Bixby and Herbert R. Heinicke for assistance in the determination of liver fat in experiments 2 and 3; Hashim Mohammed Moghrabi for assistance in experiment 1; and Miss Ruta Pokornijs for services rendered.

LITERATURE CITED

BARKI, V. H., R. A. COLLINS, C. A. ELVEHJEM AND E. B. HART 1950 The importance of the dietary level of fats on their nutritional evaluation. J. Nutrition, *40:* 383.

BECKNER, C. W. 1953 Relation of high protein diets to kidney function. Master's Thesis, Department of Biochemistry, University of Wisconsin, Madison, Wisconsin.

BIXBY, J. N., A. J. BOSCH, C. A. ELVEHJEM AND A. M. SWANSON 1954 Determination of liver fat. Comparative studies of different methods. Agr. Food Chem., *2:* 375.

CARMAN, G. G., AND H. H. MITCHELL 1926 Estimation of the surface area of the white rat. Am. J. Physiol., *76:* 380.

DEUEL, H. J., JR. 1950 Non-caloric functions of fat in the diet. J. Am. Diet. Assoc., *26:* 255.

DEUEL, H. J., JR., E. R. MESERVE, E. STRAUB, C. HENDRICK AND B. T. SCHEER 1947 The effect of fat level of the diet on general nutrition. J. Nutrition, *33:* 569.

DRUMMOND, J. C., G. P. CROWDEN AND E. L. G. HILL 1922 Nutrition on high protein diets. J. Physiol., *56:* 413.

FENTON, P. F., AND C. J. CARR 1951 The nutrition of the mouse. 10. Studies on the utilization of high and moderately low protein diets for growth in 4 strains of mice. J. Nutrition, *43:* 441.

FORBES, E. B., AND R. W. SWIFT 1944 Associated dynamic effects of protein, carbohydrate and fat. Ibid., *27:* 453.

FORBES, E. B., R. W. SWIFT, R. F. ELLIOT AND W. H. JAMES 1946a Relation of fat to economy of food utilization. 1. By the growing rat. Ibid., *31:* 203.

FORBES, E. B., R. W. SWIFT, W. H. JAMES, J. W. BRATZLER AND A. BLACK 1946b Further experiments on the retention of fat to economy of food utilization. 1. By the growing albino rat. Ibid., *32:* 387.

FRENCH, C. E., A. BLACK AND R. W. SWIFT 1948 Further experiments on the relation of fat to economy of food utilization. 3. Low protein intake. Ibid., *35:* 83.

HARPER, A. E., AND M. C. KATAYAMA 1953 The influence of various carbohydrates on the utilization of low protein rations by the white rat. 1. Comparison of sucrose and cornstarch in 9% casein rations. Ibid., *49:* 261.

HEGSTED, D. M., R. C. MILLS, C. A. ELVEHJEM AND E. B. HART 1941 Choline in the nutrition of chicks. J. Biol. Chem., *138:* 459.

HOAGLAND, R., AND G. G. SNIDER 1940 Nutritive properties of certain animal and vegetable fats. U. S. D. A. Tech. Bull., *725.*

LEATHAM, J. H. 1947 Plasma protein concentrations and organ weights of rats as related to a high protein diet. Proc. Soc. Exp. Biol. Med., *64:* 90.

MAYER, J., J. J. VITALE AND M. W. BATES 1951 Mechanism of the regulation of food intake. Nature, *167:* 562.

MCSWINEY, B. A., AND W. R. SPURRELL 1935 The effect of fat on gastric motility. J. Physiol., *84:* 41.

MUNRO, H. N. 1951 Carbohydrate and fat as factors in protein utilization and metabolism. Physiol. Rev., *31:* 449.

RATHBUN, E. N., AND N. PACE 1945 Studies on body composition. 1. The determination of total body fat by means of the body specific gravity. J. Biol. Chem., *158:* 667.

READER, V. B., AND J. C. DRUMMOND 1925 Further observations on nutrition with diets rich in protein. J. Physiol., *59:* 472.

RICHTER, C. P., AND M. E. BRAILEY 1929 Water-intake and its relation to the surface area of the body. Proc. Nat. Acad. Sci., *15:* 570.

SCHEER, B. T., E. STRAUB, M. FIELDS, E. R. MESERVE, C. HENDRICK AND H. J. DEUEL, JR. 1947 The effect of fat level of the diet on general nutrition. 4. The comparative composition of rats in relation to intake of fat and calories. J. Nutrition, *34:* 581.

SCHREIBER, M., AND C. A. ELVEHJEM 1954 The influence of flavonoid compounds on the nasal excretion of a red pigment by rats subjected to stress conditions. Ibid., *54:* 257.

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

MASONIC TEMPLE, SAN FRANCISCO, CALIFORNIA

APRIL 11–15, 1955

COUNCIL MEETINGS

Council meetings were held in the Sheraton-Palace Hotel on Sunday, April 10, and Monday, April 11. Formal actions of the Council are reported in the following minutes of the two business meetings.

SCIENTIFIC SESSIONS

The scientific program consisted of 7 half-day sessions at which 79 papers were presented; one half-day session was devoted to a symposium entitled "Lipids in Nutrition." On a trial basis, the secretaries arranged 8 half-day sessions that were designated "intersociety" in character, an attempt to bring together papers of common interest in all represented fields. Our Institute contributed three papers to the intersociety sessions in the subject areas of "atherosclerosis." Nineteen papers were read by title.

BUSINESS MEETINGS

Two business meetings were called to order by President George R. Cowgill: one at 4:15 p.m. on Tuesday, April 12 and one at 4:15 p.m. on Thursday, April 14. The following items were considered:

1. Minutes. The minutes of last year's business meetings, as published in the September 1954 issue of the *Journal of Nutrition,* were approved.

2. Election. The Secretary transmitted the ballots to the Tellers' Committee, Harold Goss and Benjamin H. Ershoff. The Committee reported election results on 232 ballots cast as follows:

President: W. H. Sebrell, Jr.
Vice-President: Harry J. Deuel, Jr.
Councillor: L. A. Maynard
Associate Editors (4-year term beginning May 1, 1955):
J. S. Dinning
J. M. Orten
C. R. Treadwell

On the basis of suggestions given on the election returns, President Cowgill appointed the following Nominating Committee for 1955–56:

Paul L. Day, Chairman
Icie Macy Hoobler
Vernon H. Cheldelin
D. Mark Hegsted
Harold H. Williams

3. Membership. The Secretary reported that as of April 1, 1955, there were 380 active members and 42 retired members, or a total of 422 members in the American Institute of Nutrition. The following members passed away during the year:

Dr. E. P. Cathcart, June 25, 1954
Dr. Henry J. Gerstenberger, June 24, 1954
Dr. D. Breese Jones, September 5, 1954

4. Treasury. The Auditing Committee, Mary E. Reid and H. P. Morris, submitted a written report that the report of the Treasurer was substantiated by the records of the Treasurer's office. The Treasurer's report and the Auditors' report were approved and dues were approved at $1.00 per member for the coming year. The Treasurer was instructed

to reinvest the $500.00 savings bond that is now in the custody of the Treasury office in Chicago if the bond is not of a series that continues to accrue interest after maturity date.

5. Journal of Nutrition. The Journal Editor, G. R. Cowgill, submitted an annual report, a summary of which follows:

Volumes of the *Journal of Nutrition* for 1954	Nos. 52, 53, 54
Number of papers submitted	192
Number of papers published	172
Percentage of papers rejected	10.4
Number of pages per article	11.5
Number of biographies	3

The report included a suggestion that the third cumulative index for the Journal (the first covered volumes 1–15 (1938) while the second covered volumes 16–35 (1948)) should cover the 10-year period 1948–1958 and that final decision on this matter could be deferred for later meetings.

It was agreeable that supplements to regular issues of the Journal would be continued if authors agreed to the costs involved.

Vitamin nomenclature, as adopted by the Commission for the Reform of Nomenclature in Biological Chemistry of the International Union of Pure and Applied Chemistry, and as accepted by the Editors of the *British Journal of Nutrition,* was referred to the Joint Committee on Nomenclature.

The Editor's report was approved with a vote of appreciation to Dr. Cowgill for the excellent handling of the Journal affairs.

6. Reports of Standing Committees. Reports from the chairmen of standing committees were read by the Secretary and are summarized with actions as follows:

(a) Joint Committee on Nomenclature (C. G. King and E. M. Nelson) reported that no problems had been referred to the Committee for action during the year. A tentative report with reference to lipoic acid (thioctic acid) was discussed and action deferred pending a final detailed report from the Committee.

(b) Committee on the Registry of Pathology of Nutritional Diseases (H. Pollack) reported that due to foreign travel by the chairman this committee failed to submit a formal report in time for the meetings. The activities of the Committee are progressing satisfactorily.

(c) Representative to the Food and Nutrition Board and Division of Biology and Agriculture, N.R.C. (N. R. Ellis). The report included a summary of matters discussed by various committees at the Food and Nutrition Board meeting in the fall of 1954. The report was approved, and it was further considered desirable that our representative solicit membership cooperation in providing suggestions for appropriate symposium subject matter for the May, 1955, meeting of the Division of Biology and Agriculture.

(d) Representatives on the AAAS Council (F. J. Stare and J. H. Roe) were unable to attend the AAAS Council meetings in San Francisco in December, 1954. Marjorie M. Nelson, who was appointed as substitute representative by Dr. Cowgill, submitted a written report. Pertinent action by the AAAS Council included (1) a recommendation of adoption of uniform governmental clearance policies for grants on unclassified research and (2) a recommendation for the AAAS Committee to study dangers in thermo-nuclear fission tests. The report was approved.

(e) Representative to Nutrition Division of F.A.O. (P. E. Howe). A detailed report of the activities of the Nutrition Division of the F.A.O. at its meeting in September, 1954, has been filed with the record in the Secretary's office. The report was adopted with the recommendation that P. E. Howe be commended for his interests in keeping the Council informed on the activities of this organization.

7. Nominations for Membership. The Council reviewed 26 nominations for membership, 9 of which had been deferred from 1954. The following were approved:

Doris H. Calloway
S. L. Hansard
A. E. Harper
I. E. Liener
L. D. Matterson
Fred Rosen
S. A. Singal
R. B. Alfin-Slater
M. L. Sunde
H. C. Tidwell

8. Actions of the Federation Board. President Cowgill reported on the actions of the Federation Board in its Sunday night meeting. The main items were:

(a) Consider transferring the Headquarters of the National Society for Medical Research from Chicago to the Federation Headquarters in Bethesda.

(b) Consider reducing the abstracts to a maximum length of 200 words from the present 275 words to reduce cost of printing the abstract issue of *Federation Proceedings.*

It was considered appropriate to refer these matters to the business meeting for a vote and to concur in the action of the membership. At the business meeting the following actions, endorsed by the Council, were adopted: (1) Transfer of the Headquarters of the National Society for Medical Research should be delayed until its Board of Governors could meet with the Federation Board and establish permanent working arrangements between the two organizations and (b) continue the present policy of 275-word maximum for abstracts.

9. Plans for International Congress of Nutrition. The report by Paul György of the Ad Hoc Committee on the International Nutrition Congress was reviewed. After considerable discussion the following motions were passed:

(a) That the AIN should be the sponsoring organization and that the International Nutrition Congress, at its 1957 meeting in Paris, France, should be invited to the United States for its next meeting in 1960.

(b) That the President of the AIN should be authorized to appoint a standing committee to develop and carry out the many detailed arrangements that will be involved in sponsorship of such a Congress.

(c) That the administrative services offered by the Federation Secretariat should be accepted to expedite the planning and arrangements for an International Nutrition Congress in the United States.

10. Miscellaneous Items. The following miscellaneous items were considered:

(a) Approved the proposal that authors be invited to submit news releases with their abstracts.

(d) Endorse the proposal that consideration be given to devoting a *Yearbook of Agriculture* to the subject of Food and Nutrition. The last such subject coverage in the *Yearbook* was that of 1939 entitled "Food and Life." The Secretary was instructed to investigate how this action can be most appropriately brought to the USDA.

(c) That H. E. Robinson, society representative on the Federation Public Education Committee, consider the preparation of a statement outlining "Careers in Nutrition," such material to be at high school student-readability-level and useful in supplying high school guidance teachers with information on careers in nutrition.

(d) The matter of whether or not membership billing for journal, society dues, and Federation dues should be transferred from the Treasurer's office to the Federation office was tabled until next year's meeting.

(e) The Council adopted the following changes in the form to be used for inviting suggestions for nominations for awards. These included: require each form to be sub-

mitted in 5 copies and ask Jury of Award members to return all pertinent information on nominations to the Secretary's office as useful material in future years.

(f) Appropriate Joint-Federation-Symposium topics for the 1956 meeting, when the AIN is senior society and is responsible for the Joint Symposium, were briefly discussed. The subject "Dietary fat-cholesterol-atherosclerosis" was tentatively agreed upon as an appropriate subject. Dr. Cowgill invited further suggestions.

(g) Approved donation of five English pounds toward support of the International Union of Nutrition Sciences, as requested by Dr. Leslie J. Harris of Cambridge.

ANNUAL DINNER AND PRESENTATION OF AWARDS

The Annual Dinner of the American Institute of Nutrition was held on Wednesday, April 13, in the Sheraton-Palace Hotel and was attended by 173 members and guests. The evening was enlightened by President Cowgill's report of a statistical sampling of the convention registration file, conducted to ascertain the geographic distribution of those in attendance. A spontaneous editorial evaluation of the report by W. D. Salmon added to the enlightenment of the program and to the enjoyment of those in attendance.

The program highlight was the presentation of Awards by President Cowgill. The Borden Award in Nutrition was presented to Dr. Albert G. Hogan of the University of Missouri, "In recognition of his outstanding researches on the nutritive significance of the components of milk, with particular reference to the water-soluble vitamins, his pioneering work on vitamins in animal nutrition, and his distinguished career as a teacher and mentor of many younger investigators." Dr. Hogan's achievements were described by his first graduate student, Professor W. D. Salmon. Dr. Hogan responded urging young nutritionists to pay heed to the marked influences that can be ascribed to food and nutritional habits from one generation to the next in human populations.

The Osborne and Mendel Award was presented to Dr. E. V. McCollum, Professor Emeritus at the Johns Hopkins University, "In recognition of his outstanding discoveries concerning vitamins and mineral nutrients, and his numerous other distinguished contributions to the science of nutrition." Dr. McCollum's achievements were described by Dr. Harry G. Day. Dr. Day's remarks also included recollections of his experiences as a graduate student with Dr. McCollum. Dr. McCollum's accounts of his early experiences with Osborne and Mendel at Yale and his recollections of the Wisconsin laboratory were impressive in the detail with which they were recalled in his extemporaneous response.

COMMITTEES FOR 1955–56

The following are the standing committees beginning July 1, 1955:

Committee on Registry of Pathology of Nutritional Diseases

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

Consultants

P. R. Cannon
Paul Klemperer
W. Stanley Hartroft

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

Representatives to the American Association for the Advancement of Science

Joseph H. Roe, Section C (Chemistry) — one year
Paul B. Pearson, Section N (Medical Science) — two years

Respectfully submitted,
R. W. ENGEL, *Secretary*
American Instiute of Nutrition

BORDEN AWARD IN NUTRITION

Nominations are solicited for the 1956 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 1956. To be considered for the award, nominations must be in the hands of the Chairman of the Nominating Committee by January 1, 1956. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate consideration for the award. For details of nomination procedure, write to Chairman of the Nominating Committee.

Chairman, Nominating Committee:

DR. GLADYS EVERSON
Department of Home Economics
University of California
Davis, California

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 1956 Award, accompanied by data relative to the accomplishments of the nominee, must be sent to the Chairman of the Nominating Committee before January 1, 1956. For details of nomination procedure, write to Chairman of the Nominating Committee.

Chairman, Nominating Committee:

DR. OTTO A. BESSEY
Department of Biochemistry and Nutrition
University of Texas Medical Branch
Galveston, Texas