TOXICITY OF SULFANILAMIDE IN VITAMINS A AND E DEFICIENT RABBITS

by

NARAYANA RAO KOSURI

B. V. Sc. and A. H., Osmania University, 1959

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

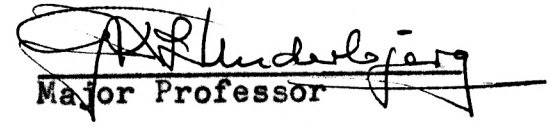
MASTER OF SCIENCE

Department of Physiology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1964

Approved by:



Major Professor

TABLE OF CONTENTS

TABLE OF CONTENTS (CONT.)

INTRODUCTION

One of the most striking advances made in the field of chemotherapy was the discovery of remarkable curative properties of the red azo dye, prontosil. This discovery led to the synthesis of a host of compounds containing the sulfanilamide group. Many workers studied the metabolism and fate of different compounds of sulfanilamide in various species (30, 65, 77, 126). It was found that the main metabolic reaction of sulfanilamide in most animal species, including man, was hepatic acetylation. It was stated that the spleen participated in acetylation in cats (123), and dogs did not acetylate sulfanilamide drugs. Marshall (76) and Shaffer and Bieter (108) showed that acetylation takes place normally in dogs and fowl. However, deacetylation occurs in dogs and fowl before urinary excretion. Krebs, et al. (65) and Marshall, et al. (77) demonstrated that the acetylated sulfanilamide was less soluble and more toxic than the parent drug. Consequently, efforts have been made to obtain a more soluble sulfanilamide, of which the acetyl derivative was also relatively soluble and less toxic than that of the earlier drugs, without loss of therapeutic efficacy in the parent drug. This seems to have been achieved in 3:4-dimethyl-5-sulfanilamidoisoxazole (grantrisin). However, interest in the metabolism of sulfanilamide remained, because some of the toxic effects of these compounds were found to be due to metabolites, particularly the acetyl derivatives. The governing factors and mechanism of acetylation has been investigated. It was shown that the degree

of acetylation was less in thiamine, riboflavin, and biotin deficient animals (79). Folic acid was illustrated as a necessary component in acetylation mechanisms (56). It has been depicted that acetate or acetyl acid derivatives influence the acetylation (79). It was demonstrated (74, 89) that vitamin A takes part in acetylation mechanism in biosynthesis of mucopolysaccharides, cholesterol and detoxication mechanisms; but how vitamin A influences sulfanilamide acetylation is not known.

There is no indication in the literature that vitamin E takes part in acetylation mechanisms, but it is closely interrelated to vitamin A in its biological activities. It is said (89) to have "a synergistic effect" on vitamin A. It has been shown that vitamin E probably plays a greater role on enzymic activities of the body (105, 107).

Nutritional deficiencies are very common among livestock, particularly of vitamins A and/or E. The role of these vitamins in metabolism and maintaining certain tissues and organs in a healthy and functional status has been studied. The toxicity of sulfanilamide has been studied, and it was reported (112) that it mainly affects the kidney, liver and blood where vitamin A and/or E has some influence.

PURPOSE OF STUDY

Sulfanilamide drugs are employed extensively in large animal practice. Nutritional deficiencies particularly avitaminosis A and/or E and related disorders are common among livestock. The effects of sulfanilamide in these conditions has not been

studied. The experiments herein reported were designed to investigate:

(a) The effect of sulfanilamide treatment in vitamin A and/or E deficient rabbits;

(b) The toxicity of sulfanilamide in deficient animals and making histopathological examination of vital organs for possible changes;

(c) The changes in certain blood and urinary constituents such as urinary quantity, urea nitrogen, creatine, creatinine, and packed cell volume values; and

(d) The effect of sulfanilamide on live body weight.

REVIEW OF LITERATURE

Metabolism and Excretion of Sulfanilamide

Sulfonamide drugs are absorbed more or less readily from the gastro-intestinal tract. The speed is varied with the compounds of different structural formulas. Oral administration of sulfanilamide is absorbed completely because of its increased solubility. Metabolism of sulfanilamides continued to be of interest, because some of the toxic effects of these compounds were found to be due to their metabolites, particularly the N^4-acetyl derivatives which are more toxic and less soluble than the parent drug.

The main metabolic reaction of sulfanilamide in most animal species, including man, was acetylation. Marshall, et al. (77), Gilligan (30), and Williams (126) reported that the acetylation occurred to a variable extent which was usually about half of the

dose. Dogs did not acetylate sulfanilamide or any of its derivatives, a point which had been investigated by Marshall (76) and Krebs, et al. (65).

Harris and Michel (40) reported that the liver of rabbits and rats was the site of acetylation of the sulfanilamides. Van Winkle and Cutting (123) reported that the spleen also participated in acetylation in cats. Martin and Rennebaum (79) studied the degree of acetylation of sulfanilamides with different chemical compounds. They noticed a decrease of acetylation in deficiency of thiamine and riboflavin. Sollmann (112) quoted that Suomalainen and Kinnunen injected thiamine into rabbits, which increased the acetylation threefold, and they suggested that the acetylation involved the diphosphothiamine redox system. Johnson (56) reported that folic acid was necessary in the acetylation mechanism. Acetylation of sulfanilamide and its derivatives in pigeon liver extracts was markedly noncompetitively inhibited by 4-aminoanalogues of folic acid and aminopterin which are folic acid antagonists.

Lipmann (69) reported that the homogenized liver pulp acetylated considerable sulfanilamide if aerated, very little if air was excluded. Addition of acetate doubled the amount of acetylation, and acetoacetate and pyruvate increased it to a lesser degree. Blondheim (9) reported that the plasma appeared to contain the precursor of the acetate. Fraenkel-Conrad and Greenburg (28) recorded an increase in acetylation of administration of thiouracil, and a decrease by thyroid administration. Hitchcock and Nelson (51) reported that paraldehyde increased the

acetylation of sulfanilamides.

Recently, the deacetylation mechanism of sulfanilamides has been studied in dogs and fowl. Marshall (76) made a comment that the dog actually acetylated the sulfa drugs which were then deacetylated before excretion. Shaffer and Bieter (108) described the acetylation of sulfanilamide occurring in the liver of the chicken, and the compound was deacetylated by an active deacetylase enzyme present in the kidney. Bray, et al. (12) have distinguished two types of deacetylating enzymes, namely, acetylglycinedeacetylase and acetanilide deacetylase, found in liver and kidney, but their occurrence depended upon species. Krebs, et al. (65) suggested that the acetylation depended upon a competition between the acetylating and deacetylating enzymes.

Davis (22) indicated from dialysis experiments that some of the sulfanilamide drug was bound by albumin of the plasma but not by globulin or lipoids. This increased the solubility, but only the unbound drug was bacteriostatic. Stowe and Sisodia (114) investigated the pharmacological properties of sulfanilamides in dairy cattle. They reported that the drug was bound to plasma protein, urine, and milk. The presence of the drug in the milk was explained on the basis of pH partition and the passive diffusion concept.

Sulfonamides are primarily excreted by the urinary system. Small amounts of sulfonamides are excreted in bile, pancreatic juice, gastric juice, intestinal juice, and in milk. However, solubility is dependent upon pH, and both sulfanilamide and its acetyl derivatives are more soluble at alkaline than at acid pH

values. As the glomerular filtrate passed through the uriniferous tubules, the reabsorption of the bicarbonate and other useful ions lowered the pH of the filtrate, favoring the precipitation of the sulfanilamides (58). Antopol and Robinson (2) have found uroliths in rats, rabbits and monkeys after feeding them the sulfanilamide for several days. Beyer, et al. (8) reported that liberal water consumption, intravenous injection of dextrose, or administering bicarbonate interfered with tubular reabsorption and, at the same time, increased the solubility of the conjugated compound of the sulfanilamide. Berliner (7) reported that a massive dose of sulfanilamide decreased the urinary acidity, presumably through inhibition of carbonic anhydrase which facilitated the excretion of sodium and potassium ions.

Sulfanilamide Toxicity

Sulfanilamides differ in kind and degree of toxicity, which depends on the compound used and on sensitization of the body. Murayama and Laeke (92) administered orally a single dose in acacia suspension to rats, which produced no fatality at 3 gm., 20 per cent at 4 gm., and 50 per cent at 10 gm. per kg. of body weight. One gm. per kg. of body weight caused gradual loss of weight and produced enlarged spleens. Four-tenths per kg. elicited no effect through intraperitonial injection into rabbits. One and one-half gm. per kg. promoted marked symptoms, such as weakness of legs, dyspnea and spasticity. Some rabbits showed chromatolysis of the central nervous system, especially in the spinal cord, with 2 gm. The surviving rabbits demonstrated

alterations in the liver and kidney. Kohn-Richards (61) reported that the toxic effects resemble those of picrotoxin and urethane.

Mendenhall and Shreeve (82) administered various sulfanilamides to dogs and observed no effect on the heart muscle. Excised frog heart was stimulated utilizing 0.25 per cent and slowed or arrested employing 0.5 per cent of sulfanilamide. Litchfield (70) reported that the skeletal muscle of frog was depressed by immersion in sulfanilamide solution.

Keilen and Mann (60) and Benesch, et al. (5) observed that the calcification of the eggshell of birds was inhibited by feeding sulfanilamide. Inhibition of calcification of eggshell occurred without other symptoms and has been attributed to inhibition of carbonic anhydrase, which was presumably necessary for shell formation.

Haerews (38) reported that sulfanilamide appeared to have a low-incidenced carcinogenic tendency in rats and mice, regardless of method of administration. Greisheimer, et al. (34) showed different effects on the blood sugar level, liver glycogen formation and storage by various sulfanilamides. Mackenzie and Mackenzie (72) reported that the basal metabolic rate of rats dropped sharply when sulfanilamides were fed. This was accompanied by thyroid hyperemia and hyperplasia (thyroid deficiency), including changes in the cells of the anterior pituitary. Astwood, et al. (3) remarked that the hyperplasia was due to failure of thyroxine synthesis.

Kracke (63) reported that cyanosis occurred in most patients with sulfanilamide. Richardson (102) cited that the mechanism

of cyanosis was due to the formation of sulfhemoglobin, which was dependent on the sulfur content of the diet, and that it was increased by giving thiosulfate, sulfur, and potassium sulfonate. James (54) showed that the oxidation of sulfanilamide by hydrogen peroxide also resulted in colored oxidation products which were absorbed by the fat of the red corpuscles envelope without affecting the oxygen carrying capacity. Oxidation products of sulfanilamide have been isolated from the urine and have been attributed to the development of cyanosis in the absence of methemoglobin and sulfhemoglobin.

Hemolytic anemia, jaundice, and secondary anemias have been noticed by many workers (49, 61). Higgines and Machella (49) administered large doses (1 gm.) of sulfanilamide in white rats, which elicited a marked decrease of the red blood cells and other macrocytic anemia changes. Southworth (113) reported that sulfanilamide lowered the carbon dioxide combining power of the blood in man. Mann and Keilen (73) attributed that the cause was the specific inhibition of carbonic anhydrase, which have been demonstrated in sulfanilamide alone. Kracke and Townsend (62) reported a decrease of blood platelets in sulfanilamide therapy. A decrease of the prothrombin levels by inhibition of the bacterial flora of the intestine by sulfanilamides was observed by Daft, et al. (19).

Herbut and Scaricacottoli (45) and Lodge and Woodcock (71) noticed diffused necrosis of the liver in sulfanilamide toxicity. Histological liver injury was studied by Menten and Andersch (83). Histopathological studies of long duration administration

of sulfanilamide in rats (3 months to 1 year) were made by Gray (32).

The most important toxic reactions produced by sulfanilamide therapy in domestic animals are chronic in nature. The most significant reaction is renal toxicity. Vilter and Blankenhorn (124) reported that sulfanilamide promoted direct renal lesions, tubular or interstitial, which were not dependent upon renal obstruction. Jones (58) described that the sulfanilamides were concentrated about fifty times while passing from the renal glomeruli through the uriniferous tubules of the normal kidney. The tendency for crystallization to occur was increased by the normal reabsorption of the alkaline sodium ions in the renal tubules, which lowered the pH of the urine and solubility of the sulfanilamide. The needle-like crystals of precipitated sulfanilamides punctured and ruptured the mucosal cells of the urinary tract. Crystals became sufficiently numerous to form calculi which obstructed the collective tubules, the renal pelvis, or the ureters. Histopathological studies were investigated by Antopol and Robinson (2) and Prien (98). They observed nephrosis with an absence of crystalluria.

In addition to these toxic reactions, certain other toxic symptoms, such as nausea, vomiting, skin eruptions, conjunctivitis, nervous disturbances, drug fever, suppression of egg production and acidosis have been noted after the administration of sulfanilamides (112).

Vitamin A and Its Relation to Vitamin E

The quantities of vitamin A absorbed and stored by mammals vary widely between species and individuals of the same species. Age is one factor which influences vitamin A, since the liver of most newly born animals contains only small amounts of the vitamin.

The value for the liver reserve vitamin A of a rabbit was 150 I.U./gm. liver weights (88). It was evident that the highest vitamin A reserve was found in some species of carnivorous animals, which presumably derived large amounts of preformed vitamin A from liver or other tissues. Herbivorous animals usually ingest provitamins in amounts which would rapidly saturate their livers, if they were efficiently converted to vitamin A. In practice, however, the rate of wastage through failure in mal-absorption and other causes was very high. Sheep accumulated the most vitamin A (600 I.U./gm. liver). This difference was believed to be related to a greater conversion efficiency of carotene to vitamin A in the intestinal mucosa of sheep, as evidenced by the absence of yellow pigments in their blood plasma and body fat.

White body fat, however, does not insure that a species of animals would be able to accumulate large stores of vitamin A when given a provitamin diet. Bentley and Morgan (6) found that most guinea pigs, in spite of their colorless fat, accumulated notably low reserves of vitamin A when they were given a diet containing green vegetables. They were quite efficient, however, in storing preformed vitamin A. They also stated that guinea

pigs contained only about 10 I.U./gm. as compared with 150 I.U./gm. in rabbits receiving the same diet.

In 1929, Moore (85) found that the removal of the usual fat components from a basal diet of rats did not noticeably affect the biological activity of a liberal dose of carotene, which was administered in a drop of archis oil. Wilson, Ahamad, and Majunder (127) confirmed the above and reported that the inclusion or exclusion of fat had little effect on growth, but the reserves of vitamin A in the liver tended to be higher in those groups receiving fat. Russell, et al. (106) reported that hens absorbed less carotene when their diet contained only 0.1 per cent of fat than 4.0 per cent. Carotene produced better growth responses when the diet contained 5 per cent lard than when the diet contained no fat.

Kraybill and Shrewsbury (64) compared the growth promoting power of graded doses of carotene in rats, administered either in cottonseed oil or butterfat, which had been decolorized by Lloyd's reagent. The provitamin was 2-3 times more effective employing the cottonseed oil as diluent. Sherman (110) pointed out that his findings on different natural oils could usually be explained by a physiological antagonism between unsaturated fatty acids and vitamin E influencing the metabolism of carotene.

Moore, et al. (90) observed that a deficiency of vitamin E in rats elicited less storage of vitamin A. It was reported that vitamin E promoted less effect on the storage of vitamin A derived from carotene than on the storage of preformed vitamin A (86).

Hickman and his colleagues (47, 48) stated that tocopherol

acted by reason of its antioxidant power on stabilizing carotene in the intestinal tract. Thus, free tocopherols were more effective than esterified tocopherols, which agreed with the findings that the tocopherols only protected fats from oxidation *in vitro* when they were added in free form. Tests utilizing human volunteers suggested that tocopherol increased the excretion of carotene in the feces due to diminished absorption (48).

Quackenbush, et al. (99) examined the effect of alpha tocopherol and other antioxidants, in relation to increasing the stability and growth promoting power of carotene. Alpha tocopherol and hydroquinone were equally effective as antioxidants *in vitro*.

Guggenhein (36) studied the efficiency of absorption of carotene from various vegetables as measured by storage of vitamin A in the liver of rats. He concluded that the efficiency of storage depended upon the tocopherol content of the vegetable. Tomarelli and Györgyi (119) observed that rice bran extracts contained a factor which could further support the action of tocopherol by increasing the growth response with small doses of carotene. Rao (100) found that the difference between the growth responses produced by solutions of carotene in arachis oil, coconut oil and olive oil were greatly reduced when the vitamin E contents of the oil were equalized by adding tocopherol to the coconut and olive oils.

Hickman, et al. (48) noted that small doses of tocopherol increased the utilization of carotene, as measured by growth responses, and larger doses promoted the reverse effect. Johnson

and Barman (57) found that small doses of tocopherol had little effect on the utilization of carotene, as measured by storage of vitamin A; on the other hand, larger doses considerably decreased the storage of vitamin A. Swick and Baumann (116) indicated that high doses of alpha-tocopherol acetate and gamma tocopherol had the same effect as alpha-tocopherol.

According to High, et al. (50) other antioxidants might vary in their effect on carotene metabolism, according to dosage. Thus, in rats, daily dosage of 0.5 mg. acetylhydroquinone promoted the storage of vitamin A accumulation following administration of 50 I.U. doses of carotene, but 10 mg. produced decreased storage. Moore (87) cited that Bernhard and associates claimed the bile pigments, bilrubin and biliverdin acted as antioxidants for vitamin A.

Moore (87) noticed that the vitamin A reserves of rats which had been maintained for long periods on deficient diets of vitamin E were invariably lower than in rats treated with vitamin E. Advanced deficiency of vitamin E accelerated the loss of vitamin A which had been stored in the liver, when fed on deficient diet. This finding explained the failure of the liver preserving power rather than the intestinal absorbing power.

The protection of vitamin E on preformed vitamin A during its absorption from the intestinal tract was studied by Hickman and his colleagues (47).

The effect of mixed tocopherols in aiding growth and increasing storage of vitamin A in rats was confirmed by Lemley, et al. (67). They emphasized that the action of tocopherol

could only be demonstrated under suitable experimental conditions. Thus, growth was promoted at low dosages of vitamin A, but depressed at higher levels. Liver storage of vitamin A was not influenced when vitamin E was given with or without tocopherol for 3 days, but considerable differences were found when treatment was continued for several months. Davis and Moor (21) stated that vitamin A reserves were decreased with prolonged deficiency of vitamin E, and vitamin A deficiency occurred as a secondary effect to vitamin E deficiency.

Goerner (31) reported that carcinogenic agents--like 1,2; 5,6-dibenzanthracene, 2-amino-5-azotoluene and methyl-chrolanthrene--decrease the liver vitamin A.

Work by Bacharach (4), in which rats were maintained on an avitaminosis E regimen for a comparatively short period of 14 weeks, treating with vitamin E elicited an improvement of 50 per cent in the storage of vitamin A. Burgess, et al. (13) reported that the addition of penicillin to the diet of chicks promoted blood and liver vitamin A content, whereas aureomycin had little effect on vitamin A. Atropine was found by Ingefinger, et al. (52) to reduce the absorption of vitamin A, as evidenced by partial or complete suppression of the increased blood vitamin A following massive vitamin dosage. They commented that the drug produced a reduction of the motor activity of the small intestines. Guggenhein (37) reported that when rats were given prolonged treatment utilizing antimalarial drug (atabrine) liver storage capacity for vitamin A was adversely affected. However, even toxic levels of atabrine did not apparently

accelerate the appearance of deficiency symptoms in rats which were deprived of vitamin A.

More extensive studies confirmed the presence of vitamin A in the liver, lungs, kidneys, and suprarenal glands (96, 97). The authors stated that vitamin A was distributed in several liver areas of normally nourished animals. Goerner (31) reported the presence of vitamin A in the liver cell mitochondria of rabbits. The work of Ganguly, et al. (29) suggested that in the liver cell, free vitamin A was associated with protein and esterified vitamin A with fat.

Young and Wald (134) observed the mobilization of vitamin A by stimulating the splanchnic nerve but not the cervical sympathetic nerves. Young and his colleague (134) noted that injections of adrenaline were effective in raising blood plasma vitamin A of rabbits. Chapman, et al. (15) observed that estradiol strengthened the power of the liver to absorb vitamin A, and that testosterone would depress the action. Crowley and Allen (18) indicated that ethyl alcohol increased the blood vitamin A of calves and goats.

Lewis, et al. (68) and Sherman and Trupp (109) studied the growth rate of rats with different allowances of vitamin A. Davis and Moor (20) demonstrated that rapid loss of vitamin A occurred during the first week of avitaminosis A with much slower losses during later weeks.

Vitamin A and Metabolism

The function of vitamin A in metabolism is not known. In

early work, Wolf and Johnson (130) cited that Von Euler and Schmidt reported the principle role of vitamin A was the stimulation of the building of cells through possible role in the nucleic acid metabolism. They also suggested a possible connection of vitamin A with oxidation reactions, which increased oxygen consumption in liver tissue with increased amounts of vitamin A. Ray and Sadhu (101) attributed their findings to a wide-spread depression in metabolism induced by hypervitaminosis A. Rosenberg (103) stated that vitamin A seems to exert some specific function concerning fat and carbohydrate metabolism. This is, fat, cholesterol, and glycogen content of the organism decreased during vitamin A deficiency, whereas excess doses promoted an increase. Ray and Sadhu (101) noted that hypervitaminosis A caused a decrease in liver glycogen and increase in liver fat.

With radioactive studies (130) it was found that vitamin A deficiency was without effect on the tricarboxylic acid cycle in the rate of acetate oxidation to carbon dioxide. It was also found (130) that there was no effect of vitamin A deficiency on the connecting link between the tricarboxylic acid cycle and glycolysis (representative of pyruvate and oxalacetate, respectively). Biosynthesis of glycogen was markedly depressed in the severely deficient animals. In <u>in vitro</u> studies (130) it was found that glucose-6-phosphate dehydrogenase, hexokinase, phosphorylase, and transdehydrogenase in the liver were unaffected by vitamin A deficiency.

Vitamin A and Detoxication

Manville (74) examined the pathological effects of interference in mucous formation by dosing rabbits with methanol. Methanol was detoxified by combining with glucuronic acid. Frequent administration of methanol produced lesions resembling avitaminosis A.

Moore (89) cited that Meunier and co-workers studied the detoxication of sodium benzoate with different dosages of vitamin A and observed that rats died from vitamin A deficiency. Moore (89) quoted that Ferrando investigated the effect of vitamin A deficiency on the urinary components of rats given sodium benzoate. Total nitrogen, urea, creatinine, and creatine was increased, while glucuronic acid decreased in the deficient animals when compared to the control group. He regarded the conjugation of benzoate with glucuronic acid as a "sortie de secours" additional to its detoxication as hippuric acid by combining with glycine; and apparently this mechanism was severely affected by the absence of vitamin A.

Moore (89) mentioned that Haley and Samuelsen and Meunier and his colleagues studied the deterioration of monobromobenzene employing various daily doses of vitamin A, which was very effective.

Moore (89) cited that Lasch noticed the cholesterol (sterol) level was increased after administering massive doses of vitamin A. Green, et al. (33) found no abnormality in blood plasma and liver cholesterol levels of avitaminosis A rats.

Vitamin A and Mucopolysaccharides

The first manifestation of vitamin A deficiency is a disruption and atrophy of all the epithelial tissues of the respiratory, intestinal and urinary tracts, and genital system that function in mucus secretion. Ultimately the mucus-secreting tissue is replaced by keratinization. Chondroitin sulphate of cartilage is affected by the deficiency of vitamin A (84).

Meyer (84) isolated three different chondroitin sulphates. Chondroitin sulfate A is a polymer of D-glucuronic acid and sulfated N-acetylgalactosamine with an N-acetylchondrosine sulfate I, chondroitin sulfate B, and chondroitin sulfate C. Meyer (84) quoted that Warner extracted three main types from pig colon mucosa, which he called (1) fucomucin contained N-acetylglucosamine, N-acetylgalactosamine and D-galactose; (2) sialomucin contained N-acetylgalactosamine; and (3) the identity was in question.

The pathway of biosynthesis of acid mucopolysaccharides was recently cleared by Markovitz, et al. (75). They showed that the acetylation by coenzyme A produced N-acetylglucosamine 6-phosphate from glucosamine-6-phosphate. Wolf and Varandani (131) investigated the localization of the function of vitamin A in a specific step in mucopolysaccharide biosynthesis. They also observed <u>in vitro</u> that vitamin A demonstrated an influence on mucopolysaccharide--bound hexosamine (acetylation of hexosamine).

Vitamin A and the Urinary Tract

In addition to the pathology of the respiratory tract, intestinal tract, and salivary glands, the urinary tract should be given important consideration. Wolbach and Howe (129) found that in their deficient rats the kidney's pelvis was replaced by keratinized epithelium. The ureters, pelvis, and bladder showed a down-growth of epithelium and formations of dermoid-like cysts in the bladder. Tyson and Smith (120) commented that the first stage appeared to be a piling up of the epithelium with infiltration of the subepithelial tissues with neutrophiles, and the second stage produced keratinized epithelium. Harris, et al. (41) noted hyperplasia in the kidney pelvis.

Van Leersum (122) followed with histological and radial studies after confirmation that calculi could readily be produced by giving a vitamin A deficient diet. Calculi were present in 130 of 297 males and 67 of 348 females. He found that the urine was usually acid. There was no infection, but hematuria was frequent. The deposits appeared to originate in the calcification of keratinized epithelium in the kidney tubules. Casts were dislodged and served as foci for calcification in the kidney pelvis, ureters, and bladder. Most of the calculi consisted of calcium, ammonium or magnesium phosphates, but concrements of calcium oxalate also were common. The question of specificity of the relationship between avitaminosis A and urolithiasis has given rise to controversy. Moore (88) cited that McCarrison and Raganathan found stones when excesses of calcium were given in

conjunction with a low protein diet but adequate in vitamin A. McCollum and Simmonds (80) confirmed the effect on stone formation of a high calcium to protein ratio. It seemed that urolithiasis may arise from different defects.

Vitamin A and Liver

Particular interest in avitaminosis A effects on the liver may arise from its importance as the main storage depot of vitamin A. Early investigations gave no indication that the liver was seriously affected by deprivation of the vitamin. Wolfe and Salter (132) reported a reduction in liver size, which they attributed to the loss of glycogen and fat. Gross (35) noted liver congestion. Thatcher and Sure (118) observed that the area around the portal space had become fibrous. Moore (88) quoted that De Ruyter described abnormalities in the reticulo-endothelial system (Kupffer cells of the liver). Bile duct injury by lack of vitamin A has been reported with formation of stones in the bile ducts or gall bladder. Hamre (39) found dilated bile ducts, accompanied by some degree of jaundice in avitaminosis A animals. Metaplasia of the epithelial lining was observed. The ducts became obstructed, and calculi of varying sizes and composition were formed but entailed a nucleus of detached epithelial cells. The obstruction of the bile ducts resulted in a mild hypertrophy of the portal tract tissues and moderate degenerative changes of the peripheral parenchymal tissues of the hepatic lobules.

Vitamin A and Hematological Abnormalities

Information on the effect of vitamin A deficiency on hematological abnormalities is not known.

Sure, et al. (115) found that rats suffering in early stages of vitamin A deficiency elicited a slight anemic condition, which was characterized by a reduction in either hemoglobin or erythrocytes. After xerophthalmia developed, the values for hemoglobin and erythrocytes were raised (probably as the result of anhydremia). Observations have been reported on avitaminosis A in relation to white blood cells. Crimm and Short (17) observed that the injections of foreign protein in vitamin A deficient animals promoted a high elevation of the leucocyte count (which suggested that the reticuloendothelial system was working inefficiently).

Vitamin E

Various metabolic relationships of vitamin E have been noted to be influenced with other factors which ranged from the nutritional to the biochemical. From the nutritional point of view, one of the most important of these was the intimate relationship with vitamin A. The vitamin A-E relationship is apparently very complicated, as the factors of this relationship have been review previously.

Several experiments have been conducted to investigate the role of tocopherol in metabolism. It was assumed (107) that vitamin E was not merely an agent which prevented peroxidation,

but rather that it fulfilled a truly catalytic function in enzyme systems which was essential for life. Dietary necrotic liver degeneration was noted in vitamin E deficiency (107). Cowin and Schwarz (16) reported that all areas of substrate utilization in the citric acid cycle appeared normal except oxalacetate. The impairment of oxalacetate metabolism became evident when malate or succinate was employed as substrate in the presence of diphospho-pyridine nucleotide. Accumulation of oxalacetate elicited a negative feedback inhibition of the oxidation of succinate and malate (107). Adenosine triphosphate, acetyl co-enzyme A, manganese, and magnesium ions were found to prevent the condition.

It was suggested (107) that the liver damage caused by bromobenzene was due to removal of methionine and cystine. Vitamin E could prevent the liver damage but not the intoxication.

A variety of papers, including those of a conflicting nature, are present in the literature relating to the effects of a vitamin E deficiency of different enzyme systems. Jacobi and co-workers (53) observed no changes in malic dehydrogenase, cytochrome oxidase, succinic dehydrogenase, lactic dehydrogenase, fumarese and adenosinetriphosphatase activities of muscle from rabbits suffering from muscular dystrophy. Rosenkrantz (105) found a decrease in the activity of ketoglutaric dehydrogenase and isocitric dehydrogenase in the terminal stage of dystrophic muscle of rabbits. Addition of vitamin E inhibited liver esterase (121) and liver acid phosphatase (53).

Hemolysis of erythrocytes (107), decrease oxygen consumption

(104), and muscular dystrophy (10) have been reported in avitaminosis E.

Oppenheimer and associates (93) showed that in E deficient rabbits there was a decrease in alpha-lipoprotein and serum albumin, but an increase in beta-lipoprotein and beta-globulin. Protein-bound carbohydrates were decreased. Moore (91) reported that vitamin E prevented renal histolysis. Tamura and co-workers (117) indicated that vitamin E protected the liver of rats from carbon tetrachloride intoxication.

Urea Nitrogen

It has been illustrated (14) that urea is formed in the liver from ammonia derived from amino acid digestion during the process of deamination. The end product of the catabolism of amino acids was mainly excreted in the urine. Urea excretion was considered to be a good index of the functional status of the liver and kidney in the animal. About 40 per cent of the urea which left the blood in the glomerular filtrate was passively reabsorbed by diffusion, in the tubules under normal conditions. Increased blood urea nitrogen was noted in glomerular nephritis, inflammation of the kidney, pyelonephritis, sulfanilamide therapy, urinary tract obstruction, hepatic or biliary tract disease, and excessive protein catabolism, while decreased blood urea was noted in acute hepatic insufficiency (14).

Urinary urea was directly proportional to increased blood urea (14). McGaughey (81) reported that vitamin E deficient mice excreted higher amounts of urea in urine. He stated that

the administration of adrenocortical hormones and thyroxine increased urea excretion. Urinary urea was lowered in renal impairment, hepatic insufficiency, and in severe acidosis due to decreased formation of urea. Woelfel, et al. (128) reported that vitamin A deficient calves excreted less urea nitrogen with increased volume of urine. Herrin and Nicholes (46) noticed decreased inulin and urea clearances in young vitamin A deficient dogs, which are turned to normal following administration of vitamin A.

Creatine and Creatinine

Creatine is derived from glycine, arginine and methionine, and is important in the phenomenon of muscle contraction as phosphocreatine. Creatinine, the anhydride of creatine and usually of endogenous origin, is excreted largely by the kidneys. It was noted (14) that creatine was not excreted in the urine but was absorbed completely by the renal tubules. Its determinations provided a good index of the functional state of the muscle.

Elevated blood creatinine occurred in any condition in which urea was increased. Higher blood creatinine was noticed in nephritis or renal functional impairment, in urinary obstruction and in cardiac decompensation (14).

Allen (1) and Borgman (10) recorded augmented creatine levels in urine of vitamin E deficient rabbits but not creatinine. It was also found in the urine during starvation, febrile and wasting diseases, and in certain myopathies. The elimination

of increased creatinine was noted in tissue catabolism and muscular atrophy.

Woelfel, et al. (128) reported a slight lowering of creatinine output in avitaminosis A calves. Pizzolato and Beard (95) showed that the injections of vitamin A, thiamine, pyridoxine, riboflavin, nicotinic acid, biotin, 2-methylnaphthoquinine, and calcium pentothenate did not influence the excretion of creatine and creatinine. Injection of cocarboxylase and cebione produced a retention in creatinine. Deatherage (23) observed elevated excretion of creatine in high fat and protein diets and depressed excretion in high carbohydrate diets.

MATERIALS AND METHODS

Preparation of Assay Animals

Sixteen New Zealand white rabbits of 4 to 6 weeks of age were utilized in the investigations and were divided at random into 8 groups, each group consisting of 2 animals. The groups were designed as delineated in Table 1. These groups were maintained on basal diet for a period of 15 days to acclimate them to the regimen. Each group of 2 animals was kept in a metabolic cage for the collection of urine. After 15 days, the rabbits were maintained on their respective experimental diets. Water and feed were provided ad libitum. Groups II, IV, VI, and VIII were administered sulfanilamide orally in a pill form at the dosage of 250 mg./kg./day for 60 days.

Table 1. Experimental design with number of rabbits on diets.

Diet	Group number	Number of rabbits
Balanced basal diet	I	2
Balanced basal diet plus oral administration of sulfanilamide	II	1*
Vitamin A deficient diet	III	2
Vitamin A deficient diet plus sulfanilamide orally	IV	2
Vitamin E deficient diet	V	2
Vitamin E deficient diet plus sulfanilamide orally	VI	2
Vitamin A and E deficient diet	VII	2
Vitamin A and E deficient diet plus sulfanilamide orally	VIII	1*

*One rabbit died during pre-experimental period.

Basal Diet

A modification of a semi-purified vitamin A and E deficient diet used by Wooley (133) and Borgman (10) was used. The diet was freshly prepared every 8 days and kept under refrigeration. The deficient diets were prepared from the same basal diet, omitting vitamin A and/or vitamin E. The ingredients were as follows:

Vitamin-free casein	30.00%
Alphacel (Non-nutritive bulk)	15.00%
Molecularily distilled lard	20.00%
Corn starch	6.70%
Sucrose	9.25%
Dextrose	8.00%
Salt mixture[1]	6.00%
Potassium acetate	2.50%
Magnesium oxide	0.50%
Linoleic acid	0.60%
Choline chloride	0.30%
Inositol	0.40%
Ascorbic acid	0.50%
P-amino benzoic acid	0.25%

Vitamin mixture:

	Mg./100gm. of diet
Alpha tocopherol acetate	10.0
Vitamin A acetate	0.6
Vitamin D_3	0.004
2 Methyl 1,4 napthoquinone	0.2
Thiamine hydrochloride	1.6
Pyridoxine hydrochloride	1.6
Riboflavin	1.6
Nicotinic acid	20.0
Folic acid	0.6
Biotin	0.6
Vitamin B_{12}	0.004

[1]Salt mixture employed was H. M. W. Salt Mixture.

Experimental Procedures

After the rabbits were placed on their respective diets, they were weighed every third day during the 60-day experimental period. Twenty-four hour urine samples were obtained every

third day by manually expressing the bladder. Blood samples were obtained from the ear vein of each rabbit the day prior to the initiation of feeding the respective experimental diets, and on the 30th day. Samples of blood were obtained from the heart on the last day of the experimental period or on the 60th day when rabbits were sacrificed for histological studies.

Packed cell volume determinations were made by use of the Adams' Micro-hematocrit Technique[1] at 0, 30, and 60 days. The pH of the urine samples were estimated with pHydrion (B) paper.

Biochemical Techniques

Determination of Sulfanilamides in Urine. Determinations of urinary sulfanilamides were made by means of a spectrophotometric technique (78, 11). Each urine sample was analyzed as follows: One ml. of urine was diluted with distilled water in a 50 ml. volumetric flask, mixed, and 2 ml. of the diluted urine were placed in another flask containing 30 ml. of water. Eight ml. of 15 per cent trichloracetic acid were then added into the flask, the solution mixed and filtered.

Free Sulfanilamides. Ten ml. of the trichloracetic acid filtrate were transferred into a test tube containing 1 ml. of 0.1 per cent sodium nitrate and mixed. After 3 minutes, 1 ml. of 0.5 per cent ammonium sulphate was added to the contents and the tube left standing for 2 minutes. One ml. of 0.1 per cent

---

[1] Adams' Micro-hematocrit Centrifuge, Clay-Adams, Inc., New York.

N-(1-naphthyl) ethylenediamine dihydrochloride was then added into the tube for color development. The volume was diluted to 20 ml. with distilled water.

Total Sulfanilamide. Ten ml. of trichloracetic acid filtrate were put in a test tube, 0.5 ml. of 4N hydrochloric acid was added and the contents mixed thoroughly. The test tube was then placed in boiling water for 1 hour, cooled, and made up to 10 ml. with distilled water. Sodium nitrate, ammonium sulphamate, and ethylenediamine dihydrochloride were then added as mentioned under Free Sulfanilamides, and diluted to 20 ml.

The Coleman Model 14 Spectrophotometer[1] was set at 100 per cent transmission employing a standard of 8 ml. distilled water and 2 ml. of 3 per cent trichloracetic acid utilizing 545 mu wavelength. The determinations were calculated by employing a graph prepared from utilizing the same technique with known quantities of sulfanilamide.

The average value of the 2 rabbits for each group was taken, and the total sulfanilamide excreted in 24 hours was calculated with the amount of urine excreted in 24 hours. (The quantity of the level given was already known.)

$$\frac{\text{Total amount of drug excreted in 24 hrs.}}{\text{Amount of drug administered}} \times 100 =$$

Per cent of total drug excreted in 24 hours.

The free drug excreted was calculated as mentioned above. Per cent of acetylated sulfanilamide was obtained by subtracting

---

[1]Coleman Instruments, Inc., Maywood, Illinois.

the free form from the total drug excreted.

Determination of Urea Nitrogen in Urine. Urea nitrogen in urine was determined employing a modification of two methods (27, 135) which was as follows:

One ml. of urine was diluted to 2 ml. with distilled water. One ml. of alcoholic urease solution and 1 drop of sodium phosphate buffer were added to 1 ml. of diluted urine in a test tube. (The alcoholic urease solution was prepared by placing 3 gm. of permutite in a flask, washed once with 2 per cent acetic acid and twice with distilled water, with 5 gm. of fine jack-bean meal and 100 ml. of 30 per cent alcohol. This was shaken gently and continuously for 15 minutes and filtered.) This mixture was digested in warm water at 42 degrees C. for 10 minutes. The contents of the flask were then transferred to a 100 ml. volumetric flask and diluted to a volume of about 80 ml. A known standard was prepared in a second flask containing 0.5 mg. of nitrogen and 1 ml. of urease solution, and water was added to a volume of 80 ml. One ml. of gum ghathi solution was added to each flask. Ten ml. of Nessler's reagent were added to the swirled flask contents and diluted immediately to 100 ml., mixed, and allowed to stand for 5 minutes.

The Coleman Model 14 Spectrophotometer was adjusted to 100 per cent transmission employing distilled water blank prepared similarly to the standard, except that the standard nitrogen solution was omitted and the per cent transmission readings were recorded at 480 mu wavelength for each sample.

The average reading of each group was determined and

calculated with the amount of urine excreted per kg. body weight per day. (As ammonia was insignificantly present in urine, the amount of ammonia nitrogen present was not deducted from urea.)

Creatine and Creatinine Determination in Urine. Urinary creatine and creatinine were determined by Folin's method (24). One ml. of urine was placed in a 100 ml. volumetric flask, and 20 ml. of 1 per cent picric acid solution were added and followed by 1.5 ml. of 10 per cent sodium hydroxide solution. The contents were mixed gently and allowed to stand 15 minutes. The solution was diluted to 100 ml. with distilled water and mixed by inversion. Total creatinine determination was measured by placing 1 ml. of urine in a 500 ml. volumetric flask, 20 ml. of 1 per cent picric acid solution, a few small pebbles, and the flask was weighed to the nearest gm. Then, 150 ml. of water were added to the contents of the flask and the contents boiled for 1 hour. After that, water was added to the flask to the original weight of the flask contents and cooled to room temperature. One and one-half ml. of 10 per cent sodium hydroxide were added and allowed to stand for 15 minutes. The contents were diluted to 100 ml. with distilled water and mixed by inversion. A blank with water and a standard with known amount of creatinine were prepared as mentioned above.

The Coleman Model 14 Spectrophotometer was set at 100 per cent transmission with the blank at 520 mu wavelength, and then the standard and other sample values were recorded.

The average readings of each group were determined and calculated for the amount of urine excreted per kg. body weight per

day. The creatine levels were recorded by subtracting the urinary creatinine level from the total level of urinary creatinine.

Determination of Sulfanilamide in the Blood. Sulfanilamide in the blood was determined by the Bratton and Marshall method (11). One ml. of oxalated blood was added to 31 ml. of water in a 125 ml. Erlenmeyer flask, mixed, and 8 ml. of 15 per cent trichloracetic acid solution were added, mixed thoroughly, and filtered. The remaining procedure for total and free sulfanilamide was followed as described previously in the determination of sulfanilamide in urine. The total sulfanilamide and free sulfanilamide present in the blood was calculated per 100 ml. of blood. Acetylated form of sulfanilamide was calculated by subtracting the free form of drug from the total sulfa drug.

Determination of Blood Urea Nitrogen. This was described by Folin and Svedburg (25), Karr (59), and Sobel, Mayer, and Gottfield (111). One ml. of oxalated blood was placed in a test tube, and 7 ml. of diluted phosphate buffer solution were added. A piece of urease paper was then placed in the test tube and the contents were mixed thoroughly. The contents were incubated at 37 degrees C. for 30 minutes, shaking the tube at five-minute intervals. After the tube was removed from the incubator, 1 ml. of 10 per cent sodium tungstate solution and 1 ml. of 0.9 N sulfuric acid were added and allowed to stand for 10 minutes. The tubes were centrifuged at 2000 r.p.m. for 10 minutes, 2 ml. of the supernatant fluid were pipetted into labeled test tubes, 8 ml. of water, one drop of gum ghatti solution and 4 ml. of

Nessler's solution were added and mixed by inversion. The procedure was followed by taking 1 ml. of water for blank and 1 ml. of standard urea solution for standard.

The Coleman Model 14 Spectrophotometer was set at 100 per cent transmission with the distilled water blank at 480 mu wavelength standard, and the samples were read between 14 to 16 minutes. The concentration of the unknowns was calculated in milligrams of urea nitrogen per 100 ml. of blood.

Determination of Blood Creatinine and Creatine. Protein-free blood filtrate was prepared as described by Folin and Wu (26). Two ml. of oxalated blood were placed in a volumetric flask and 16 ml. of N/12 sulfuric acid were added and mixed. Two ml. of 10 per cent sodium tungstate solution were added drop by drop with constant shaking of the flask. The stoppered flask and contents were shaken vigorously and filtered after 15 minutes. Ten ml. of the protein-free blood filtrate were placed in a test tube, 5 ml. of freshly prepared alkaline picrate solution were added and the tube allowed to stand for 15 minutes (94). A blank with distilled water and a standard with a known quantity were also prepared.

The Coleman Model 14 Spectrophotometer was set at 100 per cent transmission with a blank at 520 mu wavelength. The concentration of the unknowns was calculated in milligrams of creatinine per 100 ml. of blood.

The total creatinine was determined as follows: 15 ml. of protein-free blood filtrate were placed in a test tube, and 1 ml. of normal hydrochloric acid was added, boiled for about 15

minutes at 150 degrees C. and cooled. Then 5 ml. of alkaline picric acid solution were added and diluted to 25 ml. with distilled water. The samples were calculated as mentioned under Blood Creatinine Determination. The creatine readings were determined by subtracting the blood creatinine reading from the total blood creatinine readings and are expressed in milligrams per 100 ml. of blood.

Preparation of Histological Sections. After 60 days treatment with sulfanilamide, the rabbits were sacrificed, autopsied, and liver, kidney, urinary bladder, and ureter specimens were collected and fixed immediately in 10 per cent buffered formalin. Histological sections were prepared employing hematoxylin and eosin stain and were examined.

RESULTS

In the pre-experimental period, one rabbit from each group No. II and No. VIII died. The remaining rabbit in group VIII died on the 27th day of the experimental period. To compare the histological sections of the said rabbit, a rabbit from group No. VII was sacrificed at the same time. Results of the experiment are delineated in Tables 2-9 and Figures 1 to 8, inclusive. Table 10 shows the averaged values of the various constituents of the blood and urine during the 60-day experimental period for the different groups of rabbits. In order to discuss whether the blood and urine constituents of the treated groups were increased or decreased over the basal groups, the data were converted to percentages, and these data are presented in Table 11.

Body Weights

The body weights, as recorded in Table 2 and shown in Fig. 1, indicate a great difference in body weights, and the rate of growth varied from group to group. Group I, normal basal diet rabbits, gained weight uniformly with an average of 27 gm. per day--that is, about 182 per cent over their initial weight--during the 60-day experimental period. The rabbits administered sulfanilamide, group II, gained an average of 20.8 gm. per day in the 60-day period, which was a decrease of 55 per cent as compared to group I.

Vitamin A deficient rabbits, group III, gained an average of 17.4 gm. per day during the period, which was a decrease of 69 per cent in relation to control group I. Group IV, vitamin A-deficient and sulfanilamide administered rabbits, gained an average of 14.8 gm. per day during the treatment, which was a decrease of 73 per cent over the control group and 50 per cent over group II receiving sulfanilamide.

The vitamin E deficient rabbits, group V, did not grow as well as the vitamin A deficient rabbits. They gained 13.3 gm. per day, which was a decrease of 103 per cent over group I and 34 per cent over group III. Sulfanilamide administered rabbits, group VI, gained 10.1 gm. per day, a decrease of 116 per cent over group I and 61 per cent over group II, with a difference between groups IV and VI of 43 per cent.

In vitamin A and E deficient animals, group VII, the growth

Table 2. Body weights in grams of the experimental rabbits.

	Days: 0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60
Group I																					
Basal diet																					
Rabbit 1	910	1210	1306	1374	1528	1590	1678	1730	1820	1938	1990	2060	2104	2166	2210	2300	2350	2400	2484	2562	2604
Rabbit 2	880	1064	1230	1308	1444	1480	1572	1684	1794	1868	1902	1950	2060	2082	2130	2204	2230	2280	2300	2410	2428
Group II[1]																					
Basal diet + Sulfanilamide																					
Rabbit 1	993	1101	1260	1245	1320	1393	1400	1438	1507	1600	1649	1716	1776	1853	1894	1945	2005	2098	2106	2172	2242
Group III																					
Vit. A deficient diet																					
Rabbit 1	972	1160	1300	1374	1440	1508	1568	1576	1590	1630	1660	1752	1760	1780	1820	1844	1864	1904	2000	2012	2050
Rabbit 2	904	1116	1224	1238	1320	1370	1418	1488	1540	1532	1608	1640	1644	1646	1714	1740	1776	1874	1896	1908	1922
Group IV																					
Vit. A deficient diet + Sulfanilamide																					
Rabbit 1	882	1260	1268	1370	1420	1324	1384	1404	1390	1470	1450	1537	1558	1650	1600	1624	1700	1620	1680	1702	1772
Rabbit 2	780	960	1012	1170	1172	1120	1216	1268	1382	1458	1550	1575	1570	1670	1632	1620	1600	1580	1640	1680	1672
Group V																					
Vit. E deficient diet																					
Rabbit 1	1206	1300	1412	1470	1400	1352	1408	1424	1510	1500	1520	1520	1570	1608	1606	1606	1678	1798	1810	1866	1870
Rabbit 2	856	1002	1114	988	1192	1260	1300	1376	1446	1448	1460	1546	1604	1660	1600	1610	1658	1712	1720	1782	1794
Group VI																					
Vit. E deficient diet + Sulfanilamide																					
Rabbit 1	1030	1124	1162	1180	1256	1290	1248	1228	1270	1346	1300	1250	1280	1304	1368	1454	1500	1540	1604	1612	1616
Rabbit 2	918	900	862	960	1000	1106	1189	1246	1240	1240	1254	1242	1260	1300	1320	1382	1412	1460	1570	1552	1588
Group VII																					
Vit. A & E deficient diet																					
Rabbit 1	914	970	1108	1222	1224	1336	1414	1506	1340	1440	1456	1418	1514	1562	1660	1620	1656	1676	1666	1676	1686
Rabbit 2	1112	1036	1088	1076	1022	1226	1292	1264	1274	1384	--[2]	--	--	--	--	--	--	--	--	--	--
Group VIII[1]																					
Vit. A & E deficient diet + Sulfa.																					
Rabbit 1	1066	1122	1224	1260	1324	1386	1416	1390	1260	1118	--[2]	--	--	--	--	--	--	--	--	--	--

[1]No second rabbit in groups II and VIII.
[2]Rabbits died.

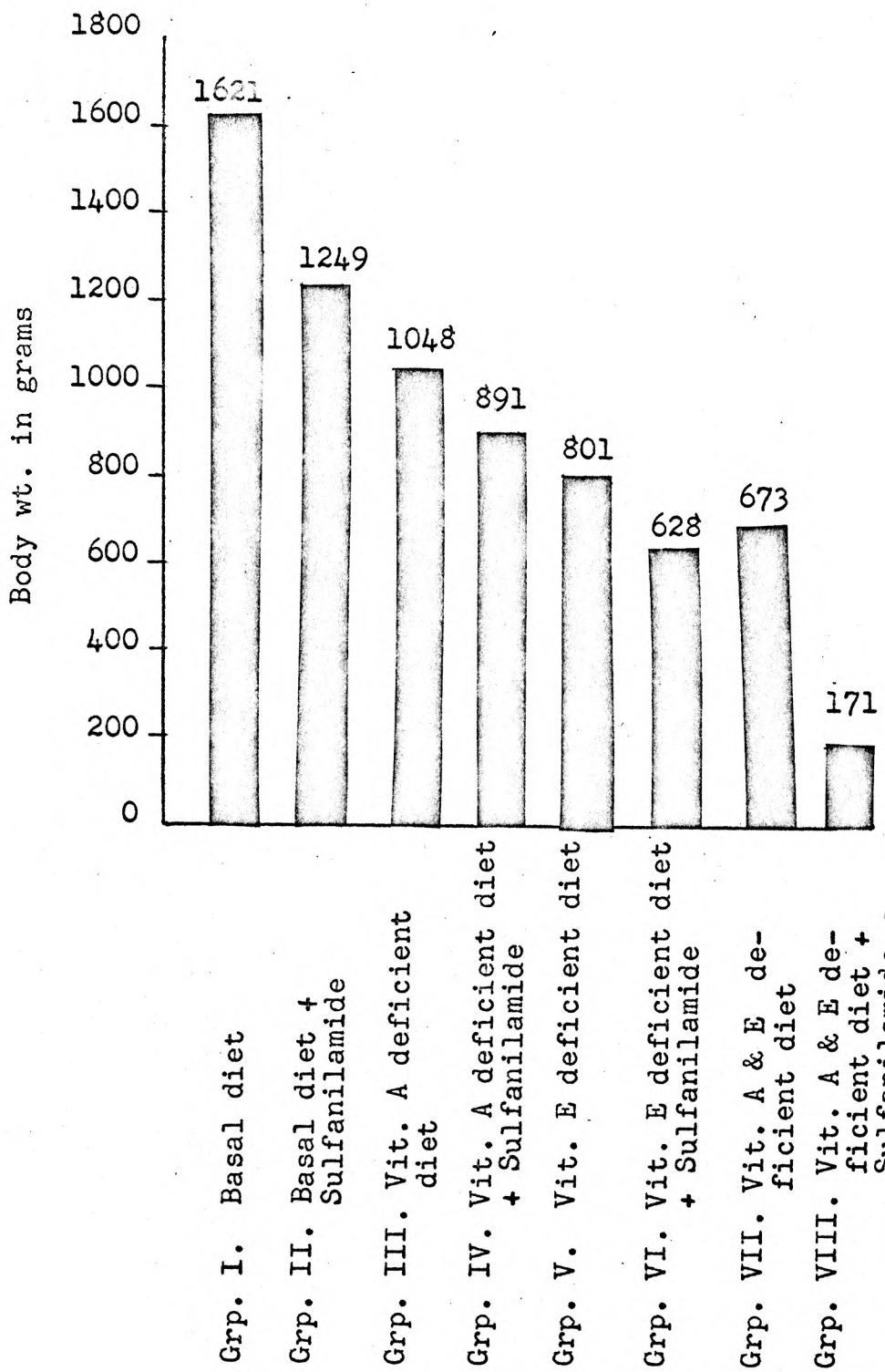


Figure 1. Average Body Weight Gain Over Initial Body Weight of Experimental Groups.

rate was 11.2 gm. per day, a decrease of 114 per cent over group I. The sulfanilamide administered rabbit, group VIII, died on the 27th day. The growth rate was 17.1 gm. per day up to that time.

Quantity of Urine

Quantity of urine was recorded in ml. per kg. body weight per 24 hours, as shown in Table 3 and Fig. 2. The urinary excretion was not uniform. Group I, control rabbits, excreted an average of 80.6 ml. of urine/kg. body weight per day, but the sulfanilamide administered rabbit, group II, produced 11 per cent less urine. Urinary excretion was increased from the 15th to the 30th day in group II, and thereafter, a decrease was noted.

In group III, vitamin A deficient rabbits, there was a slight increase in excretion of urine, 12 per cent over group I. Sulfanilamide treatment in vitamin A deficient rabbits, group IV, excreted 14 per cent less urine than group III, but 8.3 per cent more than group II. Increased excretion of urine from the 12th to the 21st day was noticed, and a decrease thereafter.

Vitamin E deficient rabbits, group V, excreted less urine than groups I and III, by 6.9 per cent and 19 per cent, respectively. Sulfanilamide administration in vitamin E deficient rabbits, group VI, produced less urinary excretion than groups II and IV, by 3.3 and 12 per cents, respectively. After 48 days of the experiment there was increased urine excretion noted in groups V and VI.

Table 3. Urine quantity excreted by various experimental groups.[1]

	Collection day																				
	0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60
Group I Basal diet	80	101	118	78	104	95	87	65	72	87	60	71	75	68	70	102	74	66	82	76	62
Group II Basal diet + Sulfanilamide	62	81	74	85	66	81	110	112	98	62	88	52	58	50	62	58	55	69	60	58	58
Group III Vit. A deficient diet	71	82	66	98	70	68	86	78	100	96	110	86	120	85	106	95	136	82	101	74	88
Group IV Vit. A deficient diet + Sulfanilamide	80	74	68	60	92	103	115	98	90	88	56	48	72	64	130	62	72	52	71	66	53
Group V Vit. E deficient diet	65	81	76	50	69	72	65	80	56	51	54	41	57	62	84	53	129	113	110	101	111
Group VI Vit. E deficient diet + Sulfanilamide	92	45	53	74	62	44	60	54	46	68	30	54	46	50	65	68	71	84	92	110	106
Group VII Vit. A & E deficient diet	84	76	59	55	56	62	88	71	81	95	102	110	80	122	108	88	72	86	95	92	109
Group VIII Vit. A & E deficient diet + Sulfa.	72	52	48	44	58	52	48	36	40	20	--[2]	--	--	--	--	--	--	--	--	--	--

[1]Average ml./kg. body weight/day.
[2]Rabbit died.

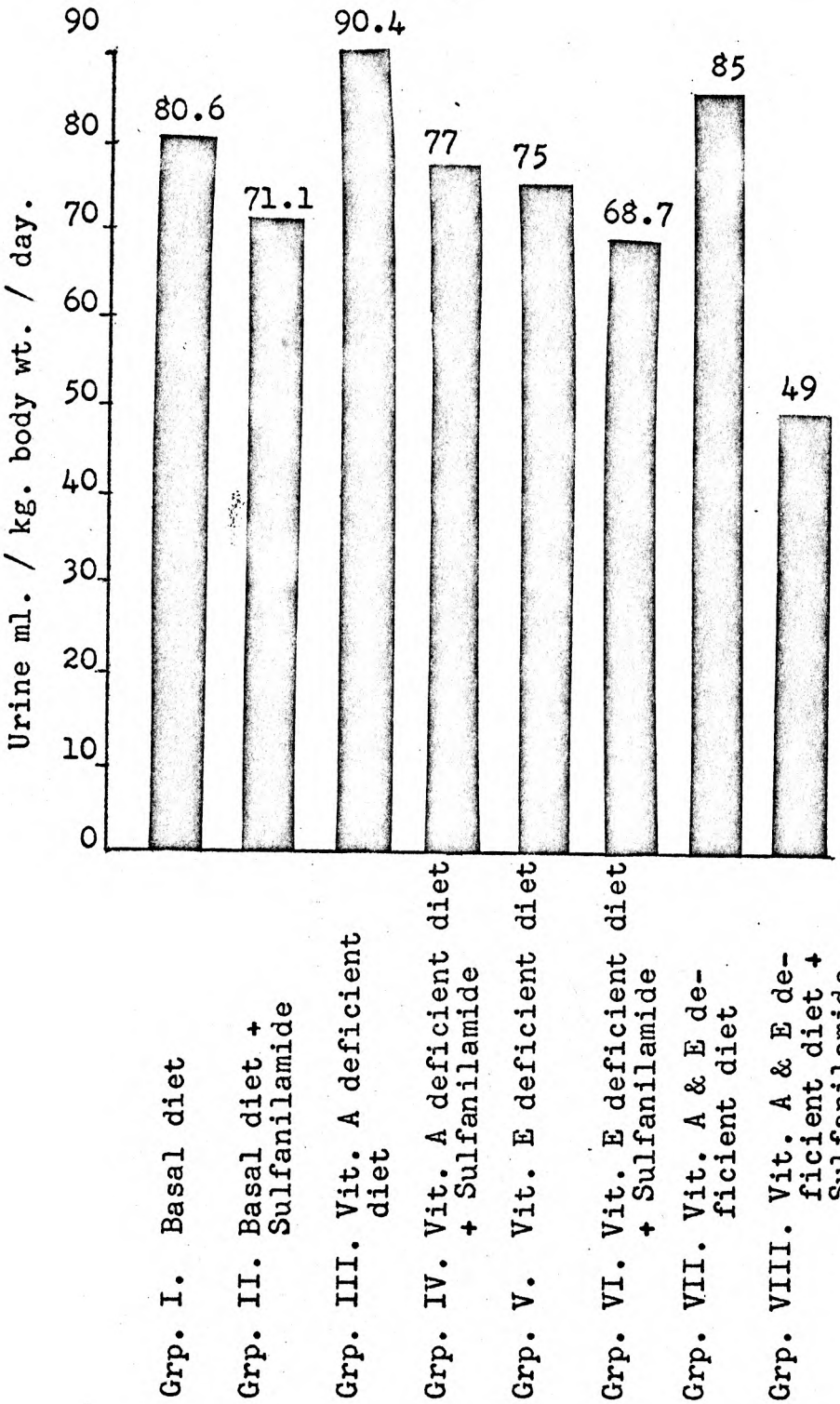


Figure 2. Average Urine Quantity Excreted by Experimental Groups.

Group VII, vitamin A and E deficient rabbits, excreted slightly more urine than group I, by 6.3 per cent. Sulfanilamide administration in vitamin A and E deficient rabbits elicited a marked decrease in excretion of urine.

Sulfanilamide in Urine

The per cent of total excretion of sulfanilamide includes free and acetylated form of sulfanilamide in urine, as shown in Table 4 and Fig. 3. Control group, No. II, excreted an average of 75.9 per cent of the total drug administered, of which 42.3 per cent was in free form and 33.6 per cent was in acetylated form.

Sulfanilamide administration in vitamin A deficient rabbits, group IV, produced excretion of 10.4 per cent less total sulfanilamide than group II. There was an increase of 12.6 per cent in the free form and a decrease in the acetylated form of 12.7 per cent as compared to controls.

Sulfanilamide administration in E deficient rabbits, group VI, promoted excretion of 5.4 per cent total sulfanilamide over group II. There was a decrease of 15.6 per cent of the free form and an increase of 13.6 per cent of the acetylated form of the drug, over the controls.

Sulfanilamide administration in A and E deficient rabbits, group VIII, caused excretion of 29.3 per cent less of the total drug than the controls, with a decrease in free and acetylated forms of drug during the 27 days.

Table 4. Urinary sulfanilamide excretion by various experimental groups.[1]

		Collection day																			
	0[2]	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60
Group II																					
Basal diet + Sulfanilamide																					
Free form	---	39	40	32	55	40	56	42	54	49	36	56	50	32	36	33	30	40	36	50	40
Acetylated form	---	23	41	25	35	31	30	32	28	28	34	26	29	36	44	32	44	42	40	31	42
Total drug	---	62	81	57	90	71	86	74	82	77	70	82	79	68	80	65	74	82	76	81	82
Group IV																					
Vit. A deficient diet + Sulfanilamide																					
Free form	---	37	45	42	35	41	43	34	54	57	46	46	41	51	57	52	50	42	52	47	56
Acetylated form	---	47	32	36	33	40	32	31	16	21	16	18	15	10	12	11	16	13	8	14	12
Total drug	---	84	77	78	68	81	75	65	70	78	62	64	56	61	69	63	66	55	60	61	68
Group VI																					
Vit. E deficient diet + Sulfanilamide																					
Free form	---	47	39	31	24	33	25	41	39	34	40	30	38	32	40	36	27	28	28	36	25
Acetylated form	---	41	39	41	56	53	47	43	56	58	53	50	58	48	42	45	50	41	37	34	37
Total drug	---	88	78	72	80	86	72	84	95	92	93	80	96	80	82	81	77	69	65	70	62
Group VIII																					
Vit. A & E deficient diet + Sulfanilamide																					
Free form	---	42	40	53	29	16	21	32	42	43	--[3]	--	--	--	--	--	--	--	--	--	--
Acetylated form	---	29	36	32	34	40	31	22	18	12	--	--	--	--	--	--	--	--	--	--	--
Total drug	---	71	76	85	63	56	52	54	60	55	--	--	--	--	--	--	--	--	--	--	--

[1]Average percentage excretion of the Sulfa administered.

[2]No administration of Sulfa drug.

[3]Rabbit died.

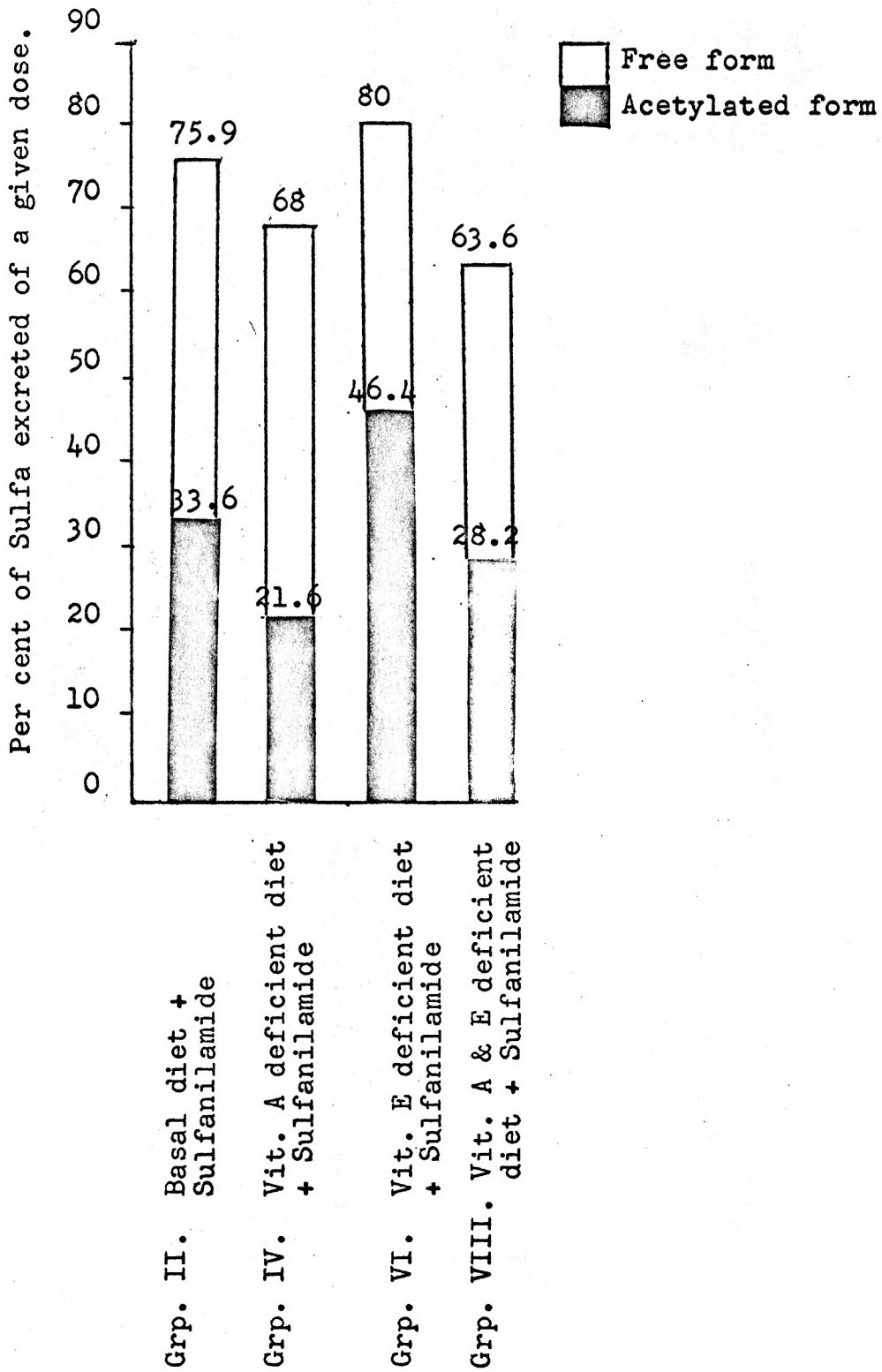


Figure 3. Average Urinary Sulfanilamide Excretion of Experimental Groups.

Urinary Urea Nitrogen

The amounts of urinary urea nitrogen are illustrated in Table 5 and Fig. 4. The excretion of urea nitrogen was not uniform, but there was increase in groups II, IV, and VI in the terminal days of the experiment.

Group I rabbits excreted an average of 306.5 mg./kg./day, but the rabbits administered sulfanilamide, group II, produced an average of 30.6 per cent more urea nitrogen than group I.

Group III, vitamin A deficient rabbits, produced low levels of urea nitrogen amounting to 24.6 per cent less than group I. Group IV, sulfanilamide administered rabbits, excreted 30 per cent more urea nitrogen than group III, but 22.4 per cent less than group II.

Group V, vitamin E deficient rabbits, produced only 1.6 per cent more urea nitrogen than group I, and group VI, administered sulfanilamide, excreted 32.5, 35.3, and 30.3 per cents more urea nitrogen than groups I, IV, and V, respectively.

In group VII, vitamin A and E deficient rabbits, less urea nitrogen was excreted than groups I and III by 34.7 and 15.5 per cents less, respectively; and the sulfanilamide administered rabbits, group VIII, produced 40 per cent more urea nitrogen than group VII and 29.8 per cent less than group II.

Creatine and Creatinine in Urine

The amounts of creatine and creatinine excreted in urine are depicted in Table 6 and Fig. 5, and are expressed in mg./kg./day.

Table 5. Urinary urea nitrogen excretion by various experimental groups.[1]

	Collection day																				
	0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60
Group I Basal diet	462	314	218	360	248	310	256	308	342	208	384	236	196	262	297	314	356	362	280	368	356
Group II Basal diet + Sulfanilamide	346	309	242	436	402	341	388	494	522	452	488	442	316	374	377	508	236	442	468	311	586
Group III Vit. A deficient diet	352	322	190	220	251	188	210	215	198	202	172	132	145	186	200	212	256	185	112	206	308
Group IV Vit. A deficient diet + Sulfanilamide	296	430	239	402	388	342	232	342	332	185	161	182	200	112	162	254	416	376	486	310	386
Group V Vit. E deficient diet	327	308	276	310	368	374	260	388	281	206	196	231	216	374	206	416	321	370	378	420	316
Group VI Vit. E deficient diet + Sulfanilamide	386	302	376	410	410	204	378	360	346	368	390	406	305	466	488	460	546	416	562	500	562
Group VII Vit. A & E deficient diet	312	366	410	301	211	251	150	138	155	141	120	174	202	183	168	170	87	138	218	143	182
Group VIII Vit. A & E deficient diet + Sulfa.	394	380	365	406	374	218	138	181	182	171	---[2]	---	---	---	---	---	---	---	---	---	---

[1]Average mg./kg. body weight/day.
[2]Rabbit died.

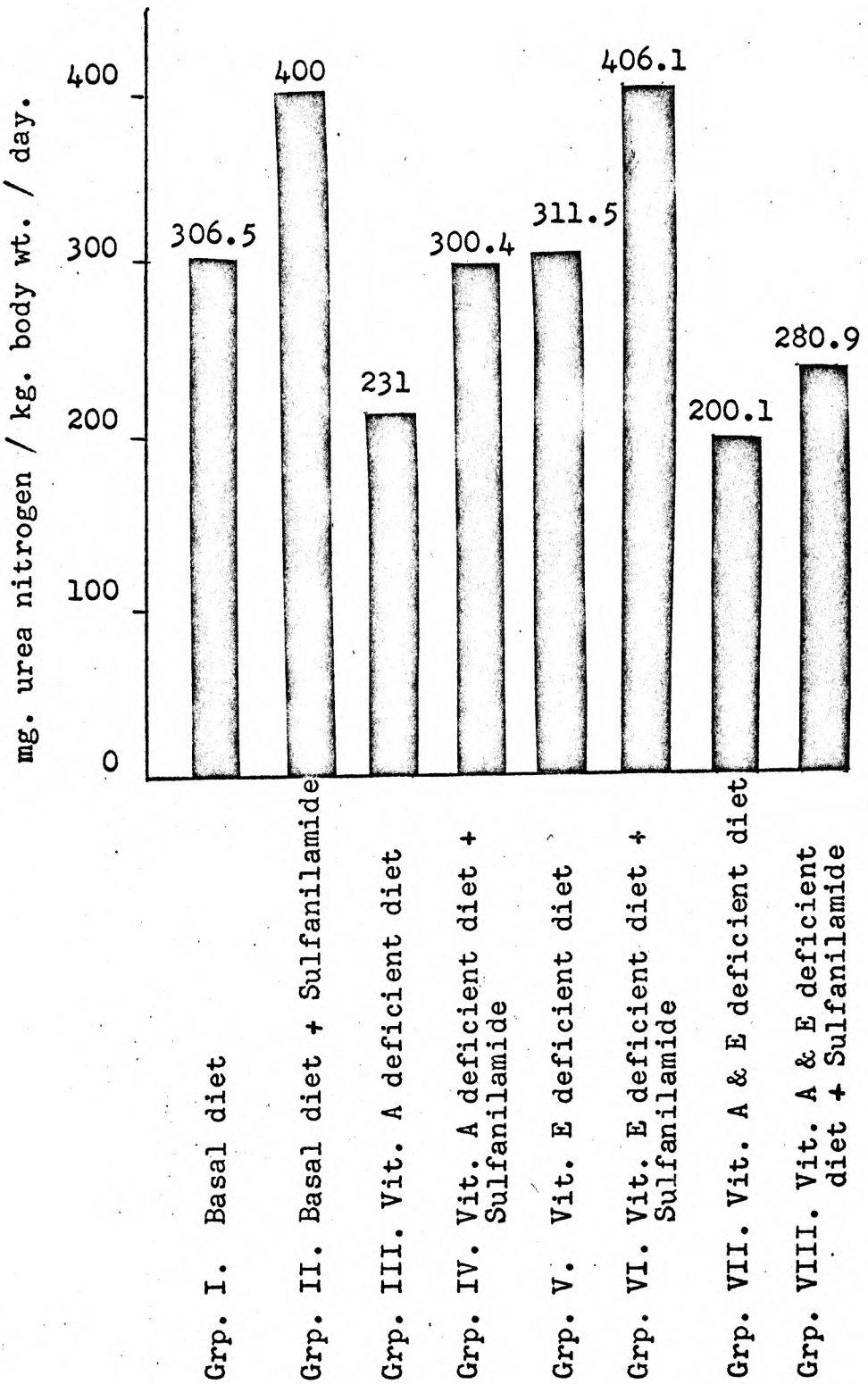


Figure 4. Average Urinary Urea Nitrogen Excretion of Experimental Groups.

Table 6. Urinary creatinine and creatine excretion by various experimental groups.[1]

	Collection day																				
	0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60
Group I																					
Basal diet																					
Creatinine	41	36	38	35	42	55	30	48	48	36	31	42	35	58	37	40	42	36	48	52	42
Creatine	0	2	0	4	2	4	0	2	3	0	0	4	6	3	0	0	2	4	2	4	2
Group II																					
Basal diet + Sulfanilamide																					
Creatinine	46	45	42	74	68	82	92	88	112	55	75	74	111	87	80	108	78	82	88	94	75
Creatine	2	2	4	2	2	2	8	0	12	2	4	2	7	2	0	10	2	8	6	16	2
Group III																					
Vit. A deficient diet																					
Creatinine	50	45	36	46	46	33	36	46	44	40	48	46	49	41	40	48	49	42	53	55	58
Creatine	2	2	2	4	2	2	4	2	4	2	0	2	6	2	2	4	4	2	4	2	4
Group IV																					
Vit. A deficient diet + Sulfanilamide																					
Creatinine	56	54	48	52	78	86	87	92	95	112	107	85	111	49	108	114	118	100	96	78	86
Creatine	2	0	2	4	2	4	2	12	4	8	4	2	8	8	6	10	2	8	2	2	2
Group V																					
Vit. E deficient diet																					
Creatinine	48	49	48	52	57	40	55	62	102	105	114	108	68	78	86	85	92	86	116	126	112
Creatine	2	2	2	0	8	6	2	4	16	8	22	4	10	7	26	0	26	10	32	28	29
Group VI																					
Vit. E deficient diet + Sulfanilamide																					
Creatinine	51	46	52	56	69	78	77	80	85	106	128	118	86	94	105	110	101	136	126	116	130
Creatine	2	0	6	2	4	0	2	8	4	27	12	18	10	6	2	4	16	46	41	32	18
Group VII																					
Vit. A & E deficient diet																					
Creatinine	55	67	56	54	72	76	78	80	86	92	105	82	88	110	106	87	84	91	112	115	122
Creatine	0	4	2	6	2	6	0	12	12	16	6	14	16	22	8	16	20	12	28	30	34
Group VIII																					
Vit. A & E deficient diet + Sulfa.																					
Creatinine	50	54	46	80	96	102	136	155	150	160	--[2]	--	--	--	--	--	--	--	--	--	--
Creatine	2	4	0	11	21	8	12	58	22	28	--	---	--	--	--	--	--	--	--	--	--

[1] Average mg./kg. body weight/day.

[2] Rabbit died.

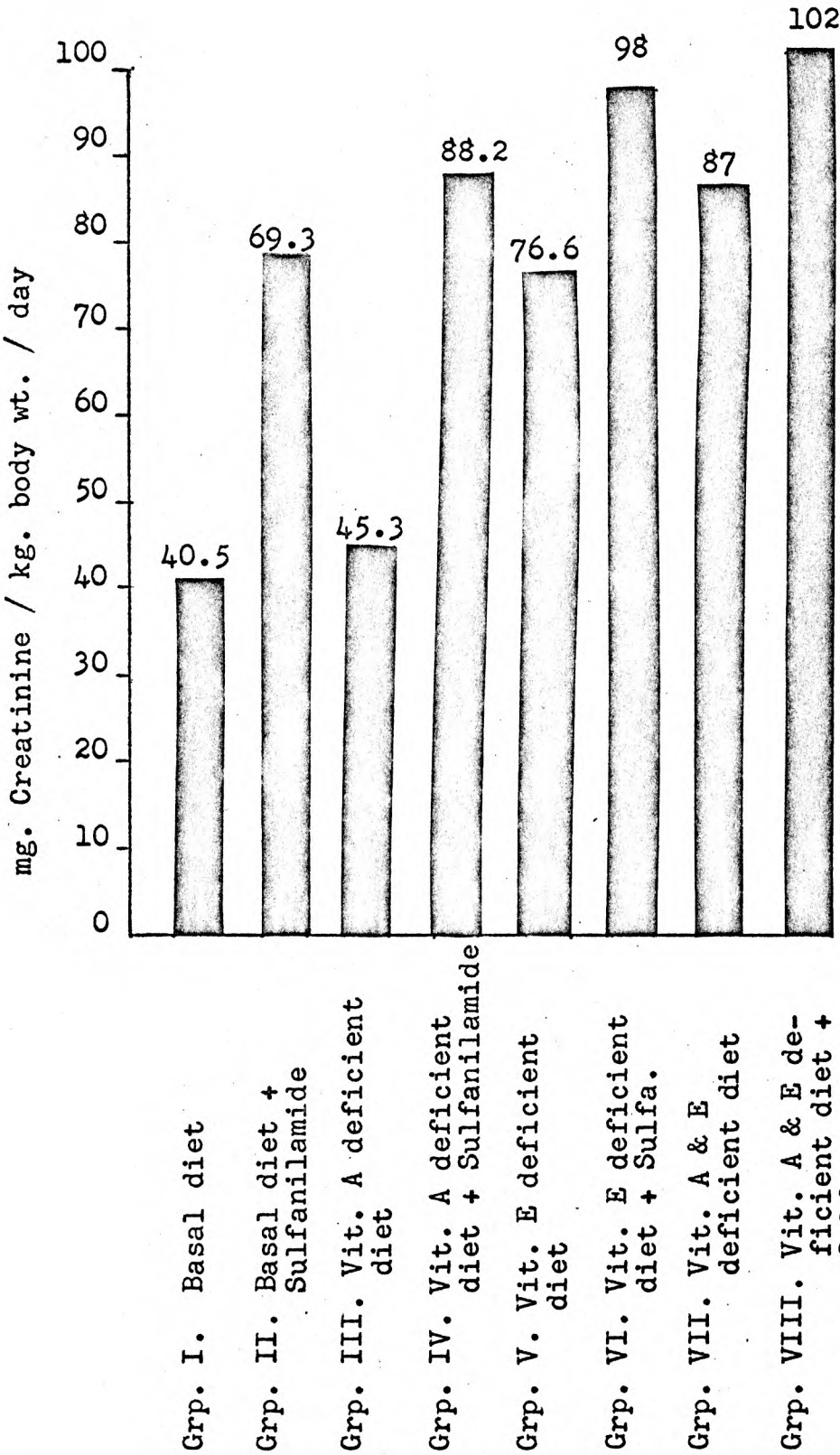


Figure 5. Average Urinary Creatinine Excretion of Experimental Groups.

Sulfanilamide administered rabbits, group II, on the average, excreted more creatinine (95.8 per cent) than group I.

There was not much change in creatinine and creatine levels in vitamin A deficient rabbits, group III, but in the sulfanilamide administered rabbits, group IV, there was an increase in creatinine levels: 105.9 per cent over group I and 11 per cent over group II.

Vitamin E deficient rabbits, group V, excreted more creatinine and creatine (189 and 44 per cents, respectively) than group I. In sulfanilamide administered rabbits, group VI, 142 per cent more creatinine was produced than in group I, and 28 per cent more than in group V, but it produced no effect on creatine levels.

Group VII, vitamin A and E deficient rabbits, excreted 13 and 10 per cents more creatinine and creatine, respectively, than group V; and the sulfanilamide administered rabbits, group VIII, produced more creatinine and creatine than groups VI or VII in the 27 day period. The greater excretion was elicited prior to death.

The creatine and creatinine levels were augmented in later stages of deficiency of vitamin E and in sulfanilamide administrated groups.

Sulfanilamide in Blood

The number of mg. present in 100 ml. of blood is depicted in Table 7 and Fig. 6. Sulfanilamide administration in groups

Table 7. Blood sulfanilamide of various experimental rabbits.[1]

Group	Collection day			
	0		60	
	Rabbit No. 1	Rabbit No. 2	Rabbit No. 1	Rabbit No. 2
Group II				
Basal diet + Sulfanilamide				
Free form	--[2]	--[2]	32	--[3]
Acetylated form	--	--	8	--
Total drug	--	--	40	--
Group IV				
Vit. A deficient diet				
Free form	--[2]	--[2]	54	52
Acetylated form	--	--	5	4
Total drug	--	--	59	56
Group VI				
Vit. E deficient diet + Sulfanilamide				
Free form	--[2]	--[2]	21	30
Acetylated form	--	--	33	22
Total drug	--	--	44	52
Group VIII				
Vit. A & E deficient diet + Sulfa.				
Free form	--[2]	--[2]	61	--[3]
Acetylated form	--	--	6	--
Total drug	--	--	67	--

[1] mg./100 ml. of blood of each rabbit

[2] No drug had been given on zero day (blood collected before treatment started).

[3] Rabbit died in pre-experimental period.

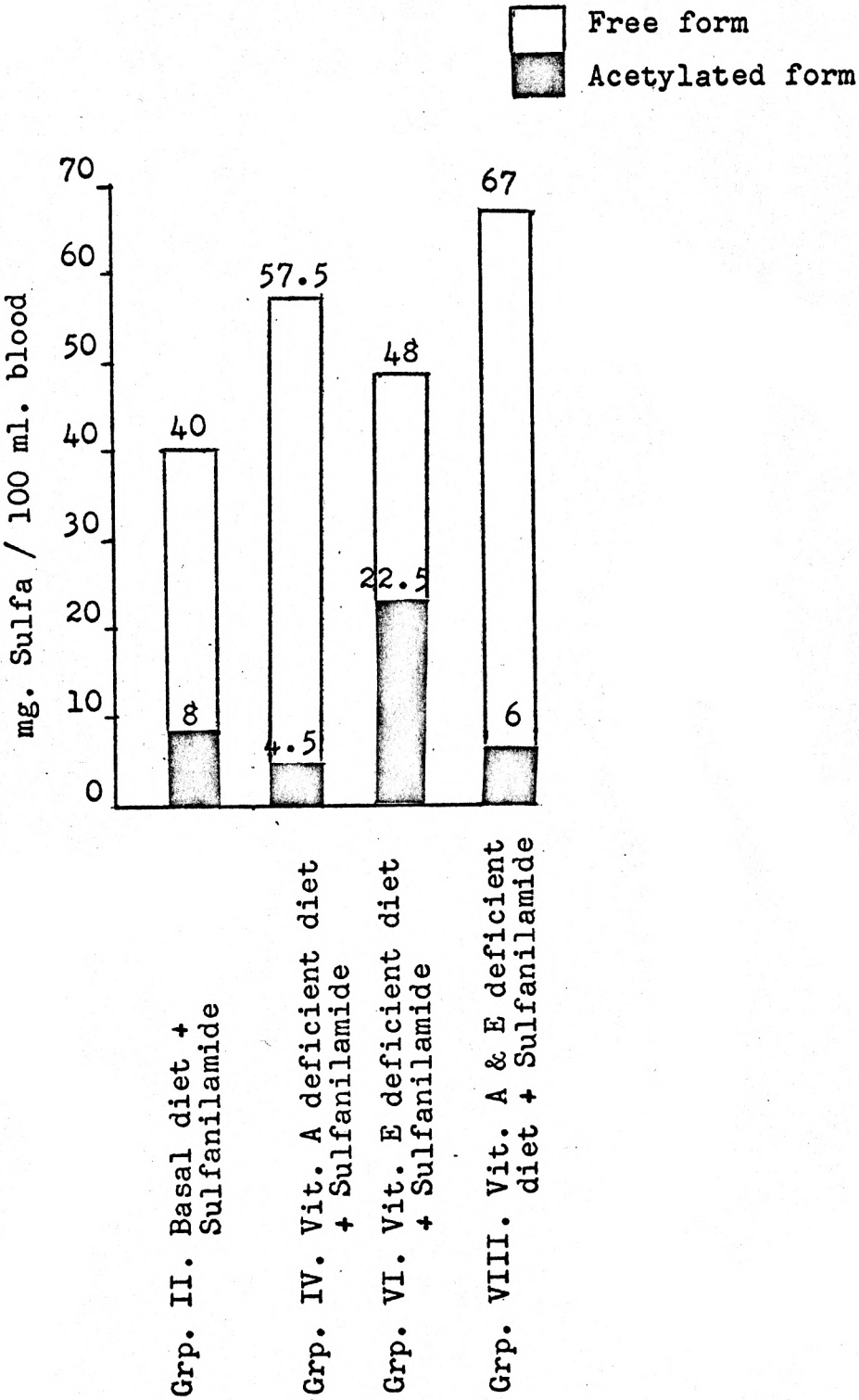


Figure 6. Blood Sulfanilamide of Experimental Groups.

IV, vitamin A deficient rabbits, VI, vitamin E deficient rabbits, and VIII, vitamin A and E deficient rabbits, demonstrated elevated levels of the total drug in the blood by 43.7, 20, and 67.5 per cents, respectively, over the control. Increased free form of the drug was noted in groups IV and VIII by 12.1 and 11 per cents, respectively, over the control group (II). Decreased acetylated form of the drug was seen in groups IV and VIII by 12.2 and 11 per cents, respectively, over the control group (II). In E deficient animals, group VI, the acetylated form was increased and the free form decreased by 27 per cent.

Blood Urea Nitrogen

Blood urea nitrogen in blood is depicted in Table 8 and Fig. 7. Blood urea nitrogen was lower in groups III and VII by 20.4 and 31.8 per cents, respectively, than control group I. Groups II, IV, V, VI, and VIII had higher blood urea nitrogen by 55, 68.1, 52.3, 77.2, and 173 per cents, respectively, than the control group II.

Blood Creatinine and Creatine

Determinations of blood creatinine and creatine were not completed due to coagulation of the blood sample.

Packed Cell Volume

The results of P.C.V. values are illustrated in Table 9 and Fig. 8. The P.C.V. values were affected progressively with the treatment of sulfanilamide.

Table 8. Blood urea nitrogen of various experimental rabbits.[1]

Group	Collection day			
	0		60	
	Rabbit No. 1	Rabbit No. 2	Rabbit No. 1	Rabbit No. 2
Group I Basal diet	48	38	42	46
Group II Basal diet + Sulfanilamide	36	--[2]	68	--[2]
Group III Vit. A deficient diet	52	42	32	38
Group IV Vit. A deficient diet + Sulfanilamide	44	36	82	66
Group V Vit. E deficient diet	42	50	64	70
Group VI Vit. E deficient diet + Sulfanilamide	55	44	84	72
Group VII Vit. A & E deficient diet	35	40	66	30[3]
Group VIII Vit. A & E deficient diet + Sulfa.	50	42	112[3]	--[2]

[1]mg./100 ml. of blood of each rabbit.
[2]Rabbit died in pre-experimental period.
[3]Rabbit died on 27th day.

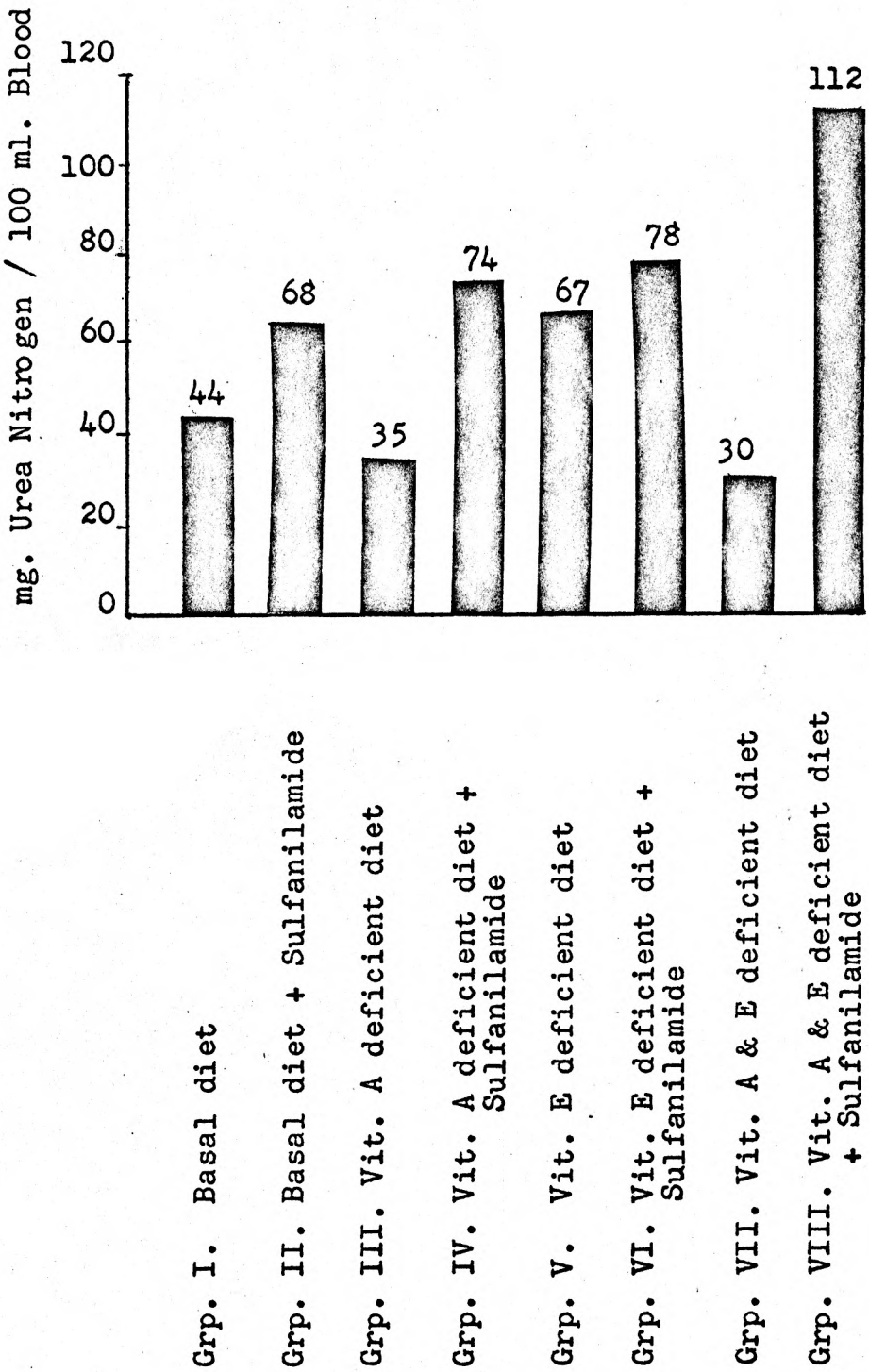


Figure 7. Average Blood Urea Nitrogen of Experimental Groups.

Table 9. Packed cell volume of various experimental rabbits.[1]

Group	Collection day					
	0		30		60	
	Rabbit No. 1	2	1	2	1	2
Group I Basal diet	46	45	45	44	45	45
Group II Basal diet + Sulfanilamide	47	--[2]	39	--[2]	36	--[2]
Group III Vit. A deficient diet	46	45	41	40	40	38
Group IV Vit. A deficient diet + Sulfanilamide	46	44	39	31	34	28
Group V Vit. E deficient diet	48	45	40	36	39	38
Group VI Vit. E deficient diet + Sulfanilamide	48	48	37	34	28	31
Group VII Vit. A & E deficient diet	44	44	35	40	36	--[3]
Group VIII Vit. A & E deficient diet + Sulfa.	46	--[2]	26[3]	--[2]	--[3]	--[2]

[1]Ml. of red blood cells/100 ml. blood.
[2]Rabbits died in pre-experimental period.
[3]Rabbits died on 27th day.

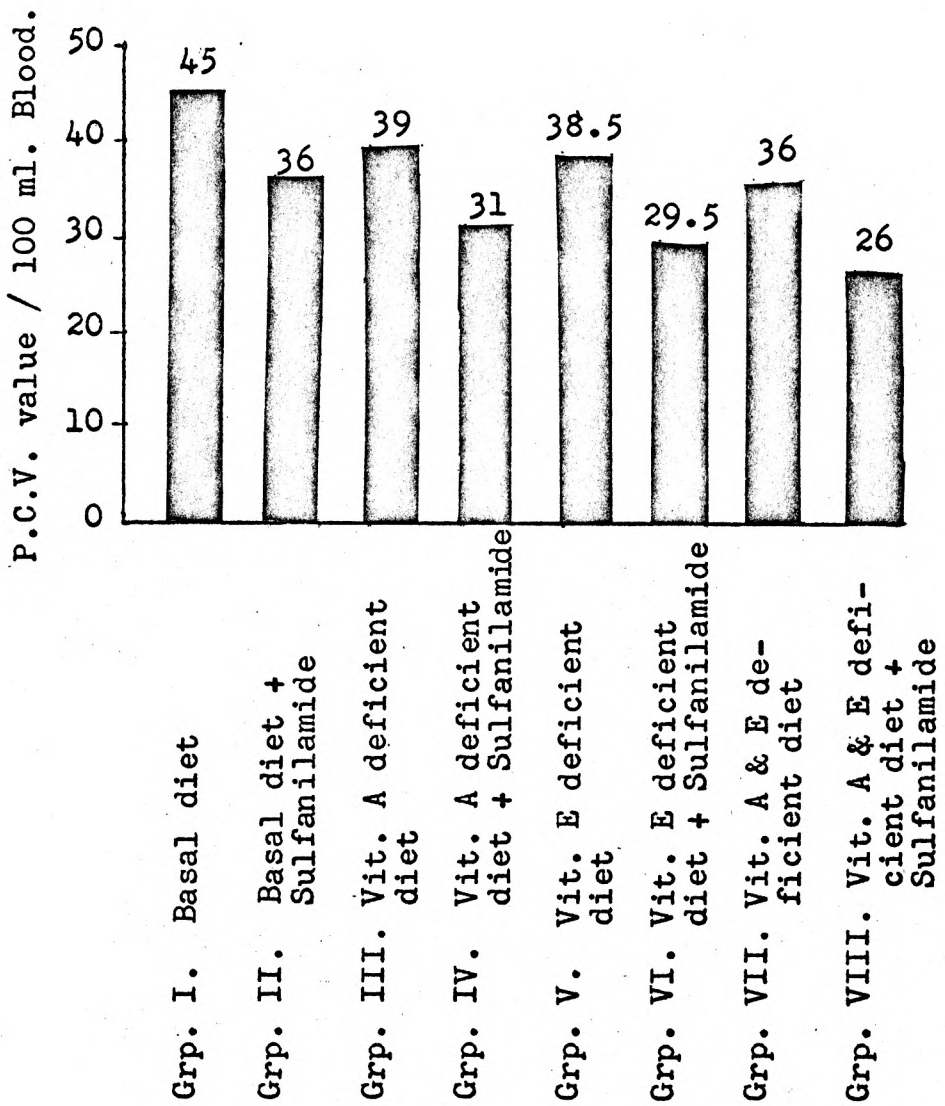


Figure 8. Average Packed Cell Volume Values of Experimental Groups.

Table 10. Averaged values of the various constituents of blood and urine for the 60 day treatment.

	Group I Basal diet	Group II Basal diet + Sulfanilamide	Group III Vit. A deficient diet	Group IV Vit. A deficient diet + Sulfanilamide	Group V Vit. E deficient diet	Group VI Vit. E deficient diet + Sulfanilamide	Group VII Vit. A & E deficient diet	Group VIII Vit. A & E deficient diet + Sulfa.
Gain of body weight in grams/day	27	20.8	17.4	14.8	13.3	10.1	11.2	17.1
Quantity of urine excreted[1]	80.6	71.1	90.4	77.0	75.0	68.7	85.0	49.0
Per cent of total sulfanilamide excreted in urine	---	75.9	---	68.0	---	80.0	---	63.6
Per cent of free form of drug in urine	---	42.3	---	46.4	---	33.6	---	35.4
Per cent of acetylated form in urine	---	33.6	---	21.6	---	46.4	---	28.2
Amount of urea nitrogen excreted[2]	306.5	400.0	231.0	300.4	311.5	406.1	200.1	280.9
Amount of creatinine excreted[2]	40.5	79.3	45.3	88.2	76.6	98.0	87.0	102.0
Amount of creatine excreted[2]	2.1	4.4	2.6	4.3	11.4	11.3	12.6	16.6
Packed cell volume[4]	45	36	39	31	38.5	29.5	36	26
Total sulfanilamide in blood[3]	---	40	---	57.5	---	48	---	67
Free form in blood[3]	---	32	---	53	---	25.5	---	61
Acetylated form in blood[3]	---	8	---	4.5	---	22.5	---	6
Urea nitrogen in blood[3]	44	68	35	74	67	78	30	112

[1]Ml./kg. body weight/day.
[2]Mg./kg. body weight/day.
[3]Mg./100 ml. blood.
[4]RBC/100 ml. blood.

Table 11. The various constituents of the blood and urine of the experimental groups, expressed in percentage increase (+) or decrease (-) over the two basal groups.

	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
	Basal diet	Basal diet + Sulfanilamide	Vit. A deficient diet	Vit. A deficient diet + Sulfanilamide	Vit. E deficient diet	Vit. E deficient diet + Sulfanilamide	Vit. A & E deficient diet	Vit. A & E deficient diet + Sulfa.
Body weight	1621	-55	-69	-73	-103	-116	-114	---
Volume of urine	80.6	-11	+12	-4.4	-6.9	-14.7	+6.3	-39.2
	---	71.1	---	+8.3	---	-3.3	---	-3.1
Total sulfanilamide in urine	---	75.9	---	-10.4	---	+5.4	---	-29.3
Free form in urine	---	42.3	---	+12.6	---	-15.6	---	-44.2
Acetylated form in urine	---	33.6	---	-12.7	---	+13.6	---	-0.1
Total sulfa in blood	---	40	---	+43.7	---	+20	---	+67.5
Free form in blood	---	32	---	+12.1	---	-27	---	+11
Acetylated form in blood	---	8	---	-12.2	---	+27	---	-11
Urinary urea nitrogen	306.5	+30.6	-24.6	-2.0	+1.6	+32.5	-34.7	-8.2
		400.0	---	-22.4	---	+1.5	---	-29.8
Blood urea nitrogen	44	+55	-20.4	+68.1	+52.3	+77.2	-31.8	+173
		68	---	+8.8	---	+14.6	---	+80
Creatinine in urine	40.5	+54.5	+11.8	+105.9	+189.1	+141.9	+114.8	+203.7

Note: A + or - sign appearing prior to any value indicates the value was compared with the respective basal groups.

Histopathological Examination

Histopathological investigations of ureters were eliminated because the sectioning was not satisfactory.

Group I, basal diet control group of rabbits, revealed no changes in kidney, liver, and bladder sections; but rabbit 1 exhibited a slight inflammatory change from the liver section. Sulfanilamide administered rabbits, group II, showed no changes but an edema in the kidney tubules.

Group III, vitamin A deficient rabbits sections, exhibited focal areas of necrosis and hydropic degeneration of the liver cord cells. Sections from kidney revealed degenerative changes in the tubules. Group IV, sulfanilamide administered rabbits, showed a cloudy swelling, areas of fibrosis, and a slight congestion in the liver sections. The kidney tubules demonstrated casts in collecting tubules in the lumen, degenerative changes, pycknotic nuclei, and necrosis associated with toxic tubular nephritis.

Group V, vitamin E deficient rabbits, revealed a cloudy swelling of liver and areas of mild fibrosis of the bile duct in liver sections. Sections from kidney demonstrated marked fibrosis in cortex area with infiltration of mononuclear cells in the tubules, and some pink color proteinosis casts in the lumen associated with chronic interstitial nephritis. Sections from urinary bladder revealed desquamation of epithelial cells associated with hydropic degeneration. Group VI, sulfanilamide administered rabbits, showed in liver sections, fibrosis of the bile

duct with some focal areas of neutrophils. Sections from kidney revealed casts and congestive areas in collecting tubules associated with interstitial nephritis. Rabbit No. 2 of group VI showed scattered areas of irregular deeply blue stained calcification in the kidney sections.

Group VII, vitamin A and E deficient rabbit which was sacrificed on the 27th day, revealed cloudy swelling of the liver. The kidney section exhibited edema and colloid material in the tubules with intact glomeruli in Bowmen's capsules. The other rabbit (No. 2), which was alive for 60 days, demonstrated cloudy swelling of the liver and mononuclear cell infiltration in liver sections. The kidney section showed casts in the convoluted tubules, reticuloendothelial cell hyperplasia, and pycknotic nuclei leading to chronic interstitial nephritis. Sulfanilamide fed rabbit, group VIII, which died on the 27th day, showed hyperplasia of bile duct and proliferation of Kupffer cells in liver sections. Kidney sections revealed casts in tubules, glomeruli were intact in Bowmen's capsules, and degenerative changes. Sections from bladder revealed diffuse hemorrhage and desquamation of epithelial cells.

DISCUSSION

Acetylation as a mode of detoxication of sulfanilamide drugs has been known for a long time. Marshall (76) and Krebs, et al. (65) have shown in their experiments that dogs do not acetylate sulfanilamides. Later work of Marshall (76) has shown that deacetylation takes place before urinary excretion. This

demonstrated that there is a species difference in metabolism of sulfanilamides. The liver as the site of acetylation in rabbits and rats was demonstrated by Harris and Michel (40), while spleen appeared to take part in cats (123).

Acetylation is accelerated by the presence of acetic acid or acetic acid precursors in the body (51, 79). In thiamine deficiency, pyruvic acid cannot be converted to acetyl co A and, hence, acetylation is depressed as evidenced by the work of Martin and Rennebaum (79) and mentioned by Sollmann (112), who also cited that the diphosphothiamin redox system is involved. How acetylation is decreased in riboflavin deficiency has not been sufficiently explained. Folic acid has been proved (56) to be necessary in acetylation mechanism. Acetylation of sulfanilamides and their derivatives in pigeon liver extracts were markedly inhibited noncompetetively by 4-aminoanalogues of folic acid and aminopterin, which are folic acid antagonists.

Krebs, et al. (65) were of the opinion that the extent of acetylation also depended upon the competition between acetylation and deacetylation enzymes. Cholesterol is synthesized in the liver by coenzyme of acetic acid fragments. There is a controversial opinion regarding the role of vitamin A in cholesterol synthesis. However, Meyer (84) has shown that in a deficiency of vitamin A, there was a depressed formation of chondroitin sulphate and other mucopolysaccharides which contain acetylated derivatives of glucosamine or chondrosinine. Thus, it is evident that vitamin A has a function in acetylation reactions. There

is no evidence cited in literature that vitamin E takes part in acetylation.

The results of the present experiments showed that acetylation of sulfanilamide decreased in vitamin A deficient animals. In E deficiency where vitamin A was provided in the diet, acetylation was augmented. This increase in acetylation may be due to the presence of vitamin A. No conclusions are drawn in vitamin A and E deficient animals, as they died prematurely. In the light of previous findings (84, 89) and the results of the present experiments, it may be concluded that vitamin A takes part in acetylation, whether in detoxication or in the synthesis of mucopolysaccharides and in cholesterol.

From the results obtained, the excretion of total sulfanilamide was decreased showing a greater retention of sulfanilamide in blood of vitamin A deficient animals. Comparative to control animals with sulfanilamide there was an increase of 43.7 per cent in retention of total sulfanilamide in blood, and 60.3 per cent of free form of drug was retained in avitaminosis A. There was a depression of about 43.7 per cent of the acetylated form in blood. The results indicate that there was a greater retention of total sulfa and a rise in retention of free form of sulfanilamide. The fall in the acetylated sulfanilamide in blood and urine in vitamin A deficient animals suggests that the acetylation mechanism of sulfanilamide itself deficient, and the decreased elimination was not due to retention of the acetylated form in blood. It was assumed that the elimination of sulfanilamide sources other than urine was equal in all groups. The

urine pH of all groups remained alkaline in which the free and acetyl derivatives are soluble.

It was proved by Marshall (76) that sulfanilamide after acetylation in the body had no therapeutic affect. Inasmuch as acetylation of sulfanilamide was decreased in vitamin A deficiency, the level of free sulfanilamide in the body will be elevated. It can be deduced from this that in avitaminosis A condition, the effective dosage of sulfanilamide can be reduced.

Woelfel, et al. (128) reported that vitamin A deficient calves excreted excess urine. In the present experiment the vitamin A deficient and vitamin A and E deficient rabbits excreted larger quantities of urine when compared to controls. Administration of sulfanilamide to controls, vitamin A deficient, vitamin E deficient, and vitamin A and E deficient animals depressed the volume of urine compared to the normal controls.

Jones (58) mentioned that the administration of sulfanilamides produced an elevated urinary volume in the beginning and a depressed volume thereafter, producing anuria. In the present experiment there was a tendency that urine volume would increase with slight fluctuations up to the 30th day then decrease. In vitamin E deficient animal the volume of urine was increased after 48 days.

In the present experiments the urine pH remained alkaline, at which free and acetylated form of sulfanilamide could not be precitipated. Thus, the possibility of crystallization of sulfanilamide in renal tubules and consequent blocking may not be a factor for decreased urine elimination. In sulfanilamide therapy,

it was reported (73, 112), acidosis results as a result of inhibition of carbonic anhydrase. Acidosis decreases the blood pressure, and as a result, the glomerular filtration is decreased, and consequently, there was less formation of urine.

Woelfel, et al. (128) reported that there was a fall in excretion of urea nitrogen in calves deficient in vitamin A, due to renal dysfunction. Increased urinary urea nitrogen in vitamin E deficient mice was reported by McGaughey (81). The results of the present experiment coincide with the above workers.

In the present experiments a similar depression of urinary urea nitrogen was found in vitamin A deficient and vitamin A and E deficient animals; however, there was an increase in sulfanilamide fed groups. The decrease of urea nitrogen in the vitamin A deficient animal was about 24.6 per cent more than group I, whereas in the sulfanilamide fed animals there was also a fall, 22.4 per cent less than group II. The blood urea nitrogen was also depressed in group III and group VII by 20.4 and 31.8 per cents, respectively, as compared to group I; in other groups it was elevated.

In vitamin A deficiency, the depressed urea formation may be ascribed to decreased absorption of amino acids and consequent lowered deamination in the liver, or it may be due to pathological changes taking place in the liver or kidney.

The concentration of creatinine and creatine did not vary much between controls and avitaminosis A rabbits in the present experiments. There was a great increase in creatine and creatinine elimination in vitamin E deficient animals. These findings

agree with the results obtained by previous workers (1) and (10). In avitaminosis E animals the creatine excretion was higher. This suggested that conversion of creatine to creatinine in vitamin E deficient animals was low. However, with the administration of sulfanilamide to control, vitamin A deficient, vitamin E deficient, and vitamin A and E deficient animals, both creatine and creatinine elimination was promoted.

Growth rate in vitamin A and/or E deficient animals was decreased. In sulfanilamide fed groups the growth rate was lower than in their controls. Group VI showed the lowest rate of growth. The depressed growth rate in sulfanilamide administered groups may be attributed to an increased excretion of creatinine and creatine. However, there was no correlationship between avitaminosis A and in creatine and creatinine excretion. The increase in creatinine elimination in vitamin A deficient animals compared to controls was about 12 per cent, whereas the sulfanilamide fed group was 11 per cent over group II.

Packed cell volume values were altered in all groups of animals, but they were greatly affected in the sulfanilamide fed groups, which indicated that it might be due to anemia as reported by other workers (49, 61).

The avitaminosis A animals, as compared to sulfanilamide administration in vitamin A deficient animals, elicited exaggerated and progressive degenerative changes in the liver and kidney sections. Liver sections demonstrated cloudy swelling and fibrosis, and the kidney showed tubular casts, degenerative changes and pyknotic nuclei which were suggestive of toxic

tubular nephritis.

The kidney changes in avitaminosis E deficient rabbits were suggestive of chronic interstitial nephritis. Administration of sulfanilamide in vitamin E deficient group, in addition to chronic interstitial nephritis, also revealed congestive areas in renal collecting tubules. The liver revealed cloudy swelling and mild fibrosis of bile duct and some focal areas of neutrophil infiltration.

The lesions in vitamin A and E deficient group were more severe as evidenced by edema, colloid material in kidney tubules with mononuclear cell infiltration and hyperplasia of reticuloendothelial cells associated with chronic interstitial nephritis. Sulfanilamide administration in A and E deficient animal revealed proliferation of liver Kupffer cells and hyperplasia of bile duct accompanied by other degenerative changes in kidney and liver sections. The bladder section showed a diffuse hemorrhage and desquamation of epithelial cells.

The progressive deterioration of the liver and kidney of vitamin A and/or E deficiency with sulfanilamide administration was suggestive of toxic effects on these tissues. The changes in blood and urine discussed above might be the result of degenerative changes taking place in liver and kidney. No deposition of crystals of sulfanilamide could be detected in the sections.

SUMMARY AND CONCLUSIONS

The experiment was conducted to determine the effect of sulfanilamide toxicity and excretion employing vitamin A de-

ficient, vitamin E deficient, and vitamin A and E deficient animals.

Sixteen rabbits were divided into 8 groups, each containing 2 animals. A synthetic diet deficient in vitamin A and/or E was fed to various groups of rabbits, while one group serving as control was fed added vitamin A and E. Sulfanilamide was administered every day to the normal control and groups fed deficient diet of vitamin A and/or E (4 groups including control).

The growth rate, volume of urine, free and acetylated form of sulfanilamide in blood and urine, urea nitrogen in blood and urine, creatine and creatinine in urine, and packed cell volume values were determined. The animals were sacrificed after 60 days treatment and histopathological sections of liver, kidney, and urinary bladder were studied.

The administration of sulfanilamide to animals fed vitamin A deficient diet resulted in decreased elimination of acetylated form of sulfanilamide in urine while maintaining higher levels of free drug in circulation.

The volume of urine was depressed progressively in all the animals treated with sulfanilamide inclusive of group given normal diet with sulfanilamide.

The urea nitrogen excretion in avitaminosis A animals was 24.6 per cent less than the controls. However, sulfanilamide administration to vitamin A deficient animals demonstrated an increased urea nitrogen elimination with higher blood urea nitrogen levels.

The creatine and creatinine excretion was greater in vitamin

E deficient animals. Administration of sulfanilamide in all 4 groups resulted in an increase in total quantity of creatine and creatinine urinary excretion.

The packed cell volume values were lower in sulfanilamide administered groups when compared to their controls.

In the comparison of depressed growth rate of animals fed vitamin A and/or E deficient diets, the sulfanilamide administered groups lost considerable weight.

The histopathological sections revealed that vitamin A and/or E deficiency were of a cellular degenerative type, cloudy swelling of liver, mild fibrosis of bile duct, and degenerative changes in the renal tubules. Sulfanilamide administrations aggravated the histopathological changes which were attributed to the toxicity of the drug.

From the results obtained, it may be concluded that the therapeutic dosage of sulfanilamide in avitaminosis A could be profitably reduced maintaining the therapeutic efficiency.

Prolonged treatment with sulfanilamide has been shown to have a deleterious effect on growth rate, even in normal animals.

The experiment seems to indirectly prove that vitamin A takes part in detoxication mechanism by acetylating some of the foreign organic compounds like sulfanilamide.

The conclusions from this study have been drawn with considerable reservation, as the number of experimental animals employed in these experiments may be considered insufficient for sound and safe deduction.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to his Major Professor, Dr. G. K. L. Underbjerg, Head of the Department and Professor of Physiology, under whose direction these investigations have been conducted, for his skilled guidance and advice in the preparation of this thesis.

The author is indebted to Dr. N. R. Christensen, Assistant Professor, and Dr. R. N. Swanson, Assistant Professor in Physiology, for the ready help at all times during the course of these investigations.

Particular thanks are due to Dr. R. C. Piper and Mr. S. L. Issar of the Pathology Department in carrying out the histopathological studies.

The author is grateful to Mrs. Audyne E. Self, Secretary, Department of Physiology, for her suggestions and help.

The author is obliged for the kind help extended by Dr. R. A. Frey, Dr. K. S. Rao, K. L. Jeffrey, and D. W. Butts during the course of work.

BIBLIOGRAPHY

(1) Allen, J. R., et al.
Metabolic studies in vitamin E deficient rabbits. Metab. 7:646. 1958.

(2) Antopol, W., and H. Robinson.
Pathologic and histologic changes following oral administration of sulfapyridine. Arch. Path. 29:67. 1940.

(3) Astwood, E. B., et al.
Action of certain sulfonamides and of thiourea upon the function of the thyroid gland of the rat. Endocrin. 32:210. 1943.

(4) Bacharach, A. L., et al.
The chemistry and physiology of vitamin A. Vit. Horm. 2:7. 1944.

(5) Benesch, R., et al.
Inhibition of bone calcification by sulphonamides. Nature. 155:203. 1945.

(6) Bentley, L. S., and A. F. Morgan.
Vitamin A and carotene in the nutrition of the guinea pig. Jour. Nutr. 30:159. 1945.

(7) Berliner, R. W.
Renal secretion of potassium and hydrogen ions. Fed. Proc. 11:695. 1952.

(8) Beyer, K. H., et al.
The renal elimination of sulfamerazine, sulfamethazine, sulfadiazine, and sulfathiazole by the dog. Jour. Pharm. Expt. Therap. 82:239. 1944.

(9) Blondheim, S. H.
Acetylation by blood cells. Arch. Biochem. and Biophy. 55:365. 1955.

(10) Borgman, R. F.
Avitaminosis E and nutritional muscular dystrophy in relation to induced stress. Ph. D. Thesis. K.S.U. 1959.

(11) Bratton, A. C., and E. K. Marshall, Jr.
A new coupling component for sulfanilamide determination. Jour. Biol. Chem. 128:537. 1939.

(12) Bray, H. G., et al.
Deacetylation of acetamido compounds by tissue extracts. Biochem. Jour. 47:483. 1950.

(13) Burgess, R. C., et al.
Effect of dietary penicillin on liver vitamin A and serum carotenoids in the chick. Arch. Biochem and Biophy. 33:339. 1951.

(14) Cantarow, A., and M. Trumper.
Clinical Biochem. Philadelphia: W. B. Saunders Co., 6th Ed. pp. 166-170, 419-423. 1962.

(15) Chapman, D. G., et al.
Sex hormones on vitamin A storage. Canad. Jour. Res. D27:200. 1949.

(16) Cowin, L. M., and K. Schwarz.
An effect of vitamin E on the regulation of succinate oxidation in rat liver mitochondria. Jour. Biol. Chem. 234:191. 1959.

(17) Crimm, P. D., and D. M. Short.
Qualitative blood cell changes in the rat due to vitamin A. Am. Jour. Physiol. 111:397. 1935.

(18) Crowley, J. W., and N. N. Allen.
The effect of ethyl alcohol on the plasma vitamin A levels of calves and goats. Jour. Dairy Sci. 36:156. 1953.

(19) Daft, F. S., et al.
Biotin deficiency and other changes in rats given sulfanylguanidine or succinyl sulfathiozolein purified diets. Sci. 96:321. 1942.

(20) Davis, A. W., and T. Moor.
Vitamin A and carotene. Biochem. Jour. 29:147. 1935.

(21) Davis, A. W., and T. Moor.
Interaction of vitamins A and E. Nature. 147:794. 1941.

(22) Davis, B. D.
The binding of sulfonamide drugs by plasma proteins. Jour. Clin. Invest. 22:753. 1943.

(23) Deatherage, F. F., et al.
The effect of dietary changes on the nitrogen retention in hyperthyroids. Jour. Biol. Chem. 140:32. 1941.

(24) Folin, O.
On the determination of creatinine and creatine in urine. Jour. Biol. Chem. 17:469. 1914.

(25) Folin, O., and A. Svedberg.
Micromethods for the determination of non-protein nitrogen, urea, uric acid, and sugar in unlaked blood. Jour. Biol. Chem. 88:85. 1930.

(26) Folin, O., and H. Wu.
A system of blood analysis. Jour. Biol. Chem. 38:81. 1919.

(27) Folin, O., and G. E. Youngburg.
Note on the determination of urea in urine by direct nesslerization. Jour. Biol. Chem. 38:111. 1919.

(28) Fraenkel-Conrad, J., and D. M. Greenburg.
Acetylation of sulfanilamide as influenced by the thyroid. Proc. Soc. Expt. Biol. Med. 63:537. 1946.

(29) Ganguly, J., et al.
Studies of the distribution of vitamin A as ester and alcohol and of carotenoids in plasma proteins of several species. Arch. Biochem. Biophys. 38:275. 1952.

(30) Gilligan, R. D.
Comparative studies of the chemical changes occurring in sulfonamide drugs during therapy in man. Jour. Clin. Invest. 24:301. 1945.

(31) Goerner, A.
Effect of dibenzanthracene on vitamin A and total lipid of mitochondria. Jour. Biol. Chem. 122:529. 1938.

(32) Gray, E. T.
Naturally occurring and sulfonamide induced lesions in rats during a one-year toxicity study. Am. Jour. Vet. Res. 24:1044. 1963.

(33) Green, B., et al.
The effect of vitamin A deficiency on the cholesterol levels of the plasma and liver of the rat. Biochem. Jour. 61:447. 1955.

(34) Greisheimer, E. M., et al.
Sulfonamides on blood sugar. Med. Times. 16:238. 1941.

(35) Gross, L.
The effects of vitamin deficient diets on rats, with special reference to the motor functions of the intestinal tract in vivo and in vitro. Jour. Path. Bact. 27:27. 1924.

(36) Guggenhein, K.
The biological value of carotene from various sources and the effect of vitamin E on the utilization of carotene and of vitamin A. Biochem. Jour. 38:260. 1944.

(37) Guggenhein, K.
Studies of the effect of atabrine on vitamin A metabolism. Jour. Nutr. 46:141. 1952.

(38) Haerews, A. T.
Carcinogenic effects of sulfanilamides. Proc. Soc. Expt. Biol. Med. 68:330. 1948.

(39) Hamre, C. J.
Dilation of the bile ducts and intrahepatic lesions with obstructive jaundice in rats fed diets deficient in vitamin A. Am. Jour. Med. Sci. 220:183. 1950.

(40) Harris, J. S., and H. O. Michel.
The formation of methehemoglobin and sulfhemoglobin during sulfanilamide therapy. Jour. Clin. Invest. 18:507. 1939.

(41) Harris, L. J., et al.
Pathogenesis of avitaminosis A. Lancet. 223:614. 1932.

(42) Harris, P. L., et al.
Covitamin studies: II. The sparing action of natural tocopherol concentrates on carotene. Jour. Biol. Chem. 152:313. 1944.

(43) Hawking, F.
Pharmacological actions of sulphanilamide. Lancet. 233:1019. 1937.

(44) Heaton, F. W., et al.
Aspects of vitamin A deficiency in the rat. Biochem. Jour. 67:208. 1957.

(45) Herbut, P. A., and T. M. Scaricacottoli.
Diffuse hepatic necrosis caused by sulfadiazine. Arch. Path. 40:94. 1945.

(46) Herrin, R. C., and H. J. Nicholes.
The influence of vitamin A upon urea and insulin clearances in the dog. Am. Jour. Physiol. 125:786. 1939.

(47) Hickman, K. C. D., et al.
Interrelationship of vitamins A and E. Nature. 150:91. 1942.

(48) Hickman, K. C. D., et al.
Covitamin studies: III. The sparing equivalence of the tocopherols and mode of action. Jour. Biol. Chem. 152:321. 1944.

(49) Higgines, G. M., and T. E. Machella.
The bone marrow of rats made anemic by administration of sulfanilamide. Ant. Record. 75:529. 1939.

(50) High, E. G., et al.
Effects of some alkyl hydroquinones on the utilization of carotene by the rat. Jour. Biol. Chem. 195:787. 1952.

(51) Hitchcock, P., and E. E. Nelson.
The metabolism of paraldehyde. Jour. Pharm. Expt. Therap. 79:286. 1943.

(52) Ingefinger, F. J., et al.
The effect of atropine upon the absorption of vitamin A. Jour. Clin. Invest. 22:699. 1943.

(53) Jacobi, H. P., et al.
Some effects of alpha-tocopheryl phosphate on enzymatic activity. Fed. Proc. 6:136. 1947.

(54) James, G. V.
The oxidation of sulphanilamide and sulphapyridine by hydrogen peroxide. Biochem. Jour. 34:636. 1940.

(55) Jensen, O. J.
The prevention of renal precipitation of sulfadiazine. Am. Jour. Med. Sci. 206:746. 1945.

(56) Johnson, W., et al.
Effect of 4-amino folic acid antagonists on biological acetylations. Proc. Soc. Expt. Biol. Med. 99:677. 1958.

(57) Johnson, R. M., and C. A. Barman.
The effect of alpha-tocopherol on the utilization of carotene by the rat. Jour. Biol. Chem. 175:811. 1948.

(58) Jones, L. M.
Veterinary pharmacology and therapeutics. Ames, Iowa. Iowa State Univ. Press, p. 387. 1962.

(59) Karr, W. G.
A method for the determination of blood urea nitrogen. Jour. Lab. Clin. Med. 9:329. 1924.

(60) Keilen, D., and T. Mann.
Carbonic anhydrase. Purification and nature of the enzyme. Biochem. Jour. 34:1163. 1940.

(61) Kohn-Richards, R.
Acute toxic effects of sodium sulfapyridine treatment--experimental observations. Am. Jour. Physiol. 129:399. 1940.

(62) Kracke, R. R., and E. W. Townsend.
The effect of sulfonamide drugs on the blood platelets. Jour. Am. Med. Assoc. 122:168. 1943.

(63) Kracke, R. R.
The effects of sulfonamide drugs on the blood. Am. Jour. Clin. Path. 14:191. 1944.

(64) Kraybill, H. R., and C. L. Shewsbury.
The relative vitamin A potency of carotene fed in butter fat and cottonseed oil. Jour. Nutr. 11:103. 1936.

(65) Krebs, H. A., et al.
Acetylation and deacetylation of the p-amino group of sulfonamide drugs in animal tissues. Biochem. Jour. 41:622. 1947.

(66) Laidman, D. L., et al.
Substance Sc (ubichromenol): A naturally-occurring cyclic isomeride of ubiquinone-50. Biochem. Jour. 74:541. 1960.

(67) Lemley, J. M., et al.
The effect of mixed tocopherols on the utilization of vitamin A in the rat. Jour. Nutr. 34:205. 1947.

(68) Lewis, J. M., et al.
Vitamin A requirements in the rat. The relation of vitamin A intake to growth and to concentration of vitamin A in the blood plasma, liver and retina. Jour. Nutr. 23:351. 1942.

(69) Lipmann, F.
Acetylation of sulfanilamide by liver homogenates and extracts. Jour. Biol. Chem. 160:173. 1945.

(70) Litchfield, J. T., Jr.
The effects of sulfanilamide on the lower vertebrates. Jour. Pharm. Expt. Therap. 67:212. 1939.

(71) Lodge, K. V., and A. S. Woodcock.
Widespread acute necrosis of the liver following sulfonamide therapy in patients with leukemia. Am. Jour. Path. 30:361. 1954.

(72) Mackenzie, J. B., and C. G. Mackenzie.
Effect of prolonged and intermittent sulfonamide feeding on the basal metabolic rate, thyroid and pituitary. Bull. John Hopkins Hosp. 74:85. 1944.

(73) Mann, T., and D. Keilen.
Sulfanilamide as a specific inhibitor of carbonic anhydrase. Nature. 146:164. 1940.

(74) Manville, I. A.
The interrelationship of vitamin A and glucuronic acid in mucine metabolism. Sci. 85:44. 1937.

(75) Markovitz, A., et al.
The biosynthesis of hyaluronic acid by group A streptococcus: VI. Biosynthesis from uridine nucleotides in cell-free extracts. Jour. Biol. Chem. 234:2343. 1959.

(76) Marshall, E. K., Jr.
Acetylation of sulfonamides in the dog. Jour. Biol. Chem. 211:499. 1954.

(77) Marshall, E. K., Jr., et al.
Acetylation of para-aminobenzene-sulfonamide in the animal organism. Sci. 85:202. 1937.

(78) Marshall, E. K., Jr., and J. T. Litchfield, Jr.
The determination of sulfanilamide. Sci. 88:85. 1938.

(79) Martin, G. J., and E. H. Rennebaum.
Sulfanilamide acetylation as influenced by various chemicals and by vitamin deficiencies. Jour. Biol. Chem. 151:417. 1943.

(80) McCollum, E. V., and N. Simmonds.
New knowledge of nutrition. New York. The Macmillan Co., 4th Ed. p. 171. 1929.

(81) McGaughey, C.
Excretion of alpha-ketoglutarate, urea and amino acids by normal and muscular dystrophic mice. Proc. Soc. Expt. Biol. Med. 103:730. 1960.

(82) Mendenhall, W. L., and K. E. Shreeve.
Effect of sulfanilamide on the isolated frog heart. Proc. Soc. Expt. Biol. Med. 42:317. 1939.

(83) Menten, M. L., and M. Andersch.
Sulfanilamide on liver. Ann. Int. Med. 19:609. 1943.

(84) Meyer, K.
"Polysaccharides in Biology": G. F. Springer, Ed. New York. Josiah Macy, Jr. Foundation. pp. 11-56. 1958.

(85) Moore, T.
The effect of various dietary modifications upon the vitamin A activity of carotene. Biochem. Jour. 23:1270. 1929.

(86) Moore, T.
The effect of vitamin E deficiency on the vitamin A reserves of the rat. Biochem. Jour. 34:1321. 1940.

(87) Moore, T.
Vitamin A. London, Elsevier Publ. Co. p. 194. 1957.

(88) Moore, T.
Vitamin A. London, Elsevier Publ. Co. pp. 308-310. 1957.

(89) Moore, T.
Vitamin A. London, Elsevier Publ. Co. pp. 536-541, 1957.

(90) Moore, T., et al.
Vitamin E symposium. Cambridge, England. Heffer, 41. 1939.

(91) Moore, T., et al.
Kidney changes in vitamin E deficient rats. Jour. Nutr. 65:183. 1958.

(92) Murayama, F., and C. Leake.
The acute and chronic toxicity of sulfanilamide in rats. Jour. Pharm. Expt. Therap. 63:29. 1938.

(93) Oppenheimer, H., et al.
Serum proteins, lipoproteins and glycoproteins in muscular dystrophy of vitamin E deficiency. Proc. Soc. Expt. Biol. Med. 97:882. 1958.

(94) Peters, J. H.
The determination of creatinine and creatine in blood and urine with the photo-electric colorimeter. Jour. Biol. Chem. 146:179. 1942.

(95) Pizzolato, P., and H. H. Beard.
Creatine and creatinine metabolism and the vitamins II. Jour. Biol. Chem. 140:102. 1941.

(96) Popper, H.
Histological demonstration of vitamin A in rats by means of fluorescence microscopy. Proc. Soc. Expt. Biol. Med. 43:133. 1940.

(97) Popper, H., and R. Greenberg.
Visualization of vitamin A in rat organs by fluorescence microscopy. Arch. Path. 32:11. 1941.

(98) Prien, E. L.
Sulfanilamide renal toxicity; the mechanism of renal complications in sulfonamide therapy. New Engl. Jour. Med. 232:63. 1945.

(99) Quackenbush, F. W., et al.
Tocopherol and the stability of carotene. Jour. Biol. Chem. 145:169. 1942.

(100) Rao, S. D.
Influence of vitamin E on the utilization of carotene from oils. Nature. 156:449. 1945.

(101) Ray, A., and D. P. Sadhu.
Carbohydrate metabolism in hypervitaminosis A. Nature. 184:Sup 17, 1323. 1959.

(102) Richardson, A. P.
Effects of continued administration of sulfanilamide on the blood. Jour. Pharm. Expt. Therap. 70:370. 1940.

(103) Rosenberg, H. R.
"Chemistry and Physiology of the Vitamins." New York, Interscience. p. 42. 1942.

(104) Rosenkrantz, H.
Studies in vitamin E deficiency I. The oxygen consumption of various tissue from the rabbit. Jour. Biol. Chem. 214:789. 1955.

(105) Rosenkrantz, H.
Dehydrogenase levels in normal and vitamin E deficient rabbits. Fed. Proc. 17:299. 1958.

(106) Russell, W. C., et al.
The absorption and retention of carotene and vitamin A by hens on normal and low fat rations. Jour. Biol. Chem. 140:109. 1941.

(107) Schwarz, K.
Vitamin E, trace elements and sulfhydryl groups in respiratory decline. Vita. and hormones. 20:463. 1962.

(108) Shaffer, J. M., and R. N. Bieter.
Conversion of acetylsulfonamides to the unconjugated form by the chicken kidney. Jour. Pharm. Expt. Therap. 100:192. 1950.

(109) Sherman, H. C., and H. Y. Trupp.
Long-term experiments at or near the optimum level of intake of vitamin A. Jour. Nutr. 37:467. 1949.

(110) Sherman, W. C.
Activity of alphatocopherol in preventing antagonism between lineolic and lineolinic esters and carotene. Proc. Soc. Expt. Biol. Med. 47:199. 1941.

(111) Sobel, A. E., A. M. Mayer, and S. P. Gottfrield.
A convenient titrimetric ultramicromethod for the estimation of urea and kjeldahl nitrogen. Jour. Biol. Chem. 156:355. 1944.

(112) Sollmann, T.
A Manual of Pharmacology. Philadelphia. W. B. Saunders Co. pp. 756-766. 1957.

(113) Southworth, H.
Acidosis associated with administration of para-amino-benzene-sulfonamide. Proc. Soc. Expt. Biol. Med. 36:58. 1937.

(114) Stowe, C. M., and C. S. Siscdia.
The pharmacologic properties of sulfadimetoxine in dairy cattle. Am. Jour. Vet. Res. 24:525. 1963.

(115) Sure, B., et al.
The effect of avitaminosis on hematopoietic function (I. Vitamin A deficiency). Jour. Biol. Chem. 83:375. 1929.

(116) Swick, R. W., and C. A. Baumann.
Effect of certain tocopherols and other antioxidants on the utilization of beta-carotene for vitamin A storage. Arch. Biochem. Biophy. 36:120. 1952.

(117) Tamura, V., et al.
Effect of vitamin E upon the liver of rats intoxicated with carbon tetrachloride. Med. Jour. Osaka Univ. 10:91. 1958.

(118) Thatcher, H. S., and B. Sure.
Avitaminosis. Pathologic changes in tissues of the albino rat during early stages of vitamin A deficiency. Arch. Path. 13:756. 1932.

(119) Tomarelli, R. M., and P. György.
The antioxygenic synergism of tocopherol and rice bran extract in the preservation of carotene. Jour. Biol. Chem. 161:367. 1945.

(120) Tyson, M. D., and A. H. Smith.
Tissue changes associated with vitamin A deficiency in the rat. Am. Jour. Path. 5:57. 1929.

(121) Van der Meer, C., and H. T. M. Nieuwerkerk.
Influence of vitamin E on liveresterase and cholinesterase. Biochem. & Biophy. Acta. 7:263. 1951.

(122) Van Leersum, E. C.
Vitamin A deficiency and calcification of the epithelium of the kidney. Jour. Biol. Chem. 79:461. 1928.

(123) Van Winkle, W., and W. C. Cutting.
Acetylation of sulfanilamide and sulphapyridine in the cat. Jour. Pharm. Expt. Therap. 69:40. 1940.

(124) Vilter, C. F., and M. A. Blankenhorn.
The toxic reactions of the newer sulfonamides. Jour. Am. Med. Assoc. 126:691. 1944.

(125) Wang, P., et al.
Serum vitamin A and carotene levels in children with rheumatic fever. Am. Jour. Dis. Child. 87:659. 1954.

(126) Williams, R. T.
The metabolism of sulphonamides. Biochem. Jour. 40:219. 1946.

(127) Wilson, H. E. C., B. Ahamad and B. N. Majunder.
Further observation on the metabolism of carotene. Indian Jour. Med. Res. 24:399. 1936.

(128) Woelfel, C. G., et al.
Some biochemical constituents in urine of vitamin A deficient holstein calves. Jour. Dairy Sci. 46:947. 1963.

(129) Wolbach, S. B., and P. R. Howe.
Tissue changes following deprivation of fat soluble A vitamin. Jour. Expt. Med. 42:753. 1925.

(130) Wolf, G., and C. Johnson.
The function of vitamin A in carbohydrate metabolism. Vita. and hormones. 18:457. 1960.

(131) Wolf, G., and P. T. Varandani.
Studies on the function of vitamin A in mucopolysaccharide biosynthesis. Biochem. and Biophy. Acta. 43:501. 1960.

(132) Wolfe, J. M., and H. P. Salter, Jr.
Vitamin A deficiency in the albino mouse. Jour. Nutr. 4:185. 1931.

(133) Wooley, J. G.
Growth of three to four weeks old rabbits fed purified and stock rations. Jour. Nutr. 52:39. 1954.

(134) Young, G., and G. Wald.
The mobilization of vitamin A by the sympathico-adrenal system. Am. Jour. Physiol. 131:210. 1940.

(135) Youngburg, C. E.
The removal of ammonia from urine preparatory to the determination of urea. Jour. Biol. Chem. 45:391. 1921.

TOXICITY OF SULFANILAMIDE IN VITAMINS A AND E DEFICIENT RABBITS

by

NARAYANA RAO KOSURI

B. V. Sc. and A. H., Osmania University, 1959

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Physiology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1964

Sulfanilamide drugs are employed extensively in large animal practice. Nutritional deficiencies, such as avitaminosis A and/or E and related disorders, are common among livestock. The effects of sulfanilamide administration in these conditions have not been studied. These experiments were designed to investigate certain effects of administering sulfanilamide to rabbits receiving diets deficient in vitamin A or vitamin E, and vitamins A and E.

Sixteen New Zealand white rabbits were divided into eight groups, each containing two animals. A synthetic diet deficient in vitamin A and/or E was fed to various groups, while one group serving as the controls received added vitamins A and E. Sulfanilamide was administered daily (250 mg./kg./day) to the normal control and groups fed deficient diet of vitamin A and/or E. The animals were sacrificed after sixty days treatment, and histopathological sections of liver, kidney, and urinary bladder were studied. Urine samples were collected and volume measured every third day; and blood samples were collected on 0 and 60th days for analysis of free and acetylated form of sulfanilamide, urea nitrogen, creatine, and creatinine. Packed cell volume determinations were made on 0, 30th, and 60th days, and body weights recorded every third day.

The administration of sulfanilamide to animals fed vitamin A deficient diet resulted in a decreased elimination of acetylated form of sulfanilamide in urine while maintaining higher levels of free drug in circulation.

The volume of urine was depressed progressively in all the

animals treated with sulfanilamide, inclusive of group given basal diet with sulfanilamide.

The urea nitrogen excretion in avitaminosis A rabbits was less than the controls. However, sulfanilamide administration to vitamin A deficient animals demonstrated an increased urea nitrogen elimination with higher blood urea nitrogen levels.

The creatine, creatinine excretion was greater in vitamin E deficient animals. Administration of sulfanilamide in all four groups resulted in an increase in total quantity of creatine and creatinine urinary excretion.

The packed cell volume values were lower in sulfanilamide administered groups when compared to their controls.

In the comparison of depressed growth rate of animals fed vitamin A and/or E deficient diets, the sulfanilamide administered groups lost considerable weight.

The histopathological sections revealed that vitamin A and/or E deficiency was of a cellular degenerative type cloudy swelling of liver, mild fibrosis of bile duct, and degenerative changes in the renal tubules. Sulfanilamide administration aggravated the histopathological changes.

From the results obtained it may be concluded that the therapeutic dosage of sulfanilamide in avitaminosis A could be profitably reduced, maintaining the therapeutic efficiency.

Prolonged treatment with sulfanilamide has been shown to have a deleterious effect on growth rate, even in normal animals.

The experiments seem to indirectly prove that vitamin A takes part in detoxication mechanisms by acetylating some of the

foreign organic compounds like sulfanilamide.

The conclusions from this study have been drawn with considerable reservation, as the number of experimental animals employed in these experiments may be considered insufficient for sound and safe deductions.