ABSTRACT

PATHOLOGIC AND BIOCHEMICAL INFLUENCES OF A VIRAL ENTERIC INFECTION IN PROTEIN-CALORIE MALNUTRITION IN PIGS

By

Turner T. Isoun

Protein-calorie malnutrition (PCM) is a serious nutritional disease in children in many areas of the world, particularly in the developing countries of Africa, Asia, and Latin America. However, under field conditions, protein-calorie malnutrition is believed to be a complex of malnutrition and infection. Three experiments, utilizing 35 pigs, were conducted to elucidate the role played by infectious diarrhea, caused by the transmissible gastroenteritis (TGE) virus, in precipitating protein-calorie malnutrition in baby pigs.

The pigs were divided into 6 groups. Pigs in Group I were fed 6% soyprotein and 30% fat (high fat) diet; and pigs in Group II were fed the same level of protein but 5% fat (low fat). Pigs in Groups III and IV were fed the same diet as pigs in Groups I and II, respectively, and served as uninfected controls. Pigs in Groups I and II were infected with the TGE virus orally after they had consumed the diet for 3 weeks. Pigs in Group V were allowed to nurse the sow and served as uninfected well-nourished controls. A sixth group of pigs was allowed to nurse the sow and was infected orally with TGE virus at 4 weeks of age.

The effect of enteric infection on the protein-calorie malnourished pigs was evaluated by the parameters of food consumption, growth rate,

clinical signs, hematology, gross and microscopic lesions, plasma amino acid pattern, serum electrophoretic values, plasma potassium and sodium values, vitamin A content in the liver, and ultrastructural changes in the zona fasciculata of the adrenal gland.

TGE virus infection in pigs resulted in a decrease in feed consumption, diarrhea, apathy, and a retardation in growth in comparison to the controls.

There was a significant decrease in hemoglobin and packed cell volume in the infected as compared to uninfected control pigs fed the same diet. Enteric infection induced a significant decrease in plasma potassium and sodium values compared to uninfected control pigs on similar diets. The alpha globulin was significantly raised in the presence of an enteric infection. Furthermore, there was a marked decrease in the values of vitamin A in the liver in the infected pigs.

Lesions of protein-calorie malnutrition included subcutaneous edema, fatty liver, parakeratosis of the skin and severe emaciation. The lesions were most marked in the infected pigs fed the deficient rations. In addition, the TGE virus infection was characterized by jejunal and ileal atrophy of the villi.

Plasma amino acid analysis revealed a decrease of isoleucine, alanine, valine, arginine, glutamic acid, proline glycine, serine and methionine with time in the pigs infected with the TGE virus when compared to the uninfected pigs fed the same diets. However, plasma lysine, histidine and phenylalanine values did not change due to infection. The nonessential to essential amino acid ratio decreased with time in the infected pigs. However, the ratio of nonessential to essential amino acids increased with time in the uninfected pigs fed the same diet. The glycine to valine ratio increased in both infected and in uninfected pigs.

The alanine to arginine ratio was higher in the infected than uninfected pigs fed the same diet.

The adrenal gland of infected, protein-calorie malnourished pigs, had evidence of hyperactivity characterized ultrastructurally, by depletion of lipid, vesicular appearance of endoplasmic reticulum, the presence of triple or double complexes comprising mitochondria, lipid droplets and "myelin bodies." An enteric infection superimposed on malnourished animals would appear to be more than just additive.

PATHOLOGIC AND BIOCHEMICAL INFLUENCES OF A VIRAL ENTERIC INFECTION IN PROTEIN-CALORIE MALNUTRITION IN PIGS

By

Turner T. Isoun

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Pathology

1970

Dedicated to

my wife, Miriam, and my son, Diseye

ACKNOWLEDGEMENTS

The author wishes to express sincere gratitude and appreciation to Dr. C. K. Whitehair, my major professor, for advice and encouragement. My thanks are due to members of my guidance committee for counsel in the conduct of this research and the preparation of the manuscript.

The author wishes to thank Dr. W. G. Bergen of the Department of Animal Husbandry for help in the determination of values of plasma amino acids.

I am grateful to Dr. N. J. Unakar of the Department of Biological Sciences, Oakland University, Rochester, Michigan, for help in electron microscopy.

My appreciation is due to Dr. N. J. Gatzemeyer for the use of his laboratory for the determination of values of vitamin A in the liver.

The author wishes to express his appreciation to all members of the Department of Pathology for technical and professional aid and guidance.

I wish to thank the Rockefeller Foundation for generous financial support.

Special acknowledgement is made to my wife, Miriam, for support and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Protein-calorie malnutrition is a generic name covering a spectrum of clinical, pathologic and nutritional manifestations: at one clinical extreme is kwashiorkor, and at the other, marasmus. However, in field conditions, the two may blend into one another. Perhaps one principal factor which has complicated the precise definition of the role of diet in the pathogenesis of protein-calorie deficiency, is the part played by infections and infestations. Clinically, it is accepted that the presence of infectious diarrhea does precipitate an acute phase of malnutrition. However, the precise biochemical and morphologic changes which accompany the interaction between infectious diarrhea and protein-calorie malnutrition have not been completely determined.

OBJECTIVES

The objective of this study was to determine the influence of a viral enteric infection (using transmissible gastroenteritis virus, TGE) in young pigs fed protein-calorie deficient diets. This objective was determined by:

1. The plasma amino acid pattern
2. The total serum protein
3. The serum protein electrophoretic pattern
4. Plasma potassium and sodium values
5. Gross and microscopic lesions
6. Concentration of vitamin A in the liver
7. Clinical signs.

REVIEW OF LITERATURE

A complete review of all the available literature in human protein-calorie malnutrition is beyond the scope of this dissertation. However, the pathologic and the experimental aspects of protein-calorie malnutrition will be emphasized.

Nature of disease

The Joint FAO/WHO Expert Committee (1962) coined the term protein-calorie malnutrition for a class of diseases in man which included marasmus, marasmic-kwashiorkor and kwashiorkor. The hyphen in protein-calorie malnutrition indicated that the two dietary factors were always interrelated. Williams (1933) gave the name kwashiorkor - a term from the Ga language of the Gold Coast (now Ghana) - which indicated "the disease that the deposed baby gets when the next one is born." The disease was commonly seen between the ages of 6 months and 4 years. Williams suggested that the children were weaned to a predominantly maize diet. Jelliffe (1959) described kwashiorkor as insufficient intake of protein but an adequate intake of calories. Edema, apathy and skin lesions were typical clinical signs. In contrast, marasmus was characterized by an insufficient intake of both calories and protein. This entity is clinically marked by emaciation. In addition, he suggested that, in field conditions, the two syndromes of kwashiorkor and marasmus could blend together to form marasmic-kwashiorkor. Protein-calorie malnutrition is found primarily in developing countries of

Africa, Asia, and Latin America (Chanda, 1958; Senecal, 1958; Waterlow and Wills, 1960). However, protein-calorie malnutrition has also been reported in technically developed countries including the United States (Wolf, 1961).

The Anemia of Protein-Calorie Malnutrition

McLaren (1968), a physician in Beirut, Lebanon, mentioned that children with protein-calorie malnutrition had hemoglobin values below 10 g./100 ml. and packed cell volumes below 30%. Viteri *et al.* (1968) reported that serum iron values in control subjects ranged from 30 to 135 mg./100 ml.; whereas in children with kwashiorkor, the values ranged from 12 to 100 mg./100 ml. Adams *et al.* (1967) suggested that protein deficiency by itself led to bone marrow hypoplasia, probably because of the high protein turnover in the bone marrow. Viteri *et al.* (1968) hypothesized that

> "in pure, uncomplicated protein-calorie malnutrition, the lowering in hemoglobin concentration is a consequence of decrease in active tissue mass and dilution anemia due to a relatively preserved intravascular space."

Lanowsky *et al.* (1967), using Cr^{51} labeling, described a series of protein-calorie malnourished children who had a reduced red blood cell life span.

Platt *et al.* (1964) carried out extensive experiments with pigs. They observed that when pigs were given low protein diets, the animals developed anemia, but when a low protein diet with extra carbohydrate was given, the anemia was more pronounced. Sood *et al.* (1965) provided evidence that rhesus monkeys fed protein deficient diets consistently developed anemia. The anemia was normocytic and hypochromic. Woodruff *et al.* (1968) fed a group of dogs a low protein diet. They determined

that Fe^{59} bound to plasma was cleared from the circulating plasma in the deficient animals more slowly than the controls. They also injected Fe^{59} into protein deficient and well-nourished dogs. Incorporation of Fe^{59} into new erythrocytes was slower and the amount incorporated was less in the depleted than in the well-nourished dogs. That is, the transit time of the Fe^{59} through the bone marrow was more prolonged in the depleted animals, and the total amounts of Fe^{59} which their marrow was able to utilize was less than that of the controls.

Electrolyte and Water Metabolism

Garrow (1965), by using K^{40}, recorded a remarkable depletion of potassium in 10 malnourished infants. The children with kwashiorkor were much more deficient in potassium than protein. The potassium levels of the non-edematous marasmic infants were not significantly depleted. Kahn (1959) reocrded that children with protein-calorie malnutrition had evidence of dehydration, as evidenced by low serum sodium and potassium values. The depletion of electrolytes, Kahn concluded, contributed at least in part, to the high mortality rates among such infants despite intravenous electrolyte replacement therapy. He stated that depletion of potassium in protein-calorie malnutrition was related to failure in the renal conservation of potassium and a deficient intake of potassium due to anorexia. Children who died with kwashiorkor also had low concentrations of potassium in brain tissue taken during postmortem examination (Garrow, 1967). Widdowson (1960) recorded a reduction in the levels of potassium in the brain of malnourished pigs. Scrimshaw *et al.* (1956), working in Guatemala, reported low values for serum calcium in infants with kwashiorkor.

Serum Proteins in Protein-Calorie Malnutrition

Dean and Schwartz (1953) reported that in umcomplicated cases of kwashiorkor in children there was a reduction in the total serum proteins from a normal of 6 to 7 g./100 ml. to 4.5 g./100 ml. The gamma, alpha and beta globulins were relatively and absolutely higher in children with kwashiorkor than children without the disease. They reported that the albumin/globulin ratio was near unity instead of a normal value of 2.2. However, Whitehead (1968) reported a reduction in the levels of both serum albumin and globulin in kwashiorkor. Coles (1960), Dean and Schwartz (1953) and Cohen and Hansen (1962) indicated that there was a marked decrease in serum albumin in patients with kwashiorkor. They reported that the total albumin in malnourished children was reduced to 50%, the circulating albumin to 60% and extravascular albumin to 40% of the value observed after refeeding. Rothschild *et al.* (1969) reported that in children with kwashiorkor, albumin synthesis as measured with administration of I^{131} albumin, was decreased to half of the normal rate. Albumin synthesis took place in the liver. The mechanism of adaptation to low protein intake in children (Waterlow, 1968) was the reduction in the rate of breakdown of plasma albumin. Waterlow pointed out that muscle should be accepted as a dynamic tissue capable of giving up its protein and amino acids to plasma in states of protein malnutrition.

Donoso *et al.* (1965) observed that marasmic children, even in very serious cases, had normal plasma proteins. Consequently, edema was not seen. In addition, they noted a prolonged half-life of plasma albumin in marasmic infants. Rothschild *et al.* (1969) pointed out that up to 30% of the endogenous loss of albumin occurred via the feces in gastro-enteritis. In normal subjects, less than 10% of the loss of albumin occurred in the stool.

Amino Acid Metabolism in Protein-Calorie Malnutrition

Saunders *et al.* (1967), Anasuya and Rao (1968), Truswell *et al.* (1966), and Dean and Whitehead (1964) agreed in that in the uncomplicated form of kwashiorkor, there was a remarkable reduction in the essential amino acids. In particular, leucine, isoleucine, valine and methionine, were affected. Of the nonessential amino acids, tyrosine was the only amino acid that was reduced. In contrast, they found the nonessential amino acids, particularly glycine and serine, were increased. Whitehead and Dean (1964) developed the concept of plasma nonessential to essential plasma amino acid ratios and concluded that an elevated ratio can be used as a reliable index of early protein malnutrition. Anasuya and Rao (1968) stated that the plasma levels of all amino acids were higher in cases of marasmus than in kwashiorkor. Of particular interest, was the elevation in the plasma alanine and the basic amino acids. In addition, there were decreased plasma glutamine, asparagine, threonine, glutamic acid and methionine values. Furthermore, in contrast to Dean and Whitehead (1964), they found that the nonessential to essential amino acid ratios were an unreliable index of protein calorie malnutrition. Edozien, Phillips and Collis (1960) recorded ethanolamine and beta-amino isobutyric acid in the plasma of 30% of the cases of kwashiorkor. They mentioned that when methionine was given to 3 of these cases, there was a disappearance of plasma ethanolamine.

Grimble and Whitehead (1969), working in Uganda, recorded that the amino acid ratio of protein-deficient pigs became elevated when growth was impaired and total serum protein and albumin concentration began to decrease. In addition, the magnitude of the plasma amino acid ratio was statistically correlated with the rate of growth, appetite, serum protein and albumin concentration and hydroxyproline excretion. Platt *et al.*

(1964), experimenting with pigs, suggested that the plasma nonessential to essential amino acid ratio did not differentiate the protein-calorie deficient pigs from control pigs at ages of 8 and 13 weeks, but did at 17 weeks. Grimble and Whitehead (1969), at Cambridge, established that tryptophan, lysine, threonine, methionine, leucine, isoleucine, histidine, arginine, phenylalanine and valine were essential amino acids for the pig.

Factors Affecting Amino Acid Levels

Boomgaardt and McDonald (1969) claimed fasting was a factor affecting plasma amino acid patterns in the pig, rat and chicken. There was more variability in the plasma amino acid pattern in 5- to 6-week-old pigs subjected to fasting than older pigs. Furthermore, the fasting plasma amino acid levels in pigs reached steady-state values for most amino acids between 12 and 20 hours postprandial. Snyderman *et al.* (1968), and Richardson *et al.* (1965) reported that the level of protein intake affected the plasma aminogram. There was also an increase in the branched chain amino acids and valine and methionine in both infants and pigs on high protein diets. Various energy levels also affect the plasma free amino acids (Potter *et al.*, 1968). These latter workers infused glucose into sheep and the depression in essential amino acids was greater than the decreases in the nonessential amino acids. Leung (1968) reported an elevation of plasma amino acid concentration due to administration of cortisol into rats. Landau and Lugibihl (1969) noted that glucagon administration to humans resulted in lowered plasma concentrations of threonine, proline, glycine, alanine, lysine, serine, cystine and histidine. Squibb (1964) reported that in Newcastle disease virus infection, lysine, histidine, arginine, aspartic acid, alanine, valine and leucine were significantly reduced in the liver.

Amino Acid Deficiency and Fatty Liver

Lyman *et al.* (1964) and Sidransky and Verney (1968) reported an excessive periportal accumulation of triglycerides in the liver of rats force-fed diets devoid of isoleucine or threonine.

Role of Vitamin A

Tiong (1956), in Indonesia, described keratomalacia as characterized by cloudiness, ulceration and softening of the cornea in severe protein-calorie malnutrition. The underlying cause was severe vitamin A deficiency. Williams and Tsvetkov (1969) described a bilateral spontaneous expulsion of the lens in a case of kwashiorkor in a female African child. In this child, there was apparently no cornea except necrotic tags that were everted and attached to the limbus. Arroyave *et al.* (1961) observed that children, with clinical kwashiorkor, given therapeutic diets of proteins and calories had an increase in serum vitamin A levels. There was a close correlation between serum protein levels and serum vitamin A levels. Arroyave *et al.* (1959) found that severe protein-calorie malnutrition (kwashiorkor) in children interfered with vitamin A absorption. Deshmukh *et al.* (1964) claimed that with diets containing 5, 10, or 20% casein, the efficiency of absorption of vitamin A by rats after a single massive dose increased progressively with the increase in dietary protein. Goodman *et al.* (1967) reported that pro-vitamin A compounds (beta-carotene, for example), all of which contain one unsubstituted beta-ionone ring, were cleaved in the intestinal mucosa by a non-particulate cleavage enzyme called carotene 15-15'-dioxygenase which required molecular oxygen. Vitamin A, present in food as the retinyl ester, was hydrolyzed in the small intestine by vitamin A ester hydroxylase enzyme of the pancreatic juice. Furthermore,

Goodman noted that vitamin A was absorbed exclusively as the free alcohol, retinol. Olson (1969), in an extensive review article, reported that the vitamin A alcohol crossed the mucosal cell membrane in the upper small intestine and was re-esterified with palmitic acid inside the cell. The reaction was catalyzed by retinyl ester synthetase. In addition, Olson noted that in protein-calorie malnutrition, all the enzymes involved in the absorption of vitamin A were decreased. Kanai *et al.* (1968) isolated a retinol-binding protein from the plasma. The protein had the mobility of an alpha-2-globulin with a molecular weight of 21,000. Certain acidic proteins in the plasma combined with retinol binding protein to form a complex with a molecular weight of 70,000 to 80,000 which migrates in the pre-albumin fraction. Kanai stated that the plasma concentration of retinol binding protein was about 4 mg./ 100 ml. Veen and Beaton (1966) indicated that plasma vitamin A was statistically correlated to plasma albumin and to alpha-2-globulin. In addition, the retinol binding protein was quite sensitive to protein deficiency.

Olson (1969) indicated that within the liver, retinyl ester from the chylomicron in the blood was hydrolyzed, and re-esterified to yield the major storage form - retinyl palmitate - which formed a part of a very low density lipoprotein. Only 20 to 30% of a dose of retinol was excreted quickly in the bile. Faraque and Walker (1970) reported that there was a relationship between the level of protein in the diet and the degree of vitamin A storage in the liver in lambs. Lambs given a high protein diet with either a large supplement of retinyl palmitate on the first day of life, or a small daily supplement, showed significantly greater liver retinol reserves and stored a significantly higher proportion of the supplement than pair-fed lambs given a low protein

diet. Furthermore, the lambs given casein as the source of protein had a decreased efficiency in storing dietary retinyl palmitate in the liver as compared with lambs fed diets containing primarily skim milk protein. In addition, the rate of depletion of the reserves of liver vitamin A was determined by liver concentration at any given time. Finally, they suggested that the amount of enzyme involved in the hydrolysis of stored ester of vitamin A to retinol for release into the blood stream was controlled by the amount and quality of dietary protein.

Olson (1967) postulated that the lack of synthesis of mucopolysaccharides might be the biochemical basis for the remarkable keratinization of epithelial cells of the reproductive, urinary, and respiratory systems and that of the skin. Olson (1969) emphasized the synergistic nature of protein and vitamin A deficiencies. He observed that the mortality rate in children with protein-calorie malnutrition in Jordan was 60% higher when the children had xerophthalmia due to vitamin A deficiency. He thought that the immediate precipitating cause of death was infection, as resistance to infection was seriously impaired in vitamin A deficiency.

Gross and Histologic Lesions of Protein-Calorie Malnutrition

Lesions in infants. Scrimshaw (1956) and Jelliffe (1959) have summarized the characteristic features of the lesions of kwashiorkor in children. Edema of the subcutaneous tissue was a constant finding. The hair was sparse, thin and easily pulled out. In some infants the hair acquired a reddish tint. Histologically, there was severe fatty metamorphosis involving almost every cell in the liver. A proliferation of portal reticulum was observed. Furthermore, they described an atrophy of the acini, a reduction in the secretory granules of the pancreas, and

hyper- and parakeratosis of the skin. Finally, they described atrophy and loss of lipid of the adrenal cortex, fatty degeneration of the kidney tubules, and atrophy of the follicles of the thyroid gland.

Tejada (1957) observed abundant amphophils of the pituitary gland, in malnourished children. He reasoned that the lipoid depletion seen in many adrenals and the remarkable thymic atrophy in infants with kwashiorkor might be due to excessive adrenocorticotrophic hormone production. Higginson (1964) suggested that kwashiorkor had a marked delaying effect on bone development. A reduction in the zone of endochondral ossification in the costochondral junction of the fifth rib was present in Bantu children with kwashiorkor. In addition, there was a decrease in the area of calcified cartilage and a reduction in the number of osteoblasts. The prominent features of marasmus (Scrimshaw *et al.*, 1956) were the severe muscular wasting, loss of subcutaneous fat and absence of edema.

Lesions in experimental protein-calorie malnutrition in animals. Platt *et al.* (1964b) described the histologic changes in the gastrointestinal tract of protein deficient pigs. The surface epithelium of the stomachs of these animals was less columnar and contained an increased number of leukocytes. The parietal cells stained less intensely with stain. In addition, the villi in the small intestine, notably the jejunum, were reduced in height with an increased width and bulbous tips. Furthermore, the bulbous villi were occasionally denuded of epithelium indicating that cell proliferation had not kept up with desquamation. Platt (1964b) fed rats a protein-free diet and carried out labeling experiments on the intestinal mucosal cells with P^{32}. There was a 36% reduction of P^{32} incorporation into the mucosal DNA. Kirsch

(1968) observed marked periportal (occasionally centrilobular) fatty infiltration in young rats fed 5% protein.

Deo *et al.* (1965) observed that in protein deficient rhesus monkeys there was a focal loss of cytoplasmic zymogen granules and basophilic chromidial material from the acini cells of the pancreas. In addition, they reported changes in the salivary glands comprising loss of cytoplasmic material in young malnourished monkeys. Furthermore, they described atrophy of the spleen characterized by depletion of lymphocytes from the germinal centers. McLaren (1968) fed a low protein cassava diet (a common West African food) to rats and pigs, and produced cataracts, corneal vascularization, keratinization of the corneal epithelium and a reduction in the thickness of the cornea.

The Adrenal Gland

Ultrastructural, histological and biochemical changes of the adrenal in protein-calorie malnutrition. Castellanos and Arroyave (1961) reported that there was greater 17-hydroxysteroid excretion and a lower eosinophil count in children with marasmus than kwashiorkor. From this data, they concluded that the increased glucocorticoid production in marasmus contributed to the extreme muscle wasting in this syndrome. Platt *et al.* (1964) reported that histologically, there was a reduction in the amount of lipid in the cells and an increase in the thickness of the zona fasciculata in protein-calorie deficient pigs. Munro *et al.* (1962) indicated that in protein deficiency in the rat, there was a reduction in the weight and a decrease in the protein, ribonucleic acid and the total content of phospholipids of the adrenal gland. They explained these changes on the basis of reduced secretion of adrenocorticotrophic hormone (ACTH) by the anterior pituitary gland in protein-calorie

malnutrition. Svoboda *et al.* (1966) did not find ultrastructural changes in the adrenals of protein deficient rats at 12 weeks of age. However, at 24 weeks of age, they observed enlargement of the mitochondria in the cells of the zona fasciculata. Occasionally, some of the enlarged mitochondria had focal loss of the limiting membrane.

The ultrastructure of the adrenal cortex under ACTH stimulation. Idelman (1970) reported that the zona fasciculata, unlike the zona glomerulosa, was very sensitive to ACTH stimulation in the rat. He noted a reduction in the amount of lipids in the zona fasciculata. There was an increase in the smooth endoplasmic reticulum as evidenced by an increase in the number of smooth vacuoles. Furthermore, he described double and triple complexes: namely, (a) mitochondria and vacuoles, (b) mitochondria and liposomes, and (c) mitochondria, vacuoles, and liposomes. In addition, there was proliferation of mitochondria under prolonged ACTH stimulation. Other changes described by Idelman (1970) due to ACTH stimulation included: proliferation of microvilli in zona fasciculata cells, appearance of coated vesicles originating from the cellular membranes by a process known as endopinocytosis and an increase in the size of the nucleus. Solomon (1969) reported that there was an increased ACTH production in parasitic infections and that adrenal exhaustion contributed, at least in part, to the cause of death.

Protein and Caloric Requirements in the Baby Pig

Wintrobe (1939), summarizing the available data, reported that baby pigs consumed 20 to 34 grams of protein per kilogram of body weight daily at birth, but at 3 weeks of age, ate 7 to 11 grams per kilogram body weight daily. Reber *et al.* (1953) noted that rations, with a composition of 41% protein, fed to baby pigs, produced maximum weight gains and feed

efficiency. Pigs at 8 weeks of age fed a 20% protein diet performed as efficiently as those fed higher levels of protein. Wyllie *et al.* (1969) noted a marked increase in water and protein and a decrease in fat content of the carcass of baby pigs and as protein level was increased from 17% to 24%. They also noted that the age and size of pigs influence urinary nitrogen excretion. As the pigs grew older and heavier, there was a definite trend towards loss of nitrogen in the urine at all levels of protein.

The addition of 5% corn oil to a ration which had 3% ether extract resulted in an increase in gain although there was no appreciable change in feed intake (Crampton and Ness, 1954). They attributed the increase in weight gain to the greater caloric value due to the addition of corn oil. DeMan and Bowland (1963) reported that lard and the fat from sow's milk were similar in chemical composition and since sow's milk is efficiently utilized, lard on the basis of chemical structure, could promote rapid and efficient gains. More recently, Frobish *et al.* (1970) found that the total gain of baby pigs given lard was greater than that of pigs given other sources of fat. Five percent fat in the diet was reported to be optimum for maximum weight gains. Digestion of fat was significantly improved with age. They also reported that the calculated energy needed per unit of weight gain was much higher for pigs fed added fat than those without extra fat in the diet.

Wintrobe (1939) reported that pigs at birth, fed a milk diet, consumed 254 to 329 calories daily per kilogram of weight. However, by the end of the fourth week, the food intake was reduced to 70 to 150 calories per kilogram daily. He noted that sucrose was as good a source of carbohydrates as lactose, dextrin or whey powder.

Experimental Production of Protein-Calorie Malnutrition

Pond *et al.* (1966), at Cornell University, recommended the baby pig as an experimental animal in the production of protein-calorie malnutrition because of its voracious appetite, for its adaptibility to weaning to dry diets at an early age, and for its ability to undergo tests of learning behavior. They also suggested that an initial weight of less than 3 kg. was correlated with high death loss when fed low protein, low calorie diets; and above 7 kg. was associated with resistance to the development of severe protein deficiency signs. They also reported the production of protein deficiency signs including edema and hypoproteinemia by feeding diets containing 3% protein for about 8 weeks. The caloric density was adjusted by varying the percentage of fat in the diet. Pigs fed diets low in soybean protein developed more severe signs of edema than those fed a casein diet at the same level of protein. In additional research at Cornell, Lowrey *et al.* (1962) reported that signs of protein-calorie malnutrition were more severe in pigs fed wheat gluten with 25% fat than those fed casein with 3% fat. The pigs were fed the experimental diets for 14 weeks.

Edozien (1968) fed male Sprague-Dawley rats weighing 100 to 130 grams a diet containing .5% lactalbumin. In another group, he fed a diet containing 1% lactalbumin but the diet was given in restricted amounts in order to supply approximately the same amount of protein but half the total caloric intake of the other. The diets were fed for a period of 8 weeks. Edozien's rationale for making up such diets was that diets producing kwashiorkor in children in developing countries usually consisted of 2% to 4% protein, almost all of which is derived from vegetable protein sources. He concluded that rats fed the .5% lactalbumin diet had signs resembling kwashiorkor while those fed 1% lactalbumin

diet given with restriction had signs resembling marasmus. However, in contrast, Kirsch *et al.* (1968) pointed out that rats on experimental protein deficient diets rarely show edema, one of the principal signs of kwashiorkor. In addition, they suggested that due to anorexia, it may require forced tube feeding of very low protein diets to produce clinical signs resembling marasmus or kwashiorkor, the extremes of protein-calorie malnutrition in children. Sidransky (1960) force-fed groups of young rats corn, rice, wheat, cassava and milo as the sole source of protein but supplemented with other essential nutrients. Fatty liver, one of the signs of protein-calorie malnutrition, was most marked with rice, corn and cassava diets and less severe with wheat and milo diets. Sidransky and Baba (1960) force-fed rats diets devoid of valine and lysine and claimed that they produced lesions that resembled those described for kwashiorkor. Follis (1957) fed a diet consisting primarily of maize to monkeys and produced signs which had many of the features of kwashiorkor. Ramalingaswami and Deo (1968) force-fed growing monkeys diets almost devoid of protein but supplied 100 calories per kg. body weight and all other nutrients in adequate amounts. They concluded that the monkeys on the experimental diet had signs that resembled all the essential features of kwashiorkor.

Transmissible Gastroenteritis (TGE) in Pigs

Transmission, etiology and metabolic changes. The disease is easily transmitted orally with ground suspensions of intestine, kidney, spleen, liver, and brain of infected pigs (Lee *et al.*, 1954; Hooper and Haelterman, 1966). Transmissible gastroenteritis in pigs is caused by a virus (Bay *et al.*, 1949; Young *et al.*, 1955; Lee *et al.*, 1954; Haelterman and Hooper, 1967). Clinically, the syndrome is characterized by vomiting, diarrhea,

loss of weight and high death rate in baby pigs (Whitehair *et al.*, 1948; Lee *et al.*, 1954). Reber and Whitehair (1953) reported that the nitrogen retained in TGE virus infected pigs was about one-third that retained in uninfected pigs. In earlier studies, Whitehair *et al.* (1950) indicated that blood urea values increased from 25 to 215 mg./100 ml. in young pigs infected with TGE virus. The total protein and hemoglobin values were higher in TGE virus infected pigs than controls owing primarily to dehydration (Yusken *et al.*, 1959).

Gross and histologic lesions. The usual gross lesions in TGE virus infection in pigs are atonic small intestines, and excess intestinal fluid, varying in color from whitish to yellow green (Bay *et al.*, 1951). Histologically, the prominent lesion in TGE virus infection in pigs is marked jejunal villous atrophy (Maronpot and Whitehair, 1967; Haelterman and Hooper, 1967). In addition, hydropic degeneration of the epithelial cells of the jejunum of TGE virus infected pigs has been reported (Trapp *et al.*, 1966). The villous changes in TGE virus infection were due to increased cell destruction and improper maturation of epithelial cells (Thake, 1968).

TGE in pigs as a model for the study of enteric disease. Haelterman and Hooper (1967) recommended the use of TGE in pigs as a model for the study of enteric infections for its reproducibility and for the ease in demonstrating the lesions of villous atrophy. In addition, the disease affects young pigs and is therefore a model for the study of infantile diarrhea.

Interaction Between Nutrition and Infection

Salomon *et al.* (1968) have emphasized that the interrelationship between infectious diseases and malnutrition was of special relevance in

developing countries. They suggested that the combined effects of malnutrition and infection on a given individual were more than additive. Specifically, kwashiorkor was precipitated in many instances by measles or acute diarrheal disease. In Capetown, South Africa, Wittman *et al.* (1967) reported that *Giardia* infections were found in 80% of children with kwashiorkor. The coexistence of malnutrition and infections was a common feature in Ethiopian children (Vahlquist, 1967).

Ogbeide (1968), in Nigeria, reported that marasmus or the marasmic-kwashiorkor form of protein-calorie malnutrition was commonly seen in the large provincial towns such as Lagos. In most of these children he found debilitating infections such as malaria, tuberculosis, hookworm infestations, measles, acute respiratory infections and gastroenteritis. Furthermore, Ogbeide indicated that gastroenteritis, brought about by mothers feeding unwholesome contaminated milk to their infants, precipitated marasmus or marasmic-kwashiorkor.

Gordon *et al.* (1963) pointed out that enteroviruses and adenoviruses caused weanling diarrhea in children. They further stated that "kwashiorkor without diarrhea is likely not to be kwashiorkor."

Scrimshaw *et al.* (1965), in a critical review article, introduced the terms synergism and antagonism in referring to interrelationships between nutrition and infection. In synergism "the combination of infection and malnutrition often results in a severity greater than the sum of the two individual disease processes." They suggest that synergism was common in the interactions between bacterial, rickettsial, helminthic and sometimes viral and protozoal infections and protein and several vitamin deficiencies.

In contrast, antagonism referred to the condition in which a specific deficiency of a given nutrient inhibited the development of an infectious

agent. Scrimshaw *et al.* (1965) suggest that this condition was seen mainly in experimental conditions and rarely in clinical conditions. However, Hendrickse (1967) reported antagonism between protein-calorie malnutrition and *Plasmodium falciparum* infections in children. Hendrickse reported the results of a study on two groups of children from Yoruba speaking parts of Nigeria; one group from well-to-do families and the other from poor families living in a traditional environment. The incidence of measles in these two groups of children was the same. However, 9 children from the poor group died from the measle infection and there were no deaths in the children from wealthy families. He concluded that the interaction between measles and malnutrition was synergistic and ascribed to malnutrition the major cause of the poor prognosis of measles in West Africa.

Platt (1957), a physician and world authority in protein-calorie malnutrition, reported that there was increased nitrogen excretion in the urine of infants during infection. He stated that it required 10 to 20 grams of protein to furnish a leukocytosis of 30,000 white blood cells per cu. mm. elicited due to infections. Scrimshaw (1965) indicated that in diarrhea in infants, there was a decrease of 10 to 15% in the absorption of nitrogen. In malarial infection in monkeys, decreased intestinal absorption of amino acids was a noticeable feature (Maegraith, 1967). Woodward, Sbarra and Holtman (1954) found a depression in plasma amino acids in rats infected with *Bacterium tularense*. Oomen (1958) recognized that diarrheal disease precipitated xerophthalmia and keratomalacia in infants fed low vitamin A diets. Rapaport *et al.* (1947) observed that in infants with diarrhea there was severe loss of sodium, chloride, potassium and phosphorus. He reasoned that the electrolyte imbalance caused death in some infants. Mata *et al.* (1967) and Salomon *et al.*

(1968) agreed that infants with measles, severe diarrhea, bronchopneumonia and tonsillitis failed to gain weight.

Axelrod (1958) reported that nutritional deficiencies of proteins and B-complex vitamins resulted in decreased antibody production in rats. Scrimshaw (1965) mentioned that severe undernutrition or protein depletion reduced the phagocytic ability of leukocytes. In addition, he emphasized that malnutrition affected the integrity of epithelial linings of respiratory, digestive, and urinary tracts. Furthermore, nonspecific factors such as lysozymes and interferon production were reduced in malnutrition.

Summary of Literature Review

Protein-calorie malnutrition is a world-wide important nutritional deficiency in children and is especially prevalent in developing countries. An enormous amount of data has been accumulated on the clinical aspects of the disease. However, very little experimental work has been done that is analagous to the disease in children. The baby pig has been used to study protein-calorie malnutrition without the complications of infection. Since, under field conditions, protein-calorie malnutrition is a complex of malnutrition and infection, experimental work incorporating malnutrition and infection in the baby pig would seem to be a fruitful area of research important to public health.

MATERIALS AND METHODS

Animals

Three experiments were conducted, one in the summer of 1969, one in the winter of 1970, and the last in the summer of 1970. In the first experiment, 12 baby pigs 9 to 12 days of age were used. Of the 12 pigs, 6 were littermates of the Yorkshire breed and the other 6 pigs were littermates but were crossbred between Hampshire and Yorkshire. In the second experiment, one litter of 8 Hampshire pigs and 2 Yorkshire littermate pigs from another litter were used. In the third experiment, one litter of 7 Hampshire and 6 Yorkshire littermate pigs were used. The pigs were all purchased from a local producer, who indicated no previous history of TGE infection in the herd. No clinically detectable infections or gross abnormalities were present in the pigs when the experiments were initiated.

Care of Animals

All pigs were housed in individual, grated bottom, galvanized metabolic cages designed for quantitative collection of urine, feces, and spilled food. The pigs that were infected with transmissible gastroenteritis (TGE) virus were kept in a separate room and handled by a separate caretaker from the uninfected control pigs. The ambient temperature in the rooms housing the pigs was about 21 C. Records of daily feed consumption, weekly changes in body weight and clinical signs were recorded in both experiments.

Rations

In the first experiment, all 12 pigs were fed a commercial, canned, sterile milk* for an adjustment period of 2 days. For the following 2 days, the pigs were fed the experimental diets in liquid form prepared by mixing 50 g. of diet and 100 ml. of water in a Waring blender. Pigs were then fed solid feed for 3 weeks. The pigs were fed 3 times a day: 8 a.m., 1 p.m. and 6 p.m. In the second experiment the baby pigs were also fed SPF milk for 2 days. The following 4 days they were fed a mixture of 3 parts of soybean protein, 7 parts of cerelose and 1 part of SPF milk. This was followed by feeding the experimental diet in the solid form for 3 weeks. The experimental diets in the two experiments were the same (Table 1). In Experiment 3, the protocol was the same as in Experiment 1.

Experiment 1

Twelve pigs from the 2 litters of pigs were randomly divided into 4 groups of 3 pigs each. Group I was fed the 6% soyprotein and a high (30%) fat diet and Group III was fed the 6% soyprotein and a low (5%) diet. The pigs in Groups III and IV were fed the same diets as Groups I and II, respectively, and served as uninfected controls. Blood samples were collected from all pigs from the anterior vena cava, after they had been fed the experimental diet for 2 weeks and at necropsy. Each blood sample was allotted between 3 tubes: one containing heparin for plasma; the second, containing EDTA, for hemoglobin and packed cell volume determinations; and the third, for serum. Five days after inoculation of TGE virus, one pig from each of the 4 groups was killed by electric shock and necropsied. The remainder of the pigs in the 4 groups were

*SPF lac, Borden Co., New York, N.Y.

Table 1. Composition of experimental diets

Component	Low Protein High Fat (Energy) (%)	Low Protein Low Fat (Energy) (%)
Isolated soyprotein*	6	6
Cerelose	58	83
Lard	30	5
Phillips and Hart Salt Mix**	5	5
Vitamin Mix***	1	1

*Nutritional Biochemicals.

**Phillips and Hart (1935).

***Vitamin mixture supplied per kg. of diet in mg.: α-tocopheryl acetate, 110; menadione, 49.5; thiamine HCl, 22; riboflavin, 22; inositol, 110; p-aminobenzoic acid, 11; folic acid, 0.2; biotin, 0.44; ascorbic acid, 990; and choline chloride, 1.65 g.; vitamin B_{12}, 30 μg; and vitamin A, 1980 I.U., vitamin D_3, 220 I.U. (Vitamin Diet Fortification Mixture in Dextrose, Nutritional Biochemicals Corporation, Clevealand, Ohio).

killed 6 days after inoculation of TGE virus. At necropsy, tissues were examined and selected sections were preserved for histologic and electron microscopic examination.

Experiment 2

The 8 Hampshire littermate pigs were divided into 4 groups of 2 pigs each. The 2 Yorkshire pigs constituted a fifth group. Dietary treatment of Groups I to IV was the same as in Experiment 1. Group V was allowed to nurse the sow until they were 4 weeks of age. This group served as well nourished uninfected controls for plasma amino acid analyses. Pigs in Group I and II were infected with 10^5 infective doses of TGE virus when they were 5 weeks of age. The pigs in Groups III and IV served as uninfected malnourished controls.

Blood samples were collected at 3 periods during the course of the experiment. The first samples were taken after the pigs had been fed the experimental diets for 2 weeks. The second and third samples were collected 3 and 7 days, respectively, after TGE virus inoculation. Each blood sample was treated as in Experiment 1 for whole unclotted blood and serum. However, the blood for plasma was specially processed for free plasma amino acid analyses. One pig from each group was killed 72 hours after TGE virus inoculation. The rest were killed 6 days after the virus inoculation. At necropsy, tissues were collected for histologic examination and for analysis of liver for vitamin A.

Experiment 3

One litter of 7 Hampshire and another litter of 6 littermate Yorkshire pigs were used. The pigs were divided into 4 groups (Groups I to IV) as in Experiments 1 and 2. An additional group (Group VI) of 3 Hampshire piglets was allowed to nurse the sow and infected with TGE

virus. Dietary treatment of Groups I to IV was the same as in Experiment 1. However, pigs in Group VI were allowed to nurse the sow until 4 weeks of age. Pigs in Groups I, II and VI were infected with 5×10^5 infective dose of TGE virus after they had consumed the diet for 3 weeks. The pigs in Groups III and IV served as uninfected, malnourished pigs.

Blood samples for plasma free amino acid analyses were taken at 2 periods during the course of the experiment. The first samples were taken 12 hours after TGE virus inoculation and the last samples 100 hours after infection. Blood samples were treated as in Experiment 2.

Analyses

Blood analysis. Hemoglobin determinations were carried out by the cyanmethemoglobin method (Benjamin, 1964) on EDTA preserved blood from the anterior vena cava by the method of Carle and Dewhirst (1942). Packed cell volume was determined by the micro-capillary tube method. White blood cell counts were done using the electronic Coulter Counter.

Sodium and potassium determinations. Plasma sodium and potassium concentrations were determined using the Coleman Flame Photometer (operating directions for the Coleman Model 21-900 Flame Photometer D-332-C, 1968). A.R.A.C.S. grade of potassium and sodium and Sterox SE* were used as reagents.

Total serum proteins. Total serum protein was determined by refractometry.

---

*Hartmann-Leddon Co.

Serum protein electrophoresis. The serum protein electrophoresis was performed in a Spinco-Drumin cell.* The electrolyte used was pH 8.6 Veronal Buffer** of .075 ionic strength. Sepraphore III paper was used. At one end of the Sepraphore III, .1 ml. of serum was applied. A maximum of 8 strips of paper were run at one time. Electrophoresis was carried out for 60 minutes at 150 volts at 21 C. Fixation and staining were carried out according to Spinco Model R paper electrophoresis system operating instructions 656 R24. The relative intensities of the separated proteins were determined by scanning on the Analytrol by using the Gelman Scan-a-Tron.***

Determination of vitamin A in liver. Vitamin A in the liver was determined by the method of Neeld and Pearson (1963) with the following modifications: (a) aqueous liver homogenates were prepared in a Waring blender, (b) the homogenates were saponified for 10 minutes at 40 C. with an alkaline solution of 50% KOH and 10% ascorbic acid, and (c) a measured amount of saponified aqueous homogenate was substituted for serum.

Histologic technique. Tissues were preserved in Zenker's and 10% formalin solutions. Liver and brain tissues were fixed in Carnoy's solution. Liver, fixed in Carnoy's solution, was stained with Best's Carmine for determination of glycogen. Oil red O fat stain was used on frozen sections from the 10% formalin fixed tissues. Zenker's fixed tissues were stained with hematoxylin and eosin. The histologic

---

*Spinco Division, Beckman Instruments, Inc., Belmont, Calif.

**Buffer B-2, Spinco Division, *Ibid.*

***Beckman Instruments Co., Belmont, Calif.

procedures were as described in the *Manual of Histologic and Special Staining Techniques of the Armed Forces Institute of Pathology*, Washington, D.C. (1957).

Electron microscopic techniques. At necropsy, small sections of the adrenal gland were fixed in 4% glutaraldehyde for 4 hours at 4 C. The tissues were washed in 4 to 5 changes of Sorenson's buffer at pH 7.2 and stored in this buffer for no longer than 1 month. The middle portion of the adrenal cortex, the zona fasciculata, was obtained by trimming off the outer and inner portions of the adrenal cortex. Pieces of the zona fasciculata were placed in a drop of osmium tetroxide (OsO_4) and fixed for 1 hour. The tissues were dehydrated in 50%, 70%, 95% and absolute alcohols. The specimens were cleared in propylene oxide (Luft, 1961) and infiltrated for 1 hour in a 1:1 mixture of propylene oxide and Epon.*

A modified method (Luft, 1961) was followed for embedding the specimens in Epon in gelatin capsules. An accelerator, 2,4,6-tridimethyl amino methyl phenol (DMP 30**) at a concentration of 1.5% was added to the resin mixture. The Epon was cured in an oven for 36 hours at 60 C. Thin sections, 400 to 600 Å, were cut with a diamond knife on a Porter Blum MT2*** ultramicrotome.

The sections were mounted on carbon-coated 100 mesh copper grids or on uncoated 300 or 400 mesh grids. The grids were stained with lead citrate for 15 to 25 minutes and counterstained with uranyl acetate for 30 minutes. The sections were dried in a vacuum chamber. Examination

---

*Epon 812, Shell Chemical Corp., San Francisco, Calif.

**Rohn and Haas Co., Philadelphia.

***Ivan Sorvall, Inc., Norwalk, Conn.

of the grids and taking of photomicrographs were performed with a Philips 200 electron microscope.*

Plasma free amino acids. Ten milliliters of blood were collected from the anterior vena cava of the pigs and placed in heparinized tubes which were then placed in a container with ice cubes. The tubes were centrifuged** (refrigerated) at 6500 rpm, for 20 minutes. The plasma was removed with a Pasteur pipet and placed in clean test tubes. For every 1 ml. of plasma, .1 ml. of 36% sulfosalicylic acid (SSA) and .1 ml. norleucine were added to serve as an internal control standard. The SSA filtrate was obtained by centrifuging the tubes at 15,000 rpm in a refrigerated centrifuge for 15 minutes. The supernatant, containing the free amino acids, was stored at -40 C. The amino acid content of the supernatant was determined with a Technicon Auto Analyzer.***

*Philips, Mount Vernon, N.Y.

**Ivan Sorvall, Inc., Norwalk, Conn.

***Technicon Autoanalyzer, Ardsley, N.Y.

RESULTS

The 3 experiments in this research were actually triplicates involving the same rations, the same virus, and the same species of animal of about the same age. Consequently, the data on feed intake, growth, clinical signs, hematology, vitamin A values in the liver, serum protein values and gross and microscopic changes, in Experiments 1 and 2, are summarized together. However, the data on the ultrastructure of the adrenal gland and on serum electrolytes were obtained only from pigs in Experiment 1. The analysis of the free amino acids in the plasma was performed on 48 samples obtained from pigs in Experiments 2 and 3.

Feed Intake

Feed intake data for TGE virus infected and control groups of pigs in both experiments are summarized (Table 2). There was a statistically significant decrease in feed intake in the TGE virus infected pigs in comparison to the control groups. During the 3-day period after virus inoculation, the uninfected (control) pigs ate an average of 180 g. of feed per day. The infected pigs consumed about 1/3 of this amount. While this is a significant difference in feed consumption, it must be considered that the control pigs were limited in feed consumption and actually may have consumed more if they had been allowed to do so.

Growth

The data on growth are summarized (Figure 1) and the clinical signs are illustrated (Figures 2 and 3). There were weight gains in both

Table 2. Feed intake (in grams) of TGE virus infected and control pigs fed 6% soyprotein and high or low fat diets

Group	Ration	No. pigs	Feed Intake Before Infection		Feed Intake After Infection		
			Day 2	Day 1	Day 1	Day 2	Day 3
				Infected			
I	high fat	5	173 ± 11.5**	173 ± 10.3	92 ± 1.4*	66 ± 24*	63 ± 5.6*
II	low fat	5	180 ± 0	180 ± 0	86 ± 29.9*	65 ± 18.7*	63 ± 24.7
				Uninfected			
III	high fat	5	173 ± 11.5	167 ± 21.6	173 ± 11.5	180 ± 0	180 ± 0
IV	low fat	5	180 ± 0	180 ± 0	180 ± 0	180 ± 0	180 ± 0

*t tests between infected and control groups are significant.

**values = mean plus standard deviation.

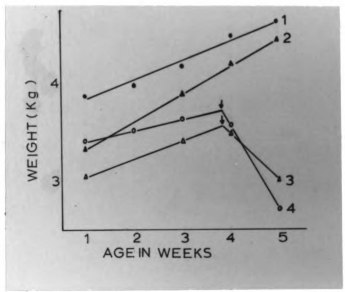

Figure 1. Growth rate of TGE virus infected and control pigs fed 6% soyprotein and high or low fat diet.

1. Uninfected control; 6% soyprotein, high fat diet.
2. Uninfected control; 6% soyprotein, low fat diet.
3. TGE infected; 6% soyprotein and high fat diet.
4. TGE infected; 6% soyprotein and low fat diet.

Arrow indicates time of infection.

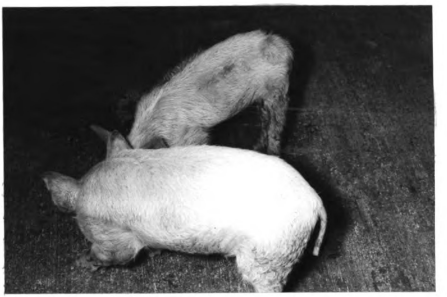

Figure 2. Note rough hair coat and small size of TGE infected pig (above) as compared with control pig (below). Both pigs fed 6% soyprotein and high fat (energy).

Figure 3. Note rough hair coat and small size of TGE virus infected pig (left) as compared with control (right). Both pigs were fed 6% soyprotein and low fat (energy).

control and experimental groups on the protein deficient diets. However, there was a depression of growth rate and a loss of the weight previously gained before the pigs were inoculated with TGE virus.

Clinical Signs

There was vomition and diarrhea in the infected pigs, starting 19 to 24 hours after TGE virus inoculation. Diarrhea (Figure 4) was still present in the infected pigs 154 hours later. However, one of the inoculated pigs had diarrhea for only 24 to 36 hours.

Analyses

Hemoglobin and packed cell volume. There was a significant decrease in hemoglobin and packed cell volume (Table 3) in pigs fed 6% soyprotein and low fat (energy) diet with superimposed TGE virus infection as compared with uninfected controls fed the same diet. The same differences were observed in the paired samples taken from pigs fed 6% soyprotein and low fat (energy) diets before and after TGE virus inoculation. However, there were no significant differences in hemoglobin and packed cell volume of pigs fed 6% soyprotein and high fat (energy) diets with superimposed TGE virus infection and the uninfected control pigs on the same diet.

Plasma potassium and sodium. There was a significant decrease in plasma potassium values (Table 4) of paired samples collected from pigs fed 6% soyprotein and high fat diets before and after TGE virus infection. In addition, there was a significant decrease in plasma potassium values between pigs fed 6% soyprotein and high fat (energy) diets with TGE virus infection and the uninfected control pigs on the same diet. However, there were no significant differences between the infected and

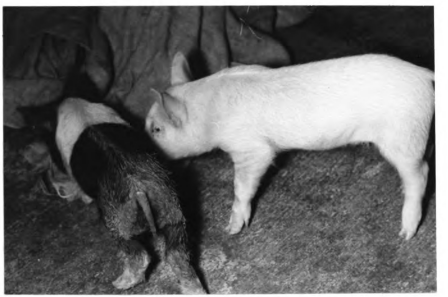

Figure 4. Note soiled hindquarters owing to infectious diarrhea in TGE infected pig (left). Control pig (right) and infected pig fed 6% soyprotein and high fat.

Table 3. Hemoglobin and PCV of TGE virus infected and control pigs fed 6% soyprotein and high fat or low fat diets

Group	Ration	No. pigs	Preinfection		Six Days After Virus Inoculation	
			Hb.	PCV	Hb.	PCV
				Infected		
I	high fat	5	9 ± 1.4	30.25 ± 1.4	9.2 ± 1.3	30.6 ± 1.73
II	low fat	5	9.3 ± 1.0[a]	30.0 ± .18[b]	6.1 ± 1.0[a,d]	21.0 ± 3.4[b,c]
				Uninfected		
III	high fat	5	9.5 ± 1.0	30.25 ± 1.4	9.50 ± 2.23	30.0 ± 6.0
IV	low fat	5	9.7 ± 0.18	29.6 ± 0.17	9.0 ± 0.86[d]	27.0 ± 2.5

*values = mean plus standard error.

[a,b]t test for Hb. and PCV applied to paired sample Group II significant $P < .05$.

[c,d]t test for Hb. and PCV between Groups II and IV significant $P < .05$.

Table 4. Serum potassium and sodium values (mEq./L.) of TGE virus infected and control pigs fed 6% soy-protein and high or low fat diets

Group	Ration	No. pigs	Preinfection		Six Days After Virus Inoculation	
			Potassium	Sodium	Potassium	Sodium
				Infected		
I	high fat	3	5.16 ± .31*[a]	136 ± 2.82[c]	3.31 ± .34[a,b]	128 ± 0[c,d]
II	low fat	3	5.86 ± .6	138 ± 2.82[c]	4.83 ± .38	131 ± 4.8[c,d]
				Uninfected		
III	high fat	3	5.15 ± 1.05	138 ± 0	4.5 ± 0.26	137.6 ± 3.7[d]
IV	low fat	3	5.8 ± 0.76	138 ± 0	4.5 ± 0.26	137.6 ± 3.7[d]

*values = mean plus standard error.

[a]t test applied to paired potassium values Group I. Significant $P < .05$.

[b]t test applied to paired potassium values between Groups I and III. Significant $P < .05$.

[c,d]t test applied to sodium values between paired and unpaired samples. Significant $P < .05$.

control groups of pigs, both fed 6% soyprotein and low fat diets. There was a significant decrease in plasma sodium values (Table 4) between TGE virus infected and uninfected control pigs fed the same diet.

Serum total protein and serum protein electrophoretic values. The average total serum protein values of 4-week-old pigs allowed to nurse exclusively on sow's milk were 6.05 g./100 ml. The average serum total protein and albumin values (Table 5) were greater in the pigs fed 6% soyprotein and high or low fat (energy) diets with superimposed TGE virus infection than uninfected pigs fed the same diet. Furthermore, beta globulin and gamma globulin levels (Table 6) of pigs fed 6% soyprotein and high fat or low fat (energy) diets with TGE virus infection were greater, though not significantly, than the level in uninfected control pigs on the same diets.

Vitamin A content in the liver. There was a significantly lower amount of vitamin A in the liver (Figure 5) in infected pigs fed 6% soyprotein and high or low fat diets than uninfected pigs fed identical diets.

Gross Pathology

There was distention of the stomach and small intestine with gas in the TGE infected pigs. Diarrhea characterized by yellow and watery fluid was present in the infected pigs. The mesenteric blood vessels were congested in the TGE infected pigs. In addition, 2 TGE infected pigs had dry, scaly appearing skin. Control pigs had a healthy appearing skin.

There was marked edema in the subcutaneous tissues and the spiral colon of pigs fed 6% soyprotein and high fat or low fat diets and

Table 5. Serum total protein and albumin (g./100 ml.) values of virus infected and control pigs fed 6% soyprotein and high or low fat diets

Group	Ration	No. pigs	Preinfection		Six Days After Virus Inoculation	
			Total Protein	Albumin	Total Protein	Albumin
				Infected		
I	high fat	5	3.94 ± 0.54*	2.11 ± 0.3	3.8 ± 0.95**	1.47 ± .5
II	low fat	5	4.13 ± 0.82	2.26 ± 0.51	3.5 ± 0.17	1.77 ± .38
				Uninfected		
III	high fat	5	4.83 ± .56	2.66 ± .114	2.8 ± .17	1.26 ± .026
IV	low fat	5	3.93 ± .12	2.02 ± .25	3.13 ± .26	1.31 ± .24

*values = mean plus standard error.

**One pig with diarrhea for 1 day had .9 g./100 ml. albumin. Clinically, edema was present.

Table 6. Serum alpha, beta and gamma globulin values (g./100 ml.) of TGE virus infected and control pigs fed 6% soyprotein and high or low fat diets

Group	Ration	No. pigs	Preinfection			Six Days After Virus Inoculation		
			Alpha	Beta	Gamma	Alpha	Beta	Gamma
					Infected			
I	high fat	5	.74 ± 26	.51 ± .15	.54 ± 1.5	1.36 ± .22	.53 ± .2	.52 ± .14
II	low fat	5	.77 ± .01	.47 ± .01	.61 ± .26	.80 ± .06	.37 ± .11	.56 ± .17
					Uninfected			
III	high fat	5	.94 ± .31	.47 ± .02	.55 ± .25	.75 ± .10[a]	.32 ± 1.0	.46 ± .02
IV	low fat	5	.62 ± .01	.50 ± .03	.75 ± .15	.79 ± .01	.40 ± 1.0	.62 ± .17

*values = mean plus standard error.

[a]t test for alpha globulin between groups is significant $P < .05$.

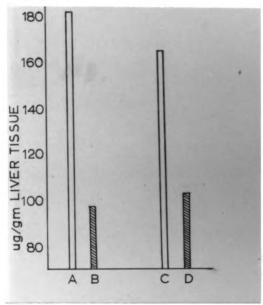

Figure 5. Liver content of vitamin A (mean of 5 pigs) in TGE infected and control pigs fed 6% soyprotein and high fat or low fat diets.

A: High fat control
B: High fat TGE infected
C: Low fat control
D: Low fat TGE infected

infected with TGE virus (Figures 6 and 7). Uninfected control pigs fed the same diet did not exhibit this edema. The livers of pigs fed 6% soyprotein and high or low fat diets were yellow and friable, indicative of fatty metamorphosis (Figure 8).

Microscopic Lesions

There were atrophic villi with cuboidal cells at the apex (Figure 9) in the jejunum and ileum of TGE virus infected pigs fed 6% soyprotein and high or low fat (energy) diets. However, the uninfected control pigs, fed the same diets, had tall villi with columnar epithelium (Figure 10).

The zone of mature cartilage on the epiphyseal plate of the rib was characterized by a narrow 3-cell-thick area in the TGE infected pigs fed 6% soyprotein and high or low fat diets (Figure 11). The uninfected control pigs fed the same diets had a wide, 7-cell-thick zone of maturing cartilage (Figure 12).

There was depletion of lipid vacuoles in the zona fasciculata of the adrenal gland of pigs infected with TGE virus and fed 6% soyprotein and a high or low fat diet (Figure 13). The uninfected control pigs fed the same diets had numerous lipid vacuoles in the zona fasciculata of the adrenal glands (Figure 14).

The skin from the abdominal and back regions of TGE virus infected pigs fed 6% soyprotein and high or low energy diets was characterized by hyperkeratosis with pustule formation and acanthosis (Figure 15). The uninfected control pigs fed the same diet had only hyperkeratosis (Figure 16). There was disorganization of acini and loss of cytoplasm of acinar cells (Figure 17) of the pancreas in the TGE virus infected pigs fed 6% soyprotein and high or low fat diets. Uninfected control pigs fed the

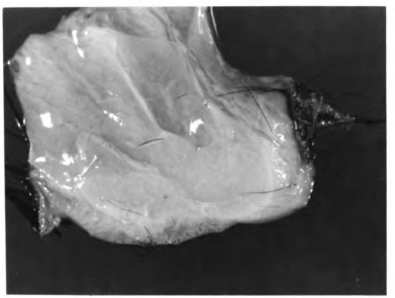

Figure 6. Note subcutaneous edema in tissue from lower abdomen in TGE infected pig fed 6% soyprotein and high fat diet.

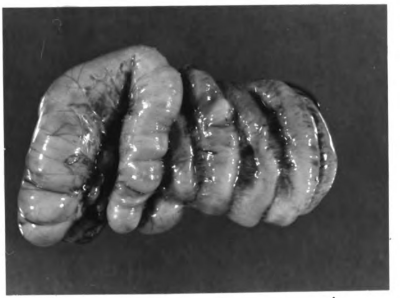

Figure 7. Note edema in spiral colon of TGE infected pig fed 6% soyprotein and high fat diet.

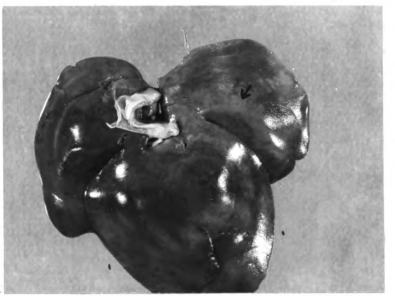

Figure 8. Pale friable liver indicative of fatty metamorphosis. TGE infected pig fed 6% soyprotein and high fat (energy) diet.

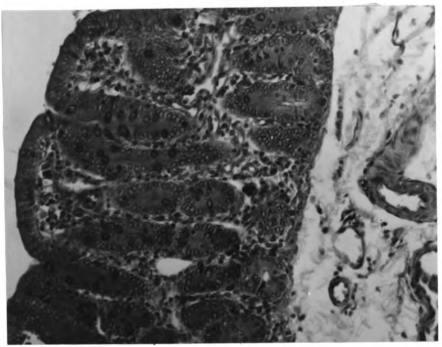

Figure 9. Villous atrophy in jejunum of TGE infected pig fed 6% soyprotein and low fat diet. Hematoxylin and eosin. x 120.

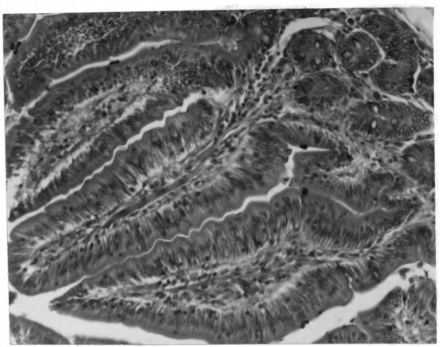

Figure 10. Well preserved normal villi in jejunum from uninfected control pig fed 6% soyprotein and low fat diet. Hematoxylin and eosin. x 120.

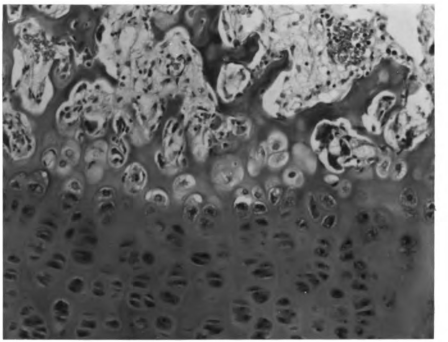

Figure 11. Narrow 3-cell-thick zone of maturing cartilage in epiphyseal plate of the sixth rib bone of TGE infected pig fed a 6% soyprotein and high fat diet. Hematoxylin and eosin. x 120.

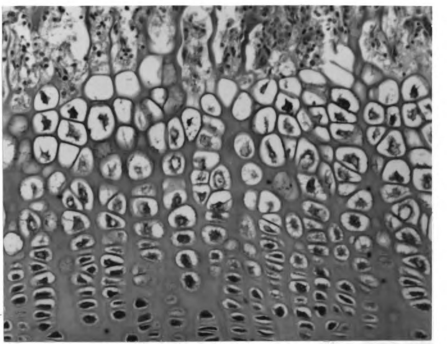

Figure 12. Wide, 7-cell-thick zone of maturing cartilage in epiphyseal plate of the sixth rib bone of uninfected control pig fed 6% soyprotein and high fat diet. Hematoxylin and eosin. x 120.

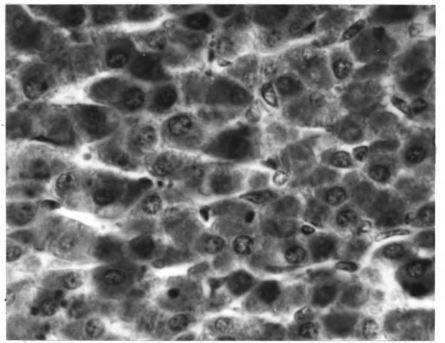

Figure 13. Depletion of vacuoles (lipids) in cytoplasm of cells in zona fasciculata of the adrenal of TGE infected pig fed 6% soyprotein and low fat diet. Hematoxylin and eosin. x 450.

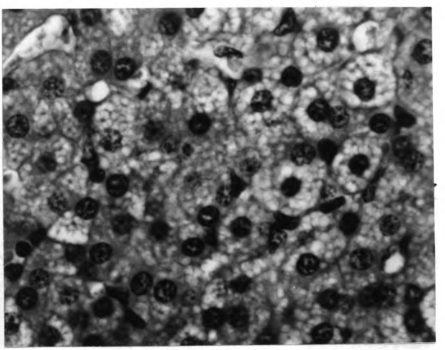

Figure 14. Several vacuoles (lipid) in cytoplasm of zona fasciculata of the adrenal of uninfected control pig fed 6% soyprotein and low fat diet. Hematoxylin and eosin. x 450.

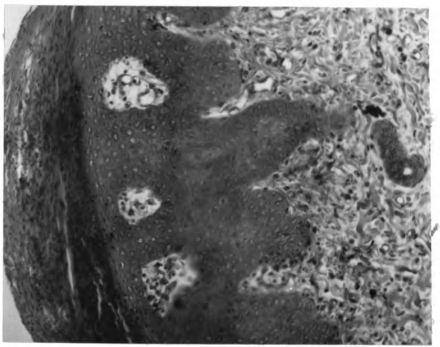

Figure 15. Hyperkeratosis with pustule formation (A) and acanthosis (B) in the skin of TGE virus infected pig fed 6% soyprotein and high fat diet. Hematoxylin and eosin. x 120.

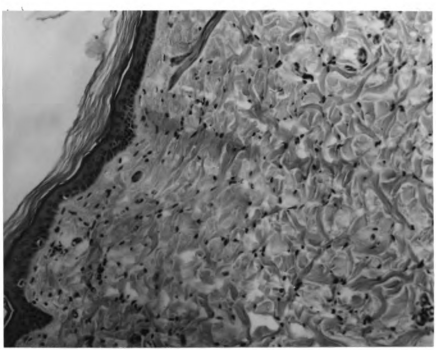

Figure 16. Hyperkeratosis of skin of uninfected control pig fed 6% soyprotein and high fat diet. Hematoxylin and eosin. x 120.

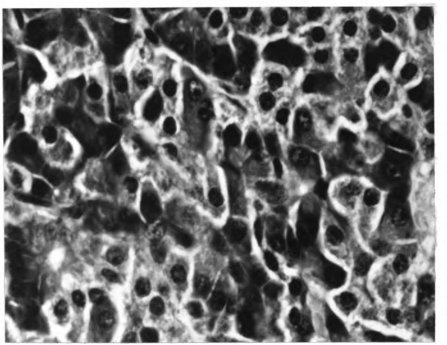

Figure 17. Disorganization of acini and loss of cytoplasm of acinar cells in pancreas of TGE virus infected pig fed 6% soyprotein and low fat diet. Hematoxylin and eosin. x 450.

same diet did not have these lesions. There was marked depletion of mature lymphocytes from the germinal centers (Figure 18) of the spleen of infected animals as compared with pigs fed 6% soyprotein and high or low fat diets (Figure 19).

There was fatty metamorphosis involving the whole hepatic lobule of the liver (Figure 20) in TGE infected pigs fed a 6% soyprotein and high or low fat (energy) diet. The uninfected control pigs fed the same diets had only periportal fatty changes (Figures 21 and 22). Glycogen (Figure 23) was present in the hepatocytes of both the infected and control pigs fed 6% soyprotein and high fat (energy) diets. However, there was more glycogen in the liver of the infected than uninfected pigs. The differences in glycogen content were not noted in the infected and uninfected pigs fed 6% soyprotein and low fat (energy) diet. Inflammatory cells, predominantly lymphocytes, were present in the mucosa of the fundic stomach of TGE virus infected pigs on a 6% soyprotein and high fat or low fat (energy) diet. Uninfected control pigs fed similar diets appeared normal (Figure 25). Glial nodules (Figure 26) were in the thalamic region of the brain of TGE infected pigs fed 6% soyprotein and high or low fat (energy) diets. Uninfected pigs on similar diets did not have glial nodules in this region of the brain.

Ultrastructural Changes in the Zona Fasciculata of the Adrenal Cortex

Mitochondria. There was hypertrophy of the mitochondria (Figure 27) in the pigs fed 6% soyprotein and infected. The cristae were replaced by circular to elongated, sometimes called tubulosaccular (Idelman, 1970), structures attached to the mitochondrial membranes. In addition, there was proliferation of mitochondria with inner structures that appeared vesicular (Figure 28). Triple complexes composed of

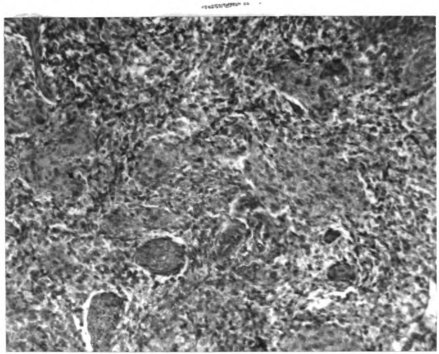

Figure 18. Spleen of TGE virus infected pig fed 6% soy-protein and high fat diet. Note depletion of mature lymphocytes in germinal centers. Hematoxylin and eosin. x 120.

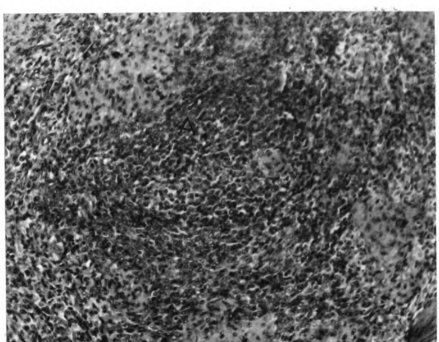

Figure 19. Spleen of uninfected control pig fed 6% soy-protein and high fat diet. Note abundance of mature lymphocytes in germinal center. Hematoxylin and eosin. x 120.

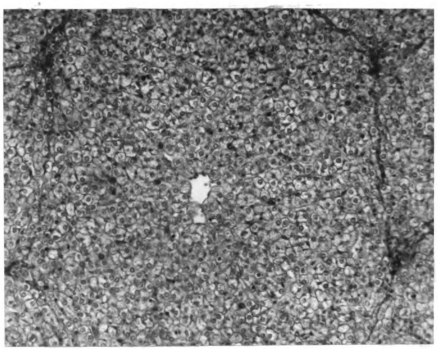

Figure 20. Vacuole formation of hepatocytes involving the whole lobule, in liver of TGE virus infected pig fed 6% soyprotein and high fat (energy) diet. Hematoxylin and eosin. x 120.

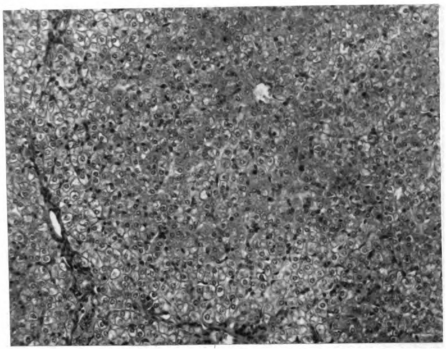

Figure 21. Vacuole formation of hepatocytes confined to periphery of the lobule in liver of uninfected control pig fed 6% soyprotein and high fat (energy) diet. Hematoxylin and eosin. x 120.

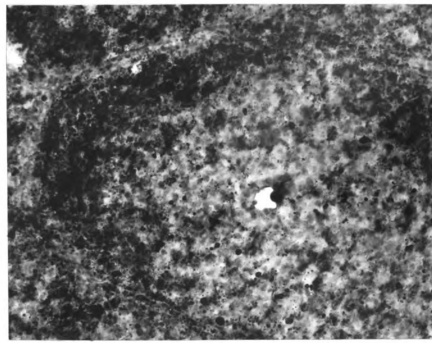

Figure 22. Liver of TGE infected pig fed 6% soyprotein and high fat (energy) diet (same section as in Figure 21). Note presence of fat in hepatic lobule. Oil red O. x 120.

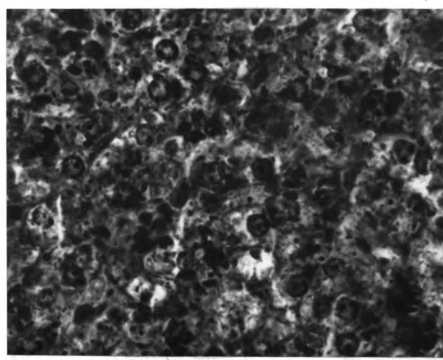

Figure 23. Note presence of glycogen in liver of TGE infected pig fed 6% soyprotein and high fat (energy) diet. Best's Carmine. x 120.

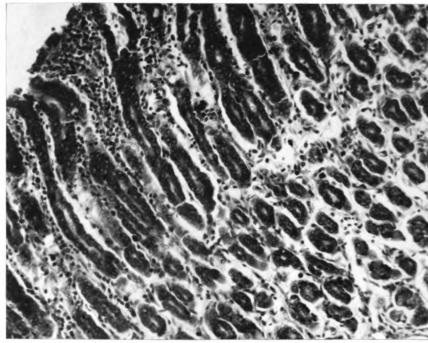

Figure 24. Inflammatory cells in the mucosa of the stomach of pig infected with TGE virus and fed 6% soyprotein and high fat (energy) diet. Hematoxylin and eosin. x 120.

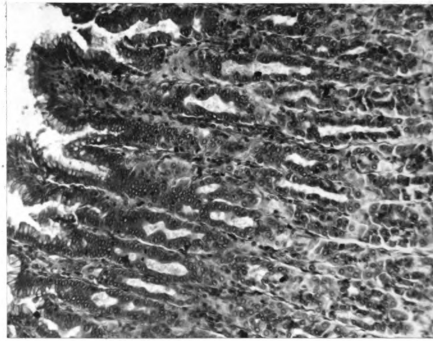

Figure 25. Normal mucosa of uninfected control pig fed 6% soyprotein and high fat. Hematoxylin and eosin. x 120.

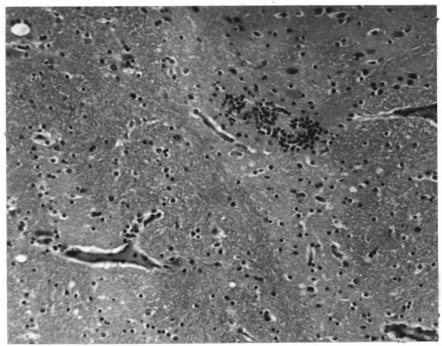

Figure 26. Note glial nodule in thalamus region of brain. Pig infected with TGE virus and fed 6% soyprotein and high fat (energy) diet. Hematoxylin and eosin. x 120.

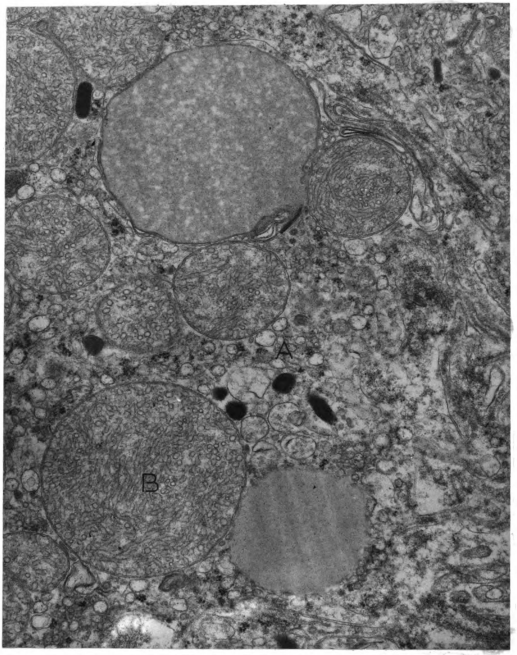

Figure 27. Adrenal zona fasciculata. Vesicular smooth endoplasmic reticulum (A). Enlarged tubulosaccular mitochondria (B). Experimental pig fed 6% soyprotein and high fat; killed 6 days after TGE infection. x 19,800

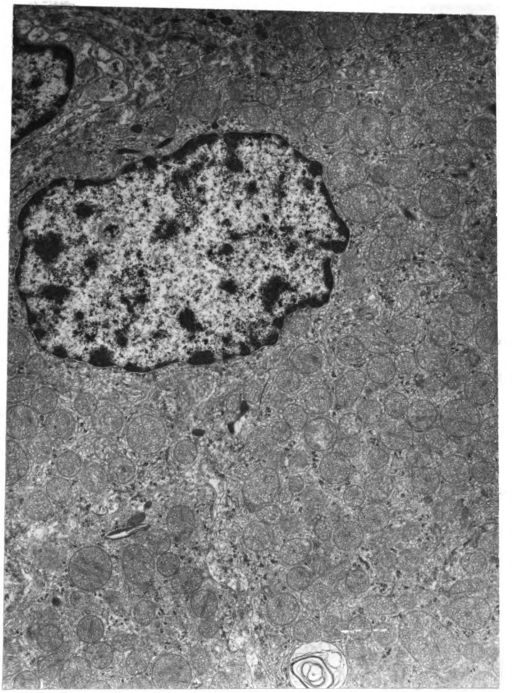

Figure 28. Adrenal zona fasciculata. Proliferation of vesicular mitochondria. Experimental pig fed 6% soyprotein and high fat; killed 6 days after TGE virus inoculation. x 11,800.

mitochondria, myelin figures and dense bodies; and double complexes characterized by the association of lipid droplets (liposomes) and mitochondria (Figure 29) were present in the infected pigs fed 6% soyprotein and low fat diets. Furthermore, some mitochondria enveloped lipid droplets (liposomes) and in a few instances lipid droplets were found within the mitochondria (Figure 30). Uninfected pigs on the same diets had no changes.

Endoplasmic reticulum. There was a vesicular appearance to the endoplasmic reticulum in the infected pigs fed 6% soyprotein and high fat diets (Figure 31). The endoplasmic reticulum was predominantly of the smooth type. In addition, a few of the endoplasmic vesicles were in direct contact with the mitochondria (Figure 31). Furthermore, a membrane lined structure, characterized by a closely packed vesicular smooth endoplasmic reticulum, mitochondria and a lipid droplet, was present in the periendothelial space (Figure 32). The structure was in direct contact with a precapillary cell and 3 other parenchymal cells of the zona fasciculata (Figure 32). In yet another section from an infected pig fed 6% soyprotein and high fat diet, there was degeneration of smooth endoplasmic reticulum of the parenchymal cells of the zona fasciculata (Figure 33). Uninfected pigs fed the same diets had no changes.

Dense bodies or lysosomes. In one of the infected pigs fed 6% soyprotein and high fat diet there was vomition and diarrhea for only 2 days. The cells of the zona fasciculata, in this pig, had a large number of dense bodies (lysosomes) (Figure 34). These structures were occasionally in contact with the vesicular smooth endoplasmic reticulum (Figure 34). A precapillary cell lay very close to the parenchymal cell

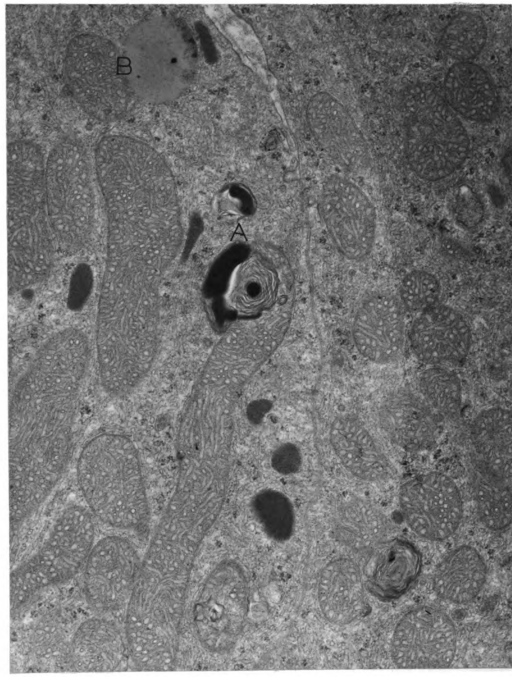

Figure 29. Adrenal zona fasciculata. Note triple complex of mitochondria, myelin-like body and a dense body (A) and a double complex of mitochondria and liposome (lipid droplet) (B). Experimental pig fed 6% soyprotein and low fat and killed 6 days after TGE infection. x 25,000.

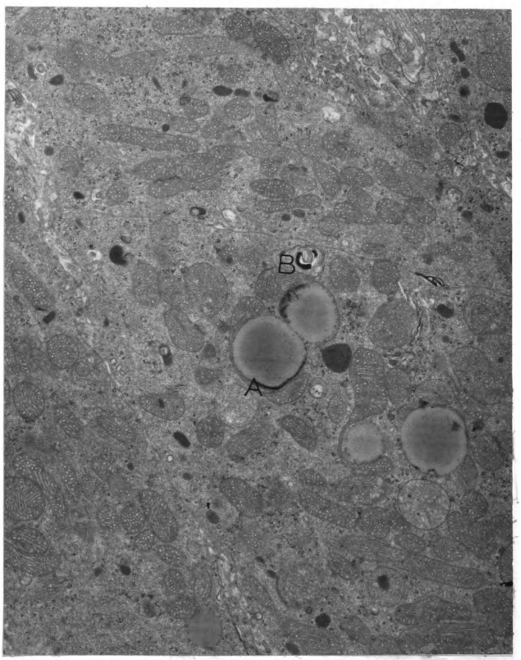

Figure 30. Adrenal zona fasciculata. Mitochondria enveloping lipid droplet (liposome) (A); myelin-like body and dense bodies (B, C). Experimental pig fed 6% soyprotein and low fat and killed 6 days after TGE virus infection. x 11,800.

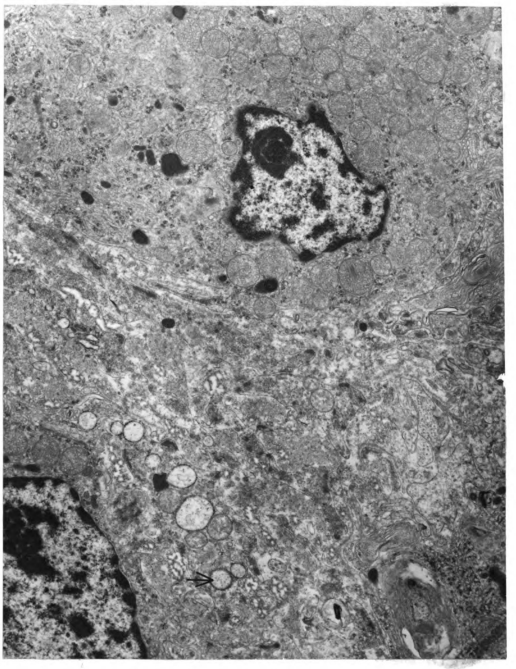

Figure 31. Adrenal zona fasciculata. Vesicular endoplasmic reticulum (arrow). Experimental pig fed 6% soyprotein and high fat and killed 6 days after TGE infection. x 11,800.

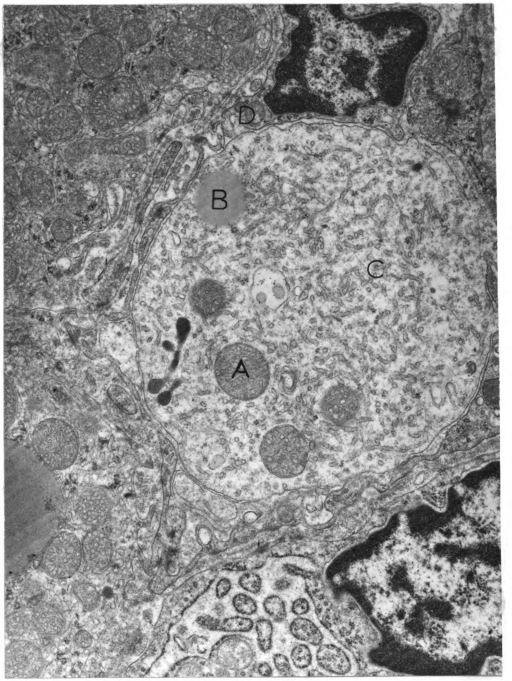

Figure 32. Adrenal zona fasciculata. Membrane lined structure consisting of mitochondria (A), lipid droplet (B), and vesicular smooth endoplasmic reticulum (C). Note precapillary cell (D). Pig fed 6% soyprotein and high fat and killed 6 days after TGE infection. x 19,800.

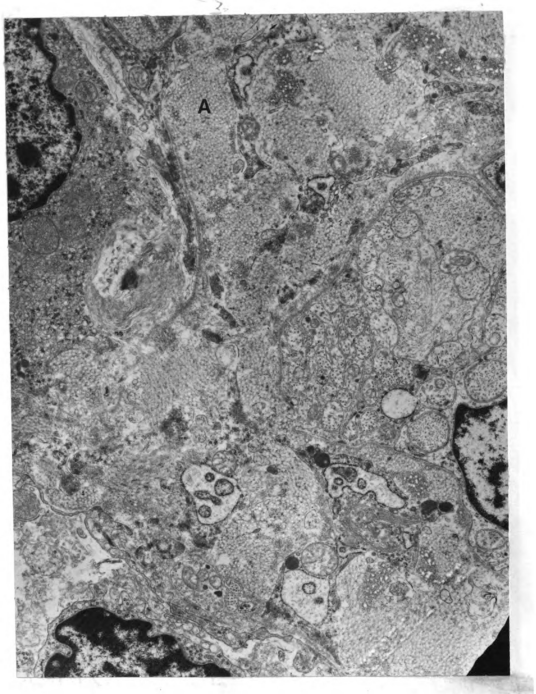

Figure 33. Adrenal zona fasciculata. The smooth endoplasmic reticulum is undergoing degeneration (A). Infected pig fed 6% soyprotein and high fat and killed 6 days later. x 11,800.

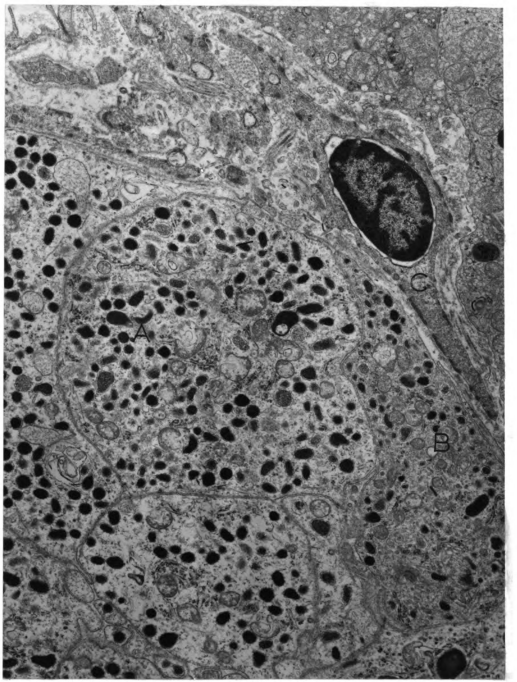

Figure 34. Adrenal zona fasciculata. Large number of dense bodies (lysosomes) (A) and vesicular endoplasmic reticulum (B); precapillary cell (C). Infected pig fed 6% soyprotein and high fat and killed 6 days later. x 11,800.

containing the large number of lysosome dense bodies (Figure 34). Furthermore, there was a myelin figure in close association with a dense body, probably a lysosome (Figure 35). Uninfected pigs did not have such changes.

Microvilli. There was an increase in the number of microvilli (Figure 36) projecting from the cells in the zona fasciculata of infected pigs fed 6% soyprotein and low fat diets, as compared to uninfected pigs fed the same diet.

Lipid droplets or liposomes. A panoramic view of 4 parenchymal cells of an infected pig fed 6% soyprotein and high fat (energy) diet is illustrated (Figure 37). There are only 2 lipid droplets in the latter illustration. The uninfected pigs fed the same diet had more lipid droplets (7) in a cell in the zona fasciculata (Figure 38). In contrast, the uninfected pigs fed 6% soyprotein and low fat (energy) diet had 8 fat droplets in a given cell in the zona fasciculata (Figure 39). A few lipid droplets appeared to be undergoing dissolution or degeneration, characterized by a wavy membranous outline, loss of lipid contents and the appearance of crystalline-like structures (Figure 40) in the uninfected pig fed 6% soyprotein and low fat.

Plasma Free Amino Acid

There was a statistically significant decrease in plasma threonine, tyrosine, leucine, valine, arginine, aspartic acid and proline; but a significant increase in plasma glycine, serine and alanine in the uninfected pigs fed 6% soyprotein and high fat diet for 3 weeks compared to pigs of similar age fed exclusively on sow's milk (Table 7). The decrease in plasma phenylalanine, lysine, methionine, isoleucine, histidine and

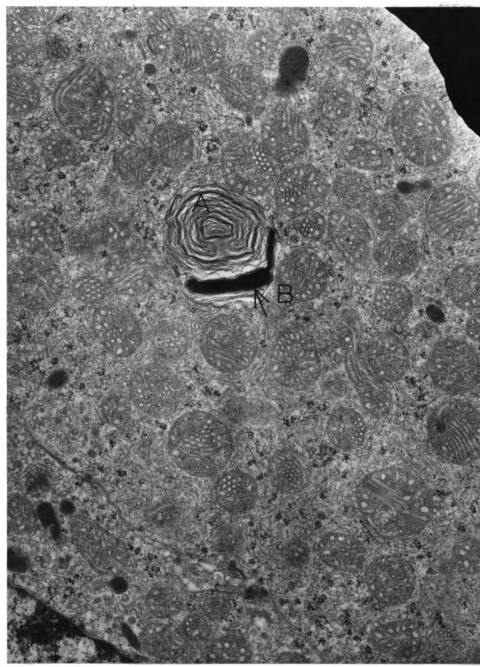

Figure 35. Adrenal zona fasciculata. Myelin-like structure (A) and dense body lysosome (B). Infected pig fed 6% soyprotein and high fat. Killed 6 days later. x 25,000.

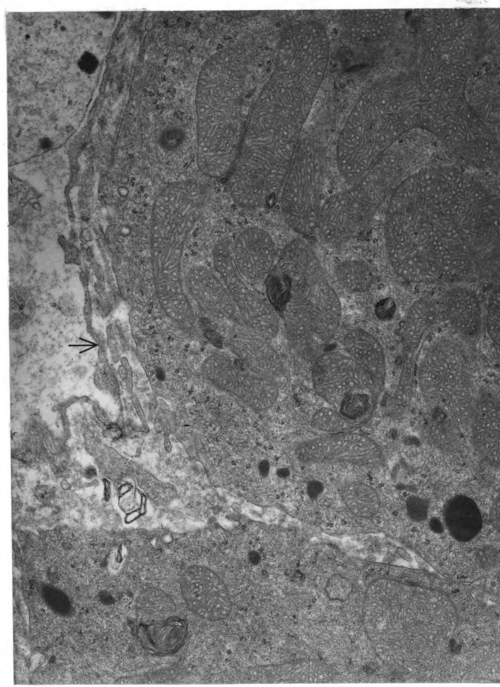

Figure 36. Adrenal zona fasciculata. Note proliferation of microvilli. Experimental pig fed 6% soyprotein and high fat; killed 6 days after TGE virus inoculation. x 18,800.

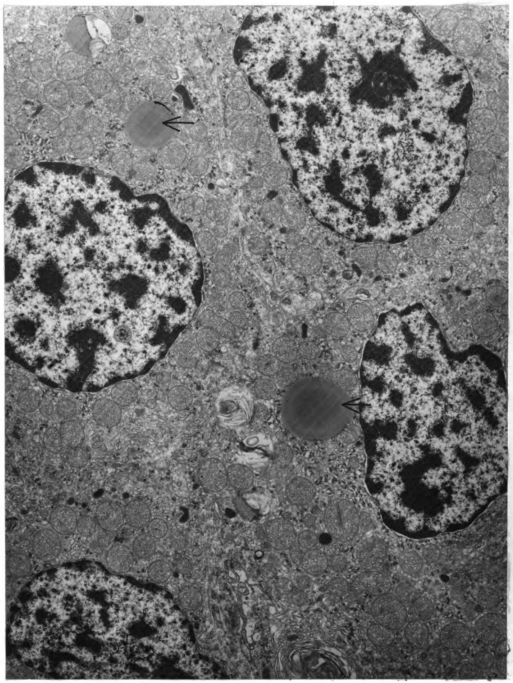

Figure 37. Adrenal zona fasciculata, panoramic view. Note only 2 lipid droplets (liposomes) on a group of 4 cells. Animal fed 6% soyprotein and high fat; killed 6 days after infection. x 9,000.

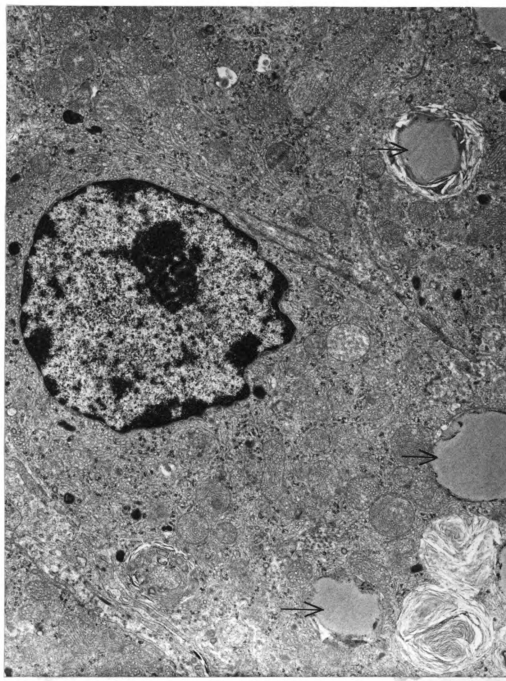

Figure 38. Adrenal zona fasciculata. Note several lipid droplets from uninfected pig fed 6% soyprotein and high fat diet. x 11,800.

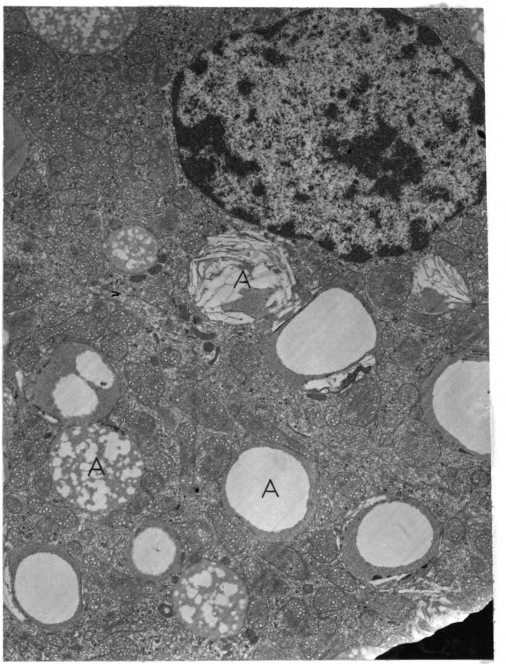

Figure 39. Adrenal zona fasciculata. Note several lipid droplets or liposomes (A). Uninfected pig fed 6% soyprotein and low fat diet. x 11,000.

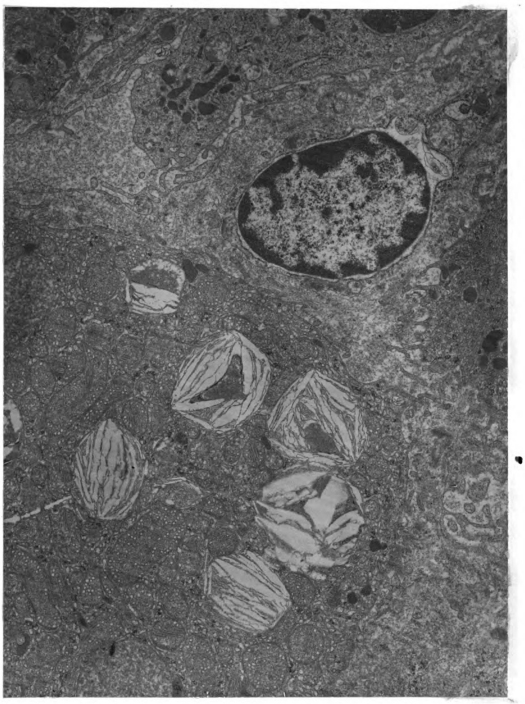

Figure 40. Adrenal zona fasciculata. Note degeneration of fat droplets. Uninfected pig fed 6% soyprotein and low fat. x 25,000.

Table 7. Plasma amino acid pattern (μ moles/liter) of pigs fed exclusively on sow's milk and pigs fed 6% soyprotein and a high or low fat diet

	Milk-fed*	High Fat Fed	Low Fat Fed
Phenylalanine	76.07	41.60 ± 4.32	43.71 ± 6.89++
Lysine	109.43 ± 1.72	85.01 ± 9.24	151.10 ± 33.47
Methionine	32.16 ± 4.46	10.12 ± 2.87	19.42 ± 5.73
Threonine	160.69 ± 4.9	17.91 ± 1.99+	56.98 ± 14.42++
Tyrosine	132.27 ± 15.0	29.50 ± 3.36+	30.31 ± 1.19++
Isoleucine	66.53 ± 8.05	100.87 ± 9.57	95.61 ± 21.98
Leucine	112.19 ± 10.61	57.87 ± 4.50	68.95 ± 8.68++
Valine	161.42 ± 10.93	100.54 ± 7.43	104.29 ± 7.13
Arginine	117.62 ± 13.47	57.89 ± 6.38	68.53 ± 14.26
Aspartic acid	24.83 ± 0.87	15.22 ± 0.93	28.74 ± 6.08
Glycine	512.81 ± 46.36	740.99 ± 33.82	863.79 ± 124.54
Histidine	46.18 ± .74	43.33 ± 3.59	46.56 ± 2.47
Alanine	294.10 ± 7.84	465.48 ± 23.75	468.59 ± 43.52++
Proline	417.49 ± 25.47	129.59 ± 26.51+	208.36 ± 37.19++
Glutamic acid	117.49 ± 13.14	175.29 ± 27.77	206.65 ± 26.44++
Serine	99.55 ± 3.78	135.00 ± 4.03+	150.54 ± 12.05

*mean of 2 animals plus standard error.

**mean of 4 animals plus standard error.

+t test between milk-fed and high fat fed, significant $P < .05$.

++t test between milk-fed and low fat fed, significant $P < .05$.

glutamic acid in the high fat fed pigs, was not significantly different from the milk fed pigs of the same age (Table 7). There was a significant decrease in plasma phenylalanine, threonine, tyrosine, leucine, valine, and proline, but a significant increase in plasma serine, alanine and glutamic acid in the pigs fed 6% soyprotein and low fat compared to pigs fed sow's milk at the same age (Table 7). However, the decrease in plasma lysine, methionine, isoleucine, arginine, aspartic acid, and histidine was not significantly different from those of pigs fed sow's milk of the same age.

Samples of plasma taken from pigs 72 hours after infection (fed 6% soyprotein and high fat diet) had significantly reduced isoleucine, arginine, serine and alanine values from preinfection values, taken 1 week prior to infection, in pigs fed the same diet (Table 8). Seventy-two hours following infection there was a decrease, though not significant, of isoleucine, valine, aspartic acid, glycine, serine, alanine, proline, and glutamic acid, and significant decrease of arginine as compared to values of samples taken concurrently from uninfected pigs fed the same diet (Table 8).

There was a significant decrease in plasma tyrosine, arginine, aspartic acid and histidine, in the samples taken 72 hours postinfection in pigs fed 6% soyprotein and low fat diet as compared to preinfection samples, taken 1 week prior to infection (Table 9). There was a decrease, though not significant, of plasma lysine, tyrosine, valine, histidine, serine, and proline; and a significant decrease in threonine and arginine in the samples taken 72 hours postinfection of pigs fed 6% soyprotein and low fat as compared to samples taken concurrently from uninfected pigs fed the same diets (Table 9). In contrast, there was an increase, though not significant, of plasma isoleucine, leucine, glycine, alanine,

Table 8. Plasma amino acid pattern (μ moles/liter) of TGE virus infected and control pigs fed 6% soyprotein and high fat diet

	Preinfection Control	72 Hours Postinfection	
		Control**	Infected**
Phenylalanine	41.60 ± 4.32	25.25 ± 5.80	36.73 ± 4.14
Lysine	85.01 ± 9.24	62.09 ± 2.76	65.96 ± 8.55
Methionine	10.12 ± 2.87	13.55 ± 0.53	13.91 ± 2.67
Threonine	17.91 ± 1.99	18.78 ± 5.64	28.86 ± 5.08
Tyrosine	29.50 ± 3.36	21.36 ± 5.51	22.54 ± 2.32
Isoleucine	100.87 ± 9.57	58.75 ± 1.40	49.12 ± 18.76[b]
Leucine	57.87 ± 4.50	26.19 ± 2.06	42.80 ± 9.67
Valine	100.54 ± 7.43	64.77 ± 1.01	39.02 ± 19.97
Arginine	57.89 ± 6.38	28.18 ± 2.39	7.12 ± 4.47[b,c]
Aspartic acid	15.22 ± 0.93	11.15 ± 1.04	10.71 ± 1.70
Glycine	740.99 ± 33.82	564.98 ± 94.89	443.59 ± 207.28
Histidine	43.33 ± 3.59	32.96 ± 1.67	38.53 ± 2.93
Serine	135.12 ± 4.03	96.00 ± 14.94	82.42 ± 13.62[b]
Alanine	465.48 ± 23.75	369.16 ± 55.27	208.96 ± 55.13[b]
Proline	129.59 ± 26.51	131.50 ± 3.65	88.09 ± 27.84
Glutamic acid	175.29 ± 27.77	123.88 ± 1.67	111.39 ± 29.78

*mean of 4 values plus standard error.

**mean of 2 values plus standard error.

[b]t test between 72 hour postinfection and preinfection values, significant $P < .05$.

[c]t test between 72 hour postinfection and sample taken concurrently from uninfected control, significant $P < .05$.

Table 9. Plasma amino acid pattern (μ moles/liter) of TGE virus infected and control pigs fed 6% soyprotein and low fat diet

	Preinfection Control	72 Hours Postinfection	
		Control**	Infected**
Phenylalanine	43.71 ± 6.89	26.90 ± 0.90	40.46 ± 1.66[c]
Lysine	151.10 ± 33.47	87.44 ± 5.66	78.69 ± 22.44
Methionine	19.42 ± 5.73	10.56 ± 3.77	25.18 ± 0.73[c]
Threonine	56.98 ± 14.42	42.31 ± 2.21	29.13 ± 1.96[c]
Tyrosine	30.31 ± 1.19	25.66 ± 6.26	23.93 ± 0.88[b]
Isoleucine	95.61 ± 21.98	52.21 ± 0.90	69.44 ± 6.59
Leucine	65.95 ± 8.68	55.81 ± 4.56	61.00 ± 3.57
Valine	104.29 ± 7.13	88.93 ± 2.40	85.32 ± 15.10
Arginine	68.53 ± 14.26	70.07 ± 2.24	16.34 ± 9.36[b,c]
Aspartic acid	28.74 ± 6.08	22.20 ± 8.14	11.56 ± 0.45[b]
Glycine	836.79 ± 124.54	454.35 ± 40.85	574.75 ± 178.38
Histidine	46.56 ± 2.47	44.30 ± 10.87	33.41 ± 3.70[b]
Serine	150.54 ± 12.05	107.58 ± 8.92	100.31 ± 25.84
Alanine	468.59 ± 43.52	272.99 ± 19.17	366.97 ± 165.25
Proline	208.36 ± 37.19	119.83 ± .20	109.92 ± 19.16
Glutamic acid	206.65 ± 26.44	144.16 ± 49.78	152.76 ± 10.20

*mean of 4 values plus standard error.

**mean of 2 values plus standard error.

[b]t test between 72 hour postinfection and preinfection values, significant $P < .05$.

[c]t test between 72 hour postinfection sample and sample taken concurrently from uninfected control pigs, significant $P < .05$.

and glutamic acid but a significant increase of phenylalanine and methionine in the samples taken 72 hours after infection of pigs on low fat from samples taken concurrently from uninfected pigs on the same diets (Table 9).

Samples of plasma taken from pigs 144 hours postinfection (fed 6% soyprotein and high fat) had marked reduction of isoleucine, methionine, valine, arginine, glycine, serine, alanine, proline, and glutamic acid from pair-fed, uninfected pigs fed the same diet (Figure 41). However, marked differences were not noted in phenylalanine, lysine, threonine, tyrosine, leucine, aspartic acid and histidine in the samples of plasma taken 144 hours postinfection of high fat fed pigs compared to paired uninfected pigs fed the same diet (Figure 41).

There was a more precipitous decline of alanine, valine, arginine (Figure 42), glutamic acid and proline (Figure 43), glycine and serine (Figure 44) and methionine (Figure 45), with time in the infected pigs fed 6% soyprotein and high fat than in the uninfected control pigs fed the same diets. However, plasma lysine (Figure 45), aspartic acid and histidine (Figure 46) did not decline faster with time in the TGE virus infected high fat fed pigs as compared to uninfected pigs fed the same diet. Indeed, plasma aspartic acid and lysine and phenylalanine (Figure 47) increased with time in the infected pigs fed high fat, as compared to uninfected controls fed the same diet.

The nonessential to essential amino acid ratio declined with time (Table 10) in the infected pigs fed high fat diets; however, the ratio increased with time in the uninfected pigs fed the same diet (Table 10). There was marked increase in the ratio of alanine to arginine with time in the infected pigs fed high or low fat diets; the same ratio declined with time in the uninfected pigs fed 6% soyprotein and high or low fat

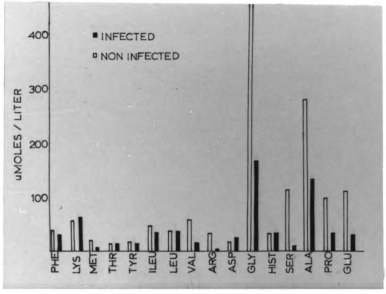

Figure 41. Pattern of plasma amino acids of samples taken 144 hours after infection of pigs fed 6% soyprotein and high fat and uninfected pigs fed the same diet.

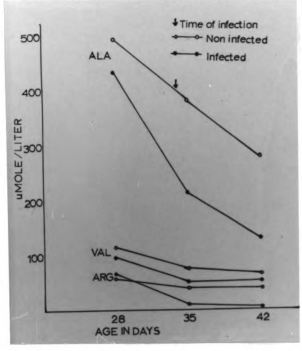

Figure 42. Changes in plasma alanine, arginine and valine in TGE virus infected and control pigs fed 6% soyprotein and high fat.

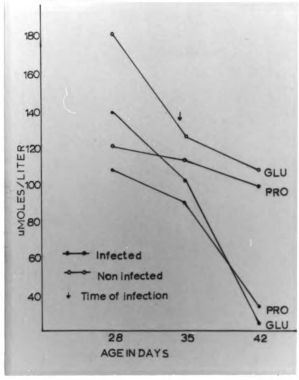

Figure 43. Changes in plasma proline and glutamic acid in TGE virus infected and control pigs fed 6% soyprotein and high fat diets.

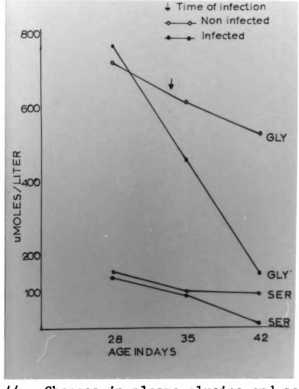

Figure 44. Changes in plasma glycine and serine in TGE virus infected and control pigs fed 6% soyprotein and high fat diets.

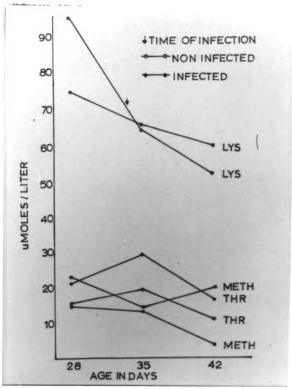

Figure 45. Changes in plasma lysine, threonine and methionine in TGE virus infected and control pigs fed 6% soyprotein and high fat diets.

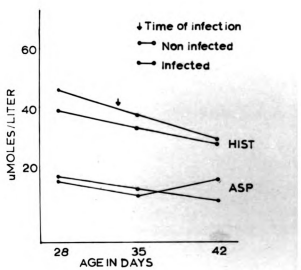

Figure 46. Changes in plasma histidine and aspartic acid in TGE virus infected and control pigs fed 6% soyprotein and high fat diets.

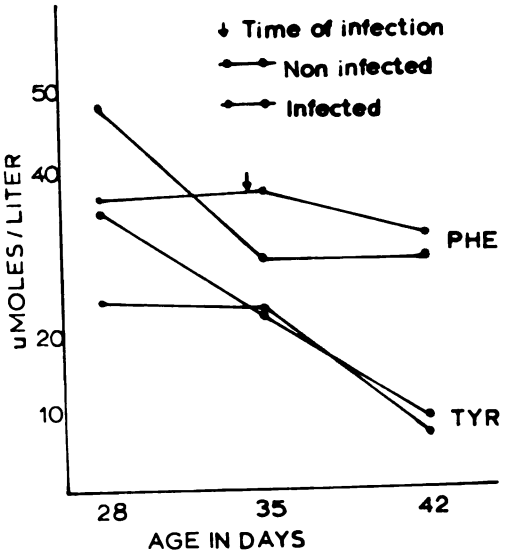

Figure 47. Changes in plasma phenylalanine and tyrosine in TGE virus infected and control pigs fed 6% soyprotein and high fat diet.

Table 10. Plasma amino acid ratios of TGE virus infected and control pigs fed 6% soyprotein and high fat diets

Ratios	NEAA* / EAA	Glycine / Valine	Phenylalanine / Tyrosine	Alanine / Arginine
		Infected		
Preinfection	3.63	8.34	1.48	8.30
72 hours postinfection	3.07	11.36	1.62	40.89
144 hours postinfection	1.94	13.44	1.93	75.70
		Uninfected		
Milk-fed pigs at 4 weeks of age	1.58	3.26	.58	2.53
Preinfection	3.48	7.04	.90	10.14
72 hours	3.85	8.36	1.74	11.66
144 hours	3.90	9.58	2.05	9.11

*Nonessential amino acids/essential amino acids.

diets (Table 10). The glycine to valine ratio, and the phenylalanine to tyrosine ratio, increased with time in both the infected and uninfected pigs fed 6% soyprotein and high fat diets. However, the increase in the ratio of glycine to valine was higher in the infected than uninfected pigs on the same level of protein and a high fat diet (Table 10).

The phenylalanine to tyrosine ratio of plasma samples taken 72 hours after infection of pigs fed 6% soyprotein and low fat diet was higher than that of a sample taken concurrently from uninfected control pigs fed the same diet. There were no marked differences in the nonessential to essential amino acid ratio and the glycine to valine ratio in the sample of plasma taken 72 hours after TGE virus infection of low fat fed pigs compared to a sample taken concurrently from uninfected control pigs on a similar diet (Table 11).

In the pigs allowed to nurse the sow, there was a significant decrease of the essential amino acids: arginine, isoleucine, leucine, lysine, phenylalanine, threonine and valine within 12 and 100 hours after TGE virus inoculation as compared to uninfected pigs of the same age, nursing a sow (Tables 12 and 13). In contrast, the essential amino acid, methionine, in the infected pigs remained unchanged after 12 and 100 hours of virus infection as compared to the uninfected pig. The nonessential amino acids were reduced, though not significantly, 12 and 100 hours after TGE infection, as compared to the uninfected pigs on the same diet (Tables 12 and 13). The values, after 100 hours, of arginine, isoleucine, and tyrosine in the plasma of pigs allowed to nurse the sow, were further reduced from the 12-hour values of the same animals. However, the values of other amino acids in the plasma of the pigs 100 hours after infection were unchanged from the values in pigs recorded 12 hours after infection.

Table 11. Plasma amino acid ratios of TGE virus infected and control pigs fed 6% soyprotein and low fat diets

Ratios	NEAA* / EAA	Glycine / Valine	Phenylalanine / Tyrosine	Alanine / Arginine
			Infected	
Preinfection	3.20	8.35	1.83	9.61
72 hours postinfection	3.34	6.56	1.69	24.80
			Uninfected	
Preinfection	3.11	8.04	1.06	6.26
72 hours	2.53	5.11	1.10	3.40

*Nonessential amino acids/essential amino acids.

Table 12. Pattern of plasma amino acids (μ moles/liter) of TGE virus infected and uninfected pigs allowed to nurse the sow. Samples taken 12 hours postinfection

Amino Acid	Uninfected	Infected
Phenylalanine	142.14 ± 0.00	73.53 ± 8.59[a]
Lysine	218.86 ± 1.72	92.90 ± 28.94[a]
Methionine	64.32 ± 4.46	85.18 ± 22.76
Threonine	321.38 ± 4.90	135.91 ± 15.58
Tyrosine	264.55 ± 15.00	86.15 ± 8.85[a]
Isoleucine	133.06 ± 8.05	73.16 ± 22.85[a]
Leucine	224.38 ± 10.61	134.50 ± 25.04[a]
Valine	322.84 ± 10.93	155.98 ± 26.42[a]
Arginine	235.24 ± 13.47	65.47 ± 17.51[a]
Aspartic acid	49.66 ± 0.87	24.96 ± 1.17
Glycine	1025.62 ± 46.36	577.69 ± 41.74
Histidine	92.35 ± 0.74	71.51 ± 20.43
Serine	199.10 ± 3.78	114.16 ± 15.99
Alanine	588.20 ± 7.84	625.40 ± 98.68
Proline	834.49 ± 25.47	489.66 ± 64.11[a]
Glutamic acid	234.98 ± 13.14	208.01 ± 42.45

*Mean of 2 pigs.

**Mean of 3 pigs.

[a]t test between infected and uninfected sample significant $P < .05$.

Table 13. Plasma amino acid pattern (μ moles/liter) of TGE virus infected and uninfected pigs fed exclusively sow's milk. Samples taken 100 hours postinfection

Amino Acid	Uninfected	Infected
Phenylalanine	142.14 ± .00	90.94 ± 3.76[a]
Lysine	218.86 ± 1.72	175.25 ± 35.90
Methionine	64.32 ± 4.46	67.81 ± 8.46
Threonine	321.38 ± 4.90	137.06 ± 31.01[a]
Tyrosine	264.55 ± 15.00	72.18 ± 18.40[a]
Isoleucine	133.06 ± 8.05	81.39 ± 2.90[a]
Leucine	224.38 ± 10.61	125.29 ± 8.13[a]
Valine	322.84 ± 10.93	154.02 ± 8.19[a]
Arginine	235.24 ± 13.47	52.41 ± 13.13[a]
Aspartic acid	49.66 ± 0.87	26.23 ± 3.64
Glycine	1025.62 ± 46.36	493.94 ± 131.88
Histidine	92.36 ± .74	68.88 ± .74
Serine	199.10 ± 3.78	109.05 ± 12.42
Alanine	588.20 ± 7.84	519.94 ± 157.88
Proline	834.49 ± 25.47	379.48 ± 96.92
Glutamic acid	234.98 ± 13.14	274.94 ± 35.87

*Mean of 2 pigs.

**Mean of 3 pigs.

[a]t test between infected and uninfected sample significant $P < .05$.

DISCUSSION

General Observations

The baby pig is a good model on which to conduct research on the interrelations of nutrition and infection. It has a voracious appetite and readily consumes deficient diets. The investigator is saved the problem of force feeding which is actually unphysiological. However, much time coupled with frustration is involved in caring for pigs on experiments. In addition, it is not always possible to have an infectious agent that is uniform in pathogenicity. Nevertheless, the results of research into the interaction between nutrition and infection are rewarding, since this synthesis is the closest model of what happens naturally in animals and in man.

Food Consumption and Growth

The results on food consumption indicate that the TGE virus infection induces anorexia in pigs in protein-calorie malnutrition. There was a retardation of growth and loss of weight in the infected pigs with suboptimal levels of protein. The data confirm the work of Doyle and Hutchings (1946). However, uninfected pigs had good food consumption and growth in spite of the low protein diets. The implication of this is that early exposure of animals to infection results in stunting with the possibility that these animals might not attain the inherited size of the species even with prolonged periods of adequate feeding (Schultze, 1955).

The data are essentially in agreement with the clinical data of Collis and Janes (1967), Behar (1967) and Mata *et al.* (1967), which indicate that children in developing countries with infectious diarrhea caused by entero- and adenoviruses, *Giardia*, and hookworms fail to grow. In addition, the data corroborate the report of Ogbeide (1968) that anorexia and retardation of growth caused by infection may contribute to the precipitation of marasmus in children.

Clinical Signs

Vomition, depression, diarrhea, and distention of the gastrointestinal tract with gas are consistent with the signs of TGE virus infection (Doyle and Hutchings, 1946; Hopper and Haelterman 1966). Edema and skin lesions, observed in infected pigs fed either the high or low fat, 6% soyprotein diet are similar to those reported in children with kwashiorkor (Jelliffe, 1959).

Hemoglobin and Packed Cell Volume

The hemoglobin and packed cell volume of protein-calorie malnourished pigs were within normal range for well nourished pigs (Miller *et al.*, 1961a). The values of hemoglobin and packed cell volume of the 6% soyprotein and high fat fed pigs infected with TGE virus were also within normal range (Miller *et al.*, 1961b). In contrast, however, the decreased hemoglobin and packed cell volume of pigs fed 6% soyprotein and low fat was consistent with the data of Keahey (1963). Hemoconcentration (Schalm, 1965) may explain, at least in part, the absence of a decrease in hemoglobin and packed cell volume in the infected pigs fed the high fat diet.

Serum Proteins

Serum total protein and albumin values in pigs fed high or low fat, 6% soyprotein were lower than values for pigs of about the same age, established by Miller *et al.* (1961a). The decrease in serum total protein agrees with the findings of Dean and Schwartz (1953) in cases of kwashiorkor in children. There was an increase in beta, gamma, and alpha globulins in the infected pigs fed 6% soyprotein and high or low fat diets. Of particular interest is the significant increase in alpha globulin in the high and low fat fed infected pigs. These data are in agreement with that of Platt and Heard (1965), Keahey (1963) and Williams and Newberne (1970). The increase in plasma albumin in the high or low fat fed infected pigs could be due to dehydration. However, albumin synthesis occurs in the liver (Rothschild *et al.*, 1969) and infection which enhances protein synthesis (Williams and Newberne, 1970) could result in a greater output of albumin to the plasma.

Serum Sodium and Potassium

The values for serum potassium and sodium of uninfected protein-calorie malnourished pigs were within normal values, as summarized by Coles (1967). However, infection decreased serum potassium and sodium values in the infected pigs fed high or low fat and 6% soyprotein. The corollary to this data may be that severe depression of serum potassium and sodium in protein-calorie malnutrition (Kahn, 1959) occurs in the face of complicating infection. Reber and Whitehair (1953) did not find decreased serum sodium in TGE infected pigs, although there were heavy fecal losses. However, their pigs were well nourished when they were infected.

Vitamin A

The vitamin A values in the liver of uninfected pigs fed 6% soyprotein and high or low fat diets were lower than those of well fed pigs reported by Maronpot (1965). Furthermore, vitamin A values in the liver were lower in infected pigs fed high or low fat and 6% soyprotein than uninfected pigs fed the same diet. The data are consistent with the fact that low protein diets and infection act synergistically to lower vitamin A values in the liver (Faraque and Walker, 1970; Kanai *et al.*, 1968; Olson, 1969; Arroyave *et al.*, 1959). However, Maronpot (1965), using a few pigs, found increased vitamin A values in the liver in the presence of TGE infection. This could be due to the fact that his experimental pigs were adequately nourished when they were infected.

Histologic Lesions

Villous atrophy was marked in infected pigs fed low fat and 6% soyprotein diet. However, villous atrophy was not noted in the uninfected pigs fed these rations. These data are at variance with that of Platt *et al.* (1964), who reported villous atrophy in uninfected protein deficient pigs. However, in their experiments the pigs were fed protein deficient diets for a longer time.

Periportal fatty changes in the liver of uninfected pigs were consistently present in both high or low fat and 6% soyprotein diets. However, high or low fat fed pigs on 6% soyprotein and infected with TGE virus had fatty metamorphosis involving the whole lobule characteristic of the liver of children with kwashiorkor (Jelliffe, 1959). The decrease in the zone of maturing cartilage found in infected pigs fed high or low fat and 6% soyprotein and infected with TGE virus was reported in children with kwashiorkor (Higginson, 1964), and might partially explain

the further retardation of growth in the face of infection in subclinical protein-calorie malnutrition.

Glial nodules in the thalamic region of the brain were greater in number in the infected pigs than in the uninfected pigs, regardless of diet. These data corroborate those of Stewart and Platt (1967), who reported increased glial cell population in spinal cord of pigs on low protein diets. Mental retardation may be a sequela of protein-calorie malnutrition in children (Cravioto, 1967). However, the significance of glial nodules in central nervous function is not precisely known. The marked atrophy of the pancreas, the depletion of mature lymphocytes in the spleen, and the parakeratosis with pustule formation in the skin, in infected pigs fed either high or low fat diets and 6% soyprotein, have been reported to be features of protein-calorie malnutrition in children (Jelliffe, 1959).

Adrenal: Histology and Ultrastructure

The adrenal gland plays an important role in mammalian protein, calorie and electrolyte metabolism. This organ, therefore, plays a vital role in the control of homeostasis in the face of stress. The combination of malnutrition and infection is more than an additive stressful condition to an animal.

The presence of an increased lipid content in the cells of the zona fasciculata in the uninfected pigs fed high or low fat and low protein diets may be due to the reduction in the secretion of adrenocorticotrophic hormone by the pituitary (Munro *et al.*, 1962). This results in a decreased stimulation of the adrenal gland; hence the presence of excess lipid content. However, the marked depletion of lipids in the zona fasciculata in the infected pigs fed high or low fat and low protein

might be due to excess ACTH stimulation induced by acute infections (Wilbur and Rich, 1953; Redmond and Koysto, 1970).

The presence of tubulosaccular mitochondria of the adrenal gland of the domestic pig is consistent with mammalian adrenal ultrastructure (Zealander, 1959). Triple complexes consisting of mitochondria, myelin-like bodies and dense bodies; double complexes comprising mitochondria and lipids; vesicular nature of the smooth endoplasmic reticulum; and hypertrophy and hyperplasia of mitochondria in the infected pigs fed high or low fat and 6% soyprotein have been reported (Idelman, 1970; Yoshimura *et al.*, 1968) to be characteristic features of the mammalian adrenal gland under excessive ACTH stimulation. Myelin-like bodies or concentric lamellar formations, noted in the infected pigs fed the low protein diet, have been reported to arise from the endoplasmic reticulum (Nickerson and Curtis, 1969). Myelin-like bodies may result from proliferation of the smooth endoplasmic reticulum involved in the synthesis of glucocorticoids (Garren, 1968), and probably indicate increased steroid production (Davis and Medline, 1970) in the infected pigs fed high or low fat and low protein diets. However, Nickerson and Curtis (1969) speculated that myelin figures are degenerative products of infection. Increase in lysosomes in the infected pigs fed high or low fat and low protein diets may also indicate excess ACTH stimulation (Szabo *et al.*, 1967). Lysosomes selectively accumulate precursors of cholesterol and adrenocorticosteroids (Dietert and Scallen, 1969). The degenerative changes of the smooth endoplasmic reticulum seen in a few of the infected pigs fed protein-calorie deficient diets may be the result of excessive ACTH stimulation which ultimately leads to adrenal exhaustion (Wilbur and Rich, 1953). The presence of membrane lined structures consisting of mitochondria, dilated smooth endoplasmic

reticulum and lipid-like droplets in the precapillary space could be morphological expressions of possible discharge of steroid hormones into this space - a process sometimes called endopinocytosis (Idelman, 1970).

The histologic and ultrastructural changes of the adrenals in the pigs under the double jeopardy of protein-calorie malnutrition and infection indicate overactive glands. Hyperactive adrenal glands have been reported in children with marasmus (Castellanos and Arroyave, 1961).

Plasma Amino Acids

The decrease in plasma leucine, valine, tyrosine and threonine in the preinfection samples of pigs fed high or low fat and low protein diets for a period of 3 weeks is similar to the decrease of these amino acids in children with kwashiorkor (Saunders *et al.*, 1967; Anasanya and Rao, 1968; Dean and Whitehead, 1964). Furthermore, the significant increase of plasma glycine, serine and alanine values in pigs fed high fat diets and the increase of plasma serine, glutamic acid and alanine in the pigs fed low fat diets for the same period corroborates the data of Saunders *et al.* (1967) and Snyderman *et al.* (1968) in children with kwashiorkor and Grimble and Whitehead (1970) in pigs fed low protein diets. The data on plasma free amino acids of TGE infected pigs allowed to nurse the sow indicate increased utilization of essential amino acids during infection as these amino acids were significantly decreased from the plasma pool of amino acids. Of the essential amino acids, only arginine in the pigs fed low fat and low protein diets decreased significantly 72 hours postinfection. Arginine appears to be particularly sensitive to the stress of infection in protein-calorie malnourished pigs. Arginine may be utilized maximally in increased protein synthesis (Williams and Newberne, 1970) which results from infection. With severe infectious

diarrhea, the essential amino acids, valine, methionine, isoleucine and arginine, were significantly depressed. Infection and protein deficiency appear to act synergistically to depress the plasma pool of amino acids. However, infection appeared to have no effect and in some animals increased the plasma phenylalanine in the protein-calorie malnourished pigs. Williams and Newberne (1970) noted increased phenylalanine values in dogs infected with *Salmonella typhimurium* and Laudau and Lugibihl (1969) reported no changes in plasma phenylalanine values in glucagon administration. The reason for the abnormal metabolism of phenylalanine values is not known. However, Whitehead and Dean (1964) reported in children with kwashiorkor, decreased catabolism of phenylalanine was due to decrease in phenylalanine hydroxylase enzyme. Infection might put extra strain on an already existing abnormality in phenylalanine metabolism.

Of the nonessential amino acids, serine, alanine and glycine in infected pigs fed high fat diets and aspartic acid in the low fat diet were decreased significantly in 72 hours. The decrease in serine, alanine, glutamic acid and proline became more marked with the severity of diarrhea in the pigs fed high fat diets. The amino acids, alanine and glycine, glutamic acid (Potter *et al.*, 1968) and proline (Ray, 1968) may be used as substrates in the process of gluconeogenesis which is enhanced during infection (Scrimshaw, 1968). The infection decreased the nonessential to essential amino acid ratio. An increased ratio is reported to be a sensitive indication of protein-calorie malnutrition (Dean and Whitehead, 1964). This is probably because infection causes greater depression of the nonessential amino acids, particularly glycine, alanine, glutamic acid and proline, than the essential amino acids. Since the glycine to valine ratio is consistently raised both in the uninfected

and infected pigs, this ratio may be used to monitor subclinical and clinical protein-calorie malnutrition. The elevation of the glycine to valine ratio has also been reported in children with protein-calorie malnutrition (Snyderman *et al.*, 1968). The alanine to arginine ratio, raised markedly during infection, could be used to monitor complications of infection in protein-calorie malnutrition. Furthermore, the phenylalanine to tyrosine ratio, known to be elevated during infection (Williams and Newberne, 1970), was not as greatly increased as the alanine to arginine ratio.

Summary

The data are consistent with the hypothesis that the stress of infection stimulates the higher centers of the brain, especially the hypothalamus, resulting in marked output of adrenocorticotrophic hormone. The histologic and ultrastructural changes in the adrenal cortex corroborate this concept. As a result of the marked stimulation of the adrenal cortex by ACTH, there is an increased production of glucocorticoids involved in mobilizing amino acids from peripheral tissues, particularly muscles. The amino acids may be transferred to the liver where glucocorticoids enhance protein synthesis, in particular, the gluconeogenic enzymes. In addition, alanine, serine, glutamic acid and proline may be channeled by glucocorticoids to the path of gluconeogenesis in the liver, thus lowering these amino acids in the plasma. Furthermore, infectious diarrhea may interfere with the absorption of, and enhance the excretion of, amino acids. Retardation of growth resulting from infection may be due to the diversion of amino acids to gluconeogenesis and catabolism. Of equal importance are changes of sodium, potassium and vitamin A metabolism in infection. Further

studies in the interrelationship between nutrition and infection might provide the needed answers to the host-parasite relationship and mammalian metabolic regulation.

SUMMARY

Protein-calorie malnutrition (PCM) is a serious nutritional disease in children in many areas of the world, particularly in the developing countries of Africa, Asia, and Latin America. However, under field conditions, protein-calorie malnutrition is believed to be a complex of malnutrition and infection. Three experiments, utilizing 35 pigs, were conducted to elucidate the role played by infectious diarrhea, caused by the transmissible gastroenteritis (TGE) virus, in precipitating protein-calorie malnutrition in baby pigs.

The pigs were divided into 6 groups. Pigs in Group I were fed 6% soyprotein and 30% fat (high fat) diet; and pigs in Group II were fed the same level of protein but 5% fat (low fat). Pigs in Groups II and IV were fed the same diet as pigs in Groups I and II, respectively, and served as uninfected controls. Pigs in Groups I and II were infected with the TGE virus orally after they had consumed the diet for 3 weeks. Pigs in Group V were allowed to nurse the sow and served as uninfected well-nourished controls. A sixth group of pigs was allowed to nurse the sow and was infected orally with TGE virus at 4 weeks of age.

The effect of enteric infection on the protein-calorie malnourished pigs was evaluated by the parameters of food consumption, growth rate, clinical signs, hematology, gross and microscopic lesions, plasma amino acid pattern, serum electrophoretic values, plasma potassium and sodium

values, vitamin A content in the liver, and ultrastructural changes in the zona fasciculata of the adrenal gland.

TGE virus infection in pigs resulted in a decrease in feed consumption, diarrhea, apathy, and a retardation in growth in comparison to the controls.

There was a significant decrease in hemoglobin and packed cell volume in the infected as compared to uninfected control pigs fed the same diet. Enteric infection induced a significant decrease in plasma potassium and sodium values compared to uninfected control pigs on similar diets. The alpha globulin was significantly raised in the presence of an enteric infection. Furthermore, there was a marked decrease in the values of vitamin A in the liver in the infected pigs.

Lesions of protein-calorie malnutrition included subcutaneous edema, fatty liver, parakeratosis of the skin and severe emaciation. The lesions were most marked in the infected pigs fed the deficient rations. In addition, the TGE virus infection was characterized by jejunal and ileal atrophy of the villi.

Plasma amino acid analysis revealed a decrease of isoleucine, alanine, valine, arginine, glutamic acid, proline, glycine, serine and methionine with time in the pigs infected with the TGE virus when compared to the uninfected pigs fed the same diets. However, plasma lysine, histidine and phenylalanine values did not change due to infection. The nonessential to essential amino acid ratio decreased with time in the infected pigs. However, the ratio of nonessential to essential amino acids increased with time in the uninfected pigs fed the same diet. The glycine to valine ratio increased in both infected and in uninfected pigs. The alanine to arginine ratio was higher in the infected than uninfected pigs fed the same diet.

The adrenal gland of infected, protein-calorie malnourished pigs had evidence of hyperactivity characterized ultrastructurally by depletion of lipid, vesicular appearance of endoplasmic reticulum, the presence of triple or double complexes comprising mitochondria, lipid droplets and "myelin bodies." An enteric infection superimposed on malnourished animals would appear to be more than just additive.

REFERENCES

REFERENCES

Adams, E. B., J. N. Scragg, B. J. Naidoe, S. K. Liljestrand, and V. I. Cockram: Observations on the aetiology and treatment of anaemia of kwashiorkor. Brit. Med. J., 3, (1967): 451-454.

Anasuya, A., and B. S. N. Rao: Plasma amino acid pattern in kwashiorkor and marasmus. Amer. J. Clin. Nutr., 21, (1968): 723-732.

Armed Forces Institute of Pathology: *Manual of Histologic and Special Staining Technics*. Washington, D.C., 1957.

Arroyave, G., F. Viteri, M. Behar, and N. S. Scrimshaw: Impairment of intestinal absorption of vitamin A palmitate in severe protein malnutrition (kwashiorkor). Am. J. Clin. Nutr., 7, (1959): 185-190.

Arroyave, G., D. Wilson, J. Mendez, M. Behar, and N. S. Scrimshaw: Serum and liver vitamin A and lipids with severe protein malnutrition. Am. J. Clin. Nutr., 9, (1961): 180-185.

Axelrod, A. E.: The role of nutritional factors in the antibody responses of the anamnestic process. Am. J. Clin. Nutr., 6, (1958): 119-125.

Bay, W. W., L. M. Hutchings, L. P. Doyle and D. E. Bunnell: Transmissible gastroenteritis in baby pigs. J.A.V.M.A., 115, (1949): 245-248.

Bay, W. W., L. P. Doyle and L. M. Hutchings: The pathology and symptomatology of transmissible gastroenteritis. Am. J. Vet. Res., 12, (1951): 215-218.

Behar, M.: Prevalence of malnutrition among pre-school children of developing countries. In *Malnutrition, Learning and Behavior*. Edited by N. S. Scrimshaw and J. E. Gordon. M.I.T. Press, Cambridge, Mass., 1967. pp. 30-41.

Benjamin, M. E.: *Outline of Veterinary Clinical Pathology*. The Iowa State University Press, Ames, Iowa, 1961.

Boomgaardt, J., and B. E. McDonald: Comparison of fasting plasma amino acid pattern in the pig, rat and chicken. Can. J. Phsyiol. Pharmacol., 47, (1969): 392-395.

Carle, B. N., and W. H. Dewhirst: A method for bleeding swine. J.A.V.M.A., 101, (1942): 495-496.

Castellanos, H., and G. Arroyave: Role of the adrenal cortical system in the response of children to severe protein malnutrition. Am. J. Clin. Nutr., 9, (1961): 187-195.

Chanda, N.: Pathological study of the liver in kwashiorkor. Brit. Med. J., 1, (1958): 1263.

Cohen, S., and J. D. L. Hansen: Metabolism of albumin and gamma globulin in kwashiorkor. Clin. Sci., 23, (1962): 351-359.

Coles, B. L.: Serum protein pattern in infants with nutritional disorders. Arch. Dis. Child., 35, (1960): 271-274.

Coles, E. B.: *Veterinary Clinical Pathology*. W. B. Saunders, Philadelphia, Pa., 1967.

Collis, W. R. F., and M. Janes: Multifactorial causation of malnutrition and retarded growth and development. In *Malnutrition, Learning and Behavior*. Edited by N. S. Scrimshaw and J. E. Gordon. M.I.T. Press, Cambridge, Mass., 1967, pp. 55-71.

Crampton, E. W., and O. M. Ness: A meal mixture suitable as the entire ration to be self-fed dry to pigs weaned at ten days of age. J. Animal Sci., 13, (1954): 357-364.

Cravioto, J., and E. R. DeLicardie: Intersensory development of school-age children. In *Malnutrition, Learning and Behavior*. Edited by N. S. Scrimshaw and J. E. Gordon. M.I.T. Press, Cambridge and London, 1967, pp. 252-269.

Davis, A. D., and N. M. Medline: Spironolactone (aldactone) bodies: concentric lamellar formation in the adrenal cortices of patients treated with spironolactone. Am. J. Clin. Path., 54, (1970): 22-32.

Dean, R. F. A., and R. Schwartz: The serum chemistry in uncomplicated kwashiorkor. Brit. J. Nutr., 7, (1953): 131-146.

Dean, R. F. A., and R. G. Whitehead: Plasma aminogram in kwashiorkor. Lancet, II, (1964): 98-99.

Deo, M. G., S. K. Sood and V. Ramalingaswami: Experimental protein deficiency: Pathologic features in the rhesus monkey. Arch. Pathol., 80, (1965): 14-23.

Deshmukh, D. S., and J. Ganguly: Effect of dietary protein contents on the intestinal conversion of beta-carotene to vitamin A in rats. Indian J. Biochem., 1, (1964): 204-207.

Dietert, S. E., and T. J. Scallen: An ulctrastructural and biochemical study of the effects of three inhibitors of cholesterol biosynthesis upon murine adrenal gland and testis. Histochemical evidence for a lysosome response. J. Cell Biol., 40, (1969): 44-58.

Donoso, G., O. Brunser and F. Monckeberg: Metabolism of serum albumin in marasmic infants. J. Pediat., 67, (1965): 306-311.

Doyle, L. P., and L. M. Hutchings: A transmissible gastroenteritis in pigs. J.A.V.M.A., 108, (1946): 257-259.

Edozien, J. C., and M. E. Obasi: Protein and amino acid metabolism in kwashiorkor. Clin. Sci., 29, (1965): 1-24.

Edozien, J. C., E. J. Phillips and W. R. F. Collis: The free amino acids of plasma and urine in kwashiorkor. Lancet, I, (1960): 615-618.

Edozien, J. C.: Experimental kwashiorkor and marasmus. Nature, 220, (1968): 917-919.

Farague, O., and D. M. Walker: Vitamin A and protein interrelationships in the milk-fed lamb. British J. Nutr., 24, (1970): 11-22.

Follis, R. H.: A kwashiorkor-like syndrome observed in monkeys fed maize. Proc. Soc. Exp. Biol. Med., 96, (1957): 523-528.

Frobish, L. T., V. W. Hays, V. C. Speer and R. C. Ewan: Effect of fat source and level on utilization of fat by young pigs. J. Animal Sci., 30, (1970): 197-202.

Garren, L. D.: The mechanisms of action of adrenocorticotropic hormone. Vitamins and Hormones, 26, (1968): 119-145.

Garrow, J. S.: Total body potassium in kwashiorkor and marasmus. Lancet, II, (1965): 455-458.

Garrow, J. S.: Loss of brain potassium in kwashiorkor. Lancet, II, (1967): 643-645.

Goodman, D. S., H. S. Huang, M. Kanai, and T. Shiratori: The enzymatic conversion of all-trans beta-carotene into retinal. J. Biol. Chem., 242, (1967): 3543-3554.

Gordon, J. E., I. D. Chitkara and J. B. Wyon: Weanling diarrhea. Am. J. Med. Sci., 245, (1963): 345-377.

Grimble, R. F., and R. G. Whitehead: The relationship between elevated serum amino acid ratio and the development of other biological abnormalities in the experimentally malnourished pig. Brit. J. Nutr., 23, (1969): 791-804.

Grimble, R. F., and R. G. Whitehead: Changes in the concentration of specific amino acids in the serum of experimentally malnourished pigs. Brit. J. Nutr., 24, (1970): 557-564.

Guzman, M. A.: Impaired physical growth and maturation in malnourished populations. In *Malnutrition, Learning and Behavior*. Edited by N. S. Scrimshaw and J. E. Gordon. M.I.T. Press, Cambridge and London, 1967, pp. 42-54.

Haelterman, E. O., and B. E. Hooper: Transmissible gastroenteritis of swine as a model for the study of enteric disease. Gastroenterol., 53, (1967): 109-113.

Hendrickse, R. G.: Interactions of nutrition and infection: Experience in Nigeria. In *Nutrition and Infection*. Edited by G. E. W. Wolstenholme. CIBA Foundation Study Group No. 31, Little, Brown and Co., Boston, 1967, pp. 98-111.

Higginson, J.: Studies on human bone from South African Bantu subjects. II. Histopathological changes in the ribs of South African infants. Metabolism, 3, (1964): 392-399.

Hooper, B. F., and E. O. Haelterman: Growth of transmissible gastroenteritis virus in young pigs. Am. J. Vet. Res., 27, (1966): 286-291.

Idelman, S.: Ultrastructure of the mammalian adrenal cortex. International Review of Cytology, 27, (1970): 181-281.

Jelliffe, D. B.: Protein-calorie malnutrition in tropical pre-school children. A review of recent knowledge. J. Pediat., 54, (1959): 227-256.

Jensen, A. H., D. E. Becker, H. W. Norton and S. W. Terris: Protein requirements for pigs weaned at two weeks of age. J. Animal Sci., 16, (1957): 389-395.

Joint FAO/WHO Expert Committee on Nutrition. Report of 6th Session. WHO Tech. Rep. Ser. No. 245, 1962.

Kahn, E.: Prognostic criteria of severe protein malnutrition. Am. J. Clin. Nutr., 7, (1959): 161-165.

Kanai, M., A. Raz, and D. S. Goodman: Retinol-binding protein: The transport protein for vitamin A in human plasma. J. Clin. Invest., 47, (1968): 2025-2044.

Keahey, K. K.: Pathology of Protein Malnutrition and Infection. Ph.D. Thesis, Michigan State University, East Lansing, 1963.

Kirsch, R. E., J. F. Brock, and S. J. Saunders: Experimental protein-calorie malnutrition. Am. J. Clin. Nutr., 21, (1968): 820-826.

Landau, R. L., and K. Lugibihl: Effect of glucogon on concentration of several free amino acids in plasma. Metabolism, 18, (1969): 265-275.

Lanzkowsky, P., D. McKenzie, S. Katz, R. Hoffenberg, R. F. Friedman and E. Black: Eruthrocyte abnormality induced by protein malnutrition. II. ^{51}Chromium labelled erythrocyte studies. Brit. J. Haemat., 13, (1967): 639-649.

Lee, K. M., M. Moro, and J. A. Baker: Transmissible gastroenteritis in pigs. Am. J. Vet. Res., 15, (1954): 364-372.

Leung, P. M. B., A. R. Rogers, and A. E. Harper: Effect of cortisol on growth, food intake, dietary preference and plasma amino acid pattern of rats fed amino acid imbalanced diets. J. Nutr., 96, (1968): 139-151.

Lowrey, R. S., W. G. Pond, R. H. Barnes, L. Krook and J. K. Loosli: Influence of caloric level and protein quality on the manifestations of protein deficiency in the young pig. J. Nutr., 78, (1962): 245-253.

Luft, J.: Improvements in epoxy resin embedding methods. J. Biophys. and Biochem. Cytol., 9, (1961): 409-414.

Lyman, R. L., C. R. Cook and M. A. Williams: Liver lipid accumulation in isoleucine-deficient rats. J. Nutr., 82, (1965): 432-438.

Maegraith, B. G.: Interaction of nutrition and infection. In *Nutrition and Infection.* Edited by G. E. W. Wolsten and M. O'Connor. Little, Brown and Co., Boston, Mass., 1967, pp. 41-55.

deMan, J. M., and J. P. Bowland: Fatty acid composition of sow's colostrum, milk and body fat as determined by gas-liquid chromatography. J. Dairy Res., 30, (1963): 339-343.

Maronpot, R. R.: *The Influence of a Viral Enteric Infection on Carotene Metabolism.* Master's Thesis, Michigan State University, East Lansing, 1965.

Maronpot, R. R., and C. K. Whitehair: Experimental sprue-like small intestinal lesions in pigs. Canad. J.Comp. Med. and Vet. Sci., 31, (1967): 309-316.

Mata, L. J., J. J. Urrutia and B. Garcia: Effect of infection and diet on child growth: Experience in a Guatemalan village. In *Nutrition and Infection.* Edited by G. E. W. Wolstenholme and M. O'Connor. Little, Brown and Co., Boston, Mass., 1967, pp. 112-126.

McLaren, D. S.: Vitamin deficiencies complicating the severe forms of protein-calorie malnutrition, with special reference to vitamin A. In *Calorie Deficiencies and Protein Deficiencies.* Edited by R. A. McCance and E. M. Widdowson. Little, Brown and Co., Boston, Mass., 1968, pp. 191-201.

Miller, E. R., D. E. Ullrey, I. Ackerman, D. A. Schmidt, J. A. Hoefer and R. W. Luecke: Swine hematology from birth to maturity. I. Serum proteins. J. Animal Sci., 20, (1961a): 31-35.

Miller, E. R., D. E. Ullrey, I. Ackerman, D. A. Schmidt, R. W. Luecke, and J. A. Hoefer: Swine hematology from birth to maturity. II. Erythrocyte population size and hemoglobin concentration. J. Animal Sci., 20, (1961b): 890-897.

Munro, H. N., W. C. Hutchison, T. R. Ramaiah and F. J. Neilson: The influence of diet on the weight and chemical constituents of the rat adrenal gland. Brit. J. Nutr., 16, (1962): 387-395.

Needld, J. B., and W. N. Pearson: Macro and micromethod for the determination of serum vitamin A using trifluoroacetic acid. J. Nutr., 79, (1963): 454-462.

Nickerson, P. A., and J. C. Curtis: Concentric whorls of rough endoplasmic reticulum in adrenocortical cells of the Mongolian gerbil. J. Cell Biol., 40, (1969): 859-862.

Ogbeide, M. I.: The influence of protein-calorie malnutrition on the pattern of preschool childhood diseases. Observations in Lagos, Nigeria. Clin. Ped., 7, (1968): 425-431.

Olson, J. A.: The metabolism of vitamin A. Pharmacol. Rev., 19, (1967): 559-596.

Olson, J. A.: Metabolism and function of vitamin A. Fed. Proc., 28, (1969): 1670-1677.

Oomen, H. A. P. C.: Hypovitaminosis A. III. Clinical experience on hypovitaminosis A. Fed. Proc., 17, (1958): 111-124.

Phillips, P. H., and E. B. Hart: The effect of organic dietary constituents upon chronic fluorine toxicosis in the rat. J. Biol. Chem., 109, (1935): 657-663.

Platt, B. S.: Protein malnutrition and infection. Am. J. Trop. Med. and Hyg., 6, (1957): 773-779.

Platt, B. S., C. R. C. Heard, and R. J. C. Stewart: Experimental protein-calorie deficiency. In *Mammalian Protein Metabolism*, Vol. II. Edited by H. N. Munro and J. B. Allison. Academic Press, New York, 1964, pp. 466-514.

Platt, B. S., G. R. D. Heard and R. J. C. Stewart: The effects of protein-calorie deficiency on the gastrointestinal tract. In *The Role of the Gastrointestinal Tract in Protein Metabolism.* Edited by H. N. Munro. Blackwell Scientific Publishers, Oxford, 1964b, pp. 227-238.

Platt, B. S., and C. R. C. Heard: The contribution of infections to protein-calorie deficiency. Trans. Roy. Soc. Trop. Med. and Hyg., 59, (1965): 571-581.

Pond, W. G., R. H. Barnes, I. Reid, L. Krook, E. Kwong and A. U. Moore: Protein deficiency in baby pigs. In *Swine in Biomedical Research.* Edited by L. K. Bustad and R. O. McClellan. Frayn Co., Seattle, Wash., 1966, pp. 213-224.

Porta, E. A., and W. S. Hartroft: Protein deficiency and liver injury. Am. J. Clin. Nutr., 23, (1970): 447-461.

Potter, E. L., D. B. Purser and J. H. Cline: Effect of various energy sources upon plasma free amino acids in sheep. J. Nutr., 95, (1968): 655-663.

Ramalingaswami, V., and M. G. Deo: Experimental protein-calorie malnutrition in the rhesus monkey. In *Calorie Deficiencies and Protein Deficiencies*. Edited by R. A. McCance and E. M. Widdowson. Little, Brown and Co., Boston, Mass., 1968, pp. 265-275.

Rapaport, S., K. Dodd, M. Clark, and I. Syllm: Postacidotic state of infantile diarrhea. Symptoms and chemical data. Postacidotic hypocalcemia and associated decreases in levels of potassium, phosphorus and phosphatases in the plasma. Am. J. Dis. Child., 73, (1947): 391-441.

Ray, P. D.: Adrenal glucocorticoids and gluconeogenesis. In *Functions of the Adrenal Cortex*. Edited by K. W. McKerns. Appleton Century Crofts, New York, 1968, pp. 1137-1176.

Reber, E. F., C. K. Whitehair, and R. MacVicar: The effect of level of protein fed baby pigs. J. Nutr., 50, (1953): 451-458.

Redmond, A. F., and J. L. Koysto: Acute effects of adrenocorticotropin on the levels of various lipids in the adrenals of hypophysectomized rats. Endocrinol., 86, (1970): 440-443.

Richardson, L. R., F. Hale, and S. J. Ritchie: Effect of fasting and level of dietary protein on free amino acids in pigs plasma. J. Animal Sci., 24, (1965): 368-372.

Rothschild, M. A., M. Oratz and S. S. Schreiber: Serum albumin. Am. J. Digestive Dis., 14, (1969): 711-744.

Sabatini, D. D., and E. D. P. DeRobertis: Ultrastructural zonation of adrenocortex in the rat. J. Biophys. Biochem. Cytol., 9, (1961): 105-119.

Saunders, S. J., A. S. Truswell, G. O. Barbetzat, W. Wittman, and J. D. L. Hansen: Plasma free amino acid pattern in protein-calorie malnutrition. Lancet, II, (1967): 795-797.

Schalm, O. W.: *Veterinary Hematology*. Bailliere Tindall and Cassell. Second edition, London, 1965.

Schultze, M. O.: Effects of malnutrition in early life on subsequent growth and reproduction of rats. J.Nutr., 56, (1955): 25-33.

Scrimshaw, N. S., M. Behar, G. Arroyave, F. Viteri, and C. Tejada: Characteristics of kwashiorkor. Fed. Proc., 15, (1956): 977-985.

Scrimshaw, N. S.: Malnutrition and infection. Borden's Review of Nutrition Research, 26, (1965): 17-29.

Scrimshaw, N. S., C. G. Taylor and J. E. Gordon: *Interactions of Nutrition and Infection*. World Health Organization, Geneva, Switzerland, 1968.

Senecal, J.: Treatment and prevention of kwashiorkor. Ann. N. Y. Acad. Sci., 69, (1958): 916-953.

Shank, R. E., A. F. Coburn, L. V. Moore, and G. L. Hoagland: The level of vitamin A and carotene in the plasma of rheumatic subjects. J. Clin. Invest., 23, (1944): 289-295.

Sidransky, H., and T. Baba: Chemical pathology of acute amino acid deficiencies, morphologic and biochemical changes in young rats fed valine or lysine devoid diets. J. Nutr., 70, (1960): 463-471.

Sidransky, H.: Chemical pathology of nutritional deficiency induced by certain plants. J. Nutr., 71, (1960): 387-395.

Sidransky, H., and E. Verney: Chemical pathology of acute amino acid deficiencies: Morphologic and biochemical changes in young rats force fed a threonine-deficient diet. J. Nutr., 96, (1968): 349-358.

Snyderman, S., L. E. Holt, P. M. Norton, E. Roitman, and S. V. Phansalker: The plasma aminogram. I. Influence of the level of protein intake and comparison of whole protein and amino acid diets. Pediat. Res., 2, (1968): 131-144.

Salomon, J. B., L. J. Mata, and J. E. Gordon: Malnutrition and the common communicable diseases of childhood in rural Guatemala. Am. J. Pub. Health, 58, (1968): 505-516.

Solomon, G. B.: Host hormones and parasitic infection. International Rev. Trop. Med., 3, (1969): 101-158.

Sood, S. K., M. G. Deo and V. Ramalingaswami: Anemia in experimental protein deficiency in the rhesus monkey with special reference to iron metabolism. Blood, 26, (1965): 421-432.

Squibb, R. L.: Nutrition and biochemistry of survival during Newcastle disease virus infection. II. Relation of clinical symptoms and starvation to nucleic and free amino acids of avian liver. J. Nutr., 83, (1964): 423-426.

Squibb, R. L.: Nutrition and biochemistry of survival during Newcastle disease virus infection. III. Relation of dietary protein to nucleic and free amino acids of avian liver. J. Nutr., 82, (1964): 427-431.

Stewart, R. J. C., and B. S. Platt: Nervous system damage in experimental protein-calorie deficiency. In *Malnutrition, Learning and Behavior*. Edited by N. S. Scrimshaw and J. E. Gordon. M.I.T. Press, Cambridge, Mass., 1967, pp. 168-180.

Svoboda, D., H. Grady, and J. Higginson: The effects of chronic protein deficiency in rats. II. Biochemical and ultrastructural changes. Lab. Invest., 15, (1966): 731-749.

Szabo, D., E. Stark and B. Varga: The localization of acid phosphatase activity changes in lysosomes in the adrenal zona fasciculata of intact and hypophysectomized rats following ACTH administration. Histochemie, 10, (1967): 321-328.

Tejada, C., and R. B. Russfield: Preliminary report on the pathology of the pituitary gland in children with malnutrition. Arch. Dis. Child., 32, (1957): 343-346.

Thake, D. C.: Jejunal epithelium in transmissible gastroenteritis of swine. An electron microscope and histochemical study. Am. J. Path., 53, (1968): 149-168.

Tiong, Y. K.: Protein deficiency in keratomalacia. Brit. J. Ophth., 40, (1956): 502-503.

Trapp, A. L., V. L. Sanger and E. Stalnaker: Lesions of the small intestinal mucosa in transmissible gastroenteritis infected germ-free pigs. Am. J. Vet. Res., 27, (1966): 1695-1702.

Truswell, A. S., P. Wannenburg, W. Wittmann and J. D. L. Hansen: Plasma amino acids in kwashiorkor. Lancet, I, (1966): 1162-1163.

Vahlquist, B.: Malnutrition and infection in Ethiopia. In *Nutrition and Infection*. Edited by G. E. W. Wolstenholme. CIBA Foundation Study Group, No. 31, Little, Brown and Co., Boston, Mass., 1967, pp. 90-96.

Veen, M. J., and G. H. Beaton: Vitamin A transport in the rat. Can. J. Physiol. Pharmacol., 44, (1966): 521-527.

Viteri, F. E., J. Alvarado, D. G. Luthringer, and R. P. Wood: Hematological changes in protein-calorie malnutrition. Vitamins and Hormones, 26, (1968): 573-615.

Waterlow, J. C., and V. G. Wills: Balance studies in malnourished Jamacican infants. I. Absorption and retention of nitrogen and phosphorus from human milk and a cow's milk mixture. Brit. J. Nutr., 14, (1960): 199-205.

Waterlow, J. C.: Observations on the mechanism of adaptation to low protein intakes. Lancet, II, (1968): 1090-1097.

Whitehair, C. K., R. H. Grummer, P. H. Phillips, G. Bohstedt and S. H. McNutt: Gastroenteritis in pigs. Cornell Vet., 38, (1948): 24-39.

Whitehair, C. K., A. B. Watts, and O. B. Ross: Urea N.P.N. and blood sugar values of baby pigs exposed to transmissible gastroenteritis. J. Animal Sci., 9, (1950): 672.

Whitehead, R. G., and R. E. A. Dean: Serum amino acids in kwashiorkor. I. Relationship to clinical conditions. Am. J. Clin. Nutr., 14, (1964): 313-319.

Whitehead, R. G.: Biochemical changes in kwashiorkor and marasmus. In *Calorie Deficiencies and Protein Deficiencies*. Edited by R. A. McCance and E. M. Widdowson. Little, Brown and Co., Boston, Mass., 1968, pp. 109-117.

Widdowson, E. M., and J. W. T. Dickerson: The effect of growth and function on the chemical composition of soft tissues. Biochem. J., 77, (1960): 30-43.

Wilbur, O. M., and A. R. Rich: A study of the role of adrenocorticotropic hormone (ACTH) in the pathogenesis of tubular degeneration of the adrenals. Bull. Johns Hopkins Hosp., 93, (1953): 321-335.

Williams, C. D.: A nutritional disease of childhood associated with maize diet. Arch. Dis. Child., 8, (1933): 423-433.

Williams, P. H., and V. L. Tsvetkov: Bilateral spontaneous expulsion of the lens in a case of kwashiorkor. J. Trop. Med. and Hyg., 72, (1969): 47-48.

Williams, G., and P. M. Newberne: Effects of infection on selected clinical and biochemical parameters in dogs. Brit. J. Exp. Path., 51, (1970): 253-264.

Wintrobe, M. M.: Nutritive requirements of young pigs. Am. J. Physiol., 126, (1939): 375-387.

Wittman, W. A., D. Hansen, and J. F. Brock: Studies on protein-calorie malnutrition and infection. In *Nutrition and Infection*. CIBA Foundation Study Group, No. 31, Little, Brown and Co., Boston, Mass., 1967, pp. 73-89.

Woodruff, A. W.: Anaemia associated with protein-calorie malnutrition. In *Calorie Deficiencies and Protein Deficiencies*. Edited by R. A. McCance and E. M. Widdowson. Little, Brown and Co., Boston, Mass., 1968, pp. 165-173.

Wolf, G. B.: Kwashiorkor on the Navajo Indian Reservation. Henry Ford Hosp. Med. Bull., 9, (1961): 566.

Woodward, J. M., A. J. Sbarra and D. F. Holtman: The host parasite relationship in tularemia. I. A study of the influence of bacterium tularense on the amino acid metabolism of white rats. J. Bact., 67, (1954): 58-61.

Wyllie, D., V. C. Speer, R. C. Ewan and V. W. Hays: Effects of starter protein level on performance and body composition of pigs. J. Animal Sci., 29, (1969): 433-438.

Yoshimura, F., K. Harumiya, M. Watanabe, T. Omoto and T. Sekiguchi: Functional significance of the tubular agranular endoplasmic reticulum in the adrenocortical cells of albino rats. Endocrinol. Japon., 15, (1968): 145-169.

Young, G. A., R. W. Hinz, and N. R. Underdahl: Some characteristics of transmissible gastroenteritis TGE in disease-free antibody devoid pigs. Am. J. Vet. Res., 20, (1955): 529-535.

Yusken, J. W., P. Ho, E. F. Reber, and H. W. Norton: The effects of infecting newborn pigs with transmissible gastroenteritis virus. Am. J. Vet. Res., 20, (1959): 585-588.

Zealander, T.: Ultrastructure of mouse adrenal cortex. An electron microscopical study in intact and hydrocortisone trated male adults. J. Ultrastructural Res. Suppl., 2, (1959).

VITA

The author was born on September 2, 1938, in Odi in the Rivers State of Nigeria. He attended high school at Okrika Grammar School and Hope Waddell Training Institute.

He received his B.S. with Honors in 1963 and the D.V.M. in 1965 from Michigan State University.

From September 1965 to June 1967 the author was a lecturer in Veterinary Science at the University of Nigeria at Nsukka. He was a Ford Foundation postdoctoral Research Fellow in pathology at the University of Ibadan, Nigeria, from August 1967 to August 1968.

He was awarded a Rockefeller Foundation Fellowship, in 1968, to study for the degree of Ph.D. in pathology. Research conducted during this period forms the data for this thesis.

The author has published or co-authored the following papers:

Isoun, T. T.: The pathology of *Trypanosoma simiae* infection in pigs. Annals of Tropical Medicine and Parasitology, 62: 188-192, 1968.

Carter, G. R., T. T. Isoun, and K. K. Keahey: Occurrence of *Mima* and *Herellea* species in clinical specimens from various animals. J.A.V.M.A., 156: 1313-1318, 1970.

Isoun, T. T., C. K. Whitehair, O. Mickelsen, R. Schemmel and D. Cederquist: Dietary obesity in rats: Effect on gastrointestinal tract. Fed. Proc., 29: 299, 1970.