THE PIG AS A BIOMEDICAL MODEL TO STUDY HUMAN PROTEIN CALORIE MALNUTRITION

by

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ABSTRACT

Two experiments were undertaken to evaluate the baby pig as a biomedical model with which to study Protein-Calorie malnutrition. In the first experiment, 32 Yorkshire and Yorkshire X Landrace pigs weaned at 21 days were fed either an 18% or 4% protein ration. Blood samples were taken biweekly from the anterior vena cava and the serum samples analyzed for calcium, phosphorus, glucose, cholesterol, lactic dehydrogenase, glutamic oxaloacetic transaminase, amylase, alkaline phosphatase, total protein, albumin and blood urea nitrogen. Significant ($p \leq .01$) treatment effects were observed for total protein, albumin, amylase, alkaline phosphatase, lactic dehydrogenase, cholesterol, calcium and phosphorus.

In the second experiment, 40 Yorkshire and Yorkshire X Landrace pigs weaned at 28 days were fed rations containing 18%, 10%, 8%, 6%, and 4% protein. Blood samples were again taken biweekly and serum samples were analyzed for the same parameters as in trial one. In addition, serum copper, iron, magnesium, and zinc were measured. The livers of any animals which died on the low protein diets, were fat extracted, and the level of fat compared to that obtained from livers of animals killed as suckling pigs at a slaughter plant. Total body water was determined on three animals on the 18% ration and three on the 4% ration utilizing tritiated water as a tracer.

Total protein, albumin, amylase, lactic dehydrogenase, calcium, phosphorus, copper, iron and magnesium correlated well with dietary protein intake. Significant treatment effects were observed for total body water and fat content of the liver. An attempt was made to find a biochemical

parameter which might be used in diagnosing developing protein calorie malnutrition. The results of the study would indicate that serum phosphate and amylase are the most sensitive parameters to dietary protein intake.

Not every lesion or biochemical serum change occurring in man was reproduced in the present study. Nevertheless, characteristic symptoms such as the development of fatty liver, growth retardation, abnormal hair texture, hypoalbuminemia, and apathy were reproduced in the protein deficient swine. The baby pig would therefore appear to be a good model for the study of protein-calorie malnutrition.

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INTRODUCTION

Protein-Calorie malnutrition (PCM) is one of the most important public health problems in underdeveloped countries. PCM is largely responsible for the fact that in many areas of the world up to one-half the children born do not survive to the age of five years. Death rates in these children may be 20-50 times higher than the rate in rich and prosperous communities in North America and Europe.

Protein-Calorie malnutrition, describes a spectrum of clinical disorders. At one end, marasmus is due to a continued restriction of both dietary energy and protein. At the other end, is kwashiorkor, due to a quantitative and qualitative deficiency of protein, but in which energy may be adequate. These two syndromes are the extremes. Between them are forms in which the clinical features are due to varying combinations of protein and energy deficiency together with deficiencies of vitamins and minerals and with associated infections.

The study of disease in the human has frequently been advanced by the development of an experimental model system in animals which mimicks the condition in the human. Attempts at reproducing PCM in research animals are numerous. To-date, the majority of research projects have utilized the monkey (Coward and Whitehead, 1972; Deo et al., 1965), the dog (Heard, 1968; Stewart, 1968) and the rat (Kirsch et al., 1968a; Anthony and Edozien, 1975). Considerable differences of opinion have been voiced concerning the adequacy of these animal models and extrapolation of results from these sources to man. The most common complaints against these models are that they fail to develop severe edema, extensive skin and hair changes and

sprue-like bowel changes as seen in man (Madi et al., 1970; Blackburn and Vinijchaikul, 1969).

During the last decade, many workers have elaborated on the potential of swine as an ideal experimental animal. Glauser (1966) has reviewed some of the advantages of pigs as experimental animals. Most importantly, the pig has many physiological analogies with man. Kirsch et al., (1968b); Bustad (1966) and Douglas (1972) have emphasized the similarities between swine and man.

Piglet litter size may run between 12-16 in number enabling the use of statistically significant numbers of piglets from the same litter. Considering that the same sow has two litters per year and sometimes three, a large number of subjects of the same parentage are available for study during a year. It is therefore possible to run entire experiments from the progeny of one sow. This practice leads to less variability in the environmental, gestational and other genetic factors affecting experimental parameters than occurs with other species.

Swine readily accept experimental diets at an early age which reduces the time spent in feeding and handling animals. Other large animals such as the dog or monkey generally refuse experimental diets and must be force fed.

In animal experimentation, the cost of buying experimental animals is significant. In comparison with dogs or monkeys, piglets are relatively inexpensive.

One theoretical limitation to the use of the pig is that growth is relatively more rapid than in man who has a very long juvenile period between weaning and puberty (Evans and Miller, 1968). Despite this

consideration, it is believed that the pig is a more ideal animal model than others for research in PCM.

In human PCM, various biochemical parameters are altered as a consequence of the insufficient supply of energy and amino acids to the tissues. In order to evaluate the pig as a model to study PCM, the biochemical changes occurring in the protein deficient pig will be compared to those occurring in malnourished children as reported in the literature. If the pig proves to be a suitable model, results might be obtained utilizing this model to further the knowledge of the aetiology of PCM and ultimately enable suitably pretreated animals to be used for trials on the examination of theraputic agents.

The prognosis of PCM is often difficult to assess on clinical grounds alone, and various attempts have been made to find an accurate biochemical index of severity or response to treatment (Barclay, 1973; Stephens, 1974; MacFarlane, 1969; Bradfield, 1973; Whitehead and Dean, 1964). In addition to evaluating the pig as a model to study PCM, an attempt will be made to investigate a number of biochemical variables which may reflect severity and response to treatment of subjects with PCM.

LITERATURE REVIEW

A. BIOCHEMICAL ASPECTS OF PCM IN MALNOURISHED INFANTS

1. Protein Metabolism in PCM

a. Total Proteins

One of the most consistent serum biochemical alterations observed in PCM is lowered total proteins. Ismadi et al. (1971) reported values of 6.73 ± .17 g/100 ml in well nourished children, compared with a mean value of 3.43 ± .50 g/100 ml in malnourished children. Several workers have considered total proteins to be a reliable indicator of developing PCM (Baertl et al., 1974; Haddad and Harfouche, 1971) but this is not a commonly accepted view.

The lowering of total proteins is due to a great extent to the reduction of the albumin fraction (Scrimshaw and Behar, 1961). This reduction in albumin is partially obscured by changes in the globulin fraction (Scrimshaw and Behar, 1961). During the early phases of malnutrition, there is a reciprocal relationship between the albumin and globulin concentrations, with decreasing albumin and increasing globulins (Coward et al., 1972). Analysis of the individual globulin components show that the rise in globulin concentration is due mainly to increases in gamma globulin, and to a lesser extent alpha globulins. The rise in alpha globulin is thought to be related to cellular destruction, while the high levels of gamma globulin are believed to be related to infection (Viteri et al., 1964).

During the later pathological stages of PCM, total globulin concentrations fall mainly because of a reduction in the alpha and beta globulin fraction (Coward et al., 1972).

It is interesting to note that the immuno globulins are the only group of proteins in plasma normally synthesized outside the liver. Therefore, a serious derangement in the functional capacity of the liver might explain the final falls in albumin and alpha and beta globulins (Coward et al., 1972). The increase in gamma globulins suggests the preferential use of amino acids for the synthesis of these proteins when infection is present and this demand for amino acids may actually aggrevate the protein deficiency (Cohen and Hansen, 1962).

b. Albumin

Hypoalbuminemia is a consistent finding in PCM. Ismadi et al. (1971) observed albumin levels of 1.54 ± .30 g/100 ml in 14 malnourished children which was considerably lower than the mean 3.89 ± .13 g/100 ml of the control group.

Several workers have suggested that serum albumin levels are a good index with which to estimate the severity and prognosis of recovery from PCM (Whitehead et al., 1973; Baertl et al., 1974). However, McFarlane (1970), showed that serum albumin levels were not a guide to the subsequent progress of the malnourished child. He observed no differences in the serum albumin levels of children who survived and those who died. Reeds and Laditan (1976) concluded that serum albumin concentrations are poor indicators of a marginal reduction in nutritional status.

Whitehead et al. (1973) proposed that the concentrations of serum albumin could be used as an indicator of the development of edema. Their preliminary observations indicated that 3.0 g/100 ml was the critical serum albumin concentration as far as the appearance of early edematous signs was concerned. Approximately 75% of the total colloid osmotic pressure of plasma is normally derived from albumin, as a result of its relatively low molecular weight and high net charge (Searcy, 1969).

Albumin plays an important role in the transportation of metabolic products. Albumin is essential for the transport of unesterified fatty acids, hormones such as estrogens, testosterone and catecholamines, and anions such as calcium. As a result of hypoalbuminemia in PCM, the transport of these products would be reduced, which could result in metabolic disturbances throughout the body.

Theoretically, the reduction in albumin levels in PCM could result either from a lower rate of synthesis or from an increased rate of catabolism. However, experimental evidence suggests that the catabolic rate is actually reduced in PCM. Nine children with PCM and nine who had recovered from malnutrition were injected with albumin ^{131}I and were then studied during consecutive periods in which the amount of dietary protein was changed (James and Hay, 1968). Malnourished children had significantly lower catabolic rates of albumin than did the recovered children on the same protein intake. Growth hormone has been shown to produce a decrease in the catabolic rate of albumin in patients with cirrhosis (Gabuzda et al., 1963). However, it is not presently known if the elevated growth hormone levels occurring in malnourished children are the cause of the lower catabolic rate of albumin (Pimstone et al., 1966).

James and Hay (1968), measured the rate of synthesis of albumin in malnourished children. Their malnourished group had a synthetic rate of 101 mg/kg/day which was significantly lower than the 148 mg/kg/day observed in their control group. It has been concluded that the diminution in the rate of synthesis of albumin is caused by a reduction in the availability of amino acids (Rothschild et al., 1969). Livers obtained from fasted rabbits synthesized 18 ± 1 mg of albumin. The addition of methionine, lysine, leucine, valine, or threonine, at 10 umoles/ml failed to alter albumin synthesis. Tryptophan increased albumin synthesis 38-75%. The addition of isoleucine resulted in an 89% increase in albumin synthesis. When the donor rabbits were fed, albumin synthesis averaged 33 mg and no increases were observed with the addition of tryptophan or isoleucine. Baertl et al. (1974) noted high correlation coefficients between tryptophan and the branched chain amino acids, and serum albumin levels.

c. Blood Urea Nitrogen

Blood urea nitrogen has been reported to be low in PCM. Arroyave et al. (1962) reported levels of 7.8 mg/100 ml in malnourished children compared with a mean of 14.9 mg/100 ml in recovered children. Similar reductions have been reported by Bjornesjo et al. (1965), Kelman et al. (1972) and Edozien et al. (1960). Van der Westhuysen et al. (1975) reported no differences in blood urea nitrogen levels between controls and malnourished children.

Ammonia, a highly toxic agent, is continuously generated in all tissues as an end product of protein catabolism. Its accumulation is prevented by the conversion of any ammonia not required for amino acid

synthesis into urea.

The quantity and quality of dietary protein are important determinants of circulating levels of urea. Ingestion of large amounts of readily absorbable protein produce substantial increases in blood urea nitrogen. Low values of blood urea nitrogen are a reflection of diminished deamination of amino acids.

An elevation in blood urea nitrogen is most often interpreted as indicative of possible renal disfunction (Searcy, 1969). In PCM, glomerular filtration rate and renal plasma flow are reduced and there is evidence of impaired tubular function as shown by an amino-aciduria, occasional renal phosphaturia and an inability to excrete an acid load (Mann et al., 1972).

Since the liver is one of the major sites of urea synthesis, the cellular damage occurring as a result of fatty infiltration could result in lower circulating urea levels. However, the liver has a great functional reserve and urea synthesis is not likely to be curtailed unless hepatic tissue is acutely damaged (Searcy, 1969). In addition, urea formation may take place in other tissues than the liver. Such mechanisms could therefore augment circulating urea levels.

Urea is highly water soluble and diffuses freely through a majority of cell membranes. As a result of the increases in extracellular body water due to the presence of edema, it is possible that circulating urea levels would be reduced.

d. Amylase

Serum amylase levels are reduced in PCM. Edozien (1961) measured the serum amylase levels in malnourished and healthy Nigerian

children. Serum amylase values of malnourished children were reduced to an average of 72.6% of the normal. The healthy group had a mean serum level of 157 somogyi units/100 ml while the malnourished group had a mean of 114 somogyi units/100 ml. Similar reductions have been reported by Srikantia et al. (1964), Mukherjee and Werner (1954), and Dean and Schwartz (1953).

In the body, amylase is present in a number of organs and tissues. The greatest concentration is present in the pancreas where the enzyme is synthesized by the acinar cells and then secreted into the intestinal tract for the digestion of starches. The salivary glands secrete a potent amylase to initiate hydrolysis of starches while the food is still in the mouth or esophagus.

The decrease in serum amylase in PCM is believed to indicate pancreatic disfunction (Viteri et al., 1964), since the major source of serum amylase is the pancreas (Wiberg and Tuba, 1952). In addition to lowered serum amylase levels, Thompson and Trowell (1952) showed lower concentrations of amylase in duodenal contents of malnourished children.

e. Alkaline Phosphatase

Alkaline phosphatase values are low in PCM. Edozien (1961), noted that serum levels were reduced to 59.2% of the normal. Malnourished children showed a mean value of 16 King-Armstrong units/100 ml compared with a normal value of 27 King-Armstrong units/100 ml. Similar reductions have been reported by Sandstead et al. (1965), Schwartz (1956), and Scrimshaw et al. (1955). A good correlation has been shown to exist between the levels of alkaline phosphatase and albumin (Edozien, 1961).

Alkaline phosphatase may be derived from a number of different organs such as liver, bone, kidney, and intestine, but that found in the serum of young children is mostly of bone origin (Waterlow and Stephen, 1969).

Alkaline phosphatase is believed to be involved in the differentiation of both osteoblasts and chondroblasts, as well as in the synthesis of fibrous protein and the formation of preosseous bone matrix (Searcy, 1969). The excretion of hydroxyproline peptides is lower in PCM (Waterlow and Stephen, 1969) and this combined with the low serum levels of alkaline phosphatase would seem to reflect a decreased rate of formation and remodelling of the bone matrix in PCM.

Rosenthal et al. (1952), observed higher concentrations of alkaline phosphatase in the livers of adult rats fed diets deficient in protein. It has been suggested that the changes in liver concentrations might be explained by the fact that the liver normally disposes of alkaline phosphatase, and that this function is impaired during protein depletion (Schwartz, 1956).

f. Serum Glutamic Oxaloacetic Transaminase

Serum glutamic oxaloacetic transaminase (SGOT) levels have been shown to be moderately increased in PCM. Sandstead et al. (1965), obtained 36 sigma units in malnourished children compared to a control value of 23 sigma units. A slight elevation was also reported by Zaki et al. (1970), Baron (1960) and Edozien (1961). Contrary to the above reports, Smith (1962) estimated SGOT activity in 60 malnourished children and showed

no alterations which could be attributed to the state of malnutrition.

The transaminases constitute a group of enzymes which catalyze the interconversion of amino acids and alpha-ketoacids by the transfer of amino groups. SGOT catalyzes the transfer of the amino group from glutamic acid to oxaloacetic acid. This provides a mechanism for redistributing nitrogen according to the particular needs of the organism.

The greatest amount of GOT is present in the liver, followed by lesser amounts in the heart muscle and skeletal muscle. A small amount is present in the kidney, pancreas, red blood cells, brain and skin. Under normal conditions, only minute amounts of the enzyme are present in the serum (Searcy, 1969). However, in conditions in which there is tissue destruction, serum levels are increased. It is thought, therefore, that elevated levels in malnourished children indicate liver damage (Waterlow, 1959). Edozien (1961), suggested that small increases were due to muscle breakdown and larger increases were due to liver necrosis.

g. Lactic Dehydrogenase

Lactic dehydrogenase (LDH) levels are high in PCM and tend to decrease during protein repletion. Zaki et al. (1970), showed LDH levels of 326.5 ± 28.1 units/ml serum in malnourished infants, compared with a level of 205.5 ± 14.5 in controls. Elevated levels were also observed by Weimer et al. (1959) in protein deficient rats and by Sandstead et al. (1965) in Egyptian malnourished children.

LDH is a hydrogen transfer enzyme which catalyzes the oxidation of L-lactate to pyruvate with the mediation of NAD as hydrogen

acceptor. Enzyme levels present in various tissues (Wroblewski-LaDue units/ml) are very high as compared to serum: liver 260,000 units/gm; heart, 160,000 to 240,000 units; kidney, 250,000 to 300,000 units; and skeletal muscle, 133,000 units. Thus, tissue levels are about 1000-fold higher than those normally found in serum, and leakage of the enzyme from even a small mass of damaged tissue can increase the observed serum level (Kachmar, 1970).

The major factor contributing to the increase LDH levels in PCM is believed to be degeneration of somatic tissue with release of the enzyme into the circulation (Weimer et al., 1959). It is well established that skeletal muscle undergoes the greatest loss in weight during depletion and contains large amounts of LDH.

2. Fat Metabolism in PCM

a. General

Fat malabsorption is often present in PCM. Gomez et al. (1956), showed that the average absorption of fat was 48% in malnourished infants on admission to hospital. After six weeks of treatment, absorption rose to 79%. There are several possible reasons for this decrease in fat absorption. Firstly, it has been shown that pancreatic lipase secretion is diminished in malnourished children (Thompson and Trowell, 1952). Secondly, it has been reported that jejunal bile salts were deconjugated in eight out of twenty South African cases of PCM (Redmond et al., 1972). This deconjugation would decrease fragmentation of the fat globules.

Finally, mucosal atrophy could result in decreased fat absorption.

A serious result of this fat malabsorption would seem to be an impairment of absorption of fat soluble vitamins such as vitamin A. (Konno et al., 1968). In addition, there is the possibility of an essential fatty acid deficiency. Naismith (1964) measured the amounts of linoleic, arachidonic, and eicosatrienoic acid in the serum of Yoruba children with PCM. The ratio of eicosatrienoic acid to arachidonic acid was 1.08 which is in excess of the .4 considered to be indicative of an essential fatty acid deficiency (Holman, 1960). The resemblance between the skin lesions in cases of PCM and those produced experimentally by diets lacking essential fatty acids is striking (Hansen et al., 1958; Dean, 1965). A deficiency of essential fatty acids has been shown to lead to an impairment in the utilization of protein in the rat (Naismith, 1962) and therefore could aggravate the protein deficiency.

b. Serum Cholesterol

The fact that total serum cholesterol is low in PCM is a generally agreed upon finding. Schendel and Hansen (1958) measured the serum cholesterol in 48 cases of PCM and observed a mean of 93 mg/100 ml. This cholesterol level was significantly lower than the 173 mg/100 ml observed in the control group. Following treatment, there was a significant increase in serum cholesterol levels. They noted a close correlation between cholesterol rise and clinical improvement and suggested that the measurement of serum cholesterol might provide a sensitive biochemical index of success or failure of treatment. Low circulating levels of cholesterol have also been reported by Matthew and Dean (1960), Jaya Rao

and Prasad (1966) and Cravioto et al. (1959).

Cholesterol serves as a precursor for many of the steroid hormones as well as the bile salts. Cholesterol esters may also play a role in the transport of fatty acids. In addition, it is believed that cholesterol may function in the production and conduction of electrical impulses (Searcy, 1969).

Several factors may account for the reduction in serum cholesterol in PCM. Exogenous sources of preformed cholesterol are limited since the majority of the pre-PCM diet is almost totally of vegetable origin. One of the sterols in such a diet, sitosterol, has also been shown to be associated with reduced cholesterol absorption and hypocholesterolemia (Friedman et al. 1956). Diarrhea, which is common in PCM, may also play a part in producing the hypocholesterolemia, either through general metabolic disturbances and stress or through increased excretion of endogenous cholesterol (Schendel and Hansen, 1958).

The liver is the main site of cholesterol synthesis, but the skin, adrenals, gonads, intestine, and aorta can carry out the biosynthesis. Acetate radicals, chiefly in the form of acetyl-coenzyme A, are all that the body requires as starting material. Consequently many amino acids, carbohydrates, and fatty acids, when supplied in excess of other metabolic needs, can contribute to the cholesterol pool. Since the diet may be deficient in protein and energy, endogenous synthesis of cholesterol may be reduced.

Under normal conditions cholesterol is released from the liver in the form of lipoproteins. Serum lipoproteins have been estimated to be reduced in PCM (Cravioto et al., 1959).

c. Fatty Liver

An extremely fatty liver is one of the most striking characteristics of PCM. However, despite the clinical importance of this fatty condition, its pathogenesis is not yet resolved.

There are several possible mechanisms which may result in a fatty liver. Firstly, it is possible that decreased oxidation of fatty acids leads to the build up. However, using very small doses of ^{14}C-palmitate, Lewis et al. (1967), discovered that plasma free fatty acids were oxidized to respiratory CO_2 more rapidly than normal in PCM. This is the opposite of what would be expected if the fatty liver resulted from decreased oxidation of the fatty acids.

The role of lipotrophic factors on fat accumulation has also been investigated. The diets of children who develop PCM may be lacking in choline and methionine (Truswell, 1975). If lipotrophic factor deficiency contributes to fatty liver, this presumably comes about by reduced synthesis of phosphatidylcholine in the liver. Phosphatidylcholine is the principal transport phospholipid in plasma. If fatty liver is caused by a deficiency of lipotrophic factors, cases with gross fatty liver would be expected to have low serum phosphatidylcholine, with total phospholipids reduced more than other lipid classes. However, serum phospholipids are not as reduced as cholesterol or triglycerides in untreated PCM, and phospholipids are not lower in cases with severe fatty liver (Truswell et al., 1969). It is therefore unlikely that lipotrophic substances have a role in the formation of fatty liver.

Another possibility is that there is increased fatty acid synthesis in the liver. Fletcher (1966) observed a striking reduction in glucose-6-phosphatase activity in the liver of malnourished children and considered it a key factor in the pathogenesis of fatty liver. He postulated that with the reduction in this enzyme, there is an impaired mechanism for secreting glucose. With a continued addition of carbohydrate from the diet, the liver in malnourished subjects receives a greater load of carbohydrate than it can handle. Some of this is deposited as glycogen while the remainder is converted by normal metabolic processes into fat. However, Lewis et al. (1964), discovered that 10% of the fat in the liver of malnourished subjects was linoleic acid. This would not occur if the fat were synthesized in the liver because linoleic acid is an essential fatty acid ultimately derived from the diet. In addition, Fletcher (1966) measured fatty acid synthesis from ^{14}C-acetate invitro in liver biopsy samples from children with PCM. The synthetic rate was reduced.

Another possible mechanism for the accumulation of liver fat is increased mobilization of free fatty acids from adipose tissue. Lewis et al. (1964), showed that the concentration of nonesterified fatty acids is increased in the plasma of children with PCM. They suggested that in the acute phase of the disease, the child becomes deprived of calories because of anorexia, vomiting and diarrhea. As a result, the plasma glucose concentrations decrease. This reduction in plasma glucose leads to an increased output of NEFA from fat depots which in turn causes deposition of excess fat in the liver. Lewis et al. (1966), went on to show that the flux of free fatty acids through plasma was increased in infants with PCM. However, mobilization of NEFA cannot be the total mechanism for fatty liver, because in marasmus, where the plasma free fatty acids are as high as

in kwashiorkor, the liver is able to cope with an excessive input of fatty acids and no fatty liver develops.

Fat accumulated or produced in the liver is secreted into the plasma in combination with protein in the form of lipoproteins. It now appears that the lipids cannot be released from the liver because of low concentrations of beta lipoproteins and that this is probably the result of reduced hepatic synthesis of the protein moiety of the lipoprotein (Truswell and Hansen, 1969).

3. Carbohydrate Metabolism in PCM

a. Blood Glucose

There is some disagreement in the literature on the question of blood glucose levels in PCM. Slone et al. (1961), investigated blood sugar levels in malnourished South African Bantu and observed a mean glucose level of 51 mg/100 ml, which was significantly lower than the 76 mg/100 ml of the control group. Hypoglycemia has also been reported by Kassem et al. (1975), Baig and Edozien (1965), and Wharton (1970). Bowie (1964) however, reported normal levels in malnourished South African children as did Jaya Rao (1965) working with malnourished Indian children.

There is considerable disagreement as to the cause of the hypoglycemia when it does occur. One possible explanation for the hypoglycemia may be intestinal malabsorption of carbohydrates. Sugar absorption was studied by jejunal perfusion in five malnourished children both on admission and after at least two months high protein feeding

(James, 1968). All children initially had poor glucose absorption, four had defective lactose hydrolysis and two had a defect in sucrose hydrolysis. The hydrolytic defects were related to low disaccharidase activities in jejunal mucosal tissue as a result of mucosal damage. Digestion of starch is presumably impaired by the severe reduction of pancreatic amylase secretion reported in PCM (Thompson and Trowell, 1952). Kerpel-Fronius and Kaiser (1967), concluded that malabsorption of carbohydrate did not play a leading role in eliciting hypoglycemia since severe hypoglycemia was also observed in cases in which the ability to split disaccharides was preserved.

Slone et al. (1961), postulated that a decrease in gluconeogenesis may be a factor leading to hypoglycemia. They cited high amino acid levels and low blood urea levels as evidence of impaired gluconeogenesis and suggested that the block may be in the deamination of amino acids. However, Arroyave et al. (1962) showed that the total amino acid level in children with PCM was approximately one-half of those in healthy children. Whitehead and Dean (1964) also reported significantly reduced levels of amino acids. Whitehead and Harland (1966) measured the blood sugar, lactate and pyruvate levels of 69 Ugandan children during treatment of PCM. The majority of untreated cases had low levels of blood glucose but high levels of lactate and pyruvate. It is therefore possible that there is a metabolic block in the gluconeogenic pathway from pyruvate via the citric acid cycle. A deficiency of thiamine may cause this, or of pantothenic acid, the precursor of coenzyme A.

The relationship between hypoglycemia and the glycogen content of the liver has also been investigated. Alleyne and Scullard (1968) showed that liver glycogen is depleted in PCM. Wayburne (1963) reported that needle biopsies taken within a few minutes of death in hypoglycemic patients have

shown a direct correlation between the presence of glycogen and blood sugar levels. Usually, when blood sugar was below 30 mg/100 ml, glycogen was absent from the liver.

In addition, it is possible that there is a defect in the mobilization mechanism of the glycogen stores. Some investigators attribute the presence of hypoglycemia to a deficiency of the enzymes glucose-6-phosphatase and phosphoglucomutase (Durbin et al., 1959; Fletcher, 1966). These findings however, are not unanimously accepted. Alleyne and Scullard (1968) reported that glucose-6-phosphatase levels were elevated in PCM.

Low glucagon levels would also result in hypoglycemia. However, Kabadi et al. (1976), showed that glucagon secretion is normal in protein deficient rats. Kassem et al. (1975), injected glucagon intramuscularly, but observed little response in PCM patients. They postulated that the hepatic enzyme systems involved in glycogenolysis are impaired in PCM.

The literature on the incidence, clinical significance and pathogenesis of hypoglycemia in PCM contains many physiological contradictions. It is difficult to reconcile the hypoglycemia with the low insulin (Becker et al., 1972) and high cortisol levels (Alleyne and Young, 1967) reported in PCM. Further research would seem to be needed in this area.

4. Water and Electrolyte Metabolism in PCM

a. Edema

One of the most striking features of PCM is the formation of

edema. Total body water, expressed as a percentage of body weight is consistently increased in PCM. Smith (1960), measured total body water in 24 Jamaican children suffering from severe chronic malnutrition and observed a mean total body water (TBW) of 84.5% of body weight in the presence of edema and a mean of 62.6% upon recovery. Similar elevated levels have been reported by Brinkman et al. (1965), Flynn et al. (1967), and Schneiden et al. (1958).

There appears to be significant alterations in body water distribution in PCM. When expressed in absolute terms or as a percentage of body weight, the extracellular body water is increased. Muscle biopsies obtained from five children on admission and before treatment was started, gave a mean value for ECW of 226 ± 26 ml and for intracellular body water of 225 ± 38 ml per 100 grams of fat free solids, compared with control values of 131 ± 5 and 236 ± 5 ml respectively (Metcoff et al.,1960). The main cause of this relative expansion of the extracellular space appears to be the result of disproportionate losses in the body structure. The skin, nervous tissue, and skeleton of the body do not change much with loss of body weight. However, these structures contain a considerable proportion of the ECW. The major losses of body weight in malnutrition result from losses of fat and protoplasmic mass which contain relatively little ECW (Kerpel-Fronius, 1960).

The basic cause of the development of edema in PCM is still not clear. A hypothesis that found wide acceptance for a long time was the classical one of Starling. This concept was that physiochemical alterations brought about by low protein levels in plasma could explain the development of edema in protein deficient states. This explanation is now recognized as failing to explain several known facts about the edema

of PCM, one of them being that children suffering from PCM shed their edema during treatment long before there is any significant increase in their serum proteins. Hypoproteinemia is probably a modifying factor, but not the basic cause of the water retention (Waterlow et al., 1960). Garrow (1965) goes so far as to say that the hypoproteinemia may be the result rather than the cause of the edema.

There is evidence that renal function may be impaired in PCM, and that these defects may be important in the production of the edema of PCM. The glomerular filtration rate has been shown to be reduced in PCM (Alleyne, 1967). This reduction in GFR was attributed mainly to a substantial fall in cardiac output and renal blood flow. Alleyne (1967), considered the possibility that glomerular membrane or capillary cell disfunction might be the cause of the fall in GFR. In the presence of a diminished GFR, with restriction in the amount of fluid presented to the distal segment of the kidney, the body would be unable to clear water maximally.

Potassium depletion may result in retention of water and salt (Black and Milne, 1952), and consequently potassium deficiency is very likely a factor in the development of the edema of PCM. Garrow (1965), observed that the edematous type of child with PCM was even more depleted in potassium than in protein. Marasmic children, characterized by a lack of edema, were not potassium deficient.

It has been reported that aldosterone, the most potent sodium retaining steroid known, might be responsible for the edema in PCM. (Hansen, 1956). Aldosterone acts on the kidney to increase tubular reabsorption of sodium and water while urinary excretion of potassium is enhanced. However, Lurie and Jackson (1962) observed no relationship

between urinary aldosterone levels and sodium retention or edema in PCM. They suggested that mechanisms other than aldosterone secretion were responsible for the water and electrolyte disturbances in PCM. However, it is possible that the secretion rate of aldosterone is not reflected in the urinary excretion.

Leonard and MacWilliam (1965) measured the percentage of bound aldosterone in the serum of six malnourished children and observed it to be almost one-half the control value. Aldosterone is bound almost exclusively to albumin in the serum. In PCM, the serum albumin level is greatly reduced, which could cause a decrease in the amount of bound aldosterone, and an increase in the physiologically active free form (Leonard and MacWilliam, 1965).

Beitins et al. (1974) measured the plasma aldosterone levels in malnourished children and observed them to be higher when compared with those of a control group. They reported that the aldosterone secretion rate remained normal and suggested that the increased plasma concentrations might be the result of an alteration in the metabolic clearance rate of the steroid. Unfortunately, they did not correlate the raised aldosterone levels with edema formation and therefore the role of aldosterone in the formation of edema is still to be determined.

In most mamalian species the major control over aldosterone secretion is exerted by angiotensin, which is derived from a plasma protein when the kidney releases the enzyme renin. Plasma renin activity was measured by bio-assay in 100 children with PCM and in 20 healthy children (Kritzinger et al., 1974). Renin activity was significantly increased in children with PCM. This increased plasma renin activity must be the result

of either an increase in renin secretion by the kidney or a decrease in renin clearance by the liver with the latter more likely (Kritzinger et al., 1974). The renin-angiotensin system opposes salt and water elimination by direct action on the kidney (DeBono et al., 1963) and also by releasing vasopressin (Bonjour and Malvin, 1970), and aldosterone (Mulrow et al., 1962). The renin-angiotensin system also increases water consumption by inducing thirst (Fitzsimmons and Simons, 1969).

In a further study, increased plasma renin activity was found in PCM, but there was no strict relationship to the degree of edema (Van der Westhuysen et al., 1975). Many children with high renin activity did not develop edema and vice versa. In addition, work on malnourished pigs has shown that the increase in renin activity occurs after the edema develops (Van der Westhuysen et al., 1977). These findings suggest that the renin-angiotensin system is not responsible for the initial fluid retention and the development of edema in PCM.

It has recently been observed that during PCM, there are elevated levels of antidiuretic hormone (ADH) in the circulation (Srikantia and Mohanran, 1970). ADH is secreted from the neurohypophysis and acts on the distal and collecting tubules of the kidney, thereby increasing the reabsorption of water. There are two possible explanation for the increased ADH levels in the circulation. Firstly, it has been shown that in PCM, the ability of the liver to inactivate ADH is considerably impaired (Srikantia and Gopalan, 1958). Secondly ferritin, the iron-protein complex, has been shown to exert considerable antidiuretic activity (Baez et al., 1952). Ferritin is mainly present in the liver, spleen, and bone marrow, and is not normally observed in the circulation. However, probably as a result of

liver damage, active ferritin has been observed in the circulation of children with PCM (Srikantia, 1958). The elevated levels of plasma ADH may therefore be explained on the basis of defective inactivation of the hormone as a result of liver damage, and an increased secretion of the hormone as a result of stimulation of the neurohypophysis by ferritin.

It has been shown that children with marasmus do not have elevated ADH. In addition, following therapy, and disappearance of edema, levels of the hormone return to normal (Srikantia and Mohanram, 1970). These results suggest that the high levels of ADH are the cause of the edema in PCM.

b. Serum Calcium

Studies on calcium metabolism in PCM have shown variable results. Jayalakshmi et al. (1957), reported that serum calcium concentrations were subnormal in malnourished children. They observed a mean of 8.25 mg/100 ml in 14 malbourished children compared with a mean of 10.50 mg/100 ml after six weeks of treatment. Hypocalcaemia has also been reported by Sandstead et al. (1965), and Senecal (1958). They attribute the lower serum levels to a lowering of the protein bound fraction secondary to hypoproteinemia.

Khalil et al. (1974), reported that serum calcium values in children with PCM did not differ significantly from controls. Erythrocyte levels however, were significantly reduced. They thought that the maintenance of this normal concentration in spite of deficient intake and inadequate absorption, was a reflection of increased freely diffusible calcium through bone mobilization.

Shenolikar and Narasinga Rao (1968) demonstrated reduced calcium accretion rates in the bones of protein deprived rats using ^{45}Ca as a tracer. This reduction of calcium accretion was associated with a reduced incorporation of proline into hydroxyproline and suggests an impairment in bone collagen synthesis.

Le Roith and Pimstone (1973), demonstrated a significant reduction in intestinal calcium absorption in protein deficient rats. Kalk and Pimstone (1974), observed that in the protein deficient rat, there was a significant reduction in intestinal calcium binding protein activity. They felt that as a consequence of deficient amino acid substrate, synthesis of calcium binding protein was reduced.

Shenolikar and Narasinga Rao (1968) observed a higher fecal calcium excretion in rats on a low protein diet. They observed that the major component of this fecal calcium was of endogenous origin. Endogenous calcium is contributed by bile, pancreatic juice and the secretion of epithelial cells along the intestines. It is not known which factors contribute to the increased fecal excretion.

Leonard et al. (1968), reported significantly higher calcium levels in nail clippings from malnourished children. They suggested that the analysis of nail electrolytes may be of considerable value as a guide to alterations in tissue electrolytes.

c. Serum Copper

Serum copper levels have been shown to be reduced in PCM. Lahey et al. (1958), studied 10 Guatemalan children with PCM and indicated

that the concentration of copper in serum was substantially reduced. Gopalan et al. (1963), observed low serum copper in kwashiorkor but reported normal levels in marasmic infants. Edozien and Udeozo (1960), reported a reduction in serum copper concentration from 180 ± 47 ug/100 ml in normal children to 86 ± 23 ug/100 ml in children with PCM.

The possibility exists that a dietary deficiency of copper occurs concomitantly with a deficiency of protein. The daily requirement for copper is approximately 50 ug/kg body weight. Dried cassava, which is a staple food of most developing countries, contains approximately 140 ug/100 gm. However, it has been established that only about 5% of the copper in an ordinary diet is retained. If such is the case in children with PCM, it would appear likely that the copper intake would be below normal. However, a study of the copper content of the home diet of children with PCM revealed that the dietary intake was not inadequate (Gopalan et al., 1963).

It is known that copper is excreted in the bile (Cartwright and Wintrobe, 1964), and it is therefore possible that diarrhea resulting in an excessive loss of bilary contents may be a factor resulting in low circulating copper levels.

Lahey et al. (1958), and Sanstead et al. (1965) attribute the reduced serum copper levels to a reduction in serum proteins. Over 95% of the copper in serum is bound to the ceruloplasmin component of the alpha-2-globulin fraction of serum protein. Gopalan et al. (1963) observed a highly significant correlation between serum copper levels and ceruloplasmin.

Warren et al. (1969), determined the concentration of copper in the livers of children suffering from PCM. This study showed that there

was a decrease in the concentration of copper in the livers of PCM patients.

The pigmentary changes in the hair commonly noted in PCM may be due to a deficiency of copper at the tissue level (Lahey et al., 1958). A copper containing enzyme, tyrosinase, is known to be involved in the production of melanin from tyrosine (Flesch, 1949). Gopalan et al. (1963), measured the copper content in the hair of malnourished and normal children. The copper content of the hair in PCM was low and averaged only 9.1 gamma/gm of hair compared to 19.3 gamma/gm of hair in the normal group. However, among cases of PCM, the copper content of hair was uniformly low, irrespective of whether the hair was normal or not.

Copper is known to play a role in erythropoiesis but whether the anemia in PCM is in any way related to the abnormalities in the metabolism of this element is not known. However, it should be noted that the anemia in copper deficient animals is characteristically microcytic and hypochromic, while the anemia of PCM is rarely this type (Edozien and Rahim-Khan, 1968).

d. Serum Iron

Serum iron and iron binding capacity are low in PCM. Edozien and Udeozo (1960) showed that 35 Nigerian children with PCM had a serum iron concentration of 40.6 $\pm$ 21.5 ug/100 ml compared with 62.7 $\pm$ 17.0 ug/100 ml in 37 controls. The iron binding capacity was further reduced from a control value of 274.0 $\pm$ 59.0 ug/100 ml to 119.1 $\pm$ 54.4 ug/100 ml in malnourished children. These findings have been confirmed by Lahey et al. (1958), in malnourished Mexican children and by El-Sholmy et al. (1962) in malnourished Egyptian children.

Sood et al. (1965) studied the absorption of iron using Fe^{59} in nine protein deficient and four control monkeys. At 8-10 weeks of protein deficiency, there was a fall in iron absorption ranging from 9-23% over the basal value in seven out of nine animals. The average value for iron absorption in the deficient group was 39.9% compared with the basal value of 50.8%. A possible reason for malabsorption of iron in PCM relates to the changes that occur in the intestinal mucosa of the small bowel. Extensive atrophy, as well as functional abnormalities have been demonstrated (Barbezat et al., 1967).

It is generally accepted that ferrous iron only is transported across the intestinal wall. The reduction of ferric to ferrous iron is facilitated by the reducing powers of ascorbic acid. Andersson et al. (1956) reported a low dietary intake of ascorbic acid in malnourished South African Bantu. In addition, Edozien and Rahim-Khan (1968) reported low serum ascorbic acid in over 75% of malnourished cases examined.

Amino acids such as histidine and lysine assist in iron absorption (Van Campen and Gross, 1969). It is suggested that a direct reaction between iron and histidine occurs and that an amino acid-iron chelate may be formed and absorbed. In PCM the level of histidine in the gastrointestinal tract would be low and therefore iron absorption may be impaired.

Losses of iron from the bodies of normal infants occur from the gastrointestinal tract, primarily in the form of desquamated intestinal epithelial cells, from the skin and in urine. It is possible that fat losses in steatorrhea could be accompanied by increased desquamation of gastrointestinal mucosal cells.

Malnourished children are also subject to parasitic infestations, which can act as further drains on the very meager supplies of dietary iron.

The iron binding protein resides in the beta globulin, transferrin. El-Sholmy et al. (1962), reported a close correlation between the iron binding capacity and beta globulin. It is therefore probable that the reduction in serum iron and iron binding capacity are due to profound metabolic alterations produced by protein depletion.

El Shobaki et al. (1972) observed that the liver non-heme iron concentration was higher in malnourished children than in controls. Chattopadhyay and Banerjee (1975) reported that the synthesis of hemoglobin was impaired in PCM. Since excretion of iron is very limited, the only way to get rid of the non-utilized part is to store it in the liver.

e. Serum Magnesium

Magnesium deficiency appears to be a relatively frequent occurrence in PCM. Evidence of magnesium depletion has been derived from analysis of muscle biopsy material, balance studies, and estimations of urinary, plasma and serum magnesium.

A gross tissue depletion of magnesium was first reported in PCM by Montgomery (1960). He performed muscle biopsies on 12 Jamaican children suffering from PCM and observed significantly lower levels of magnesium in muscle. Caddell and Goddard (1967) also reported low magnesium values in muscle samples of Nigerian patients. Linder et al. (1963) showed that cumulative magnesium retention in the first 21 days of treatment was

2.1 meq/g N with and .94 meq/g N without magnesium supplementation. Increased magnesium retention persisted for as long as six weeks, showing that repletion takes a long time.

There appears to be a discrepancy regarding serum magnesium levels in PCM. Linder et al. (1963) reported low magnesium values in over two-thirds of their observations, while Montgomery (1960) reported unaltered levels. A large proportion of body magnesium is in the skeleton and muscle, and therefore blood levels may be normal.

Urinary magnesium is extremely low and is probably a better index of magnesium deficiency than is the serum level (Linder et al., 1963).

Leonard et al. (1968) reported that normal plasma electrolyte levels are frequently observed in association with decreased tissue levels rendering measurement of the former of little value in assessing the cellular electrolyte status. They reported significantly reduced magnesium levels in nail clippings from PCM patients and suggested that the analysis of nail clippings may be of considerable value as a guide to alterations in tissue electrolytes.

According to Caddell and Goddard (1967), the magnesium deficiency in malnutrition results from prolonged losses of magnesium through the GI tract during diarrhea and vomiting coupled with a low magnesium intake.

Children on high milk protein therapy often die suddenly and unexpectedly just as they are beginning to recover and feed themselves. It has been shown that protein, calcium, phosphorus and potassium increase the metabolic demand for magnesium and this therapy may actually increase the magnesium deficiency syndrome. In addition, children recovering from PCM grow at a fantastic rate and this must entail a need for large amounts

of magnesium. For these reasons, it has been suggested that supplemental magnesium be given routinely to all severely malnourished children during treatment (Caddell, 1966).

Caddell (1967) conducted a double-blind paired sequential trial in malnourished Nigerian children to assess the efficacy of parenteral magnesium therapy. Her preliminary findings indicated that this form of therapy was of such significant value that the trial was stopped before completion in order to give the control patients the benefit of the therapy. The clinical symptoms attributed to magnesium depletion included weakness, anorexia, tremors, sleeplessness, hyperirritability hypotension, and hypothermia, all of which improved rapidly after magnesium administration. Another trial of magnesium therapy was undertaken in South Africa (Rosen et al. 1970). Although initial plasma magnesium values were commonly lower than normal and tended to fall transiently in untreated cases, the trial failed to demonstrate any theraputic benefit of magnesium supplementation. In no instance was it possible to recognize clinically the children who had the lowest plasma magnesium values; nor were there any specific symptoms identifiable with magnesium depletion. Possibly the South African children were less severely depleted than are those described by Caddell (1967); their staple diet being maize which has a higher magnesium content than does cassava, the main substance of diet in Nigeria.

A magnesium deficiency would help to explain many of the biochemical lesions associated with PCM. A deficiency of magnesium would help to explain the fatty liver commonly observed in PCM. Severe fatty infiltration, with obliteration of cells by fatty changes and marked fibrosis, has been described in patients with depletion of serum magnesium (Waterlow, 1962). Oxidative phosphorylation, which is dependant on

magnesium activated enzyme systems, is severely decreased in fatty livers (Griffiths and Rees, 1957). Magnesium is also an activator in alpha keto fatty acid metabolism (Vallee, 1960).

The human bone is thought to be a highly labile reserve for magnesium. The marked bone changes in PCM (Higginson, 1954) might be related to a very substantial bone deficit (Montgomery and Chir, 1961).

Absorption of carbohydrate has been shown to be depressed in PCM (James, 1968). The active absorption of glucose, galactose, and fructose involves the hexokinase reaction. The hexokinases for glucose and fructose are activated by magnesium (Cori and Slein, 1947).

Magnesium is an activator for all the enzymes that require thiamine pyrophosphate as a cofactor (Brown, 1962). This coenzyme is responsible for alpha keto fatty acid metabolism and for oxidative decarboxylation of pyruvate in muscle and brain. Pyruvic acid is a key substance in intermediary metabolism, being a stage reached by glycerol, many amino acids, and all carbohydrates. PCM patients manifest disturbances in pyruvate metabolism; the blood pyruvate is raised and pyruvate decarboxylation is reduced (Whitehead and Harland, 1966).

Pyridoxine phosphate requires magnesium for optimal activity. In addition, magnesium is needed for the phosphorylation of riboflavin to form its coenzyme. Since magnesium is required for coenzyme formation of riboflavin and for activation of the coenzyme of pyridoxine, it might be that the skin lesions of PCM, so similar to those of riboflavin and pyridoxine deficiencies, are related to disturbed metabolism of these vitamin B coenzymes in magnesium deficient subjects (Caddell, 1965).

f. Serum Phosphorus

Hypophosphataemia has been observed in PCM. Sandstead et al. (1965), measured the serum phosphorus levels in 39 malnourished Egyptian children. Upon admission, they had a mean serum level of 2.8 ± 1.1 mg/100 ml compared with a level of 4.7 ± 1.1 mg/100 ml upon clinical cure. This is in agreement with the findings of Bjornesjo et al. (1965). Smith (1960) reported that serum phosphorus levels tended to be lower in those infants who died within seven days of admission compared with those who survived. In all fatal cases, the mean serum phosphorus level was 3.7 ± 1.6 mg/100 ml, but among 51 survivors the mean was 4.39 ± 1.43 mg/100 ml.

In PCM, inorganic and organic phosphate are decreased in muscle (Waterlow and Mendes, 1957). Upon recovery, both kinds of phosphate increase. In cases ending in death, inorganic phosphate increase at the expense of organic phosphate.

g. Serum Zinc

Zinc deficiency is manifested by the occurrence of skin lesions, retarded growth, diarrhea, vomiting, alopecia, disturbed protein metabolism, and a decrease in blood alkaline phosphatase and pancreatic amylase. Histological studies have revealed hyperkeratinization, thickening of the epidermis and intra and intercellular edema. It becomes apparent that a striking similarity exists between the signs and symptoms observed in animals in a zinc deficient state and the corresponding findings reported by various workers investigating PCM.

Kumar and Jaya Rao (1973), estimated the plasma and erythrocyte zinc levels in children suffering from PCM. Plasma zinc levels were low on admission and returned to normal levels after nutritional rehabilitation. Erythrocyte zinc concentrations were unaltered. These observations are in line with those reported earlier by Smit and Pretorius (1964), and by Sandstead et al. (1965).

The low levels of serum zinc during the acute phase of PCM may be due to several factors. Plant products particularly cereals, are not satisfactory sources of zinc because of their phytic acid content and its binding effects on the zinc ion (O'Dell and Savage, 1960). The pre-PCM diet of malnourished children is high in cereals and other vegetable products and very low in animal protein. Therefore, because of the presence of phytate and other biological chelators, the availability of zinc for absorption is probably low.

The protein level of the diet has been shown to affect zinc absorption. To assess some of the effects of protein malnutrition on zinc metabolism, rats were fed diets with 5 or 15% casein and 9 or 33 mg/kg zinc (Van Campen and House, 1974). Rats on the 5% protein diet retained less of a single oral dose of Zn^{65} than those on the 15% protein diet with either amount of protein.

Sandstead et al. (1965) ascribe the zinc deficiency to the presence of infections or to intestinal loss of zinc due to diarrhea.

Ninety percent of circulating zinc is bound to serum proteins. It is therefore not surprising that in PCM where serum proteins are low, that the total zinc levels should be low.

The loss of zinc into the interstitial compartment is yet another factor which might contribute to the low levels of serum zinc in PCM. Edema fluid was found to contain substantial amounts of zinc (Kumar and Jaya Rao, 1973).

In PCM cases the zinc content of the liver has been observed to be markedly reduced (Warren et al., 1969).

It is conceivable that some of the changes in skin and hair observed in PCM are related to zinc deficiency. Alopecia, coarseness and dispigmentation of the hair occurs in zinc deficient rats. Zinc was estimated in the hair of 43 Peruvean Andean Indian children on admission to hospital and again after recovery (Bradfield et al., 1969). There was no significant difference between admission and recovery.

Impaired insulin secretion is a characteristic finding in PCM (Becker et al., 1972). Recent findings indicate that zinc deficiency results in the impairment of insulin secretion from the pancreas (Sullivan et al., 1974). Huber and Gershoff (1973) fed rats diets containing 1, 20, or 1200 mg/kg zinc. Feeding of high or low zinc diets did not alter the insulin content of the pancreas, but immunoreactive serum insulin and total serum insulin like activity were significantly reduced in the zinc deficient group.

B. BIOCHEMICAL ASPECTS OF PCM IN SWINE

1. Protein Metabolism

a. Serum Proteins

Tumbleson et al. (1972b) measured serum proteins in malnourished swine. Mean serum total proteins and albumin concentrations were lower for the undernourished pigs than for the controls. From 4-32 weeks of test, the undernourished pigs had higher serum total globulins as a percentage of total proteinthan did control pigs. Mean serum beta globulin concentration for the pigs fed the low protein diet was lower than for the controls.

b. Serum Amino Acids

Badger and Tumbleson (1974), reported that normal relationships among concentrations of amino acids were altered in young miniature swine fed 5% protein diets. Mean concentrations of 11 free amino acids were altered significantly in the serum of malnourished piglets. Taurine, threonine, glutamic acid, valine, isoleucine, leucine, tyrosine, and phenylalanine were reduced, while alanine, alpha amino-butyric acid and methionine were increased. Similar findings were reported by Grimble and Whitehead (1970).

c. Serum Enzymes

Tumbleson (1972), reported little difference in the alkaline phosphatase, glutamic oxaloacetic transaminase and lactic dehydrogenase levels of control and malnourished pigs. Alkaline phosphatase and lactic dehydrogenase were slightly reduced. Surprisingly, SGOT levels were also slightly lower. Heard et al. (1957) observed lower plasma amylase levels in pigs on a 5% protein diet.

d. Antibodies

Hook et al. (1972) measured antibody responses in undernourished Sinclair miniature swine. There were no differences in the antibody responses of the two dietary groups tested after 0, 4, or 8 weeks. After 12 or 16 weeks on test, the appearance of maximumtitres of serum antibody was delayed in undernourished swine. Serum antibody not only appeared later in undernourished swine tested after 20-24 weeks, but the antibodies also failed to attain the levels reached in corresponding control swine. In addition, there was a decrease in the number of antibody producing cells obtained from the lymph nodes of undernourished swine.

2. Water and Electrolyte Metabolism in PCM

a. Edema

Tumbleson et al. (1969) utilized deuterium oxide to measure

total body water and sodium thiocyanate to measure extracellular body water in malnourished pigs. There was little difference in total body water but extracellular body water was slightly elevated. Payne and Done (1959) however, reported significantly higher total body water in a pig fed a low protein diet with carbohydrate supplement.

b. Electrolytes

Tumbleson et al. (1972a) measured serum electrolytes in undernourished Sinclair miniature swine. Mean serum calcium and phosphorus concentrations were lower from two through twenty-eight weeks on test for the undernourished pigs compared with the controls. The undernourished group had a lower mean sodium level from 14 through 24 weeks on test. Mean serum concentrations of potassium, urea nitrogen, and creatinine were not altered. The undernourished pigs had significantly higher mean serum chloride levels compared with the controls. Platt and Frankul (1962) reported lower serum levels of iron and zinc in their malnourished group of pigs.

3. Fat Metabolism in PCM

a. Fatty Liver

Gupta (1973a) conducted a study on the quantitative changes in the liver lipids of Indian pigs suffering from severe protein malnutrition. The livers of all pigs fed low protein showed periportal to diffuse fatty changes. There was a two fold increase in the total lipid content and an

eleven fold increase in the triglyceride content of the livers of pigs fed the low protein diet. The low protein diet also increased the cholesterol content in the liver. Protein deficiency resulted in marked reduction of hepatic phospholipids in malnourished pigs.

b. Serum Cholesterol

Tumbleson et al. (1969) measured the serum cholesterol values in undernourished Hormel miniature swine. They found no significant difference due to dietary treatment after 12 weeks on test.

4. Hormones in PCM

a. General

Platt and Stewart (1967), reported that the endocrine glands of PCM pigs were smaller than those of pigs fed normally. However, relative to body weight, the adrenals were large, the hypophysis within the normal range, the thymus small and the pancreas and thyroid showed wide variations attributable to different degrees of edema.

Baldijao et al. (1976) measured corticosteroids in malnourished and control pigs. No significant differences were observed for total plasma corticosteroids. Free cortisol however, was significantly higher in the protein depleted group compared to controls.

Atinmo et al. (1976b) measured immunoreactive growth hormone levels in protein depleted pigs. Post weaning protein deprivation resulted in higher growth hormone levels during the restriction period compared with controls.

Atinmo et al. (1976a) conducted an experiment to investigate changes in plasma insulin levels during PCM in swine. Protein restriction after weaning resulted in persistently low insulin levels during the depletion and rehabilitation period.

5. Miscellaneous Aspects of PCM in Swine

a. Organ Weights

Badger et al. (1972) conducted a study to determine the effects of PCM on body and organ weights. Malnourished pigs weighed significantly less at 63 days than did controls and consumed significantly less feed. Organ weights were lower in the malnourished group. The order or vulnerability from the least to the most affected was as follows: cerebrum, cerebellum, eye, heart, kidney and liver. Tumbleson et al. (1969) reported significantly lower kidney, gastroenemius muscle, spleen, liver, lungs, adrenals, heart, tibia, thyroid, and brain weights in their malnourished group. Gupta (1973b) reported similar changes in malnourished Indian pigs.

b. Behavior

Barnes et al. (1970) studied behavioral changes in baby pigs malnourished for a period of eight weeks by restricting protein or energy

intake. An apparatus was designed for the measurement of changes in the level of excitement or emotionality under conditions of stress, as well as changes in learning performance in a conditioned avoidance situation. The most striking behavioral change due to early malnutrition was the heightened excitement of the pigs when exposed to adverse stimuli, although there was also an indication of decreased learning ability.

c. Hematology

Burks et al. (1974) performed a hematological study in malnourished swine. Mean erythrocyte count was lower from 12 through 28 weeks on test for malnourished pigs. From 4 through 28 weeks on test, mean packed cell volumes and hemoglobin concentrations were lower for pigs fed the 4% protein diet. Malnourished pigs had lower mean corpuscular volumes and lower mean corpuscular hemoglobin from 4 through 26 weeks on test. Mean percent of neutrophils was greater for malnourished pigs from 8 through 16 weeks; mean percent lymphocytes was lower from 8 through 18 weeks on test.

d. Rehabilitation

Pigs have also been used in studies on rehabilitation from PCM. Pond et al. (1971) conducted a study to compare the adequacy of casein, isolated soy protein, or fish concentrate for rehabilitation from PCM. A similar study was performed by Barnes et al. (1966). They concluded that fish concentrate and casein were superior to isolated soy protein in promoting growth, feed intake, and regeneration of serum proteins and pancreatic enzymes.

EXPERIMENT 1

A. MATERIALS AND METHODS

a. Objectives

Trial 1 was undertaken to determine the suitability of the pig as a biomedical model with which to study protein-calorie malnutrition. Various biochemical parameters are altered in the malnourished human and the object of this trial was to determine if similar changes occur in the malnourished pig.

b. Experimental Procedures

Thirty-two Yorkshire and Yorkshire X Landrace pigs were selected at approximately the same age and weight for the study. The experimental animals were weaned at twenty-one days of age and assigned to a dietary treatment on the basis of weight, litter, and sex. Each treatment was further divided into two pens with eight pigs in each (four barrows and four gilts). The duration of the trial was ten weeks.

The experiment was conducted at the Swine Research Unit at the University of British Columbia. The building was insulated and supplemental infrared heat lamps were used to maintain the air temperature at approximately 25^{o}C. The building was ventilated with thermostatically controlled air exhaust fans. The pens had concrete floors, partially slatted and a total area of 4.8 square meters.

Pigs were fed ad libitum from a portable wooden feed trough. Feed consumption records were kept daily and weekly totals were recorded. The composition of the experimental rations is shown in Table 1. The experimental rations were formulated to meet NRC nutrient requirements for growing pigs (National Academy of Sciences, 1973). The only exception was the protein level of the PCM ration. The rations were formulated to be similar in energy level and utilized adjustments in the levels of cassava and soybean in contributing to the protein deficiency. Water was supplied ad libitum by drinking nipple.

The experimental animals were weighed biweekly and weight gains were recorded. Following weighing, blood samples were collected from the anterior vena cava, utilizing the method of Mackenzie (1961). A twenty millilitre blood sample was obtained through a twenty gauge heparinized needle. The pigs were fasted for a period of twelve hours prior to sampling, and the samples were taken at the same time of day to minimize diurnal variations. Samples were allowed to clot, centrifuged and the serum obtained. The samples were then frozen for later analysis.

c. Feed Analysis

Dry matter determinations were carried out on triplicate samples of feed, by drying to a constant weight in a forced draught oven at 100°C.

Nitrogen was determined in triplicate according to the macrokjeldahl method (A.O.A.C., 1975). Nitrogen content of the feed and feces was converted to crude protein using the factor of 6.25 and results were expressed as a percentage of the initial sample dry matter.

Acid Detergent Fibre was determined in triplicate, utilizing the micro-digestion procedure of Waldern (1971).

Ether extract was determined on triplicate samples of feed according to the methods of the A.O.A.C. (1975), utilizing a goldfisch fat extractor.

Ash was determined on triplicate samples of feed by heating a known weight of sample in a muffle furnace at 600°C for six hours.

Gross energy was determined on feed and feces using a Gallenkamp Adiabatic bomb calorimeter and the result expressed as kilocalories per kilogram of sample.

The apparent digestibility of the feeds was determined using a modified method of McCarthy et al. (1974). Fecal samples were collected for a period of four days, three times during the trial and a feed sample was also obtained. Fecal samples were dried for 48 hours at 60°C and then ground in a hammer mill. Five grams of finely ground feed or feces were boiled in 100 ml of 4 N HCl for thirty minutes in a 250 ml erlenmeyer flask. They were then filtered through sintered glass filtering crucibles which had been oven dried, desiccated and weighed. They were then washed free of acid with boiling water and ashed at 600° C for six hours. The samples were then reweighed and acid insoluble ash was expressed as a percentage of the original sample dry matter. Digestibility was calculated by determining the ratio of the concentration of the reference substance to that of a given nutrient in the feed and the same ratio in the feces resulting from that feed. The results of the proximate analysis and determination of digestible energy are presented in Table 2.

Mineral content of the experimental rations was determined utilizing a new block digestion procedure developed in the U.B.C. Animal Science Laboratory by Mr. Edward B. Cathcart. The procedure involved mixing .15 g of dried ground sample with 6 mls of reagent grade H_2SO_4 and pre-digesting with 1 ml 30% H_2O_2. After the solution cleared, 3 grams of catalyst consisting of reagent grade K_2SO_4 and reagent grade HgO in a ratio of 22.4g:1g were added. The solution was then allowed to digest on a block heater pre-set to a range between 410°C and 425°C. Following digestion, the samples were allowed to cool and made up to volume with demineralized water. Calcium, magnesium, iron, zinc and copper were determined by atomic absorption spectrophotometry while phosphorus was determined utilizing the Technicon Autoanalysis method number 327-74W. Results of the mineral analysis are presented in Table 3.

d. Biochemical Analysis

Serum calcium, phosphate, total proteins, albumin, cholesterol, glucose, lactic dehydrogenase, alkaline phosphatase, glutamic-oxaloacetic transaminase, and blood urea nitrogen were determined using a survey model sequential multiple auto analyzer (SMA-12-90). Amylase was determined on a single channel auto analyzer at the same commercial laboratory as the multi-channel analysis (B.C. Biomedical Lab).

e. Statistical Analysis of Data

The data were subjected to analysis of variance using the computer program UBC BMD:10V (Bjerring et al., 1975). Sex, litter, and

Table 1. Composition of Experimental Diets (Trial 1).

Ingredient (%)	Dietary Protein Level 18%	4%
Corn	21.70	21.70
Cassava	35.00	72.25
Soybean	32.30	.85
Premix*	4.00	4.00
Corn Oil	7.00	1.20

* Vitamin-Mineral Premix supplied/kg: Vitamin A, 4545 I.U.; Vitamin D, 363 I.U.; Vitamin E, 5.5 I.U.; Calcium, 9.0 gm.; Phosphorus, 4.5 gm.; Salt, 7.5 gm.; Iron, 120 mg.; Iodine, 160 mg.; Zinc, 120 mg.

Table 2. Proximate Analysis of Experimental Rations (Trial 1).

Component	Dietary Protein Level 18%	4%
Moisture	10.96	11.86
Crude Protein	20.74	6.36
Acid Detergent Fibre	5.90	8.37
Ether Extract	6.60	1.61
Ash	7.91	7.94
Nitrogen Free Extract	47.89	63.86
Acid Insoluble Ash	0.86	2.10
Gross Energy (kcal/kg)	4481.0	3989.7
Digestible Energy (kcal/kg)	3955.2	3359.0

Table 3. Mineral Analysis of Rations (Trial 1).

Mineral	Dietary Protein Level 18%	4%
Calcium (%)	1.23	1.07
Phosphorus (%)	.54	.42
Magnesium (%)	.18	.13
Iron (%)	.01	.01
Copper (mg/kg)	27.10	27.80
Zinc (mg/kg)	121.80	104.20

treatment were the factors taken into account in the analysis of variance table. Since there were empty cells, no interaction terms were included.

B. RESULTS

a. General

Data for body weight gains are presented in Table 4. Pigs fed the 18% protein ration gained an average of 24.1 kg during the duration of the trial compared with only 2.1 kg for the 4% group. There was a significant treatment effect four weeks on trials ($p < .01$). There was no significant sex or litter effect during the trial.

Weekly feed consumption is presented in Table 5. The animals on the low protein diet consumed considerably less feed than animals on the control diet. However, the difference is not as great when feed consumption is calculated per kilogram of body weight. The low protein group were very apathetic in their feeding habits and consumed less feed as the trial progressed.

Animals in the low protein group had sparse hair, which was brittle and thinner than normal and seemed to lack the lustre of the control group. Some animals showed abnormal gait, their legs became stiff, the hind legs being more severely affected than the fore legs. In addition, as the trial progressed, there were frequent outbreaks of diarrhea in the low protein group.

b. Protein Metabolism

Values for total serum proteins are given in Table 6. Total proteins were significantly lower ($p \leq .01$) in pigs after four weeks on the protein deficient diet. Values fell from a mean of 5.16 g/100 ml at the beginning of the trial to 4.03 g/100 ml after ten weeks. There was a significant litter effect at the beginning of the trial ($p \leq .01$), while sex effect was not significant during the entire trial.

Serum albumin levels are given in Table 7. After four weeks on trial, values for serum albumin were significantly lower ($p \leq .01$) for pigs on the low protein diet. The levels fell from a mean of 2.85 g/100 ml at the beginning of the trial to a level of 1.76 g/100 ml after eight weeks. Sex and litter showed no effect on serum albumin levels.

Blood urea nitrogen values are given in Table 8. There were no significant differences for sex, litter or treatment.

Values for serum amylase are presented in Table 9. Serum amylase levels were significantly lower ($p \leq .01$) in the protein deficient group after four weeks on trial. There was a significant litter effect ($p \leq .01$) at the beginning of the trial and after four weeks. Sex had no effect on levels of serum amylase through out the feeding trial.

Serum alkaline phosphatase values are given in Table 10. Serum alkaline phosphatase levels were significantly lower ($p \leq .01$) in the protein deficient pigs after four weeks on trial. At the beginning of the trial, alkaline phosphatase values averaged 135.5 m I.U./ml and this value fell to 61.2 m I.U./ml on the four percent ration. Sex and litter had no influence on alkaline phosphatase levels.

Serum glutaminc oxaloacetic transaminase levels are given in Table 11. A significant difference ($p \leq .01$) was obtained after six weeks on trial but this difference was not maintained during the balance of the trial. Litter had a significant influence at the beginning of the trial and after six weeks ($p \leq .01$). However, no differences were observed for sex.

Lactic dehydrogenase values are given in Table 12. Lactic dehydrogenase levels were significantly higher ($p \leq .01$) in the protein deficient pigs after ten weeks on trial. Values rose from a mean of 321.6 Wroblewski units/ml at the beginning of the trial to a mean of 519.7 Wroblewski units/ml after ten weeks. Sex and litter had no significant effect on lactic dehydrogenase levels during the trial.

c. Fat Metabolism

Values for serum cholesterol are given in Table 13. Protein deficient pigs had significantly lower ($p \leq .01$) serum cholesterol values after eight weeks on trial. Sex and litter effects were not significant during the entire trial.

d. Carbohydrate Metabolism

Values for serum glucose are given in Table 14. No significant differences were observed for sex, litter, or treatment.

Table 4. Body Weights (kg) of Swine Fed Varying Levels of Protein (Trial 1)[1,2].

Weeks	Dietary Protein Level 18%	4%
0	5.18 ± .25[a]	5.45 ± .23[a]
2	6.93 ± .49[a]	5.46 ± .29[a]
4	9.56 ± .77[a]	5.45 ± .37[b]
6	15.11 ±1.19[a]	5.73 ± .44[b]
8	21.96 ±1.51[a]	6.10 ± .54[b]
10	29.26 ±2.00[a]	7.57 ±1.36[b]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 5. Weekly Feed Consumption (kg) in Trial 1.

Weeks	Dietary Protein Level 18%	4%
1	26.40 (16)	24.50 (16)
2	36.30 (16)	20.90 (13)
3	54.00 (16)	19.80 (13)
4	59.50 (16)	16.70 (13)
5	77.20 (16)	20.50 (12)
6	99.50 (16)	16.00 (12)
7	117.80 (16)	16.30 (11)
8	137.50 (16)	18.90 (11)
9	152.60 (16)	14.80 (8)
10	171.50 (16)	6.20 (6)

* Values in parenthesis denote the average number of pigs on trial during a week.

Table 6. Serum Total Protein (g/100 ml) in Swine Fed Varying Levels of Protein (Trial 1).[1,2]

Weeks	Dietary Protein Level	
	18%	4%
0	5.31 ± .24[a]	5.16 ± .15[a]
2	4.96 ± .10[a]	4.76 ± .12[a]
4	4.76 ± .19[a]	4.18 ± .09[b]
6	4.99 ± .10[a]	3.91 ± .10[b]
8	5.83 ± .10[a]	3.89 ± .15[b]
10	5.87 ± .16[a]	4.03 ± .20[b]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 7. Serum Albumin Levels (g/100 ml) in Swine Fed Varying Levels of Protein (Trial 1).[1,2]

Weeks	Dietary Protein Level	
	18%	4%
0	2.95 ± .14[a]	2.85 ± .12[a]
2	2.96 ± .06[a]	2.91 ± .04[a]
4	2.31 ± .08[a]	1.98 ± .06[b]
6	2.98 ± .06[a]	2.55 ± .03[b]
8	3.18 ± .10[a]	1.76 ± .07[b]
10	3.41 ± .12[a]	2.05 ± .16[b]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 8. Blood Urea Nitrogen Levels (mg/100 ml) in Swine Fed Varying Levels of Protein (Trial 1).[1,2]

Weeks	Dietary Protein Level	
	18%	4%
0	16.31 ± 1.14[a]	16.25 ± .85[a]
2	17.20 ± 1.14[a]	13.46 ± .91[a]
4	16.94 ± 0.97[a]	13.31 ±1.13[a]
6	16.56 ± 0.79[a]	16.83 ±1.10[a]
8	15.44 ± 0.86[a]	16.45 ± .92[a]
10	13.69 ± 0.53[a]	16.75 ±3.61[a]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 9. Serum Amylase Levels (Somogyi Units/100 ml) in Swine Fed Varying Levels of Protein (Trial 1).[1,2]

Weeks	Dietary Protein Level	
	18%	4%
0	320.2 ± 21.1[a]	335.1 ± 29.9[a]
2	305.9 ± 23.2[a]	314.8 ± 28.9[a]
4	310.8 ± 15.9[a]	234.3 ± 19.9[b]
6	255.2 ± 11.2[a]	186.7 ± 13.6[b]
8	245.9 ± 13.8[a]	184.0 ± 18.5[a]
10	307.4 ± 17.2[a]	166.3 ± 41.1[b]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 10. Serum Alkaline Phosphatase Levels (m I.U./ml) in Swine Fed Varying Levels of Protein (Trial 1). [1,2]

Weeks	Dietary Protein Level 18%	4%
0	125.6 ± 9.0[a]	135.5 ± 7.2[a]
2	125.5 ± 6.5[a]	122.8 ± 6.8[a]
4	136.8 ± 8.9[a]	87.8 ± 9.9[b]
6	143.9 ± 9.3[a]	77.5 ± 6.4[b]
8	130.6 ± 6.6[a]	64.6 ± 9.8[b]
10	122.9 ± 8.8[a]	61.2 ±17.5[b]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 11. Serum Glutamic Oxaloacetic Transaminase Levels (Karmen Units/ml) in Swine Fed Varying Levels of Protein (Trial 1).[1,2]

Weeks	Dietary Protein Level 18%	4%
0	39.47 ± 2.60[a]	41.50 ± 2.71[a]
2	41.13 ± 3.50[a]	43.00 ± 2.56[a]
4	57.75 ± 4.53[a]	43.62 ± 3.71[a]
6	58.00 ± 2.05[a]	49.08 ± 1.65[b]
8	47.25 ± 1.50[a]	41.20 ± 2.13[a]
10	47.56 ± 2.17[a]	43.50 ± 6.25[a]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 12. Serum Lactic Dehydrogenase Levels (Wroblewski Units/ml) in Swine Fed Varying Levels of Protein (Trial 1).[1,2]

Weeks	Dietary Protein Level	
	18%	4%
0	397.3 ± 44.7[a]	321.6 ± 19.9[a]
2	550.4 ± 65.0[a]	698.2 ± 51.6[a]
4	552.9 ± 74.7[a]	488.0 ± 38.5[a]
6	320.6 ± 15.1[a]	324.9 ± 25.7[a]
8	372.1 ± 19.7[a]	387.9 ± 21.4[a]
10	401.6 ± 15.2[a]	519.7 ± 28.3[b]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 13. Serum Cholesterol Levels (mg/100 ml) in Swine Fed Varying Levels of Protein (Trial 1).[1,2]

Weeks	Dietary Protein Level	
	18%	4%
0	82.87 ± 5.74[a]	81.75 ± 8.17[a]
2	95.53 ± 2.70[a]	98.62 ± 3.82[a]
4	85.37 ± 3.25[a]	86.00 ± 4.27[a]
6	108.30 ± 3.90[a]	98.67 ± 3.68[a]
8	103.10 ± 2.10[a]	87.82 ± 6.92[b]
10	115.30 ± 3.42[a]	91.75 ± 6.15[b]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 14. Serum Glucose Levels (mg/100 ml) in Swine Fed Varying Levels of Protein (Trial 1).[1,2]

Weeks	Dietary Protein Level	
	18%	4%
0	132.5 ± 7.7[a]	113.1 ± 4.4[a]
2	85.3 ± 2.7[a]	79.1 ± 5.0[a]
4	80.6 ± 4.0[a]	91.2 ± 3.5[a]
6	87.5 ± 1.8[a]	89.5 ± 5.9[a]
8	93.2 ± 2.9[a]	84.6 ± 5.2[a]
10	94.6 ± 3.0[a]	100.0 ±12.1[a]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 15. Serum Calcium Levels (mg/100 ml) in Swine Fed Varying Levels of Prtein (Trial 1).[1,2]

Weeks	Dietary Protein Level	
	18%	4%
0	9.16 ± .22[a]	8.95 ± .24[a]
2	9.90 ± .12[a]	9.84 ± .15[a]
4	9.26 ± .22[a]	8.86 ± .18[a]
6	8.97 ± .13[a]	7.82 ± .17[b]
8	10.03 ± .13[a]	8.16 ± .31[b]
10	9.85 ± .10[a]	8.13 ± .25[b]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 16. Serum Phosphate Levels (mg/100 ml) in Swine Fed Varying Levels of Protein (Trial 1).[1,2]

Weeks	Dietary Protein Level	
	18%	4%
0	6.87 ± .48[a]	6.73 ± .24[a]
2	8.19 ± .25[a]	7.28 ± .14[b]
4	7.53 ± .23[a]	5.85 ± .19[b]
6	8.04 ± .17[a]	6.34 ± .31[b]
8	8.64 ± .13[a]	6.25 ± .18[b]
10	8.64 ± .25[a]	6.70 ± .41[b]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

e. Mineral Metabolism

Serum calcium levels are listed in Table 15. Values for serum calcium were significantly lower ($p \leq .01$) after six weeks on the low protein diet. There was a significant litter effect after four weeks ($p \leq .01$), while sex effect was not significant during the entire trial.

Serum phosphate levels are given in Table 16. Protein deficient pigs had significantly lower ($p \leq .01$) serum phosphate values after two weeks on trial. Sex and litter had no significant effect on serum phosphate levels during the entire trial.

C. DISCUSSION

a. Protein Metabolism

One of the most consistent biochemical alterations found in PCM is lowered total serum proteins. Several workers have considered total proteins to be a reliable indicator of developing PCM (Baertl et al., 1974; Haddad and Harfouche, 1971).

In the present study, serum total protein was significantly lower ($p \leq .01$) in the malnourished group. This is in agreement with earlier work by Tumbleson et al. (1972b), in malnourished Sinclair miniature swine. The control values are similar to those obtained by Miller et al. (1961) for swine of a similar age.

There are several explanations for the reduced level of serum protein in the malnourished pig. Owing to the protein deficiency, not only is the total concentration of amino acids low, but in addition, the pattern of amino acids is distorted (Whitehead and Dean, 1964). Also, as a result of fatty infiltration, the functional capacity of the liver is reduced, resulting in lower protein synthesis.

Hypoalbuminemia is another constant finding in human PCM. In the present study, albumin levels in the protein deficient group were reduced to 60% of those of the controls. Similar results have been obtained in swine by Tumbleson et al. (1972b).

The reason albumin levels drop is not clear. Theoretically, the reduction in albumin levels could arise either from a lower rate of synthesis or an increased rate of catabolism of serum albumin. Research has indicated that cortisone enhances albumin destruction (Searcy, 1969). Alleyne and Young (1967), observed high levels of circulating cortisone in malnourished children. However, work by James and Hay (1968), indicate that the catabolic rate of albumin is reduced in PCM. It therefore seems more likely that a reduction in albumin synthesis is the cause of the reduction of circulating albumin.

James and Hay (1968), observed that the rate of albumin synthesis was significantly lower in malnourished children. This diminution in the rate of albumin synthesis is believed to be caused by a reduction in the availability of amino acids (Rothschild et al., 1969).

Blood urea nitrogen levels have been reported to be low in human PCM (Arroyave et al., 1962). However, in this study, dietary treatment did not affect blood urea levels. Tumbleson (1972), also failed to note any changes in blood urea levels in his malnourished pigs.

The quantity and quality of dietary protein are important determinants of circulating levels of urea. Addis et al. (1947), showed that the level of serum urea is proportional to the protein intake. As a result of their observations, it was expected that serum urea levels would be low in the present trial. The higher values observed may be explained by increased catabolism of body proteins due to a very small supply of proteins and calories.

Serum amylase levels have been reported to be reduced to 72.6% of normal in Nigerian children with PCM (Edozien, 1961). The results of the present study indicate that swine are more severely affected. Serum amylase in the malnourished group levels were reduced to almost 50% of the control. Heard et al. (1957) reported similar reductions in their malnourished group of pigs.

The reduction in serum amylase in PCM is believed to reflect pancreatic disfunction (Viteri et al., 1964). The pathophysiologic alterations of the pancreas in PCM have been studied extensively (Pitchumoni, 1973; Barbezat and Hansen, 1968). The turnover of protein in the pancreas is among the highest of any organ (Wheeler et al., 1949), and therefore it is not surprising that the pancreas is rapidly affected in states of protein deprivation. An early paper on the essential pathology of PCM goes so far as to suggest that it is primarily a pancreatic disorder secondary to malnutrition, resulting in cirrhosis of the liver, pancreatic cirrhosis and a form of nephritis (Davis, 1948). Histological studies in PCM patients have revealed atrophy of acinar cells, with a diminution in the number of secretory granules (Pitchumoni, 1973).

Reductions in serum alkaline phosphatase have been reported in human PCM. Edozien (1961) noted that serum levels were reduced to 59.2% of normal. In the present trial, after ten weeks on test, alkaline phosphatase values were reduced to 49.8% of normal.

Although the alkaline phosphatases are found in most tissues, the source of the activity in serum is still underfined. The location of alkaline phosphatase in the growing individual is in the osteoblasts and the condroblasts of the skeleton. Thus, most of the alkaline phosphatase is present in the bones of this age group. In the adult, the gastointestinal mucosa and liver contain the largest amount of enzyme, while lung, spleen, thyroid and placenta, also contain the enzyme.

It is believed that the low serum levels of alkaline phosphatase in PCM reflect a decreased rate of formation and remodelling of the bone matrix (Waterlow and Stephen, 1969). It is possible that the disturbances of gait exhibited by the malnourished pigs in the present study were a result of this altered bone metabolism.

Diminished serum alkaline phosphatase values have been noted in children with underactive thyroid glands (Cassar and Joseph, 1969). Experimental work with pigs has shown that the activity of the procine thyroid gland is reduced by PCM (Platt and Stewart, 1962). It was suggested that PCM affects the thyroid gland in two ways; by reducing the supply of thyrotrophin and by reducing the supply of proteolytic enzymes required for the breakdown and release of the stored product (Platt and Stewart, 1967).

Another possible explanation for the low serum levels of alkaline phosphatase could be increased urinary excretion. It is possible that the urinary output of this enzyme is influenced by adrenal cortical

hormones, since the urinary activity of this enzyme is high in Cushing's disease (Searcy, 1969). Alleyne and Young (1967) reported high plasma cortisol levels in children with PCM.

Magnesium is one of the ions necessary for the function of alkaline phosphatase, and a magnesium deficiency results in lowered serum alkaline phosphatase (Wolf and Williams, 1973). Linder et al. (1963), reported lower serum magnesium levels in PCM patients.

Lactic dehydrogenase values are elevated in human PCM (Zaki et al., 1970). In the present study, malnourished pigs had a mean LDH activity 20% higher than the controls after ten weeks on trial.

Lactic dehydrogenase acts in the glycolytic cycle to catalyze the conversion of lactic acid to pyruvic acid. It is widely distributed throughout the body with levels in tissues about 1000 fold higher than those normally observed in serum. Therefore leakage of the enzyme from even a small mass of damaged tissue can increase the observed serum level.

In man, elevated serum levels of LDH occur in myocardial infarction, pernicious anemia, leukemia, hepatic necrosis, renal disease and tropical sprue (Wolf and Williams, 1973). The major factor contributing to the increase in circulating LDH levels in PCM is believed to be degeneration of somatic tissue (Weimer et al., 1959).

There was no apparent dietary effect on serum levels of glutamic oxaloacetic transaminase in the present trial. The significant difference obtained after week six is difficult to explain. In light of data obtained in experiment two, it is believed that a real difference did not occur. Tumbleson et al. (1969) also did not observe any differences in SGOT levels between controls and malnourished pigs.

There is considerable controversy in the literature regarding SGOT levels in malnourished children. Elevated levels have been reported by Sandstead et al. (1965), while others (Edozien, 1961; Smith, 1962) have reported no elevation of SGOT in PCM.

SGOT catalyzes the transfer of the alpha amino group from glutamic acid to oxaloacetic acid. The carbon skeleton so formed, can be used for energy synthesis. The greatest amounts of SGOT are present in the liver, followed by lesser amounts in the heart, and skeletal muscle. A small amount is present in the kidney, pancreas, red blood cells, brain and skin. Normally, almost all the transaminases are located within the cell and relatively small amounts of the enzyme circulate in the serum. Therefore, hypertransaminasemia is an expression of a release of the enzyme through cellular destruction. It is thought that in PCM, the circulating enzymes arise from the foci of necrosis in liver or muscle. It is also possible that enzyme might leak out from intact cells whose cell membranes may have suffered structural damage induced by protein deficiency (Edozien and Rahim-Khan, 1968).

The coenzyme pyridoxal phosphate is necessary for the action of transaminase enzymes. Pyridoxine disorders have been reported in PCM (Theron et al., 1961).

b. Fat Metabolism

Children with PCM havebeen reported to have low serum cholesterol levels (Schendel and Hansen, 1958). Similar results were discovered in the present trial. This however, is in contrast to the animal

studies of Tumbleson (1969) who found no differences between control and malnourished pigs, and Tumbleson (1972) who reported elevated serum cholesterol in his malnourished group.

The initial step in the synthesis of cholesterol involves the combination of acetate with coenzyme A. Consequently, many amino acids, carbohydrates and fatty acids, when supplied in excess of other metabolic needs can contribute to the cholesterol pool. Whitehead and Harland (1966), on the basis of elevated blood pyruvate levels, postulated that there was a depression of pyruvate oxidation and impaired entry of pyruvate into the Krebs cycle of PCM patients. Therefore, it would appear that acetate levels may be lower in PCM, which may be reflected in reduced synthesis of cholesterol.

c. Carbohydrate Metabolism

Dietary treatment did not appear to have any effect on serum glucose levels in the present study.. This is in agreement with work done by Bowie (1964) and Jaya Rao (1965) in human studies and Tumbleson (1972) in pigs.

d. Mineral Metabolism

Although studies on calcium metabolism in human PCM have shown variable results, work with swine appears to be more conclusive. Tumbleson et al. (1972) and Tumbleson et al. (1969) reported reduced serum calcium in malnourished swine. This has been confirmed in the present study.

There are many factors which could contribute to the diminished calcium levels. Prolonged administration of adrenalcortical hormones or adrenal hyperfunction is often followed by skeletal rarefaction (Sissons, 1971). Alleyne and Young (1967) have shown elevated levels of adrenalcortical hormones in PCM.

Severe inflammatory attacks of pancreatic disease are often accompanied by an impressive decline in serum calcium levels (Searcy, 1969). It has been suggested that PCM is primarily a pancreatic disease secondary to malnutrition (Davis, 1948).

Large quantities of calcium can be lost in cases of diarrhea, particularly those involving excessive fecal excretion of fat (Thomas and Howard, 1964). Shenolikar and Narasinga Rao (1968) observed higher fecal calcium excretion in rats on a low protein intake.

Serum calcium is rapidly reduced to a low level in conditions of hypoparathyroidism (Searcy, 1969). The effects caused by PCM upon endocrine functions are not well known. There are no published reports concerning the activity of the parathyroids in PCM. However, PCM has been shown to adversely affect other endocrine glands (Heard and Stewart, 1971; Godard, 1974) and it is possible that the parathyroids might also be affected.

It is possible that the reduced alkaline phosphatase levels observed in the present trial result in less bone calcium being mobilized. In addition, it has been shown that calcium binding protein is reduced in the intestine of protein deficient rats (LeRoith and Pimstone, 1973). If the same is true in pigs, this reduction could help to explain the lower serum calcium levels observed.

Hypophosphataemia was observed in the protein deficient pigs. This is in accord with the human studies of Sandstead et al. (1965), and Bjornesjo et al. (1965) as well as the animal studies of Tumbleson (1972).

Circulating phosphorus levels could be reduced by an impairment in intestinal absorption or through some impairment in the renal excretory mechanism. Phosphorus absorption correlates positively with the concentration of calcium present and it is reduced in conditions involving an impairment in the intestinal uptake of calcium (Searcy, 1969). Thus the lowered calcium binding protein in PCM (LeRoith and Pimstone (1973) may be reflected in lower absorption of phosphorus.

The renal tubular mechanism for phosphate reabsorption, operates to maintain circulating levels of the mineral within normal limits. The reabsorption process is variable and is influenced by the state of the body reserves of the mineral, parathyroid hormone, insulin, vitamin D, adrenocortical hormones as well as protein intake (Searcy, 1969).

The lower serum alkaline phosphatase levels observed in the present trial could result in less bone phosphorus being mobilized.

EXPERIMENT 11

A. MATERIALS AND METHODS

a. Objectives

The results of trial one would seem to indicate that the pig is a good model relative to the human with which to study protein-calorie malnutrition. However, it is not known at what level of protein intake PCM develops. The object of this trial was to study the effects of protein intake on the development of the symptoms of PCM. In addition, it was intended that this experiment would provide some insight into which parameter is the most sensitive index of the nutritional state of the animal.

b. Experimental Procedures

Forty Yorkshire and Yorkshire X Landrace pigs chosen as closely as possible to be of the same age and weight were used in this study. The experimental animals were weaned at 28 days of age and assigned to a dietary treatment on the basis of sex, litter and weight. Five treatments were used with one pen per treatment. Each pen contained four barrows and four gilts.

Details of research facilities, feeding methods, bleeding methods, and weighing procedure are as given for experiment one.

The composition of the experimental rations is shown in Table 17. The experimental rations were formulated to meet NRC requirements for growing pigs with the exception of the protein level. In this trial, the different protein levels were obtained by diluting the basal ration (18%)

with corn starch. These diets allowed the amino acid ratios presented to the experimental animals to be constant among treatments. Different levels of animal tallow were added to keep the diets isocaloric. Rations were formulated to contain 18%, 10%, 8%, 6%, and 4% protein. Actual levels of protein are presented in Table 18.

c. Analytical Methods

Analytical methods for proximate analysis, digestible energy, and mineral analysis are given in trial one. The results of the proximate analysis are given in Table 18 while mineral analysis is presented in Table 19.

d. Biochemical Analysis

Biochemical analysis for parameters repeated in experiment two are as given for experiment one.

Serum copper, iron, magnesium, and zinc, were determined by atomic absorption, utilizing a Unicam SP 90 spectrophotometer. For the determination of magnesium, samples were diluted fifty times and for serum zinc the samples were diluted five times. Copper and iron were determined on undiluted serum samples.

The livers of any animals which died on trial were removed and frozen for later analysis. Control livers were obtained from pigs of approximately the same weight, killed as suckling pigs at a slaughter plant. The livers were dried at 60^{o}C for 48 hours and ground in a hammer

mill. Fat was extracted on triplicate one gram samples utilizing a goldfisch fat extractor. The final results were expressed as a percentage of the dried sample weight.

Total body water was determined using the tritiated body water technique of Brinkman et al. (1965). Three animals selected at random from the 18% and 4% treatments were used in this study.

Tritiated water was purchased from New England Nuclear with a specific activity of one millicurie per millilitre. It was considered desirable to maintain the tracer in a concentrated form. Therefore, 2.5 millilitres of the tracer was diluted with physiological saline to make an injectable dose of 100 uc/ml. Ten microcuries per kilogram body weight were then injected intraperitoneally. Approximately five and six hours post injection, five millilitres of blood was obtained by vena cava puncture. The samples were then allowed to clot, centrifuged and the serum obtained.

In the past, it has been deemed necessary to obtain water free of pigment and protein in order to achieve adequate counting efficiency. Several methods have been used including benzene distillation (Werbin, 1959), deproteinization with TCA (Langhan et al. 1956) and vaccum sublimation (Vaughan and Boling, 1961). Recently, several new scintillation cocktails have come on the market which promise much higher counting efficiencies at much higher water contents (i.e. Handifluor or Aquasol). A preliminary trial was run to compare counting water obtained by lyphilization to direct counting of serum. The difference was not significant and therefore for this trial vaccum sublimation was not used.

Triplicate one-tenth millilitre serum samples were added to ten millilitres of the scintillation cocktail Handifluor (Mallinckrodt).

An aliquot of the injected dose was diluted 1/200 with distilled water and 1/10 millilitre of this sample was counted in the same manner as the samples. A quench correction curve was obtained by counting quenched standards from Amershan/Searle and plotting counting efficiency vs channels net count rate ratio. Background was determined by counting a reference background vial from Amershan/Searle. Mean serum activity was used to calculate body water according to the equation of Kay, Jones and Smart (1966).

$$\text{Body Water (ml)} = \frac{\text{Dose injected (dpm)}}{\text{Equilibrium activity/ml serum}}$$

e. Statistical Analysis

The data were subjected to analysis of variance using the computer program UBC BMD:10V (Bjerring et al., 1975). Sex, litter, and treatment were the factors taken into account in the analysis of variance table. Since there were empty cells, no interaction terms were included. Means from comparisons showing a significant "F" were tested using Tukey's test (1953).

Table 17. Composition of Experimental Diets (Trial 2).

Ingredients (%)	Dietary Protein Level 18%	10%	8%	6%	4%
Corn	21.70	12.05	9.64	7.23	4.82
Cassava	35.00	19.44	15.55	11.66	7.77
Soybean	32.30	17.94	14.35	10.76	7.17
Corn Starch	0.00	40.57	50.71	60.85	70.74
Corn Oil	7.00	0.00	0.00	0.00	0.00
Tallow	0.00	6.00	5.75	5.50	5.50
Premix*	4.00	4.00	4.00	4.00	4.00

* Vitamin-Mineral Premix supplied/kg: Vitamin A, 4545 I.U.; Vitamin D, 363 I.U.; Vitamin E, 5.5 I.U.; Calcium, 9.0 gm; Phosphorus, 4.5 gm; Salt, 7.5 gm; Iron, 120 mg; Iodine, 160 mg; Zinc, 120 mg.

Table 18. Proximate Analysis of Experimental Rations (Trial 2).

Component	Dietary Protein Level 18%	10%	8%	6%	4%
Moisture	10.96	12.76	12.57	14.04	13.65
Crude Protein	20.74	11.84	10.70	8.07	5.80
A.D.F.	5.90	3.35	2.77	2.08	1.28
Ether Extract	6.60	5.50	5.19	6.21	5.76
Ash	7.91	6.33	6.19	5.33	4.71
N.F.E.	47.89	60.22	62.58	64.27	68.80
A.I.A.	0.86	0.42	0.39	0.41	0.15
G.E. (kcal/kg)	4481.6	4324.9	4274.9	4309.9	4288.3
D.E. (kcal/kg)	3955.2	4035.1	3940.9	4052.0	4147.3

Table 19. Mineral Analysis of Rations (Trial 2).

Mineral	Dietary Protein Level 18%	10%	8%	6%	4%
Calcium (%)	1.23	1.03	1.10	0.96	0.96
Phosphorus (%)	0.54	0.46	0.47	0.44	0.42
Magnesium (%)	0.18	0.11	0.10	0.07	0.07
Iron (%)	0.01	0.01	0.01	0.01	0.01
Copper (mg/kg)	27.10	29.60	27.10	31.40	27.80
Zinc (mg/kg)	121.80	50.50	94.70	112.40	103.50

B. RESULTS

a. General

Weekly feed consumption data is recorded in Table 20. There was a linear relationship between feed consumption and percent dietary protein (r^2 = .98 at week 10).

Body weights are recorded in Table 21. There was a significant ($p \leq .01$) treatment effect after two weeks on trial. In addition, there was a significant ($p \leq .01$) litter effect up to six weeks on trial. Sex had no influence on body weight gain during the trial.

As in trial one, the animals on the low protein diets exhibited disturbances of gait, apathy, diarrhea, and sparse hair growth. There were no visual symptoms of edema.

b. Protein Metabolism

The results of the analysis for serum total proteins are presented in Table 22. There was a significant treatment effect after four weeks on trial ($p \leq .01$). Serum total proteins declined with decreasing dietary protein. There was no significant sex or litter effect during the trial.

Mean values for serum albumin are presented in Table 23. There was a significant ($p \leq .01$) treatment effect after four weeks on trial. Serum albumin declined with decreasing dietary protein. There was a significant ($p \leq .01$) litter effect after four weeks and at the beginning of the trial. There was no significant sex effect during the entire trial.

Blood urea nitrogen (BUN) values are presented in Table 24. There was a significant ($p \leq .01$) treatment effect after six and eight weeks on trial, but this disappeared at week ten. The eighteen percent ration had significantly higher BUN levels than did the other treatments. There was a significant ($p \leq .01$) litter effect after four weeks on trial, while sex had no influence on BUN values.

Mean values for serum amylase are presented in Table 25. There was a significant treatment effect after four weeks on trial ($p \leq .01$). Serum amylase values declined with decreasing dietary protein. There was a significant ($p \leq .01$) litter effect at the beginning of the trial and after four and ten weeks on experiment. Sex had no effect.

The results of the analysis for alkaline phosphatase are presented in Table 26. There was a significant ($p \leq .01$) treatment effect after four weeks on trial. At four and six weeks, alkaline phosphatase values declined with decreasing dietary protein. However, at eight and ten weeks, there was a quadratic effect, with the eight percent protein ration being higher than the other groups. Litter effect was significant ($p \leq .01$) at the beginning of the trial and after four weeks. Sex had no influence on alkaline phosphatase levels during the trial.

Values for serum glutamic oxaloacetic transaminase are presented in Table 27. There was no significant effect for sex, litter, or treatment.

The results of serum lactic dehydrogenase analysis are presented in Table 28. There was a significant ($p \leq .01$) treatment effect after ten weeks on trial. The four percent protein ration had significantly higher LDH levels than did the other treatments. Sex and litter had no influence on LDH levels during the trial.

c. Fat Metabolism

The results of the analysis for serum cholesterol are presented in Table 29. There was a significant ($p \leq .01$) treatment effect at two weeks and at ten weeks. At two weeks, the 18% ration was lower than the other treatments. At ten weeks the four percent ration was significantly lower than the other treatments. There was no significant effect for sex or litter.

Livers obtained from those animals on the low protein ration which died during the experiment were smaller and contained significantly more fat ($p \leq .01$) than livers obtained from animals killed at the slaughter plant. Malnourished animals had a mean of 31.24% liver fat compared with a mean of 11.83% for the controls.

d. Carbohydrate Metabolism

Serum glucose levels are presented in Table 30. There was a significant ($p \leq .01$) treatment effect after four, six, and eight weeks, but this disappeared at week ten. After weeks four and six, serum glucose levels appeared to increase with decreasing dietary protein. After week eight, glucose levels were lowest on the 8% ration and highest on the 6% ration. There was a significant ($p \leq .01$) sex effect after two weeks on trial, while litter was not significant during the trial.

e. Water and Electrolyte Metabolism

Mean values for serum calcium are presented in Table 31. There was a significant treatment effect after six weeks on trial. Serum calcium declined with dietary protein level. Sex and litter were not significant.

The results of serum copper analysis are presented in Table 32. No differences in serum copper were picked up until after ten weeks on the trial. After ten weeks, there was a significant treatment effect ($p \leq .01$). Serum copper levels declined with decreasing dietary protein. There was a steady decline until the 8% level was reached, thereafter, serum copper values levelled off. There was a significant ($p \leq .01$) litter effect after ten weeks on trial, while sex effect was not significiant during the entire trial.

Values for serum iron are presented in Table 33. No significant differences were observed for sex, litter, or treatment.

The results of serum magnesium analysis are presented in Table 34. There was a significant treatment effect after six weeks on trial ($p \leq .01$). Serum magnesium values declined with decreasing protein level. There was no significant sex or litter effect.

Mean values for serum phosphate are presented in Table 35. There was a significant treatment effect after two weeks on trial ($p \leq .01$). Serum phosphate values declined with decreasing dietary protein. There was a significant litter effect at the beginning of the trial, while sex effect was not significant during the entire trial.

Mean values for serum zinc are presented in Table 36. There was a significant treatment effect after ten weeks on trial ($p \leq .01$). The eight percent protein ration had significantly higher serum zinc levels than did the other treatments. No sex or litter effect was observed.

The animals on the four percent ration had significantly greater total body water ($p \leq .01$) than did those pigs fed the 18% ration. The three animals on the 4% ration had a mean total body water of 78.71% compared with a mean of 67.84% for the three animals fed the 18% ration.

Table 20. Weekly Feed Consumption (kg) in Trial 2.

Weeks	Dietary Protein Level 18%	10%	8%	6%	4%
1	14.80(8)	12.00(8)	10.60(8)	11.30(8)	12.10(8)*
2	23.20(8)	17.30(8)	14.60(8)	14.50(8)	10.50(8)
3	29.90(8)	19.90(8)	16.30(8)	13.40(8)	11.20(8)
4	37.20(8)	24.70(8)	19.70(8)	16.50(8)	13.30(8)
5	59.80(8)	34.10(8)	24.40(8)	21.40(8)	16.70(8)
6	64.70(8)	37.30(8)	25.60(8)	18.80(8)	11.20(8)
7	73.40(8)	46.10(8)	35.40(8)	22.00(8)	11.40(7)
8	87.90(8)	47.80(8)	35.80(8)	21.00(8)	10.20(7)
9	115.10(8)	58.20(8)	47.10(8)	26.40(8)	9.10(6)
10	89.30(8)	48.70(8)	29.80(8)	17.20(7)	3.60(5)

* Values in parenthesis are average number of animals per pen during the week.

Table 21. Body Weights (kg) of Swine Fed Varying Levels of Protein (Trial 2).[1,2]

Weeks	Dietary Protein Level 18%	10%	8%	6%	4%
0	7.38 ± .22[a]	7.27 ± .24[a]	6.74 ± .39[a]	6.90 ± .49[a]	7.00 ± .50[a]
2	8.79 ± .18[a]	8.06 ± .23[ab]	7.44 ± .45[ab]	7.41 ± .51[ab]	7.04 ± .45[b]
4	12.80 ± .45[a]	9.80 ± .36[b]	8.50 ± .51[b]	7.64 ± .51[c]	7.30 ± .75[c]
6	19.26 ± .85[a]	11.90 ± .51[b]	9.45 ± .50[c]	8.05 ± .56[c]	7.03 ± .59[c]
8	28.53 ±1.22[a]	16.25 ±1.01[b]	12.12 ± .49[c]	8.75 ± .65[d]	7.43 ± .45[d]
10	34.37 ±1.58[a]	20.72 ±1.57[b]	14.45 ± .46[c]	8.80 ± .76[cd]	6.78 ± .55[d]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 22. Serum Total Protein (g/100 ml) in Swine Fed Varying Levels of Protein (Trial 2).[1,2]

Weeks	Dietary Protein Level 18%	10%	8%	6%	4%
0	4.94 ± .05[a]	4.95 ± .11[a]	4.86 ± .04[a]	4.91 ± .07[a]	5.01 ± .11[a]
2	4.44 ± .08[a]	4.23 ± .09[a]	4.24 ± .10[a]	4.28 ± .13[a]	4.15 ± .11[a]
4	4.73 ± .10[a]	4.18 ± .12[bc]	4.15 ± .08[bc]	4.23 ± .12[b]	3.71 ± .07[c]
6	5.21 ± .13[a]	4.25 ± .16[b]	4.21 ± .11[b]	4.20 ± .13[b]	3.67 ± .11[b]
8	5.64 ± .11[a]	4.35 ± .15[b]	4.25 ± .11[b]	4.08 ± .20[bc]	3.40 ± .08[c]
10	5.31 ± .17[a]	4.26 ± .16[b]	4.31 ± .16[b]	3.93 ± .33[b]	3.28 ± .13[b]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 23. Serum Albumin Levels (g/100 ml) in Swine Fed Varying Levels of Protein (Trial 2).[1,2]

Weeks	Dietary Protein Level				
	18%	10%	8%	6%	4%
0	3.18 ± .08[a]	3.18 ± .13[a]	3.04 ± .12[a]	3.15 ± .10[a]	3.21 ± .10[a]
2	2.46 ± .09[a]	2.29 ± .07[a]	2.30 ± .09[a]	2.31 ± .08[a]	2.28 ± .09[a]
4	2.26 ± .11[a]	1.86 ± .11[b]	1.77 ± .08[b]	1.76 ± .08[b]	1.67 ± .08[b]
6	2.41 ± .11[a]	1.51 ± .09[b]	1.44 ± .09[b]	1.28 ± .06[b]	1.24 ± .08[b]
8	3.17 ± .10[a]	1.83 ± .08[b]	1.61 ± .08[bc]	1.40 ± .07[c]	1.23 ± .06[c]
10	2.70 ± .19[a]	1.86 ± .11[b]	1.45 ± .08[b]	1.26 ± .11[b]	1.12 ± .11[b]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 24. Blood Urea Nitrogen Levels (mg/100 ml) in Swine Fed Varying Protein Levels (Trial 2).[1,2]

Weeks	Dietary Protein Level 18%	10%	8%	6%	4%
0	11.75 ± 1.21[a]	11.87 ± 1.04[a]	11.62 ± 1.22[a]	12.25 ± 1.32[a]	12.75 ± 1.28[a]
2	12.62 ± 1.31[a]	9.25 ± 0.78[c]	11.12 ± 0.96[a]	9.63 ± 0.78[a]	10.00 ± 0.73[a]
4	12.00 ± 0.54[a]	13.62 ± 0.97[a]	13.71 ± 0.77[a]	13.00 ± 0.95[a]	13.43 ± 1.36[a]
6	12.87 ± 0.58[a]	10.62 ± 1.00[ab]	9.86 ± 0.85[ab]	8.63 ± 0.63[b]	8.86 ± 0.80[ab]
8	16.14 ± 0.85[a]	12.00 ± 1.24[ab]	10.37 ± 0.78[b]	14.37 ± 1.17[ab]	9.83 ± 0.65[b]
10	11.25 ± 1.27[a]	13.00 ± 1.87[a]	9.75 ± 0.92[a]	11.83 ± 1.11[a]	9.60 ± 1.16[a]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 25. Serum Amylase Levels (Somogyi Units/100 ml) in Swine Fed Varying Levels of Protein (Trial 2).[1,2]

Weeks	Dietary Protein Level				
	18%	10%	8%	6%	4%
0	228.6±17.1[a]	242.1±21.6[a]	236.9±25.7[a]	276.4±29.1[a]	271.2±29.1[a]
2	348.8±18.6[a]	367.6±31.6[a]	356.8±20.2[a]	325.8±31.5[a]	330.7±26.9[a]
4	379.0±12.7[a]	336.4±16.1[ab]	347.9±31.6[ab]	292.8±26.4[b]	283.1±33.6[b]
6	446.7±16.8[a]	406.9±26.3[ab]	377.8±25.7[ab]	325.7±22.8[b]	285.0±28.5[b]
8	374.0±19.3[a]	333.4±26.0[abc]	342.3±15.6[ab]	257.4±22.0[bc]	217.4±24.3[c]
10	444.1±34.5[a]	438.5±22.0[ab]	448.9±26.6[a]	309.4±29.9[bc]	228.6±21.9[c]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 26. Serum Alkaline Phosphatase (m I.U./ml) in Swine Fed Varying Levels of Protein (Trial 2).[1,2]

Weeks	Dietary Protein Level 18%	10%	8%	6%	4%
0	183.1±16.6^{a}	190.6±20.3^{a}	179.4±17.4^{a}	184.9±19.8^{a}	184.4±15.4^{a}
2	130.6± 7.2^{a}	152.7±13.5^{a}	125.9± 9.2^{a}	125.0± 5.1^{a}	115.9± 7.9^{a}
4	134.7± 9.6^{a}	124.2± 9.9^{ab}	119.6± 9.5^{ab}	91.6± 9.7^{bc}	76.9± 5.2^{c}
6	138.5± 9.0^{a}	134.1± 9.9^{a}	123.9±13.4^{ab}	79.6± 9.1^{bc}	49.1± 6.1^{c}
8	119.3± 5.9^{ab}	145.1± 9.4^{a}	149.9±13.6^{a}	95.0±11.7^{bc}	49.67±4.3^{c}
10	97.9± 9.7^{ac}	177.9±13.8^{a}	272.9±26.73^{b}	112.8±21.9^{ac}	43.8± 5.1^{c}

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 27. Serum Glutamic Oxaloacetic Transaminase (Karmen Units/ml) in Swine Fed Varying Levels of Protein (Trial 2).[1,2]

Weeks	Dietary Protein Level 18%	10%	8%	6%	4%
0	71.3 ± 0.1[a]	68.6 ± 5.0[a]	68.0 ± 5.9[a]	66.1 ± 3.2[a]	82.9 ± 5.8[a]
2	41.6 ± 2.9[a]	50.3 ± 2.6[a]	45.1 ± 2.6[a]	42.3 ± 3.9[a]	46.4 ± 4.4[a]
4	57.1 ±10.2[a]	63.5 ±10.5[a]	49.3 ± 4.7[a]	45.5 ± 2.5[a]	60.7 ± 6.0[a]
6	51.1 ± 4.1[a]	52.2 ± 5.8[a]	48.3 ± 4.8[a]	48.1 ± 7.0[a]	55.7 ±13.8[a]
8	52.6 ± 4.0[a]	44.9 ± 3.8[a]	44.1 ± 2.9[a]	43.4 ± 2.5[a]	40.17 ± 5.41[a]
10	48.1 ± 4.1[a]	50.4 ± 2.8[a]	49.8 ± 6.1[a]	38.7 ± 3.6[a]	60.0 ± 16.85[a]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 28. Serum Lactic Dehydrogenase (Wroblewski Units/ml) in Swine Fed Varying Levels of Protein (Trial 2).[1,2]

Weeks	Dietary Protein Level 18%	10%	8%	6%	4%
0	727.5±86.0[a]	600.2±46.3[a]	711.9±56.2[a]	810.7±100.9[a]	740.2±33.3[a]
2	386.4±21.6[a]	445.5±18.9[a]	464.5±16.1[a]	450.7±23.0[a]	402.1±9.5[a]
4	393.4±25.2[a]	661.6±126.9[a]	426.9±22.8[a]	430.0±21.8[a]	518.9±60.5[a]
6	393.0±17.3[a]	429.1±21.7[a]	404.3±26.9[a]	454.6±48.4[a]	462.0±74.2[a]
8	376.9±23.2[a]	395.6±15.7[a]	431.7±17.6[a]	408.6±23.7[a]	410.2±33.2[a]
10	415.2±25.3[a]	473.5±16.7[a]	666.7±81.4[a]	598.7±63.2[a]	736.4±124.3[b]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 29. Serum Cholesterol Levels (mg/100 ml) in Swine Fed Varying Levels of Protein (Trial 2).[1,2]

Weeks	Dietary Protein Level				
	18%	10%	8%	6%	4%
0	94.00 ± 6.62[a]	95.62 ± 5.49[a]	104.70 ±10.68[a]	87.50 ±4.56[a]	101.70± 11.38[a]
2	81.50 ± 3.88[a]	87.75 ± 2.96[ab]	91.37 ± 5.03[ab]	105.50± 5.03[b]	98.25± 4.05[ab]
4	95.62 ± 4.78[a]	105.00± 6.32[a]	105.30± 4.88[a]	113.00± 7.53[a]	109.10± 4.40[a]
6	101.00± 3.52[a]	117.10± 5.24[a]	112.30± 6.66[a]	118.70± 6.21[a]	109.10± 6.80[a]
8	117.10± 6.01[a]	133.4 ± 6.67[a]	129.90± 8.73[a]	117.60± 7.84	103.2 ± 7.45[a]
10	105.70± 2.94[a]	115.10± 6.68[a]	123.40± 3.27[a]	103.80± 5.90[a]	65.20 ± 6.47[b]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 30. Serum Glucose Levels (mg/100 ml) in Swine Fed Varying Levels of Protein (Trial 2).[1,2]

Weeks	Dietary Protein Level 18%	10%	8%	6%	4%
0	109.5±3.7[a]	102.4±5.8[a]	106.1±5.9[a]	99.8±4.2[a]	108.0±3.9[a]
2	86.5 ±5.3[a]	75.5±2.6[a]	88.3±6.5[a]	88.4±5.4[a]	89.9±4.9[a]
4	66.8 ±2.9[a]	63.6±2.8[a]	76.6±3.7[ab]	94.0±8.4[b]	85.1±5.3[ab]
6	75.5 ±2.0[ab]	71.5±2.0[ab]	66.3±2.1[a]	73.9±3.0[ab]	83.1±4.6[b]
8	79.4 ±3.9[ab]	70.9±2.4[ab]	62.3±2.2[a]	83.2±5.5[b]	72.0±6.83[ab]
10	77.8 ±2.7[a]	70.4±4.1[a]	68.4±7.6[a]	67.2±3.8[a]	70.8±9.6[a]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 31. Serum Calcium Levels (mg/100 ml) in Swine Fed Varying Levels of Protein (Trial 2).[1,2]

Weeks	Dietary Protein Level 18%	10%	8%	6%	4%
0	9.54±.07[a]	9.48±.11[a]	9.41±.17[a]	9.34±.10[a]	9.51±.10[a]
2	9.46±.08[a]	9.03±.09[a]	9.14±.17[a]	8.98±.20[a]	9.21±.16[a]
4	9.72±.15[a]	9.34±.15[a]	9.33±.14[a]	9.46±.16[a]	9.11±.16[a]
6	9.95±.12[a]	8.95±.12[b]	8.77±.06[bc]	8.25±.17[c]	8.39±.20[bc]
8	10.69±.12[a]	9.25±.12[b]	9.00±.15[b]	8.77±.14[bc]	8.14±.14[c]
10	10.04±.27[a]	9.10±.06[b]	8.64±.05[bc]	8.52±.22[b]	7.72±.28[c]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 32. Serum Copper Levels (ug/100 ml) in Swine Fed Varying Levels of Protein (Trial 2).[1,2]

Weeks	Dietary Protein Level 18%	10%	8%	6%	4%
0	162.0±8.8[a]	146.1±8.6[a]	157.0±13.1[a]	161.9±10.2[a]	166.7±16.2[a]
6	154.9±5.8[a]	107.9±4.2[a]	130.4±18.3[a]	119.5±10.6[a]	133.0±24.9[a]
10	220.4±9.1[a]	158.0±12.0[b]	93.3±13.5[c]	121.0±14.6[bc]	136.8±15.3[bc]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 33. Serum Iron Levels (ug/100 ml) in Swine Fed Varying Levels of Protein (Trial 2).[1,2]

Weeks	Dietary Protein Level 18%	10%	8%	6%	4%
0	217.7±15.2[a]	215.6±21.9[a]	177.0±17.4[a]	215.4±16.3[a]	239.8±28.1[a]
6	170.6±11.1[a]	124.5±8.5[a]	146.0±13.1[a]	169.4±26.5[a]	106.5±4.0[a]
10	181.0±19.4[a]	148.7±7.3[a]	151.6±24.1[a]	142.3±20.7[a]	141.4±31.4[a]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 34. Serum Magnesium Levels (mg/100 ml) in Swine Fed Varying Levels of Protein (Trial 2).[1,2]

Weeks	Dietary Protein Level				
	18%	10%	8%	6%	4%
0	2.37±.18[a]	2.96±.16[a]	2.81±.11[a]	2.85±.13[a]	2.56±.17[a]
6	2.51±.12[a]	2.28±.15[ab]	1.79±.20[abc]	1.74±.11[bc]	1.39±.11[c]
10	2.75±.17[a]	2.68±.04[a]	2.27±.09[a]	2.15±.25[ab]	1.33±.08[b]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 35. Serum Phosphate Levels (mg/100 ml) in Swine Fed Varying Levels of Protein (Trial 2).[1,2]

Weeks	Dietary Protein Level 18%	10%	8%	6%	4%
0	7.49±.16[a]	7.74±.13[a]	7.64±.17[a]	7.54±.13[a]	7.76±.22[a]
2	8.03±.27[a]	7.85±.17[ab]	7.30±.17[ab]	7.16±.19[ab]	7.03±.16[b]
4	9.05±.28[a]	8.13±.18[b]	8.10±.10[b]	7.53±.26[bc]	6.90±.16[c]
6	8.74±.14[a]	7.75±.11[b]	7.10±.16[bc]	6.71±.27[c]	6.40±.16[c]
8	8.81±.25[a]	8.19±.12[ab]	7.66±.21[bc]	7.06±.26[c]	5.78±.17[d]
10	8.74±.20[a]	8.51±.12[a]	8.13±.22[a]	6.52±.48[b]	5.90±.11[b]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

Table 36. Serum Zinc Levels (ug/100 ml) in Swine Fed Varying Levels of Protein (Trial 2).[1,2]

Weeks	Dietary Protein Level				
	18%	10%	8%	6%	4%
0	186.6±20.7[a]	198.9±32.7[a]	193.7±21.8[a]	214.0±23.6[a]	214.0±20.4[a]
6	213.0±11.0[a]	200.3±22.9[a]	176.0±21.6[a]	141.4±15.0[a]	149.7±18.9[a]
10	188.5±27.4[ab]	176.9±14.3[a]	260.0±21.5[b]	179.3±14.1[a]	106.0±6.6[a]

1 Values are Means ± Standard Error of the Mean.

2 Values within rows with the same superscripts are not significantly different ($p \leq .01$).

C. DISCUSSION

This trial had two objectives. The first was to try and determine at what level of protein intake PCM develops. Utilizing the clinical criteria of growth retardation, abnormal hair texture, emaciation, and apathy it was possible to class animals on the 4% and 6% rations as having developed PCM. The second objective was to find the most sensitive biochemical index of the nutritional state of the animal. To be of value as an index of the nutritional state of the animal, biochemical parameters must fulfill three criteria. First, it must have a high correlation with dietary protein intake. Second, it must be sensitive enough to protein deficiency to allow significant treatment effects to be observed relatively early. Finally, it must be able to discriminate between those animals on a marginal protein intake (8% and 10%) and those animals with PCM (4% and 6%).

In this trial, many of the same parameters were measured as in trial one, and for the most part, similar results were obtained. Discussion of the physiological significance of these parameters and the changes that occur in PCM were presented following data for trial one and will not be repeated here. Discussion of these parameters will be limited to the effect of protein intake on the development of symptoms of PCM. However, several new parameters were measured in this trial, and the significance of these results will be discussed in the following pages.

a. Protein Metabolism

Total serum proteins declined with decreasing dietary protein intake (r^2 = .92 at week ten). Significant differences between treatments

for serum total proteins occurred early in the trial. However, the measurement of serum total proteins was not sensitive enough to allow one to differentiate between animals on the 10%, 8%, 6% or 4% rations. Therefore, total serum proteins would not appar to be a good indicator of the nutritional status of the animal.

There was a significant linear relationship between dietary protein intake and serum albumin levels (r^2 = .98 at week ten). However, as with total proteins, serum albumin did not appear to be sensitive enough to allow one to differentiate between PCM animals and those on marginal protein intake.

There was little correlation between levels of BUN and dietary protein intake (r^2 = .09 at week ten). This is rather surprising as it is believed that the level of serum urea is proportional to the protein intake (Addis et al., 1947). However, similar results were obtained in trial one, and it is possible that increased catabolism of body proteins could explain the observed values.

Serum amylase levels declined with decreasing dietary protein intake (r^2 = .50 at week ten). Significant treatment differences appeared as early as four weeks. After ten weeks, it was possible to differentiate between animals on the 8%, 6% and 4% rations. It is felt that the measurement of serum amylase is a good indicator of the nutritional status of the animal.

The results of the analysis for alkaline phosphatase are very difficult to explain. There was no correlation between alkaline phosphatase levels and dietary protein intake (r^2 = .001 at week ten). However, as in trial one, the four percent ration had significantly lower

($p \leq .01$) alkaline phosphatase levels than did the eighteen percent group. Why the eight percent ration had significantly higher alkaline phosphatase activity after ten weeks is not known. However, serum zinc levels were also elevated in animals on the eight percent ration. Li (1966) reported that zinc was required for the activity of alkaline phosphatase and it is possible that the elevated zinc levels are responsible for the elevation in serum alkaline phosphatase.

SGOT did not correlate with dietary protein intake ($r^2 = -.03$ at week ten). No significant differences were picked up between treatments and therefore, SGOT would be of little value as an indicator of the nutritional status of the animal.

Lactic dehydrogenase levels correlated fairly well with dietary protein intake ($r^2 = -.75$ at week ten). However, no significant treatment effects were observed until after ten weeks on trial. It was felt that LDH was not sensitive enough to be of value as an indicator of protein status.

b. Fat Metabolism

Serum cholesterol levels did not correlate well with dietary protein intake ($r^2 = .17$ at week ten). No significant treatment effects were observed until after ten weeks on trial. It was therefore felt that serum cholesterol was not a good indicator of the protein status of the animal.

Fatty infiltration of the liver is an important feature of human PCM. The pattern of fat distribution is quite characteristic of the syndrome (Davis, 1948). Quantitative lipid analysis in the present study

showed significantly higher fat concentrations in the malnourished pigs. This is in agreement with the work of Gupta (1973a) in malnourished Indian pigs.

The mechanism of this fatty condition is not clearly understood. One of the factors associated with the increase in the liver lipid is an increase in the serum free fatty acids (Jaya Rao and Prasad, 1966). Synthesis of triglycerides from carbohydrates may be another factor (Fletcher, 1966). However, it now appears that the lipids cannot be released from the liver because of low concentrations of beta lipoproteins, and that this is probably the result of reduced hepatic synthesis of the protein moiety of the lipoprotein (Truswell and Hansen, 1969).

c. Carbohydrate Metabolism

Serum glucose levels did not correlate with dietary protein intake (r^2 = .048 at week eight). In addition, no significant differences for treatments were picked up after ten weeks. Some treatment effects were observed at weeks 4, 6 and 8 but they would appear to be the result of something other than protein intake. The measurement of serum glucose is of little value as an indicator of the protein status of the animal.

d. Water and Electrolyte Metabolism

Total body water, expressed as a percentage of body weight, is consistently increased in PCM (Smith, 1960; Brinkman et al., 1965; Flynn et al., 1967). In the present study, pigs on the 4% protein ration

had significantly higher total body water than did those animals on the 18% ration. The actual significance of this increase is difficult to ascertain because of differences in body fat between the two treatments. It was unfortunate that determinations on extracellular water were not performed.

Serum calcium levels correlated extremely well with dietary protein intake (r^2 = .96 at week ten). Significant treatment effects were observed after six weeks on trial. However, it was not felt that the measurement of serum calcium was sensitive enough to allow one to differentiate between PCM animals and those on marginal protein intake.

Serum copper levels have been shown to be reduced in human PCM (Lahey et al., 1958; Gopalan et al., 1963). In the present study there was a moderate correlation between the levels of serum copper and dietary protein intake (r^2 = .67 at week ten).

It is possible that copper absorption on the lower protein diets was poor. Copper is absorbed mainly from the small intestine and colon in pigs (Bowland et al., 1961), and there is evidence of morphological alterations in the intestinal mucosa of experimentally malnourished pigs (Platt et al., 1964).

A copper binding protein has been demonstrated by Starcher (1969) in the mucosal cells of the duodenum of the chick which plays a role in copper absorption. If a similar mechanism exists in swine, reduced synthesis is possible in much the same manner as the calcium binding protein in protein deficient rats (Kalk and Pimstone, 1974).

It is known that copper is excreted in the bile (Cartwright and Wintrobe, 1964) and there was a possibility of excessive loss of bilary

contents in the presence of diarrhea, resulting in lower circulating copper levels.

It is interesting to note the low serum copper levels in the 8% protein group in combination with the high serum zinc levels. Zinc is believed to have an antagonistic effect on copper absorption (Van Campen and Scaife, 1967).

Although serum copper correlated relatively well with dietary protein intake, it was not possible to differentiate between those animals exhibiting clinical signs of PCM and those on a marginal protein intake. Therefore, serum copper is not a reliable indicator of the nutritional status of the animal.

Many workers have reported lower iron and iron binding capacity in the serum of human PCM patients (Edozien and Udeozo, 1960; Lahey et al., 1958; El Sholmy et al., 1962). In the present study, serum iron correlated extremely well with the dietary intake of protein (r^2 = .92). However, although there was a good correlation, there were no significant treatment effects. The lack of significance would seem to be explained by the relatively high standard errors associated with the treatment means. In addition there are several other explanations for this apparent discrepancy between human and animal studies.

There is evidence that some of the human pre-PCM diets are deficient in iron (Metz and Stein, 1959). In the present study, NRC recommendations for dietary iron in growing swine were followed and therefore a true iron deficient diet did not exist.

Iron is transported in serum completedly bound to transferrin (Holmberg and Laurell, 1947). In normal individuals, only 30-40% of the

transferrin carries iron, the remainder of the transferrin being known as the latent iron binding capacity (Underwood, 1971). In light of the reduced serum proteins observed, it would appear as though the protein deficient pigs had a higher percentage iron saturation.

The animal body has a relatively large storage capacity for iron in the form of either ferritin or hemosiderin. It is possible that due to the short duration of the trial, serum iron levels were still being maintained at the expense of body stores.

There is evidence that vitamin C increases the efficiency of iron absorption in man (Pirzio-biroli et al., 1958). It is believed that ascorbic acid aids in the reduction of ferric to ferrous iron. Andersson et al. (1956) reported a low dietary intake of ascorbic acid in malnourished South African Bantu children and it was suggested that this low intake might explain the lower serum iron levels in PCM. It is interesting to note that pigs do not have a dietary requirement for vitamin C as it is believed that they can synthesize adequate amounts of vitamin C for their needs (National Academy of Science, 1973).

Malnourished children are subject to parasitic infestations which act as additional drains on the bodies supply of iron. As a result of the concrete floor in the swine unit and periodic deworming as a management technique, the existence of parasites in the test animals is not likely.

Magnesium deficiency appears to occur relatively frequently in human PCM. Linder et al. (1963) reported low serum magnesium values in patients with PCM. In the present study, magnesium values declined with decreasing dietary protein intake (r^2 = .64 at week ten). However, it was

not felt that serum magnesium was sensitive enough to protein intake to be of much value as an indicator of nutritional status.

According to Caddell and Goddard (1967) the magnesium deficiency in malnutrition results from prolonged losses of magnesium through the GI tract during diarrhea and vomiting coupled with a low magnesium intake.

In serum, magnesium is bound to albumin and alpha globulin with a majority associated with the former. In addition, a small part of the serum magnesium is also combined with phospholipids in the form of a colloidal phosphate complex. In light of the lower serum proteins observed in the present trial, it is possible that this could account for the lowering of serum magnesium observed.

An over production of aldosterone is accompanied by hypomagnesemia and a negative magnesium balance (Milne et al., 1957). It is believed that the negative balance is due to renal losses that cause a reduction in circulating magnesium levels. Beitins et al. (1974) measured the plasma aldosterone levels in malnourished children and reported that they were higher than those of a control group.

Lower serum zinc levels have been observed in human protein calorie malnutrition (Kumar and Jaya Rao, 1973; Sandstead et al., 1965). In addition, Platt and Frankel (1962) observed lower serum zinc levels in malnourished pigs. The results of the analysis for serum zinc in the present trial are difficult to explain. Van Campen and House (1974) reported that the protein level of the diet affected zinc absorption. For this reason, it was believed that serum zinc levels would decline with decreasing protein intake. Contrary to expectations, there was very little correlation

between serum zinc and dietary protein intake (r^2 = .09 at week ten). The 8% group had significantly higher serum zinc than did the other treatments, though while not significant, the four percent ration appeared to have lower serum zinc levels.

The loss of zinc into the intersitial compartment is a factor which may have contributed to the lower serum zinc values on the 4% ration (Kumar and Jaya Rao, 1973). Animals on the 4% diet had significantly higher total body water than did those animals on the 18% ration. Unfortunately, extracellular body water was not determined in the present study. However, it is probable that at least a portion of the observed increase in total body water was extracellular.

Skeletal abnormalities are a regular and conspicuous feature of zinc deficiency (Miller et al., 1968). It is possible that the disturbances of gait observed in the low protein diets are related to the lower serum zinc levels observed.

SUMMARY AND CONCLUSIONS

There is general agreement by workers engaged in the study of protein calorie malnutrition that two distinct clinical forms can be recognized. Kwashiorkor is primarily a condition of early life (1-3 years) but older children and adults may be affected whenever there is prolonged consumption of a diet low in protein and high in carbohydrate. Kwashiorkor is characterized clinically by retardation of growth and development, loss of weight with muscular wasting, edema, fatty liver, abnormal hair texture, and apathy. Marasmus is primarily a disease of infancy (less than one year) and is associated with diets that are low in protein and energy. Marasmus is characterized by retardation of growth and development, loss of weight with severe muscular wasting, and a minimum of changes in blood composition.

An attempt was made in this study to reproduce the symptoms of the "kwashiorkor" type of PCM in the baby pig. Not every lesion occurring in man was reproduced in the present study. However, because of the multifaceted nature of the disease, it is doubtful if the entire gamut of lesions could ever be reproduced. It is difficult to expect a research animal to develop all of the known lesions, when man from one geographical area often does not exhibit the same lesions which may be characteristic of the disease in another region or country. PCM is a disease compounded by deficiencies of vitamins and minerals, which in combination with concurrent infections, can alter the clinical and metabolic pattern of PCM in an endless array of patterns.

Nevertheless, characteristic symptoms such as the development of fatty liver, growth retardation, abnormal hair texture and apathy were reproduced in protein deficient swine. In addition, most of the biochemical parameters which are altered in the human condition were altered in the protein deficient swine. It is therefore concluded that the baby pig is a good model with which to study protein-calorie malnutrition.

The prognosis of PCM is often difficult to assess on clinical grounds alone and various attempts have been made to find an accurate biochemical index of developing PCM. It was hoped that this experiment would provide some insight into the most sensitive index of the nutritional state of the animal. Such parameters as zinc, alkaline phosphatase, serum glutamic oxaloacetic transaminase, cholesterol, glucose, and blood urea nitrogen can be discarded as indicators since no correlation was observed between these parameters and dietary protein intake. Lactic dehydrogenase and iron did not appear to be sensitive enough to protein deficiency since significant treatment effects occurred only after ten weeks of trial. Copper, total protein, albumin, calcium and magnesium all correlated well with dietary protein intake but failed to differentiate between those animals on marginal protein intake and those developing PCM. This leaves amylase and serum phosphate as being the only parameters which met the three criteria for a diagnostic index.

Serum phosphate was the first parameter to be affected by diminishing dietary protein intake and as such these results were rather unexpected. Unfortunately, hypophosphatemia occurs in other conditions such as Addison's disease, Fanconi's syndrome and multiple myeloma. This holds true for other parameters as well and it is doubtful if any one index can be considered as being characteristic of a certain disease.

Under the conditions of the current trial, serum phosphate and serum amylase would appear to be the best biochemical indices of the nutritional status of the protein deprived pig. However, because of the many factors affecting the serum levels of these parameters, it is important that they be used in conjunction with clinical criteria in diagnosing developing PCM.

It is concluded that the young pig could be effectively used for research on the PCM snydrome as it occurs in humans. For example, studies of therapeutic dietary regimes for treating the condition might well be undertaken with this species.

BIBLIOGRAPHY

Addis, T., E. Barrett, L.J. Poo and D.W. Yeun. 1947. The relationship between serum urea concentrations and the protein consumption of normal individuals. J. Clin. Invest. 26: 869-874.

Alleyne, G.A. 1967. The effect of severe protein-calorie malnutrition on the renal function of Jamaican children. Pediatrics 39: 400-411.

Alleyne, G.A. and V.H. Young. 1967. Adrenocortical function in children with severe protein-calorie malnutrition. Clin. Sci. 33:189-200.

Alleyne, G.A. and G.H. Scullard. 1968. Alterations in carbohydrate metabolism in Jamaican children with severe malnutrition. Clin. Sci. 37: 631-641.

Andersson, M., A.R. Walker and H.C. Falcki. 1956. An investigation of the rarity of infantile scurvy among South African Bantu. Brit. J. Nutr. 10: 101-105.

Anthony, L.E. and J.C. Edozien. 1975. Experimental protein and energy deficiencies in the rat. J. Nutr. 105: 631-648.

Arroyave, C., D. Wilson, C. De Funes and M. Behar. 1962. The free amino acids in blood plasma of children with kwashiorkor and marasmus. Amer. J. Clin. Nutr. 11: 517-524.

Association of Official Agricultural Chemists. 1975. Official Methods of Analysis. 12th Edition, Washington, D.C.

Atinmo, T., C. Baldijao, W.G. Pond and R.H. Barnes. 1976a. Plasma insulin levels in weaned pigs fed protein or energy restricted diets. J. Nutr. 106: 1654-1658.

Atinmo, T., C. Baldijao, W.G. Pond and R.H. Barnes. 1976b. Immunoreactive growth hormone levels in pigs fed protein or energy restricted diets during the postweaning period. J. Nutr. 106: 947-951.

Badger, T.M., M.E. Tumbleson and D.P. Hutcheson. 1972. Protein-Calorie Malnutrition in young miniature swine. Growth 36: 235-245.

Badger, T.M. and M.E. Tumbleson. 1974. Protein Calorie Malnutrition in young miniature swine. Serum free amino acids. J. Nutr. 104: 1339-1347.

Baertl, J.M., R.P. Placko and G.C. Graham. 1974. Serum proteins and plasma free amino acids in severe malnutrition. Amer. J. Clin. Nutr. 27: 733-742.

Baez, S., A. Mazur and E. Shorr. 1952. Role of the neurohypophysis in ferritin induced antidiuresis. Amer. J. Physiol. 169: 123-133.

Baig, H.A. and J.C. Edozien. 1965. Carbohydrate metabolism in kwashiorkor. Lancet 2: 662-665.

Baldijao, C., T. Atinmo, W.G. Pond and R.H. Barnes. 1976. Plasma adrenocorticosteroid levels in protein and energy restricted pigs. J. Nutr. 106: 952-957.

Barbezat, G.O., M.D. Brown, R.O. Kascula and J.D. Hansen. 1967. Studies on the intestinal mucosa of children with protein-calorie malnutrition. S. Afr. Med. J. 41: 1031-1036.

Barbezat, G.O. and J.D. Hansen. 1968. The exocrine pancreas and protein-calorie malnutrition. Pediatrics 42: 77-92.

Barclay, G.P. 1973. Pseudocholinesterase activity as a guide to prognosis in malnutrition. Amer. J. Clin. Pathol. 59: 712-716.

Barnes, R.H., A.U. Moore and W.G. Pond. 1970. Behavioral abnormalities in young adult pigs caused by malnutrition in early life. J. Nutr. 100: 149-155.

Barnes, R.H., W.G. Pond, E. Kwong and I. Reid. 1966. Effect of severe protein-calorie malnutrition in the baby pig upon relative utilization of dietary proteins. J. Nutr. 89: 355-364.

Baron, D.N. 1960. Serum transaminases and isocitric dehydrogenases in kwashiorkor. J. Clin. Pathol. 13: 252-255.

Becker, D.J., B.L. Pimstone, J.D. Hansen, B. MacHutchon and A. Drysdale. 1972. Patterns of insulin response to glucose in protein-calorie malnutrition. Amer. J. Clin. Nutr. 25: 499-500.

Beitins, I.Z., G.C. Graham, A. Kowarski and C.J. Nigeon. 1974. Adrenal function in normal infants and in marasmus and kwashiorkor. Plasma aldosterone concentrations and aldosterone secretion rate. J. Pediat. 84: 444-451.

Bjerring, J.H., M. Grieg and J. Halm. 1975. UBC BMD:10V. General Linear Hypothesis. Computing Centre. The University of British Columbia, Vancouver.

Bjornesjo, K.B., M. Belew and B. Zaar. 1965. Biochemical study of advanced protein malnutrition in Ethiopia. Scad. J. Clin. Lab. Invest. 18: 591-602.

Black, D.A. and M.D. Milne. 1952. Experimental potassium depletion in man. Clin. Sci. 11: 397-415.

Blackburn, W.R. and K. Vinijchaikul. 1969. The pancreas in kwashiorkor. An electron microscopic study. Lab. Invest. 20; 305-318.

Bonjour, J.P. and R.L. Malvin. 1970. Stimulation of ADH release by the renin angiotensinn system. Amer. J. Physiol. 218; 1555-1559.

Bowie, M.D. 1964. Intravenous glucose tolerance in kwashiorkor and marasmus. S. Afr. Med. J. 38; 328-329.

Bowland, J.P., R. Braude, R.F. Chamberlain, R.F. Glascock and K.G. Mitchell. 1961. The absorption, distribution, and excretion of labelled copper in young pigs given different quantities as sulfate or sulfide, orally or intravenously. Brit. J. Nutr. 15; 59-72.

Bradfield, R.B., T. Yee and J.M. Raertl. 1969. Hair zinc levels of Andean Indian children during Protein Calorie Malnutrition. Amer. J. Clin. Nutr. 22; 1349-1353.

Bradfield, R.B. 1973. Protein-calorie malnutrition diagnosis by hair tissue; a review. Prep. Prediat. 19; 370-375.

Brinkman, G.L., M.D. Bowie, B. Friss-Hansen and J.D. Hansen. 1965. Body water composition in kwashiorkor before and after loss of edema. Pediatrics 36; 94-103.

Brown, C.M. 1962. Enzymic synthesis of thiamine. Ann. N.Y. Acad. Sci. 98; 479-485.

Burks, M.F., P.S. Evans, and M.E. Tumbleson. 1974. Hematologic values of malnourished sinclair miniature swine. Lab Anim. Sci. 24; 84-89.

Bustad, K.K. 1966. Pigs in the laboratory. Sci. Amer. 214; 94-100.

Caddell, J.L. 1965. Magnesium in the therapy of Protein Calorie Malnutrition of childhood. J. Pediat. 66; 392-413.

Caddell, J.L. 1966. Magnesium therapy in Protein Calorie Malnutrition. J. Trop. Pediat. 12; 23-25.

Caddell, J.L. 1967. Studies in Protein Calorie Malnutrition. II. A double-blind clinical trial to assess magnesium therapy. N. Eng. J. Med. 276; 535-540.

Caddell, J.L. and D.R. Goddard. 1967. Studies in Protein Calorie Malnutrition. I. Chemical evidence for magnesium deficiency. N. Eng. J. Med. 276; 533-535.

Cartwright, G.E. and M. Wintrobe. 1964. Copper metabolism in normal subjects. Amer. J. Clin. Nutr. 14; 224-232.

Cassar, J. and S. Joseph. 1969. Alkaline phosphatase levels in thyroid disease. Clin. Chim. Acta. 23; 33-37.

Chattopadhyay, S. and P.K. Banerjee. 1975. Serum iron and iron binding capacity in Protein Calorie Malnutrition. J. Indian Med. Assoc. 65; 101-102.

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

Cori, G.T. and M.W. Slein. 1947. Gluco-and fructokinase in mammalian tissues. Fed. Proc. 6; 245-246.

Coward, W.A., R.G. Whitehead and D.G. Coward. 1972. Quantitative changes in serum protein fractions during the development of kwashiorkor and in recovery. Brit. J. Nutr. 28; 433-441.

Coward, W.A. and R.G. Whitehead. 1972a. Changes in serum beta lipoprotein concentrations during the development of kwashiorkor and in recovery. Brit. J. Nutr. 27; 383-393.

Coward, D.G. and R.G. Whitehead. 1972b. Experimental protein-energy malnutrition in baby baboons. Brit. J. Nutr. 28; 223-237.

Cravioto, J., C.L. De la Pina and G. Burgos. 1959. Fat mobilization in chronic, severe malnutrition. Lipoprotein in children with kwashiorkor. Metab. Clin. Exp. 8; 722-729.

Davis, J.M. 1948. Essential pathology of kwashiorkor. Lancet 1: 317-320.

Dean, R.F. 1965. Kwashiorkor. P. 234-265. In: D. Gairdner (ed.) Recent Advances in Pediatrics. Little, Brown and Co., Boston.

Dean, R.F. and R. Schwartz. 1953. Serum chemistry in uncomplicated kwashiorkor. Brit. J. Nutr. 7; 131-147.

De Bono, E., G. Lee, F.R. Nottram, G.W. Pickering, J.J. Brown, H. Keen, W.S. Beart and P.M. Sanderson. 1963. The action of angiotensin in man. Clin. Sci. 25; 123-157.

Deo, M.G., S.K. Sood and V. Ramalingaswami. 1965. Experimental protein deficiency. Arch. Pathol. 80; 14-23.

Douglas, W.R. 1972. Of pigs and men and research. Space life Sci. 3; 226-234.

Durbin, P., C. Heard and S. Platt. 1959. Glycogen and glucose-6-phosphatase in the liver of normal and protein deficient pigs. Proc. Nutr. Soc. 19; xxxv.

Edozien, J.C. 1961. Enzymes in serum in kwashiorkor. Pediatrics 27: 325-333.

Edozien, J.C. and M.A. Rahim-Khan. 1968. Anemia in protein malnutrition. Clin. Sci. 34: 315-326.

Edozien, J.C., E.J. Philips, and W.R. Collis. 1960. The free amino acids of plasma and urine in kwashiorkor. Lancet 1: 615-618.

Edozien, J.C. and I.O. Udeozo. 1960. Serum copper, iron, and iron binding capacity in kwashiorkor. J. Trop. Pediat. 6: 60-64.

El-Shobaki, F.A., M.F. El-Hawary, S.P. Morocos, M.K. Abdelkhalek, K. El-Zawahry and R. Sakr. 1972. Iron metabolism in Egyptian infants with protein calorie deficiency. Brit. J. Nutr. 28: 81-89.

El-Sholmy, A., Y.W. Aboul-Dahab, M. El-Essawi, Y. Abdel-Rahman and A. Malek. 1962. Biochemical studies on anemia in malnourished infants: a comparison of marasmus and kwashiorkor cases. J. Trop. Med. Hyg. 65: 64-67.

Evans, E. and D.S. Miller. 1968. Comparative nutrition, growth, and longevity. Proc. Nutr. Soc. 27: 121-129.

Fitzsimons, J.T. and B.J. Simons. 1969. The effect of drinking in the rat of intravenous infusion of angiotensin, given alone or in combination with another stimuli of thirst. J. Physiol. 203: 455-457.

Flesch, P. 1949. The role of copper in mammalian pigmenetation. Proc. Soc. Exp. Biol. Med. 70: 79-83.

Fletcher, K. 1966. Observations on the origin of liver fat in infantile malnutrition. Amer. J. Clin. Nutr. 19: 170-174.

Flynn, M.A., F.M. Hanna, R.Y. Asfour and R.N. Lutz. 1967. Estimation of body water compartments of preschool children. ii. Undernourished children. Amer. J. Clin. Nutr. 20: 1129-1133.

Friedman, M., S.O. Byers, and S.S. George. 1956. Cholesterol metabolism. Ann. Rev. Biochem. 25: 613-640.

Gabuzda, T.G., H. Jick, and T.G. Chalmers. 1963. Human growth hormone and albumin metabolism in patients with cirrhosis. Metab. Clin. Exp. 12: 1-10.

Garrow, J.S., K. Fletcher and D. Halliday. 1965. Body composition in severe infant malnutrition. J. Clin. Invest. 44: 417-425.

Garrow, J.S. 1965. Total body potassium in kwashiorkor and marasmus. Lancet 2: 455-458.

Glauser, E.M. 1966. Advantages of piglets as experimental animals in pediatric research. Exp. Med. Surg. 24: 181-190.

Godard, C. 1974. Review: The endocrine glands in infantile malnutrition. Helv. Paediat. Acta. 29: 5-26.

Gomez, F., R. Ramog-Galvan, J. Cravioto, S. Frenk, J. Vasquez Santolla and C. De la Pina. 1956. Fat absorption in chronic severe malnutrition in children. Lancet 2: 121-122.

Gopalan, C., V. Reddy and V.S. Mohan. 1963. Some aspects of copper metabolism in protein calorie malnutrition. J. Pediat. 63: 646-649.

Griffiths, D.P. and K.R. Rees. 1957. Human liver metabolism. Brit. Med. J. 2: 971-974.

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

Gupta, P.P. 1973a. A study on quantitative changes in the liver lipids of Indian pigs suffering from severe protein malnutrition. Indian J. Anim. Sci. 43: 854-858.

Gupta, P.P. 1973b. Effect of low protein diet on haematological, biochemical and morphological tissue changes in Indian pigs. Indian J. Anim. Sci. 43: 746-755.

Haddad, M.E. and J.K. Harfouche. 1971. Serum proteins in Lebonese infants. J. Trop. Pediat. 17: 91-93.

Hansen, J.D. 1956. Electrolyte and nitrogen metabolism in kwashiorkor. S. Afr. J. Lab. Clin. Med. 2: 206-231.

Hansen, A.E., M.E. Haggard, A.N. Boelsche, D.J. Adams and H.F. Wiese. 1958. Essential fatty acids in infant nutrition. III Clinical manifestations of linoleic acid deficiency. J. Nutr. 66: 565-576.

Heard, C.R. 1968. Biochemical disturbances in experimental protein calorie deficiency. P. 249-256. In: R.A. McCance and E.M. Widdowson (ed) Calorie Deficiencies and Protein Deficiencies. Little, Brown and Co., Boston.

Heard, C.R., R.S. Platt and R.J. Stewart. 1957. The effects on pigs of a low protein diet with and without additional carbohydrate. Proc. Nutr. Soc. 17: xli.

Heard, C.R. and R.J. Stewart. 1971. Protein-Calorie deficiency and disorders of the endocrine glands. Hormones 2: 40-61.

Higginson, J. 1954. Studies on human bone from South African Bantu subjects. ii. Histological changes in ribs of South African Bantu infants. Metab. Clin.Exp. 3: 392-399.

Holman, R.T. 1960. The ratio of trienoic: tetraenoic acids in tissue lipids as a measure of essential fatty acid requirement. J. Nutr. 70: 405-410.

Holmberg, C.G. and C.B. Laurell. 1947. Investigations in serum copper. I. Nature of serum copper and its relation to the iron binding protein in human serum. Acta. Chem. Scand. 1: 944-950.

Hook, R.R., K.M. Brose and M.E. Tumbleson. 1972. Antibody responses of undernourished Sinclair miniature swine. Clin. Biochem. 5: 62-72.

Huber, A.M. and S.N. Gershoff. 1973. Effect of zinc deficiency in rats on insulin release from the pancreas. J. Nutr. 103: 1739-1744.

Ismadi, S.D., T.P. Susheela and B.S. Narasinga Rao. 1971. Usefulness of plasma ceruloplasmin and transferrin levels in the assessment of protein calorie malnutrition among pre-school children. Indian J. Med. Res. 59: 1581-1587.

James, W.P. 1968. Intestinal absorption in protein calorie malnutrition. Lancet 1: 333-335.

James, W.P. and A.M. Hay. 1968. Albumin metabolism. Effect of the nutritional state and the dietary protein intake. J. Clin. Invest. 47: 1958-1972.

Jayalakshmi, V.T., M.K. Ramanathan and C. Gopalan. 1957. Some observations on the effects of treatment of cases of kwashiorkor. Indian J. Med. Res. 45: 431-437.

Jaya Rao, K.S. 1965. Kwashiorkor and marasmus. Blood sugar levels and response to epinephrine. Amer. J. Dis. Child. 110: 519-522.

Jaya Rao, K.S. and K.P. Prasad. 1966. Serum triglycerides and nonesterified fatty acids in kwashiorkor. Amer. J. Clin. Nutr. 19: 205-209.

Kabadi, U.M., A.B. Eisenstein and I. Strack. 1976. Decreased plasma insulin but normal glucagon in rats fed low protein diets. J. Nutr. 106: 1247-1253.

Kachmar, L. 1970. Enzymes. P. 362-473. In: N.W. Tietz (ed) Fundamentals of Clinical Chemistry. W.B. Saunders, Philadelphia.

Kalk, W.J. and B.I. Pimstone. 1974. Calcium binding protein and vitamin D metabolism in experimental protein malnutrition. Brit. J. Nutr. 32: 569-578.

Kassem, A.S., M. Hafez, M.K. Badr-El-Din and A.I. Hassan. 1975. The liver and carbohydrate metabolism in protein calorie malnutrition. J. Trop. Pediat. 21: 3-6.

Kay, M., A.S. Jones and R. Smart. 1966. The use of tritiated water, 4-aminoantipyrine, and N-acetyl-4-aminoantipyrine for the measurement of body water in living pigs. Brit. J. Nutr. 20: 439-447.

Kelman, L., S.J. Saunders, L. Firth, S. Wicht and A. Corrigal. 1972. Effects of dietary protein restriction on albumin synthesis, albumin catabolism and the plasma aminogram. Amer. J. Clin. Nutr. 25: 1174-1178.

Kerpel-Fronius, E. 1960. Volume and composition of the body fluid compartments in severe infantile malnutrition. Pediatrics 56: 826-833.

Kerpel-Fronius, E. and E. Kaiser. 1967. Hypoglycemia in infantile malnutrition. Acta. Pediat. Scand. Sup. 172: 119-127.

Khalil, M., A. Kabiel, S. El-Khatub, K. Aref, M. El-Lozy, S. Jain and F. Nasr. 1974. Plasma and red and cell water and elements in protein calorie malnutrition. Amer. J. Clin. Nutr. 27: 260-267.

Kirsch, R.E., J.F. Brock and S.J. Saunders. 1968a. Experimental protein calorie malnutrition. Amer. J. Clin. Nutr. 21: 820-825.

Kirsch, R.E., S.J. Saunders and J.F. Brock. 1968b. Animal models and human protein calorie malnutrition. Amer. J. Clin. Nutr. 21: 1225-1228.

Konno, T., J.D. Hansen, A.S. Truswell, R. Woodd-Walker and D. Becker. 1968. Vitamin A deficiency and protein calorie malnutrition in Cape Town. S. Afr. Med. J. 42: 950-955.

Kritzinger, E.E., E. Kanengoni and J.J. Jones. 1974. Plasma renin activity in children with protein energy malnutrition. S. Afr. Med. J. 48: 499-501.

Kumar, S. and K.S. Jaya Rao. 1973. Plasma and erythrocyte zinc levels in protein calorie malnutrition. Nutr. Metab. 15: 364-371.

Lahey, M.E., M. Behar, F. Viteri and N.S. Scrimshaw. 1958. Values for copper and iron binding capacity in the serum in kwashiorkor. Pediatrics 22: 72-78.

Langhan, W.H., W.J. Eversole, F.N. Hayes and T.T. Triyillo. 1956. Assay of tritium activity in body fluids with use of a liquid scintillation system. J. Lab. Clin. Med. 47: 819-825.

Leonard, P.J. and K.M. MacWilliam. 1965. The binding of aldosterone in the serum in kwashiorkor. Amer. J. Clin. Nutr. 16: 300-302.

Leonard, P.J., W.T. Morris and R. Brown. 1968. Sodium, Potassium, Calcium, and Magnesium contents in nails of children with kwashiorkor. Biochem. J. 110: 22P-23P.

LeRoith, D. and B.L. Pimstone. 1973. Bone metabolism and composition in the protein deprived rat. Clin. Sci. 44: 305-319.

Lewis, B., G. Barbezat, I.H. Krut and J.D. Hansen. 1967. Oxidation of free fatty acids of plasma in infants with protein calorie malnutrition. S. Afr. Med. J. 41: 1103-1105.

Lewis, B., J.D. Hansen, W. Wittman, L.H. Krut and F. Stewart. 1964. Plasma free fatty acids in kwashiorkor and the pathenogenesis of the fatty liver. Amer. J. Clin. Nutr. 15: 161-168.

Lewis, B., W. Wittman, L.H. Krut, J.D. Hansen and J.F. Brock. 1966. Free fatty acid flux through plasma in protein malnutrition of infants. Clin. Sci. 30: 371-375.

Li, T.K. 1966. The functional role of zinc in metalloenzymes. P. 48-68. In: A.S. Prasad (ed) Zinc Metabolism. Charles C. Thomas, Springfield.

Linder, G.C., J.D. Hansen and C.D. Karabus. 1963. The metabolism of magnesium and other inorganic cations and of nitrogen in acute kwashiorkor. Pediatrics 31: 552-568.

Lurie, A.O. and P.U. Jackson. 1962. Aldosteronuria and the edema of kwashiorkor. Amer. J. Clin. Nutr. 11: 115-126.

Mackenzie, A. 1961. Observations on anterior vena cava venepuncture in the pig. Vet. Rec. 73: 435-437.

Madi, K., H.R. Jervis, P.R. Anderson and M.R. Zimmerman. 1970. A protein deficient diet. Effect on the liver, pancreas, stomach and small intestine of the rat. Arch. Pathol. 89: 38-52.

Mann, W.D., M.D. Bowie and J.D. Hansen. 1972. Potassium in protein-calorie malnutrition. S. Afr. Med. J. 46: 2062-2064.

Matthew, C.E. and R.F. Dean. 1960. The serum lipids in kwashiorkor. J. Trop. Pediat. 5: 135-138.

McCarthy, J.F., F.X. Aherne and D.B. Okai. 1974. Use of HCl insoluble ash as an index material for determining apparent digestibility with pigs. Can. J. Anim. Sci. 54: 107-109.

McFarlane, H., S. Reddy, K.J. Adcock, H. Adeshina, A.R. Cooke and J. Akene. 1970. Immunity, transferrin and survival in kwashiorkor. Brit. Med. J. 4: 268-270.

McFarlane, H., K.J. Adcock, A. Cooke, M.I. Ogbeide, H. Adeshina, G.O. Taylor, S. Reddy, J.M. Curney and J.A. Mordie. 1969. Biochemical assessment of protein calorie malnutrition. Lancet 1: 392-394.

Metcoff, Frenk, S., I. Antonowicz, G. Gordillo and E. Lopez. 1960. Relation of intracellular ions to metabolic sequences in muscle in kwashiorkor. Pediatrics 26: 960-972.

Metz, J. and H. Stein. 1959. Iron deficiency anemia in Bantu infants, and its association with kwashiorkor. S. Afr. Med. Ass. J. 33: 624-627.

Miller, E.R., R.W. Luecke, D.E. Ullrey, B.V. Baltzer, B.L. Bradley and J.A. Hoefer. 1968. Biochemical, skeletal and allometric changes due to zinc deficiency in the baby pig. J. Nutr. 95: 278-286.

Miller, E.R., D.E. Ullrey, I.M. Ackermann, D.A. Schmidt, J.A. Hoefer and R.W. Luecke. 1961. Swine hematology from bith to maturity. 1. Serum proteins. J. Anim. Sci. 20: 31-35.

Milne, M.D., R.C. Muehrake and I. Aird. 1957. Primary aldosteronism. Quart. J. Med. 26: 317-334.

Montgomery, R.D. 1960. Magnesium metabolism in infantile protein malnutrition. Lancet 2: 74-76.

Montgomery, R.D. and B. Chir. 1961. Magnesium balance studies in marasmic kwashiorkor. J. Pediat. 59: 119-123.

Mukherjee, K.L. and G. Werner. 1954. Enzyme activity and protein concentration in the serum of patients with malnutrition. J. Lab. Clin. Med. 43: 727-731.

Mulrow, P.J., W.F. Ganong, G. Cera and A. Kuljian. 1962. The nature of the aldosterone-stimulating factor in dog kidneys. J. Clin. Invest. 41: 505-518.

Naismith, D.J. 1962. The role of dietary fat in the utilization of protein. 11. The essential fatty acids. J. Nutr. 77: 381-386.

Naismith, D.J. 1964. The role of the essential fatty acids in the aeitiology of kwashiorkor. Proc. Nutr. Soc. 23: viii.

National Academy of Sciences. 1973. Nutrient requirements of swine. 7th edition, Washington, D.C.

O'Dell, B.L. and S.E. Savage. 1960. Effect of phytic acid on zinc availability. Proc. Soc. Exp. Biol. Med. 103: 304-305.

Payne, P.R. and J. Done. 1959. The equilibration of water and albumin labelled with tritium in the body fluids of pigs maintained on normal, low protein, and low protein plus carbohydrate diets. Proc. Nutr. Soc. 18: vi.

Pimstone, B.N., W. Wittman, J.D. Hansen and P. Murray. 1966. Growth hormone and kwashiorkor. Lancet 2: 779-780.

Pirzio-biroli, G., T.H. Bothwell and C.A. Finch. 1958. Iron absorption. ii. The absorption of radioiron administered with a standard meal in man. J. Lab. Clin. Med. 51: 37-48.

Pitchumoni, C.S. 1973. Pancreas in primary malnutrition disorders. Amer. J. Clin. Nutr. 26: 374-379.

Platt, B.S. and W. Frankul. 1962. The influence of a low protein diet on the level of zinc and iron in the serum and liver of the pig. Proc. Nutr. Soc. 21: viii.

Platt, B.S. and R.S. Stewart. 1962. The thyroid glands of protein malnourished pigs. Proc. Nutr. Soc. 21: v.

Platt, B.S., C.R. Heard and R.J. Stewart. 1964. Experimental protein-calorie deficiency. P. 445-521. In: H.N. Munroe (ed) Mammalian Protein Metabolism. Vol. 11. Academic Press, New Y.rk.

Platt, B.S. and R.J. Stewart. 1967. Experimental protein calorie deficiency: histopathological changes in the endocrine glands of pigs. J. Endocrinol. 38: 121-143.

Pond, W.G., W. Synder, J.T. Snook, E.F. Walker, D.A. McNeill and B.R. Stillings. 1971. Relative utilization of casein fish protein concentrate and isolated soybean protein for growth and pancreatic enzyme regeneration of the protein caloric malnourished baby pig. J. Nutr. 101: 1193-1200.

Redmond, A.O., J.D. Hansen and B. McHutchon. 1972. Abnormal bile salt metabolism in kwashiorkor. S. Afr. Med. J. 46: 617-618.

Reeds, P.J. and A.A. Laditan. 1976. Serum albumin and transferrin in protein energy malnutrition. Brit. J. Nutr. 36: 255-263.

Rosen, E.U., P.G. Campbell and G.M. Moose. 1970. Hypomagnesemia and magnesium therapy in protein calorie malnutrition. J. Pediat. 77: 709-719.

Rosenthal, C., J.C. Fahel and H.M. Vars. 1952. Response of alkaline phosphatase of rat liver to protein depletion and inanition. J. Biol. Chem. 194: 299-309.

Rothschild, M.A., M. Oratz, J. Mongelli, L. Fishman and S.S. Schreiber. 1969. Amino acid regulation of albumin synthesis. J. Nutr. 98: 395-403.

Sandstead, H.M., S. Shuky, A.S. Prasad, M.K. Gabr, A. El-Mifney, N. Mokhtar and W.J. Darby. 1965. Kwashiorkor in Egype. 1. Clinical and biochemical studies with special reference to plasma zinc and serum lactic dehydrogenase. Amer. J. Clin. Nutr. 17: 15-26.

Schendel, H.E. and J.D. Hansen. 1958. Studies on fat metabolism in kwashiorkor. 1. Total serum cholesterol. Metab. Clin. Exp. 7: 731-741.

Schnieden, H., R.G. Hendrickse and C.P. Haigh. 1958. Studies in water metabolism in clinical and experimental malnutrition. Trans. Roy. Soc. Trop. Med. Hyg. 52: 169-175.

Schwartz, R. 1956. Alkaline phosphatase activity of the serum in kwashiorkor. J. Clin. Pathol. 9: 333-341.

Scrimshaw, N.S. and M. Behar. 1961. Protein malnutrition in young children. Science 133: 2038-2047.

Scrimshaw, N.S., M. Behar, C. Perez and F. Viteri. 1955. Nutritional problems of children in Central America and Panama. Pediatrics 16: 378-397.

Searcy, R.I. 1969. Diagnostic Biochemistry. McGraw-Hill Book Company, New York, New York.

Senecal, J. 1958. The treatment and prevention of kwashiorkor in French West Africa. Ann. N.Y. Acad. Sci. 69: 916-953.

Shenolikar, I.S. and B.S. Narasinga Rao. 1968. Influence of dietary protein on calcium metabolism in young rats. Indian J. Med. Sci. 56: 1412-1421.

Sissons, H.A. 1971. The growth of bone. P. 145-180. In: G.H. Bourne (ed) The Biochemistry and Physiology of Bone. Academic Press, New York.

Slone, D., L.S. Taitz and G.S. Gilchrist . 1961. Aspects of carbohydrate metabolism in kwashiorkor with special reference to spontaneous hypoglycemia. Brit. Med. J. 1: 32-34.

Smit, Z.M. and P.J. Pretorius. 1964. Studies in metabolism of zinc. ii. Serum zinc levels and urinary zinc excretions in South African Bantu kwashiorkor patients. J. Trop. Pediat. 9: 105-112.

Smith, R. 1960. Total body water in malnourished infants. Clin. Sci. 19: 275-285.

Smith, R. 1962. Serum transaminases in kwashiorkor. J. Pediat. 61: 617-619.

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

Srikantia, S.G. 1958. Ferritin in nutritional edema. Lancet 1: 667-677.

Srikantia, S.G. and C. Gopalan. 1958. Role of ferritin in nutritional edema. J. Appl. Physiol. 14: 829-833.

Srikantia, S.G., C.M. Jacob and V. Reddy. 1964. Serum enzyme levels in protein calorie malnutrition. Amer. J. Dis. Child. 107: 256-259.

Srikantia, S.G. and M. Mohanram. 1970. Antidiuretic hormone values in plasma and urine of malnourished children. J. Clin. Endocrinol. 31: 312-314.

Starcher, B. 1969. Studies on the mechanism of copper absorption in the chick. J. Nutr. 97: 321-326.

Stephens, A.J. 1974. A points system, using clinical and biochemical parameters to identify malignant kwashiorkor. Ann. Trop. Med. Parasitol. 68: 453-466.

Stewart, R.J. 1968. Pathological changes in the central nervous system in experimental protein-calorie deficiency. P. 257-264. In: R.A. McCance and E.M. Widdowson (ed) Calorie Deficiencies and Protein Deficiencies. Little, Brown and Co., Boston.

Sullivan, J.F., R.E. Burch, N.J. Quigley and D.F. Magee. 1974. Zinc deficiency and decreased pancreatic secretory response. Amer. J. Physiol. 227: 105-108.

Theron, J.J., P.J. Pretorius, H. Wolf and C.P. Joubert. 1961. The state of pyridoxine nutrition in patients with kwashiorkor. J. Pediat. 59: 439-450.

Thomas, W.C. and J.B. Howard. 1964. Disturbances in calcium metabolism. P. 445-482. In: C.I. Comar (ed) Mineral Metabolism. Academic Press, New York.

Thompson, M.D. and H.C. Trowell. 1952. Pancreatic enzyme activity in duodenal contents of children with a type of kwashiorkor. Lancet 262:1031-1035.

Truswell, A.S. 1975. Carbohydrate and lipid metabolism in protein calorie malnutrition. P. 119-141. In: R.E. Olsen (ed) Protein Calorie Malnutrition. Academic Press, New York.

Truswell, A.S. and J.D. Hansen. 1969. Fatty liver in protein calorie malnutrition. S. Afr. Med. J. 43: 280-283.

Truswell, A.S., J.D. Hansen, C.E. Watson and P. Wannenburg. 1969. Relation of serum lipids and lipoproteins to fatty livers in kwashiorkor. Amer. J. Clin. Nutr. 22: 568-576.

Tukey, J.W. 1953. Some selected quick and easy method of statistical analysis. Trans. N.Y. Acad. Sci. 16: 88-97.

Tumbleson, M.E. 1972. Protein calorie undernutrition in young sinclair miniature swine: Serum biochemic and hematologic values. Advances in Automated Analysis, Vol. 7 : 51-71.

Tumbleson, M.E., M.F. Burks, P.S. Evans and D.P. Hutcheson. 1972a. Serum electrolytes in undernourished sinclair miniature swine. Amer. J. Clin. Nutr. 25: 476-479.

Tumbleson, M.E., M.F. Burks and R.R. Hook. 1972b. Serum proteins in undernourished sinclair miniature swine. Clin. Biochem. 5: 51-61.

Tumbleson, M.E., O.W. Tinsley, I.A. Corwin, R.E. Flatt and M.A. Flynn. 1969. Undernutrition in young miniature swine. J. Nutr. 99: 505-518.

Underwood, E.J. 1971. Trace Elements in Human and Animal Nutrition. Academic Press, New York.

Vallee, B.L. 1960. Metal and enzyme interactions: Correlation of composition, function, and structure. P. 225-276. In: P.D. Boyer (ed) The Enzymes. Academic Press, New York.

Van Campen, D. and W.A. House. 1974. Effect of a low protein diet on retention of an oral dose of Zn^{65} and on tissue concentrations of zinc, iron, and copper in rats. J. Nutr. 104: 84-90.

Van Campen, D. and P.U. Scaife. 1967. Zinc interference with copper absorption in rats. J. Nutr. 91: 473-476.

Van Campen, D. and E. Cross. 1969. Effect of histidine and certain other amino acids on the absorption of Iron-59 by rats. J. Nutr. 99: 68-74.

Van Der Westhuysen, J.M., E. Kanengoni, M. Mbizvo and J.J. Jones. 1977. The effect of protein energy malnutrition on plasma renin and edema in the pig. S. Afr. Med. J. 51: 18-20.

Van Der Westhuysen, J.M., F. Kanengoni, J.J. Jones and C.H. Van Niekerk. 1975. Plasma renin activity in edematous and marasmic children with protein energy malnutrition. S. Afr. Med. J. 49: 1729-1731.

Vaughan, B.E. and E.A. Boling. 1961. Rapid assay for tritium labelled water in water fluids. J. Lab. Clin. Med. 57: 159-164.

Viteri, F., M. Behar, G. Arroyave and N.S. Scrimshaw. 1964. Clinical aspects of protein malnutrition. P. 523-568. In: H.N. Munroe (ed) Mammalian Protein Metabolism. Vol. II. Academic Press, New York.

Waldern, D.E. 1971. A rapid micro-digestion procedure for neutral and acid detergent fibre. Can. J. Anim. Sci. 51: 67-69.

Warren, P.J., J.D. Hansen and B.H. Lehmann. 1969. The concentrations of copper, zinc, and manganese in the liver of African children with marasmus and kwashiorkor. Proc. Nutr. Soc. 28: 6A.

Warton, B. 1970. Hypoglycemia in children with kwashiorkor. Lancet 1: 171-173.

Waterlow, J.C. 1959. Protein nutrition and enzyme changes in man. Fed. Proc. 18: 1143-1155.

Waterlow, J.C. 1962. Oxidative phosphorylation in human infants. Nutr. Rev. 20: 150-152.

Waterlow, J.C. and C.B. Mendes. 1957. Composition of muscle in malnourished human infants. Nature, 180: 1361-1362.

Waterlow, J.C., J. Cravioto and J.M. Stephen. 1960. Protein malnutrition in man. Advan. Protein Chem. 15: 131-238.

Waterlow, J.C. and J.M. Stephen. 1969. Enzymes and the assessment of protein nutrition. Proc. Nutr. Soc. 28: 234-242.

Wayburne, S. 1963. Hepatic failure in malnutrition. Lancet 1: 447-447.

Weimer, H.P., A.W. Carpenter, A.W. Nayor-Foote, R.W. McKee and H. Nishihari. 1959. Effects of inanition, protein depletion and repletion on serum lactic acid dehydrogenase levels in rats. Proc. Soc. Exp. Biol. Med. 101: 344-345.

Werbin, H.B., M.R. Chaikoff and M.R. Imada. 1959. Rapid sensitive method for determining tritiated water in body fluids by liquid scintillation spectrometry. Proc. Soc. Exp. Biol. Med. 102: 8-12.

Wharton, B. 1970. Hypoglycemia in children with kwashiorkor. Lancet 1: 171-173.

Wheeler, J.E., F.D. Lukens and P. Gyory. 1949. Studies on the localization of tagged methionine within the pancreas. Proc. Soc. Exp. Biol. Med. 70: 187-189.

Whitehead, R.G. and R.F. Dean. 1964. Serum amino acids in kwashiorkor. 1. Relationship to clinical condition. Amer. J. Clin. Nutr. 14: 313-319.

Whitehead, R.G. and P.S. Harland. 1966. Blood glucose, lactate, and pyruvate in kwashiorkor. Brit. J. Nutr. 20: 825-831.

Whitehead, R.G., W.A. Coward and P.G. Lunn. 1973. Serum albumin concentrations and the onset of kwashiorkor. Lancet 1: 63-66.

Wiberg, G.S. and J. Tuba. 1955. On rat serum amylase. III The contribution by various tissues to serum amylase activity. Can. J. Biochem. 33: 817-825.

Wolf, P.L. and Williams. 1973. Practical Clinical Enzymology. John Wiley and Sons, New York.

Zaki, A.H., B. El-Kammah, I. Fayad, A.H. Shehata and S. Mahmoud. 1970. Serum enzymes in protein malnutrition. Acta. Biol. Med. Germ. 24: 137-140.

APPENDIX TABLES 1A - 30A

Table 1A. Analysis of Variance for Body Weight in Trial 1.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	0.4637 1	0.3686 1	2.4009 1	0.7341 1	3.0496 1	3.0157 1
Litter	1.4099 3	1.9450 3	4.8739 3	18.8840 3	44.2650 3	98.8950 3
Treatment	0.2430 1	15.3520 1	118.1000* 1	546.0300* 1	1391.5000* 1	1016.0000* 1
Error	0.8187 21	2.6693 22	5.9732 23	13.8620 22	20.7330 21	48.4780 14

* $p \leq .01$

Table 2A. Analysis of Variance for Serum Total Protein in Trial 1.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	0.0156 1	0.3957 1	0.0001 1	0.0105 1	0.0049 1	0.4408 1
Litter	2.0642 3	0.2651 3	0.6487 3	0.0796 3	0.1646 3	0.3982 3
Treatment	0.1616 1	0.2540 1	2.6311* 1	7.2164* 1	22.7770* 1	6.1917* 1
Error	0.4286 21	0.1469 22	0.3419 23	0.1816 22	0.2308 21	0.3949 14

* $p \leq .01$

Table 3A. Analysis of Variance for Serum Albumin in Trial 1.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	0.2861 1	0.1776 1	0.2512 1	0.0022 1	0.0005 1	0.1046 1
Litter	0.7412 3	0.0329 3	0.0051 3	0.0139 3	0.0938 3	0.1933 3
Treatment	0.1760 1	0.0185 1	0.8498* 1	1.1537* 1	11.2380* 1	3.6143* 1
Error	0.1714 21	0.0420 22	0.0836 23	0.0529 21	0.1507 21	0.2442 14

* $p \leq .01$

Table 4A. Analysis of Variance for Blood Urea Nitrogen in Trial 1.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	54.457 1	10.808 1	1.737 1	0.0204 1	29.308 1	12.783 1
Litter	33.639 3	8.598 3	5.130 3	13.782 3	0.458 3	13.564 3
Treatment	8.594 1	100.170 1	86.392 1	0.031 1	8.278 1	39.603 1
Error	10.793 19	16.881 22	17.345 23	12.375 22	11.454 21	12.033 14

* $p \leq .01$

Table 5A. Analysis of Variance for Serum Amylase in Trial 1.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	584130.0 1	2451700.0 1	7728.7 1	1601.5 1	1157.6 1	1173000.0 1
Litter	3513300.0* 3	732010.0 3	2084100.0* 3	510870.0 3	646290.0 3	758440.0 3
Treatment	65248.0 1	5654.3 1	4480600.0* 1	3919200.0* 1	2055900.0 1	5213300.0* 1
Error	450830.0 20	883140.0 22	244800.0 23	171600.0 22	301920.0 20	384070.0 14

* $p \leq .01$

Table 6A. Analysis of Variance for Serum Alkaline Phosphatase in Trial 1.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	6132.7 1	452.4 1	308.9 1	619.2 1	1011.2 1	2020.2 1
Litter	2473.4 3	1541.9 3	2124.1 3	1773.6 3	1272.8 3	1323.4 3
Treatment	492.8 1	184.7 1	16605.0* 1	30957.0* 1	32189.0* 1	10089.0* 1
Error	523.3 21	501.2 22	1122.8 23	928.0 22	764.4 21	1118.8 14

* $p \leq .01$

Table 7A. Analysis of Variance fo Serum Glutamic Oxaloacetic Transaminase in Trial 1.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	26.02 1	401.88 1	581.07 1	9.34 1	0.19 1	393.40 1
Litter	402.38* 3	7.40 3	57.64 3	229.89* 3	83.48 3	92.56 3
Treatment	10.01 1	34.20 1	1421.20 1	639.06* 1	201.35 1	0.50 1
Error	56.21 21	144.43 22	261.16 23	31.09 22	36.67 20	70.31 14

* $p \leq .01$

Table 8A. Analysis of Variance for Lactic Dehydrogenase in Trial 1.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	13810.0 1	113330.0 1	3.4 1	187.8 1	235.6 1	73.9 1
Litter	54465.0 3	24987.0 3	10514.0 3	5997.7 3	5082.8 3	5693.1 3
Treatment	46697.0 1	179140.0 1	34769.0 1	455.3 1	3036.8 1	44488.0* 1
Error	14389.0 21	51048.0 21	64397.0 21	5638.4 22	6058.2 21	3437.7 14

* $p \leq .01$

Table 9A. Analysis of Variance for Serum Cholesterol in Trial 1.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	916.19 1	61.48 1	841.84 1	305.42 1	341.95 1	62.67 1
Litter	347.04 3	134.65 3	201.34 3	519.31 3	235.90 3	71.26 3
Treatment	25.41 1	29.70 1	1.72 1	591.54 1	1989.70* 1	2091.90* 1
Error	666.83 21	153.03 22	170.28 23	152.25 22	248.95 21	131.34 14

* $p \leq .01$

Table 10A. Analysis of Variance for Serum Glucose in Trial 1.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	7.45 1	95.32 1	6.59 1	32.80 1	324.40 1	190.17 1
Litter	998.25 3	160.15 3	218.94 3	411.99 3	347.57 3	89.80 3
Treatment	2678.50 1	357.09 1	633.47 1	158.84 1	512.26 1	0.35 1
Error	576.42 21	224.60 22	206.39 23	190.57 22	175.42 21	243.44 14

* $p \leq .01$

Table 11A. Analysis of Variance for Serum Calcium in Trial 1.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	1.3481 1	0.0492 1	0.0531 1	0.1914 1	0.4605 1	0.1739 1
Litter	1.6332 3	0.7750 3	2.8038* 3	0.0109 3	0.2751 3	0.0994 3
Treatment	0.3662 1	0.1334 1	1.2437 1	8.3812* 1	23.1400* 1	6.5797* 1
Error	0.5704 21	0.2150 22	0.3101 22	0.3834 22	0.6662 21	0.1913 14

* $p \leq .01$

Table 12A. Analysis of Variance for Serum Phosphate in Trial 1.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	8.2598 1	1.9846 1	0.1416 1	0.4590 1	1.0451 1	0.2876 1
Litter	6.5122 3	0.9511 3	2.0520 3	1.4786 3	0.2188 3	1.9648 3
Treatment	0.6522 1	5.0232* 1	19.6620* 1	17.0860* 1	35.3770* 1	10.6320* 1
Error	1.5307 21	0.5508 22	0.5485 23	0.6901 22	0.2822 20	0.8382 14

* $p \leq .01$

Table 13A. Analysis of Variance for Body Weight in Trial 2.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	0.8027 1	0.4267 1	0.0659 1	0.0000 1	6.9606 1	13.9750 1
Litter	4.7546* 5	4.1672* 5	5.1212* 5	8.2966* 5	7.4171 5	16.9540 5
Treatment	0.5733 4	4.1250* 4	40.0040* 4	189.2400* 4	497.2900* 4	822.3900* 4
Error	0.5905 29	0.7197 29	1.1946 27	2.0803 27	4.6085 26	9.0858 24

* $p \leq .01$

Table 14A. Analysis of Variance for Serum Total Protein in Trial 2.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	0.0239 1	0.0395 1	0.0049 1	0.2588 1	0.2125 1	0.1908 1
Litter	0.0428 5	0.0676 5	0.1450 5	0.2303 5	0.2436 5	0.2521 5
Treatment	0.0240 4	0.0889 4	0.9673* 4	2.3235* 4	4.3502* 4	3.1681* 4
Error	0.0614 29	0.0856 29	0.0664 27	0.1061 27	0.1286 26	0.2636 24

* $p \leq .01$

Table 15A. Analysis of Variance for Serum Albumin in Trial 2.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	0.0474 1	0.0402 1	0.1021 1	0.0310 1	0.0167 1	0.0095 1
Litter	0.4555* 5	0.1224 5	0.1901* 5	0.1211 5	0.1271 5	0.1283 5
Treatment	0.0385 4	0.0471 4	0.3961* 4	1.7928* 4	4.0972* 4	2.6533* 4
Error	0.0434 29	0.0431 29	0.0455 27	0.0516 27	0.0332 26	0.1281 24

* $p \leq .01$

Table 16A. Analysis of Variance for Blood Urea Nitrogen in Trial 2.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	3.2111 1	0.4812 1	0.4247 1	2.0876 1	9.0137 1	38.7620 1
Litter	27.3820 5	3.4022 5	21.3910* 5	2.7848 5	6.9594 5	6.6551 5
Treatment	1.6625 4	13.8700 4	4.4935 4	22.9830* 4	50.2470* 4	17.1630 4
Error	9.4288 29	7.7616 29	4.2228 27	5.1917 27	7.7239 26	13.0940 24

* $p \leq .01$

Table 17A. Analysis of Variance for Serum Amylase in Trial 2.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	124200.0 1	77910.0 1	883770.0 1	31426.0 1	142640.0 1	491290.0 1
Litter	2006100.0* 5	1416600.0 5	1825200.0* 5	902830.0 5	386480.0 5	1695500.0* 5
Treatment	366280.0 4	252690.0 4	1077200.0* 4	2799200.0* 4	2811800.0* 4	5515400.0* 4
Error	247150.0 29	416360.0 29	221840.0 27	370430.0 27	354400.0 26	386600.0 24

* $p \leq .01$

Table 18A. Analysis of Variance for Serum Alkaline Phosphatase in Trial 2.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	504.10 1	25.24 1	1.61 1	59.71 1	65.69 1	47.08 1
Litter	13386.00* 5	1170.80 5	2096.10* 5	1878.70 5	1897.80 5	4394.40 5
Treatment	131.65 4	1497.10 4	4238.10* 4	10748.00* 4	10928.00* 4	51447.00* 4
Error	686.22 29	574.76 29	384.25 27	571.31 27	577.89 26	2906.70 24

* $p \leq .01$

Table 19A. Analysis of Variance for Serum Glutamic Oxaloacetic Transaminase in Trial 2.

Source	Mean Square ÷ Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	220.90 1	13.22 1	2.65 1	1995.40 1	278.96 1	37.14 1
Litter	341.03 5	220.45 5	718.33 5	385.06 5	53.37 5	185.97 5
Treatment	357.56 4	70.73 4	446.76 4	123.40 4	143.84 4	301.21 4
Error	208.66 29	70.86 29	396.85 27	433.73 27	100.04 26	355.05 24

* $p \leq .01$

Table 20A. Analysis of Variance for Serum Lactic Dehydrogenase in Trial 2.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	6024.3 1	1676.1 1	97016.0 1	21625.0 1	10293.0 1	24.6 1
Litter	80464.0 5	3187.1 5	31063.0 5	16966.0 5	6405.5 5	34114.0 5
Treatment	46181.0 4	8838.3 4	82326.0 4	7864.0 4	3339.0 4	123560.0* 4
Error	32181.0 29	2660.2 29	29932.0 27	12740.0 27	3039.0 26	281.40.0 24

* $p \leq .01$

Table 21A. Analysis of Variance for Serum Cholesterol in Trial 2.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	1207.60 1	10.94 1	10.21 1	4.10 1	165.27 1	185.59 1
Litter	1307.60 5	303.52 5	310.60 5	451.87 5	702.68 5	102.57 5
Treatment	366.78 4	706.48* 4	339.17 4	395.06 4	821.00 4	2522.40* 4
Error	367.63 29	121.44 29	255.89 27	217.07 27	376.56 26	188.07 24

* $p \leq .01$

Table 22A. Analysis of Variance for Serum Glucose in Trial 2.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	529.66 1	1167.20* 1	237.33 1	74.69 1	1.16 1	648.97 1
Litter	109.95 5	421.87 5	127.17 5	58.27 5	212.60 5	122.91 5
Treatment	129.71 4	383.90 4	1329.60* 4	282.47* 4	550.51* 4	115.08 4
Error	159.57 29	130.25 29	209.17 27	65.95 27	123.03 26	233.73 24

* $p \leq .01$

Table 23A. Analysis of Variance for Serum Calcium in Trial 2.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	0.1493 1	0.0027 1	0.1412 1	0.3002 1	0.01899 1	0.2236 1
Litter	0.2589 5	0.2660 5	0.0427 5	0.1142 5	0.0692 5	0.4832 5
Treatment	0.0535 4	0.2630 4	0.3682 4	3.5738* 4	5.8099* 4	4.7033* 4
Error	0.0787 29	0.1752 29	0.2171 27	0.1622 27	0.1573 26	0.2134 24

* $p \leq .01$

Table 24A. Analysis of Variance for Serum Copper in Trial 2.

Source	Mean Square + Degree of Freedom		
	0 Weeks	6 Weeks	10 Weeks
Sex	757.54 1	2508.00 1	1792.80 1
Litter	3940.10 5	1485.10 5	4251.30* 5
Treatment	173.02 4	1981.80 4	18565.00* 4
Error	604.58 27	1463.00 27	612.91 25

* $p \leq .01$

Table 25A. Analysis of Variance for Serum Iron in Trial 2.

Source	Mean Square + Degree of Freedom		
	0 Weeks	6 Weeks	10 Weeks
Sex	3956.60 1	916.93 1	3178.10 1
Litter	3978.60 5	3410.90 5	2330.50 5
Treatment	4739.80 4	5265.90 4	2330.50 4
Error	2339.20 22	1566.30 25	2915.40 24

* $p \leq .01$

Table 26A. Analysis of Variance for Serum Magnesium in Trial 2.

Source	Mean Square + Degree of Freedom		
	0 Weeks	6 Weeks	10 Weeks
Sex	0.2171 1	0.0463 1	0.2223 1
Litter	0.2712 5	0.3592 5	0.0678 5
Treatment	0.4631 4	0.3558* 4	1.6924* 4
Error	0.1894 28	0.1132 26	0.1804 25

* $p \leq .01$

Table 27A. Analysis of Variance for Serum Phosphate in Trial 2.

Source	Mean Square + Degree of Freedom					
	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Sex	0.5377 1	0.0278 1	0.0716 1	0.6287 1	0.0127 1	0.2253 1
Litter	0.6682* 5	0.7204 5	0.9026 5	0.5255 5	0.6691 5	0.6327 5
Treatment	0.1160 4	1.4684* 4	4.5256* 4	6.5366* 4	8.0350* 4	8.9269* 4
Error	0.1582 29	0.2416 29	0.2546 27	0.1879 27	0.2767 26	0.4174 24

* $p \leq .01$

Table 28A. Analysis of Variance for Serum Zinc in Trial 2.

Source	Mean Square + Degree of Freedom		
	0 Weeks	6 Weeks	10 Weeks
Sex	256.87 1	1078.70 1	2304.40 1
Litter	7093.40 5	1573.30 5	8056.60 5
Treatment	1116.10 4	7176.50 4	20541.00* 4
Error	4013.00 27	2346.80 24	1954.00 25

* $p \leq .01$

Table 29A. Analysis of Variance for Total Body Water.

Source	Mean Square + Degree of Freedom
Treatment	177.12* (1)
Error	9.77 (4)

* $p \leq .01$

Table 30A. Analysis of Variance for Fatty Livers.

Source	Mean Square + Degree of Freedom
Treatment	2097.21* / 1
Error	182.13 / 24

* $p \leq .01$