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Contents

No. 1 JANUARY 1968

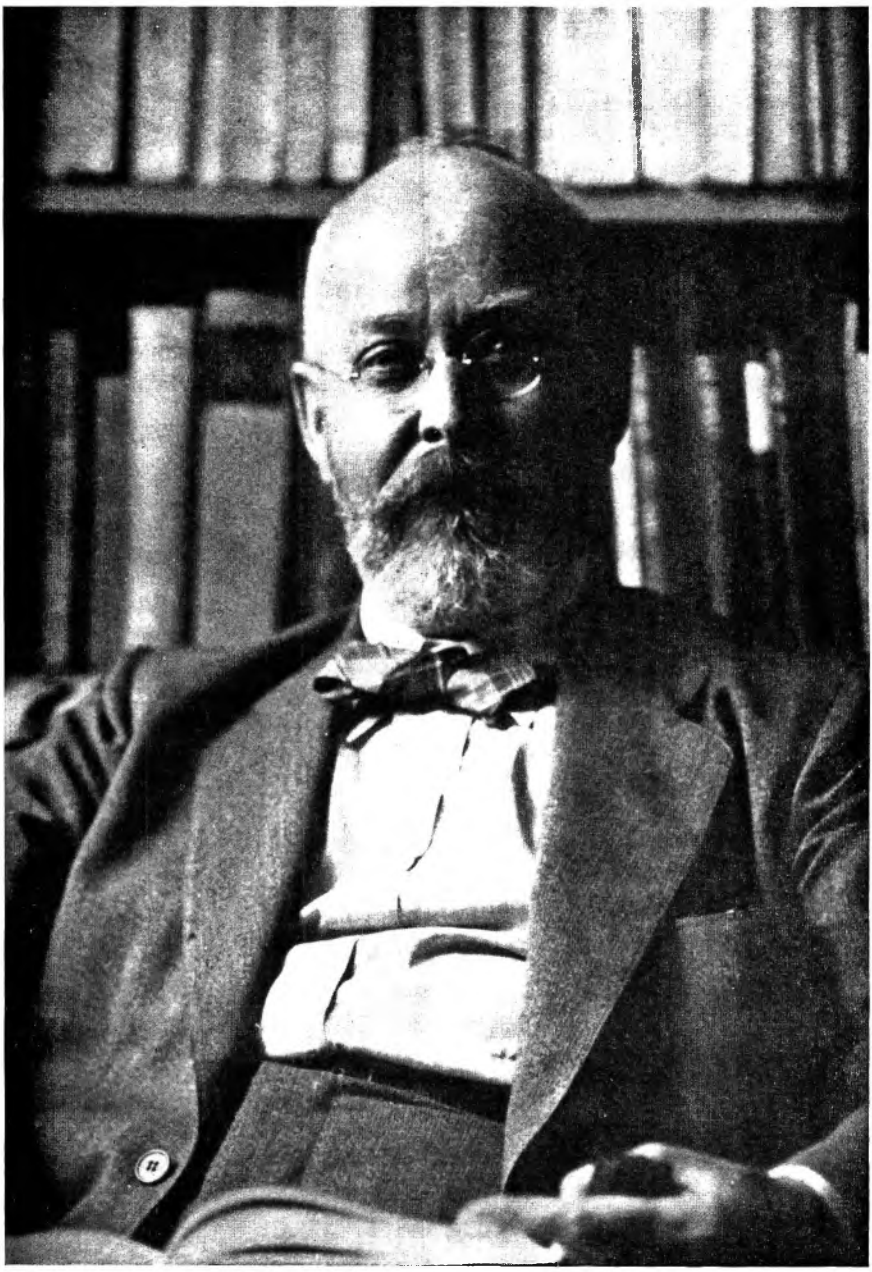
No. 2 FEBRUARY 1968

No. 3 MARCH 1968

No. 4 APRIL 1968

LAWRENCE J. HENDERSON

(*1878–1942*)



LAWRENCE J. HENDERSON

Lawrence J. Henderson

— A Biographical Sketch

(1878–1942)

This year (1967) marks the twenty-fifth anniversary of the death of L. J. Henderson. It seems fitting that the *Journal of Nutrition* should pay tribute to a man who did as much as any scientist in the history of the United States to orient universities toward areas of useful contact between Science and Society — nutrition being, of course, a prime example of such contact.

Lawrence J. Henderson (L. J. to generations of his friends and — though not to his face — of his students) was born on June 3, 1878, in Lynn, Massachusetts, the son of Joseph and Mary Bosworth Henderson. He was educated in Salem, at Harvard College, and at Harvard Medical School. He went on to study for two years with Hofmeister in whose laboratory, says Professor C. C. Brenton, the author of his Harvard memorial minute, he acquired "a dislike for German metaphysics, German push, and German fondness for giving and taking orders." He came back to Harvard, where he stayed for the rest of his academic career, mostly as Abbott and James Lawrence Professor of Chemistry (except for a half year as Harvard exchange professor to France in 1921 and for shorter periods as visiting lecturer with other universities, notably at Yale where he gave the Sillman Lectures). As foreign secretary of the National Academy of Sciences he also spent in his later years much time in Washington.

Dr. Henderson's early research is basic to the understanding of the acid-base balance of the organism. Strongly influenced by Willard Gibbs and d'Ocagne, and more interested in theoretical thinking than in elaborate experimentation, he became fascinated by the manner in which the organism maintains its neutrality and eventually developed the famous Henderson-Hasselbach equation representing the buffering action of blood constituents. He went on to study some of the mathematical relationships describing oxygen transport and release and eventually summarized these conditions in his famous book, *Blood — A Study in General Physiology,* which appeared in 1928.

While he taught biological chemistry at Harvard for 35 years (1904 to 1939) (with a brief interruption during World War I when he was involved in chemical warfare and in research on bread manufacture and conservation for the troops), and in spite of the importance of this theoretical research in biochemistry, it was through his concern with areas other than his field of scientific research that he exerted his greatest influence. This concern was first translated into an officially scheduled University function when in 1911 L. J. Henderson first gave a half course in the history of science. This course, which evolved into a discussion of the strategy of science, was continued for 30 years. L. J. Henderson's philosophical interest was crystallized in two books, *The Fitness of the Environment,* published in 1913, and *The Order of Nature,* published in 1916, the theme of which was that environment and fitness are in a sort of reciprocal relationship to each other so that the finding of an order in nature is something that the human mind cannot fail to find. Going from the natural to the social environment, Henderson became increasingly interested in the application of physiological thinking to the study of sociological events. It is perhaps unfortunate that he became so impressed by Pareto, which he first read in 1926, that his sociological views became to a certain extent frozen in the technocratic, amoral, authoritarian mold provided by this writer; this was all the more curious in that L. J. Henderson's own bent was one of intellec-

tual tolerance, of recognition of the diversity of human contributions, and that he conserved to the end a nostalgia for the familiar virtues quite at variance with Pareto's scorn for ideals.

This nostalgia was best expressed in his yearly lectures on medical ethics, an interesting and curious exercise for a man who had never practiced medicine and whose conscious and articulate creed was that values and ideals ought to be questioned rather than followed. Those who knew him well felt that he was actually giving more of himself in these lectures than in his more elaborate courses.

Henderson's influence on Harvard University was enormous. He established the Laboratory of Physical Chemistry at Harvard Medical School. Under the leadership of his protégé, Professor Edwin J. Cohn, this laboratory was to make a considerable contribution to our knowledge of the protein fractions of blood — the body constituent which Henderson had always been most interested in. While the Laboratory of Physical Chemistry was discontinued in the fifties, shortly after Cohn's death, its members — among whom were John Edsall, George Scatchard, Lawrence Oncley, I. Fankuchen, and John Ferry — went on to develop similar laboratories all over this country.

Even more original was the creation of the Fatigue Laboratory at the Harvard Business School. L. J. Henderson was preoccupied by the ignorance of the "human factor" by U. S. industry. He was instrumental in bringing the great British sociologist, Elton Mayo, to the Business School. He also raised the money necessary to assemble a group of physiologists who would study the physiology of industrial work and fatigue. The laboratory was eventually discontinued a few years after Henderson's death, but its members — among whom are numbered many of the most distinguished American and British physiologists including at least two who later became members of the Faculty of the Harvard School of Public Health — such as Ross MacFarland and William H. Forbes — went on to create related institutions elsewhere in the United States. Scores of well-known environmental physiologists, both American and European, did some of their significant work in the Fatigue Laboratory.

In the twenties and the thirties, the Fatigue Laboratory publications included a series of important papers on blood, dealing in particular with respiratory exchanges in man (and also in such more unexpected laboratory animals as the horse, the skate, the gila monster, and the crocodile) with the mechanism of fatigue, the effects of heat on electrolyte balance, the role of myoglobin in muscular exercise, and the physiology of high altitude. More immediately significant for nutrition, the Fatigue Laboratory played a considerable role during World War II. No later than the invasion of France in 1940, and probably one or two years before this, Dr. Henderson had guessed that sooner or later the United States would be involved in the war with Germany and had decided to prepare the Fatigue Laboratory to play a useful role in the coming conflict. The bulk of the work was to be in nutrition and the Fatigue Laboratory was involved in determining nutritional requirements in man, in studying the effects of physical exercise, climate and fatigue on requirements and in testing the adequacy of various diets for keeping men fit and working under a variety of circumstances. For example, in the period 1941–1946, papers from the Fatigue Laboratory related to nutrition (and authored by such well-known physiologists and nutritionists as R. E. Johnson, S. M. Horvath, D. B. Dill, L. Brouha, R. C. Darling, G. C. Pitts, F. Consolazio, and W. H. Forbes) appeared on the influence of glycine on muscular strength, training and its effects on man at rest and at work, tests of physical fitness, experimental human vitamin A deficiency, the effects of vitamin B complex deficiency on sedentary men and on men doing manual labor, the effects of vitamin C intake on men doing heavy work; and also nutritional standards for men in tropical climates, acclimatization to work in hot climates, water-soluble vitamins and electrolytes in sweat, dietary protein and physical fitness in temperate and hot environments in man at rest and doing heavy work, the effects of hard physical work upon nutritional requirements, and others. Johnson and his collaborators elaborated on the structure and functions

of a field nutrition laboratory together with the description of rapid field methods to determine vitamin levels in body fluids and to assess the state of vitamin nutriture. A number of studies related to food technology and food acceptance under field conditions also came out of the laboratory.

While L. J. Henderson did not live to see it, the Fatigue Laboratory he had created became, through the crucial period of the early forties, a key allied scientific installation. Though neither the Fatigue Laboratory nor the Department of Physical Chemistry survived their organizer by very long, their achievements in terms of research accomplished and as training grounds for important scientists — nutritionists, in particular — constitute lasting contributions to science and to the nation by L. J. Henderson.

Oddly enough, the third creation of L. J. Henderson at Harvard, the Society of Fellows, was at the same time the most criticized and the only one to survive as an institution to this day. L. J. Henderson, impressed by the European emphasis on early selection — particularly as exemplified by the École Normale Supérieure in France and by some of the fellowship systems at Oxford — was anxious to recognize and assemble promising young men and free them of the usual academic obligations. They would receive a stipend, spend their term as "junior fellows" as they wished, and, through a schedule of social meetings and elaborate dining arrangements, be exposed to one another and to carefully selected "senior fellows." This, he felt, would prepare them for leadership in their field under the best conditions. Many of his colleagues, adverse to the creation of a small elite group on what they felt was an insufficient basis of judgment, unimpressed with the results of similar systems in Europe, and convinced that life could be counted on to provide a more solid basis of later selection, opposed this creation. Henderson's views prevailed and the society was established in 1933 with his serving as chairman until his death in 1942 (of a pulmonary embolism following an operation).

Considerable though Henderson's achievements were, his personality was even more remarkable. Those of us who were fortunate to know him well will always remember him as an impressive figure, the dignified, almost majestic, bearded scholar who enjoyed above all an honest, lively disputation on any subject of general philosophical import, the wise advisor and the generous friend who yearned for the old loyalties under the cover of a worldly cynicism. Whether at his house in Cambridge, or at his summer camp in Morgan Center, Vermont, he liked to be surrounded by students and younger colleagues and through Socratic questioning help them to better define and enlarge their ideas. A legend in his own time at Harvard, his national influence through his post as foreign secretary of the National Academy of Sciences was great. Though it took him longer than it did Walter Cannon, for example, to recognize the dangers of Fascism to the academic and humanistic traditions, his eyes were opened in the mid-thirties by a visit to German universities and he became committed to early support of the Allies. He was also extremely helpful in helping to relocate exiled fellow scientists.

To write of the history of science and of its application to man in the United States during the first half of this century without mentioning L. J. Henderson would be to have missed one of the most important elements in the academic atmosphere in which they developed.

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Autoradiographic Studies on the Distribution of Zinc-65 in Mice [1]

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ABSTRACT The distribution of zinc in various organs and tissues was studied simultaneously at various periods after a single injection of labeled zinc. ^{65}Zn as chloride in isotonic solution was injected intraperitoneally in adult mice, including pregnant females. The gross distribution patterns of labeled zinc were studied by means of whole-body autoradiography at various intervals after the injection of a single dose. The kinetics of zinc was generally found to be rapid. However, the uptake of zinc in the hard tissues and the central nervous system was slower than in other tissues. ^{65}Zn was transported through the placenta into the fetus, and at 2 hours a high uptake was visible in the fetal bones and in the liver. Accumulation of zinc was found in liver, kidney, urinary bladder, lung tissues, pancreas, gastric and intestinal mucosa, hard tissues and cartilage. A relatively high concentration was also found in the retina and choroid of the eye, testes, lactating mammary glands, salivary and lacrimal glands, spleen, hair follicles and hair. The zinc level in blood, skeletal muscles, central nervous system and pituitary gland was low. After 24 hours and longer periods the concentration of zinc in the hard tissues dominated the distribution pattern.

The presence of zinc in living organisms and its role as an essential nutrient has been reviewed comprehensively (1). The normal human intake has been reported to be 10 to 15 mg/day (2) and 15 to 20 mg/day (3). Zinc is present in vertebrates in quantities varying from 10 to 300 ppm. Most organs contain between 20 and 30 ppm of zinc in wet tissue. The highest concentrations of zinc have been found in bone, kidney, liver, muscles, testes, and prostate, about 100 ppm of the wet weight (4). High values of zinc in human teeth have been reported for dentine, 199 ppm, and for enamel, 276 ppm, on a dry weight basis (5, 6). In human mandibular bone a value of 117 ppm (dry weight) has been reported (7) and for cartilage, 29 ppm (human mandibular disc) and 98 ppm (articular fibrocartilage from the human mandibular head) expressed as dry weight (8).

Haumont (9, 10) reported high concentrations of zinc at the points of calcification in bone tissue, but he was unable to prove that zinc was necessary for calcification.

To obtain information about the uptake and disappearance of zinc in different organs and tissues, several experiments have been carried out on various mammals with the aid of labeled zinc. Studies in rats indicate that labeled zinc is in general poorly absorbed from the intestinal tract (11). Similar results were reported for mice, rats, and dogs (12). When ^{65}Zn was injected intravenously into rabbits it was removed rapidly from blood and about 90% left the blood stream in the course of 3 hours (13). The fate of intravenously injected ^{65}Zn in rats was studied and it was found that the decrease in clearance of ^{65}Zn was not in direct proportion to the decrease in specific activity (14). It was demonstrated that more than 90% of the injected ^{65}Zn was bound to protein within the first 3 minutes after injection. The most rapid uptake of ^{65}Zn after injection has been observed in liver, pancreas, spleen and intestine of rabbits (15), and in pancreas, liver and spleen of mice, rats, and dogs (12). Using ^{69}Zn in the form of zinc benzoate, a rapid uptake in the pancreas, the prostate, the pituitary gland, and

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the serous membranes of mice and rats was observed (16). In the muscles, brain, and bones the uptake and loss of ^{65}Zn was slow (12, 15). Studies on rats showed a prolonged retention in bone of intravenously injected ^{65}Zn (17–19).[2]

The present investigation was undertaken to study the distribution of zinc in various organs and tissues simultaneously. For this purpose a whole-body autoradiographic technique was found to be advantageous.

MATERIALS AND METHODS

This study was carried out on 30 adult mice, females and males, weighing about 20 g, and on 8 pregnant females, weighing about 45 g. The albino mice were of the N.M.R.I.[3] strain. The animals were housed in acrylic cages with stainless steel covers, and had free excess to tap water and a conventional mouse diet.[4]

The radioactive isotope, ^{65}Zn, used was supplied by the Radiochemical Centre, Amersham, in the form of zinc chloride in aqueous solution. The specific activity was 100 mCi/g zinc.

Each mouse was injected intraperitoneally with a single dose of approximately 50 μCi ^{65}Zn in isotonic solution. The animals were killed by immersion in a solution of solid carbon dioxide in acetone (about $-80°$) at various intervals after injection. Two mice were killed at each of the following time intervals after the injection: 1, 2, 4, 8, 16, 32 minutes, 1, 2, 4, 8, 24, 32 hours, and 2, 4, and 8 days. Two pregnant mice were killed at each of the following time intervals: 1, 2, 4, and 8 hours. Sagittal sections, 20-μ thick, were taken through the entire animals at various levels. Sectioning, drying of the sections, and autoradiographic exposures were carried out in a freezing room at $-10°$. Structurix[5] X-ray film was used. With the dosages used and the survival periods studied, it was found that the suitable exposure time was 1 to 2 weeks. Detailed descriptions of the autoradiographic techniques used are given by Ullberg (20, 21).

OBSERVATIONS

The gross distribution of ^{65}Zn in various tissues and organs as found by means of whole-body autoradiography will be described. In most organs and tissues ^{65}Zn was found to be accumulated very soon after the intraperitoneal injection. However, the uptake in the hard tissues, the central nervous system, and in the fetus was relatively slow.

At the longest surviving periods studied the concentration of zinc was low in the central nervous system, skeletal muscles, adrenal, thymus, pituitary, blood and myocardium. The highest concentration was found in hard tissues, lactating mammary glands, liver, pancreas, kidney, gastric and intestinal mucosa, retina and choroid, cartilage, hair follicles and hair, salivary and lacrimal glands, testes, lung tissue, placenta, and spleen.

At one, two and four minutes after injection the highest concentration of ^{65}Zn was observed in the lungs and blood. A high accumulation could also be detected in the cortex of the kidneys, liver, heart muscle and in the choroid and retina of the eye. It was also observed that zinc accumulated in the squamous epithelium of the nasal cavity, oral cavity, esophagus and the esophageal part of the gastric mucosa. The secretory part of the gastric mucosa had a relatively high concentration, especially in the basal part; however, no secretion of zinc into the gastric lumen was noted. The mucosa of the small intestine rapidly concentrated large amounts of the radioactivity. In the mucosa of the large intestine the concentration was lower than in the small intestine. The salivary glands, spleen, pancreas and Harder's gland accumulated radioactivity, which persisted throughout the period of the investigation. At this time an uptake was also observed in the epiphyseal parts of long bones and in other skeletal parts.

At eight and sixteen minutes after injection the distribution patterns were similar to those of the previous survival periods. Figure 1 shows the distribution after 8 minutes. However, a continuous

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[2] Ballou, J. E. 1959 Metabolism of Zn^{65} in the rat. General Electric Company, Hanford Laboratory, Richland, Washington (ref. H W.-60062).

[3] Naval Medical Research Institute, Bethesda, Maryland.

[4] Anticimex 210, Norrviken, Stockholm.

[5] Structurix, Gevaert-Agfa, Mortsel, Belgium.

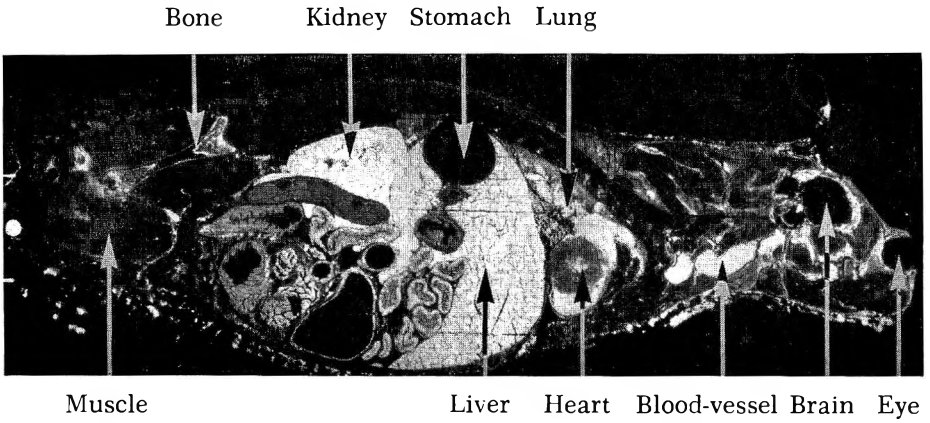

Fig. 1 Autoradiogram showing distribution of ^{65}Zn in a mouse 8 minutes after intraperitoneal injection. White areas correspond to high uptake of ^{65}Zn. (Structurix) × 2.

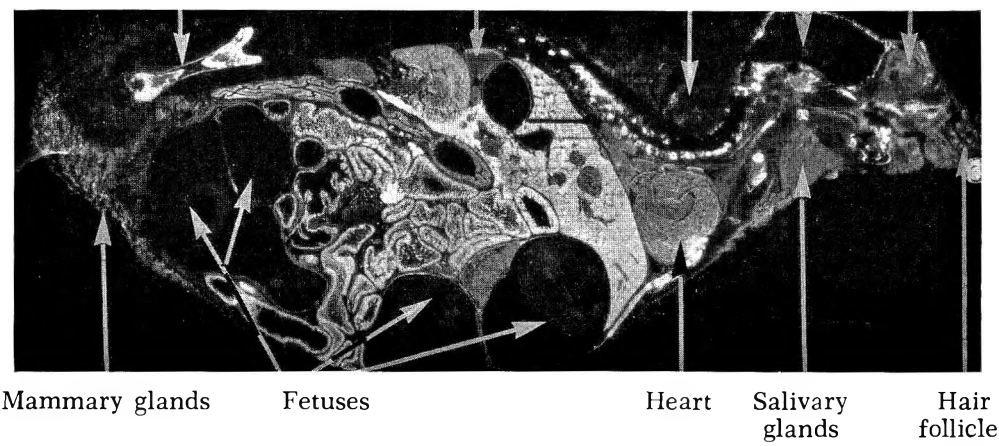

Fig. 2 Autoradiogram showing distribution of ^{65}Zn in a pregnant mouse 1 hour after intraperitoneal injection. (Structurix) × 2.

increase of the accumulation of zinc in hard tissues occurred. At these survival periods an increased uptake was also noted in the gastric and intestinal mucosa, the intestinal content, pancreas, spleen and choroid of the eye. In the central nervous system, the pituitary gland and in the skeletal muscles the concentration was low. No increase in the activity was noted in these tissues at longer survival periods.

At 32 minutes and 1 hour after injection a further increase of the uptake of ^{65}Zn was observed in the hard tissues, the liver, pancreas, the gastric and intestinal mucosa as well as in the intestinal content. A slight uptake of ^{65}Zn could then be observed in the fetuses; in the placenta the concentration was high as well as in the lactating mammary glands (fig. 2). The zinc level in the blood and in the myocardium had decreased considerably at those times.

At two, four and eight hours after injection the uptake in the hard tissues was further pronounced (figs. 3, 4 and 5). However the highest uptake was still in the liver, pancreas, spleen, kidneys and in the intestinal walls. The concentration in lactating mammary glands was also pronounced. The accumulation of ^{65}Zn in the

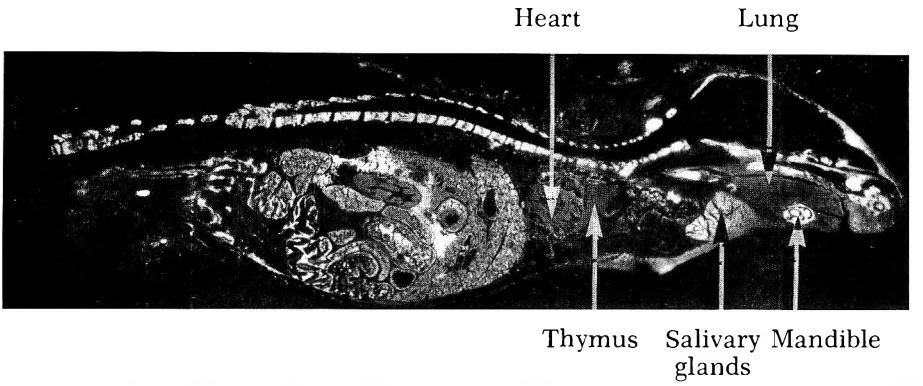

Fig. 3 Autoradiogram showing distribution of ^{65}Zn in a mouse 2 hours after intraperitoneal injection. (Structurix) × 2.

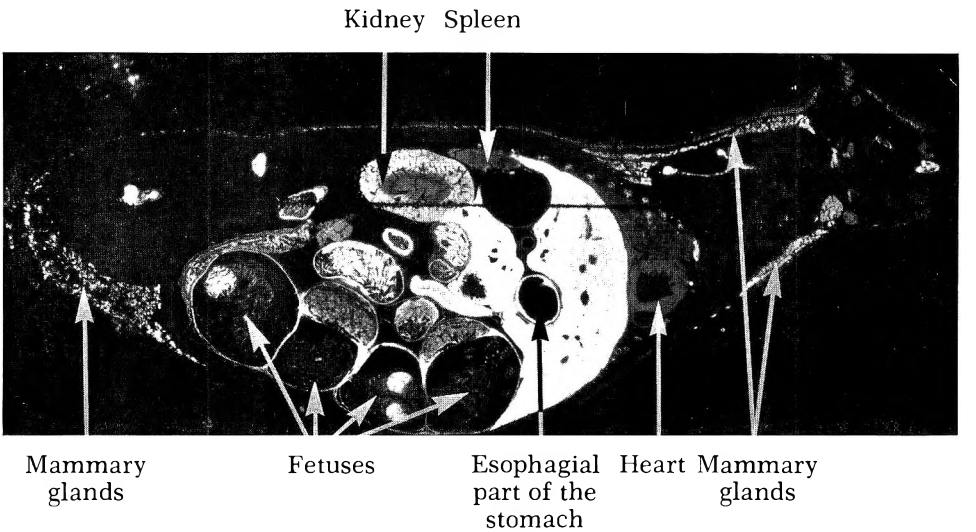

Fig. 4 Autoradiogram showing distribution of ^{65}Zn in a pregnant mouse 4 hours after intraperitoneal injection. (Structurix) × 2.

fetuses was then much higher than previously. Within the fetuses the highest concentration was noted in the liver, kidneys and fetal bones. In blood the concentration was then very low.

At 24 and 32 hours and 2 days after injection the uptake in the hard tissues was strong. There was also a pronounced uptake in the gastric and intestinal mucosa. The uptake in the liver, pancreas, spleen and in the kidneys was still strong but somewhat less pronounced than at earlier periods. In the lungs a low radioactivity was observed at these survival periods. In table 1 are shown the approximate levels of ^{65}Zn as found by autoradiograms 24 hours after injection.

At 4 and 8 days after injection there was a large uptake in the hard tissues, but in the soft tissues and the body fluids the concentration was less pronounced than previously.

DISCUSSION

The distribution of ^{65}Zn by means of autoradiographic studies of separate organs and tissues has been published previously (9, 10, 22–24). In the present study the

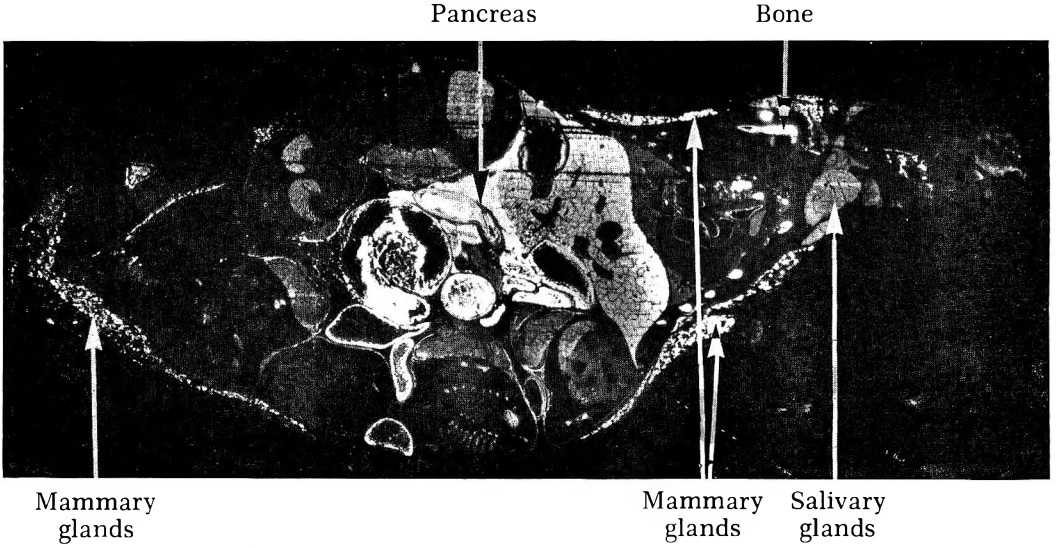

Fig. 5 Autoradiogram showing the distribution of ^{65}Zn in a pregnant mouse 8 hours after intraperitoneal injection. (Structurix) × 2.

Fig. 6 Autoradiogram showing the distribution of ^{65}Zn in the kidney of a mouse 2 minutes after intraperitoneal injection. (Structurix) × 4.

distribution of ^{65}Zn in various organs and tissues was studied simultaneously on the same film.

The results of the present investigation are in good agreement with recently reported observations obtained mainly by scintillation counting and in histochemical studies. The present study indicated a relatively high uptake of ^{65}Zn in some soft tissues as, for example, the liver, kidneys, lactating mammary glands, gastric and intestinal mucosa, pancreas and spleen. The distribution of ^{65}Zn in the kidney 2 minutes after injection is shown in figure 6. With increasing survival periods the accumulation of ^{65}Zn in the hard tissues dominated the distribution pattern to an increasing degree. These overall pictures agree well with an unpublished study where quantitative comparisons on rats between various soft and hard tissues were made by scintillation measurements.[6]

[6] Bergman, B., manuscript in preparation.

TABLE 1

Approximate levels of ^{65}Zn 24 hours after intraperitoneal injection in mice

High uptake	Intermediate uptake	Low uptake
Bone	Teeth	Hair
Cartilage	Choroid and retina of eye	Heart
Pancreas	Testes	Muscles
Kidney	Salivary and lacrimal glands	Central nervous system
Liver		Pituitary gland
Spleen		Blood
Gastric and intestinal mucosa		
Lactating mammary glands		
Intestinal contents		

The main route of excretion of zinc is by feces (12, 15). The intestinal mucosa and content showed a high accumulation of zinc, which indicated an excretion of zinc. Zinc in pancreatic juice and bile probably contributed to the fecal elimination. In the gastric mucosa the zinc concentration was also high, but no secretion of zinc into the lumen could be observed. The kidney showed a high concentration of zinc; however, this decreased slightly with time.

The high concentration of zinc observed in the lactating mammary glands indicated postnatal transfer of ^{65}Zn via the milk, which has been reported previously (11, 25).[7] It has been noted that colostrum contains a large amount of zinc and that the concentration falls significantly as lactation progresses (26). Newborn rats deprived of colostrum have been reported to show zinc deficiency symptoms (27).

The prenatal transfer of absorbed ^{65}Zn from the dam across the placenta to the fetus has been shown in rats (11, 24, 25), in rabbits (28) and in dogs (29). The survival periods in the present study were considerably shorter than in previous studies, thus making it possible to study the early transfer of ^{65}Zn across the placenta. At one hour after the administration the first signs in the autoradiograms of zinc uptake could be observed in the fetuses; later this uptake was greatly increased. The highest concentration within the fetuses was noted in the liver, kidney and in the fetal bones. At 8 hours the concentration of the fetal bone dominated the distribution pattern in the fetuses. These observations are in agreement with the results presented by Kinnamon (24), who, 4 days after administration of ^{65}Zn to pregnant rats, found the highest concentration of labeled zinc within the fetuses in the calcifying bones in those areas where mineralization occurred.

The high concentration of ^{65}Zn, at short survival periods, in the peripheral dorsal wall of the eye bulb could be related mainly to the choroid and retina and is in agreement with the high concentration of zinc demonstrated in the *tapetum lucidum* and the pigment-protein complex in the choroid in some animals, such as the fox, marten, and dog (30, 31).

Within 1 to 2 minutes after injection, it could be observed that ^{65}Zn was taken up by the hard tissues. This concentration tended to increase steadily over the time period studied. From 24 hours the ^{65}Zn accumulation in the hard tissues dominated the distribution pattern. This result appears to point in the same direction as the observations reported in scintillation studies (17, 18), showing a prolonged retention in bone of intravenously injected ^{65}Zn up to about 300 days. A prolonged retention of ^{65}Zn up to 630 days after administration of the radioisotope has also been reported (19). In the present study the concentration of ^{65}Zn in the long bones was very high in the mineralized portions. Both shafts and ends as well as the metaphyseal portions were clearly visible in the autoradiograms. The bone marrow contained only small amounts of ^{65}Zn. A very high uptake was noted in other mineralized tissues, as the skull bone, teeth, ribs, and vertebrae. In young growing rats Haumont (10) showed an incorporation of ^{65}Zn in the hard tissue that was being formed at the time of injection. The bone deposited between injection and killing showed decreasing radioactivity from a peripheral intense imprint corresponding to the injections toward the most recently deposited layer.

It has been reported that zinc benzoate accumulates in the pituitary gland (16). In the present study it was found, however, that zinc administered as zinc chloride did not accumulate in the pituitary gland. The pancreas showed a relatively high concentration of zinc. This investigation was unable to differentiate zinc content of α- and β-cells of the Islands of Langerhans. The binding of zinc to amino acids, peptides and proteins through imidazole and sulfhydryl groups has been discussed previously (32). It has been suggested that at least part of the substantial amount of zinc in the pancreas is chelated to imidazole residues of amorphous insulin and to carboxylate groups of crystalline insulin (1). It has not been shown clearly, however, that zinc is an essential component for the normal function of insulin.

[7] Ballou, J. E. 1960 Metabolism of Zn^{65} in the rat — chronic administration studies and age effects. General Electric Company, Hanford Laboratory, Richland, Washington (ref. H.W.-63047).

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Tissue Levels of Acetyl Choline and Acetyl Cholinesterase in Weanling Rats Subjected to Acute Choline Deficiency [1]

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ABSTRACT The concentrations of acetyl choline and acetyl cholinesterase were assayed in the brain, small intestine and kidneys of choline-deficient and supplemented weanling male Fischer rats. These measurements were made to explore our thesis that acute choline deficiency induces a deficiency in acetyl choline which makes the renal vasculature hyperresponsive to vasopressor amines, resulting in vasospasm, ischemia, vascular rupture, hemorrhage and renal tubular necrosis. We found that 5 days of choline deficiency resulted in a 30–35% decrease in the concentration of acetyl choline in the brain and small intestine and a 50–75% decrease in the kidney. The level of acetyl cholinesterase of the brain was unchanged; kidney acetyl cholinesterase was 18% lower in choline-deficient rats. These changes in tissue acetyl choline and acetyl cholinesterase levels occurred before there were changes in kidney weight and gross appearance or blood urea concentration. These experiments support our thesis that the nephropathy of acute choline deficiency results from vascular disturbances caused by a deficiency of acetyl choline.

Weanling rats fed diets deficient in choline develop fatty livers and bilateral renal cortical hemorrhagic necrosis within 8 days. The accumulation of liver fat is thought to be due in part to an interference with the mobilization of fats from the liver via the cytidine diphosphocholine pathway (1). Impairment of phospholipid formation (2) with decreased methylation of phosphatidyl ethanolamine has been observed (3, 4).

The basic mechanism leading to the characteristic renal pathology has not been established. Hartroft (5) observed in 1948 that the earliest histologic change seen in the kidneys of choline-deficient rats was the appearance of fat droplets in the tubular epithelial cells; subsequent tubular swelling appeared to compress the cortical capillary plexus resulting in tubular ischemia, necrosis and hemorrhage. Wolbach and Bessey (6) advanced the "cholinergic mechanism" theory in 1941 when they stated, "Although the mechanism underlying the hemorrhages is not yet clear, the function of acetyl choline as a neuromuscular mediator is suggestive of a neurovascular cause." This hypothesis was provisionally explored by Baxter (7) who abandoned this theory because of his inability to demonstrate clear differences in nephropathy when neostigmine, atropine, and dibenzyline were given to choline-deficient rats.

We set out to explore the possibility that choline deficiency leads to decreased levels of acetyl choline, vasospasm because of altered reactivity to catechol amines, and renal ischemia. To this end, as a first step, we measured the levels of acetyl choline and acetyl cholinesterase activity in the brain, intestine and kidney of normal and choline-deficient rats.

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METHODS AND MATERIALS

The diet used for the production of acute choline deficiency is detailed elsewhere (8); briefly, we modified the Salmon and Newberne diet (9) by the addition of 5% cholesterol, 0.4% cystine, certain B vitamins, menadione and inositol. The diets were steam autoclaved.

Male rats of the Fischer strain,[6] 21 days old, were used for all experiments. The rats were housed singly in stainless steel cages on a 2-mesh wire floor in a room where the temperature was controlled at 22° ± 1°.

On arrival, the rats were divided into 2 groups with similar weights, and immediately fed the experimental diet. Half the rats were given a supplement of choline chloride, 1.5 mg/ml in the drinking water. The animals were weighed 3 times each week, between 1 and 3 PM. Diet and supplement were changed at these times; the animals ate and drank ad libitum.

On the morning of day 6 of the experiment each rat was lightly anesthetized with ether and an abdominal incision was made. The small intestine was removed and transected at the midpoint; the proximal half of the small intestine was immediately flushed out with Ringer's solution containing 10^{-4} molar physostigmine by placing an 18-gauge needle in the lumen and gently forcing the solution through with a 5-ml syringe. The excess solution was removed by gentle massage, the segment weighed to 0.01 g, and placed in 5 ml fresh Ringer's solution with physostigmine in the cold room, 4°. The distal half of the small intestine was flushed out as above with Ringer's solution without physostigmine, weighed, and placed in 5 ml fresh Ringer's solution without physostigmine in the cold room. During this time, the brain and kidneys were removed by another person. The brain was bisected; after weighing each half of the brain to 0.01 g, the right side of the brain was placed in 5 ml Ringer's solution containing physostigmine in the cold room and the left half of the brain placed in 5 ml Ringer's solution without physostigmine in the cold room. The kidneys were then weighed to 0.01 g; the right kidney was placed in 5 ml Ringer's solution with physostigmine and the left kidney placed in 5 ml Ringer's solution without physostigmine, both in the cold room. The kidneys were then minced with scissors in their respective test tubes. All tissues were kept in the cold room for 1 hour. Identical procedures were carried out for rats given the choline supplement and the choline-deficient animals.

After 1 hour in the cold, the supernatant fluid from each tissue was decanted from the test tube, discarded, and the tissues quick-frozen by placing the test tube containing the tissue in a mixture of acetone and dry ice. The tissues were subsequently thawed, and ground in a motor-driven mortar and pestle (in an ice bath) in 2 ml of the same type of solution originally used at killing and subsequently diluted so that the concentration of acetyl choline ranged between 0.2 and 0.7 μg/ml.

The method of acetyl choline assay was adapted from that of Chang and Gaddum (10) as modified by Dettbarn and Rosenberg (11); the method to be described has a reproducibility of 95%. The assay involves the determination of the rate of contraction of the rectus abdominus muscle of the frog in solutions with known concentrations of acetyl choline and subsequent comparison of these with those produced by the same muscle (after washing and relaxation) when placed in solutions of unknowns. Similar measurements were made for the tissues placed in Ringer's solution without physostigmine. In each instance, the measurable activity in extracts without physostigmine was zero, indicating that all muscle contraction obtained from extracts of Ringer's solution with physostigmine were caused by acetyl choline or acetyl choline like substances, that is, substances destroyed by acetyl cholinesterase.

Statistical comparisons of the acetyl choline concentrations and cholinesterase concentrations were made between tissues from choline-supplemented and choline-deficient groups using Student's *t* test.

Assay of acetyl cholinesterase was carried out by the method of Hestrin (12); the method has a reproducibility of 97%. A suspension of brain or kidney in Ringer's solution (without physostigmine) was in-

[6] Purchased from the Charles River Breeding Laboratories, Wilmington, Massachusetts.

cubated with a known assayed concentration of acetyl choline. At given times during the incubation, samples of the mixture were removed and assayed chemically (12) for residual acetyl choline; this assay is based on the color formation of hydroxamic acid derivatives with ferric chloride. The rate of acetyl choline breakdown was taken as a reflection of the concentration of acetyl cholinesterase in the tissue suspension extract.

RESULTS

The concentrations of acetyl choline in the proximal half of the small intestine, in the brain and in the kidneys of choline deficient male weanling Fischer rats fed the modified Salmon and Newberne (9) diet for 5 to 6 days were markedly lower than those of rats fed the same diet but given supplements of choline chloride in their drinking water (table 1). The concentration of acetyl choline of the choline-deficient rats was decreased significantly in the small intestine (28–36%), the brain (32–36%) and in the kidneys (50–75%). These studies were made when the majority of the rats had near-normal blood urea nitrogen concentrations. The weights of the kidneys from the 2 groups of rats were not significantly different from one another (table 1).

The concentrations of acetyl cholinesterase (table 1) of the kidneys of choline-deficient rats were significantly lower (18%) than the concentrations of the enzyme in the kidneys of the control animals receiving the choline supplement. The levels of brain acetyl cholinesterase in the 2 groups of rats were not different. Measurements of intestinal cholinesterase were not made.

DISCUSSION

A neurovascular abnormality as a result of acute choline deficiency was postulated some 25 years ago by Wolbach and Bessey (6). Subsequently, several papers appeared which may be interpreted as favoring the vasospastic hypothesis for the development of renal damage of acute choline deficiency. In 1939, Griffith and Wade (13) reported the presence of intraocular hemorrhages in choline-deficient rats. These lesions were studied histologically by Burns and Hartroft (14) and Bellows and Chinn (15) and were thought to be due to hyaline artery vasospasm and rupture. Implication of a vascular mechanism is also suggested by the work of Engle and Salmon (16) who observed focal hemorrhages in the adrenal, lymph nodes, spleen and lungs in acute choline deficiency, and by the studies of Hartroft et al. (17) and Wilgram et al. (18) who found cardiac necrosis and aortic sclerosis during the third and fourth weeks of acute choline deficiency of young or adult rats. Recently, Baer and Hartroft (19) attempted to elucidate the basis for the intraocular hemorrhages by seeking other means of producing them, such as hemorrhagic shock, bilateral and unilateral nephrectomy, bilateral and unilateral adrenalectomy, various other surgical procedures, hypoxia, and hyperoxia. Only acute choline deficiency, unilateral adrenalectomy and bilateral nephrectomy resulted in the intraocular hemorrhages; the authors offered no unifying hypothesis to explain this. The cholinergic mechanism was explored provisionally by other investigators using atropine (5, 7, 20), dibenzyline and dibenamine (7) and renal decapsulation (5, 20, 21). Hartroft (5) and Baxter (7) were unable to protect rats from acute choline deficiency nephropathy with atropine, whereas Dessau and Oleson (20) showed that of 21 rats so treated, 10 suffered no renal lesion; we infer from the latter paper that the incidence of renal pathology in non-atropinized choline-deficient rats was close to 100%. The dose, frequency, and route of atropine injection were the same in the experiments of Hartroft and Dessau and Oleson. In Baxter's experiments, the atropine was fed rather than injected. Also, Hartroft was unable to prevent the renal lesion by previous renal decapsulation (unilateral and bilateral), but both Dessau and Oleson and Baxter with unilateral decapsulation were able to do so. In these apparently conflicting experiments, the time of decapsulation was the same. The diets used in these 3 investigations were different; Baxter's diet contained 19% lard and 20% casein; Dessau and Oleson's diet, 10% lard and 15% casein; and in the diet used by Hartroft, the protein was 3% casein and 10% beef fat. None of the diets

TABLE 1

Acetyl choline and acetyl cholinesterase in tissues from choline-deficient rats [1]

Exp. no.	Animal status (supplement)	Tissue	Acetyl choline-like material [2]		Blood urea nitrogen	Kidney wt	Acetyl cholinesterase	
				μg/g	*mg/100 ml*	*g*	*μmoles acetyl choline hydrolyzed/g tissue/hr*	
1 (7) [3]	Water	small intestine		0.89 ± 0.13	—	—		—
	Choline [4]	small intestine	$P < 0.025$	1.40 ± 0.17	—	—		—
	Water	brain		0.77 ± 0.05	—	—		—
	Choline	brain	$P < 0.01$	1.13 ± 0.08	—	—		—
2 (7)	Water	small intestine		1.09 ± 0.09				
	Choline	small intestine	$P < 0.05$	1.51 ± 0.17				
	Water	brain		0.66 ± 0.06	15(3)	0.31		328 ± 7
	Choline	brain	$P < 0.025$	0.97 ± 0.09	20(3)	0.29	ns	344 ± 26
	Water	kidney		0.08 ± 0.03				15.9 ± 1.2
	Choline	kidney	$P < 0.001$	0.27 ± 0.02			$P < 0.002$	19.5 ± 0.5
3 (6)	Water	brain		0.71 ± 0.04	—	—		277 ± 10
	Choline	brain	$P < 0.01$	1.11 ± 0.12	—	—	ns	291 ± 12
	Water	kidney		0.07 ± 0.04	22(6)	0.25		15.0 ± 1.3
	Choline	kidney	$P < 0.001$	0.28 ± 0.03	21(5)	0.26	ns	19.4 ± 2.6
4 (10)	Water	kidney		0.12 ± 0.03	28(10)	0.27		18.6 ± 1.2
	Choline	kidney	$P < 0.001$	0.23 ± 0.03	21(10)	0.24	$P < 0.05$	22.4 ± 1.4
2+3+4	Water	brain					ns	300 ± 16
	Choline	brain						315 ± 17
	Water	kidney					$P < 0.005$	17.0 ± 0.8
	Choline	kidney						20.8 ± 0.9

[1] Fischer rats, 21 days old (weanling male) at start of experimental diet, killed 6 days later.
[2] Data are presented as the mean ± SE.
[3] Numbers in parentheses indicate number of animals.
[4] Choline chloride, 1.5 mg/ml in drinking water.

contained vitamin B_{12}, added cholesterol nor cystine. The sex and ages of the rats were the same in all the experiments; whether there were strain differences could not be determined since the strain of rat used by Dessau and Oleson was not given in their paper.

Baxter also performed experiments to assess the importance of renal vasospasm and ischemia by the use of catecholamine blockers such as dibenzyline and dibenamine (blockers of α-adrenergic receptors). He found that dibenamine (0.8 μg/day, injected subcutaneously) protected 61% of the rats from developing the nephropathy of acute choline deficiency; untreated rats had a 96% incidence of renal pathology. Dibenzyline (0.2–0.4 mg/g diet) was ineffective. Among several other compounds tested by Baxter, formaldehyde was also found to be protective. Because of the dermal irritation produced by both dibenamine and formaldehyde, and the negative results with dibenzyline, Baxter attributed the protective effects of dibenamine and formaldehyde to an "alarm reaction" rather than catecholamine blockage. We offer an alternative interpretation of Baxter's findings, namely that the protective effect of dibenamine is due to its blocking effects on catecholamine-induced vasoactivity and that the protective effect of formaldehyde is due to a stimulating effect of formaldehyde on labile methyl group synthesis, the formaldehyde serving as a component of the choline synthesis (22, 23). The animals in Baxter's experiments received 12 mg of formaldehyde daily which in the presence of 27 mg methionine (that amount of methionine the animals ate as calculated by us from the estimated amount of food consumed and the concentration of casein in the diet) might lead to sufficient choline synthesis to obviate renal damage (13). The negative data obtained with dibenzyline by Baxter may be due to one or both of the following factors. The dose of dibenzyline fed these rats was 1 to 2 mg/day; the recommended dosage for rats of this size for α-adrenergic blockade to catecholamines is about 0.05 mg/day. At the doses Baxter used, acetyl choline activity may have been blocked as well (24). The second possibility underlying the ineffectiveness of dibenzyline in preventing the nephropathy may be the refractoriness that rats develop to dibenzyline as a catecholamine blocker. In experiments performed in our laboratory, we found that dibenzyline was not effective as a catecholamine blocker after 5 days, as measured by the blood pressure response of treated rats to the intravenous injection of epinephrine. During the first 5 days of the dibenzyline treatment, the rats were refractory to injections of epinephrine that would cause a 20 to 40 mm rise in blood pressure of normal rats; after 5 days of dibenzyline treatment, regardless of the time after administration of dibenzyline, a significant and equivalent rise in blood pressure to intravenous epinephrine was obtained in these treated rats as compared with nontreated rats. The dose of dibenzyline in our experiments was 0.02 mg/day. This dose was given 2 times daily by subcutaneous injection and sometimes twice as much was given twice each day after the fifth day without achieving any blockage to intravenous epinephrine.[7]

The data obtained in our experiments reported in this paper support the view that the mechanism underlying the acute nephropathy of choline deficiency reflects an acute deficiency in acetyl choline, which we believe leads to abnormal vascular reactivity to pressor amines. The level of acetyl choline of the intestine, brain and kidney were markedly reduced very soon after the start of the choline-deficient diet, at a time at which the blood urea nitrogen concentrations were almost normal. The most markedly affected tissue of those tested was the kidney, the organ most severely affected by the deficiency as judged histologically and in terms of early lethality of the choline deficiency.

Considering the possibility that such acetyl choline deficiency might reflect increased cholinesterase activity, we also assayed the kidney and brain for this enzymatic activity. Our results show that the level of acetyl cholinesterase in the brain is unaltered but that of the kidney is lowered by acute choline deficiency. The decrease of acetyl cholinesterase in the kidney, the organ showing the greatest deficiency in acetyl choline, may be a result of a compensatory mechanism.

[7] Unpublished observations, A. L. Nagler and S. M. Levenson.

The lack of effectiveness of atropine in preventing the nephropathy of acute choline deficiency found by Hartroft and Baxter is consistent with the hypothesis developed in the present work. The questionable positive effect of neostigmine described by Baxter is also consistent with this hypothesis. One would think that pilocarpine, which exerts its cholinergic action without the mediation of acetyl choline, would be more likely to improve renal function in choline-deficient rats, than a cholinergic compound such as neostigmine, which requires acetyl choline for its action. As mentioned earlier we do not know how to explain the effect of Dessau and Oleson's conflicting findings regarding the effects of atropine.

We conclude that the mechanism by which choline deficiency causes the acute nephropathy is a consequence of an acute acetyl choline-deficient state. The vascular system of choline-deficient rats is thereby more susceptible to vasoconstriction by catecholamines. This in turn leads to ischemic changes leading to necrosis and hemorrhage. Studies to be published elsewhere [8] were conducted in our laboratory to compare the microcirculation (mesoappendix) of choline-deficient and normal rats. We found that the terminal arteries supplying the microcirculatory system in the mesentery (innervated and under acetyl choline influence) of the choline-deficient rat are hypersensitive to epinephrine, a finding consistent with the view that choline deficiency leads to acetyl choline deficiency with subsequent vasospasm and ischemic damage.

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Metabolism of Proteins by Progeny of Underfed Mother Rats [1]

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ABSTRACT Fifty percent dietary restriction during gestation and lactation was reported previously to result in permanent growth-stunting of the progeny, even though they were offered an adequate diet ad libitum after weaning. Progeny from restricted mothers also showed reduced feed efficiency, low nitrogen balance and abnormal urinary nitrogen distribution. Nitrogen utilization has now been further studied by feeding the progeny from unrestricted and restricted mothers diets containing protein of different nutritive quality — egg albumin, Alpha-Protein and casein in successive periods. The restricted progeny utilized a good-quality protein as efficiently as the unrestricted progeny, for example, egg albumin, but they did not utilize a poorer quality protein such as soy protein or even casein. The specificity of abnormal urinary nitrogen distribution in progeny from restricted mothers was demonstrable after feeding the animals diets containing proteins of different quality and quantity, and also in a diet of low caloric level. Qualitative as well as quantitative analysis of urinary amino acids showed the restricted progeny excreted more free basic amino acids such as arginine, and significantly more total amino acids than the normal ones.

Maternal dietary restriction during mating, gestation and lactation (or during gestation and lactation only) results in permanent growth-stunting of the offspring. The body weight of progeny of restricted mothers is less than that of progeny of unrestricted mothers and remains so throughout the entire life span, even though both groups, after weaning, are fed a nutritionally adequate diet on an unlimited basis (1). The progeny of restricted mothers also have lower feed efficiency, more nitrogen wastage and abnormal urinary nitrogen distribution (2). In the work now reported we have extended these studies by comparing the nitrogen excretion and nitrogen retention by the progeny of unrestricted and restricted mothers when the offspring were fed diets containing proteins of different quality. We also report the urinary nitrogen distribution and the qualitative as well as quantitative analysis of urinary amino acid excretion in the 2 groups of rats.

EXPERIMENTAL

Preparation of animals. In all studies, rats from a colony of the original McCollum strain were used. The method of maternal dietary restriction has been described in detail previously (1). The following comparisons reported are between groups of rats born of mothers whose diets were not restricted and groups born of mothers whose diets were restricted. These two kinds of rats are hereafter described as UR and R, respectively, with further characterization as to sex, age, and other details as required.

Diets. 1. Stock diet: A commercial laboratory ration [2] was used throughout the study as the maternal diet. According to analyses by the manufacturer, this diet contained not less than 23.4% protein and 3.78% fat. Vitamins and minerals were present in amounts adequate to support reproduction and growth of offspring.

2. Formulated diets: (a) The standard formulated diet consisted of 72.0% sucrose, 20.0% protein, 4.0% corn oil,[3] 4.0% salt mixture,[4] 2.0 ml fat-soluble vitamin solution (table 1), and 10 ml water-soluble vitamin solution (table 1) per kg diet. The several diets contained casein, Alpha-

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[2] Purina Laboratory Rat Chow, Ralston Purina Company, Saint Louis.

[3] Mazola, Best Foods Division, Corn Products Company, New York.

[4] Hegsted Salt Mixture, General Biochemicals Inc., Chagrin Falls, Ohio; for composition see Hegsted et al., J. Biol. Chem., 138: 459, 1941.

TABLE 1
Composition of vitamin solutions

Fat-soluble vitamin solution	
α-Tocopherol, g	5
Percomorphum oil,[1] ml	50
Menadione, mg	456
Corn oil, to make 435 ml	

Water-soluble vitamin solution/liter of 50% ethyl alcohol	
	mg
Thiamine	200
Riboflavin	300
Pyridoxine	250
Folic acid	20
Vitamin B_{12} (cyanocobalamin)	5
Biotin	10
	g
Niacin	5
Inositol	10
Ca pantothenate	2
p-Aminobenzoic acid	25
Choline chloride	100

[1] Vitamin A, 3,000,000 USP units; and vitamin D, 425 USP units.

protein [5] or egg albumin, respectively, as the protein source. (b) A protein-free diet was prepared by replacing the protein component in (a) with sucrose. (c) The low caloric diet consisted of 20.0% casein, 1.0% corn oil, 7.8% sucrose and 67.2% nonnutritive fiber (cellulose). Vitamins and minerals were added as in the formulated diets. The replacement of sugar with cellulose reduced the caloric density from 3.04 kcal/g to 1.20 kcal/g.

Experimental design. There were three main studies. In each one, groups of male UR and R rats were fed ad libitum. In the first study, nitrogen balance alone was determined in animals fed the formulated diet (a). In the second study, in which there were 3 separate experiments, the urinary nitrogen distribution was examined after feeding the following diets: (a) casein-Alpha-Protein in sequence, (b) protein-free diet, and (c) low caloric diet. In the third study, urinary amino acids of R and UR animals were determined chromatographically.

1. Nitrogen-balance studies with protein of different nutritive quality. Groups of 6 UR and 6 R males, 18 months of age were first fed a weighed amount of the 20% egg albumin diet (usually 30 g daily for 16 days). Unconsumed and spilled food was collected and weighed and substracted from the total offered. Thereafter the animals were fed similarly the Alpha-Protein diet for another 16 days. Finally the casein diet was fed for 16 days. Changes in body weight, daily food intake, nitrogen excretion and nitrogen retention during the 3 periods were measured as described previously (2).

2. Specificity of abnormal urinary nitrogen distribution. To investigate whether the abnormal urinary nitrogen distribution previously observed in R progeny is diet-dependent or occurs regardless of diet, 18 UR and 18 R males were used. Six rats from each group were used for each of the 3 sets of diets: (a) dietary proteins of different quality, (b) the protein-free diet, or (c) the low caloric diet. All the diets were offered ad libitum. The distribution of the urinary nitrogen among urea, ammonia, creatinine and total amino acids was determined as previously described (2).

(a). Quality of dietary protein: Groups of 6 UR and 6 R males, 6 months of age, were fed diets of different protein quality for two 9-day periods, after having been acclimatized to metabolism cages for 8 days with the stock diet. At the end of the period, 24-hour urine samples were collected and analyzed to provide base-line data. In the first experimental period, these 6 UR and 6 R animals were divided into 2 equal subgroups. One was fed 20% casein diet (groups UR-I and R-I); and the second, 20% Alpha-Protein diet (groups UR-II and R-II) for 9 days. At the end of the period, 2 successive 24-hour urine samples were collected. During the second period, the UR-I and R-I animals were fed 20% Alpha-Protein diet, while the UR-II and R-II animals were fed the 20% casein diet. At the end of this second 9-day period, urine samples were again collected for 2 days. Urinary nitrogen components were determined in all samples.

(b). Protein-free diet: Two groups of 2.5-month-old UR and R males, six per group, were fed the casein diet for 8 days. Thereafter the animals were fed the protein-free diet for 20 days. At the end of each period, 24-hour urine samples were

[5] Alpha-Protein (industrial protein derived from soybean), Central Soya, Chicago.

collected and urinary nitrogen components determined.

(c.) Low caloric intake: Groups of 7-month-old UR and R males were fed the casein diet for 8 days followed by the low caloric diet (1.20 kcal/g) for another 8 days. At the end of each period, 24-hour urine samples were collected and urinary nitrogen components determined.

3. *Chromatographic analysis of urinary amino acids.* To determine individual amino acids in the urine of UR and R progeny fed the stock diet ad libitum, 6 males from each group, 20 months of age, were used. Each animal was placed in a metabolic cage and fed stock diet ad libitum for about one week. Then 48-hour urine samples were collected under toluene. The total volume, including cage washings, was made up to 50 ml with distilled water. Forty-milliliter aliquots were concentrated to 10 ml in a Rotary evaporator. The urine samples were hydrolyzed with 6 N hydrochloric acid at 95° for 15 to 17 hours. Free and total amino acids were determined with an amino acid analyzer (Phoenix Model K-8000) (3,4).

RESULTS

1. Nitrogen balance studies with protein of different nutritive quality. The results of the nitrogen balance studies are summarized in table 2. The mean daily food intake if calculated per unit body weight of the R animals, was as much as or more than that of the UR animals and both groups were in positive balance during all 3 periods.

During the egg albumin feeding period there were no significant differences in urinary nitrogen excretion, nitrogen balance or the ratio of urinary nitrogen to absorbed nitrogen between the 2 groups of animals. The R animals excreted more fecal nitrogen. Following the change from stock diet to the formulated egg albumin diet, the UR animals, in the first week lost an average of 4 g in body weight; the R group lost 15 g despite ad libitum feeding. After the animals had received the egg albumin diet for 16 days, the UR animals showed a gain of 7 g while the R animals gained only 1 g.

After transfer to the Alpha-Protein diet both groups excreted about twice as much

TABLE 2

Nitrogen balance studies with proteins of different nutritive quality

Group [1]	Period	Diet [2]	Body wt	Food intake	Nitrogen				Urinary N/absorbed N
					Intake	output Fecal	Urinary output	Retention	
			g	*g/day*	*mg N/kg body wt/day*				
UR	1	20% egg albumin	507	16.6	822	45	299	477 ± 38 [3]	0.383 ± 0.016
R	1	20% egg albumin	425	14.9	882	51	322	510 ± 24	0.387 ± 0.010
UR	2	20% Alpha-Protein	506	17.9	1003	60	600	343 ± 28	0.636 ± 0.028
R	2	20% Alpha-Protein	424	15.2	992	69	649	* 274 ± 11	** 0.704 ± 0.008
UR	3	20% casein	517	16.8	931	54	504	374 ± 13	0.575 ± 0.010
R	3	20% casein	433	15.4	1007	62	616	* 328 ± 12	** 0.652 ± 0.035

[1] UR indicates unrestricted; R, restricted; each group consisted of 6 males; in periods 1 and 2 the animals were 18 months old; in period 3, 19 months of age.
[2] Nitrogen content of diet: (in milligrams N/gram diet) 20% egg albumin, 25.2; Alpha-Protein (an industrial protein derived from soybean, Central Soya, Chicago), 27.7; and 20% casein, 28.3.
[3] Mean ± SE of mean.
* $P < 0.05$.
** $P < 0.01$.

cept that some basic amino acids (for example, arginine) were noted in the R group. After hydrolysis the amino acid content increased about 2.8-fold in the UR group and about 3.6-fold in the R group; the total amino acids in the R group were significantly higher, thus confirming the results of earlier studies using a chemical method for the determination of total amino acids (2). Although some histidine and arginine were excreted by UR animals, R animals excreted much greater quantities of them.

DISCUSSION

The observed growth-stunting of progeny from mothers maintained with restricted intakes during pregnancy and lactation has been considered a result of low efficiency of feed utilization in these animals. The food intake per unit body weight is consistently higher in R animals, the feed efficiency is reduced and the nitrogen balance significantly and considerably poorer at each age (2). The results failed to indicate any important impairment in digestion or absorption in R animals; therefore, poor utilization or abnormal protein metabolism becomes a plausible explanation for the observed growth stunting. This report presents further studies of nitrogen utilization when UR and R animals were fed diets containing protein with different nutritive qualities—egg albumin, Alpha-Protein, and casein in successive periods. R animals can utilize high nutritive value protein (egg albumin) as efficiently as UR animals; no significant differences were noted in urinary nitrogen excretion, nitrogen balance or the ratio of urinary nitrogen to absorbed nitrogen. However, when a poor-quality protein diet, such as the Alpha-Protein, was fed, R animals had more nitrogen wastage and therefore retained less nitrogen than UR animals. Alpha-Protein has been shown to have a relative deficiency of the essential amino acid, methionine. Perhaps R animals require more of this amino acid than UR animals. Therefore more nitrogen wastage is observed in R animals when a diet with this poor-quality protein is fed. When animals previously fed Alpha-Protein diets are given a casein diet, R animals continue to have higher urinary nitrogen excretion and lower nitrogen retention. All these results demonstrate low efficiency or poor nitrogen utilization by R animals.

Anomalous protein utilization is further suggested by the excretion of a larger proportion of ammonia, amino acid and urea nitrogen by R animals. Perhaps the higher ammonia excretion is not due to an impairment of acid-base balance, but reflects a higher level of circulating amino acids. Two possible causes of higher urinary amino acid excretion may be that 1) R animals have normal ability to digest protein but are unable to utilize the resulting amino acids fully for protein synthesis, or 2) their protein catabolism may be increased, because they maintain a high level of urinary excretion of amino acids even when fed a protein-free diet. The higher urinary urea of R animals may be a reflection of the greater food intake in this group. Urinary nitrogen distribution was investigated by feeding both R and UR animals diets containing proteins of different quality and quantity and also a diet of low caloric level. The consistent finding of significantly higher excretion of total amino acids and urea in R animals along with essentially constant creatinine excretion whether the diets were protein-free, contained protein, or were of low caloric content, shows that the abnormal urinary nitrogen partition is specifically due to the effect of maternal dietary restriction during gestation and lactation and not due to these dietary differences. Increased ammonia excretion by R animals was found in the casein, Alpha-Protein and low caloric diet groups. The excretion of ammonia is affected by the quantity of dietary protein. When the protein-free diet was fed, both groups of animals excreted the same lowered amounts of urinary ammonia.

The urinary excretion of amino acids was further investigated by qualitative as well as quantitative amino acid analysis. The findings confirm the existence of an aminoaciduria in R animals. It is still unknown whether the cause or nature of this aminoaciduria is some defect in one or more enzyme systems involved in amino acid metabolism or a secondary manifestation of some pathological condition, perhaps of the liver or kidneys. However,

it has been reported that in cystinuria, the reabsorption defect is confined to 4 amino acids—cystine, lysine, arginine and ornithine (5). Since higher excretion of cystine and arginine in the nonhydrolyzed urine from R animals was observed, there is some indication of the possibility of a disorder in the metabolism of cysteine-cystine and also abnormal tubular reabsorption as the cause of aminoaciduria. In any event the qualitative as well as quantitative differences in urinary amino acid excretion of R animals provide further evidence for some derangement in protein metabolism in these animals.

Finally it is important to note that as in previous experiments (2), UR and R progeny differ in their excretion of nitrogen in forms other than urea, ammonia, creatinine or amino acid; in every case these components account for less of the total in UR progeny. Investigation of the other urinary nitrogen components may well provide further clues to the nature of the derangement in protein metabolism sustained from congenital undernutrition.

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Effect of Protein Intake on the Excretion of Quinolinic Acid and Niacin Metabolites by Men during Vitamin B_6 Depletion [1]

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ABSTRACT The effect of the level of dietary protein on the conversion of tryptophan to niacin by man was studied. The urinary excretion of quinolinic acid, nicotinic acid, N^1-methylnicotinamide and N-methyl-2-pyridine-5-carboxamide was determined before and after tryptophan loading both when subjects were adequately nourished with vitamin B_6 and when they were depleted of the vitamin. The amount of quinolinic acid excreted in response to the tryptophan load dose was affected by the level of protein in the diet during vitamin B_6 depletion. Post-tryptophan quinolinic acid excretion of subjects fed a diet containing 150 g of protein was significantly elevated after 6 days of vitamin B_6 deprivation and the amount excreted continued to increase as the length of deficiency increased. Men fed the diet containing 54 g protein did not excrete significantly increased amounts of quinolinic acid in response to tryptophan loading until after 40 days of vitamin deprivation. The amount of nicotinic acid, N^1-methylnicotinamide and N-methyl-2-pyridone-5-carboxamide excreted in response to the tryptophan load dose was not affected by either the vitamin B_6 intake or the level of dietary protein.

Studies concerning the effect of a vitamin B_6 deficiency on the amounts of N^1-methylnicotinamide (1–7), nicotinic acid (1, 4, 6) and quinolinic acid (5, 7) excreted by rats have given conflicting results. The amount of quinolinic acid excreted by man in response to tryptophan loading was significantly increased during vitamin B_6 deficiency, but the amounts of nicotinic acid, N-methylnicotinamide and N-methyl-2-pyridone-5-carboxamide were not affected by the deficiency (8). In a previous paper from this laboratory (9) it was reported that men fed 150 g of protein daily developed abnormal tryptophan metabolism more rapidly than those fed 54 g of protein when vitamin B_6 was withdrawn from the diet; and Baker et al. (10) reported that the amounts of hydroxykynurenine and xanthurenic acid excreted by men deficient in vitamin B_6 were related to protein content of the diet.

In the present paper are reported the results of the effect of a vitamin B_6 deficiency in man as related to the level of protein intake on the urinary excretion of quinolinic acid, N^1-methylnicotinamide, nicotinic acid and N-methyl-2-pyridone-5-carboxamide.

EXPERIMENTAL

Men ranging in age from 18 to 31 years, in height from 168 to 191 cm and in weight from 62.7 to 72.9 kg participated in two human metabolic studies. The subjects have been described previously (9). The subjects of study 1 received 54 g of protein daily and the subjects of study 2, 150 g. The experimental diets contained approximately 0.16 mg of vitamin B_6 per day. A mixture of animal and vegetable fats contributed 40% of the total caloric intake. Protein supplied 7 and 19% of the respective caloric values of the low and high protein diets, whereas carbohydrate supplied 53 and 41%. Complete details concerning the composition of the diets and of the methods of preparation have been given previously (9, 11).

During study 1, five young men were fed the experimental diet low in protein supplemented with 1.5 mg of pyridoxine per day for 6 days; then the diet without

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vitamin B_6 supplementation was fed to two of these subjects for 33 days and to the other three for 40 days. The subjects deprived of vitamin B_6 for 40 days were then supplemented with 0.6 mg of pyridoxine daily for 7 days. The 6 men who participated in study 2 were fed the experimental diet high in protein supplemented with 1.5 mg of pyridoxine per day for the first 18 days. Then the supplement was withdrawn and the subjects were deprived of vitamin B_6 for 16 days. Following the period of depletion the subjects were given the pyridoxine supplement, 0.6 mg daily for 16 days and then 50 mg daily for the two final days.

Subjects were given oral doses of 2.0 g of L-tryptophan periodically during the predepletion periods, the depletion periods and postdepletion periods. Urine samples collected during the 24 hours before (basal) and after (post-tryptophan) tryptophan loading were assayed for quinolinic acid and niacin metabolites. The analyses were made by the following methods: quinolinic acid (12), nicotinic acid (13), N^1-methylnicotinamide (14) and N-methyl-2-pyridone-5-carboxamide (15).

RESULTS

The data presented in table 1 show the average amounts of nicotinic acid, N^1-methylnicotinamide, N-methyl-2-pyridone-5-carboxamide and quinolinic acid excreted within the 24 hours before and after tryptophan loading by the subjects of both studies. The basal and the post-tryptophan amounts of nicotinic acid, N^1-methylnicotinamide and N-methyl-2-pyridone-5-carboxamide excreted by the subjects could be related neither to the protein intake nor to the vitamin B_6 intake.

The basal amounts of urinary quinolinic acid appeared to be related to the protein intake but not to the vitamin B_6 intake. The average daily basal excretion of quinolinic acid by subjects fed the diet containing 54 g of protein varied among the periods from 27 to 36 μmoles, whereas that of subjects fed the high protein varied from 39 to 43 μmoles (table 1).

The yield of quinolinic acid from a 2-g loading dose of L-tryptophan by subjects fed the low protein diet is shown in figure 1; that by subjects fed the high protein is shown in figure 2. When the subjects were given 1.66 mg vitamin B_6 daily, the yield

TABLE 1

Effect of vitamin B_6 depletion on the average urinary excretion of quinolinic acid and niacin metabolites by men

Dietary vitamin B_6	Period	Length of specified vitamin B_6 intake	Quinolinic acid	Nicotinic acid	N^1-methylnicotinamide	N-methyl-2-pyridone-5-carboxamide
mg		*days*		*μmoles/24 hours*		
			Study 1			
1.66	Pre-tryptophan	1	27 ± 4.8 [1]	3.9 ± 0.6	35 ± 5.0	73 ± 21.4
	Post-tryptophan		53 ± 13.3	4.2 ± 0.7	54 ± 10.0	93 ± 47.0
0.16	Pre-tryptophan	12	30 ± 9.3			
	Post-tryptophan		63 ± 15.6			
0.16	Pre-tryptophan	39	36 ± 5.0	5.2 ± 1.1	21 ± 9.0	57 ± 11.2
	Post-tryptophan		98 ± 34.3	4.4 ± 0.9	30 ± 2.0	75 ± 35.4
0.76	Pre-tryptophan	5	34 ± 10.8	4.2 ± 0.5	24 ± 10.5	58 ± 20.4
	Post-tryptophan		70 ± 18.4	4.3 ± 0.7	34 ± 0.5	58 ± 25.5
			Study 2			
1.66	Pre-tryptophan	7	43 ± 3.5	4.9 ± 0.7	33 ± 5.9	53 ± 7.8
	Post-tryptophan		68 ± 10.2	5.3 ± 0.8	39 ± 7.9	96 ± 20
0.16	Pre-tryptophan	13	39 ± 6.6	5.5 ± 0.9	25 ± 4.4	72 ± 20.7
	Post-tryptophan		103 ± 21.2	5.1 ± 0.8	45 ± 8.0	104 ± 13.2
0.76	Pre-tryptophan	12	41 ± 5.5	5.4 ± 0.7	23 ± 7.6	70 ± 22.4
	Post-tryptophan		89 ± 12.6	5.8 ± 0.7	35 ± 14.6	101 ± 26.6

[1] SD.

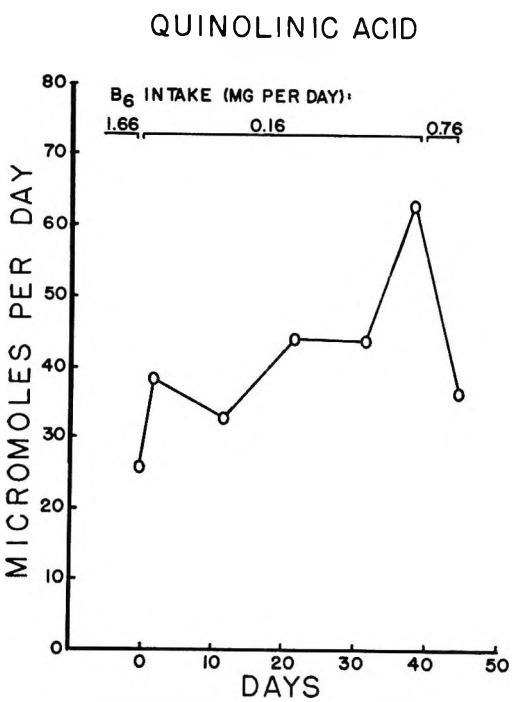

Fig. 1 Effect of vitamin B_6 depletion on the yield of quinolinic acid from a 2-g load of L-tryptophan by subjects fed the low protein diet. Yield = post-tryptophan minus pre-tryptophan values. Values given are the averages for all subjects. Day zero indicates the last day the 1.5-mg supplement of pyridoxine was given.

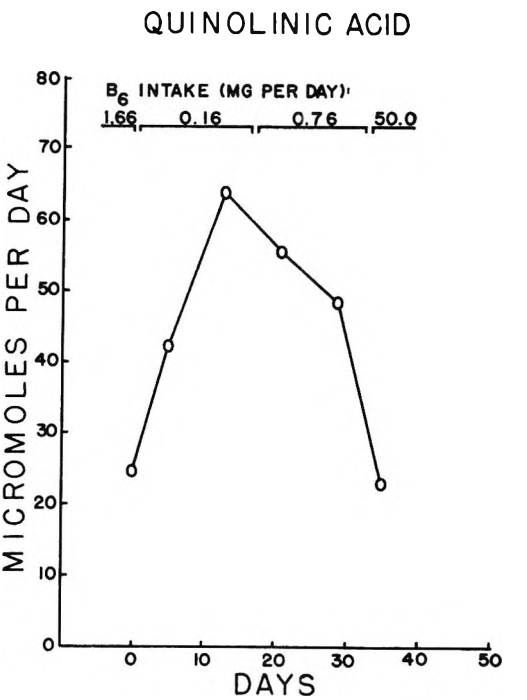

Fig. 2 Effect of vitamin B_6 depletion on the yield of quinolinic acid from a 2-g load of L-tryptophan by subjects fed the high protein diet. Yield = post-tryptophan minus pre-tryptophan values. Values given are the averages for all subjects. Day zero indicates the last day the 1.5-mg supplement of pyridoxine was given.

of quinolinic acid from the tryptophan dose was not affected by the protein intake and averaged about 25 μmoles within the 24 hours following loading. The yield of quinolinic acid increased when the subjects were fed the vitamin B_6-deficient diets, and the increase occurred much earlier for subjects fed the high protein diet (figs. 1 and 2). A significant increase in the yield of quinolinic acid from the tryptophan load by subjects fed the low protein diet did not occur until after 40 days of vitamin B_6 deprivation (fig. 1). However, subjects fed the high protein diet excreted 44 μmoles of quinolinic acid in response to tryptophan loading after 6 days of vitamin deprivation as compared with predepletion values of 25 μmoles. The difference was statistically significant ($P < 0.01$). The yield of quinolinic acid continued to increase as the period of vitamin deprivation increased, and after 14 days of depletion, subjects fed the high protein diet excreted an average of 63 μmoles within the 24 hours after the tryptophan load dose was administered, a highly significant increase ($P < 0.005$).

After the diets had been supplemented with 0.6 mg of pyridoxine for 5 days, all subjects fed the low protein diet responded normally to the tryptophan load dose. The average quinolinic acid yield was 36 μmoles per day. Subjects fed the high protein diet excreted an average of 57 and 48 μmoles in response to the tryptophan load after their diets had been supplemented with 0.6 mg of pyridoxine for 5 and 13 days, respectively. After 50 mg of pyridoxine were given for 2 consecutive days the post-tryptophan quinolinic acid excretion returned to predepletion levels.

DISCUSSION

The results reported here are in accord with those of Brown et al. (8) who reported that man excreted increased

amounts of quinolinic acid in response to tryptophan loading during vitamin B_6 deficiency. The results of the present studies along with those of Brown and co-workers indicate that the level of dietary protein affects the amount of quinolinic acid excreted during vitamin B_6 deficiency. The yield of quinolinic acid from the 2 g of L-tryptophan by the subjects of Brown et al. (8) averaged 112 μmoles within the 24 hours following loading; these subjects were deprived of vitamin B_6 for periods of time which ranged among them from 27 to 40 days, and they were given 100 g of protein daily. Subjects of the present studies who were fed 54 g of protein did not excrete abnormally high amounts of quinolinic acid in response to tryptophan loading until after 40 days of vitamin B_6 deprivation, whereas those fed 150 g of protein excreted elevated amounts of quinolinic acid after 6 days of deprivation following loading with tryptophan. The amounts of quinolinic acid excreted by the subjects of the present studies were never as great as those reported by Brown and co-workers; however, the data reported here indicate that the metabolic abnormality in quinolinic acid became more severe as the vitamin B_6 deficiency progressed. Had the subjects of the present studies been deprived of vitamin B_6 for longer periods of time, the amount of urinary quinolinic acid would probably have been increased.

In the tryptophan-niacin pathway 3-hydroxykynurenine is cleaved by a kynureninase to form 3-hydroxyanthranilic acid which in turn is converted to quinolinic acid, or the 3-hydroxykynurenine is metabolized to xanthurenic acid by a transaminase; both enzymes utilize vitamin B_6 as a coenzyme. Thus the increased excretion of quinolinic acid in response to tryptophan loading during vitamin B_6 deficiency was somewhat unexpected and is similar in situation to the elevation in urinary xanthurenic acid observed during vitamin B_6 deficiency. Ogasawara et al. (16) attempted to elucidate the phenomenon of elevated xanthurenic acid excretion. These authors reported that in vitamin B_6-deficient animals kynureninase and the supernatant kynurenine-transaminase showed markedly decreased activities, whereas the mitochondrial kynurenine-transaminase remained active; they believed it possible to explain the mechanism of increased xanthurenic acid excretion in the following way. During vitamin B_6 deficiency the conversion of kynurenine to anthranilic acid is markedly decreased, resulting in an accumulation of 3-hydroxykynurenine. Because of the low activity of kynureninase, 3-hydroxykynurenine is not rapidly metabolized to 3-hydroxyanthranilic, leading to the formation of a large amount of xanthurenic acid by the mitochondrial kynurenine-transaminase. It is not easy to reconcile this explanation with the fact that quinolinic acid is also increased in the urine during vitamin B_6 deficiency. It is true, however, that the percentage of the tryptophan loading dose which is excreted as quinolinic acid is much less than that excreted as xanthurenic acid. Men fed 150 g of protein daily who were deprived of vitamin B_6 for 14 days excreted 0.6% of the 2-g load of L-tryptophan as quinolinic acid and 6.0% as xanthurenic acid.

The elevation in the amount of quinolinic acid excreted during vitamin B_6 deficiency by the subjects reported by Brown et al. (8) led the authors to suggest that vitamin B_6 coenzymes might be necessary for the further metabolism of quinolinic acid.

These results agree with those of Brown et al. (8) who reported that vitamin B_6 depletion in adult man did not significantly affect the amount of niacin and niacin metabolites excreted. However, Snyderman et al. (17) reported that infants deprived of vitamin B_6 for 60 days lost the ability to convert tryptophan to niacin. It seems safe to assume that infants deprived of vitamin B_6 for 60 days would be more deficient in vitamin B_6 than adult men deprived of the vitamin for 16 days.

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Utilization of Biuret as a Source of Nonspecific Nitrogen in a Crystalline Amino Acid Chick Diet

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ABSTRACT Three experiments were conducted to study the effect of adding biuret to crystalline amino acid diets as a source of nonspecific nitrogen. Growth and feed consumption were measured for chicks for the experimental period, 8 to 14 days of age. The addition of 2.33% biuret to a basal diet deficient in nonspecific nitrogen depressed growth, whereas addition of isonitrogenous amounts of urea or glutamic acid resulted in increased growth. Proportionate decreases in growth and feed consumption resulted from increased levels of biuret in a diet containing adequate amino acid nitrogen. Addition of 1.165 and 2.33% biuret to a diet containing 70% of the required amount of amino acid nitrogen resulted in increased feed consumption; however, successive decreases in growth and feed intake were observed with increases of biuret from 1.165 to 4.66% in the diet containing 100% of the required amount of amino acid nitrogen. When feed intake was equalized, 4.66% biuret had no effect on growth. These studies demonstrated that biuret did not serve as a source of nonspecific nitrogen and that the effect of biuret on growth was due to its effect on feed consumption.

Previous reports (1, 2) have indicated no beneficial effect from adding biuret as supplemental nitrogen to chick diets. Similarly Kriss and Marcy (3) observed that urea was not utilized as supplemental nitrogen. Others (4, 5) have reported depression of growth rate when urea was added to diets adequate in protein. In contrast, Featherston et al. (6) observed that addition of urea to a chick diet containing only indispensable amino acids resulted in growth equal to that observed when a mixture of nonessential amino acids was added.

The purpose of the study reported here was to determine whether biuret, like urea, could serve as a source of nonspecific nitrogen for a crystalline amino acid diet deficient in nonspecific nitrogen.

MATERIALS AND METHODS

Male chicks originating from the mating of New Hampshire males and Columbian females were used. A standard procedure (7) of handling chicks was followed during the pretest and test periods. Chicks were fed a corn-soybean meal diet for the first 7 days of life. To reduce variability in initial weight because of intestinal fill, chicks were subjected to a routine of fasting, refeeding and fasting on the sixth day of age. On the seventh day, chicks were selected for uniformity of weight, fed a semipurified isolated soybean protein diet to accustom them to purified diets and fasted overnight. Chicks were weighed and allotted to treatments on the morning of the eighth day. Water was supplied ad libitum in all experiments. Each treatment included 3 groups of 10 chicks each. Weight gain and feed consumption were measured for the period of 8 to 14 days of age. The data were analyzed statistically by the method of least squares. Additions to the basal diet were made at the expense of cornstarch.

Experiment 1. This experiment was designed to compare biuret, urea and glutamic acid as sources of nonspecific nitrogen. The treatments consisted of 1) basal (reference diet minus 10% glutamic acid, table 1), 2) basal plus 10% glutamic acid, 3) basal plus 2.33% pure biuret and 4) basal plus 2.03% urea. Treatments 2, 3 and 4 were isonitrogenous.

Experiment 2. A 2 × 4 factorial design consisting of 2 levels of the amino acid mixture (table 2) and 4 levels of pure biuret was used. Treatments 1–4 and 5–8 contained 70 and 100%, respectively, of the amino acid mixture. Biuret levels consisted of 0, 1.165, 2.33 and 4.66% of the diet.

Experiment 3. The third experiment studied the effect of biuret on gain when

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TABLE 1

Composition of basal diet

	% of diet
Cornstarch	56.15
Amino acid mixture [1]	19.28
Corn oil, refined	15.00
Salt mixture [2]	5.37
Cellulose [3]	3.00
$NaHCO_3$	1.00
Choline chloride	0.20
Vitamins [4]	+
α-Tocopheryl acetate (20 mg/kg diet)	+
Antioxidant [5] (125 mg/kg diet)	+
Total	100.00

[1] See table 2 for composition.
[2] Mineral mixture as percentage of the total diet: NaCl, 0.88; $ZnCO_3$, 0.01; $CuSO_4 \cdot 5H_2O$, 0.002; H_3BO_3, 0.0009; $COSO_4 \cdot 7H_2O$, 0.0001; $Na_2MoO_4 \cdot 2H_2O$, 0.0009; KI, 0.0004; ferric citrate, 0.05; $MgSO_4 \cdot 7H_2O$, 0.35; K_2HPO_4, 0.9; $MnSO_4 \cdot H_2O$, 0.065; $CaCO_3$, 0.3; $Ca_3(PO_4)_2$, 2.8; total, 5.3629.
[3] Solka Floc (BW40), Brown Company, Chicago 60603.
[4] Klain, G. J., H. M. Scott and B. C. Johnson 1960 Poultry Sci., 39: 39 (abstract).
[5] Santoquin (6-ethoxy,1,2,dihydro,2,2,4-trimethyl-quinoline), Monsanto Company, St. Louis.

TABLE 2

Composition of crystalline amino acid mixture [1,2]

	% of diet
Arginine·HCl	1.21
Histidine·HCl·H_2O	0.41
Lysine·HCl	1.19
Tyrosine	0.45
Tryptophan	0.15
Phenylalanine	0.50
Methionine	0.35
Cystine	0.35
Threonine	0.65
Leucine	1.20
Isoleucine	0.60
Valine	0.82
Glycine	1.20
Proline	0.20
Glutamic acid	10.00
Total	19.28

[1] Only the L-isomer used.
[2] Zimmerman, R. A., and H. M. Scott. J. Nutr., 87: 13, 1965. Same as mixture B except that L-methionine replaced DL-methionine and L-isoleucine was reduced from 0.80 to 0.60% of the diet.

feed consumption was equalized. The treatments consisted of the following: 1) basal diet (table 1) fed ad libitum, 2) basal diet with feed consumption restricted to consumption of treatment 3 and 3) basal diet plus 4.66% biuret fed ad libitum.

RESULTS AND DISCUSSION

Experiment 1. This experiment was designed to determine whether biuret, like urea, is a useful source of nonspecific nitrogen for a crystalline amino acid diet deficient in nonspecific nitrogen. Results (tables 3 and 4) showed that chicks fed the basal diet supplemented with biuret gained significantly ($P < 0.05$) less than chicks fed only the basal diet. They also consumed significantly ($P < 0.05$) less feed. Iwata et al. (1) and Berry et al. (2) reported that chickens did not utilize biuret when added to less well-defined diets. They observed no harmful effects with biuret.

Gains, feed intake and efficiency of gain were significantly greater ($P < 0.01$) when urea was added to the basal diet. Performance for the urea treatment was intermediate between the basal and glutamic acid treatments and agrees with results of Featherston et al. (6) and Sullivan and Bird (8).

Experiment 2. Results of experiment 1 indicated some detrimental effect of biuret; thus, the effect of increasing levels of biuret was studied in the second experiment. Significant linear decreases of gain, feed consumption and efficiency of gain ($P < 0.01$) were observed for increasing levels of biuret (tables 4 and 5). Level of amino acid mixture significantly affected performance as the 70% level had been shown previously to be inadequate for maximal growth.

TABLE 3

Effect of feeding chicks pure biuret, urea or glutamic acid as sources of nonspecific nitrogen for a crystalline amino acid diet (exp. 1)

	Dietary treatment	Gain/ chick/ day [1]	Avg feed consumed/ chick	Gain/ feed
		g	g	
1	Basal [2] – 10% glutamic acid	3.3	64.6	0.302
2	1) + 10% glutamic acid	12.0	111.9	0.643
3	1) + 2.33% biuret	2.5	55.6	0.265
4	1) + 2.03% urea	5.8	86.7	0.399

[1] Average of triplicate groups of 10 chicks/treatment for the period of 8 to 14 days of age.
[2] See table 1 for composition.

TABLE 4

Analysis of variance of weight gain, feed consumed and gain-to-feed ratio

Source	df	Mean square		
		Gain	Feed consumed	Gain/feed
		Experiment 1		
Total	11			
Treatment [1]	3	201780.3	189680.7	0.08719
Basal vs. biuret	1	3504.2 *	12150.0 *	0.00212 **
Biuret vs. urea	1	59600.7 **	145081.5 **	0.02745 **
Glutamic acid vs. $\frac{\text{biuret} + \text{urea}}{2}$	1	447458.0 **	332656.1 **	0.19385 **
Error	8	350.5	1328.1	0.00010
		Experiment 2		
Total	23			
Treatment [1]	7	33775.1	9489.6	0.01951
1. Biuret linear	1	45511.3 **	32339.2 **	0.01183 **
2. Biuret quadratic	1	1126.0	7796.4 *	0.00000
3. Biuret cubic	1	2359.9 *	2728.7	0.00041
4. Amino acid level	1	180787.0 **	7141.5 *	0.02406 **
5. 1 × 4	1	1990.3 *	1860.1	0.00003
6. 2 × 4	1	3577.0 *	13076.4 **	0.00002
7. 3 × 4	1	1074.2	1485.0	0.00019
Error	16	412.3	986.0	0.00017
		Experiment 3		
Total	8			
Treatment [1]	2	28506.4	11664.0	0.00866
Basal vs. $\frac{\text{basal restricted} + \text{biuret}}{2}$	1	56784.5 **	23328.0 **	0.01714 **
Basal restricted vs. biuret	1	228.2	0	0.00018
Error	6	1837.6	677.0	0.00492

[1] Triplicate groups of 10 chicks/treatment.
** $P < 0.01$.
* $P < 0.05$.

TABLE 5

Effect of level of amino acid mixture and level of pure biuret on chick gain, feed consumption and efficiency of gain (exp. 2)

Dietary treatment		Gain/chick/day [2]	Avg feed/chick	Gain/feed
Amino acid mixture [1]	Pure biuret			
%	% of diet	g	g	
70	0	8.3	103.8	0.478
70	1.165	9.0	113.2	0.479
70	2.33	8.1	109.0	0.446
70	4.66	7.0	99.0	0.426
Avg		8.1	106.3	0.457
100	0	12.1	115.5	0.630
100	1.165	11.5	112.1	0.614
100	2.33	10.7	107.9	0.594
100	4.66	9.8	103.3	0.567
Avg		11.0	109.7	0.601

[1] See table 2 for composition; percentage of amino acid mixture in basal diet (table 1).
[2] Average of triplicate groups of 10 chicks/treatment for the period of 8 to 14 days of age.

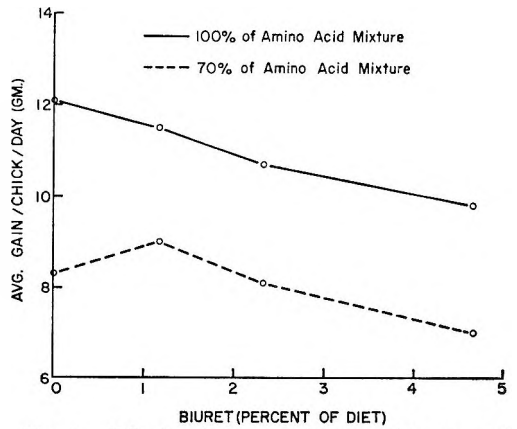

Fig. 1 Effect of level of pure biuret and amount of amino acid nitrogen on chick gain.

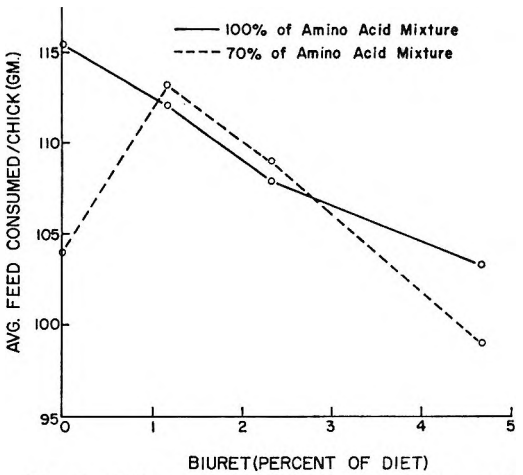

Fig. 2 Effect of level of pure biuret and amount of amino acid on feed consumption.

Response to increasing levels of biuret as affected by level of amino acid nitrogen is shown in figures 1 and 2. Gain and feed consumption for chicks fed the 100% level of amino acid mixture decreased as level of biuret increased. In contrast, gain and feed consumption of chicks fed the 70% level of amino acid mixture increased with the 1.165% level of biuret compared with that for no biuret. Additional increases in the level of biuret to 2.33 and 4.66% caused successive decreases in gain and feed consumption. There was essentially no difference in gain for chicks fed the zero or 2.33% level of biuret. Feed intake was greater for chicks fed 2.33% biuret than for chicks fed the basal diet.

The results demonstrate that addition of biuret to a diet adequate in amino acid nitrogen depresses performance of chicks. This was similar to the effect of urea reported by Finlayson and Baumann (4), Fried and DaSilva (9) and Matsumoto (5). Significant interactions observed for amino acid level and biuret (exp. 2, table 4) were associated primarily with the lower level of amino acid mixture (figs. 1 and 2).

In contrast with results of experiment 1 which indicated that biuret was not utilized when added to a diet deficient in nonspecific nitrogen, results of this experiment suggest that addition of lower levels of biuret to a diet containing the 70% level of a balanced amino acid mixture resulted in increased feed intake over that of chicks fed the basal diet. It appears likely that the increase in gain for the treatment with 1.165% biuret was a result of the increased feed intake. The effect of biuret at the lower levels may have been to dilute the energy content of the diet deficient in nitrogen, thus resulting in more feed intake.

Experiment 3. Experiments 1 and 2 suggested that the effect of biuret on gain was a result of altered feed intake. Results of experiment 3 (tables 4 and 6) indicate that 4.66% biuret in the diet did not significantly affect weight gain or efficiency of gain when feed intake of the basal diet

TABLE 6

Effect of biuret on chick gain, feed consumption and efficiency of gain (exp. 3)

	Dietary treatment	Gain/ chick/ day [1]	Avg feed/ chick	Gain/ feed
		g	*g*	
1	Basal,[2] ad libitum	12.8	117.1	0.653
2	Basal, restricted [3]	10.0	106.3	0.566
3	Basal + 4.66% biuret, ad libitum	9.8	106.3	0.555

[1] Average of triplicate groups of 10 chicks/treatment for the period of 8 to 14 days of age.
[2] See table 1 for composition.
[3] Feed intake restricted to intake of treatment 3.

was equalized to that of the diet containing biuret. Restricting intake of the basal diet significantly depressed weight gain and efficiency of gain ($P < 0.01$) as compared with ad libitum intake of the basal diet. The reduced performance of chicks receiving pure biuret apparently was due to reduced feed intake.

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Alterations in the Activities of Several Rat Liver Enzymes at Various Times after the Feeding of High Carbohydrate Diets to Rats Previously Adapted to a High Protein Regimen [1]

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ABSTRACT The effect of high carbohydrate (protein-free) diets on the activities of several rat liver enzymes was studied in rats previously adapted to a 90% casein, carbohydrate-free diet. Both, the 90% glucose and the 90% fructose diet, were found to decrease all the liver constituents and enzyme activities studied, with the exception of pyruvate kinase, the activity of which was increased temporarily by both carbohydrate diets. The possibility of a control system regulating the synthesis of this enzyme at the translation level is discussed. Phosphorylase, glucose 6-phosphatase, fructose 1,6-diphosphatase, L-α-glycerophosphate dehydrogenase, glucose 6-phosphate dehydrogenase and malic enzyme activities were decreased to variable amounts. In some cases glucose 6-phosphate dehydrogenase and malic enzyme activities were increased. Tyrosine-α-ketoglutarate transaminase activity was minimal after 1 day of carbohydrate feeding. Glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase and serine dehydrase activities declined logarithmically. In the glucose-fed animals glutamic-oxaloacetic transaminase declined with a half-life of 4 days, glutamic-pyruvic transaminase with a half-life of 2 days and serine dehydrase with a half-life of 1.7 days. Fructose feeding increased the half-life of both glutamic-oxaloacetic transaminase and glutamic-pyruvate transaminase but not the half-life of serine dehydrase. Pair-feeding experiments showed that the tendency of the fructose diet to increase liver size and the half-lives of the 2 transaminases (glutamic-oxaloacetic transaminase and glutamic-pyruvate transaminase) was not due to an increased consumption of the fructose diet.

It has been known since the first radioactive tracer experiments performed by Schoenheimer et al. (1) that some animal proteins are unstable in the physiological environment and undergo rapid degradation. Mammalian liver is particularly active in this respect; the average half-life of rat liver protein has been estimated to be 3 days (2). The use of rat liver protein, therefore, is very convenient for the study of protein synthesis and breakdown. In addition, individual enzymes or groups of enzymes of rat liver respond differentially to endocrinectomy, dietary manipulations, and hormone treatments (3). These factors have greatly stimulated research into the effect of diets and hormones on some rat liver enzymes.

The half-life of a number of these enzymes has been estimated by various methods. The half-life of tryptophan pyrrolase has been estimated to be 3 to 4 hours from the rate of loss of enzyme activity following the induction of increased synthesis by treatment with corticosteroids (4) in vivo. The half-life of tyrosine-α-ketoglutarate transaminase has been estimated to be about the same (5). Glutamic-pyruvic transaminase activity decreases with a half-time of 40 hours (6) following adrenalectomy in young rats.

Segal et al. (7) have recently examined the kinetic parameters of rat liver glutamic-pyruvic transaminase following prednisolone injection. These authors had found that prednisolone increases not only the rate of enzyme synthesis, but also the rate of its breakdown. According to Segal et al., the half-life of glutamic pyruvic transaminase is 1.2 days if calculated from data

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obtained up to 6 days subsequent to the administration of the hormone, whereas enzyme activity decreases after 6 days with a half-life of 3.5 days. However, as the authors point out, this latter figure may have been influenced by a possible delay in the disappearance of the hormone.

In a previous article we have described the induction of certain rat liver enzymes by a 90% casein diet following the depletion of these enzymes by a protein-free diet (8). It appeared desirable that we examine the rate of disappearance of these enzymes, by changing the diet from a commercial laboratory ration to a high protein diet as an inducing stimulus and changing from the high protein diet to the protein-free diet as the potential suppressor of enzyme synthesis. By using this technique we hoped to determine the half-life of a number of enzymes under conditions more physiological than endocrinectomy or massive doses of hormones. In addition it was important to ascertain whether the rate of disappearance of enzyme activities after high protein feeding were similar to those found after cortisol induction.

EXPERIMENTAL

Animals. Male rats of the Sprague-Dawley strain were fed a high protein diet consisting of 90% casein, 5% corn oil, 4% minerals (9) and 1% vitamins (10). Five days later the animals were offered a diet in which carbohydrate was substituted for casein, but which was otherwise identical to the previous diet. The animals were killed in the early morning at zero, 1, 2, 3, 5 and 7 days following the change to the protein-free diet.

In another series of experiments, the same procedure was followed, except that the animals receiving the 90% fructose diet were allowed to eat only the amount of diet which was consumed by the animals receiving the 90% glucose diet. These animals (referred to from here on as pair-fed) were killed in the early morning at 1, 2, 3, 4 and 5 days following the change to the protein-free diet.

All animals were housed individually in screen-bottom cages and were offered water ad libitum.

Procedure. The preparation of liver homogenates, the determination of liver protein and glycogen, and the determination of enzyme activities were described in a previous paper (8, 11).

Analysis of data. The half-lives of liver transaminases and serine dehydrase were estimated from the first-order decrease in enzyme activity following the change to the protein-free diet. Such decrease in enzyme activity is described by the equation:

$$\frac{-d(A)}{dt} = k_2(A).$$

If this relationship applies, then the activity of an enzyme (A) at any time (t) after the dietary change is related to the activity measured after 5 days of feeding the 90% casein diet (A_0), the time elapsed after the dietary change (t) and the rate constant of disappearance of activity (k_2). This relationship is expressed by the equation:

$$\ln(A) - \ln(A_o) = -k_2 t.$$

A plot of log (A) versus (t), therefore, should produce a straight line with a slope (b) of $-k_2/2.303$. The half-life ($T_{1/2}$) is calculated from k_2 according to the well-known equation:

$$T_{1/2} - \frac{0.693.}{k_2}$$

The values of (b) were calculated by a method described by Snedecor (12) using the mean values of enzyme activity. Since a secondary adaptation in enzyme activity can be expected after the fifth or seventh day of feeding the protein-free diet (that is, the animal adapts to the diet and the activity of an enzyme levels off or increases), producing two consecutive values of enzyme activity which may be the same; in such cases the last value was omitted in the calculation of (b).

The goodness of fit of the data to the straight-line equation was estimated by calculating the correlation coefficient (r) (13). We have tested the hypothesis that fructose-feeding increases the half-life of the transaminases and serine dehydrase. For this purpose we calculated the sample standard deviation from regression (S_b) (12). The test was performed by comparing the ratio of

$$\frac{{}^{b}\text{glucose} - {}^{b}\text{fructose}}{S_b}$$

with the appropriate expected value in the *t*-distribution. If the ratio was larger than

the expected value, the difference between the slopes was statistically significant.

RESULTS

Rats fed the high protein diet for 5 days gained a total of 10 to 30 g of weight per rat (tables 1–4). This gain in weight was rapidly lost after the dietary change (tables 3 and 4) at a rate of 4 to 6 g/day. During the last 2 days of feeding the protein-free diet, the animals receiving the high fructose diet lost less weight per day than the animals receiving the high glucose diet (table 4).

Liver glycogen values increased after the dietary change (tables 1–4). In the fructose-fed rats, however, there was a decrease in the glycogen values the day after the dietary change, which was followed by a sharp increase in glycogen deposition.

Relative liver size and liver protein (both total and soluble) also decreased after the

TABLE 1

Effect of high glucose diet on several liver constituents and enzymes of rats fed ad libitum

No. of days of feeding high glucose diet	0	1	2	3	5	7
Original body wt before high protein feeding, g	183 ± 9 [1]	149 ± 16	148 ± 13	157 ± 13	153 ± 15	163 ± 37
Body wt at time of killing, g	192 ± 6	162 ± 21	157 ± 15	162 ± 15	153 ± 17	154 ± 15
Total liver protein, mg/100 g body wt	1900 ± 100	1270 ± 37	756 ± 38	880 ± 85	796 ± 53	893 ± 69
Soluble liver protein, mg/100 g body wt	800 ± 50	566 ± 36	481 ± 27	515 ± 39	553 ± 38	546 ± 66
Liver glycogen, mg/100 g body wt	54 ± 9.1	215 ± 15	153 ± 27	148 ± 29	185 ± 14	167 ± 26
Relative liver size = (wt of liver/body wt at time of killing) × 100	5.30 ± 0.1	5.03 ± 0.2	4.49 ± 0.2	4.10 ± 0.2	4.21 ± 0.2	3.70 ± 0.3
Phosphorylase	36.8 ± 14 [2]	51.3 ± 3	41.0 ± 6	38.7 ± 4	27.6 ± 4	35.0 ± 9
Glucose 6-phosphatase	83.9 ± 7	61.2 ± 9	72.3 ± 5	75.4 ± 6	66.2 ± 4	47.6 ± 13
Fructose 1,6-diphosphatase	19.9 ± 1	17.0 ± 3	14.3 ± 3	9.52 ± 3	11.0 ± 3	13.5 ± 2
L-α-Glycerophosphate dehydrogenase	191 ± 23	165 ± 20	115 ± 19	73.5 ± 6	65.1 ± 9	61.0 ± 8
Glucose 6-phosphate dehydrogenase	28.2 ± 6	13.7 ± 3	9.15 ± 2	7.00 ± 2	7.64 ± 2	5.70 ± 1
Pyruvate kinase	63.2 ± 17	48 ± 10	89.8 ± 13	51.1 ± 10	23.2 ± 6.8	27.8 ± 11
Malic enzyme	10.8 ± 3	7.00 ± 1	9.28 ± 1	12.2 ± 3	12.9 ± 4	13.7 ± 8
Glutamic-oxaloacetic transaminase	743 ± 29	537 ± 60	474 ± 57	540 ± 38	317 ± 44	272 ± 70
Glutamic-pyruvic transaminase	162 ± 6	116 ± 10	81.8 ± 6	54.0 ± 6	33.4 ± 5	33.8 ± 2
Serine dehydrase	24.8 ± 6	13.6 ± 2	10.1 ± 2	5.80 ± 1	2.84 ± 0.4	2.03 ± 0.5
Tyrosine-α-ketoglutarate transaminase	5.46 ± 1	0.298 ± 0.1	0.647 ± 0.1	0.798 ± 0.1	1.10 ± 0.3	0.894 ± 0.2

[1] SE of mean. All values are the average of at least 4 animals.
[2] Values of enzyme activity are given as micromoles of substrate converted per minute per 100 g body weight.

TABLE 2

Effect of high fructose diet on several liver constituents and enzymes of rats fed ad libitum

No. of days of feeding high fructose diet	0	1	2	3	5	7
Original body wt before high protein feeding, g	134 ± 3 [1]	139 ± 2	136 ± 4	172 ± 4	169 ± 2	135 ± 2
Body wt at time of killing, g	164 ± 4	159 ± 3	158 ± 4	165 ± 3	159 ± 2	146 ± 3
Total liver protein, g/100 g body wt	1370 ± 21	1019 ± 53	900 ± 32	— [2]	— [2]	912 ± 27
Soluble liver protein, mg/100 g body wt	683 ± 33	618 ± 5	687 ± 32	— [2]	— [2]	704 ± 33
Liver glycogen, mg/100 g body wt	179 ± 17	107 ± 25	215 ± 56	127 ± 37	301 ± 27	374 ± 56
Relative liver size = (wt of liver/body wt at time of killing) × 100	5.29 ± 0.2	4.64 ± 0.2	4.55 ± 0.2	4.22 ± 0.1	5.20 ± 0.2	5.41 ± 0.3
Phosphorylase	81.1 ± 2 [3]	52.1 ± 4	44.3 ± 4	62.6 ± 9	53.4 ± 3	56.7 ± 4
Glucose 6-phosphatase	34.7 ± 0.8	35.0 ± 1	32.1 ± 1	25.8 ± 3	36.5 ± 2	33.2 ± 1
Fructose 1,6-diphosphatase	32.4 ± 1	23.7 ± 1	22.4 ± 0.6	20.0 ± 2	22.2 ± 0.8	18.7 ± 1
L-α-Glycerophosphate dehydrogenase	214 ± 11	194 ± 5	176 ± 7	216 ± 15	219 ± 7	235 ± 28
Glucose 6-phosphate dehydrogenase	18.3 ± 4	12.7 ± 2	16.4 ± 2	9.96 ± 0.9	9.76 ± 2	8.32 ± 1
Pyruvate kinase	65.4 ± 3	239 ± 21	113 ± 13	107 ± 13	205 ± 9	222 ± 13
Malic enzyme	3.20 ± 0.9	4.24 ± 0.8	4.83 ± 0.9	7.04 ± 2	15.2 ± 2	25.4 ± 2
Glutamic-oxaloacetic transaminase	546 ± 31	473 ± 3	352 ± 11	315 ± 11	308 ± 11	239 ± 20
Glutamic-pyruvic transaminase	136 ± 6	102 ± 6	91.4 ± 4	78.7 ± 9	41.8 ± 4	37.6 ± 5
Serine dehydrase	24.5 ± 3	20.3 ± 3	8.94 ± 0.7	6.71 ± 1	3.26 ± 0.2	3.11 ± 0.3
Tyrosine-α-ketoglutarate transaminase	3.84 ± 0.3	1.31 ± 0.1	1.05 ± 0.1	1.29 ± 0.4	1.82 ± 0.1	2.54 ± 0.2

[1] SE of mean. All values are the average of 6 animals.
[2] Experimental difficulties prevented accurate determination of these values.
[3] Values of enzyme activities are given as micromoles of substrate converted per minute per 100 g body weight.

dietary change (tables 1–4). It appears, however, that while in the glucose-fed rats the decrease was continuous, in the fructose-fed rats a leveling off and even occasional increases were observable.

The activities of phosphorylase, glucose 6-phosphatase, and fructose 1,6-diphosphatase were decreased by both protein-free diets (tables 1–4). The extent of decreases varied from group to group, and a secondary adaptation of these enzymes (that is, a stabilization or increase of activity) was observed after 2 or 3 days (tables 1 and 2).

L-α-Glycerophosphate dehydrogenase activity was unstable in the glucose-fed rats (table 1), but in the fructose-fed rats the activity of this enzyme was much more stable (table 2). This may be related to caloric intake as well as the carbohydrate in the diet.

Glucose 6-phosphate dehydrogenase activities were decreased by both protein-free

TABLE 3

Effect of high glucose diet on several liver constituents and enzymes in pair-fed rats [1]

No. of days of feeding high glucose diet	0	1	2	3	4	5
Original body wt before high protein feeding, g	176 ± 6 [2]	193 ± 1	186 ± 8	200 ± 19	185 ± 3	188 ± 7
Body wt at time of killing, g	190 ± 9	188 ± 4	188 ± 5	189 ± 12	180 ± 2	175 ± 8
Total liver protein, mg/100 g body wt	— [3]	— [3]	1370 ± 120	862 ± 40	835 ± 12	722 ± 8
Soluble liver protein, mg/100 g body wt	— [3]	— [3]	512 ± 32	517 ± 45	456 ± 14	389 ± 8
Liver glycogen, mg/100 g body wt	112 ± 19	214 ± 19	106 ± 33	195 ± 43	146 ± 10	155 ± 9
Relative liver size = (wt of liver/body wt at time of killing) × 100	5.42 ± 0.4	4.68 ± 0.1	3.98 ± 0.2	4.03 ± 0.03	3.78 ± 0.04	3.84 ± 0.2
Fructose 1,6-diphosphatase	27.9 ± 0.5 [4]	21.8 ± 0.2	21.4 ± 0.5	18.3 ± 2	16.0 ± 2	5.31 ± 0.3
L-α-Glycerophosphate dehydrogenase	— [3]	— [3]	168 ± 4	163 ± 28	133 ± 5	149 ± 8
Glucose 6-phosphate dehydrogenase	14.7 ± 1	7.44 ± 2	10.7 ± 1	6.09 ± 0.5	5.37 ± 0.4	3.83 ± 0.9
Pyruvate kinase	66.4 ± 4	93.0 ± 7	158 ± 18	125 ± 8	131 ± 7	128 ± 3
Malic enzyme	7.05 ± 1	5.61 ± 1	12.0 ± 0.8	13.6 ± 2	13.6 ± 2	16.6 ± 2
Glutamic-oxaloacetic transaminase	499 ± 35	363 ± 20	301 ± 27	312 ± 16	272 ± 9	198 ± 8
Glutamic-pyruvic transaminase	149 ± 22	109 ± 4	75.8 ± 10	43.5 ± 5	30.4 ± 4	31.6 ± 1
Serine dehydrase	25.4 ± 2	15.4 ± 0.9	11.8 ± 0.4	5.79 ± 0.5	4.38 ± 0.2	3.52 ± 0.1
Tyrosine-α-ketoglutarate transaminase	4.29 ± 0.5	0.341 ± 0.06	0.746 ± 0.3	0.877 ± 0.1	1.55 ± 0.3	1.58 ± 0.3
Change in body wt, g	+ 12.9 [5]	− 4.0	− 7.9	− 4.2	− 4.8	− 6.0
Food consumed, g/day	13.7 [6]	11.2	18.4	16.1	13.7	11.7

[1] The rats receiving the high-fructose diet were pair-fed to the animals receiving the high glucose diet, which were offered food ad libitum.
[2] SE of mean. All values are the average of 3 animals.
[3] Experimental difficulties prevented accurate determination of these values.
[4] Values of enzyme activities are given as micromoles of substrate converted per minute per 100 g body weight.
[5] The first number (12.9) refers to the average total weight gain of all animals after receiving the 90% casein diet for 5 days. The rest of the numbers in this row indicate the average weight loss of all the animals during a 24-hour period.
[6] Average amount of diet consumed per rat per day. The first number in this row (13.7) is the average daily consumption of the 90% casein diet.

diets (tables 1–4). From the data in table 1 we estimated the apparent half-life of this enzyme to be about 1.6 days. However, this was not corroborated by the rest of our data, and it appears that while the activity of this enzyme was decreased by feeding the high carbohydrate diet, the rate of decrease in activity was slower than would be expected if synthesis of this enzyme were stopped entirely. That the half-life of glucose 6-phosphate dehydrogenase may be less than a day is indicated by the nearly 50% decrease in the activity of this enzyme after the first day of starvation (11).

TABLE 4

Effect of high fructose diet on several liver constituents and enzymes in pair-fed rats [1]

No. of days of feeding high fructose diet	0	1	2	3	4	5
Original body wt before high protein feeding, g	176±6 [2]	183±3	185±8	167±9	183±4	184±7
Body wt at time of killing, g	190±9	192±1	182±8	176±8	171±5	175±8
Total liver protein, mg/100 g body wt	— [3]	1530±120	1160±311	1060±88	1020±33	775±19
Soluble liver protein, mg/100 g body wt	— [3]	598±32	525±29	548±58	532±9	546±9
Liver glycogen, mg/100 g body wt	112±19	49.6±6	121±30	245±18	188±10	195±51
Relative liver size = (wt of liver/body wt at time of killing) × 100	5.42±0.4	4.39±0.4	4.46±0.4	4.58±0.1	4.98±0.2	4.67±0.3
Fructose 1,6-diphosphatase	27.9±0.5 [4]	23.1±0.7	19.9±2	22.2±1	10.2±0.9	6.35±0.2
L-α-Glycerophosphate dehydrogenase	— [3]	171±8	174±3	193±29	228±26	232±6
Glucose 6-phosphate dehydrogenase	14.7±1	6.64±0.4	9.26±0.8	7.73±0.9	5.80±0.4	6.71±1
Pyruvate kinase	66.4±4	103±6	163±5	178±47	178±43	184±16
Malic enzyme	7.05±1	13.5±3	6.95±1	9.15±4	— [3]	9.49±3
Glutamic-oxaloacetic transaminase	449±35	476±12	354±12	343±85	328±10	283±29
Glutamic-pyruvic transaminase	149±22	117±8	82.4±10	63.1±2	79.1±10	44.2±0.7
Serine dehydrase	25.4±2	23.0±1	9.21±0.6	7.06±0.2	7.77±1	4.75±0.6
Tyrosine-α-ketoglutarate transaminase	4.29±0.5	2.07±0.2	0.489±0.11	1.69±0.2	1.66±0.3	1.96±0.2
Change in body wt, g	+12.9 [5]	−4.0	−7.6	−3.9	−3.8	−4.3
Food consumed, g/day	13.7 [6]	11.2	18.4	16.1	13.7	11.7

[1] The rats receiving the high-fructose diet were pair-fed to the animals receiving the high glucose diet, which were offered food ad libitum.
[2] SE of mean. All values are the average of 3 animals.
[3] Experimental difficulties prevented accurate determination of these values.
[4] Values of enzyme activities are given as micromoles of substrate converted per minute per 100 g body weight.
[5] The first number (12.9) refers to the average total weight gain of all animals after receiving the 90% casein diet for 5 days. The rest of the numbers in this row indicate the average weight loss of all the animals during a 24-hour period.
[6] Average amount of diet consumed per rat per day. Some of the animals receiving the high fructose diet did not eat all of it. However, the uneaten food was negligible (less than 7% of the amount given). The first number in this row (13.7) is the average daily consumption of the 90% casein diet.

Pyruvate kinase activities were relatively low after the feeding of the high protein diet and were increased temporarily after the dietary change (tables 1–4). This increase in pyruvate kinase activity was the largest following the feeding of the high fructose diet (table 2). The same tendency was observed in the pair-fed animals, though relatively smaller.

Malic enzyme activity was temporarily decreased by glucose feeding, which was followed by an increase in the activity of

this enzyme (tables 1 and 3). However, fructose feeding increased malic enzyme activity (tables 2 and 4).

The 3 transaminases studied and serine dehydrase were all decreased by the high carbohydrate diet (tables 1–4). Glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, and serine dehydrase activities were decreased at a measurable rate, while tyrosine-α-ketoglutarate transaminase activities were minimal one day after the dietary change, which is consistent with the short half-life of this enzyme (5).

Glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, and serine dehydrase activities decreased logarithmically following the dietary change. The correlation coefficients for these decreases were very close to −1 (tables 5 and 6). The $T_{1/2}$ values obtained from feeding the high glucose diet are close to the values obtained by others for glutamic-oxaloacetic transaminase (4) and glutamic-pyruvic transaminase (6, 7). Fructose-feeding increased the apparent half-life of both transaminases (in the case of glutamic-pyruvic transaminase the difference is statistically significant at the 95% confidence level) but not the apparent half-life of serine dehydrase. The observed stabilizing effect of fructose on several rat liver constituents and enzyme activities, therefore, is not uniform and differs qualitatively at least in the case of serine dehydrase.

DISCUSSION

It is assumed in these experiments that enzyme activity, as measured under the conditions of the assay, is a good approximation of the amount of enzyme. This assumption may not be always warranted. Consequently, the value of k_2 calculated from measurements of enzyme activity is a rate constant of enzyme deactivation which may or may not be the same as the rate constant for the disappearance of enzyme protein. We have assumed that the feeding of a protein-free diet to animals adapted to a 90% casein diet would cause a cessation of synthesis of at least the transaminases studied and serine dehydrase. This assumption is subject to limitations, as protein synthesis may continue after the dietary change or may be resumed shortly thereafter, although at a reduced rate.

TABLE 5

Half-life of liver transaminases and serine dehydrase in animals fed ad libitum

Treatment	90% glucose					90% fructose				
Parameter	r [1]	b	S_b	k_2	$T_{1/2}$	r	b	S_b	k_2	$T_{1/2}$
				days						*days*
Glutamic-oxaloacetic transaminase	−0.95(0–> 7) [2]	−0.0611	0.0111	0.141	4.9	−0.95(0–> 7)	−0.0478	0.0073	0.110	6.3
Glutamic-pyruvic transaminase	−0.97(0–> 5)	−0.142	0.0101	0.328	2.1	−0.84(0–> 7)	−0.0714	0.0185	0.164	4.2 *
Serine dehydrase	−0.99(0–> 5)	−0.179	0.0141	0.412	1.7	−0.92(0–> 5)	−0.171	0.0266	0.394	1.8

[1] The following symbols are used: r = correlation coefficient, b = slope of the log (enzyme activity) vs. time plot, S_b = sample standard deviation from regression; k_2 = rate constant of disappearance of enzyme activity, $T_{1/2}$ = half-life of enzyme.

[2] Numbers in the parentheses indicate whether the last value of enzyme activity (on the seventh day in ad libitum-fed rats, or on the fifth day in pair-fed rats) was considered or not. For example, in the first column glutamic-pyruvic transaminase activity levels off after the fifth day and therefore the activity on the seventh day after the dietary change is ignored in the calculation of the slope.

* Differs significantly ($P < 0.05$) from values obtained with the high glucose diet.

TABLE 6

Half-life of liver transaminases and serine dehydrase in pair-fed animals

Treatment	90% glucose					90% fructose				
Parameter	r [1]	b	S_b	k_2	$T_{1/2}$	r	b	S_b	k_2	$T_{1/2}$
					days					*days*
Glutamic-oxaloacetic transaminase	−0.95(0–> 5)	−0.0677	0.0095	0.156	4.4	−0.96(0–> 5)	−0.0494	0.0083	0.114	6.1
Glutamic-pyruvic transaminase	−0.99(0–> 4)	−0.178	0.0082	0.410	1.7	−0.93(0–> 5)	−0.0932	0.0077	0.215	3.2 *
Serine dehydrase	−0.99(0–> 5)	−0.178	0.0097	0.411	1.7	−0.94(0–> 5)	−0.148	0.0206	0.340	2.0

[1] The following symbols are used: r = correlation coefficient, b = slope of the log (enzyme activity) vs. time plot, S_b = sample standard deviation from regression; k_2 = rate constant of disappearance of enzyme activity, $T_{1/2}$ = half-life of enzyme.

[2] Numbers in the parentheses indicate whether the last value of enzyme activity (on the seventh day in ad libitum-fed rats, or on the fifth day in pair-fed rats) was considered or not. For example, in the first r column glutamic-pyruvic transaminase activity levels off after the fifth day and therefore the activity on the seventh day after the dietary change is ignored in the calculation of the slope.

* Differs significantly ($P < 0.05$) from values obtained with the high glucose diet.

However, if the degree of synthesis were considerable, the log (A) versus t plot would be a hyperbola. Since correlation coefficients of these plots were close to −1 (tables 5 and 6), it is probable that the degree of synthesis was small.

The behavior of serine dehydrase is difficult to explain, since it has been shown that force-feeding of glucose causes a cessation of increase in the activity of this enzyme (14) which appears to be due to a cessation of enzyme synthesis.[2] If we assume that the synthesis of serine dehydrase were stopped by feeding the high glucose diet, then we must conclude that the half-life of this enzyme is greater than indicated by the experiments of Peraino and Pitot (15). If, however, we assume that the synthesis of this enzyme continued during the feeding of the high glucose diet (and thus accounting for the increase in $T_{1/2}$), then it appears that glucose does not have the effect on serine dehydrase synthesis shown by Pitot and others.[3] A possible explanation may be that the increased rate of synthesis after the high protein regimen decreases as a first-order process; thus if the true half-life were comparatively short the steady-state activity would also appear to decrease as a first-order process, and it may be this decrease which we are measuring and not the true $T_{1/2}$ of the enzyme.

The feeding of the high fructose diet increased the apparent half-life of glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase (table 6), but fructose did not increase the apparent half-life of serine dehydrase. It might be reasoned that at least a part of the increase in the apparent half-life of the 2 transaminases is due to a better maintenance of liver size by fructose but in that case it must be concluded that serine dehydrase activity is less stable in the fructose-fed rats on a per gram of liver basis than the activities of the 2 transaminases. Clarification of this point requires further experimentation.

We have previously reported that the activity of pyruvate kinase was low after

[2] Khairallah, E. A., J. P. Jost and H. C. Pitot 1967 Studies on the mechanism of glucose repression of inducible enzymes in rat liver. Federation Proc., 347: 26.

[3] See footnote 2.

the feeding of a 90% glucose diet for 4 days and was temporarily elevated after changing to a 90% casein diet (8). Since this trend was different than that observed by others (16–18) with respect to the effect of dietary protein and carbohydrate on pyruvate kinase activity, we assumed that the inducing effect of a carbohydrate diet on the activity of pyruvate kinase is not expressed in the absence of protein. Specifically, we suggested that the feeding of a 90% glucose diet may increase the synthesis of messenger RNA specific for pyruvate kinase, but the absence of protein in the diet causes an inhibition of pyruvate kinase synthesis, either because of an effective lowering of amino acid concentration in the liver, or by a specific or general inhibition of protein synthesis, or both. Under such circumstances the refeeding of protein would produce a transitory elevation in pyruvate kinase synthesis and activity until the specific messenger RNA supply diminished. If this were so, then a reversal of the feeding of the 2 diets would result in low pyruvate kinase activity after feeding the high protein diet and a transitory elevation of the activity of this enzyme after changing to the high carbohydrate diet until the amino acid supply of the liver diminished. This type of transitory elevation in pyruvate kinase activity was in fact observed (tables 1 and 2), though the extent of elevation in enzyme activity was much smaller in the slightly heavier animals (tables 3 and 4).

Finally, it may be concluded that dietary manipulations of extreme nature may be a useful technique in the estimation of the half-life of some liver enzymes in vivo. Data obtained from such experiments, however, should be interpreted with caution. The reason for this caution is that enzyme activity assayed in vitro may reflect not only the true half-life of these enzymes, but also the effects of possible secondary adaptive mechanisms activated to cope with the sudden and relatively large changes in internal environment.

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Comparison of Amino Acid and Alfalfa Supplementation of Purified Diets for Ruminants [1]

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ABSTRACT A series of experiments was undertaken comparing three purified diets for growth of lambs: the basal, diet 30, contained urea as the sole source of nitrogen; diet $27V_2$ consisted of the basal plus 5% alfalfa; and diet 28, the basal plus a selected group of amino acids based on the amino acid composition of alfalfa. As observed in previous studies, the alfalfa-supplemented diet was superior for growth to the basal urea diet. Results of this investigation also showed that the basal plus the amino acid supplement was equal to that of the alfalfa-containing diet for growth of lambs. The results from nitrogen balance studies indicated that neither the amino acid supplement nor the alfalfa improved the nitrogen balance, suggesting that the effect may have been mediated via changes through the microflora of the rumen. Gram stains from the samples of rumen fluid from the animals fed the experimental diets showed a marked difference in the flora of the animals fed the amino acid supplement.

Previous work (1), investigating dietary factors in purified diets for ruminants, showed that, where urea was substituted for casein, 5% alfalfa in the diet had a significant positive effect on gains of lambs. Since 5% alfalfa did not enhance the growth when casein was the source of nitrogen in the diet, it was reasoned that the factor(s) added by the alfalfa might be associated with the amino acids supplied by this natural feed supplement. The present study was designed to determine whether a supplement of amino acids, selected on the basis of their presence in alfalfa, to the basal purified urea diet would supply the needed growth factors furnished by the alfalfa.

EXPERIMENTAL

The details of biological procedures used in these growth trials were similar to those described previously (2, 3). Lambs 2.5 to 3 months of age were divided into 6 pairs, based on weight, and then each pair was assigned at random to 2 experimental diets (table 1, diets 27V and 28) in part 1 of this experiment. This part of the experiment extended over 25 weeks. In part 2, 14 lambs similar in age and previous history to those in part 1 were used. Since the animals were not available at the same time, they were placed on experiment at varying times in the following manner: (a) 4 lambs, diet $27V_2$ and 4 lambs, diet 28; (b) 2 lambs, diet 28, and 4 lambs, diet 30. Part 2 was carried out over 28 weeks. A total of 26 animals was used in the experiment.

All experimental animals received vitamin A and D capsules (table 1). Except for the addition of 0.2% sodium sulfate, 0.1 ppm selenium as sodium selenate and 0.1 ppm Mo as sodium molybdate, these diets were similar to the urea purified diet developed previously (4). Glucose level was reduced where alfalfa or amino acid mix was added. Diet $27V_2$ was similar to diet 27V (4). Diet 28 was different only in that the alfalfa was replaced with an amino acid mixture containing a selected group of amino acids in amounts similar to those found in alfalfa (table 2). Diet 30 was an all-urea purified diet with no alfalfa or amino acid mix. Thus it contained the equivalent of 12.25% protein in contrast with 13.25% in the other 2 diets. The animals were housed in individual cages and fed twice a day as much as they would consume. The diets were mixed twice weekly

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TABLE 1

Composition of experimental diets

	Diet no.		
	$27V_2$	28	30
	%	%	%
Urea	4.6	4.6	4.6
Glucose [1]	32.0	32.0	34.5
Starch	30.6	30.6	30.6
Hydrogenated vegetable fat [2]	4.0	4.0	4.0
Cellulose	3.0	3.0	3.0
Alfalfa leaf meal [3]	5.0	—	—
$KHCO_3$	4.0	4.0	4.0
$NaHCO_3$	6.0	6.0	6.0
$CaCO_3$	1.0	1.0	1.0
$CaHPO_4$	1.8	1.8	1.8
Amino acid mix [4]	—	5.0	—
Vitamin mix [5]	5.0	5.0	5.0
Mineral mix [6]	3.0	3.0	3.0
Methionine mix [7]	—	—	2.5

[1] Crystalline glucose, Corn Products Sales Company, Norfolk, Virginia.
[2] Primex B and C (pure vegetable shortening), Procter and Gamble Company, Cincinnati.
[3] Alfalfa leaf meal (20% protein), National Alfalfa Dehydrating and Milling Company, Kansas City, Missouri.
[4] Amino acid mixture/45.36 kg diet: (in grams) monosodium glutamate, 907; DL-aspartate, 45; DL-isoleucine, 45; L-leucine, 22.7; L-arginine·HCl, 22.7; DL-tryptophan, 22.7; DL-methionine, 91; and glucose monohydrate (Cerelose, Corn Products Company), 1,111 g.
[5] Vitamin mixture/45.36 kg diet: thiamine·HCl, 400 mg; riboflavin, 850 mg; nicotinic acid, 1.13 g; Ca pantothenate, 1.42 g; pyridoxine·HCl, 570 mg; folic acid, 57 mg; *p*-aminobenzoic acid, 1.13 g; inositol, 11.35 g; biotin, 11.4 mg; choline chloride, 113.45 g; menadione (2 methyl-naphthoquinone), 115 mg; 0.1% vitamin B_{12} (with mannitol), 4.66 g; *a*-tocopheryl acetate, 570 mg; glucose, 2132 g. In addition, 4,000 IU of vitamin A (from fish liver oil) and 400 IU of vitamin D (irradiated ergosterol)/day/45.36 kg body weight were administered by capsule (contributed by R. P. Scherer Corporation, Detroit).
[6] Mineral mixture/45.36 kg diet: KCl, 273 g; NaCl, 239 g; $MgSO_4$, 204 g; Na_2SO_4, 90 g; $CuSO_4 \cdot 5H_2O$, 893 mg; $FeSO_4 \cdot H_2O$, 7648 mg; $MnSO_4 \cdot H_2O$, 1399 mg; ZnO, 2263 mg; $CoCO_3$, 9 mg; KI, 6 mg; $Na_2SeO_4 \cdot 5H_2O$, 4.5 mg; $Na_2MoO_4 \cdot 2H_2O$, 4.5 mg; and glucose, 633 g.
[7] Methionine mixture/45.36 kg diet: DL-methionine, 91 g; and glucose monohydrate (Cerelose, Corn Products Company), 1043 g.

in a food mixer.[2] Records of daily feed intakes and weekly weights were kept during the experiment. Blood samples were taken at 4-week intervals and analyzed for iron, copper, calcium, magnesium and phosphorous as described previously (2). Sodium and potassium were determined with a flame photometer.[3]

Another phase of the investigation was carried out with 6 fistulated yearling ewes. The 6 ewes were used in a study with diets $27V_2$ and 28 (table 1) and subsequently 4 of these ewes were used to study diets 28 and 30 (table 1). A digestion trial was run which included a nitrogen balance. The ewes were fed the test diet for a 30-day preliminary period followed by a 5-day collection period. Following the balance trials, a series of measurements was made.

TABLE 2

Comparison of amino acids supplied as alfalfa meal or free amino acids

Amino acid	Supplied by alfalfa meal [1]	Supplied by amino acid mix
	% in diet	*% in diet*
Glutamic	0.0750	2.00 [2]
Aspartic	0.1250	0.10 [3]
Isoleucine	0.0514	0.10 [3]
Leucine	0.0791	0.05 [4]
Arginine	0.0493	0.05 [4]
Tryptophan	0.0211	0.05 [3]

[1] Based on values reported in Sievert, C. W., and B. W. Fairbanks 1954 The Feed Bag Red Book for 1954. Editorial Service Company, Inc., Milwaukee, p. 178; and the National Research Council, Committee on Animal Nutrition 1958 Nutrient Requirements of Domestic Animals, publ. 585. National Academy of Sciences — National Research Council, Washington, D. C.
[2] Glutamic supplied as monosodium glutamate as "Accent" from Accent International, Skokie, Illinois.
[3] DL-form.
[4] L-form; arginine as arginine·HCl.

Samples of blood, urine and rumen contents were taken from each sheep. Measurements of blood ammonia and urea, urine ammonia and urea, rumen fluid fatty acid content and pH were made. Gram stains of the rumen microorganisms were made several times during the period that the animals were fed these diets. Photographs shown in figure 1 were taken at the end of the 30-day preliminary period. The rumen samples and blood samples for analytical measurements were taken in the following sequence: a zero-hour sample was taken in the morning before forced feeding of 227 g of the diet (by stomach tube in 1 liter of water) and subsequent samples were taken at 0.75, 1.5, 3 and 6 hours thereafter from the rumen fistula by syringe. Measurements of the urine were made on a zero-sample and a total 6-hour collection sample. Volatile fatty acid analyses of the samples of rumen fluid were made by gas chromatography [4] according to the procedure of Erwin et al. (5). Immediately after the rumen samples were collected, pH was determined.[5] Free ammonia values from the blood and urine

[2] Hobart Manufacturing Company, Troy, Ohio.
[3] Baird Atomic Flame Photometer, Model Ky, Baird Atomic, Inc., Cambridge, Massachusetts.
[4] Perkin Elmer Vapor Fractometer, Model 154C, modified for flame ionization operation, Perkin-Elmer Corporation, Norwalk, Connecticut.
[5] Beckman Zeromatic pH Meter, Beckman Instruments, Inc., Fullerton, California.

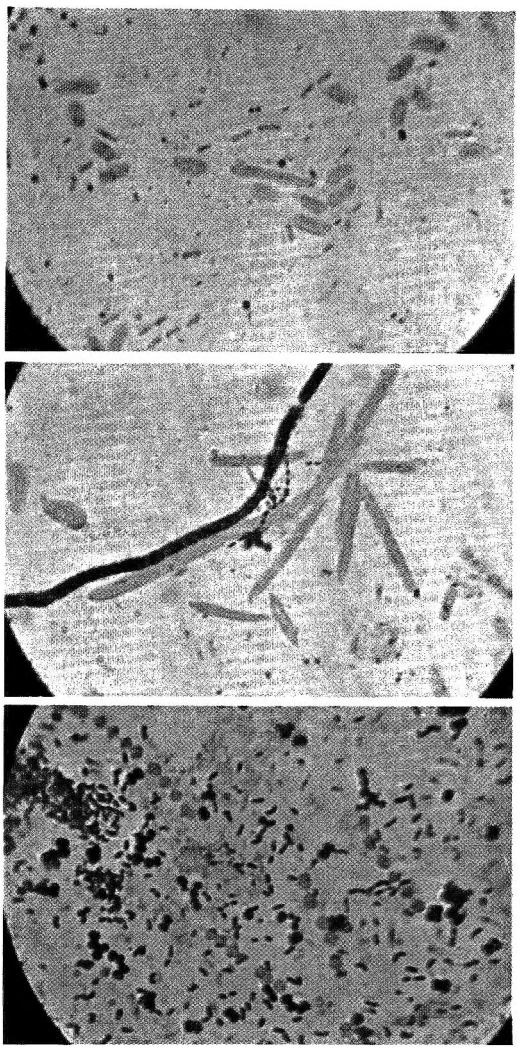

Fig. 1 Gram stains of ruminal microorganisms obtained from sheep fed diet $27V_2$ (*upper print*), diet 28 (*center print*) and diet 30 (*lower print*).

were determined by the method of Conway (6), and urea ammonia determinations were run according to Conway and O'Malley (7).

Data were subjected to analysis of variance: the 0.05 level of significance represents odds of 1:19, and the 0.01 level represents odds of 1:99 (8).

RESULTS AND DISCUSSION

The results of the growth trial are shown in table 3. Analysis of variance of the data indicated that neither the feed intakes nor the gains were significantly different between diets 28 and $27V_2$. In contrast, the feed intake and gains were significantly lower for the sheep fed diet 30 than for those fed 28 and $27V_2$.

As shown in table 3, mineral analyses of the blood show normal levels of all constituents measured. These overall results of the growth trial suggest that the basic urea purified diet supplemented with amino acids was equal to the alfalfa supplemented diet.

Presumably, amino acids could have affected the flora, resulting in a change of metabolism in the rumen, or they could have been absorbed and utilized directly by the animal itself. If the first premise held, it might be reflected in a difference in rumen flora; however, if the second alternative prevailed, one might expect to find that the supplement of the amino acids improved the nitrogen balance of the animals receiving this diet. The results of the nitrogen balance study (table 3), however, did not support the latter premise. Although the nitrogen balance values obtained for the diets were not significantly different, the highest nitrogen balance was obtained with diet 30 which gave the poorest growth performance. Reports in the literature showing that amino acids are absorbed poorly from the rumen (9) further substantiate the view that the effect of amino acids is not on the animal per se but via the microflora.

Rumen samples were taken from the experimental ewes, and gram stains were made of the rumen flora to determine whether the free amino acids brought about a change in flora. The results are shown in figure 1. A marked change in the floral population was observed in the rumen samples from the animals fed diet 28. This flora is characterized by the presence of an unusually large, curved, rod-shaped, gram-negative organism. It is tentatively identified as *Selenomonas ruminantium*,[6] an organism frequently observed in the rumen, and which can become dominant under dietary conditions of high starch or sugar such as those found here (10). The dominant organisms found in the rumen

[6] The assistance of Dr. Marvin Bryant, Department of Dairy Science, University of Illinois, Urbana, Illinois in the identification is gratefully acknowledged.

TABLE 3

Summary of results obtained with lambs and ewes

	Diet $27V_2$	Diet 28	Diet 30
	Avg weekly gains and feed intakes of lambs [1]		
Intake, g	6877 (12)	7049 (10)	5440 (4) [2]
Gain, g	780 (12)	762 (10)	490 (4) [2]
	Mineral analyses of blood serum of lambs (mean values)		
Iron, μg/100 ml	113.87(12)	156.93(10)	157.00(4)
Copper, μg/100 ml	75.53	53.19	68.04
Calcium, mg/100 ml	13.07	13.45	13.41
Magnesium, mg/100 ml	3.04	3.19	2.87
Phosphorus, mg/100 ml	8.44	8.47	7.70
Sodium, mEq/liter	160.75	158.50	158.69
Potassium, mEq/liter	6.47	6.62	6.18
	Red blood cells		
Sodium, mEq/liter	110.75	83.48	100.44
Potassium, mEq/liter	30.35	31.70	16.11
	Nitrogen balance data obtained with fistulated ewes [3]		
Nitrogen intake, g	103.0 (3)	103.1 (5)	102.6 (2)
Nitrogen balance, g	28.4	18.5	35.6

[1] Numbers in parentheses indicate number of observations in each mean.
[2] Significantly lower ($P \leq 0.05$) than for diets $27V_2$ and 28.
[3] Nitrogen balance values not significantly different ($P < 0.13$).

samples from the ewes fed diet $27V_2$, however, appear to be lactic acid- and propionic acid-producing bacteria, which are also favored by high levels of readily available carbohydrate. Since carbohydrates were not a variable in these diets, the differences in the flora of ewes fed diets 28 and $27V_2$ are presumed to be due to amino acids.

Both the time trend and average of the pH values of the rumen contents and of ammonia and urea values of blood and urine of the ewes fed the experimental diets were not significantly different. The general trend was for the pH to decrease soon after feeding and then increase. These changes were similar for all diets. The average pH value obtained for each diet over the 6-hour period of observation was 6.41 for the animals fed diet 28, 6.49 for those fed diet $27V_2$, and 6.25 for those fed diet 30.

The results of volatile fatty acid analyses were inconclusive. The most marked difference between diets $27V_2$ and 28 was in the valeric and isovaleric components of the gas chromatogram. The values expressed as percentages of the total volatile fatty acids obtained for diet $27V_2$ were 0.7% for 2-methyl butanoic and 0.7% for a mixture of *n*-valerate and 3-methyl butanoic, whereas the values for diet 28 were, respectively, 1.4% and 1.8%. Presumably the higher values for these valeric acid isomers for diet 28 arose from the supplemental amino acids. The higher levels of the branched-chain fatty acids in the rumen samples from the animals fed diet 28 may be a reflection of the higher levels of leucine and isoleucine (11, 12).

The data obtained from this investigation suggest the following possible sequence of events. Addition of supplemental amino acids to a basal urea-containing purified diet improved growth to equal that of the basal plus 5% alfalfa. Gram stains of rumen flora indicated that the amino acid-supplemented diet produced a different rumen flora from that of the other 2 diets. Nitrogen balance values indicated that the results were not mediated via direct utilization of the supplemental amino acids by the animal.

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Diet-Dependence of Rat Liver Glucose 6-Phosphate Dehydrogenase Levels [1]

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ABSTRACT The level of glucose 6-phosphate dehydrogenase in liver is altered strikingly by starvation and also by refeeding, but the steps by which increased synthesis of this enzyme is induced have not been satisfactorily identified. Recent work suggests that hormones may have a general rather than a specific role. When starved rats were refed diets varying either in amount or in carbohydrate content, their liver glucose 6-phosphate dehydrogenase increased directly with their carbohydrate intakes. Insulin, and other hormones tested, given without carbohydrate, but with adequate protein and energy intakes, had no effect on liver glucose 6-phosphate dehydrogenase levels.

The increase of liver glucose 6-phosphate dehydrogenase in response to high carbohydrate feeding and the remarkable "overshoot" of this enzyme in response to refeeding after starvation have been discussed in earlier reports (1–4). The mechanism of these responses has proved difficult to determine because of the extent of intermediate metabolism, illustrated by the delay of 48 to 72 hours before peak levels are reached. Suggestions that the level of this enzyme is regulated positively by insulin and negatively by intracellular fatty acids (5, 6) may be valid with respect to maintenance of normal levels and perhaps also with respect to fluctuations associated with feeding times (7, 8). Nevertheless, the striking and transient increases noted in the livers of healthy, intact rats that are starved acutely and refed with carbohydrate-containing diets, appear to be directly related to the amount of carbohydrate refed and to be independent of intakes of dietary fat and of the levels of insulin, hydrocortisone or thyroxine.

MATERIALS AND METHODS

Male rats of the Sprague-Dawley strain were obtained from our own breeding colony or from a recognized supplier [2] and were fed a balanced stock diet [3] until experimentation. During experiments they were caged individually with unrestricted access to water. They were decapitated at 9:00 AM. Livers were washed in normal saline (0.9% NaCl) containing 0.25 g/liter of EDTA-sodium salt, pH 5.0, and were each homogenized in 15 ml of that medium by the Potter-Elvehjem method, using 4 cycles (down, then up) of a Teflon pestle turning at 600 rev/min. Enzyme levels were measured in the 105,000 × *g* supernatants by methods described previously (9). In all diets sucrose was the only source of carbohydrate, since it had appeared in earlier work to be the most efficient producer of enzyme increases (7).

Data were calculated initially on the basis of liver fresh weight. The results are expressed on a body weight basis, as proposed by Carroll and Bright (10), in order to show any relationship with feed intake expressed similarly. This "correction" for small variations in liver size did not influence the ranking of the data as calculated initially.

EXPERIMENTS AND RESULTS

Table 1 shows the responses of groups of 4 rats to refeeding limited daily amounts of a 69% sucrose diet after 4 days of complete starvation. While 6-phosphogluconate dehydrogenase and pyruvate kinase levels

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[2] Charles Rivers, Inc., Wilmington, Massachusetts.

[3] Mouse/Rat Diet, Teklad Inc., Monmouth, Illinois.

rose according to group intake, the level of glucose 6-phosphate dehydrogenase rose only when body weight recovery was positive; then the progressive increase was relatively large.

Sucrose was substituted progressively for lard (table 2) in a series of diets refed ad libitum for 3 days to rats that had been starved for a week, and hence these animals then received sufficient protein and energy for enzyme protein synthesis; nevertheless, the enzyme levels reflected only the amount of sucrose eaten. If the enzyme levels of the "carbohydrate-free" group, no. 4, are subtracted from the respective levels in the 3 groups receiving carbohydrate, and the enzyme differences are plotted against the caloric intakes of carbohydrate alone (fig. 1), the relationship appears to be rectilinear.

Table 3 contains data from a series of experiments to study the possibilities of hormonal induction. One group (a) was allowed to feed ad libitum a pelleted stock diet in order that normal enzyme levels as at the start of experimentation might be shown. Three groups (b) were starved for 4 days, during which one group received alloxan and another alloxan and insulin replacement. Five groups (c) were

TABLE 1

Liver enzyme levels in rats[1] starved 4 days and refed restricted amounts of a 69% sucrose diet[2] for 2 days

No. rats/ group	2-day intake	Body wt recovery	6-Phospho-gluconate dehydrogenase	Glucose 6-phosphate dehydrogenase	Pyruvate kinase
	kcal/100 g body wt[3]	% [4]	*μmoles substrate converted/min in liver/100 g body wt*[3]		
3	6.3 ± 0.1 [5]	−3 ± 0.5	6.81 ± 0.43	2.30 ± 0.81	7.62 ± 1.01
4	11.8 ± 0.9	−1.5 ± 0.8	10.6 ± 1.76	2.37 ± 0.42	13.09 ± 2.89
4	22.1 ± 0.5	+0.3 ± 0.4	14.70 ± 3.48	5.05 ± 2.92	20.47 ± 0.96
4	38.2 ± 4.2	+6.0 ± 1.2	26.08 ± 4.93	19.41 ± 2.59	41.36 ± 3.83

[1] Male rats, 300–350 g.
[2] Diet no. 2 in table 2.
[3] Body weight at killing after 48 hours of refeeding.
[4] Percentage of body weight at commencement of starvation.
[5] SD.

TABLE 2

Liver enzyme levels in rats[1] starved 7 days and refed for 3 days diets with different carbohydrate-to-fat ratios

	Diet [2]	3-day intake	Body wt recovery	6-Phospho-gluconate dehydrogenase	Glucose 6-phosphate dehydrogenase	Pyruvate kinase
		kcal/100 g body wt[3]	% [4]	*μmoles substrate converted/min in liver/100 g body wt*[3]		
1	20% casein, 74% sucrose, No fat	65 ± 4.1 [5]	7 ± 2.4	25.9 ± 4.3	35.3 ± 3.7	9.5 ± 2.6
2	20% casein, 69% sucrose, 5% fat	66.5 ± 1.7	7 ± 1.2	25.0 ± 1.2	35.2 ± 3.9	11.8 ± 2.3
3	28% casein, 31% sucrose, 34% fat	95.6 ± 10.0	10 ± 0.8	14.2 ± 2.1	15.4 ± 6.5	4.0 ± 1.1
4	33% casein, No carbohydrate, 58% fat	95 ± 5.9	10 ± 1.7	9.6 ± 2.2	2.2 ± 0.6	2.1 ± 0.6

[1] Male rats, 300–350 g.
[2] All diets contained the same caloric ratio of protein to energy-source and were supplemented with vitamins and minerals in proportion to the casein (3); diets no. 2 and 4 contained 5% of corn oil, remainder of fat content as lard.
[3] Body weight at killing after 48 hours of refeeding.
[4] Percentage of body weight at commencement of starvation.
[5] SD.

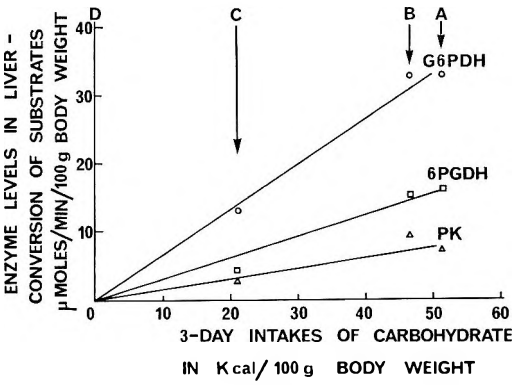

Fig. 1 Levels of three liver enzymes as functions of carbohydrate (sucrose) intake. Adult male rats were starved 7 days and refed 3 days with one of the diets described in table 2. *A* is diet no. 1; *B*, no. 2; *C*, no. 3; *D*, no. 4. All diets contained the same caloric ratio of protein to energy source. Diet D contained no carbohydrate, and the enzyme levels found therewith have been subtracted throughout. Enzymes are abbreviated: G6PDH, glucose 6-phosphate dehydrogenase, 6PGDH, 6-phosphogluconate dehydrogenase, and PK pyruvate kinase.

starved for 4 days and refed a carbohydrate-free diet (no. 4 in table 2) ad libitum for 2 days; four of these groups received supplements of either insulin, glucagon, hydrocortisone or thyroxine. Five groups (d) were similarly starved and refed a high-sucrose diet (no. 2 in table 2); one group received alloxan, a second alloxan and insulin replacement, and 2 groups received supplements of either insulin or hydrocortisone. All preparations were given intraperitoneally. These experiments were carried out at different times over a period of several months (this not applying to animals within any one group). There were no significant differences between the enzyme levels in groups *b* and c. Thus, the data in tables 1 and 2, and figure 1, and groups *b* and c (table 3) indicate that in these experiments protein was not a dietary inducer of these enzymes, fat was not a dietary inhibitor, and the hormones listed did not influence the enzyme levels

TABLE 3

Influence of hormones on some intracellular enzyme levels in rat liver [1]

Group	2-day intake	6-Phospho-gluconate dehydrogenase	Glucose 6-phosphate dehydrogenase	Pyruvate kinase
	kcal/100 g [2]	*μmoles substrate converted/min in liver/100 g body wt* [2]		
(a) Stock diet [3] fed (uninterrupted)	unobserved	15.9 ± 1.3 [4]	3.1 ± 1.3	8.7 ± 0.9
(b) Starved groups (4 days):				
Control	—	10.3 ± 1.4	8.1 ± 2.9	8.3 ± 1.6
Alloxan [5]	—	8.4 ± 0.8	4.1 ± 1.1	6.5 ± 1.0
Alloxan and insulin [6]	—	9.4 ± 1.6	5.1 ± 3.4	7.5 ± 1.0
(c) Fat-refed [7] groups:				
Control	47 ± 6	8.4 ± 1.8	2.3 ± 0.6	4.1 ± 0.3
+ insulin [8]	38 ± 6	6.4 ± 0.3	1.4 ± 0.4	2.5 ± 0.5
+ glucagon [9]	46 ± 6	9.2 ± 1.1	2.4 ± 0.8	2.5 ± 0.3
+ hydrocortisone [10]	46 ± 7	8.7 ± 0.7	2.7 ± 0.6	3.6 ± 0.4
+ thyroxine [11]	44 ± 6	7.8 ± 0.7	2.5 ± 0.4	4.2 ± 0.4
(d) Sucrose-refed [12] groups:				
Control	42 ± 3	47.2 ± 6.1	62.7 ± 12.0	46.8 ± 3.1
+ alloxan [5]	29 ± 4	23.0 ± 7.2	10.5 ± 4.7	27.1 ± 7.3
+ alloxan and insulin [6]	35 ± 7	27.6 ± 5.1	12.1 ± 3.9	29.2 ± 5.4
+ insulin [8]	43 ± 2	32.0 ± 6.7	24.8 ± 4.9	15.3 ± 3.0
+ hydrocortisone [10]	47 ± 4	40.8 ± 7.8	28.3 ± 11.1	19.1 ± 2.0

[1] Male rats of about 300 g; 4 to 6 rats/group.
[2] Body weight at killing; refed rats were killed 48 hours after start of refeeding.
[3] As described previously (3).
[4] SD.
[5] 12 mg/100 g ip 48 hours before killing.
[6] 4–6 USP units ip 24 hours before killing.
[7] Diet no. 4 in table 2 refed ad libitum.
[8] 4 USP units ip 24 hours and 16 hours before killing.
[9] 2 mg USP "for injection" ip 24 and 16 hours before killing; glucagon obtained from Eli Lilly, Indianapolis, Indiana.
[10] 3 mg/100 g ip 24 and 16 hours before killing.
[11] 5 μg ip 24 and 16 hours before killing.
[12] Diet no. 2 in table 2, refed ad libitum.

in the absence of dietary inducer, that is, of carbohydrate.

In all these groups, the lower the intake, the lower were the enzyme levels. When the data from tables 1 and 3 (4 days' starvation and 2 days' refeeding) were plotted as enzyme levels against caloric intakes of whole diet (fig. 2), it appeared that the influences of the hormonal treatments of group *d* (table 3) were indistinguishable from effects on appetite, and that in this series too, there were no signs of inhibition by dietary fat. The curve for glucose 6-phosphate dehydrogenase differed, as expected, from those for 6-phosphogluconate dehydrogenase and pyruvate kinase.

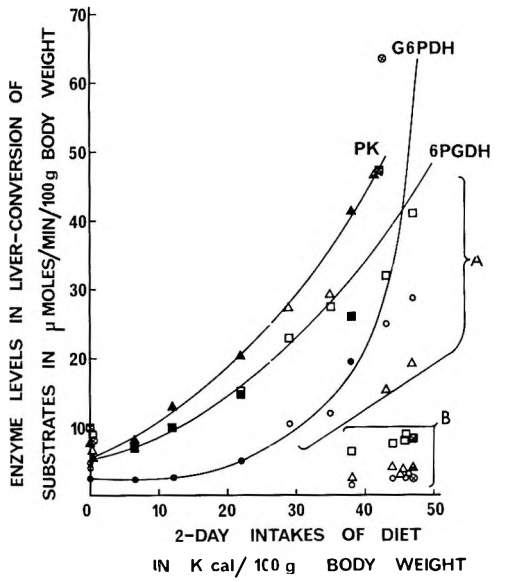

Fig. 2 Carbohydrate-dependence of three liver enzymes (identified in legend to fig. 1) in male rats starved 4 days and refed 2 days with protein-adequate diets containing either 69% sucrose (*A*) or no carbohydrate (*B*) (diets nos. 2 and 4 in table 2). Circles show group G6PDH levels, squares 6PGDH and triangles PK. Crossed symbols represent control groups refed ad libitum (table 3), closed symbols groups refed restricted amounts of diet (table 1) and open symbols groups refed ad libitum and treated with various hormones (table 3). Groups shown on the zero abscissa were killed at the end of 4 days' starvation.

These animals were all fed at 9:00 AM and were allowed to nibble for 24 hours until the next feeding time. When similar rats were allowed to eat continuously the stock or high sucrose diet, but only during the day (9 AM to 5 PM), their enzyme levels were consistently higher in relation to their caloric intakes (table 4).

DISCUSSION

Glucose 6-phosphate dehydrogenase, the first and rate-limiting enzyme of the hexose monophosphate pathway, appears to become synthesized *de novo* as an "emergency" response to the presence of excessive substrate or substrate-precursor within liver cells (1) or in adipose tissue (8, 11). This may (1) or may not [4] be preceded by glycogenolysis, and the speed at which glucose or glucose-phosphates accumulate may be important. In contrast, the response patterns of 6-phosphogluconate dehydrogenase and pyruvate kinase show no extraordinary features.

It has been proposed (5) that glucose 6-phosphate dehydrogenase levels are regu-

[4] Ruderman, N. B., V. Lauris, M. G. Herrera and G. F. Cahill 1967 Insulin regulation of rat liver glucokinase. Federation Proc., 26: 317 (abstract).

TABLE 4

Effect of altered feeding schedule on intake-based enzyme levels (non-starved rats [1])

	Feeding schedule [2]	6-Phospho-gluconate dehydrogenase	Glucose 6-phosphate dehydrogenase	Pyruvate kinase
		μmoles substrate converted/min in liver/kcal daily intake [3]		
1	Stock diet [4] continuously fed	0.74 ± 0.09 [5]	0.28 ± 0.10	0.21 ± 0.04
2	Stock diet, removed at night	1.04 ± 0.11	0.37 ± 0.14	0.23 ± 0.03
3	69% sugar diet,[6] continuously fed	1.63 ± 0.29	0.36 ± 0.20	0.51 ± 0.10
4	69% sugar diet, removed at night	2.08 ± 0.62	0.75 ± 0.27	0.99 ± 0.34

[1] Six rats, 300–350 g, in groups 1, 2 and 4; 5 in group 3.
[2] Test period, 8 days; groups 2 and 4 were fed from 9 AM to 5 PM daily, ate less, lost weight, and had smaller livers.
[3] Micromoles and kilocalories were first calculated/100 g body weight (see Methods).
[4] As described previously (3).
[5] SD.
[6] Diet no. 2 in table 2.

lated by feedback inhibition by fatty acids, and in our data (unpublished) fatty acids did inhibit this enzyme in vitro. However, Leveille (8) has pointed out that the refeeding overshoot follows the start of hyperlipogenesis and, further, it does not subside until about 4 days after first refeeding (1). No inhibitory effect of dietary fat could be shown in our studies, although such effects have been claimed (12), but then we would not expect to find immediate metabolites of dietary fat inside liver cells, in any significant quantity.

The hormones used did not appear to influence the levels of the enzymes studied in our healthy, intact rats. If extra glucose had been given along with the insulin, probably more insulin could have been administered and some increase of enzyme could have been observed in those rats possessing an adequate amino acid pool. But if so, this could have been principally a glucose effect. Our data suggested that the rise in level of these enzymes, especially glucose 6-phosphate dehydrogenase, in the liver cells of rats refed protein-adequate diets, depends chiefly on the amount of carbohydrate ingested and to a smaller extent (4) on the type of carbohydrate.

However, data in table 4 show that under other circumstances additional factors must be involved. In this case, enzyme levels relative to intakes increased when mealtimes were restricted. Similar or greater increases have been reported in rats fed on alternate nights (7) or for only 2 hours daily (8). Until more is known about the regular diurnal fluctuation of these enzyme levels such findings should probably be interpreted with caution with respect to regulatory mechanisms.

Although basal levels of glucose 6-phosphate dehydrogenase in rat liver cells may be regulated by a balance of hormone release and fatty acid level, as claimed by others, and although such factors may perhaps operate during enzyme increases associated, for example, with disturbance of species-normal mealtimes, nevertheless, the remarkable transient increases noted in carbohydrate-refed intact rats appear to depend upon the amount of carbohydrate present in a protein-adequate diet. The mechanism by which this increase is induced remains unknown, but may involve an "escape" from the normal regulatory mechanisms.

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Bioassay of Vitamin K by Intracardial Injection in Deficient Adult Male Rats [1]

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ABSTRACT The biological activity of vitamin K was determined by intracardial injection in partially depleted adult male rats. Prothrombin levels rose rapidly after the injection of phylloquinone. Response to menadione was delayed for several hours. The relative molar activity of several forms of vitamin K was determined. Menaquinones with side chains containing more than six isoprene units were up to 25 times as active as phylloquinone.

Bioassay of vitamin K by intravascular injection in deficient animals is a useful technique for the analysis of purified preparations of the vitamin without the limitations and uncertainties imposed by the absorptive process. In severely deficient chicks vascular penetration is impractical because of resulting hemorrhage; however, in rats it is possible to obtain a reproducible intermediate state of deficiency (1) permitting the administration of vitamin K by intracardial injection. The bioassay of vitamin K by this technique is the subject of this report.

MATERIALS AND METHODS

The conditions of deficiency were those obtained previously with adult male rats fed for 2 weeks a diet containing 21% soy protein, 43% cornstarch, 22% glucose monohydrate,[2] 5% corn oil, and an adequate supplement of minerals and vitamins except for vitamin K (1). Rats were selected from the St. Louis University colony at 13 ± 1 weeks of age. They were housed individually in suspended stainless wire cages and given fresh food at 2- or 3-day intervals to permit ad libitum feeding. After one week, samples of blood were taken by cardiac puncture and the plasma was analyzed by the method of Hjort et al. (2) using Russell's viper venom as an extrinsic activator. At 2 weeks the rats were divided into groups of three in such a manner that the average prothrombin levels of each group closely approximated the overall average. As observed earlier (1) this value was about 40% of normal rat prothrombin activity. For intracardial injection, phylloquinone [3] and menaquinones were dissolved in 5% or less of polyoxyethylene sorbitan monooleate (Tween 80) in saline; menadione and menadiol disphosphate were dissolved in saline.[4]

RESULTS

Initial dose-response data were obtained 18 hours after intracardial administration of the vitamin (fig. 1). On a molecular basis, injected phylloquinone is 10 times as active as menadione. Menadiol diphosphate has the same activity as the parent quinone.

The time of prothrombin response to phylloquinone and menadione was determined similarly in groups of 3 rats injected and sampled at intervals up to 42 hours (fig. 2). Each group received an amount of vitamin K sufficient to stimulate prothrombin concentration to about 80% of normal at 18 hours. Response to phylloquinone was rapid, reaching a maximum about 3 hours after injection. Response to menadione was delayed for at least 3 hours, but occurred linearly thereafter to

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[1] These studies were supported in part by Public Health Service Research Grants no. AM-09909 and no. AM-11311 from the National Institute of Arthritis and Metabolic Diseases.

[2] Cerelose, Corn Products Company, New York.

[3] Compounds are designated according to the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (3), for example, the K_2 vitamins are menaquinones (abbreviated MK) and vitamin K_1 is phylloquinone (abbreviated K).

[4] Most of the vitamins were donated by Hoffmann-LaRoche, Inc., Nutley, New Jersey, through the generosity of Dr. O. Isler. Menaquinone-7 (MK-7) was donated by Dr. E. A. Doisy. Samples of MK-9 (H) were donated by Dr. M. Weber and Dr. C. Coscia.

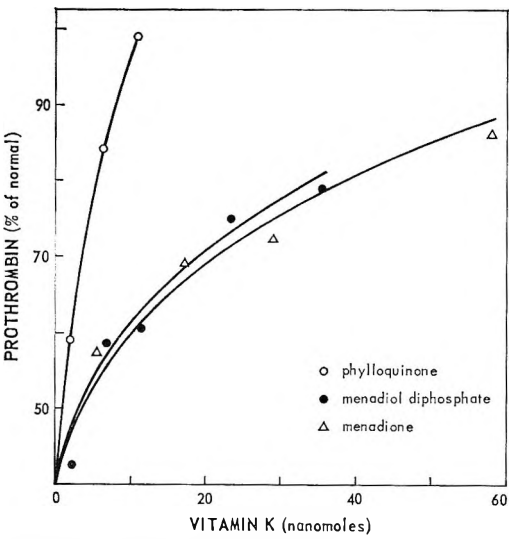

Fig. 1 Intracardial repletion of vitamin K in adult male rats.

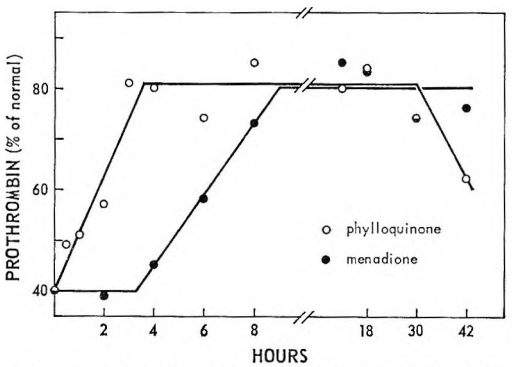

Fig. 2 Timed response to vitamin K in adult male rats.

a maximum about 9 hours after injection. These results are similar to those reported by Dam and Søndergaard (4) in the chick; although in the rat the delay in response to menadione appears to be more pronounced. Prothrombin levels remained high 42 hours after injection of menadione, whereas 42 hours after injection of phylloquinone prothrombin levels had dropped significantly.[5]

The relative biological activities of phylloquinone and menadione shown in figure 1 are not in agreement with earlier results obtained after oral administration (5,6). Further differences in biological activity were observed in the present study after intracardial injection of other forms of vitamin K. Each vitamin was assayed at several concentrations to determine the minimum amount required to produce normal prothrombin levels in injected rats. This amount and the relative molar activity of each vitamin are shown in table 1. After intracardial injection, lipophilic forms of vitamin K are highly active; menaquinones with side chains containing less than 30 carbon atoms have comparatively little activity. Phylloquinone has approximately the same molar activity as menaquinone-6 but higher isoprenologs have as much as 25 times the activity of phylloquinone.

TABLE 1

Relative molar activities of intracardially injected vitamin K

Vitamin [1]	M.N.D.[2]	Relative molar activity [3]
	nmoles	
MK-1	—[4]	< 1
K-2	—[4]	< 2
MK-2	—[4]	< 2
K-4	10	100
MK-4	80	13
MK-5	65	15
MK-6	6	170
MK-7	0.6	1700
MK-9	0.4	2500
MK-9(H)	0.7	1400
MK-10	0.6	1700

[1] Compounds are designated according to the recommendation of the IUPAC-IUB Commission on Biochemical Nomenclature (3); for example, the K_2 vitamins are menaquinones (abbreviated MK) and vitamin K_1 is phylloquinone (abbreviated K).
[2] Minimum normalizing dose in nanomoles.
[3] Phylloquinone (K-4) was arbitrarily assigned an activity of 100.
[4] Insufficient activity to estimate minimum normalizing dose. Relative molar activity estimated on the basis of the results of the largest amount of vitamin injected.

DISCUSSION

The administration of vitamin K by intravascular injection offers many advantages for studies of metabolism and function of the vitamin. Earlier attempts to standardize the effect of single doses of vitamin K by mouth or by intraperitoneal administration gave erratic results and required larger amounts of vitamin. Since vitamin K is poorly absorbed, we expected that samples injected intracardially would be more active and give more dependable results. In

[5] The standard errors of the values shown in figures 1 and 2 were approximately 10% of the mean. The prothrombin level shown 42 hours after injection of phylloquinone (64 ± 4) was significantly lower than that observed 18 hours after injection of the same vitamin (84 ± 5) ($P < 0.05$).

this way metabolic data may be correlated with changes in coagulation proteins under conditions of high physiological significance.

Despite these predeterminations, the extreme biological activity of menaquinones larger than MK-6 was unexpected (5). Quick and Collentine (7) made the original observation in dogs that injected phylloquinone is more active than injected menadione and Fisher et al. (8) observed the greatest dose-response with injected vitamin K_2 (MK-7); but there was no basis on which to predict the magnitude of the biological activities observed in the present report.

The difference between the relative molar activities shown in table 1 and those reported earlier after oral administration is probably due to the difficulty with which the lipophilic vitamins are absorbed. Some comparative data on the absorption and distribution of fat-soluble vitamins in the rat are available from recent studies of Wiss and Gloor (9, 10). Following a single oral dose, menaquinone-4 reached a higher concentration in the blood more rapidly than phylloquinone, indicating that menaquinone-4 is the more readily absorbed form. Under similar conditions, a compound as lipophilic as ubiquinone-9 reached only very low blood concentrations. In addition to differences in absorption, these investigators also reported the retention of these compounds by various tissues. Although MK-4 reached higher concentrations in blood and other tissues (particularly the heart), the concentrations of MK-4 and phylloquinone in liver were similar, indicating a relative affinity of phylloquinone for hepatic tissue. This affinity was demonstrated to a remarkable degree by ubiquinone-9 which accumulated almost exclusively in the liver after 8 hours. Similar studies will have to be conducted with several naphthoquinines to correlate biological activity with structure and cellular localization; but the present data suggest that lipophilic character is a stimulating factor in the function of vitamin K.

The high biological activity of menaquinones larger than MK-6 gives physiological significance to the occurrence of highly lipophilic forms of vitamin K recently identified in beef liver (11). Although poorly absorbed, bacterial vitamins with relatively high molecular weight probably represent nutritionally significant forms of vitamin K.

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Serum Lipid Responses in Dogs to Coconut and Sunflower Oils, before and after Thyroidectomy and with Thyroid Hormone Replacement [1]

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ABSTRACT The effects on the serum lipids of feeding either coconut oil or sunflower oil (40% of the total calorie intake) was studied in dogs before and after thyroidectomy and with thyroid hormone replacement with D- and with L-thyroxine (4.0 mg and 0.3 mg/dog/day, respectively). The elevations of serum cholesterol and triglycerides caused by feeding coconut oil were significantly greater after thyroidectomy than before, but the elevation of serum phospholipids was smaller after thyroidectomy. The serum lipid changes produced by coconut oil after the thyroidectomy were comparable to those observed before when the thyroidectomized dogs were treated with the thyroid hormones. Feeding sunflower oil produced significant elevations of serum cholesterol and phospholipids, but no significant change of serum triglycerides. These responses were not affected by thyroidectomy or by thyroid hormone replacement. Administration of 4.0 mg/dog/day of D-thyroxine to thyroidectomized dogs fed a low fat diet decreased serum cholesterol concentration to the level observed before thyroidectomy. L-Thyroxine at the dose of 0.3 mg/dog/day had a smaller effect. The basal oxygen consumption of the thyroidectomized dogs was elevated by the hormonal treatment but the values attained were lower than those observed in normal dogs.

We have recently reported (1) that thyroidectomized dogs respond to the addition of coconut oil to the diet with significantly greater elevations of serum cholesterol than normal dogs—elevations which are not entirely explained by the higher initial serum cholesterol level of the thyroidectomized animals.

Because of the lack of information about the effects of other dietary fats on the serum lipids of thyroidectomized animals it was considered of interest to extend this study using 2 fats of different degrees of saturation, known to have different effects on the serum lipids of normal dogs. It was our aim to determine whether the influence of thyroidectomy on the effects of these 2 dietary fats on the blood lipids was the same. Further, it was considered of interest to study whether the changes in serum lipid responses to the administration of dietary fat caused by thyroidectomy are prevented by replacement with thyroxine.

The present paper reports the results of experiments designed to study the effects on the dog's blood lipids (cholesterol, phospholipids, triglycerides) of 2 oils of different degrees of saturation (coconut oil and sunflower oil) under 4 experimental conditions: before thyroidectomy, after thyroidectomy without thyroid hormone treatment, and after thyroidectomy and hormone replacement with either D-thyroxine or L-thyroxine.

METHODS

Male, adult, mongrel dogs (16 to 22 kg) housed in individual cages were fed a commercial low fat dog food [2] for one month before the beginning of the experiments and throughout the study, except for the periods when the experimental diets were fed. The low fat diet (control diet) had by analysis a fat content of 1.6% (approximately 4% of the total caloric value). During the experimental periods the dogs received each morning weighed amounts of food, and the daily intake was determined by weighing the

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[1] Supported by Public Health Service research Grant no. HE-09897 from the National Heart Institute, the John A. Hartford Foundation, Inc. New York and the Mount Sinai Hospital Research Fund.
[2] Kibbies, Morton Dog Food Company, Minneapolis.

TABLE 1

Fatty acid composition of the two oils used in the preparation of the experimental diets

Fatty acids	Coconut oil	Sunflower oil
	% of total fatty acids	
Saturated		
6:0	1.6	0.0
8:0	8.0	0.0
10:0	6.7	0.0
12:0	49.0	0.0
14:0	18.1	0.0
16:0	8.2	7.3
18:0	2.0	3.7
Monoene		
14:1	0.0	0.0
16:1	0.0	0.0
18:1	5.2	16.0
Polyene		
16:2	0.0	0.0
18:2	1.2	72.4
18:3	0.0	0.6
Total saturated	93.6	11.0
Total monoene	5.2	16.0
Total polyene	1.2	73.0

food remaining in the feeders 24 hours later.

The experimental diets consisted of a mixture of 80% of the low fat diet and 20% of either coconut oil or sunflower oil. Approximately 40% of the caloric value of these diets was provided by fat. The samples of coconut oil and sunflower oil used were analyzed by gas-liquid chromatography as described elsewhere (2). The fatty acid composition of the oils is given in table 1.

Each of the 4 experiments consisted of 2 control periods and 2 experimental periods. During control period 1 the animals were observed for 3 weeks while fed the low fat diet. At the end of this period the animals were divided into 2 equal groups matched as to body weight and total serum cholesterol concentration. During the first experimental period, one group received the coconut oil diet and the other group the sunflower oil diet. At the end of 2 weeks the diets were interchanged for another 2 weeks (second experimental period). Finally, the dogs were returned to the low fat diet and observed for another 3 weeks (control 2). The experimental design is summarized in table 2.

Fasting blood samples were taken weekly throughout the control and experimental periods and twice on 2 consecutive days at the end of each period. The food was removed from the cage 16 hours before the blood sampling time. The animals were weighed weekly throughout the experiments.

This experimental pattern was repeated 4 times: 1) before thyroidectomy; 2) after thyroidectomy, without administration of thyroid hormone; 3) and 4) after thyroidectomy with administration of thyroid hormone. Experiment 2 was started one month after the thyroidectomy. Experiment 3 was started 6.5 months after the thyroidectomy and experiment 4, six weeks after the end of experiment 3.

The thyroid hormones used were D- and L-thyroxine.[3] The doses given were 4.0 and 0.3 mg/dog per day, respectively, throughout the duration of the corresponding experiments. In experiment 3 half of the dogs in each group received D-thyroxine and the other half received L-thyroxine. At the end of this experiment the treatment was discontinued for one month; the hormone treatment was then reversed and after 2 weeks experiment 4 was carried out. The hormones were given orally as pills placed inside a bolus made with a small piece of soft bread. The mouth of

[3] Sodium D-thyroxine (Choloxin), 4.0 mg tablets; kindly supplied by the Travenol Laboratories, Inc. Morton Grove, Illinois; sodium L-thyroxine (Synthroid), 0.3-mg tablets; kindly supplied by the Flint Laboratories, Morton Grove, Illinois.

TABLE 2

Design of the dietary experiments with 2 groups of dogs, using low fat, coconut oil (CNO) and sunflower oil (SFO) diets

Period	Control 1	Experimental 1	Experimental 2	Control 2
Time diet fed	3 weeks	2 weeks	2 weeks	3 weeks
Group 1	low fat	CNO	SFO	low fat
Group 2	low fat	SFO	CNO	low fat

the dog was carefully inspected to make sure that the bolus had been swallowed.

After completion of experiment 1, the dogs were thyroidectomized under sodium pentobarbital [4] anesthesia as described previously (1).

The chemical methods for the estimation of total cholesterol, lipid phosphorus, and triglycerides were those described in previous publications (1, 2). Phospholipids (mg/100 ml) were calculated as lipid phosphorus (mg/100 ml) × 25.

Basal metabolic rate was determined in the thyroidectomized dogs before and during thyroid hormone treatment. The dogs were anesthetized with sodium pentobarbital (30 mg/kg iv) and connected to a closed circuit basal metabolic rate machine [5] by means of an intratracheal tube provided with an inflatable cuff. The data presented are the mean values of 2 determinations, each representing a period of one hour.

Standard methods of statistical analysis were applied. Since the comparisons reported were made with the same animals in different experimental situations Student's *t* test for paired variates was used to test the significance of the differences. The probability values were calculated using Crow's chart (3).

RESULTS

1. Effect of coconut oil and sunflower oil on the serum lipids, before and after thyroidectomy. The mean serum lipid values of 18 dogs at the end of each of the control and experimental periods before and after the thyroidectomy are presented in table 3.

The highest serum lipid levels were observed when the animals received the coconut oil diet, both before and after the thyroidectomy. Except for the triglycerides, the serum lipid levels with the sunflower oil diet were lower than those observed with the coconut oil diet but higher than the control values. For a given diet all the lipid levels were higher after thyroidectomy, with the exception of the phospholipid values when the coconut oil diet was fed. Before thyroidectomy all the lipid values approached the initial control levels at the end of the second control period; however, after thyroidectomy the values at the end of the second control period were consistently higher than those observed at the end of the first control period.

The changes of the serum lipids associated with the addition of coconut oil or sunflower oil to the diet are described in table 4.

The effects of the experimental diets on each of the lipid fractions are presented as the differences between the levels at the end of the corresponding dietary periods and the mean of the 2 control periods. The change in the serum lipid

[4] Nembutal, Abbott Laboratories, North Chicago, Illinois.
[5] Sanborn Company, Cambridge, Massachusetts.

TABLE 3

Serum lipids at the end of each of the dietary periods, before and after thyroidectomy [1]

	Dietary periods			
	Control 1	Experimental		Control 2
	Low fat	Coconut oil	Sunflower oil	Low fat
	mg/100 ml			
	Before thyroidectomy			
Total cholesterol	114 ± 7.7 [2]	311 ± 16.8	208 ± 12.8	134 ± 7.6
Phospholipids	264 ± 17.6	597 ± 28.5	426 ± 19.6	332 ± 13.0
Triglycerides	48 ± 2.2	73 ± 4.9	47 ± 2.5	52 ± 2.1
	After thyroidectomy			
Total cholesterol	252 ± 14.2	596 ± 50.6	353 ± 17.4	317 ± 21.2
Phospholipids	391 ± 22.7	604 ± 31.2	575 ± 20.5	482 ± 22.9
Triglycerides	74 ± 3.6	150 ± 9.1	86 ± 4.6	113 ± 9.8

[1] The individual values used for the computation of the means were the means of duplicated determinations on 2 blood samples taken on the last 2 days of each period, as described.
[2] Means ± SE for 18 dogs.

TABLE 4

Blood lipid changes observed when feeding coconut oil and sunflower oil before and after thyroidectomy

	Serum lipid differences [1]			
	Coconut oil minus mean control		Sunflower oil minus mean control	
		P value		P value
	mg/100 ml		*mg/100 ml*	
Total cholesterol				
Before thyroidectomy	187 ± 13.0 [2]	< 0.0001	84 ± 8.4	< 0.0001
After thyroidectomy	312 ± 41.4	< 0.0001	68 ± 11.2	< 0.0001
Difference after thyroidectomy minus before thyroidectomy	125 ± 43.0	0.01	−16 ± 11.8	0.17
Phospholipids				
Before thyroidectomy	299 ± 27.6	< 0.0001	128 ± 16.8	< 0.0001
After thyroidectomy	168 ± 20.8	< 0.0001	138 ± 27.3	< 0.00015
Difference after thyroidectomy minus before thyroidectomy	−131 ± 34.3	0.0015	10 ± 34.4	0.75
Triglycerides				
Before thyroidectomy	23 ± 4.6	0.00015	−3 ± 2.5	0.23
After thyroidectomy	56 ± 8.9	< 0.0001	−8 ± 7.3	0.28
Difference after thyroidectomy minus before thyroidectomy	33 ± 7.4	0.0007	−5 ± 7.2	0.50

[1] Serum lipid differences were calculated by subtracting from the values at the end of the experimental periods the mean of the 2 values at the end of each of the 2 control periods.
[2] Means ± SE for 18 dogs.

responses associated with the removal of the thyroid gland are presented in table 4 as the difference: after thyroidectomy minus before thyroidectomy.

The increase of serum cholesterol and triglyceride concentration, associated with feeding the coconut oil diet was significantly greater after thyroidectomy than before. The increase of serum phospholipids when feeding the coconut oil diet was significantly less after thyroidectomy than before. Thyroidectomy, therefore, resulted in an increased response of serum total cholesterol and triglycerides to the administration of coconut oil, but a decreased response of serum phospholipids.

The effect of sunflower oil was in striking contrast with that of coconut oil. Feeding the sunflower oil diet caused significant elevations of serum cholesterol and phospholipids above the levels observed when feeding the control low fat diet, but these elevations were smaller than those caused by coconut oil and the lipid responses were not changed by thyroidectomy. As shown in table 4, the difference (after thyroidectomy minus before thyroidectomy) for total cholesterol and phospholipids was not statistically significant when the sunflower oil was fed. Feeding the sunflower oil diet caused no significant changes in serum triglycerides before or after the thyroidectomy. The data therefore indicate that the effects on the serum lipids of the unsaturated sunflower oil were not modified by removal of the thyroid gland.

2. Effect of D- *and* L-*thyroxine on the serum cholesterol levels of thyroidectomized dogs fed the low fat diet.* Before testing the effects of coconut and sunflower oils on the serum lipid levels of thyroidectomized dogs treated with thyroid hormones, the doses of the hormones administered were compared as to their effects on serum cholesterol levels while the animals were fed the low fat diet. This study was made with 12 dogs after completion of experiment 2. At this time the animals had been thyroidectomized for

about 5 months. Half of the dogs received 4.0 mg daily of D-thyroxine and the other half 0.3 mg daily of L-thyroxine. The treatment was continued for 3 weeks and then reversed for another 3 weeks. The results are shown in table 5.

TABLE 5

Effect of D-thyroxine (4.0 mg/day) and of L-thyroxine (0.3 mg/day) on total serum cholesterol concentration of dogs fed the low fat diet [1]

	Serum cholesterol
	mg/100 ml
Before thyroidectomy	126 ± 7.0 [2]
After thyroidectomy	
Before treatment (5 months after thyroidectomy)	332 ± 32.8
1 week after D-thyroxine	164 ± 11.8
2 weeks after D-thyroxine	124 ± 7.5
3 weeks after D-thyroxine	127 ± 6.5
1 weeks after L-thyroxine	202 ± 26.8
2 weeks after L-thyroxine	184 ± 19.6
3 weeks after L-thyroxine	168 ± 17.4
Difference 3 weeks L-thyroxine minus 3 weeks D-thyroxine	41 ± 15.5 ($P = 0.02$)

[1] Mean weight of the dogs was 20.8 kg.
[2] Mean values ± SE for 12 dogs.

The data show that both treatments caused marked decreases of serum cholesterol levels in the thyroidectomized dogs. D-Thyroxine reduced the serum cholesterol in 2 weeks to the pre-thyroidectomy level. L-Thyroxine also produced a marked decrease of serum cholesterol, but the values did not return to the pre-thyroidectomy level. The mean cholesterol level observed after 3 weeks of treatment with L-thyroxine was, on the average, 41 mg/100 ml (± 15.5) higher than that observed after 3 weeks on D-thyroxine treatment ($P = 0.02$).

3. *Effects of thyroidectomy and thyroid hormone replacement with* D- *and* L-*thyroxine on the responses of serum lipids to administration of coconut oil and sunflower oil.* The results obtained in 12 dogs are summarized in tables 6, 7, and 8. The serum lipid values for the experimental dietary periods before thyroidectomy and after thyroidectomy with and without thyroid hormone replacement are shown. These dogs were part of the group of 18

TABLE 6

Effect of feeding the coconut oil (CNO) and the sunflower oil (SFO) diets on total serum cholesterol concentration, before and after thyroidectomy and under thyroid hormone replacement

	Total serum cholesterol					
	Dietary periods				Differences [1]	
	Control 1	Experimental		Control 2	ΔCNO	ΔSFO
	Low fat	CNO	SFO	Low fat		
	mg/100 ml				*mg/100 ml*	
Before thyroidectomy	115 ± 9.8 [2]	316 ± 22.9	209 ± 15.3	140 ± 10.0	189 ± 17.0 ($P < 0.0001$)	82 ± 8.9 ($P < 0.0001$)
After thyroidectomy (no treatment)	239 ± 14.6	576 ± 41.0	364 ± 21.9	326 ± 17.8	294 ± 32.6 ($P < 0.0001$)	82 ± 14.2 ($P < 0.0001$)
After thyroidectomy (4.0 mg D-thyroxine daily)	122 ± 7.3	277 ± 18.0	187 ± 13.4	132 ± 8.0	150 ± 14.2 ($P < 0.0001$)	60 ± 9.9 ($P < 0.0001$)
After thyroidectomy (0.3 mg L-thyroxine daily)	134 ± 6.8	294 ± 20.6	191 ± 11.5	135 ± 9.9	160 ± 17.9 ($P < 0.0001$)	57 ± 8.3 ($P < 0.0001$)

[1] ΔCNO = mean of differences between value at the end of the coconut oil period and mean of the 2 control values.
ΔSFO = mean of differences between value at the end of the sunflower oil period and mean of the 2 control values.
[2] Means ± SE for 12 dogs. The individual values used for the computation of the means were the means of determinations on 2 blood samples taken on the last 2 days of each period, as described.

animals presented in tables 3 and 4. The mean values for the 12 dogs before and after thyroidectomy in the 4 dietary situations were comparable to the corresponding values for 18 dogs shown in tables 3 and 4.

Table 6 presents the data on serum total cholesterol. The effects of feeding coconut oil and sunflower oil are shown in the columns headed ΔCNO and ΔSFO. The results indicate that hormone replacement with D- or with L-thyroxine returns the serum cholesterol response of thyroidectomized dogs to the administration of coconut oil, to the level observed before thyroidectomy.

The serum cholesterol response to the administration of sunflower oil was the same before and after thyroidectomy. When sunflower oil was given to the thyroidectomized dogs treated with thyroid hormones the serum cholesterol responses were smaller; however, only the difference observed during L-thyroxine treatment was statistically significant ($P = 0.005$).

The experimental design allows a critical comparison of the difference between the effects of coconut oil and sunflower oil on the serum lipids. Before thyroidectomy the serum cholesterol level was higher by 107 ± 12.9 mg/100 ml ($P = 0.0001$) when feeding the coconut oil diet than when feeding the sunflower oil. The corresponding differences after thyroidectomy without treatment and after thyroidectomy with D-thyroxine and with L-thyroxine replacement were 212 ± 36.8, 90 ± 8.0 and 103 ± 14.4 mg/100 ml, respectively. All these differences were highly significant ($P < 0.00015$).

The serum cholesterol difference between the coconut oil and the sunflower oil dietary periods was higher by 105 ± 39.4 mg/100 ml after the thyroidectomy than before ($P = 0.02$). The value of this difference, however, was not significantly different from that observed before thyroidectomy when the thyroidectomized dogs were treated with either D- or L-thyroxine ($P = 0.2$ and 0.7, respectively). These observations show that thyroidectomy increased the difference in cholesterol levels between the coconut oil and sunflower oil dietary periods, and that the difference returned to the values observed

TABLE 7

Effect of feeding the coconut oil (CNO) and the sunflower oil (SFO) diets on total serum phospholipids, before and after thyroidectomy and under thyroid hormone replacement

	Total serum phospholipids					
	Dietary periods				Differences [1]	
	Control 1	Experimental		Control 2	ΔCNO	ΔSFO
	Low fat	CNO	SFO	Low fat		
	mg/100 ml				*mg/100 ml*	
Before thyroidectomy	272 ± 21.6 [2]	604 ± 38.2	431 ± 22.2	338 ± 14.7	269 ± 35.2 ($P < 0.0001$)	126 ± 21.3 ($P < 0.0001$)
After thyroidectomy (no treatment)	392 ± 19.3	613 ± 27.1	561 ± 23.4	504 ± 28.0	165 ± 21.8 ($P < 0.0001$)	113 ± 29.0 ($P = 0.003$)
After thyroidectomy (4.0 mg D-thyroxine daily)	324 ± 16.5	565 ± 19.8	436 ± 19.0	288 ± 15.6	259 ± 17.7 ($P < 0.0001$)	130 ± 15.3 ($P < 0.0001$)
After thyroidectomy (0.3 mg L-thyroxine daily)	338 ± 12.3	589 ± 18.9	451 ± 14.0	309 ± 20.2	265 ± 16.1 ($P < 0.0001$)	127 ± 11.9 ($P < 0.0001$)

[1] ΔCNO = mean of differences between value at the end of the coconut oil period and mean of the 2 control values.
ΔSFO = mean of differences between value at the end of the sunflower oil period and mean of the 2 control values.
[2] Means ± SE for 12 dogs. The individual values used for the computation of the means were the means of determinations on 2 blood samples taken on the last 2 days of each period, as described.

in the pre-thyroidectomy period when the animals were treated with D- and with L-thyroxine.

The serum phospholipid levels and the changes associated with feeding of coconut oil (CNO) or sunflower oil (SFO) are presented in table 7. The increase of serum phospholipids produced by feeding coconut oil was smaller after thyroidectomy than before by 134 ± 44.9 ml/100 ml ($P = 0.011$). The serum phospholipid responses to the administration of sunflower oil were essentially the same in the 4 experimental situations.

Comparison of the effects of coconut oil and sunflower oil showed that the CNO-SFO phospholipid differences were statistically, not significantly different from each other before the thyroidectomy and after the thyroidectomy with either of the 2 treatments. The CNO-SFO phospholipid difference after the thyroidectomy without treatment, however, was only 52 ± 39.2 mg/100 ml ($P = 0.18$).

The triglyceride data are presented in table 8. Feeding the coconut oil diet caused a significantly greater elevation of triglycerides after the thyroidectomy ($P = 0.0003$). Thyroid hormone replacement returned the triglyceride response to the pre-thyroidectomy level. Thus thyroidectomy increased the elevation of serum triglycerides associated with the administration of coconut oil, and this effect was corrected by thyroid hormone replacement.

Administration of sunflower oil had no significant effect on the serum triglycerides in any of the 4 experimental situations. The serum triglyceride differences between the coconut oil and the sunflower oil dietary periods was 22 ± 6.4 mg/100 ml before the thyroidectomy and 60 ± 11.7 mg/100 ml after the thyroidectomy without hormone replacement. Both differences were statistically significant ($P = 0.006$ and 0.0003, respectively) as was the difference between these 2 mean values (38 ± 10.5, $P = 0.004$). The corresponding differences between coconut oil and sunflower oil with hormone replacement were not significantly different from those observed before the thyroidectomy.

4. Body weight and food intake. Body weight of the animals showed little change during the 4 periods of each experiment. All the animals gained some weight after

TABLE 8

Effect of feeding the coconut oil (CNO) and the sunflower oil (SFO) diets on serum triglycerides, before and after thyroidectomy and under thyroid hormone replacement

	Serum triglycerides					
	Dietary periods				Differences [1]	
	Control 1	Experimental		Control 2	ΔCNO	ΔSFO
	Low fat	CNO	SFO	Low fat		
	mg/100 ml				*mg/100 ml*	
Before thyroidectomy	46 ± 2.3 [2]	65 ± 4.9	43 ± 2.7	50 ± 2.9	17 ± 4.2 ($P = 0.0002$)	−5 ± 1.8 ($P = 0.10$)
After thyroidectomy (no treatment)	71 ± 4.2	145 ± 13.2	85 ± 5.3	114 ± 11.9	53 ± 12.1 ($P = 0.01$)	−7 ± 10.8 ($P = 0.5$)
After thyroidectomy (4.0 mg D-thyroxine daily)	65 ± 3.3	86 ± 3.3	69 ± 5.3	64 ± 3.4	22 ± 2.7 ($P < 0.0001$)	5 ± 4.5 ($P = 0.25$)
After thyroidectomy (0.3 mg L-thyroxine daily)	64 ± 2.2	92 ± 5.2	64 ± 3.2	66 ± 3.5	27 ± 4.7 ($P = 0.00015$)	1 ± 2.8 ($P = 0.10$)

[1] ΔCNO = mean of differences between value at the end of the coconut oil period and mean of the 2 control values.
ΔSFO = mean of differences between value at the end of the sunflower oil period and mean of the 2 control values.

[2] Means ± SE for 12 dogs. The individual values used for the computation of the means were the means of duplicated determinations on 2 blood samples taken on the last 2 days of each period, as described.

thyroidectomy but their weights were constant by experimental period 2 and remained practically unchanged thereafter. The mean body weight for the 18 dogs in tables 3 and 4 before thyroidectomy was 18.6, 18.2, 18.2 and 18.8 kg at the end of the control 1, CNO, SFO, and control 2 periods, respectively. After the thyroidectomy the corresponding values were 20.4, 20.7, 20.6 and 21.0 kg. For the 12 dogs in tables 6, 7, and 8 the corresponding weights were 19.0, 18 1, 18.1, and 18.8 kg before the thyroidectomy, 20.6, 20.8, 20.8 and 21.1 kg after the thyroidectomy without treatment, 20.6, 19.8, 19.8 and 20.1 kg after thyroidectomy and D-thyroxine treatment, and 21.0, 21.4, 20.6 and 20.4 kg after thyroidectomy and L-thyroxine treatment. The mean food intake for the 18 dogs before thyroidectomy varied between 80 and 85 kcal/kg per day and decreased to 70 kcal/kg per day after the thyroidectomy. The mean food intake values for the 12 dogs studied under the 4 experimental situations and for the 2 experimental diets are given in table 9.

5. *Effect of thyroid hormone replacement on the oxygen consumption of anesthetized thyroidectomized dogs.* Measurements of oxygen consumption under sodium pentobarbital anesthesia were made between experimental periods 3 and 4 in 10 thyroidectomized dogs fed the low fat diet with and without thyroid hormone replacement. The determinations were first made when the animal had been given thyroid hormone continuously for 3 months and were repeated after thyroid hormone had been discontinued for 3 weeks. The mean oxygen consumption for the 10 dogs on thyroid hormone treatment was 4.42 ± 0.14 ml O_2/kg per minute and after discontinuing treatment dropped to 3.41 ± 0.15 ml O_2/kg per minute. The mean difference (1.01 ± 0.18 ml O_2/kg per minute) was significant ($P = 0.0005$). Since half of the dogs were treated with D-thyroxine and the other half with L-thyroxine the effects of these 2 hormones were compared. The mean decrease in oxygen consumption after discontinuing D-thyroxine in 5 dogs was 1.35 ± 0.27 ml O_2/kg per minute as compared with a decrease of 0.67 ± 0.15 ml O_2/kg per minute observed in the other 5 dogs when L-thyroxine was discontinued. These 2 differences were significant ($P = 0.007$ and 0.012, respectively) but not significantly different from each other when tested by the t test for non-paired variates ($P = 0.06$). Although the administration of thyroid hormones produced an increase in oxygen consumption of the thyroidectomized dogs, the values were lower than the mean value of 5.97 ± 0.16 ml O_2/kg per minute observed in 8 normal dogs fed the low fat diet.

TABLE 9

Mean food intake of 12 dogs for each of the experimental situations

Experimental situation	Diet	
	Coconut oil	Sunflower oil
	kcal/kg/day	
Before thyroidectomy	85	83
After thyroidectomy (no treatment)	72	71
After thyroidectomy (4.0 mg D-thyroxine/dog/day	73	74
After thyroidectomy (0.3 mg L-thyroxine/dog/day	73	75

DISCUSSION

That serum lipid levels, cholesterol in particular, become elevated in human hypothyroidism and after thyroidectomy in some animals has been known for many years (4–7); however, there have been few studies of the effects of thyroidectomy upon the responses of serum lipids to the administration of dietary fat. It has been said (8) that the endocrine influence on cholesterol metabolism is quantitatively of a lower order of magnitude than the influence of dietary changes. The present study demonstrates that removal of the thyroid gland in dogs causes large changes of serum cholesterol concentration in the absence of any dietary change. It further shows that the changes in serum lipid concentration caused by some dietary fats are modified by the level of thyroid function, and that different lipid fractions are affected differently by thyroidectomy.

The results reported here demonstrate that thyroidectomy enhances the elevation of serum cholesterol and triglycerides pro-

duced by feeding coconut oil and that this effect is abolished by thyroid hormone replacement.

There has been considerable disagreement as to the influence of thyroid function on the serum triglyceride levels (9–12) and the relationships between serum cholesterol and triglycerides in hypothyroidism (13, 14). Our results show that thyroidectomy increases the serum triglycerides in dogs fed a low fat diet. However, statistical analysis of our data revealed no significant correlation between serum cholesterol and serum triglycerides before or after thyroidectomy, in this dietary situation.

The response of the serum phospholipids of the thyroidectomized dog to the administration of coconut oil was markedly different from that of cholesterol and triglycerides. Coconut oil caused a smaller elevation of the serum phospholipids after the thyroidectomy than before and the values returned to pre-thyroidectomy levels when the thyroidectomized animals were given thyroid hormones (1). This observation indicates that the circulating lipoproteins of the thyroidectomized dog fed coconut oil have a higher proportion of cholesterol and triglycerides and a lower proportion of phospholipids than those of the normal dog, which maintains a remarkably constant proportion between cholesterol and phospholipids under a variety of dietary conditions (15). That serum cholesterol increases proportionally more than the serum phospholipids has been observed in thyroidectomized dogs (7, 16) and in hypothyroid patients (5, 17). A marked increase of β-lipoprotein has been noted in thyroidectomized dogs (16).

Thyroidectomy had no significant influence on the responses of the serum lipids to the administration of sunflower oil. The changes observed when the thyroidectomized dogs, with or without treatment, were fed sunflower oil were essentially the same as observed before the thyroidectomy. This result could be interpreted as reflecting a difference in the mechanisms by which coconut oil and sunflower oil influence the serum lipid levels. Our observations support the concept that the mechanism by which coconut oil influences serum lipid levels involves some rate-limiting step (or steps) controlled by the activity of the thyroid gland, whereas sunflower oil influences the concentration of serum lipids by mechanisms largely independent of the activity of the thyroid.

The hormones of the thyroid gland have been reported to influence a number of reactions in lipid metabolism (8, 18–20) but the relevance to the findings discussed here is still obscure. Most investigators believe that the changes in serum cholesterol concentration associated with changes in thyroid activity are mainly dependent on the effects of thyroid hormone upon the degradation of cholesterol to bile acids and the excretion of sterols (8, 18–21). It is unlikely that the effect on serum cholesterol can be explained by alteration of intestinal absorption of exogenous cholesterol and that the increased serum cholesterol response to coconut oil in the thyroidectomized dogs is related to an increased absorption of exogenous cholesterol. The cholesterol content of the diets used was very low (15.0 mg/100 g in the low fat diet) and we have demonstrated that the effect of coconut oil on the serum cholesterol concentration of normal dogs is practically the same whether the coconut oil is added to a cholesterol-free diet or to a diet of the same cholesterol content as that used in the present experiment.[6]

Since thyroid hormones stimulate phospholipid synthesis (22), it could be postulated that the lack of elevation of serum phospholipids above the level of the low fat diet, when thyroidectomized dogs are fed coconut oil, is due to deficient phospholipid synthesis. Such an explanation appears to be incompatible with our observation that with feeding of sunflower oil the thyroidectomized dog shows an elevation of the serum phospholipids similar to that observed when feeding this oil before the thyroidectomy. However, since the effects of coconut oil and sunflower oil on the serum lipids are quantitatively different, is is possible that the discrepancy between cholesterol and phospholipid changes observed in the thyroidectomized dogs fed coconut oil, might

[6] Grande, F. 1965 Effect of saturated fat on dog serum lipids in the absence of dietary cholesterol. Federation Proc., 24: 227 (abstract).

also be observed by feeding higher proportions of sunflower oil than those used here. It is worth noting in this respect that the cholesterol-to-phospholipid ratios observed in our experiments were higher after the thyroidectomy without thyroid hormone replacement than in any of the other 3 experimental situations, for each of the 3 dietary conditions. The changes of serum phospholipid concentration were correlated with the changes of serum cholesterol when feeding coconut oil, both before and after thyroidectomy. The correlation coefficients for 18 dogs were 0.79 before the thyroidectomy and 0.61 afterward and both were significant ($P < 0.01$). The slopes of the regression equations were 1.68 before the thyroidectomy and 0.33 afterward, and they were significantly different from each other ($P < 0.001$). This indicates that a given increase of serum cholesterol concentration is associated with a greater increase of phospholipids before than after thyroidectomy, but that the thyroidectomized dog still responds with some increase of serum phospholipids to the administration of coconut oil. It appears that the thyroidectomized dog has a limited ability to produce or mobilize phospholipids to maintain the normal cholesterol-to-phospholipid ratio when serum cholesterol concentration increases. The discrepancy between cholesterol and phospholipids is apparent with the low fat diet and becomes exaggerated when feeding coconut oil because of the high levels of serum cholesterol observed in this dietary situation.

While no explanation of the mechanism by which thyroid hormones influence the responses of serum lipids to changes of fat in the diet can be offered at this time, the results clearly indicate that lack of thyroid hormone has a marked effect on serum lipid responses to dietary fat and that this influence depends on the nature of the fat fed. Whether the differences between the effects of coconut oil and sunflower oil reported here are due to the different degree of saturation of these 2 oils, must await more extensive work with other dietary fats. It is apparent from our data that the D-thyroxine and L-thyroxine in the dosage used in these experiments had similar effects. It appears, however, that 0.3 mg/dog per day of L-thyroxine had less effect than 4.0 mg/dog per day of D-thyroxine in reducing serum cholesterol concentration when the thyroidectomized dogs received the low fat diet.

Administration of thyroid hormones to the thyroidectomized dogs produced a consistent increase of oxygen consumption, as well as a decrease of serum cholesterol concentration. The serum cholesterol levels of these animals returned to values close to those observed before the thyroidectomy, but as noted the oxygen consumption was still well below the values observed in normal dogs. This is in agreement with other reports showing that low doses of thyroid hormones lower serum cholesterol levels before producing a noticeable increase of oxygen consumption (19, 23–25).

Because of the differences between dog and man with respect to the effects of dietary fat on the blood lipid levels (15), it is difficult to assess the relevance of these observations for the human species. That thyroidectomy not only increases the serum lipid levels, but also modifies the responses to dietary fat suggests that the level of thyroid activity may be a factor to be considered in explaining the differences in serum lipid responses observed between supposedly comparable individuals subjected to identical dietary change (26). There is evidence that hypothyroid individuals may show some differences in response to dietary changes as compared with normal individuals. Thus the data reported by O'Hara et al. (14) indicate that hypothyroid individuals failed to show the expected decrease of serum cholesterol (27) when changing from a fat-free diet to a diet containing a high proportion of corn oil. This response, however, was not modified by thyroxine administration.

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Effects of Starch and Sucrose on the Serum Lipids of Dogs before and after Thyroidectomy [1]

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ABSTRACT The effect of dietary starch and sucrose on the serum lipids was studied in 18 male dogs before and after total thyroidectomy. The diets consisted of a mixture of a low fat, low cholesterol dog food (55 parts) and either starch or sucrose (45 parts). The starch diet provided 47.6% of the calories as starch and the sucrose diet 49.0% of the calories as sucrose. The diets were fed for 2 weeks in a reversal design. Total serum cholesterol, phospholipids and triglycerides were the same for the 2 dietary situations, before thyroidectomy. After thyroidectomy the sucrose diet caused serum triglyceride levels 13 mg/100 ml (±4.3) higher than the starch diet. This difference was statistically significant ($P = 0.006$). The difference in triglyceride level between the sucrose and the starch diets was 11 mg/100 ml (±5.2) higher after the thyroidectomy than before and this difference has a probability of chance occurrence of $P = 0.05$. The absence of a difference between the serum lipid levels caused by feeding either starch or sucrose to normal dogs may be related to the low cholesterol content of the diets used.

A number of reports in the literature indicate that the nature of the dietary carbohydrates influences the serum lipid levels in various animal species (1). In 1956 Portman et al. (2) reported a comparison of the effects of cornstarch, sucrose and fructose as the sole sources of dietary carbohydrates, on the serum cholesterol levels of rats fed diets with added cholesterol and cholic acid. These studies indicated that the starch diets caused lower serum cholesterol levels than either sucrose or fructose. Rabbits and chickens fed diets with added cholesterol have also been reported to have higher serum cholesterol levels when the diet contained sucrose than when it contained glucose (3).

The dog responds with marked changes of the serum lipid levels to changes in the amount and kind of fat in the diet (4), but little is known about the effect of the dietary carbohydrates on the serum lipids of the dog.

The experiments described here offer a comparison of the effects of starch and sucrose on the serum lipid levels of dogs before and after total thyroidectomy.

METHODS

The animals used in this study were the same as described in our previous paper (5). The experimental diets were prepared by mixing 55 parts of a low fat commercial dog food [2] with 45 parts of either cornstarch or sucrose. From the analysis of the components the following approximate distribution of dietary calories was calculated: (in percent) protein, 12; fat, 1; carbohydrates from the commercial diet, 39; and experimental carbohydrate, 48. Due to the differences in water content and caloric value between sucrose and starch there was a small discrepancy between the 2 diets with respect to the distribution of calories, sucrose providing 49% of the calories and starch, 47.6%.

The animals were fed the commercial (control) diet except for the periods of experimental feeding. The experimental diets were fed for 2 weeks each in a reversal pattern. Half of the dogs first received the starch diet, and the other half the sucrose diet. At the end of 2 weeks the diets were interchanged for another 2 weeks. The serum lipid values reported are means of 2 fasting blood samples taken on the last 2 days of each experimental period.

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[2] Kibbies, Morton Dog Food Company, Minneapolis.

TABLE 1

Serum lipid values of dogs fed the sucrose and starch diets before and after thyroidectomy

	Diets		Difference	
	Starch	Sucrose	Sucrose minus starch	
				P value
	mg/100 ml		*mg/100 ml*	
Before thyroidectomy				
Total cholesterol	137± 7.5 [1]	137± 8.0	0± 3.7	—
Phospholipids	310±15.7	314±12.2	4±13.7	0.80
Triglycerides	47± 2.3	50± 2.0	2± 1.9	0.30
After thyroidectomy				
Total cholesterol	304±21.4	292±20.9	−12±20.8	0.55
Phospholipids	468±17.1	442±11.4	−26±17.6	0.15
Triglycerides	100± 6.0	113± 5.9	13± 4.3	0.006

[1] Means ± SE for 18 dogs.

The methods of analysis used were those described in our previous paper (5).

RESULTS

The serum lipid values at the end of each of the 2 dietary periods are given in table 1. The differences between starch and sucrose are shown in the last column of the table, which also presents the corresponding values after thyroidectomy. Before thyroidectomy there were no significant differences between the sucrose and starch dietary periods for any of the three serum lipid fractions.

When the experiment was repeated with the same dogs 2 months after thyroidectomy the sucrose diet caused somewhat lower values of cholesterol and phospholipids than the starch diet, but the differences were not statistically significant (see table 1). The serum triglycerides, however, were slightly higher when the animals ate the sucrose diet than when they ate the starch diet, and the difference was statistically significant, as shown in the table. The triglyceride elevation associated with the substitution of sucrose for starch in the diet of the thyroidectomized dogs (13 mg/100 ml ± 4.3) was higher by 11 mg/100 ml (± 5.2) than that observed before the thyroidectomy. This difference has a probability of chance occurrence of $P = 0.05$.

All the serum lipid values were higher after thyroidectomy than before. Serum cholesterol showed a proportionally greater increase after thyroidectomy than the phospholipids. The cholesterol-to-phospholipid ratio before thyroidectomy was 0.44 and after thyroidectomy 0.66 for both diets. The data in table 1 show therefore that there is no significant difference for any of the 3 lipid fractions between the sucrose and starch diets in the normal dog, and that sucrose causes a small but significant elevation of the serum triglycerides above the level with the starch diet in the thyroidectomized animal.

The weight of the animals was constant throughout the 4 weeks of each experiment, but all the dogs showed some increase of weight after the thyroidectomy as previously reported (5).

DISCUSSION

The absence of any significant difference in the serum lipid levels when normal dogs are fed either starch or sucrose is at variance with observations in other animals which indicate that starch (or glucose) diets produce lower serum cholesterol levels than sucrose diets. The different behavior of the dog may, of course, be due to species difference but it is also possible that this finding is the consequence of the low cholesterol content of the diets used. Several reports in the literature recently reviewed (1) indicate that the serum cholesterol differences between diets containing different carbohydrates in various animal species tend to disappear in the absence of dietary cholesterol. The cholesterol content of our experimental diets was of the order of 8 mg/100 g, whereas the diets used in most of the experiments just mentioned had added cholesterol in much

higher proportions. Thus Grant and Fahrenbach (3) used 3% of cholesterol and Portman et al. (2), 5% If the absence of a serum cholesterol difference between the sucrose and starch dietary periods is in fact due to the low cholesterol content of our diets it would underline a striking difference between carbohydrates and fats with respect to their effects on the serum cholesterol concentration of the dog. We have recently shown that the elevation of serum cholesterol produced in this animal by administration of coconut oil also takes place when coconut oil is added to a cholesterol-free diet.[3]

The serum cholesterol levels observed at the end of the periods of feeding starch and sucrose were similar to those observed when the animals were eating the control diet (5), showing that an increase in the proportion of dietary carbohydrates from 75 to 87% of the total caloric intake for 2 weeks had no effect on the serum lipids of the dog.

Thyroidectomy caused the well-known elevation of the serum lipid levels (6), and the elevations of cholesterol and phospholipids were of the same order of magnitude as those observed when the animals were fed the commercial dog food.

As previously noted (5), the cholesterol level shows a greater increase after thyroidectomy than the serum phospholipid level, as evidenced by the higher cholesterol-to-phospholipid ratios. Thus in the thyroidectomized animal, the serum phospholipid level does not parallel the elevation of the serum cholesterol mentioned in our previous publication and this is also apparent with the high carbohydrate diets examined here.

The only difference observed between the starch and the sucrose diets was in the serum triglyceride levels. While no difference between these dietary periods was noted in the euthyroid animals, the sucrose diet caused significantly higher levels of triglycerides than the starch diet after thyroidectomy. It appears, therefore, that in the absence of thyroid, sucrose has a small but significant effect, increasing the level of serum triglycerides in the dog above the levels observed with the starch diet. In view of the small magnitude of this effect it appears unlikely, however, that the large differences in serum lipids between starch and sucrose diets observed in hyperlipemic subjects can be explained by a functional deficiency of the thyroid gland (1, 7).

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Effect of Feeding DL-Ethionine on the Polyunsaturated Fatty Acids of Rat Liver Phospholipids [1]

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ABSTRACT An experiment was carried out to investigate the effect of dietary ethionine on the essential fatty acid composition of rat liver phospholipids. Male and female rats were fed, for 14 days, a 9% casein diet supplemented with either 0.25% DL-ethionine, 0.5% DL-ethionine or 0.25% DL-ethionine plus 0.5% DL-methionine. At the end of the feeding period, livers were removed, their lipids were extracted and separated into triglycerides and phospholipid fractions, and their fatty acids were determined. While ethionine caused variable concentrations of triglyceride in both sexes, it had very little effect on the fatty acid composition of that fraction. Hepatic phospholipid concentrations, however, were much more consistent and similar for both sexes. The percentage of arachidonic acid in this lipid was significantly reduced in both sexes by ethionine, whereas the proportions of linoleic and oleic acids were increased. In both sexes, the largest phospholipid fraction, the phosphatidyl cholines showed the same changes as did the whole phospholipid, but they were more pronounced. The ethionine also decreased the percentage of stearic acid in this fraction, but the change was only significant in females. Only arachidonic acid and a pentaenoic acid were reduced in the phosphatidyl ethanolamines of both sexes by ethionine. Supplementation of the ethionine diet with methionine prevented most of the fatty acid changes in the phospholipid fractions. The results showed, therefore, that dietary ethionine exerts an influence on the metabolism of the phospholipid polyunsaturated fatty acids of both sexes by decreasing the relative amounts of arachidonic acid at the same time that linoleic acid is being increased. Possible reasons for the observed fatty acid changes are discussed.

A wide range of metabolic lesions is produced in animals administered ethionine, the ethyl analogue of methionine, either by injection or by feeding in the diet (1). Prominent among them are defects in lipid metabolism, characterized by accumulation of triglyceride in the liver (2, 3), decreased fatty acid oxidation (4) and decreased serum lipids and lipoproteins (5, 6). The depressed fatty acid oxidation and hepatic lipid accumulation are sex-dependent, and occur only in female rats. Farber and Segaloff (7) have reported that liver lipid accumulation may be completely prevented in castrated rats by testosterone administration. Thus, the male hormone appears to be the protective agent.

Most, if not all, of the ethionine-induced changes in normal lipid metabolism have been considered secondary to inhibition of protein or lipoprotein synthesis (8–10). Recently, considerable evidence has indicated that ethionine, at least under certain conditions of administration, may inhibit protein synthesis by depleting concentrations of available adenosine triphosphate (11–14).

During recent studies (15) on the effect of dietary ethionine and ATP on pancreatic exocrine function, we observed that the essential fatty acid patterns of liver phospholipids were altered when relatively small concentrations of DL-ethionine (0.25%) were included in a low protein diet. The experiment described here was therefore conducted to confirm this observation and to find out how the major liver phospholipids — the phosphatidyl cholines and phosphatidyl ethanolamines — were affected. Both male and female rats were used, to determine whether the effect of ethionine on this parameter was influenced by sex.

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MATERIALS AND METHODS

Animals and diets. Male and female rats (Sprague-Dawley strain), weighing between 100 to 110 g, were housed individually in raised screen-bottom cages. The animals were fed the low protein, 9% casein basal diet (table 1) before start of the experiment, or until body weight had stabilized. The animals were then divided into groups, and each group was fed its respective diet for 14 days. The pair-fed control groups of both sexes were not truly pair-fed, but had their daily food intake restricted to the average intake (that of the previous day) of the group fed 0.25% DL-ethionine. All other groups were allowed free access to food and water at all times. After an overnight fast, the animals were killed, and bled by decapitation. The livers were removed, weighed, frozen on solid carbon dioxide and lyophilized.

Extraction of liver lipids. The dehydrated livers were pulverized and extracted twice with 100-ml portions of chloroform: methanol (2:1, v/v) containing 0.1 mg hydroquinone. The extract was taken to dryness on a rotary evaporator, and the lipid residue was re-extracted into petroleum ether (boiling range 30°–35°).

Lipid analyses. Total lipid was determined on the extract by the method of Bragdon (16), and lipid phosphorus by the procedure of Sumner (17). Triglycerides and phospholipids were initially separated from the neutral lipids by silicic acid-column chromatography (18). Lipid and phosphorus recoveries from this procedure averaged about 95%. Fatty acids of the fractions were transmethylated (19) and determined by gas chromatography.

Phospholipids were separated further, into phosphatidyl choline and phosphatidyl ethanolamine fractions, by thin-layer chromatography (TLC) (20). The phopholipid bands were visualized under ultraviolet light after being sprayed with 2′,7′ dichlorofluoroscein. The silica gel containing the phospholipid fraction was scraped into centrifuge tubes and transmethylated directly. Methyl esters were eluted with methanol, then treated as described previously (19). Phosphorus was analyzed in the aqueous phase. Total recovery of the phosphorus averaged 90% or more. Only phosphorus was determined on the sphingomyelins, lyso-, inositol-, and serine-phosphatides. These contained an average of 25 to 30% of the total phospholipid phosphorus, but less than 10% of the total polyunsaturated fatty acids. Gas chromatography was performed on an F&M (Model 810) and an Aerograph (Model 204) instrument equipped with hydrogen flame. Details of columns and standardization have been reported previously (21). Chain lengths of the major fatty acids greater than arachidonic acid were determined by hydrogenation and comparison of retention time with behenic acid (22:0). The number of double bonds was determined by com-

TABLE 1

Composition of diets

	Basal	0.25% DL-ethionine	0.5% DL-ethionine	0.25% DL-ethionine 0.5% DL-methionine
	%	%	%	%
Casein	9.0	9.0	9.0	9.0
Sucrose	79.85	79.6	79.35	79.1
Corn oil	4.0	4.0	4.0	4.0
DL-Ethionine [1]	—	0.25	0.50	0.25
DL-Methionine [1]	—	—	—	0.50
Salts, USP XIV [2]	5.0	5.0	5.0	5.0
Fat-soluble vitamins [3]	1.0	1.0	1.0	1.0
Vitamin mix [4]	1.0	1.0	1.0	1.0
Choline chloride	0.15	0.15	0.15	0.15

[1] Obtained from Cal Biochem, Los Angeles.
[2] Obtained from Nutritional Biochemicals Corporation, Cleveland.
[3] Corn oil fortified so that 1 g provided, per 100 g diet; vitamin A acetate, 400 IU; vitamin D_2, 200 IU; α-tocopherol, 10.0 mg.
[4] Mixed in sucrose so that 1 g mix provided, in mg/100 g diet; thiamine·HCl, 0.5; riboflavin, 0.5; nicotinic acid, 2.5; Ca pantothenate, 2.0; pyridoxine·HCl, 0.25; menadione, 0.05; folic acid, 0.02; biotin, 0.01; and vitamin B_{12} (as 0.1% mannitol triturate), 2.0.

parison of relative retention times with a 22:6ω3 standard. Because of insufficient material, no definitive characterization of the position of the double bonds was obtained. However, estimation, on semilog plots, of retention time versus carbon-chain length, which, according to Ackman and Burgher (22), separates fatty acid homologues into certain families, indicated the 22:6 to be 4,7,10,13,16,19-docosahexaenoic acid and the 22:5 to be 4,7,10,13,16-docosapentaenoic acid. In the shorthand nomenclature proposed by Holman (23), these would be 22:6ω3 and 22:5ω6.

Statistical comparisons were made using Student's *t* test as outlined by Snedecor (24). Differences were significant if $P < 0.05$.

RESULTS AND DISCUSSION

Table 2 shows some of the overall effects of feeding ethionine on growth, liver weight and total lipid and phospholipid content. Although a relatively small amount of ethionine was fed (0.25% ethionine may be compared with the approximately 0.3% methionine supplied by the 9% casein diet), it severely depressed food intake and growth in both sexes. While liver size in relation to body weight appeared to be increased by the metabolic antagonist, the differences were not significant. There were also no significant differences in liver lipid concentrations in the male animals because of high concentrations of liver lipid in control groups and the wide variation in the experimental groups. Only the group fed the low level of ethionine had liver phospholipid concentrations significantly higher ($P < 0.01$) than those of the pair-fed control. Pair-feeding apparently had as much effect on the liver phospholipids as did the ethionine, since pair-fed controls had more phospholipid than the ad libitum controls. Consequently, most comparisons will be made between pair-fed controls and experimental groups.

Female rats showed similar changes except that the liver lipid concentrations in the pair-fed group were lower and very near the values obtained previously with female rats fed the same diet (15). Ethionine appeared to increase the accumulation of lipid in livers of the female rats, as compared with the controls. Wide individual variation in this group, however, vitiated any significance. However, animals fed ethionine diets supplemented with methionine had the most liver fat of any of the groups. Reasons for this are not clear, but the greater growth of this group and the possible interference of methionine utilization in the synthesis of choline by ethionine (25) may have increased choline requirements and precipitated a moderate choline deficiency. The ethionine and choline content of our diet and the length of the ex-

TABLE 2

Body weight and liver lipids of rats fed a 9% casein basal diet with and without DL-*ethionine*

Experimental groups	Final body wt	Liver wt	Liver lipid	Liver phospholipid [1]
	g	*g/100 g body wt*	*mg/g*	*mg/g*
		Males		
Ad libitum [2] control(3)	100±5 [3]	3.5±2.0	69.3± 8.9	29.7±0.5
Pair-fed control(3)	92±2	3.6±2.7	90.7±14.0	33.8±0.9
0.25% DL-ethionine(4)	84±2	4.8±2.2	87.7±17.5	40.5±1.2
0.5% DL-ethionine(4)	71±1	4.5±1.9	56.6± 8.8	36.4±1.7
0.25% DL-ethionine + 0.5% DL-methionine(4)	94±2	4.6±0.9	86.6± 5.5	34.1±2.0
		Females		
Pair-fed control (4)	103±2	3.7±1.4	46.3± 1.3	35.6±0.8
0.25% DL-ethionine (3)	81±7	4.5±0.4	65.3±14.2	37.5±0.6
0.25% DL-ethionine + 0.5% DL-methionine (4)	111±1	4.2±0.7	186.0±37.0	41.8±9.9

[1] Phosphorus x 25.
[2] Values in parentheses indicate number of rats/diet group.
[3] Mean ± SE.

periment were similar to those of Seidel and Harper (5), who found no beneficial effects of supplementary choline on growth nor on serum lipid changes induced by ethionine. They did not report liver fat, however, which may be a more sensitive indicator. This point perhaps deserves further investigation. Ethionine, with or without methionine, had no significant effect on liver phospholipid concentrations in the female rats, and the values for this liver lipid were comparable to those of the male animals.

Since liver lipid infiltration induced by ethionine is confined almost entirely to triglycerides (2, 3), it appeared advisable to determine whether the fatty acid composition of this liver lipid fraction was affected under the conditions of our experiment. Liver lipid extracts from 3 rats from each group were chromatographed, the triglycerides were isolated and their fatty acids determined. The results of these analyses are shown in table 3. Triglyceride concentrations in the male rats varied widely and were not significantly increased above those of either the pair-fed or ad libitum controls. Unexpectedly, female rats accumulated no more triglyceride in their livers than the males, and although the average increase was nearly 3 times that of their controls, the small number of animals analyzed and the wide variation negated any significance. Triglyceride accumulation in those animals fed ethionine diets supplemented with methionine was, however, significantly increased ($P < 0.01$) — a result that had been indicated by the total lipid measurements.

The most abundant fatty acids in the hepatic triglycerides (table 3) in all the animals were oleic, linoleic and palmitic, regardless of ethionine feeding. Despite the variation in triglyceride concentration, the relative proportions of most of the fatty acids in the different groups were similar. The only significant difference produced by ethionine was a reduction in the proportion of palmitic acid ($P < 0.05$) for both sexes. The proportion of linoleic acid tended to be increased, but the values were not significant. A decreased percentage of palmitic acid in liver triglycerides of rats injected with a single large dose of ethionine was reported by Horning et al. (26) and by Arvidson and Olivecrona (27). Although the latter reported an apparent increase in the percentage of triglyceride linoleate, Horning et al. (26) did not observe it. While ethionine may have some effect on the composition of rat liver triglycerides, linoleic acid of this lipid fraction, although present in high concentrations, apparently is not metabolized much differently than the other fatty acids.

Table 4 shows the fatty acid composition of the liver phospholipid. The variation in concentration of this lipid among the various groups was not nearly as wide as in the triglycerides (tables 2 and 3); yet it was in this lipid that ethionine caused its most significant changes in certain fatty acids. Arachidonic, linoleic, stearic, and palmitic were the principal fatty acids in the phospholipids. Neither stearic acid nor palmitic acid was affected by ethionine, although females consistently had lower proportions of these fatty acids than the males. In both sexes, a principal effect of ethionine was to reduce the percentages of arachidonic acid significantly below those of their respective controls. Methionine supplementation, however, almost entirely prevented this change. The decrease in phosphatidyl arachidonate was apparently compensated for by an accumulation of phosphatides containing linoleic acid and, to a smaller extent, oleic acid (significant only in males).

Fatty acids of the two major components of the liver phosphatides, phosphatidyl choline and phosphatidyl ethanolamine, are shown in tables 5 and 6. The percentages of these were not affected by ethionine and were similar for both sexes (47% and 30% in males and 48% and 27% in females, respectively). Qualitatively and quantitatively, the largest changes in fatty acid composition induced by ethionine occurred in the phosphatidyl choline fraction (table 5). In both sexes, the percentages of arachidonic acid were significantly depressed below the control levels by ethionine, whereas those of linoleic acid and oleic acid were significantly increased. Stearic acid was also significantly reduced in female rats fed ethionine, but the effect in the male animals was not as evident. Methionine supplementation prevented nearly all the fatty acid changes

TABLE 3

Liver triglycerides and fatty acids from rats fed the basal diet with and without DL-*ethionine*

Experimental group	Liver triglyceride	Fatty acids						
		16:0	16:1	18:0	18:1	18:2	20:3	20:4
	mg/g	*wt %*	*wt %*	*wt %*	*wt %*	*wt %*	*wt %*	*wt %*
				Males				
Ad libitum control	16.6 ± 4.2 [1]	30.9 ± 4.7	5.3 ± 2.1	3.4	32.0 ± 1.0	20.3 ± 5.5	0.2	2.8
Pair-fed control	34.1 ± 8.0	25.8 ± 1.0	3.8	5.8	34.6 ± 2.6	24.3 ± 2.6	0.3	2.0
0.25% DL-ethionine	28.4 ± 4.9	20.2 ± 1.1	5.7	2.7	31.0 ± 1.0	30.0 ± 1.0	0.7	2.7
0.5% DL-ethionine	15.5 ± 2.3	16.8 ± 1.6	3.6 ± 1.2	3.8	36.7 ± 1.0	28.8 ± 3.8	1.2	2.2
0.25% DL-ethionine + 0.5% DL-methionine	37.5 ± 11.3	18.2 ± 2.4	4.7	3.0	41.6 ± 3.5	21.6 ± 2.3	1.5	2.7
				Females				
Pair-fed control	7.8 ± 0.5	17.9 ± 1.4	6.6 ± 1.4	4.0	34.3 ± 1.7	25.1 ± 1.3	0.6	4.1
0.25% DL-ethionine	20.6 ± 13.3	10.5 ± 2.2	2.7	4.8	31.8 ± 2.2	30.4 ± 1.2	1.7	3.8
0.25% DL-ethionine + 0.5% DL-methionine	116.3 ± 11.5	18.2 ± 1.0	5.7 ± 1.5	3.0	35.3 ± 1.0	26.5 ± 1.8	0.8	2.3

[1] Values are means ± SE from 3 animals selected at random from each group. Standard errors below 1% have been omitted. Fatty acids < 16:0 and > 20:4, averaging less than 5% of the total fraction, were calculated but not included in the table.

TABLE 4

Liver phospholipid fatty acids from rats fed the basal diet with and without DL-*ethionine*

Experimental group	Fatty acids						
	16:0	18:0	18:1	18:2	20:3	20:4	> 20:4 [1]
	wt %	*wt %*	*wt %*	*wt %*	*wt %*	*wt %*	*wt %*
			Males				
Ad libitum control	18.8 ± 0.2 [2]	21.6 ± 0.7	7.3 ± 0.6	13.8 ± 0.9	0.5 ± 0.0	27.3 ± 1.2	6.2 ± 1.4
Pair-fed control	19.3 ± 1.6	21.0 ± 0.7	7.5 ± 0.2	15.5 ± 1.1	1.1 ± 0.6	23.9 ± 0.8	7.1 ± 0.6
0.25% DL-ethionine	21.1 ± 0.7	19.3 ± 0.9	12.1 ± 0.6 [3]	20.9 ± 0.9 [3]	1.2 ± 0.2	15.4 ± 1.3 [3]	5.2 ± 0.4
0.5% DL-ethionine	15.5 ± 1.2	21.0 ± 1.6	11.2 ± 2.1	19.2 ± 0.9 [4]	1.2 ± 0.2	16.7 ± 0.4 [3]	8.7 ± 0.3
0.25% DL-ethionine + 0.5% DL-methionine	19.6 ± 0.8	17.9 ± 0.5	11.8 ± 0.8	18.0 ± 0.4	2.3 ± 0.2	20.6 ± 1.0	5.4 ± 0.4
			Females				
Pair-fed control	9.0 ± 0.5	15.2 ± 1.0	9.5 ± 0.2	14.2 ± 0.3	1.5 ± 0.02	32.7 ± 0.9	9.7 ± 0.7
0.25% DL-ethionine	10.3 ± 1.1	16.6 ± 1.3	12.5 ± 1.3	17.1 ± 1.2 [4]	2.2 ± 0.4	21.8 ± 1.6 [3]	11.7 ± 0.3
0.25% DL-ethionine + 0.5% DL-methionine	11.9 ± 1.3	18.4 ± 1.1	11.4 ± 0.9	16.1 ± 0.9	1.6 ± 0.6	28.2 ± 1.0	5.9 ± 0.7

[1] Fatty acids > 20:4 consisted mainly of 22:5 and 22:6. Fatty acids < 16:0 and 16:1 represented less than 5% of the total fatty acids, and have not been included here.
[2] Means ± SE. See table 2 for numbers of animals/diet group.
[3] Significantly different from pair-fed control, $P < 0.01$.
[4] Significantly different from pair-fed control, $P < 0.05$.

TABLE 5

Phosphatidyl choline fatty acids from livers of rats fed the basal diet with and without DL-*ethionine*

Experimental group	Fatty acids							
	16:0	18:0	18:1	18:2	20:3	20:4	22:5ω6 [1]	22:6ω3
	wt %	*wt %*	*wt %*	*wt %*	*wt %*	*wt %*	*wt %*	*wt %*
				Males				
Ad libitum control	21.3 ± 2.0 [2]	20.7 ± 0.7	9.3 ± 1.3	10.1 ± 1.5	0.2 ± 0.1	29.1 ± 0.6	1.5 ± 0.4	3.4 ± 0.2
Pair-fed control	26.1 ± 0.8	18.1 ± 2.4	10.3 ± 1.4	15.3 ± 0.5	0.8 ± 0.2	22.0 ± 1.7	1.0 ± 0.1	3.1 ± 1.4
0.25% DL-ethionine	25.5 ± 1.0	13.9 ± 0.6	16.8 ± 1.2 [3]	22.9 ± 2.0 [3]	1.0 ± 0.4	13.5 ± 1.3 [4]	—	2.2 ± 0.3
0.5% DL-ethionine	23.2 ± 1.6	15.5 ± 2.0	16.2 ± 2.1	24.8 ± 1.6 [4]	1.2 ± 0.5	12.9 ± 2.9 [5]	—	3.0 ± 0.5
0.25% DL-ethionine + 0.5% DL-methionine	23.2 ± 0.2	18.2 ± 0.8	15.4 ± 0.8	17.0 ± 0.7	2.1 ± 0.1	19.2 ± 0.5	0.3 ± 0.1	2.0 ± 0.4
				Females				
Pair-fed control	19.6 ± 1.3	20.2 ± 1.0	9.3 ± 0.8	11.3 ± 0.7	0.9 ± 0.1	29.1 ± 0.9	1.8 ± 0.1	3.9 ± 0.6
0.25% DL-ethionine	25.4 ± 1.0 [3]	12.6 ± 0.4 [4]	19.4 ± 1.4 [4]	24.8 ± 0.3 [4]	0.8 ± 0.2	10.3 ± 0.9 [4]	—	2.6 ± 0.6
0.25% DL-ethionine + 0.5% DL-methionine	21.4 ± 1.3	18.4 ± 1.0	12.4 ± 1.2	16.6 ± 0.4	1.1 ± 0.9	24.6 ± 1.6	0.8 ± 0.1	2.7 ± 0.5

[1] Shorthand nomenclature proposed by Holman (23) to indicate carbon number of first double bond, counting from the methyl end of the fatty acid. Fatty acids < 16:0 and palmitoleic acid (< 3% of total fatty acids) showed no consistent trends and have not been included here.
[2] Mean ± SE. Numbers of animals analyzed per group were the same as shown in table 2 except that the values for the ad libitum controls were from only 2 rats.
[3] Significantly different from pair-fed control, $P < 0.02$.
[4] Significantly different from pair-fed control, $P < 0.01$.
[5] Significantly different from pair-fed control, $P < 0.05$.

TABLE 6

Phosphatidyl ethanolamine fatty acids from livers of rats fed the basal diet with and without DL-*ethionine*

Experimental group	Fatty acids							
	16:0	18:0	18:1	18:2	20:3	20:4	22:5ω6 [1]	22:6ω3
	wt %	*wt %*	*wt %*	*wt %*	*wt %*	*wt %*	*wt %*	*wt %*
				Males				
Ad libitum control	13.9 ± 0.6 [2]	24.5 ± 0.6	7.6 ± 3.5	6.2 ± 1.1	0.3 ± 0.0	25.2 ± 2.1	5.8 ± 0.3	10.2 ± 0.8
Pair-fed control	13.6 ± 1.2	25.5 ± 1.8	5.6 ± 0.7	12.8 ± 0.8	—	22.3 ± 0.5	2.6 ± 0.3	12.3 ± 3.2
0.25% DL-ethionine	13.8 ± 0.1	26.3 ± 1.1	10.5 ± 0.2 [3]	13.5 ± 1.3	1.1 ± 0.3	19.0 ± 0.9 [4]	0.9 ± 0.0 [3]	11.3 ± 1.2
0.5% DL-ethionine	13.5 ± 1.7	26.4 ± 1.2	12.2 ± 2.8	11.9 ± 1.7	0.9 ± 0.2	19.8 ± 1.2	0.8 ± 0.2 [3]	15.3 ± 1.6
0.25% DL-ethionine + 0.5% DL-methionine	14.9 ± 1.0	24.2 ± 0.5	9.0 ± 0.3	11.3 ± 0.7	1.5 ± 0.4	22.6 ± 0.9	1.4 ± 0.2	12.1 ± 1.2
				Females				
Pair-fed control	14.3 ± 0.9	26.2 ± 0.7	4.9 ± 0.3	5.1 ± 0.4	0.5 ± 0.1	29.6 ± 0.5	2.6 ± 0.8	10.7 ± 0.7
0.25% DL-ethionine	10.7 ± 1.2	26.9 ± 1.1	10.0 ± 0.5 [3]	12.9 ± 2.2 [5]	1.5 ± 0.4	20.9 ± 0.6 [3]	0.8 ± 0.1 [3]	12.4 ± 0.9
0.25% DL-ethionine + 0.5% DL-methionine	10.6 ± 0.4	28.8 ± 0.6	6.6 ± 0.5	9.7 ± 1.2	1.3 ± 0.1	27.3 ± 0.5	2.4 ± 0.4	9.4 ± 1.0

[1] See table 5 for explanation of nomenclature. Palmitoleic and fatty acids < 16:0 occurred only in trace amount and have not been included.
[2] Mean ± SE. Numbers of animals analyzed per group were the same as shown in table 2, except that the values for the ad libitum controls were from only 2 rats.
[3] Significantly different from pair-fed control, $P < 0.01$.
[4] Significantly different from pair-fed control, $P < 0.05$.
[5] Significantly different from pair-fed control, $P < 0.02$.

in both sexes. It should be emphasized that since no significant differences in liver phospholipid concentrations occurred among the female control and experimental groups, the changes in fatty acid percentages are proportional to absolute quantitative changes. Similar quantitative decreases in arachidonic acid were also induced by ethionine in male rats, but were not as pronounced as in the females because of increased concentrations of liver phospholipids. These results indicated, therefore, that in both male and female rats, ethionine increased choline phosphatides containing oleic and linoleic acids, whereas it actually decreased those containing arachidonic acid and, at least in female rats, those containing stearic acid.

In female rats, ethionine produced significant changes in arachidonic acid, linoleic acid and oleic acid in the phosphatidyl ethanolamine (table 6) that were similar to those in the phosphatidyl choline. However, stearic acid in this phospholipid was not altered. In the male animals, the ethionine-induced decrease in arachidonic acid was also evident, but the change was less than in the phosphatidyl choline. Comparisons with the pair-fed group, however, indicated that, in those animals, ethionine did not increase the linoleic acid as it had in the females.[2] Phosphatidyl ethanolamines contained the highest proportions of fatty acids with chain lengths and unsaturation greater than 20:4. In both male and female animals, feeding ethionine significantly reduced the percentages of fatty acid tentatively identified as 22:5ω6, but had no effect on the 22:6ω3 component. According to Rahm and Holman (28, 29), 22:6ω3 occurs in liver phospholipids in appreciable amounts after feeding of linolenic acid (18:3ω3), whereas 22:5ω6 increases after feeding of either linoleic acid or arachidonic acid. Since neither ethionine nor the sex of the animal had any appreciable effect on the percentage of the 22:6ω3 fatty acid in either the phosphatidyl choline or phosphatidyl ethanolamine, we feel it was probably derived from the approximately 1% linolenic acid contained in the corn oil in the diet. In other experiments in which cottonseed oil was fed, the percentage of a fatty acid resembling 22:6 was very much less.

Thus to the growing list of ethionine-induced derangements in lipid metabolism must be added an effect on the normal metabolism of the polyunsaturated fatty acids in rat liver phosphatidyl choline and phosphatidyl ethanolamine. Unlike some of the hepatic lipid changes induced by ethionine, such as triglyceride infiltration and fatty acid oxidation, which are produced only in female rats, the antagonist's effect on hepatic phospholipids occurs in both sexes. The presence of the methionine antagonist, however, does not appear to prevent incorporation of triglyceride linoleate into phosphatidyl choline and phosphatidyl ethanolamine, since these lipids contained more than control levels of this fatty acid. It appears, therefore, either that synthesis of phosphatides containing arachidonic is inhibited and the phosphatides are replaced by those containing linoleic acid or that the conversion of linoleic acid to arachidonic acid is inhibited in some way. There is some evidence for the existence of both possibilities. The first is supported by the observations that ethionine inhibits methionine transmethylase activity (30) as well as the incorporation of methionine methyl into phosphatidyl ethanolamine to form lecithins (25). Of interest in these observations was the finding that ethionine affects the reaction in both sexes. Recent evidence suggests also that lecithins containing stearic acid and arachidonic acid may, to a large extent, be formed through the methylation of phosphatidyl ethanolamine, and that this process, while active in both sexes, occurs more extensively in females than in males (31, 32). Synthesis of phosphatidyl choline containing stearic and arachidonic acids would therefore be inhibited by ethionine, and presumably those phosphatidyl cholines containing linoleic acid, oleic acid and palmitic acid, and synthesized by the diglyceride-cytidine diphosphocholine pathway (33), would be increased. Such a mechanism could help explain the simultaneous decrease in stearic acid and arachidonic

[2] This may be an artifact, since in a later experiment carried out under essentially the same conditions, PE linoleate from 4 pair-fed rats averaged 7.5%, a value comparable with that of the female and ad libitum controls of the present experiment.

acid in the phosphatidyl choline of female rats fed ethionine. Although this proposal may explain some of the changes observed when ethionine is fed, it is insufficient to account for all. Thus, if methylation of phosphatidyl ethanolamine containing arachidonic acid were being inhibited by ethionine, arachidonic acid in the phosphatidyl ethanolamine would be expected to increase or at least remain at control levels. However, from table 6, it is evident that this was not the case, and while adequate levels of linoleic acid were present in the phosphatidyl ethanolamine of rats of both sexes fed ethionine, arachidonic acid still declined. Thus the second possibility, that is, ethionine interference with the conversion of linoleic acid to arachidonic acid, must also be considered. An effect of the antagonist on this reaction is suggested by the significant decrease in the 22:5ω6 fatty acid in both the phosphatidyl choline and the phospatidyl ethanolamine. This fatty acid normally increases when linoleic acid or arachidonic acid is fed (28, 29), and is considered to be a metabolic product derived from arachidonic acid after further chain elongation and desaturation. The decrease in the pentaenoic acid at the time that arachidonic acid, its immediate precursor, decreased, even though high levels of linoleic acid were present, indicates that ethionine must interfere with some step, probably an early one, in the transformation of the linoleic acid to arachidonic. Perhaps both of the mechanisms proposed are operative in the rat fed ethionine. Final conclusions concerning the action of ethionine on the essential fatty acids of rat liver phospholipids must, however, await experiments designed to provide more direct evidence for the suggested possibilities.

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Effect of Levels of Calcium and Lysine upon the Growth of *Ascaridia galli* in Chicks [1,2]

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ABSTRACT Embryonated eggs of *Ascaridia galli* were given to one-week-old chicks to determine the impact of calcium and lysine in the diet of the host upon the parasite. As the level of calcium in the diet was increased, the length of the worms decreased. The ability of the worms to survive was generally decreased as the level of calcium was increased from 0.3% to 2.50%. When lysine was used as the variable, worm size decreased as the lysine level increased from 0.65% to 2.05%. At 4 weeks after exposure, the number of worms was significantly greater in chicks fed low levels of lysine; the number of worms in chicks destroyed at 5 weeks after exposure was not significantly different. The weights of the control (noninfected) and treated chicks were almost equal except when higher doses of eggs were administered; then exposed chicks gained less than the nonexposed. The data indicate that the lower levels of calcium and lysine were sufficient for normal maturation of embryonated eggs and growth. The results of the 6 experiments show that the needs of *A. galli* for lysine and calcium, as measured in the diet of the host, are less than 0.65% lysine and 0.3% calcium. These nutrition requirements are well below the needs of the host.

It has been reported that the natural resistance of chickens to helminthic infections may be affected by many factors, including nutrition. As early as 1926, Zimmerman et al. (1) observed that the natural resistance of chicks to parasitism by *Ascaridia galli* could be lowered by nutritional deficiencies in the diet of the host. Ackert et al. (2) reported that chickens fed a vitamin A-deficient diet harbored more and larger worms than the nondeficient controls. Brody (3) found that a simultaneous deficiency of pteroylglutamic acid and vitamin B_{12} resulted in increased length and number of worms. Similar results were found with pyridoxine. This result and others (4) have established that, in general, a deficiency of any vitamin would lower the resistance of chicks to parasitisms by *A. galli.*

Protein quality has also been shown to have an effect on the resistance of chicks to the nematode *A. galli.* Ackert and Beach (5) reported that chickens receiving meat scraps and skim milk, in addition to a basal cereal ration, contained fewer and shorter worms than chickens fed the basal plus meat scraps and peanut meal diets. Riedel and Ackert (6) found that chickens fed a supplement of soybean oil meal and skim milk every second day harbored fewer worms than chickens fed either a supplement of soybean oil meal or meat scraps. In a subsequent paper, Riedel and Ackert (7) demonstrated that quantity of protein is important in the resistance of the host to the nematode.

Todd (8) noted more and larger worms in chickens fed a 0.9% methionine-supplemented diet than in birds fed the basal ration only. Fewer worms were found in birds fed the 1.8% methionine-supplemented diet than in the birds fed 0.9% methionine.

Riedel (9–12) and Riedel and West (13) conducted a series of experiments using different amino acids to study their effects on the resistance of chicks to the fowl nematode. Lysine was not found to be essential in the diet to maintain the resistance of the chicks to parasitism by *A. galli.*

In another aspect of nutrition and its effect upon the host and nematode parasites, Clapham (14) observed that a calcium-deficient diet resulted in the estab-

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lishment of more *Heterakis gallinae* than in the controls.

Gaafar and Ackert (15) reported that fewer and shorter worms were found in chickens fed a low calcium diet than in the control groups fed adequate diets.

Deo and Srivastava (16) observed that calcium deficiency in the diet of chicks seriously affected resistance to *A. galli*. They recovered more worms per bird fed the calcium-deficient diet than the control diet, although the length of the worms was approximately equal.

In a subsequent paper, Deo and Srivastava (17), working with *H. gallinae*, reported that chicks fed calcium-deficient diets harbored more worms than those fed a control diet; the worms were shorter than those in the control chick groups. Chubb and Wakelin (18) presented a summary of the effects of nutritional deficiencies on helminth infections in chickens.

Since the information of the effect of additions of lysine and calcium on *A. galli* is confusing, it was desired to study these nutrients and their effects upon the host and the parasite. The criteria used to measure the resistance of chicks to *A. galli* were the number and length of the worms.

PROCEDURE

One-day-old New Hampshire × S. C. White Leghorn chicks were reared in electrically heated batteries and given feed and water ad libitum. The experimental diets were given to the chicks when they were one day old. The chicks were weighed individually at the beginning of the experiment and every week of the 5-week experimental period. Feed consumption was recorded weekly. Ten, fourteen or thirty chicks were used per treatment.

At the end of the first week, the chicks were exposed to 110 ±, 200 ±, or 500 ± embryonated eggs. Four weeks later the chicks were killed and the small intestine removed; the interior was washed and cleaned to collect the worms, which were counted later and measured under a calibrated microscope. In experiment 4, this procedure was carried out after 3 weeks because of problems with cannibalism in the low lysine group.

TABLE 1
Basal diets

	Ca study	Lysine study
	%	%
Corn	54.000	57.000
Sesame	—	34.000
Soybean meal (44% protein)	34.000	—
Fish solubles	3.000	3.000
White grease [1]	3.000	—
Methionine	0.050	—
Vitamin mixture [2]	0.250	0.250
Mineral mixture [3]	0.550	0.550
Dicalcium phosphate	0.275	1.100
Sucrose	2.875	3.950
K_2HPO_4	2.000	—
$CaCO_3$	—	0.150
Lysine	—	—

[1] A commercial feeding fat mixture similar to lard.
[2] Supplied the following in mg/kg of diet thiamine, 100; niacin, 100; riboflavin, 16; Ca pantothenate, 20; vitamin B_{12}, 0.02; pyridoxine·HCl, 6; biotin, 0.6; folic acid, 4; inositol, 100; menadione $NaHSO_3$, 10; vitamin A acetate, (250,000 IU/g), 40; vitamin D_3 (1,500 ICU/g), 800; choline chloride, 1,000; α-tocopheryl acetate, 10; and penicillin, 11.
[3] Supplied: (in percent) $MnSO_4 \cdot H_2O$, 0.033; $ZnCO_3$, 0.010; and NaCl, 0.500.

RESULTS AND DISCUSSION

In experiments in which calcium was used as a dietary variable, the basal diet used is shown in table 1. To have a low level of Ca in the diet and to maintain the level of phosphorus at 0.75%, it was necessary to add K_2HPO_4. The levels of Ca were increased with dicalcium phosphate and $CaCO_3$ to bring the calcium to the desired level. Additions were made at the expense of sucrose.

In the first experiment, 4 levels of Ca were used and the chicks were exposed to 110 ± embryonated eggs. The number of worms that developed in each group of chicks is almost equal (table 2). The length of the worms was similar with the lower Ca levels but in the 1.15% diet the length was significantly reduced. A weight comparison between the control and exposed chicks shows equal weights with the two lower Ca diets and a slight reduction in weight of the exposed chicks fed the two higher Ca levels. Feed efficiency was slightly better in the noninfected groups. The weights of chicks fed only 0.4% Ca were much higher than anticipated.

In a second experiment, a wider range of levels of Ca was used and the chicks were exposed to 200 ± embryonated Ascaridia eggs (table 3). The number of worms increased with a level of 1.4% Ca

TABLE 2

Effect of calcium level on length and number of worms in chicks exposed to Ascaridia galli (exp. 1) [1]

Ca	Wt of chicks, 5 weeks	Feed/gain	Avg length	Avg no. worms
%	*g*		*mm*	
		Non-exposed		
0.40	402 [a 2]	2.22		
0.55	431 [ab]	2.24		
0.85	447 [b]	2.14		
1.15	451 [b]	2.19		
		Exposed		
0.40	400 [a]	2.32	19.0 [a]	2.1 [a]
0.55	432 [ab]	2.21	16.5 [a]	1.6 [a]
0.85	433 [b]	2.31	19.9 [a]	2.4 [a]
1.15	430 [ab]	2.22	5.7 [b]	2.6 [a]

[1] Ten chicks/treatment; 110 ± embryonated eggs.
[2] Numbers with different superscript letters are significantly different ($P < 0.05$) (18).

TABLE 3

Effect of calcium level on length and number of worms in chicks exposed to Ascaridia galli (exp. 2) [1,2]

Ca	Wt of chicks, 5 weeks	Feed/gain	Avg length	Avg no. worms
%	*g*		*mm*	
		Non-exposed		
0.27	171 [c 3]	3.47		
0.80	447 [ab]	2.30		
1.40	461 [ab]	2.17		
2.00	479 [a]	2.18		
		Exposed		
0.27	166 [c]	3.52	9.04 [a]	2.3 [a]
0.80	426 [b]	2.20	8.30 [a]	3.0 [a]
1.40	472 [ab]	2.20	5.83 [a]	8.5 [b]
2.00	436 [ab]	2.21	2.81	4.2 [ab]

[1] Ten chicks per treatment; 200 ± embryonated eggs.
[2] Statistical analysis was made by the use of Duncan's multiple range and multiple *F* test (Duncan, D. B. 1955 Multiple range and multiple *F* tests. Biometrics, *11*: 1).
[3] Numbers with different superscript letters are significantly different ($P < 0.05$).

TABLE 4

Effect of calcium level on length and number of worms in chicks exposed to Ascaridia galli (exp. 3) [1]

Ca	Wt of chicks, 5 weeks	Feed/gain	Avg length	Avg no. worms
%	*g*		*mm*	
		Non-exposed		
0.30	162 [a 2]	3.56		
1.00	463 [b]	2.23		
2.50	460 [b]	2.24		
		Exposed		
0.30	179 [a]	3.25	27.79 [a]	7.7 [a]
1.00	498 [c]	2.19	25.02 [a]	3.6 [a]
2.50	427 [b]	2.36	8.09 [b]	2.5 [a]

[1] Thirty chicks per treatment (3 replicates of 10 each); 200 ± embryonated eggs.
[2] Numbers with different superscript letters are significantly different ($P < 0.05$).

but the other levels did not affect the number appreciably. The length of the worms decreased as the amount of Ca increased. The decrease was pronounced with the 2% calcium. Chick weights and feed efficiency are almost equal in the control and exposed chicks.

To further substantiate the finding that a deficiency of Ca allowed the chicks to harbor larger worms than when fed a normal or higher level of Ca, another experiment was undertaken. The results are shown in table 4. Again, as the amount of Ca increased, the length of the worm decreased. This finding was more marked when a higher level of Ca was used. These results are similar to the previous ones in relation to length. However, the number of worms decreased as the Ca increased rather than remaining about the same.

In general, the results concerning the length of the worms are in agreement with those of Deo and Srivastava (16); however, they do not agree with those of Gaafar and Ackert (15). These authors reported shorter worms from groups of chicks fed a low Ca ration than from the control groups fed adequate diets. The data show that the diets were deficient enough in Ca to markedly reduce the growth rate of the chickens, but even at those low levels of Ca, worm growth was not reduced.

The results concerning the number of worms have not been consistent since in the first experiment the number was almost equal, in the second the number increased as the Ca increased except at the highest level, and in the third experiment the number decreased as the Ca increased. This decrease, however, was not significant.

The basal diet used in the lysine experiments is shown in table 1. In the fourth experiment 4 levels of lysine were used to study the effect of this amino acid upon the host and the nematode *A. galli*. In this experiment 500 ± embryonated eggs were used. The duration of the experiment was 4 weeks because the chicks fed the lowest lysine level diet started to pick each other and two of them died; it was decided to end the experiment and avoid further mortality.

As the level of lysine increased (table 5), better growth and feed efficiency were obtained. The control chicks in each treatment weighed more than those exposed to the worms, although these differences were not significant. In general, larger and more numerous worms were found in the deficient diets.

In view of the results in the fourth experiment, a fifth experiment was undertaken but the chicks were debeaked at one week of age to avoid picking. The results are shown in table 6 and are in agreement with the previous experiment in relation to weights of chicks and feed efficiency. These results showed more clearly that the length of the worms decreased as the amount of lysine increased, although the results were not statistically significant.

TABLE 5

Effect of lysine levels on length and number of worms in chicks exposed to Ascaridia galli (exp. 4) [1]

Lysine	Wt of chicks, 4 weeks	Feed/gain	Avg length	Avg no. worms
%	*g*		*mm*	
		Non-exposed		
0.65	98 [a 2]	3.48		
0.85	189 [b]	2.27		
1.05 [3]	321 [c]	1.88		
1.25	334 [c]	1.85		
		Exposed		
0.65	92 [a]	4.65	3.14 [a]	19.1 [ab]
0.85	177 [b]	2.48	1.47 [a]	23.5 [a]
1.05	284 [c]	2.05	1.83 [a]	4.8 [c]
1.25	318 [c]	1.98	1.46 [a]	9.4 [bc]

[1] Ten chicks per treatment; 500 ± embryonated eggs.
[2] Numbers with different superscript letters are significantly different ($P < 0.05$).
[3] This level is 0.05% higher than the NRC figure (19) and 0.05% lower than the NRC figure (20).

TABLE 6

Effect of lysine levels on length and number of worms in chicks exposed to Ascaridia galli (exp. 5) [1]

Lysine	Wt of chicks, 5 weeks	Feed/gain	Avg length	Avg no. worms
%	*g*		*mm*	
		Non-exposed		
0.65	132 [a 2]	1.99		
0.85	259 [b]	2.16		
1.05	412 [c]	1.99		
1.25	430 [c]	1.91		
		Exposed		
0.65	113 [a]	2.63	6.01 [a]	3.8 [a]
0.85	233 [b]	2.33	3.79 [a]	7.1 [a]
1.05	405 [c]	1.84	2.46 [a]	6.0 [a]
1.25	429 [c]	1.82	2.32 [a]	4.7 [a]

[1] Fourteen chicks per treatment (2 duplicates of 7 each); 500 ± embryonated eggs.
[2] Numbers with different superscript letters are significantly different ($P < 0.05$).

TABLE 7

Effect of lysine levels on length and number of worms in chicks exposed to Ascaridia galli (exp. 6) [1]

Lysine	Wt of chicks, 5 weeks	Feed/gain	Avg length	Avg no. worms
%	*g*		*mm*	
		Non-exposed		
0.70	352 [a 2]	2.40		
1.05	508 [b]	1.93		
2.05	517 [b]	1.99		
		Exposed		
0.70	365 [a]	2.27	34.39 [a]	3.4 [a]
1.05	516 [b]	1.97	27.52 [a]	2.2 [a]
2.05	509 [b]	2.03	32.45 [a]	2.6 [a]

[1] Thirty chicks per treatment (3 replicates of 10 each); 200 ± embryonated eggs.
[2] Numbers with different superscript letters are significantly different ($P < 0.05$).

The number of worms in each treatment was variable.

A sixth experiment was conducted with three widely different levels of lysine (up to nearly twice the amount normally recommended). The results are shown in table 7. In this experiment, 60 chicks were used per treatment — 30 as control and 30 exposed to 200 ± embryonated eggs. The results showed no difference in the weights of chicks fed the same lysine level. Feed efficiency was almost the same. All 3 diets resulted in approximately the same number and length of worms.

The results of the lysine experiments are generally in agreement with those of Riedel (6, 9) who reported equal length and number of worms with a low and high lysine diet. He autopsied his birds at 5 weeks and found no difference in the number of worms. In other experiments he completed the test at 51 to 61 days of age. One would not expect much difference, however, in his trials because the diet was not very deficient in lysine. This is shown by the chick weights which averaged 318 and 323 g for the deficient and control groups, respectively. In the experiments reported here, a significant weight difference existed between the birds deficient in lysine and the controls at 4 weeks (98 g vs. 334 g in table 5). While the weight differences were also present at 5 weeks (tables 6 and 7) the differences in length and number of worms were not significant. A level of lysine as low as 0.65% was still not low enough in any of the experiments to interfere with normal development of the Ascaridia. This

means that the needs of the Ascaridia for lysine as measured in the diet received by the host are less than 0.65% for normal growth and infestation.

It is of interest to note some differences in the length of the worms between experiments; for instance, in experiments 4 and 5 the same numbers of embryonated eggs were given to the chicks (tables 5 and 6). In experiment 4, a new culture of embryonated eggs was used. In experiment 5, the same culture was used and was about 6 weeks older. When the older culture was used, the length of the worms was much shorter than when the same culture was new. This phenomenon is not understood.

The data with both calcium and lysine indicate that a level of 0.3% calcium and 0.65% lysine in the diet of the host chicken is sufficient for normal growth of worms and for proper maturation of embryonated eggs to the adult stage.

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Abnormal Glucose Tolerance in Manganese-deficient Guinea Pigs [1,2]

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ABSTRACT The effects of low dietary manganese during pre- and postnatal development on glucose utilization of young adult guinea pigs is reported. Glucose tolerance tests were performed on deficient and control guinea pigs, using both oral and intravenous glucose administration. Deficient guinea pigs showed decreased utilization of glucose and in consequence had a diabetic-like glucose curve in response to glucose loading. Control animals and deficient guinea pigs, given dietary manganese equivalent in amount to that supplied control animals for 2 months, showed normal responses to glucose administration.

In our studies of the effects of low dietary manganese on the prenatal and postnatal development of the guinea pig (1–3) it was noted that the pancreatic gland in neonatal animals was often absent or disproportionately reduced in size. Animals having the most extreme pancreatic abnormalities at birth died within the first few postnatal hours or days. Others, less handicapped, survived to adult life. A description of the morphological changes in the pancreatic tissues of manganese-deficient animals has been presented in another report by the authors (4).[2]

These observations have been followed by a study of glucose tolerance of surviving young adult animals, which were the offspring of females fed a diet of less than 3 ppm manganese throughout pregnancy. Before these animals were tested for glucose utilization, they were maintained postnatally on the same low manganese regimen. Certain of the deficient animals were subsequently fed the control diet for a 2-month period and retested to determine the reversibility of the defective glucose tolerance noted previously for manganese-deficient animals. This present paper reports the physiological response of the manganese-deficient, deficient-supplemented, and control guinea pigs to glucose loads administered both orally and intravenously.

EXPERIMENTAL

The animals used in this study were the offspring of female guinea pigs fed diets containing either less than 3 ppm manganese or 125 ppm manganese in the form of manganese sulfate. The composition of the ration and the management of the colony have been presented previously by Tsai and Everson (2).

Oral glucose tolerance tests were performed on 15 pairs of young adult animals. Each pair consisted of a deficient and a control animal of the same sex and age. Initial blood samples were taken after a 20-hour fast. Venous blood was obtained by clipping the nail bed of a toe, as described by Vallejo-Freire (5). A glucose load equivalent to 200 mg/100 g of body weight was given as a 20% aqueous solution. This was fed by dropper and was consumed in less than 2 minutes. Animals were anesthetized using sodium pentobarbital and subsequent sampling was performed at 30 minutes, and 1, 2, 3, and 4 hours. Glucose was determined by Nelson's modification of the Somogyi method (6).

Intravenous glucose tolerance tests were made on eight matched pairs of young adult animals. Using a modified Popovic technique (7, 8), a sterile no. 10 polyethylene cannula [3] was placed in the descending aorta via the carotid artery. The exterior-

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[3] Intramedic polyethylene tubing no. 10. Clay-Adams, Inc., New York.

ized cannula filled with sterile heparinized saline was heat-sealed and coiled on the back of the animal's neck. Complete post-surgical recovery, including return to normal body weight usually occurred within 5 to 10 days. The animals were then fasted for 18 hours and initial blood samples were removed via the cannula, using a 27-gauge needle and a disposable syringe. Unanesthesized animals were given a test load of glucose, equivalent to 100 mg/100 g of body weight. This was administered as 40% glucose in sterile physiological saline and was infused for 3 minutes. Samples were withdrawn at 15, 30, and 45 minutes and 1, 2, 3, and 4 hours. Animals were unrestrained and showed no adverse reactions to the manipulations performed during testing. Glucose was determined by both the Somogyi and glucose oxidase methods.[4] Following evaluation of the glucose tolerance, approximately one half of the deficient animals were killed to obtain tissue samples. The remaining animals (hereafter referred to as supplemented animals), were fed the control diet for 2 months. Intravenous glucose tolerance tests were then repeated on these animals using the procedures outlined above.

Peripheral utilization of glucose was calculated by the method of Amatuzio et al. (9), and Student's *t* test was applied to determine the significance of differences observed (10).

RESULTS

Oral glucose tolerance tests showed a distinctly different type of response between the manganese-deficient and control guinea pigs. Representative curves are illustrated in figure 1. The fasting glucose values of deficient animals averaged 40 mg/100 ml higher than those of control animals (table 1). Blood glucose remained elevated for the entire test period in all manganese-deficient guinea pigs and in several cases the 4-hour level exceeded the fasting value by more than 50 mg /100 ml blood. The control animals showed a more normal response to oral administration of glucose with the peak

[4] Glucostat, Worthington Biochemical Corporation, Freehold, New Jersey.

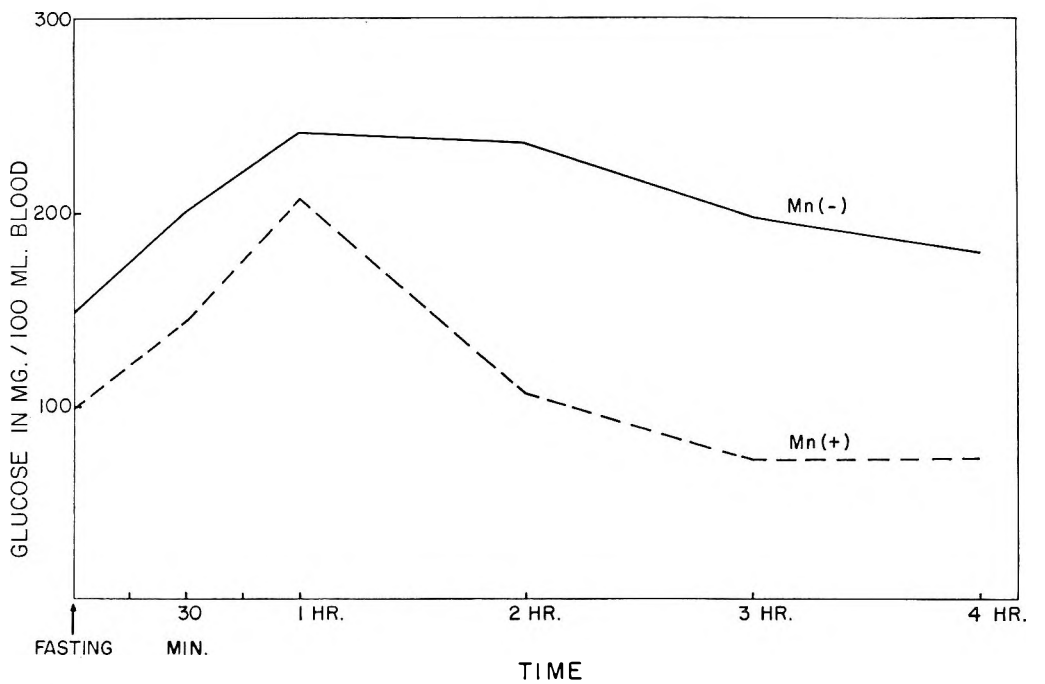

Fig. 1 Glucose tolerance curves of guinea pigs. (Oral administration of glucose, sodium pentobarbital anesthesia, blood sampling by nail-bed clipping.)

TABLE 1

Fasting blood sugar levels in manganese-deficient and control guinea pigs

Regimen of animals	Method of blood sampling			
	Nail-bed clipping		Cannulation	
	Mean	Range	Mean	Range
	mg/100 ml blood		*mg/100 ml blood*	
With manganese	130	95–165	95	85–105
Without manganese	165	130–200	125	110–140

elevation occurring at 30 minutes or 1 hour after glucose intake. Two or three hours following glucose administration, blood glucose values were approximately equal to fasting levels for control animals.

Ambiguous responses to oral glucose tolerance tests were shown by supplemented animals. Incomplete reversal of abnormal patterns appeared in all but a few of the animals tested and no clear-cut pattern of response could be determined. Curves suggestive of improvement when compared with initial test results were observed for several animals.

While there was an unmistakable difference in glucose utilization between deficient and control guinea pigs tested by oral feeding of glucose and nail-bed clipping of anesthetized animals, aberrant values resulting from trauma were obtained at varying times throughout the testing period when the above procedures were used. It was also recognized that the use of an anesthetic introduced possible variables in alimentary absorption and peripheral utilization (11). Sampling of blood for glucose concentrations using nail-bed clipping without anesthesia proved unsatisfactory in the guinea pig. The recognition of these procedural defects led to the repetition of the experiments using animals having indwelling cannulas. Mean fasting values and ranges found for nail-bed clipped and cannulated deficient and control guinea pigs are presented in table 1.

The results of the intravenous glucose tolerance tests are presented in figure 2. Data given in table 1 showed lower mean fasting blood glucose values for deficient and control animals when glucose was administered and blood sampling carried out by cannulation. Less variation in fasting values was noted with intravenous testing. The effect of diet was similar in the 2 methods of testing, in that the manganese-deficient animals had higher glucose values at the end of an 18- or 20-hour fast than controls.

Glucose tolerance curves illustrated in figure 2 confirmed the evidence obtained first by oral testing, that there is a marked difference between the two ration groups in ability to utilize glucose. Manganese-deficient animals maintained elevated glucose concentrations throughout the 4-hour test period. The effect of 2 months of adequate dietary intake of manganese on the ability of these previously deficient animals to handle glucose is demonstrated in figure 2. The broken line in this figure represents the normal glucose tolerance curve of the supplemented cannulated animals given intravascular glucose. Supplementation appeared to correct previously identified abnormalities of glucose utilization in these animals. To determine whether additional glucose loading would show any functional inadequacies, a duplicate load of glucose was administered to several supplemented cannulated animals at the end of the first test period. A typical response curve following this procedure is shown in figure 3. The curves obtained from both control and supplemented animals subjected to repeated doses of glucose appeared to be normal.

The rate of disappearance of glucose from the blood has been expressed in terms of percentage decrease per minute (9, 12, 13). Glucose decreased in the blood of normal subjects at a rate of 1 to 3% per minute. Peripheral underutilization, as in diabetes, is indicated by values of less than 1 % decrease per minute (14–16). Data from the 3 groups of cannulated guinea pigs under investigation show that the deficient animals lost glucose from

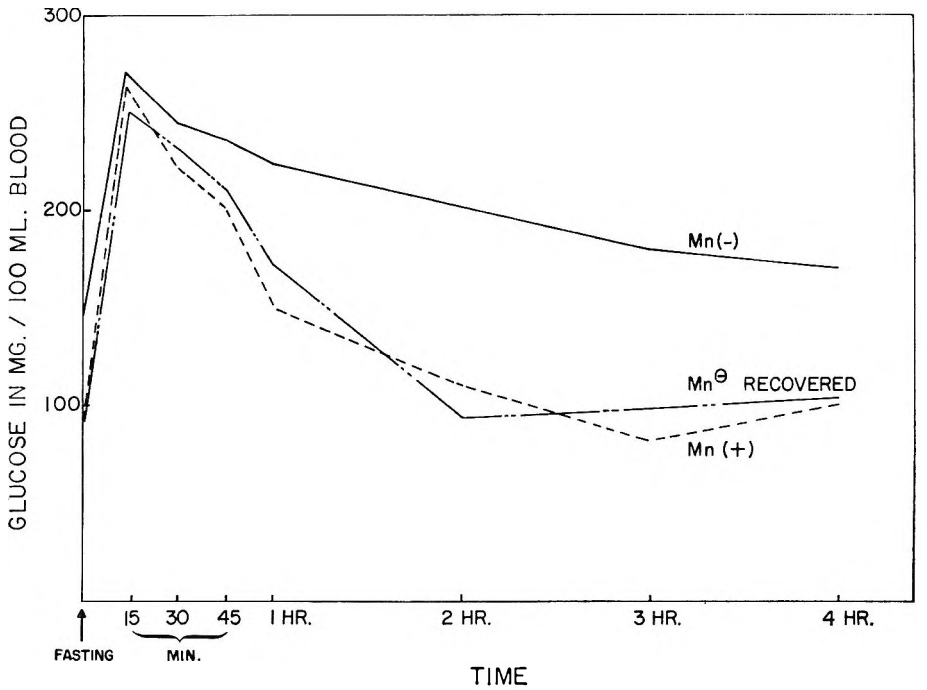

Fig. 2 Glucose tolerance curves of cannulated guinea pigs.

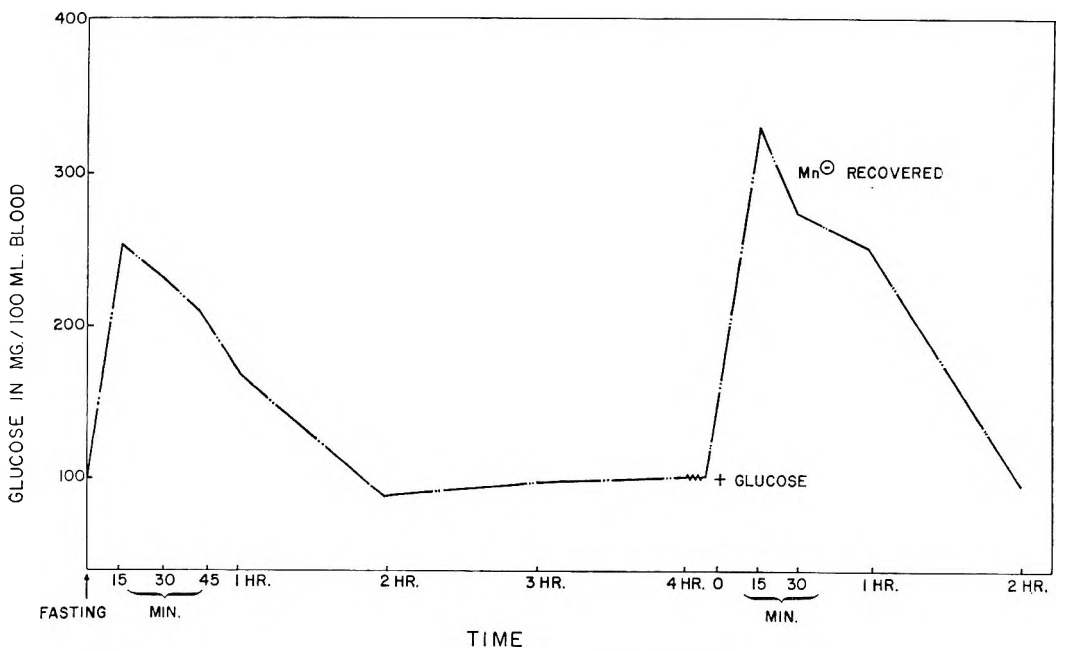

Fig. 3 Glucose tolerance curves of supplemented guinea pigs following repeated administration of glucose via cannula placed in the carotid artery.

the blood at less than 0.5% per minute, whereas the rate of loss for control and supplemented animals exceeds 1.7% per minute. These differences were found to be significant at the 0.1% level.

DISCUSSION

Manganese has been reported by other investigators to influence glucose utilization. Its administration to diabetic subjects has been found to have a hypoglycemic effect (17, 18). "Toxic" levels of dietary manganese have been observed to result in hypoglycemina in normal rabbits (19) and dogs (20). Pancreatectomy and diabetes have been correlated with decreased manganese levels in blood and tissues (21–24). The precise nature of the involvement of manganese is not clear and whether the metal is related to insulin synthesis, need, or secretion is not known.

Our previous studies of manganese deficiency in the guinea pigs have shown that congenitally deficient animals have reduced amounts of mucopolysaccharides in cartilage tissue. It is of interest in this respect that decreased concentrations of stainable mucopolysaccharides have been reported in the skin of young rats born to diabetic mothers (25). Schiller and Dorfman (26) have suggested that insulin may regulate utilization of glucose in the synthesis of mucopolysaccharides. Whether the reduced glucose utilization found in the manganese-deficient guinea pig is directly or indirectly related to the connective tissue defect established in manganese-deficient animals, is still under investigation.

It appears from these studies that manganese supplementation completely reversed the reduced glucose utilization reported.

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Effect of Glucose and Fructose Administration on Lipid Metabolism in the Rat [1]

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ABSTRACT The effect of glucose and fructose on serum triglycerides was studied after prolonged feeding or under acute loads. In the rat, administration of fructose caused hypertriglyceridemia, but no change in serum triglyceride was found in the guinea pig in which fructose is said to be absorbed mostly in the form of glucose. This finding, as well as the low yield of labeled triglyceride in the chyle after fructose-^{14}C administration, and the lack of stimulation of lipogenesis in the intestine indicate that the absorption of unchanged fructose is operative in the induction of hypertriglyceridemia. In the liver more fructose than glucose was converted to triglycerides and secretion of triglycerides into the serum was higher after fructose than after glucose administration to Triton-treated rats. In addition, fructose, unlike glucose, did not stimulate lipoprotein lipase activity in adipose tissue. The sequence of events occurring in the rat after fructose feeding could be summarized as follows: When owing to low activity of glucose 6-phosphatase in the intestine, fructose is absorbed as such into the portal circulation and reaches the liver, it is converted to α-glycerophosphate. At the same time no repression of the outflow of free fatty acid from adipose tissue occurs, leading to increased triglyceride formation in the liver and its secretion into the serum. As feeding of fructose does not induce lipoprotein lipase activity in adipose tissue the egress of triglyceride from the serum and thus the homeostatic regulation of triglyceride levels is impaired leading to its accumulation in the blood stream.

Nutritional investigations performed on human subjects, both normal (1–5) and hypertriglyceridemic (5–7), showed that when sucrose in the diet replaced starch, isocalorically, there was a rise in serum triglyceride levels. To elucidate further the hypertriglyceridemic response to sucrose in the human, isocaloric interchange of sucrose, glucose and fructose was performed (5–9). These studies showed that in normal subjects and in some hyperglyceridemic patients distinct differences in serum triglyceride levels occur, fructose feeding causing the highest response. Recently Nikkila (10–11) was able to reproduce these effects in the rat, showing that even short-term fructose supplementation may cause hypertriglyceridemia. The present investigation was initiated to evaluate the relative role of the intestine, liver and adipose tissue in fructose-induced hypertriglyceridemia.

EXPERIMENTAL

All animals were of the Hebrew University strain. Male and female albino rats and male guinea pigs were fed commercial laboratory ration [2] and were kept in air conditioned temperature-controlled rooms. Food and water were given ad libitum. The cages were cleaned daily and the animals inspected for signs of diarrhea.

Long-term administration of glucose or fructose

Experiments in vivo. Male and female rats were given glucose or fructose in their drinking water as a 10% solution. In most experiments food was withheld from the rats for 16 hours before killing. Male guinea pigs of 500 to 1000 g body weight were given glucose or fructose in drinking water as a 10% solution. The rats and guinea pigs receiving glucose were matched carefully as to body weight with those receiving fructose. All animals were exsanguinated under ether anesthesia and

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[2] Purina Laboratory Chow, Ralston Purina Company, St. Louis.

samples of liver and serum were taken for lipid analysis.

Experiments in vitro. Liver slices were prepared with a Stadie-Riggs slicer and intestinal rings were prepared free hand from the region of the jejunum. About 200 mg of liver or intestine slices were incubated in 10-ml Ehrlenmyer flasks in Krebs-Henseleit bicarbonate buffer, pH 7.4, in the presence of sodium acetate-1-^{14}C (4 μmoles) or glycerol-1-^{14}C (1 μmole) or sodium palmitate-1-^{14}C (0.2 μmole). The final volume was 2 ml, and the flasks were gassed with 95% O_2 and 5% CO_2 before incubation. Incubation time was 30 minutes at 37° in a shaking incubator. At the end of incubation the tissues were homogenized and aliquots were taken for lipid and radioactivity determination.

Acute experiments with glucose-U-^{14}C or fructose-U-^{14}C

Gastric intubation. Male rats 150 to 250 g of body weight were used. Food was withheld for 16 hours before the start of the experiment and throughout the experimental period. Glucose or fructose, 0.25 to 0.4 g/100 g body weight were administered as a single dose by stomach tube; 1 to 2 μCi of either glucose-U-^{14}C or fructose-U-^{14}C were given with the nonlabeled sugar, respectively. All rats were injected intravenously with 7 μCi of oleic acid-9, 10-^{3}H (specific activity 280 μCi/μmole) (as sodium salt), 60 to 120 minutes after the administration of the sugar. The experiment was terminated 10 minutes after the injection of the tritiated oleic acid.

Gastric intubation in Triton pretreated rat. Three groups of rats were injected intravenously with 60 to 100 mg/100 g body weight of Triton WR 1339,[3] as a 15% solution. Intragastric administration of 50% glucose-U-^{14}C or fructose-U-^{14}C was started immediately after the injection of Triton. Two groups received the sugars in a single dose (0.25–0.4 g/100 g body weight) and the rats were killed 2 hours thereafter; control animals received 2 ml of 0.9% NaCl. The third group received 7 repeated doses of labeled glucose or fructose, 1 g each, 2 hours apart and the controls in the last experiment received soluble starch[4] as a 30% suspension, 3.3 ml each dose. Food was withheld for 16 hours before and throughout the experimental period, but the rats had access to drinking water. Under ether anesthesia the rats were exsanguinated and the stomach and small intestine were removed together with their contents, homogenized in water in a Waring Blendor and aliquots were taken for radioactivity and lipid determination. Aliquots of liver and serum were taken as well.

Gastric intubation and cannulation of thoracic duct. Rats were anesthetized with ether and a polyethylene cannula (PE 50) was inserted into the abdominal portion of the thoracic duct, according to the method of Bollman et al. (12). The rats were placed in individual restraining cages and given 0.9% NaCl solution to drink, and were fed by stomach tube 1 ml of 40% solution of glucose-U-^{14}C, or fructose-U-^{14}C (containing 1–4 μCi) and chyle was collected in ice for periods up to 21 hours. Clots in the chyle were removed by winding them on a wooden stick and aliquots were taken for lipid and radioactivity determination.

Intravenous administration. Two hundred-gram rats were injected with 1 ml of 20% solution of either fructose-U-^{14}C or glucose-U-^{14}C. The rats were placed in polyethylene containers, lined with filter paper. One hour after injection the rats were exsanguinated under ether anesthesia and samples of liver and serum were taken for lipid and radioactivity determination. The filter paper soaked with the urine was extracted in water and aliquots were taken for radioactivity determination.

Induction of lipoprotein lipase. Rats weighing 190 to 230 g, from which food had been withheld for 16 hours, were given 2 ml of glucose or fructose as a 60% solution by stomach tube; control animals received 2 ml of 0.9% sodium chloride. Two and four hours later epididymal fat pads were removed under ether anesthesia. The pads were weighed and incubated in Krebs-Henseleit phosphate buffer (containing half the recommended dose of Ca^{++}) 10 ml/g wet weight for 1 hour at 37° (13). One pad of each rat was

[3] Polymeric *p*-iso-octyl polyoxyethylene phenol, obtained from Rohm and Haas, Philadelphia.

[4] Obtained from May and Baker, Dagenham, England.

incubated with heparin, the other without (3 μ/ml incubation medium). The incubation medium for the determination of enzyme activity consisted of: 0.5 ml of the enzyme solution (incubation medium), 0.1 ml of 15% Ediol [5] in water, 0.2 ml of 25% bovine albumin (free fatty acid poor)[6] in 0.05 M Tris·HCl buffer, pH 8.5, 0.5 ml of 0.05 M Tris·HCl buffer, pH 8.5, 0.05 ml of serum from fasted rats and 0.25 ml of water. After the incubation, the reaction mixture was extracted according to Dole (14) and the free fatty acids released by the enzyme were titrated. Lipoprotein lipase activity was expressed as the difference between the activity released from the pad in the presence of heparin and that released from the contralateral fat pad in the absence of heparin.

Analytical procedures

Extraction of lipids from aliquots of liver, intestine, serum and chyle was carried out in 20 volumes of chloroform: methanol (2:1, v/v) and the extracts were purified according to Folch et al. (15). Neutral lipids and phospholipids were separated on silicic acid by batch elution (16). Glyceride-glycerol was determined according to Lambert and Neish (17), total lipid phosphorus according to Bartlett (18); and total lipid esters according to Stern and Shapiro (19). Neutral lipids were separated on thin-layer silica gel plates using the solvent system of petroleum ether (30°–60°):ethyl ether: glacial acetic acid (80:20:1). In some experiments, aliquots of the chloroform extract were evaporated and the lipids were hydrolyzed in 0.4 M KOH in 70% methanol, for 2 hours at 37°. The hydrolysate was acidified, extracted in chloroform:methanol (2:1, v/v) according to Folch et al. (15).

Radioactivity determination

Radioactivity was determined on aliquots of both the aqueous and chloroform phase, using the Tri-Carb liquid scintillation spectrometer model 4322. Radioactivity of samples scraped off the plates was determined according to Snyder (20). Simultaneous counting of tritium- and carbon-labeled samples was carried out as described previously (21). Correction for quenching was performed with the aid of internal standards.

All solvents were of reagent grade. All radioactive materials were obtained from the Radiochemical Centre, Amersham, England. The significance of differences (*P* value) was tested with Student's *t* test.

RESULTS

Rats given fructose in their drinking water for 6 to 19 days did not show any adverse effects and their weight gain was even slightly greater than that of the group receiving glucose. There was a variable but significant increase in serum triglycerides following both 6 and 19 days of fructose supplementation. The serum triglycerides of rats receiving glucose did not vary or were slightly lower than those of the controls that received no sugar in their drinking water. The difference in the liver triglyceride content between the fructose and glucose groups, though suggestive, was not statistically significant. The effect of fructose on serum triglycerides was apparent in male and female rats and was not modified by a 16-hour fast which preceded the sampling of the serum for lipid determination (table 1).

In the experiments with guinea pigs, two age groups were examined. Although the serum triglycerides of the older guinea pigs were significantly higher, fructose supplementation in drinking water had no effect on the serum triglyceride level as compared with those receiving glucose (table 2).

The influence of fructose feeding on the synthesis of lipids from various precursors was studied in liver slices and rings of intestine. Fructose supplementation enhanced incorporation of acetate-^{14}C into lipids (as compared with controls), in both liver and intestine, but had no effect on the incorporation of oleic acid-^{3}H (table 3). This effect of fructose did not vary, however, from that of glucose, but for the slight difference in the incorporation of glycerol into lipids by intestine slices (table

[5] Ediol, aqueous emulsion consisting of 50% coconut oil, 12.5% sucrose, 1.5% glyceryl monostearate and 2.0% of polyoxyethylene sorbitan monostearate (obtained from Schen Labs Pharmaceuticals, New York).

[6] Obtained from Pentex, Inc.

TABLE 1

Effect of glucose or fructose supplementation in drinking water on serum and liver triglycerides in rats [1]

Exp. no.	Hexose added	Duration of experiment	Final body wt	Serum triglyceride	P value	Liver triglyceride
		days	*g*	*mg/100 ml*		*mg/g*
1 (4) [2]	Glucose	6	278 ± 8.8 [3]	73.7 ± 21.6	< 0.002	5.03 ± 0.57
1 (5)	Fructose	6	301 ± 10.0	214.4 ± 16.0		6.85 ± 0.68
2 [4](5)	Glucose	6	275 ± 4.9	63.3 ± 24.0	< 0.05	5.70 ± 0.62
2 (4)	Fructose	6	251 ± 8.9	183.7 ± 38.0		8.51 ± 1.70
3 (5)	—	6	256 ± 4.1	71.1 ± 5.8		7.50 ± 0.64
3 (5)	Glucose	6	331 ± 4.9	80.3 ± 5.8	< 0.05	4.80 ± 0.59
3 (5)	Fructose	6	327 ± 6.4	130.2 ± 17.0		7.80 ± 1.87
4 [5](5)	Glucose	6	233 ± 3.4	71.4 ± 8.7	< 0.01	5.68 ± 0.39
4 (5)	Fructose	6	249 ± 7.9	133.8 ± 15.5		6.16 ± 0.61
5 (5)	—	19	263 ± 6.9	61.8 ± 6.5		
5 (5)	Glucose	19	277 ± 10.4	45.5 ± 5.0	< 0.01	5.46 ± 0.46
5 (5)	Fructose	19	291 ± 6.5	116.1 ± 16.4		6.03 ± 0.83

[1] Male rats of similar initial body weight were fed ad libitum and glucose or fructose were given as a 10% solution in drinking water.
[2] Numbers in parentheses represent number of rats.
[3] Mean ± SE of mean.
[4] In experiment 2 female rats were used.
[5] Food was withheld from all rats before killing, except those in experiment 4.

TABLE 2

Effect of glucose or fructose supplementation in drinking water on serum triglycerides in guinea pigs [1] *(6-day experiment)*

Exp. no.	Hexose added	Final body wt	Serum triglyceride	P value
		g	*mg/100 ml*	
1	Glucose	570 ± 38.4 [2]	59.0 ± 6.7 [a]	a vs. b > 0.1
1	Fructose	622 ± 24.7	51.1 ± 5.4 [b]	a vs. c < 0.05
2	Glucose	963 ± 56.2	76.7 ± 2.2 [c]	c vs. d > 0.1
2	Fructose	998 ± 26.2	82.2 ± 10.8 [d]	b vs. d < 0.05

[1] Male guinea pigs (4/group) were given glucose or fructose in drinking water as a 10% solution and were fed ad libitum; food was withheld for 16 hours before killing.
[2] Mean ± SE of mean.

TABLE 3

Effect of glucose and fructose supplementation on lipogenesis and formation of esterified lipids in rat liver and intestine slices [1] *(5 rats/group)*

Hexose added	Liver			Intestine		
	Acetate-^{14}C	Glycerol-^{14}C	Oleic acid-^{3}H	Acetate-^{14}C	Glycerol-^{14}C	Oleic acid-^{3}H
	% incorporation into lipids [2]					
None	0.41 ± 0.01 [3]	0.90 ± 0.06	8.9 ± 1.3	0.24 ± 0.08	0.53 ± 0.05	3.8 ± 0.3
Glucose	2.62 ± 0.10	2.42 ± 0.18	9.5 ± 1.1	0.41 ± 0.03	0.66 ± 0.06	2.8 ± 0.3
Fructose	2.68 ± 0.50	2.40 ± 0.28	7.4 ± 0.7	0.34 ± 0.03	0.94 ± 0.10	3.2 ± 0.7

[1] Liver and intestine slices were derived from rats supplemented with 10% of the hexose in drinking water for 6 days and from which food was withheld during the last 16 hours before killing; conditions of incubation are given under Methods.
[2] Percentage incorporation: % of dose in incubation medium incorporated into lipids per 100 mg wet weight in 30 minutes.
[3] Mean ± SE of mean.

3). With all 3 precursors studied, 60 to 70% of the lipid radioactivity was found in neutral glycerides. These data do not explain, therefore, the higher serum triglyceride levels caused by fructose feeding.

To learn about the possible direct contribution of the fructose molecule in the intestine toward the induction of hypertriglyceridemia, fasted rats with cannulated chyle ducts were fed intragastrically with

TABLE 4

Recovery of ^{14}C-radioactivity in chyle lipids of rats fed glucose-U-^{14}C or fructose-U-^{14}C

Exp. no.	Hexose fed	Collection of chyle		Lipid-^{14}C in chyle	Lipid secreted in chyle
	g	*hr*	*ml*	*% of administered dose*	*mg/hr*
1	Glucose, 0.4	14	14	0.05	4.7
2	Glucose, 0.4	14	20	0.20	5.7
3	Fructose, 0.4	10	15	0.35	4.8
4	Fructose, 0.4	14	10	0.14	1.6
5	Fructose, 0.4	21	12	0.65	4.5

[1] Male rats 250 g in body weight were fasted 16 hours before cannulation of the thoracic duct. One milliliter of the hexose was administered intragastrically as a 40% solution containing 1–4 μCi of ^{14}C; throughout the collection period the rats were given 0.9% NaCl to drink.

TABLE 5

Incorporation of intravenously administered glucose-U-^{14}C and fructose-U-^{14}C into serum and liver lipids [1] (4 rats/group)

Hexose-^{14}C injected	Loss in urine	^{14}C-radioactivity recovered in lipids		P value
		Serum	Liver	
	% of dose	*% dose/10 ml*	*% dose/g wet wt*	
Glucose	5.0 ± 0.2 [2]	0.021 ± 0.01 [a]	0.029 ± 0.003 [c]	a vs. b < 0.05
Fructose	16.5 ± 0.4	0.108 ± 0.20 [b]	0.155 ± 0.02 [d]	c vs. d < 0.01

[1] Male rats, 200 g in body weight, were fasted for 16 hours and injected intravenously with 1 ml of 20% glucose-U-^{14}C or fructose-U-^{14}C; rats were killed 1 hour after injection and the percentage of radioactivity in lipids was calculated from the amount retained, after subtraction of radioactivity lost in urine (see Methods).

[2] Mean $\pm$ SE of mean.

labeled fructose or glucose. In all experiments the flow of chyle ranged between 0.6 to 1.5 ml per hour. During 10 to 14 hours of chyle collection not more than 0.35% of the dose of fructose fed was recovered in chyle lipids (table 4), though the chyle collected had a milky appearance and a triglyceride content ranging between 2.2 to 7.0 mg/ml. In one experiment, in which the collection of chyle was continued for 21 hours there was a slightly higher recovery of radioactivity in chyle lipids which, however, did not exceed 0.65% of the dose fed. Thus if the excess in serum triglycerides was due to direct conversion of the fructose molecule into lipids the intestine was probably not the main site of such a process. In fact, when labeled glucose or fructose was injected intravenously, significantly more of the fructose than glucose carbon was recovered in both liver and serum triglyceride one hour after injection, indicating that fructose per se, after having reached the liver, may contribute to the increase in serum lipids (table 5).

The actual amount of labeled lipid derived from fructose present in the circulation was very small, apparently owing to the continuous egress of the lipids from the circulation. The problem of the participation of the fructose molecule in the production of hypertriglyceridemia was investigated further under conditions when egress of lipid was impaired by intravenous administration of Triton (22, 23). Labeled fructose or glucose was given intragastically and about 50% of both sugars was absorbed in 2 hours (table 6). No data on absorption are given for the 24-hour period as it was assumed that as no diarrhea developed, absorption was complete 12 hours after the last dose of the sugars fed. Significantly more label was recovered in serum lipids of rats given radioactive fructose than from those given the glucose, at both time intervals and at all fructose loads. Similar results were obtained also in the liver, but no difference was found in the amount of label incorporated into intestinal lipids. Alkaline hydrolysis of the lipids extracted from serum, liver and intestine showed that radioac-

TABLE 6

Incorporation of glucose-U-^{14}C and fructose-U-^{14}C into lipids in rats injected with Triton WR-1339 [1]

Exp. no.	Triton injected	Hexose		Triglycerides		^{14}C-radioactivity in lipids		
		Fed	Absorbed	Serum	Liver	Serum	Liver	Stomach + small intestine
	mg/100 g	*g/100 g*	*%*	*mg/100 ml*	*mg/g*		*% of dose* [2]	
				2 hours after Triton injection				
1(3) [3]	60	None		307 ± 23.4 [4]				
1(3)	60	Glucose, 0.2	58.7	253 ± 16.8	4.1 ± 0.29	0.27 ± 0.03 [f]	0.53 ± 0.007 [l]	0.32 ± 0.05
1(3)	60	Fructose, 0.2	57.3	301 ± 10.1	5.3 ± 0.47	0.66 ± 0.04 [g]	0.60 ± 0.013 [m]	0.37 ± 0.08
2(4)	100	None		600 ± 32.1	4.6 ± 0.65			
2(4)	100	Glucose, 0.4	55.7	389 ± 22.2 [a 5]	3.8 ± 0.36	0.17 ± 0.02 [h]	0.29 ± 0.01 [n]	0.46 ± 0.14
2(4)	100	Fructose, 0.4	57.6	563 ± 38.1 [b]	5.4 ± 0.75	0.64 ± 0.16 [i]	0.65 ± 0.08 [o]	0.46 ± 0.13
				24 hours after Triton injection				
3(7)	60	Starch, 3.5		2525 ± 232 [c]	6.6 ± 0.70			
3(9)	60	Glucose, 3.5		1813 ± 86 [d]	5.9 ± 0.62	0.81 ± 0.11 [j]	0.50 ± 0.02 [p]	0.43 ± 0.01
3(9)	60	Fructose, 3.5		2902 ± 112 [e]	8.3 ± 0.57	1.63 ± 0.25 [k]	0.96 ± 0.07 [r]	0.52 ± 0.02

[1] Triton was injected intravenously into male rats and the hexose was given intragastrically either as a single dose (exps. 1 and 2) or in repeated doses (exp. 3); the time interval between the last dose of hexose and the termination of the experiment was either 2 hours (exps. 1 and 2) or 12 hours (exp. 3), during which period the rats had access to water only.

[2] % of dose in serum is per 10 ml; in liver, stomach and small intestine, per total organ.

[3] Numbers in parentheses represent number of rats.

[4] Mean ± SE of mean.

[5] a vs. b, $P < 0.01$; c vs. d, $P < 0.02$; d vs. e, $P < 0.001$; f vs. g, $P < 0.005$; h vs. i, $P < 0.05$; j vs. k, $P < 0.01$; l vs. m, $P < 0.01$; n vs. o, $P < 0.01$; p vs. r, $P < 0.001$.

TABLE 7

Distribution of radioactivity in glycerol and fatty acid moiety of lipids after intragastric administration of glucose-U-^{14}C or fructose-U-^{14}C to rats [1]

Exp. no.	Hexose fed	Time after Triton injection	Distribution of total lipid [2] radioactivity					
			Serum		Liver		Stomach + small intestine	
			Fatty acid	Glycerol	Fatty acid	Glycerol	Fatty acid	Glycerol
	g/100 g	*hr*	%	%	%	%	%	%
1	Glucose, 0.2	2	25	75	12	88	39	61
1	Fructose, 0.2	2	5	95	3	97	22	78
2	Glucose, 0.4	2	12	88	9	91	17	83
2	Fructose, 0.4	2	7	93	11	89	20	80
3	Glucose, 3.5	24	73	27	86	14	81	19
3	Fructose, 3.5	24	71	29	80	20	71	29

[1] Following isolation, the labeled lipids were subjected to alkaline hydrolysis; data are derived from the same experiments as in table 6.
[2] More than 90% of the radioactivity was found in neutral glycerides.

tivity at the 2-hour interval was recovered predominantly in the glycerol moiety of the molecule. The distribution was reversed at the 24-hour interval when most of the radioactivity was found in the fatty acid portion (table 7). As shown in table 6 serum triglyceride levels were elevated in both groups of rats 2 hours after Triton administration. However the rats that received glucose intragastrically as a single dose of 0.4 g/100 g body weight had serum triglyceride levels significantly lower than both those of the controls and of the fructose-fed rats. A similar trend is apparent also in the liver triglyceride content.

The serum triglycerides of all rats pretreated with Triton 24 hours previously were much higher than of those determined 2 hours after Triton injection. The control group in this experiment received starch intragastrically and no significant difference was found in the serum triglyceride levels between this group and that receiving fructose. The rats that received glucose had serum triglyceride levels significantly lower than those of the fructose and starch groups. A similar decrease in serum triglyceride levels was noted after single intragastric administration of glucose to normal rats, whereas rats receiving fructose had triglyceride levels not very different from those of controls (table 8). The incorporation of fructose and glucose carbons into serum and tissue lipids one and two hours after intragastric administration is shown in table 8. More label was recovered in serum and liver lipids 1 hour after administration of 0.25 g fructose-^{14}C and at 2 hours after 0.40 g of fructose-^{14}C than of glucose-^{14}C, respectively. In all experiments similar incorporation of glucose and fructose carbons into intestinal lipids occurred. Tritiated oleic acid injected intravenously to rats given glucose or fructose was incorporated in esterified lipids of liver and intestine and no difference was found between the 2 groups. However more tritiated esterified lipid was found in the serum of fructose-fed than in glucose-fed rats, at the 130-minute interval. The esterified lipid was more than 85% in the form of triglyceride (table 8).

The effect of glucose and fructose loads on lipoprotein lipase activity of adipose tissue is shown in table 9. The absolute values obtained for the saline controls differed between experiments. The lipoprotein lipase activity in epididymal pads of fructose-fed rats did not vary from that obtained from the saline control, whereas that in pads from glucose-fed rats was significantly higher both after 2 and 4 hours.

DISCUSSION

The role of fructose in lipid metabolism was suggested half a century ago by Higgins (24) who, from measurements of the respiratory quotient, arrived at a conclusion that fructose has a greater tendency for conversion into lipids than glucose. More recently the interest in fructose metabolism was renewed in conjunction with the studies of effects of various carbohy-

TABLE 8

Lipid radioactivity after intragastric administration of glucose-U-^{14}C or fructose-U-^{14}C and intravenous injection of oleic acid-9,10-^{3}H [1]

Exp. no.	Hexose fed	Time from incubation	Triglyceride		Lipid-^{14}C			Esterified [2] lipid-^{3}H		
			Serum	Liver	Serum	Liver	Stomach + intestine	Serum	Liver	Stomach + intestine
	g/100 g	*min*	*mg/100 mg*	*mg/g*	*% of administered dose* [3]			*% of administered dose* [3]		
1 (5) [4]	None		63.1 ± 7.5 [5]	6.7 ± 0.82						
1 (4)	Glucose, 0.25	70	30.4 ± 3.8 [a,6]	5.7 ± 0.84	0.03 ± 0.003	0.26 ± 0.03	0.22 ± 0.02	0.22 ± 0.02	25.1 ± 1.3	2.3 ± 0.07
1 (4)	Fructose, 0.25	70	59.3 ± 9.6 [b]	6.2 ± 0.57	0.05 ± 0.005	0.44 ± 0.04	0.18 ± 0.01	0.31 ± 0.07	18.0 ± 1.5	2.3 ± 0.27
2 (4)	Glucose, 0.40	130	46.4 ± 3.0 [c]	4.9 ± 0.75	0.04 ± 0.002	0.47 ± 0.08	0.42 ± 0.04	0.12 ± 0.03	20.2 ± 1.0	2.9 ± 0.98
2 (4)	Fructose, 0.40	130	88.5 ± 8.8 [d]	5.9 ± 0.52	0.11 ± 0.020	0.55 ± 0.05	0.30 ± 0.03	0.44 ± 0.10	18.3 ± 1.2	2.9 ± 0.30

[1] Rats fasted for 16 hours were given glucose-U-^{14}C or fructose-U-^{14}C intragastrically 10 minutes before killing; 7 μCi of oleic acid-9,10-^{3}H were injected intravenously.
[2] Esterified lipid was more than 85% in the form of neutral glycerides.
[3] Percentage of dose in serum is per 10 ml; in liver, and stomach and intestine, per whole organ.
[4] Numbers in parentheses represent number of rats.
[5] Mean ± SE of mean.
[6] a vs. b, $P < 0.05$; c vs. d, $P < 0.01$.

drates on lipid metabolism, both in the human (1–9) and in experimental animals (10, 11, 25–27). Thus, feeding of fructose to rats for 6 months was shown to result in a marked increase in liver fat and a higher rate of body weight gain than feeding of glucose (28). In addition, both long- and short-term administration of fructose resulted in an increase in serum triglycerides in the rat (10, 11), a finding corroborated also in the present study. The mechanism of the hypertriglyceridemic effect of fructose has not been finally elucidated, and some of the possibilities suggested were tested in the present investigation. Fructose could contribute toward the increase in serum lipids already at the site of its absorption, in the intestine. One could envisage an effect on the generation of NADPH, and thus lipogenesis, by induction of glucose formation from fructose, via sorbitol, a pathway normally present in seminal vesicles (29). However, sorbitol given to rats in their drinking water for 6 days, though tolerated poorly, did not cause hypertriglyceridemia and neither did fructose supplementation result in any rise in sorbitol dehydrogenase activity (unpublished observations). The other role of the intestine could lie in the mode of absorption of fructose which would depend on the activity of glucose 6-phosphatase. Thus in the guinea pig intestine the enzyme is very active and most of the ingested fructose is converted to glucose before absorption into the portal circulation (30). Owing to the low activity of glucose 6-phosphatase in the rat intestine (31) the greater part of fructose molecules is absorbed unchanged. The lack of induction of hypertriglyceridemia in fructose-supplemented guinea pigs provides additional evidence that absorption of unchanged fructose is essential for its hypertriglyceridemic effect. The latter might have been accounted for by increased rate of lipogenesis as a result of stimulation of the hexose monophosphate shunt and increase in α-glycerophosphate dehydrogenase activity in the liver (32). However, data obtained in the present study with liver slices of rats given supplements of either fructose or glucose, have shown equal stimulation of lipogene-

TABLE 9
Induction of lipoprotein lipase in adipose tissue of rats fed glucose or fructose [1]
(5 rats/group)

Hexose fed	Lipoprotein lipase activity [2]		P value
	2 hours	4 hours	
None	7.42 ± 0.81 [3]	1.42 ± 0.69	
Glucose	20.04 ± 2.76 [a]	5.33 ± 1.21 [c]	a vs. b < 0.002
Fructose	7.30 ± 0.67 [b]	0.94 ± 0.56 [d]	c vs. d < 0.02

[1] Labeled glucose or fructose was given intragastrically and the absorption was determined by measuring the water-soluble radioactivity found in the stomach and small intestine; 4 hours after intubation, absorption of both sugars was more than 90% of the administered dose.
[2] The lipoprotein lipase activity is expressed as the difference between the amount of fatty acid released from pad 1 (control) and pad 2 (incubated with heparin).
[3] Mean ± SE of mean.

sis by both sugars, in agreement with recent findings of Zakim et al. (33).

Evidence as to the greater contribution of the fructose molecule than of glucose toward the formation of triglycerides in the liver was obtained presently as well as by others (34), as significantly more fructose-^{14}C was recovered in the glycerol moiety of liver and serum lipids both after intragastric and intravenous administration. The intravenous route and the rather large load were used to equalize the glucose and fructose pools and thus obviate the possibility of unequal dilution. While in the present study most of the injected labeled hexose carbon was recovered in neutral glycerides, Kuo (35) has found more than 96% of the lipid radioactivity in phospholipids when human liver fragments were incubated with either glucose-^{14}C or fructose-^{14}C. These divergent results might be due to differences in species or experimental conditions and this question is under investigation.

An additional effect of glucose and fructose on serum lipid levels was proposed by Nikkila (33) who found that in acute experiments fructose stimulates and glucose depresses the rate of secretion of triglycerides into the serum. Some support for such a process could be seen in the higher percentage of labeled serum triglyceride after injection of oleic acid-^{3}H in fructose-treated than in glucose-treated rats (table 8). This aspect was investigated further in rats pretreated with Triton in which, owing to the blocking of egress of serum triglycerides, the measurement of serum triglyceride levels would provide information as to the rate of their secretion into the circulation (22, 23). The data obtained lend themselves to the interpretation that an acute glucose load depressed liver triglyceride formation and its release as reflected in the serum triglyceride levels which are lower than in rats receiving no sugar supplement or receiving fructose.

In the Triton-treated rat a "leak" of triglyceride from the plasma, owing to incomplete inhibition of lipoprotein lipase, was reported (23, 36). Such a "leak" might have been accentuated in the glucose-fed rats and thus contribute further to the lower triglyceride level found in those animals. The finding of lower serum triglyceride levels 1 and 2 hours after glucose than after fructose administration to normal rats (table 8) could indicate that activation of lipoprotein lipase by fructose is less marked than by glucose. This presumption could be substantiated in the present study by the finding of no stimulation of lipoprotein lipase by fructose in contrast with glucose.

It appears plausible, therefore, that the main differences in the effect of glucose and fructose on the blood triglyceride levels lie not in the stimulation of lipogenesis, but in the greater rate of conversion of fructose into lipids in the liver and in the rate of triglyceride secretion and removal. While glucose administration concurrently stimulates lipoprotein lipase release and depresses the inflow of fatty acids into the liver from adipose tissue, these effects are lacking after fructose feeding and the increased serum lipid load is cleared less readily in the fructose-fed rat.

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counts per minute	count/min
cubic centimeter(s)	cm^3
cubic millimeter	mm^3
degree(s)	°
degrees of freedom	df (*in tables*)
gram(s)	g
international unit(s)	IU (*to be used only when weight can not be given*)
kilogram(s)	kg
liter(s)	(spell out)
meter(s)	m
microgram(s)	μg (not γ)
micromicrogram(s)	μμg
microcurie(s)	μCi
micron(s)	μ
micromicron(s)	μμ
micromolar (unit of concn)	μM
micromole (unit of mass)	μmole
milligram(s)	mg
milligrams %	(*never use*)
milliliter(s)	ml
millimeter(s)	mm
millimicrogram(s)	mμg
millimicron(s)	mμ
millimole(s)	mmole
molar (mole per liter)	M
parts per million	ppm
per cent	%
probability (in statistics)	P
square centimeter	cm^2

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square meter	m^2
square millimeter	mm^2
standard deviation	SD
standard error	SE
t (Fisher's test)	*t*
weight (in tables)	wt

Other commonly accepted abbreviations may be found in the *Style Manual for Biological Journals*.[2] The isotope designation of a labeled compound should ordinarily appear *before* the name of the compound to which it applies. When following a symbol for a compound, it should be written as superscript (as, ^{14}C); when the name of the compound is spelled out, the isotope designation should be written on the same line (as, carbon-14).

Tables and figures. Follow form in current issues for the use of upper and lower case letters and italics. Authors are urged to economize on space used for tables and figures. These should fit one column width (2⅝ inches) or when necessary, two column widths (5½ inches). A charge will be made by the publisher for that space used for tables and figures which exceeds one-quarter of the space used for the manuscript exclusive of tables and figures. A table or figure should be constructed to be intelligible without reference to the text. Lengthy tabulation of essentially similar data can often be avoided by giving the number of experimental results and their mean values, with standard deviations or ranges within which the values fall. Statements that significant differences exist between the mean values of two groups of data should be accompanied by indications of probability derived from the test of significance applied. Units of measure should be indicated clearly two spaces above the first value in a column.

Original drawings, with two reproductions, to be sent to reviewers, or in the case of photographs, the original and two glossy prints, should accompany the manuscript. They should be marked on the back in ink with the author's name, complete address, and with the figure numbers. Such drawings and photographs must not exceed 8½ × 11 inches in size and must be at least 5¼ inches wide in order to fit the 2⅝-inch single column width when reduced by one-half. When a complicated figure requires more space for clarity, a proportionately larger illustration will be acceptable. But two copies of *prints* should be submitted on sheets of the same size as the text. Or if prints are smaller, they should be affixed to sheets of manuscript size. Oversize or undersize figures are difficult to handle in editing. Legends (including any keys to symbols or charts) should appear on a separate sheet. Drawings should be on white or blue-white paper or bristol board — not cream-white. They should be prepared for reproduction as line or halftone engravings. Letters and numbers should be uniform and large enough so that no character will be less than 2 mm high after reduction. A line 0.4 mm wide reproduces satisfactorily when reduced by one-half.

Figures should be lettered (preferably by stencil) in *black* India ink. For any charts made on cross section paper, use India ink on paper printed in light blue only. Charts drawn in India ink should be so executed throughout, with no typewritten material included. Graphs and charts should be given consecutive figure numbers as they will appear in the text.

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[2] See footnote 1.

the Journal of Nutrition, 78: 120–132, 1962.

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