# EFFECT OF VITAMIN A ON SERUM AMINO ACID PATTERNS

by

CHRISTA MARIA STANTON

B. S., Justus-Liebig-University, Giessen, Germany, 1966

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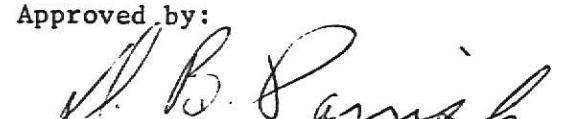

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# I. INTRODUCTION

The symptoms of deficiency diseases have been recognized since the beginning of civilization. It was only at the turn of the nineteenth century, however, that the concept of "accessory food factors" led to recognition of the causes of deficiency diseases. This, in turn, resulted in the isolation of a series of vitamins, the recognition of their chemical structure and their synthesis. Commercial manufacture has led to a relatively cheap and plentiful availability of all known vitamins, and, as a result, the classical deficiency diseases are more or less under control.

More recently, attention has been directed toward the world-wide occurrence of the disease kwashiorkor, caused by inadequate protein intake in infants. Unfortunately, kwashiorkor is not simply the result of protein deficiency. It is becoming increasingly evident that the disease involves the interaction of multiple factors. The primary causative factor is a deficit of dietary protein. A secondary factor is vitamin A deficiency, which is indicated by severe occular lesions often observed in kwashiorkor victims. The fact that protein malnutrition and vitamin A deficiency are probably the two most common nutritional deficiency diseases in very large areas of the world resulted in extensive studies of their interactions.

Observations on the relationship between vitamin A and protein in man can be summarized as follows: a) Vitamin A deficiency and protein malnutrition frequently occur simultaneously. b) The absorption of dietary vitamin A is impaired in acute protein malnutrition. c) The mobilization of vitamin A from the liver is restricted in kwashiorkor. d) Dietary protein is required to mobilize liver reserves of vitamin A into the bloodstream.

The implications of these observations are of utmost importance in food

supplementation. In areas where vitamin A status is marginal, high-grade protein supplementation may mobilize the last reserves of this vitamin from the liver and thus precipitate vitamin A deficiency. Of course, this should not be interpreted as an argument against feeding high-grade protein to children to prevent kwashiorkor, but rather emphasizes the need to supplement high-grade protein with vitamin A.

With the rapid growth of world population, there are constant reminders of the serious food shortages humanity is about to face. While synthetic processes have made vitamin A cheap and abundant, we still depend on natural sources for adequate supplies of protein. Vitamin A is known to influence synthesis of both serum and muscle proteins; vitamin A deficiency also reduces the utilization of ingested protein.

Thus, there is a close link between vitamin A and protein metabolism and between dietary protein and vitamin A metabolism. However, this link is not yet fully understood.

The purpose of this study was to investigate the effect of vitamin A status on free serum amino acid patterns of chickens fed different levels of dietary protein.

## II. REVIEW OF LITERATURE

Dietary Protein and Vitamin A Metabolism. In 1959, McLaren (1) suggested that possibly the liver stores of vitamin A are used more slowly in animals whose growth has been restricted by an insufficient intake of protein. His results confirmed the previous work of Brown and Morgan (2) and Mayer and Krehl (3). Randoin and Queuille (4) and Dye et al. (5), on the other hand, noticed little or no effect of level of protein upon utilization of vitamin A, as judged by weight gain, length of the animal, and epithelial changes.

Basu and De (6) reported that when rats were given a diet containing 18% protein and daily doses of 100 IU of vitamin A, they stored much more liver vitamin A than when the same dose was given with a diet containing only 8% protein. Baumann et al. (7) also found that a low protein intake tended to reduce the storage of vitamin A in rats.

Mayer and Krehl (3) reported that feeding increased levels of dietary protein to vitamin A-deficient rats resulted in increasingly severe symptoms of deficiency. Rechcigl et al. (8) found that when rats with adequate liver reserves of vitamin A were fed different levels of dietary protein in a vitamin A-deficient diet, the depletion of liver vitamin A stores was directly proportional to the protein intake, indicating that more vitamin A was utilized by the organism as the dietary protein intake was increased. In those studies (8) large doses of vitamin A were administered prior to the beginning of the experiment thereby eliminating changes in absorption of dietary vitamin A with different levels of the dietary protein.

Esh and Bhattacharya (9) demonstrated that prolonged inadequate or suboptimal protein feeding will lower liver storage of vitamin A in rats,

implying a reduced absorption or transport of vitamin A. The results are in agreement with those of Stoewsand and Scott (10), Olsen et al. (11), Leuts'kyi et al. (12) and Bohdal and Hruba (13). Murray (14) obtained opposite results in a study of the effect of a protein-free diet on vitamin A storage and depletion. He stated that protein was not essential for storage of vitamin A in the liver but storage was not increased in rats fed no protein. Over a four-week period the total loss of vitamin A from the liver was not significantly altered by the absence of dietary protein or by the resulting cessation of growth.

Jagannathan and Patwardhan (15) studied effect of level of dietary protein on the depletion of hepatic storage of vitamin A in the rat. The authors considered "the amount of vitamin A stored in the liver is normally the sum total of the effect of two processes, both of which can vary according to the composition of the diet: 1.) the degree of absorption of the vitamin from the gastrointestinal tract, resulting in the accumulation of it in the liver and 2.) the rate of utilization of the stored vitamin by the organism for its metabolic functions resulting in its withdrawal from the storage organ." The authors concluded that the quantity of protein in the diet might influence the utilization of hepatic stores of vitamin A.

Bhattacharya and Esh (16) studied the effect of vitamin A on utilization of dietary proteins of different nutritional value and at two different levels (10% and 18%) of intake. All the vitamin A-deficient animals grew less and retained nitrogen significantly less efficiently than the supplemented ones. A high-level of vitamin A supplementation (160 IU daily) had no greater effect than less vitamin A (30 IU daily) in curing vitamin A deficiency of animals fed 18% protein. The authors suggested that an intake of vitamin A

above the amount normally required would give some protection to the organism against the effects of a low or imbalanced protein intake. Konstantinov (17) conducted a study with young and mature chickens fed a vitamin A-deficient diet and two different levels of protein (5-12% and 30-35%). Vitamin A deficiency developed faster in the group fed the protein-rich feed. When given feed low in protein, the animals survived 5-6 months despite the vitamin A deficiency.

Deshmukh et al. (18) reported that efficiency of vitamin A absorption in rats increased with increase in the dietary protein content.

Moore (19), Roels (20) and Mahadevan et al. (21) reviewed the literature on various aspects of intestinal absorption of vitamin A, its transport in blood, storage in the liver and metabolism in normal animals, with special emphasis on the enzymatic reactions involved and on the role of body proteins. Their reviews contained essentially little additional information to that already discussed on vitamin A storage in the liver and role of body proteins in vitamin A absorption and storage.

The studies of Mathews and Beaton (22) indicated that maintenance of rats on a protein-free diet produced a significant lowering of serum vitamin A and serum proteins.

Friend et al. (23) studied the relation between dietary protein deficiency and transport of vitamin A in blood of pigs and storage in the liver. They found a highly significant correlation between concentration of serum vitamin A and that of serum albumin. However, they stated: "There is no reason to expect that vitamin A had a direct effect on serum albumin." They believed that the probable explanation of their results was that food intake of the pigs was sufficiently reduced on the vitamin A-deficient diet to cause

protein deficiency and reduction in serum albumin.

Stoewsand and Scott (24) demonstrated that high-protein diets caused a significant decrease in liver vitamin A storage in chicks compared to pair-fed chicks consuming the same type of protein at moderate dietary levels. More recently Gazo et al. (25) reinvestigated this phenomenon and concluded that their results did not support the hypothesis of a higher vitamin A need in the case of a more intensive anabolism of proteins.

With the background of knowledge derived from animal research, the interrelationships of dietary protein and vitamin A in man will be reviewed. Frequently, vitamin A deficiency is associated with protein malnutrition. It is a common observation that children suffering from kwashiorkor have a low concentration of vitamin A in the blood plasma (26, 27). When adequate dietary proteins were given, serum vitamin A level rose without administration of vitamin A, provided the patients had sufficient liver reserves of the vitamin. In kwashiorkor, total serum proteins are reduced and the albumin/globulin ratio is lowered. Adequate serum proteins or adequate dietary protein are necessary to mobilize vitamin A from the liver into the bloodstream. Alternatively, the lowered or imbalanced protein intake, which gives rise to kwashiorkor, may reduce the requirement for vitamin A and mobilization from the liver. Arroyave et al. (27) did not find a rise in serum vitamin A when they fed skim milk to kwashiorkor patients whose liver vitamin A reserves were initially low. In a further study, Pereira et al. (28) investigated the mode of action of water-miscible vitamin A palmitate and vitamin A palmitate in oil in patients suffering from severe protein deficiency. The water-miscible vitamin A reached the circulating plasma, particularly when injected intramuscularly, but the vitamin A palmitate in oil did not. The findings

indicate that the impairment was in the processes of ester hydrolysis and reesterification necessary for normal absorption and mobilization of the naturally occurring vitamin. The authors suggested that in severe protein deficiency vitamin A of the diet does not reach the liver and, furthermore, that reserves remaining in the hepatic tissue are not available to other tissues of the body for utilization. They postulated that, independently of both the dietary intake and liver stores of vitamin A, a functional deficiency of this vitamin may occur in kwashiorkor and other forms of severe protein deficiency as a consequence of the reduced plasma proteins.

Arroyave (29) concluded from a study of children in El Salvador that subsistence on a diet limited primarily in protein, but also in vitamin A, hinders the development of gross clinical lesions of the vitamin deficiency by decreasing protein metabolism, growth, and, thereby, the requirement for vitamin A. The animal experiments described earlier support this concept. Several other investigators studied the interrelationship of protein deficiency and vitamin A (30, 31, 32) with results in agreement with those of Arroyave (29) and co-workers (27) and Pereira et al. (28).

Good reviews on vitamin A and protein metabolism were published by McLaren (33), Roedel (34), Malathi (35), Olson (36) and Ganguly (37). The reviews by Olson and Ganguly placed primary emphasis on vitamin A metabolism; McLaren, Roedel and Malathi discussed dietary protein and vitamin A metabolism.

Vitamin A and Endogeneous Protein Metabolism. In 1913, McCollum and Davis (38) at the University of Wisconsin and Osborn and Mendel (39) at Yale discovered vitamin A. They studied the nutritive value of fats and found a mysterious dietary factor present in butterfat, but not in lard, which was

responsible for defective dark adaptation and for xerophthalmia in the rat. They called the factor vitamin A.

In 1925, Morgan and Osburn (40) were the first to investigate the effect of vitamin A deficiency upon nitrogen metabolism. They suggested that, in the absence of vitamin A, the animal organism failed to produce purine-containing compounds from the ordinary sources (possibly arginine and histidine) but continued to reutilize such portions of the discarded purine ring containing substances that were ordinarily excreted in the form of allantoin. Animals fed a vitamin A-deficient diet showed a decrease in the relative amount of allantoin and increased loss of body weight.

Mason and Ellison (41) suggested that vitamin A might have a role in protein metabolism. They discovered that vitamin A-deficient rats were not able to produce normal epithelial tissue. Since then several groups of workers have attempted to demonstrate a relationship between vitamin A and protein metabolism.

Emerique (42) studied avitaminosis A and nitrogen metabolism. He concluded that as the amount of fixed nitrogen decreased during avitaminosis A, the nitrogen balance remained positive and the nitrogen production remained normal. The avitaminosis seemed to consist essentially of a cessation of the synthesis of "special" proteins.

An extensive study of the influence of vitamin A on the utilization of energy and protein by calves was conducted by Ritzman et al. (43). Calves deficient in vitamin A failed to gain weight and showed a 25% decrease in protein utilization compared to the control animals. Digestion, absorption and metabolism of protein also were depressed.

Brown and Morgan (2) studied the effect of vitamin A deficiency on the

nitrogen balance of rats. Weanling rats receiving a vitamin A-deficient diet grew less and retained nitrogen more poorly than their pair-fed controls. This effect could not be observed in adult animals, from which it was concluded that vitamin A could be considered essential for growth of tissue protein but not for its maintenance.

Diaz et al. (44, 45) investigated the intervention of vitamins in protein metabolism in young and adult rats. In spite of the demonstrated relationship of vitamin A and protein they concluded that lack of vitamin A had no appreciable effect on protein utilization in rats of all ages. Moore et al. (46) investigated vitamin A and resistance of rats to protein deficiency. They found that a liberal allowance of vitamin A enabled rats, at least partially, to resist the ill effect of severe protein deficiency. The results obtained by Moore et al. were confirmed by Peretianu and Lupu (47).

Gebhardt (48) confirmed that vitamin A had a pronounced influence on nitrogen balance, particularly in endogeneous metabolism. He conducted nitrogen balance studies on rats receiving different doses of vitamin A, carotene and other vitamins. Useful results were obtained only if carotene was supplied at a level of 5 to 10 μg per 100 g of body weight. The influence of vitamin A on protein synthesis in the organism was studied by Leutskaya (49), who concluded that vitamin A deficiency in chickens caused a decrease in protein synthesis.

Anderson et al. (50) investigated the influence of protein depletion on vitamin A and carotene utilization by vitamin A-deficient sheep. In sheep fed the protein-deficient ration there were significant decreases in serum albumin, albumin/globulin ratio, plasma vitamin A and body weight at the end of the protein-depletion period and also at the termination of the 4-week

injection period. When vitamin A acetate was injected intraruminally and vitamin A storage was expressed in terms of total liver storage, the sheep fed the 10.4% crude protein ration stored more than twice as much vitamin A (66.6 mg) as those fed the 5.9% crude protein ration (32.1 mg).

Kryukova (51) studied changes in proteins of blood serum and liver of rats given a surplus supply of vitamin A. Vitamin A was administered orally in doses of 2000 to 20,000 IU daily for 30-70 days. Total serum proteins were determined and the protein fractions were separated by paper electrophoresis. In liver tissues, the amount of water- and alkali-soluble proteins were determined. The vitamin A treatment, the authors concluded, decreased the concentration of total protein, albumin, and beta-globulins in blood serum and decreased the concentration of alkali-soluble proteins in liver. A single dose of 113,000 IU vitamin A had a similar but slighter effect.

Vakil et al. (52) studied the effect of vitamin A deficiency in rats, alone or in combination with protein deficiency. Lack of vitamin A resulted in a lowering of the albumin fraction in both the protein-supplemented and the protein-deficient animals; withdrawal of protein and vitamin A simultaneously caused the most pronounced decrease. Gamma-globulins were low in both protein-deficient and vitamin A-deficient rats but the double deficiency did not further lower the concentration of gamma-globulins. Beta-globulins did not appear to be influenced by the vitamin A deficiency and the percentage of serum alpha-globulin even increased. In this experiment, the rats were fed ad libitum and part of the effect may have come from the reduced food intake. In a subsequent pair-feeding design, vitamin A deficiency lowered albumin and increased beta- and gamma-globulins, with no change in total serum protein concentration.

De Luca et al. (53) studied vitamin A and protein synthesis by rat intestinal mucosa. They concluded that protein synthesis by membrane-bound, but not by free, polyribosomes of intestinal mucosa was depressed under conditions of vitamin A deficiency and that the vitamin was involved, directly or indirectly, in protein synthesis at the translational level. The fact that protein synthesis was depressed in the intestine but not in the liver led the authors to suggest a tissue specificity with respect to the mechanism of vitamin A action. Another possible explanation was the difference in the rate of cell renewal between liver and intestinal mucosal cells. The rate of cell renewal is slow in the liver but rapid in the intestine. Thus, vitamin A deficiency might be expected to more severely affect function in the intestinal mucosa (or other rapidly renewing tissues) because of the more rapid cell loss. The findings of De Luca et al. (53) were summarized and it was concluded (54) that intestinal mucosa, which is a rapidly proliferating tissue, may represent a more sensitive system than liver for the study of defects arising from vitamin A deficiency.

Vitamin A and Amino Acid Metabolism. Abdulnabi and Bieri (55) studied the pattern of distribution of amino acids in glands and in blood serum of vitamin A-deficient and normal rats. In vitamin A-deficient, as compared to normal, pair-fed rats, tyrosine and phenylalanine levels in the testes were increased, and threonine concentration of the liver decreased with no changes of these amino acids in blood serum. The results suggested that vitamin A might be involved in the metabolism of those three amino acids. If this were so, the authors concluded, one might expect vitamin A to be implicated in some reaction characteristic of protein metabolism. The authors extended their studies (56) and investigated the glutamic-aspartic transaminase

system. They found no difference in the reactivity of such systems in homogenates of the liver, kidney and heart of normal and vitamin A-deficient rats.

Malathi et al. (57) compared the free amino acid composition in the blood, liver and kidneys of vitamin A-deficient and normal, pair-fed rats. Their results, obtained by paper chromatography, confirmed the earlier observations of Abdulnabi and Bieri (55) which were obtained by microbiological assays; however, Malathi et al. (57) found more consistent changes in the three amino acids in all the tissues examined. They suggested that accumulation of phenylalanine and tyrosine in the tissues of vitamin A-deficient rats was due to impairment of the hydroxylation reaction required for the production of adrenaline from phenylalanine in the adrenal glands.

Sastry et al. (58) were able to correct the increased levels of phenylalanine and tyrosine and the decreased threonine of vitamin A-deficient rats by oral supplementation with retinol or retinoic acid. The relationship of ascorbic acid to the metabolism of phenylalanine and tyrosine also was studied (58), and it appeared possible that the increases in tissue levels of free phenolic amino acids might have been due to a reduction in the synthesis of ascorbic acid. Ascorbic acid neutralized with sodium bicarbonate injected into deficient rats led to a correction of the increased concentrations of the phenolic amino acids and left the threonine levels unchanged. Rats maintained on a vitamin A-depletion diet and given supplements of ascorbic acid lived much longer and in better health, compared to controls receiving the depletion diet but no ascorbic acid. The authors concluded that the ability of a rat to synthesize ascorbic acid was directly dependent upon the vitamin A nutritional status. They hypothesized that some of the

symptoms observed in vitamin A-deficient animals might have been due to a secondary deficiency of ascorbic acid superimposed on the direct effect of vitamin A deficiency.

The incorporation of specific labeled amino acids has been studied by several investigators with varying results. Roels et al. (59) found, in vitro, that the rate of incorporation of $^{14}$C-phenylalanine and $^{14}$C-methyl-labeled methionine was higher or lower in the diaphragm of vitamin-deficient rats than in pair-fed controls, depending on whether low or high alpha-tocopherol levels also were fed. They suggested that an abnormal permeability of the diaphragm muscle-cell membrane might have caused this phenomena. Narasinga (60) observed increased incorporation of amino acids in blood, liver and kidney of vitamin A-deficient animals. A possible reason might have been an accelerated mobilization of amino acids into the cells of these tissues during vitamin A deficiency because of alterations in cellular permeability. The free amino acid concentrations of tissues were not increased, however, except for tyrosine and phenylalanine in the blood, liver and kidneys of vitamin A-deficient rats.

Chagovets and Dusheiko (61) found that intraperitoneal injection of $^{14}$C-labeled methionine resulted in a lower specific activity of the serum proteins (50%) in vitamin A-deficient rats compared to control animals. Since mechanisms were not studied, it was impossible to determine whether decreased anabolism or increased catabolism were responsible.

De Luca et al. (62) used rough endoplasmic reticulum and a pH 5 fraction from rat intestinal mucosa to show that vitamin A deficiency led to a lower incorporation of $^{14}$C-leucine into proteins. They traced the lesion to the pH 5 fraction. "Amino acid-charging" activity of vitamin A-normal and

vitamin A-deficient preparations was investigated; leucine, proline, lysine, phenylalanine and glutamic acid incorporations into aminoacyl-t-RNA were 30 to 60% lower in the vitamin A-deficient preparations. Other amino acids were not affected. Vitamin A, therefore, could be involved in the synthesis of the amino acid-activating enzymes, or in the t-RNA's, or as a cofactor for the enzymes.

Transport of Vitamin A in Blood. The review on this subject was limited mainly to vitamin A-binding protein. Dzialoszynski et al. (63) studied carotene and vitamin A alcohol transport in blood plasma. They apparently were the first to suggest that carotene and vitamin A alcohol were carried in the blood by attachment to albumin.

Ganguly et al. (64) conducted studies on the plasma proteins of various animal species. They found that vitamin A esters were associated with the least soluble protein fractions and, in agreement with Dzialoszynski et al., vitamin A alcohol adhered to the more soluble fractions. They suggested that in the liver cell free vitamin A is associated with protein and esterified vitamin A with fat.

Krinsky et al. (65) investigated the same problem as Dzialoszynski et al. (63), but concluded that, although vitamin A alcohol occurred in a protein complex, the carrier was not albumin.

Roels (66) reviewed the involvement of vitamin A in biochemical systems and stated that vitamin A was transported in the blood bound to an unidentified protein carrier. Alvsaker et al. (67) suggested that tryptophan-rich prealbumin represented the unknown specific transport protein for vitamin A in humans. However, detailed information about the transport of retinol in plasma was not available.

Kanai et al. (68) purified and partially characterized the retinol-binding protein (RBP) in human plasma. The protein differed from known low- and high-density plasma lipoporteins and had a hydrated density greater than 1.21. RBP also contained an unusually high amount of aromatic amino acids. On a weight basis, tyrosine made up approximately 6.1% and tryptophan approximately 3.5% of the weight of the RBP molecule. Since retinol has certain "aromatic" characteristics and since RBP contains an unusually high amount of aromatic amino acids, it was possible that the aromatic amino acid residues played an important role in the structure of the retinol-finding 'clefs' and in the interaction between retinol and RBP.

Investigations by Raz and Goodman (69) on the interaction of thyroxine with human plasma prealbumin and with the prealbumin-retinol-binding protein complex showed that the interaction of prealbumin with thyroxine appeared to be independent of the prealbumin-retinol-binding protein interaction.

Roels (66) and Krinsky et al. (65) reviewed the transport of vitamin A in blood more extensively than does this study.

Free Amino Acids in Plasma as Affected by Dietary Protein. The free amino acids in the plasma of a normal, fasting human being were markedly uniform in concentration (70). However, surpluses or deficits of individual amino acids in the diet, or high or low dietary protein intake, lead to changes in plasma amino acid concentrations and to secondary changes in the concentration of other amino acids (70).

Holt et al. (70) have reported on plasma aminograms as affected by protein intake. Results obtained on plasma samples from 64 kwashiorkor patients in 9 countries were surprisingly uniform. The data were in agreement with experimental low-protein diet studies by Tuttle et al. (71). The striking

features of the amino acid patterns of those receiving low-protein diets were (70):

1) A decline in concentration of essential amino acids, most conspicuous in branched-chain acids and least conspicuous in phenylalanine and lysine.

2) A conspicuous decline in concentrations of 4 nonessential amino acids: tyrosine, arginine, citrulline, and butyrine.

3) A resistance to decline in the concentration of other unessential amino acids (glycine, alanine, proline, histidine, serine, aspartic acid). In some of the milder protein deficiency patterns, these amino acids were found in abnormally high concentrations, although in the more severe ones their concentrations were reduced.

It was difficult, the authors (70) remarked, to interpret the kwashiorkor pattern in terms of a single limiting essential amino acid. This led to the conclusion, in agreement with Hundley et al. (72), that the limiting factor was not an essential amino acid but nitrogen, either essential or unessential. The authors attempted to explain some of the findings of the kwashiorkor aminogram:

1) Elevated concentrations of some of the unessential amino acids in the milder protein deficiency patterns may be attributed to the fact that they can be used only to a limited extent in protein synthesis.

2) Conspicuous fall of tyrosine and the smaller decrease in phenylalanine suggests difficulty in the conversion of phenylalanine to tyrosine, a concept originally proposed on the basis of urinary features. Tyrosine may become limiting in this situation, a hypothesis supported by the depigmentation of the hair.

3) Sharp decreases in concentrations of branched-chain amino acids may

be related to the fact that organs other than the viscera are largely responsible for the oxidative deamination of these amino acids. Such organs, notably muscle, may be responsible for the more effective deamination.

4) Elevated values of histidine in the plasma, found in the milder protein deficiency patterns, suggest that these may, at least in part, explain the high excretions of histidine and of urocanic acid reported in kwashiorkor patients by Dean and Whitehead (73).

5) One apparent discrepancy between the kwashiorkor pattern and the pattern induced by low-protein diets in which the protein was supplied as milk is puzzling: the concentration of lysine tends to resist decline in kwashiorkor, an observation also made by Edozien et al. (74). However, when milk protein intake is reduced in infants, the fall in the concentration of lysine appears promptly and is as marked as the reduction in concentration of the branched-chain amino acids.

When the amino acid composition of serum albumin in patients with kwashiorkor was studied by Potgieter et al. (75), they found no statistical differences, as compared to that of normal controls, with a possible exception in the content of amide ammonia. Wannemacher and Allison (76) studied plasma amino acid concentrations in relation to protein synthesis. The serum essential-to-nonessential ratio (E/NE) of free amino acids dropped from a control value of 0.58 to 0.34 in rats fed a protein-free diet for 1 day. The ratio continued to decrease slowly as the animals lost body nitrogen until it was 0.18 in rats which had lost 50% of their body nitrogen. All essential free amino acid concentrations decreased as the loss of body nitrogen increased. Except for aspartic acid and proline, the nonessential free amino acid concentrations were elevated in mild protein depletion; in

severe depletion, nonessential free amino acid concentrations decreased, but never fell below 50% of that of the control value. The authors concluded, therefore, that free amino acids of the serum must have represented the algebraic sum of tissue contributions to, and depletions of, this free amino acid pool. When the same authors fed excess dietary protein, it did not result in an overall increased utilization of amino acids for protein metabolism or in elevation of free amino acid levels in serum.

Holt et al. (70) noticed an increase of all essential amino acids in serum when a 9-g-per-kg protein diet was fed; the greatest increase was in methionine, with somewhat lesser increases in phenylalanine, threonine, valine and leucine. In the nonessential group, tyrosine and proline increased greatly, the amino acids of the urea cycle increased somewhat less, and the others remained within normal ranges. The authors were unable to explain the peculiar pattern found in the high-protein diet and they concluded that the diet affected the plasma amino acids in ways not yet understood.

Anderson et al. (77) studied the association among food and protein intake, serine dehydratase and plasma amino acids. Serine-threonine dehydratase and plasma amino acids. Serine-threonine dehydratase was selected as representative of enzymes that showed increased activity under conditions in which amino acid catabolism was enhanced. The serine-threonine dehydratase activity was low initially but increased as protein intake rose above the amount required for rapid growth. Plasma amino acid concentrations were greatly elevated one day after animals were fed high-protein diets but, with the exceptions of leucine, isoleucine, and valine, decreased thereafter as serine-threonine dehydratase activity increased. Food intake of rats fed high-protein diets was depressed initially when serine-threonine dehydratase

activity was low and plasma amino acid concentrations were high. Subsequently as food intake rose, enzyme activity increased and plasma amino acid concentrations decreased. Ingestion of a high-protein diet apparently resulted in a series of homeostatic responses. Initially plasma amino acid concentrations rose and food intake fell; despite this, protein intake was elevated and activities of enzymes for amino acid catabolism increased; subsequently plasma amino acid concentrations decreased and food intake returned to normal.

Methods. Deproteinization of Blood Serum. Aqueous solutions of amino acids are readily separated and quantified with the automatic amino acid analyzer. Blood serum, however, must be deproteinized before analysis, because protein clogs the resin, resulting in poor resolution of the amino acids. For that reason, various methods of deproteinization have been investigated. Hamilton and Van Slyke (78) precipitated plasma proteins with 1% picric acid and found lower carbon dioxide blanks in the Van Slyke manometric procedure than when using other precipitating agents. Stein and Moore (79) introduced a method of ion-exchange chromatography for determination of free amino acids in plasma, in which they modified the deproteinization procedure to remove excess picric acid by passing the supernatant fluid through a resin column. The eluate was concentrated to a known volume before a portion of it was subjected to chromatography. For the deproteinization of plasma they compared values obtained by equilibrium dialysis, ultrafiltration, and precipitation with 1% picric acid. Equilibrium dialysis was carried out for 16 hours at 4°C according to the procedure of Hamilton and Archibald (80). Ultrafiltration was done according to the procedure of Prescott and Waelsch (81). Picric acid deproteinization was accomplished as described by

Hamilton and Van Slyke (78), with the additional step, described above, to remove excess picric acid. The authors concluded that removal of protein by precipitation with 1% picric acid was the most satisfactory of the methods tested. Removal of protein from the plasma took only 30 minutes after blood was drawn. Recoveries of added amino acids were consistently within 10% of theory. When either equilibrium dialysis or ultrafiltration was employed, difficulties were experienced in obtaining reproducible recoveries of the individual amino acids added to the plasma. Both processes are relatively slow, requiring about 16 hours at 4°, and apparently during this interval secondary changes take place. Hamilton (82) later compared 3% sulfosalicylic acid and 1% picric acid for deproteinization of serum and found no difference in the amino acid concentration of the resulting filtrates. He placed the centrifugate directly on the analytical column without removing sulfosalicylic acid. More recently, Gerritsen et al. (83) examined deproteinization of serum with picric acid (method of Stein and Moore, 79) and deproteinization with 4.5% sulfosalicylic acid. A third preparation of serum was described involving partial removal of protein with a citric acid buffer, pH 1.5. The latter procedure (83) gave the most reliable and reproducible results. Partial removal of the protein, however, could clog the resin and result in poor resolution of amino acids. Block et al. (84) compared levels of free amino acids in human plasma after precipitation of the proteins by picric acid and by 20% sulfosalicylic acid. The sulfosalicylic acid-deproteinated samples were prepared as follows: 8 ml of plasma, 4 ml of water and 4 ml of 20% sulfosalicylic acid were stirred thoroughly and centrifuged. The supernatant fluid was filtered and a portion applied to the ion-exchange column for amino acid analysis. Both methods gave values

comparable in precision and accuracy. The sulfosalicylic acid method was less time consuming, required fewer manipulations and gave higher values than the picric acid method.

DeWolfe et al. (85) reinvestigated the effect of different methods for removing proteins prior to analysis. They also compared the amino acid contents of serum and plasma and studied the effect of storage on the amino acid contents. Samples were prepared as follows:

Method I. Add 100 mg sulfosalicylic acid (SSA) to 2 ml serum. Mix thoroughly with a stirring rod and centrifuge at 10,000 rpm for 20 minutes at 4°. The supernatant solution had a pH of 2.2.

Method II. Place 3 ml serum or plasma in a 0.25-inch diameter Visking dialysis sac (Union Carbide, Lindsay, Ontario) and carry out ultrafiltration overnight at 4° under a positive nitrogen pressure of 10 lb/sq. inch. Adjust the filtrate to pH 2.2 with HCl.

Method III. Add 1 ml serum to 10 ml 1% picric acid. Mix and centrifuge. Save the supernatant solution and wash the precipitate twice with 3 ml 0.1N HCl. Combine the supernatant solutions and remove excess picric acid on Dowex 2-X8 resin, 200-400 mesh. Concentrate the picric acid-free eluate by freeze drying, adjust the concentrate to pH 2.0 with HCl and dilute to 5 ml with 0.2M citrate buffer, pH 2.2. Analyze 2.0 ml (equivalent to 0.4 ml serum) on each column.

The authors concluded that:

1. Sulfosalicylic acid was more satisfactory than picric acid or ultrafiltration for the preliminary removal of protein for automatic amino acid analysis.

2. Amino acids are more stable in heparinized plasma than in serum; the

addition of fluoride to blood plasma did not further improve stability.

3. Most amino acids in plasma are more stable at -20° than at 6°. Cystine, however, was stable only at 25°, which could not be explained.

4. Amino acids present in a sulfosalicylic acid extract of fresh plasma were stable both at 6° and at -20°.

Amino Acid Analysis. The presence of amino acid nitrogen in the non-protein nitrogen fraction of blood plasma was first established in 1912 by Van Slyke and Meyer (86). Chemical, microbiological and chromatographic techniques have subsequently been employed to determine many of the individual amino acids which contribute to the total alpha-amino nitrogen. Newer methods of amino acid analysis devised by Moore and Stein (87), using fraction collectors, have found use in many fields of research. Spackman et al. (88), however, realized the need for a method of analysis that was more rapid and less laborious than that of Moore and Stein. They constructed a recording apparatus, which in conjunction with improvements in ion-exchange chromatography, permitted determination of amino acids in protein hydrolyzates in 24 hours with the minimum of attention. In the Moore and Stein (87) ion-exchange method, the effluent from the column was collected as a large number of small fractions of known volume, in each of which the individual amino acid contents were determined by a photometric ninhydrin method. In the improved technique by Spackman et al. (88), the ninhydrin reagent is introduced at a constant rate into the effluent flowing from the column. Color development ensues as the mixture is pumped through a coil of capillary tubing immersed in a boiling water bath, after which the intensity of the blue color (diketohydrindylidene-diketohydrindamine) formed when amino acids are present is determined by continuous photometry at 570 nm. The yellow colors from

proline and hydroxyproline are determined at 440 nm. The results are plotted automatically on a recording microammeter. The continuous plot of the intensity of the blue and yellow colors is a series of peaks, each corresponding to a particular amino acid. The area under each peak is proportional to the amount of that amino acid in the sample. The automatic technique developed by Spackman et al. (88) is widely employed today.

# III. EXPERIMENTAL

General Procedures and Methods. Two-day-old Leghorn chicks were obtained from a local hatchery and distributed at random, 10 birds per lot, in wire-floor batteries housed in an animal laboratory with controlled temperature and light. Feed and water were offered ad libitum. The birds fed a vitamin A-free diet were kept in the top compartment to avoid contamination of the feed by vitamin A.

Initial studies in experiment I were comparisons of the amino acid patterns of (a) four-week-old chicks 1) normally fed, 2) vitamin A-deficient, and 3) deficient, supplemented with vitamin A for 3 days; and (b) 16-week-old chicks 1) normally fed, and 2) normally fed for nine weeks then made deficient by feeding a vitamin A-free diet for seven weeks. Experiment II was essentially a repetition of the initial studies. However, depletion of nine-week-old normally-fed chicks was omitted and an additional group fed vitamin A (30,000 IU/kg) supplementation for ten days was included. Experiment III was designed for feeding different levels of vitamin A to birds getting three levels of protein (10%, 20%, 30%).

Blood samples were taken from the neck after decapitation. The blood was allowed to clot for 30 minutes and centrifuged at 5000 rpm for 20 minutes. The serum was prepared immediately for subsequent amino acid analysis. To find a method of sample preparation most suitable for our purpose, two different methods were tried: deproteinization of the blood serum by dialysis (80) and deproteinization with 20% sulfosalicylic acid (84). After deproteinization, the sample was stored in the freezer until the filtrate could be analyzed for amino acids. The amino acids were determined by the automated ion-exchange chromatographic method of Spackman et al. (88).

Stored samples were thawed and analyzed immediately. To allow maximum use of equipment, a short-run procedure for serum amino acids was tried first[1]. For the analysis of protein hydrolzates, an approximately four hour procedure, using sodium citrate buffers was used. Operating conditions for the amino acid analysis are shown in chart I.

A 0.25-ml portion of sample was pipetted onto the basic column, and driven into the resin with nitrogen pressure (15 psi). Inside walls of the column were rinsed twice with pH 5.25 buffer. The pump for pH 5.25 buffer was fitted onto the column and analysis begun. The basic amino acids and ammonia were recorded in the following order: tryptophan, lysine, histidine, ammonia and arginine. The short-column analysis was completed in 50 minutes. Regeneration of the short column was necessary only after repacking or when proteinaceous materials in previous samples had caused deterioration in resolution.

A 0.25-ml portion of sample was pipetted into the long column and the procedure followed as described for the short column, except buffer of pH 3.25 was used. The buffer in the long column was changed automatically after 90 minutes to pH 4.30. The acidic and neutral amino acids were recorded in the following order: taurine, aspartic acid, hydroxyproline, threonine, serine, asparagine, glutamic acid, glutamine, proline, glycine, alanine, half cystine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine. Separation of asparagine, glutamine, threonine and serine was not achieved despite various manipulations tried.

Long-column analysis terminated automatically after 190 minutes. The

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[1]Beckman Manual A-TB-033, December, 1966.

OPERATING CONDITIONS, CHART I

4-Hour procedure with custom spherical resin types PA-35 and UR-30

| Operating conditions required | Analysis of basic amino acids | Analysis of acidic and neutral amino acids |
|---|---|---|
| Column size | 23 x 0.9 cm | 69 x 0.9 cm |
| **Packing** | | |
| Resin type | PA-35 | UR-30 |
| Height of resin column | 5.5 cm | 56 cm |
| Resin diluting buffer (no detergent or thiodiglycol) | pH 5.3(0.35N NaCit) | pH 3.2(0.2N NaCit) |
| **Analysis** | | |
| Duration of run | 50 Min. | 190 Min. |
| Flow rates | | |
| Buffer | 68 ml/hr | 68 ml/hr |
| Ninhydrin | 34 ml/hr | 34 ml/hr |
| First buffer | pH 5.25±0.01(0.35N NaCit) | pH 3.25±0.01(0.2N NaCit) |
| Second buffer | Same as above | pH 4.30±0.02(0.2N NaCit) |
| Buffer change (minutes after start) | None | 90 Min. |
| Operating temperatures | 55.5° ± 0.1° | 55.5° ± 0.05° |
| Approximate column pressure | 50 psi | 150 psi |
| **Regeneration** | | |
| Cleaning (NaOH volume) | Approx. 3 ml | Approx. 15 ml |
| Equilibration (buffer volume) | Approx. 40 ml | Approx. 70 ml |
| Equilibration buffer | pH 5.25±0.10(0.35N NaCit) | pH 3.25±0.10(0.2N NaCit) |

ninhydrin pump was shut off. The buffer pump was switched back to buffer pH 3.25 and allowed to pump for 35 minutes to flush ninhydrin reagent and buffer pH 4.30 from the system. The long column was regenerated after each run. Buffer was removed from the top of the column with a syringe and the NaOH (0.2N) supply line was connected. The NaOH solution entered the resin bed and was allowed to travel down one-half of its total height. The progress of NaOH was followed easily by the change in color of the resin bed to a slightly darker shade. After one hour the NaOH supply line was disconnected and the column was equilibrated with pH 3.25 buffer. Equilibration was completed when the effluent of the column had the pH of the equilibrating buffer. The long column was then ready for reuse.

Since it was not possible to separate threonine and serine by the sodium citrate method, a newer buffer system employing lithium citrate[2] was tested. This procedure permitted separation and quantitation of asparagine and glutamine as well as threonine and serine.

The lithium citrate buffer system was designed for the long-column analysis only. Operating conditions for the amino acid analysis are shown in chart II.

A 0.25-ml portion of sample was applied to the long column as described for the sodium citrate system. The buffer for elution of acidic amino acids was pH 2.80 and was changed automatically after 190 minutes to pH 4.16 for elution of neutral amino acids. The sequence of the recorded amino acids was

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[2]Beckman Manual: A-TB-044, May, 1967.

OPERATING CONDITIONS, CHART II

5-Hour procedure with custom spherical resin type UR-30

| | |
|---|---|
| Resin | UR-30 |
| Column size | 69 x 0.9 cm |
| **Packing** | |
| Height of resin column | 59 cm |
| Resin diluting buffer | pH 2.80 ± 0.01 (0.3N $Li^+$, 0.53M $Cit^-$) |
| Buffer flow rate | 70 ml/hr |
| Column jacket temperature | 38.8° ± 0.1° |
| **Analysis** | |
| Duration of run | 300 minutes |
| Flow rates | |
| Buffer | 70 ml/hr |
| Ninhydrin | 35 ml/hr |
| First buffer | pH 2.80 ± 0.01 (0.3N $Li^+$, 0.053M $Cit^-$) |
| Second buffer | pH 4.16 ± 0.01 (0.3N $Li^+$, 0.033M $Cit^-$) |
| Buffer change time (min after start) | 190 minutes |
| Operating temperature | |
| Bath tank | 39.0° ± 0.1° |
| Column jacket temperature | 38.8° ± 0.1° |
| Approximate column pressure | 240 psi |
| **Regeneration and equilibration** | |
| Regeneration (LiOH volume) | approx. 15 ml |
| Regeneration (LiOH solution) | 0.3N |
| Equilibration (Buffer volume) | approx. 120 ml |
| Equilibration (Buffer solution) | pH 2.80 ± 0.01 (0.3N $Li^+$, 0.053M $Cit^-$) |

as follows: taurine[3], aspartic acid, hydroxyproline[3], threonine, serine, asparagine[3], glutamic acid, glutamine[3], proline, glycine, alanine, valine, half cystine, methionine, isoleucine, leucine, tyrosine and phenylalanine. Half cystine followed valine when using lithium citrate buffer but preceded valine when using sodium citrate buffer. When analysis was completed, the ninhydrin pump was shut off, and pH 2.80 buffer was pumped for 35 minutes to flush pumps and lines. The long column was regenerated with LiOH for one hour and equilibrated with pH 2.80 buffer. The long column was then ready for reuse.

The basic amino acids were analyzed as described for the sodium citrate buffer system.

The area under each peak on the recorder chart, indicating the amount of that amino acid in the sample, was calculated by the height-by-width method[4]. The base line was read at both sides of the peak and the average was used. The height was the value between the average baseline and the position of the maximum of the peak. Height of the peak was multiplied by the width, which was measured at half the height. The height of the peak was determined easily from the chart. Since a logarithmic scale was used for the chart the proportional accuracy with which a height value can be determined was about the same over all of the usable portion of the chart (absorbance 0.001 to 2.0). The width of a peak was measured in terms of time by counting the number of dots printed above the half-height of the peak. To facilitate counting of the dots, every fourth dot on the curves was black.

For initial standardization of the amino acid analyzer, calibration

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[3]Amino acids added to the standard amino acid mixture for calibration runs.

[4]Section 8, part 9, of model 120C, Manual Beckman Instruments (A-IM-3).

trials with standard amino acid calibration mixture[5] were made to obtain curves from which amino acid concentrations of unknown mixtures could be calculated. Some results were calculated by a computer program but others were calculated most conveniently by use of formulas and a calculator. The equation which related peak area (HxW), concentration (in micromoles), and (HxW)-constant of the standard run ($C_{HxW}$) was:

$$\frac{HxW}{C_{HxW}} = \text{micromoles}$$

Final results were given in micromoles/ml serum

$$\frac{\text{micromoles}}{0.25 \text{ (sample aliquot)}} = \text{micromoles/ml sample}$$

$$\frac{\text{micromoles}}{0.25} \times \frac{\text{ml of sample}}{\text{ml of serum}} = \text{micromoles/ml serum}$$

% recovery values were calculated as follows:

| Sample + added standard (in micromoles/ml serum) | - Sample (in micromoles/ml serum) | = X (in micromoles/ml serum) |
|---|---|---|

X = (the standard recovered)

Standard (micromoles/ml standard) x 100 = % recovery

Total vitamin A was determined in blood serum (90) and in liver by a method used in this laboratory (91).

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[5]Beckman-Spinco-Standard (1 ml contains 2.500 ± 0.004 micromoles of each amino acid) Spinco Division, Beckman Instruments, Stanford Industrial Park, Palo Alto, California.

Experiments. Experiment I. Twenty-five, two-day old Leghorn male chickens were wing-banded and divided into two groups. Group (1) was fed with commercial (Pages) chick starter diet. Group (2) was depleted with a vitamin A-free diet (89).

a) After four weeks the animals of group 2 were believed to be depleted, as judged by vitamin A-deficiency symptoms. Blood from seven chicks of group 2 and five chicks of group 1 was taken, pooled by groups, allowed to clot for 30 minutes and centrifuged at 5000 rpm for 20 minutes. The serum samples were deproteinized both by dialysis and 20% sulfosalicylic acid. The dialysis method of Hamilton and Archibald (80) was modified as follows: an aliquot of serum was put in dialysis tubing[6], which was tied on both ends and put in a test tube containing an equal quantity of doubly-distilled water, which covered the dialysis bag. The tube was placed in a refrigerator for 16 hours. The dialysis bag was removed and the dialysate was stored in the freezer.

The sulfosalicylic acid precipitation was accomplished according to the procedure of Block et al. (84), modified as follows: 4 ml of serum, 2 ml of doubly-distilled water, and 2 ml of 20% sulfosalicylic acid[7] were placed in a centrifuge tube and mixed thoroughly with a stirring rod. The tube was centrifuged at 10,000 rpm for 20 minutes at 4°. The supernatant solution was frozen immediately and kept frozen until samples were analyzed.

Total vitamin A was determined in the liver (91) and serum (90) of four-week-old, vitamin A-deficient and control-fed chickens.

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[6]Dialyser Tubing (1-1/4" Flat Width) Fischer Scientific Co.

[7]5-Sulfosalicylic-Acid, Eastman Organic Chemicals.

b) Eight chicks of group 2, which received the vitamin A-free diet for four weeks, were given a high vitamin A-supplemented diet (30,000 IU/kg) for three days. Blood was taken for analysis. Serum from those chicks and from four control-fed birds was prepared for analysis as in (a).

c) Six, nine-week-old, normally-fed chickens (control group 1) were fed a vitamin A-free diet (89) and depleted for two months. Blood was obtained and serum was prepared for analysis as in (a).

d) Amino acid recovery studies on the modified dialysis (80) and sulfosalicylic acid methods (84) were conducted as follows: Blood from 3-month-old control-fed chickens was withdrawn, allowed to clot, and centrifuged. Serum plus standard solution were dialyzed, and also prepared by the sulfosalicylic acid method by replacing 1 ml of doubly-distilled water by 1 ml of standard solution. The rest of the procedures were as described under general procedures.

Amino acid analysis employing the sodium citrate buffer system and the method of calculation are described under general procedures and methods.

Experiment II. Thirty-two-day-old, Leghorn male chicks were fed commercial starter diet for one week and then divided into two groups: a) fifteen chicks were continued on the commercial diet, and b) fifteen chicks on the vitamin A-free diet (89).

After four weeks, group b was believed to be vitamin A-depleted, as judged by deficiency symptoms. Blood serum was prepared from four birds of group a and six birds of group b and was frozen as in experiment I. Total vitamin A was determined on serum and three livers of chicks of both groups.

The remaining chickens in group b were supplemented with a high level of vitamin A (30,000 IU/kg).

After three days of supplementation, blood was drawn from five chicks, prepared and frozen as in experiment I.

After seven more days of supplementation, blood again was taken from three of the remaining birds of group b. At the same time, blood was obtained from three control-fed birds from group a. Blood serum was prepared as in experiment I. See table 1.

Recovery studies for added amino acids were conducted on samples prepared by both precipitation methods.

Amino acid analysis employing the lithium citrate buffer system and the method of calculation proceeded as described in general procedures and methods.

TABLE 1

Experimental conditions and samples taken, Experiment II

| | Age of chicken in days | | |
|---|---|---|---|
| | 35 | 38 | 45 |
| Control | +ox | + | + |
| Deficient | +ox | - | - |
| Deficient plus vitamin A supplementation (30,000 IU/kg) | - | + | + |

+) Blood was withdrawn for analysis.

o) Total vitamin A in liver was determined.

x) Total vitamin A in serum was determined.

-) No sample taken.

Experiment III. Three different levels of protein and different amounts of vitamin A were fed to two-day-old Leghorn male chicks. Nine groups, ten birds per group, were used. A basal diet (89) was fed to all groups and modified according to the desired protein content. The composition of the diets is given in Appendix, table 1.

Times at which serum and liver samples were taken and the experimental diets used are summarized in table 2. A recovery study of added amino acids was conducted for the modified sulfosalicylic acid method.

Amino acid analysis and the method of calculation were the same as described for experiment II.

TABLE 2

Experimental conditions and samples taken, Experiment III

| | Age of chicken in days | | |
|---|---|---|---|
| | 22 | 28 | 48 |
| 20% protein, control (vitamin A, 3000 IU/kg) | +0 | - | + |
| 20% protein, vitamin A deficient | +0 | - | - |
| 20% protein, deficient plus 6-day vitamin A supplementation (10,000 IU/kg) | - | + | - |
| 20% protein, high vitamin A (10,000 IU/kg) | - | - | + |
| 10% protein, control (vitamin A, 3000 IU/kg) | +0 | - | + |
| 10% protein, vitamin A deficient | +0 | - | - |
| 10% protein, deficient plus 6-day vitamin A supplementation (10,000 IU/kg) | - | + | - |
| 10% protein, high vitamin A (10,000 IU/kg) | - | - | + |
| 30% protein, control (vitamin A, 3000 IU/kg) | +0 | - | + |
| 30% protein, vitamin A deficient | +0 | - | - |
| 30% protein, deficient plus 6-day vitamin A supplementation (10,000 IU/kg) | - | + | - |
| 30% protein, high vitamin A (10,000 IU/kg) | - | - | + |

+) Blood was withdrawn and deproteinized by the modified sulfosalicylic acid method.

0) Total vitamin A in liver was determined.

-) No sample taken.

Results, Experiment I. The results for experiment I were not extensive since few data were obtained due to various early difficulties. The free amino acid patterns in serum of chicks fed various diets are illustrated in figure 1. No pattern is shown for threonine and serine due to interference by glutamine and asparagine. The numerical values used for figure 1 are shown in Appendix, table 2. Data were obtained from samples prepared using only the modified dialysis method.

Data from experiment I can be summarized as follows:

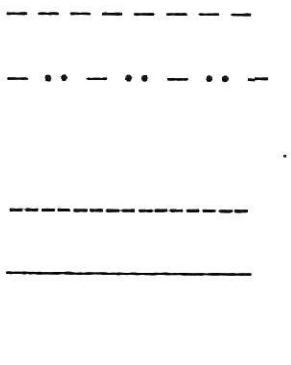
1) Four-week-old control-fed chicks were compared with 4-week-old depleted chicks supplemented with high vitamin A for 3 days. The supplementation increased the serum concentrations of 12 amino acids above those of control-fed birds and lowered lysine, cystine, tyrosine and phenylalanine.

2) Four-month-old control-fed chicks were compared with vitamin A-depleted chicks of the same age. Depleted birds had slightly higher serum amino acid concentrations (highest was lysine, about 20%) than control-fed birds.

By determining total vitamin A concentrations in serum and liver (Appendix, table 3), it was verified that deficient chicks were vitamin A depleted at the time of blood withdrawal.

Results, Experiment II. Results from experiments I and II indicated that, in general, recovery values by the modified sulfosalicylic acid precipitation method were higher and somewhat more consistent than those by the modified dialysis method (table 3). Consequently, all data reported for experiments II and III were obtained from samples prepared only by the modified sulfosalicylic acid method.

Fig. 1 Serum free amino acid patterns in chicks, Experiment I.



Control group, fed commercial diet for 4 weeks.

Depleted chicks supplemented with high vitamin A (10,000 IU/kg) for 3 days.

Control group 2 fed commercial diet for 4 months.

Chicks fed commercial diet for 9 weeks, then vitamin A-free diet for 7 weeks.

**THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.**

**THIS IS AS RECEIVED FROM CUSTOMER.**

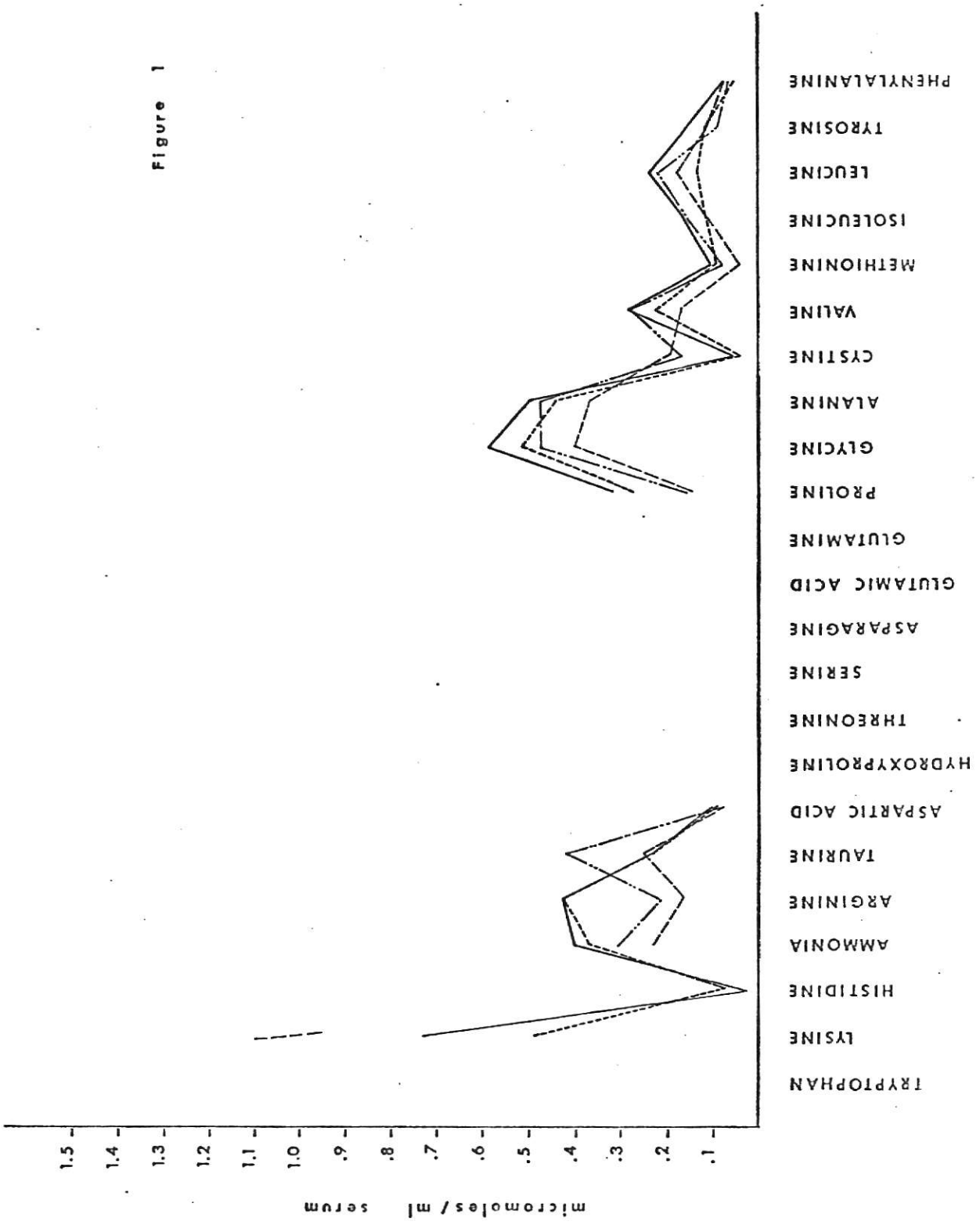

micromoles / ml serum
1.5
1.4
1.3
1.2
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
TRYPTOPHAN
LYSINE
HISTIDINE
AMMONIA
ARGININE
TAURINE
ASPARTIC ACID
HYDROXYPROLINE
THREONINE
SERINE
ASPARAGINE
GLUTAMIC ACID
GLUTAMINE
PROLINE
GLYCINE
ALANINE
CYSTINE
VALINE
METHIONINE
ISOLEUCINE
LEUCINE
TYROSINE
PHENYLALANINE

Figure 1

TABLE 3

Recovery of free amino acids added to serum and deproteinized by (a) modified dialysis and (b) modified sulfosalicylic acid precipitation.

| Amino acid | Recovery (%)* | |
|---|---|---|
| | a | b |
| Lysine | 110 | 124 |
| Histidine | 88 | 88 |
| Ammonia | 49 | 95 |
| Arginine | 120 | 123 |
| Aspartic Acid | 87 | 62 |
| Threonine | 46 | 66 |
| Serine | 51 | 61 |
| Glutamic Acid | 48 | 92 |
| Proline | 57 | 79 |
| Glycine | 66 | 68 |
| Alanine | 68 | 74 |
| Half Cystine | 65 | 78 |
| Valine | 87 | 72 |
| Methionine | 77 | 76 |
| Isoleucine | 58 | 80 |
| Leucine | 67 | 89 |
| Tyrosine | 46 | 75 |
| Phenylalanine | 70 | 68 |

*Average values for two determinations.

Figure 2 illustrates the free amino acid patterns in serum of control-fed chicks, vitamin A-deficient chicks and vitamin A-deficient chicks supplemented with high vitamin A for 3 days. Values for ammonia were not reported because sulfosalicylic acid contained ammonia which interferred sufficiently to distort values. Asparagine was absent or too low to be reported.

The results illustrated in figure 2 can be summarized as follows:

1) The concentration of methionine, isoleucine and leucine in serum of vitamin A-deficient birds increased by 40-50%. Other amino acids in serum of deficient chicks were increased much less or were lower than in serum of control-fed birds.

2) When deficient birds were supplemented with vitamin A concentrations of all amino acids in serum were lower than in that of control-fed birds.

Figure 3 illustrates the free amino acid patterns in serum of control-fed chicks and of deficient chicks supplemented with high vitamin A for 10 days. The patterns were similar in profile but the concentrations differed. Lysine, histidine, arginine, glutamic acid, glutamine, proline and glycine concentrations were lower, and tryptophan, threonine and serine concentrations were markedly higher in serum of supplemented chicks than in that of control-fed chicks. Other amino acids were approximately the same in serum of both groups.

Numerical values used for figures 2 and 3 are shown in Appendix, table 4.

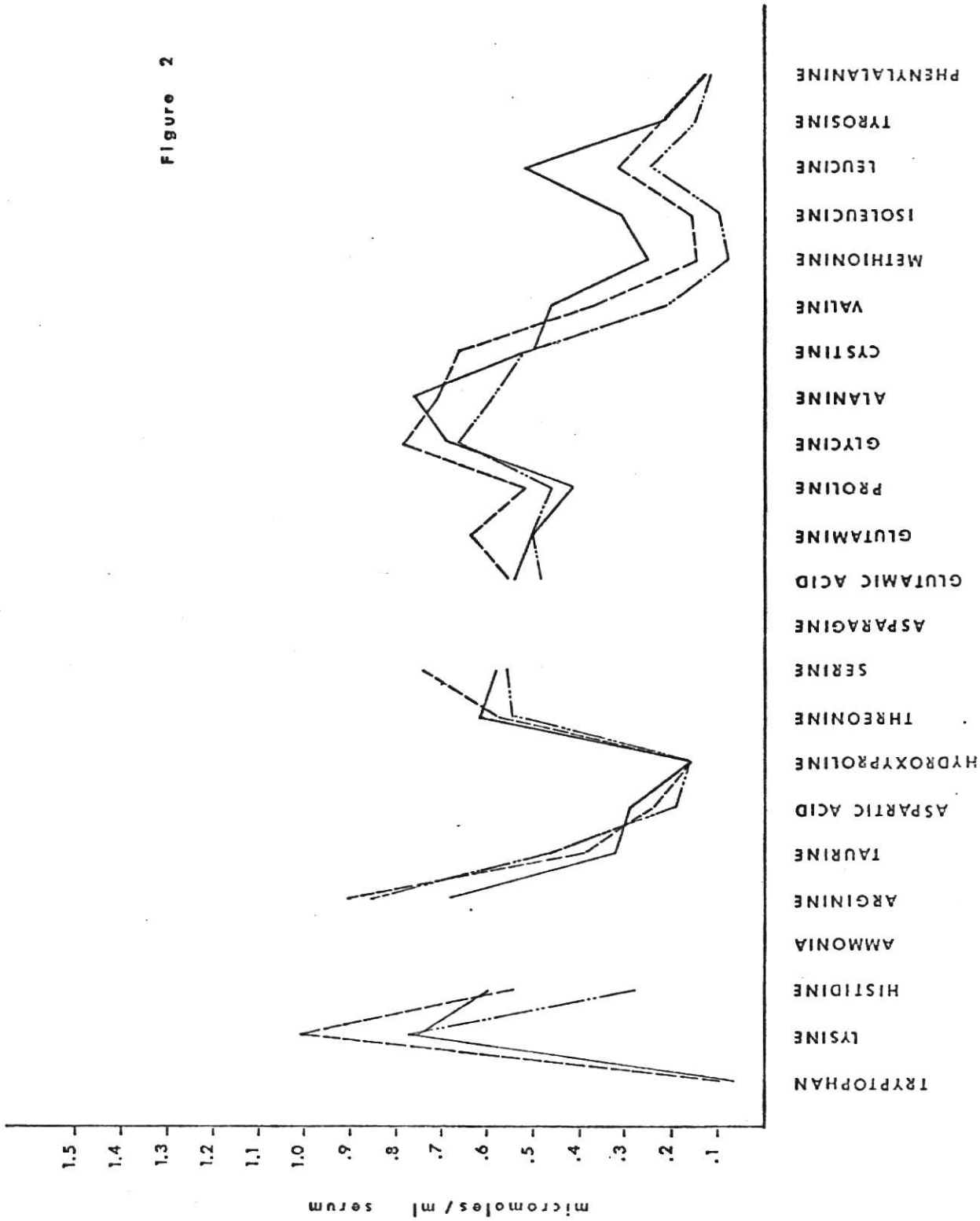
A comparison of the ratios of essential to nonessential amino acids (E/NE) was made. According to the definition of Wannemacher and Allison (76),

Fig. 2 Serum free amino acid patterns in chicks, Experiment II.

– – – – – – – – Control chicks, fed commercial diet for 35 days.

———————— Chicks fed commercial diet for 7 days, then vitamin A-free diet for 28 days.

— •• — •• — •• — Depleted chicks supplemented with high vitamin A (10,000 IU/kg) for 3 days.


micromoles / ml serum
1.5
1.4
1.3
1.2
1.1
1.0
.9
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.6
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.4
.3
.2
.1
TRYPTOPHAN
LYSINE
HISTIDINE
AMMONIA
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TAURINE
ASPARTIC ACID
HYDROXYPROLINE
THREONINE
SERINE
ASPARAGINE
GLUTAMIC ACID
GLUTAMINE
PROLINE
GLYCINE
ALANINE
CYSTINE
VALINE
METHIONINE
ISOLEUCINE
LEUCINE
TYROSINE
PHENYLALANINE

Figure 2

Fig. 3 Serum free amino acid patterns in chicks, Experiment II.

– – – – – – – – Control group, fed commercial diet for 45 days.

– •• – •• – •• – Vitamin A-depleted chicks supplemented with high vitamin A (10,000 IU/kg) for 10 days.

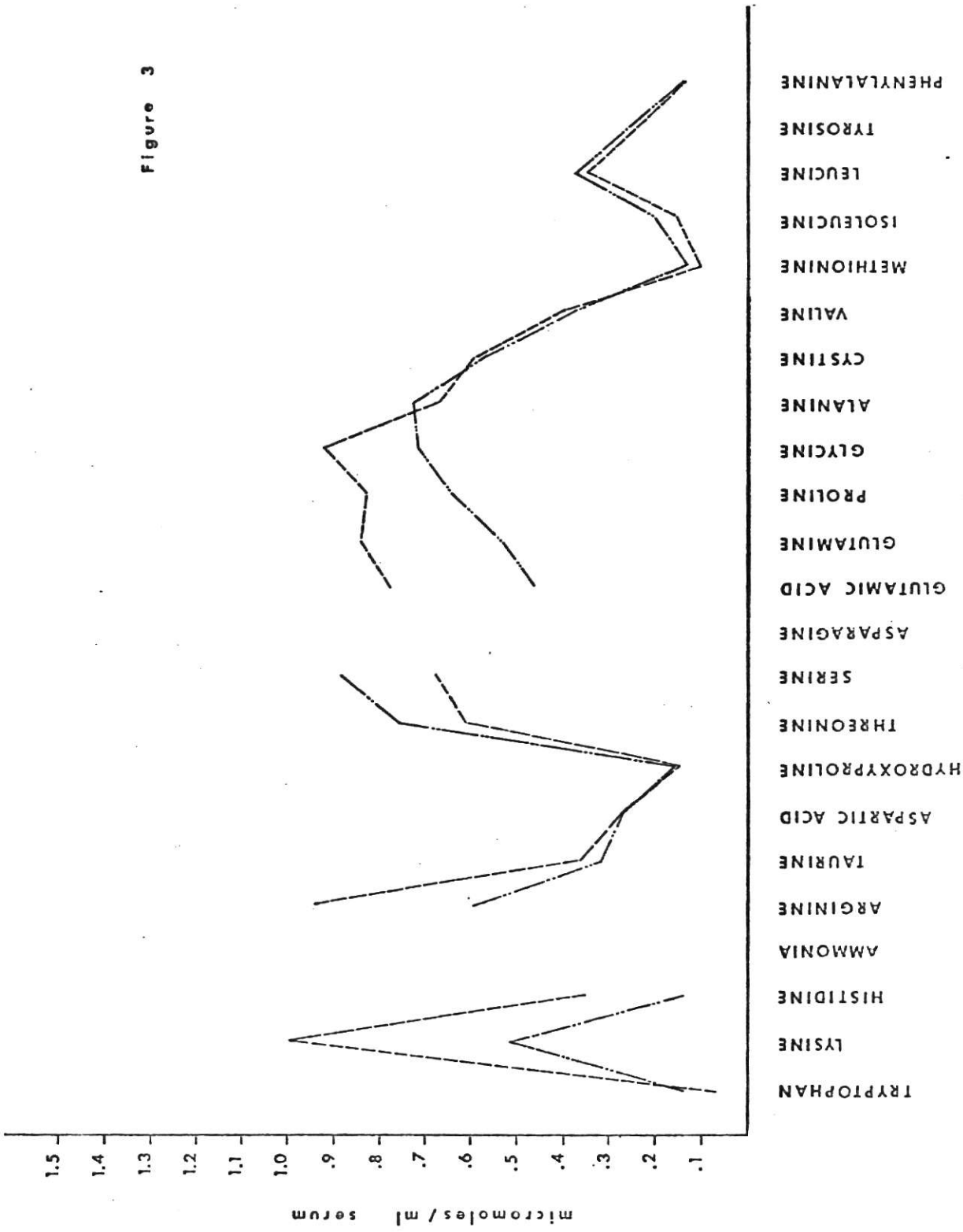


Figure 3
micromoles / ml serum
1.5
1.4
1.3
1.2
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
TRYPTOPHAN
LYSINE
HISTIDINE
AMMONIA
ARGININE
TAURINE
ASPARTIC ACID
HYDROXYPROLINE
THREONINE
SERINE
ASPARAGINE
GLUTAMIC ACID
GLUTAMINE
PROLINE
GLYCINE
ALANINE
CYSTINE
VALINE
METHIONINE
ISOLEUCINE
LEUCINE
TYROSINE
PHENYLALANINE

the following amino acids were considered in the essential group: tryptophan, lysine, histidine, arginine, threonine, valine, methionine, isoleucine, leucine and phenylalanine. Nonessential amino acids were: aspartic acid, glutamic acid, serine, proline, glycine and alanine.

Table 4 shows the ratios of E/NE amino acids in chick serum. The ratio E/NE for vitamin A-deficient chicks was higher than for control-fed chicks. The ratio for deficient chicks supplemented with high vitamin A for 3 days was approximately equal to that of the control-fed birds and the ratio for deficient chicks supplemented for 10 days was somewhat less than that for controls of the same age (45 days).

TABLE 4

Ratio of essential (E) to nonessential (NE) amino acids, Experiment II

| Experimental group | E/NE |
|---|---|
| Control (35 day-old chicks) | $\frac{4.3362}{3.7692} = 1.15$ |
| Deficient (vitamin A-free diet) (35 day-old chicks) | $\frac{4.4576}{3.3144} = 1.34$ |
| Deficient plus 3-day high vitamin A supplementation (38 day-old chicks) | $\frac{3.3616}{2.9864} = 1.13$ |
| Control (45 day-old chicks) | $\frac{4.1544}{4.1584} = 0.99$ |
| Deficient plus 10-day high vitamin A supplementation (45 day-old chicks) | $\frac{3.3976}{3.7672} = 0.90$ |

Results, Experiment III. Results for experiment III will be given first for each dietary protein group receiving various vitamin A treatments and then a comparison of the results of groups receiving the three different protein levels will follow.

Chickens fed a 20%-protein diet had free amino acid patterns as illustrated in figures 4 and 5. Numerical values used for figures 4 and 5 are shown in Appendix, table 5.

Data from figure 4 can be summarized as follows:

1) Concentrations of valine, isoleucine, leucine and tyrosine were slightly higher in serum of vitamin A-deficient chicks than in that of controls; the other 19 amino acid concentrations were lower (especially threonine, 30%).

2) Amino acid patterns in serum of deficient birds supplemented with high vitamin A were similar to that of the deficient group, except threonine, hydroxyproline and alanine were markedly increased and serine, isoleucine and leucine were decreased.

Data from figure 5 illustrate that concentrations of all amino acids decreased in serum of chicks fed high vitamin A for 48 days as compared to that of control-fed chicks.

Chickens fed a 10%-protein diet had serum free amino acid patterns as illustrated in figures 6 and 7. Numerical values for figures 6 and 7 are shown in Appendix, table 6.

Data from figure 6 can be summarized as follows:

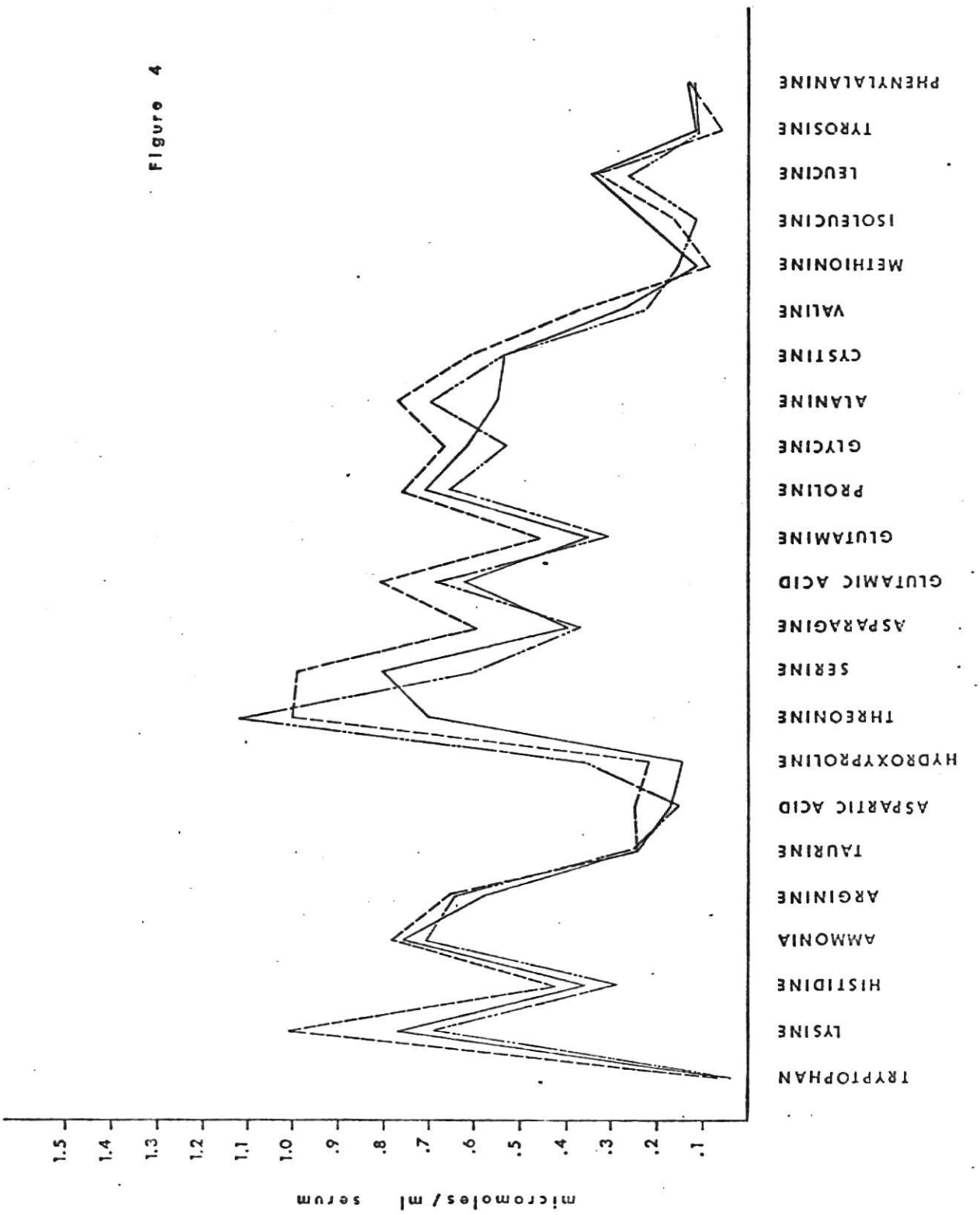
1) Vitamin A-deficient birds had a serum amino acid pattern similar to that of controls, with the exception of decreases in serine and glycine, increases in tyrosine and phenylalanine,

Fig. 4 Serum free amino acid patterns in chicks fed a 20% protein diet, Experiment III.

– – – – – – – – Control group fed basal diet with normal vitamin A (3000 IU/kg) for 22 days.

———————— Deficient group fed vitamin A-free diet for 22 days.

– •• – •• – •• – Depleted birds supplemented with high vitamin A (10,000 IU/kg) for 6 days.


micromoles/ ml serum
1.5
1.4
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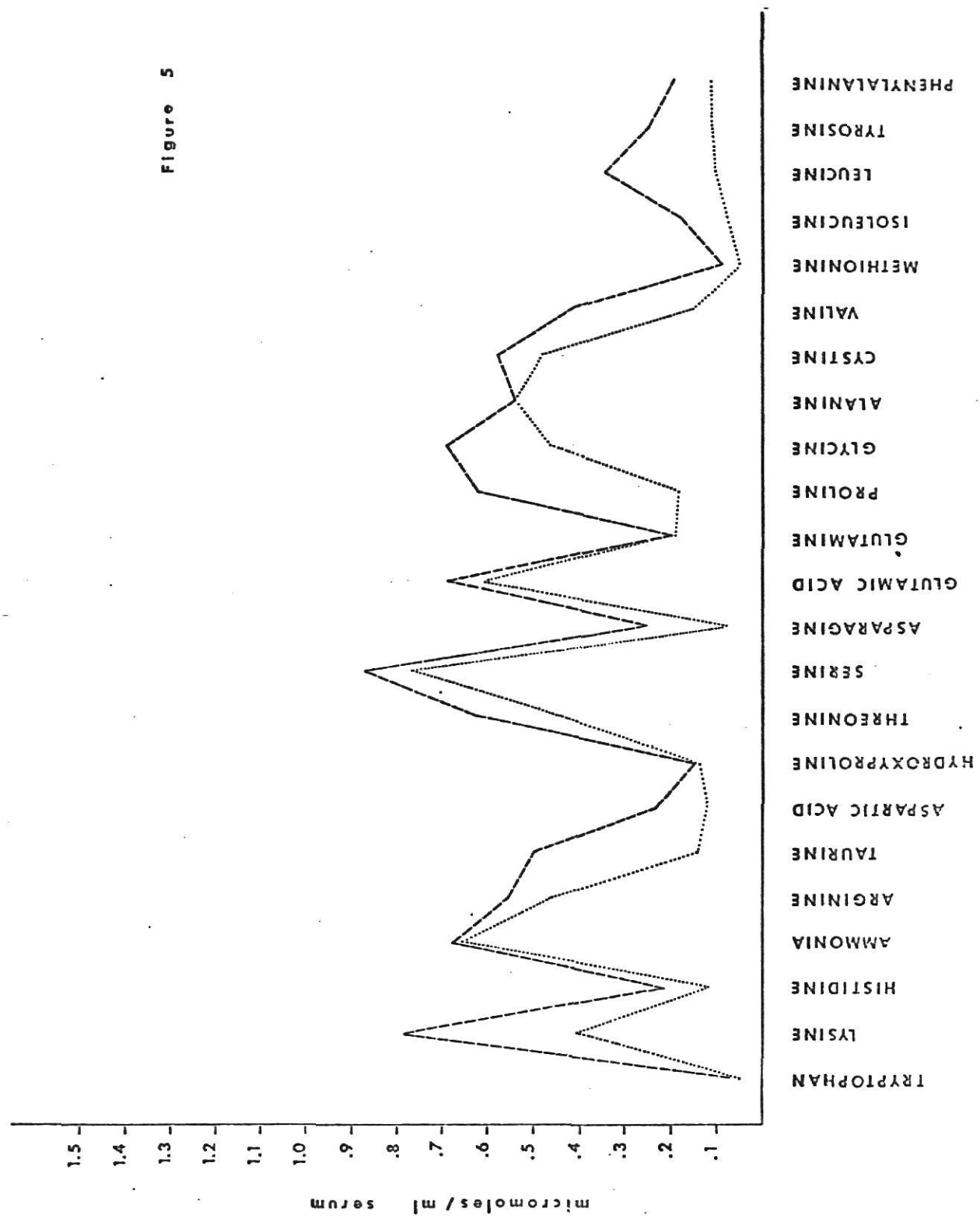
TRYPTOPHAN
LYSINE
HISTIDINE
AMMONIA
ARGININE
TAURINE
ASPARTIC ACID
HYDROXYPROLINE
THREONINE
SERINE
ASPARAGINE
GLUTAMIC ACID
GLUTAMINE
PROLINE
GLYCINE
ALANINE
CYSTINE
VALINE
METHIONINE
ISOLEUCINE
LEUCINE
TYROSINE
PHENYLALANINE

Figure 4

Fig. 5 Serum free amino acid patterns in chicks fed a 20% protein diet, Experiment III.

– – – – – – – – Control group fed basal diet with normal amount of vitamin A (3000 IU/kg) for 48 days.

•••••••••••••• High vitamin A diet (10,000 IU/kg) fed for 48 days.


micromoles / ml serum
1.5
1.4
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ASPARAGINE
GLUTAMIC ACID
GLUTAMINE
PROLINE
GLYCINE
ALANINE
CYSTINE
VALINE
METHIONINE
ISOLEUCINE
LEUCINE
TYROSINE
PHENYLALANINE

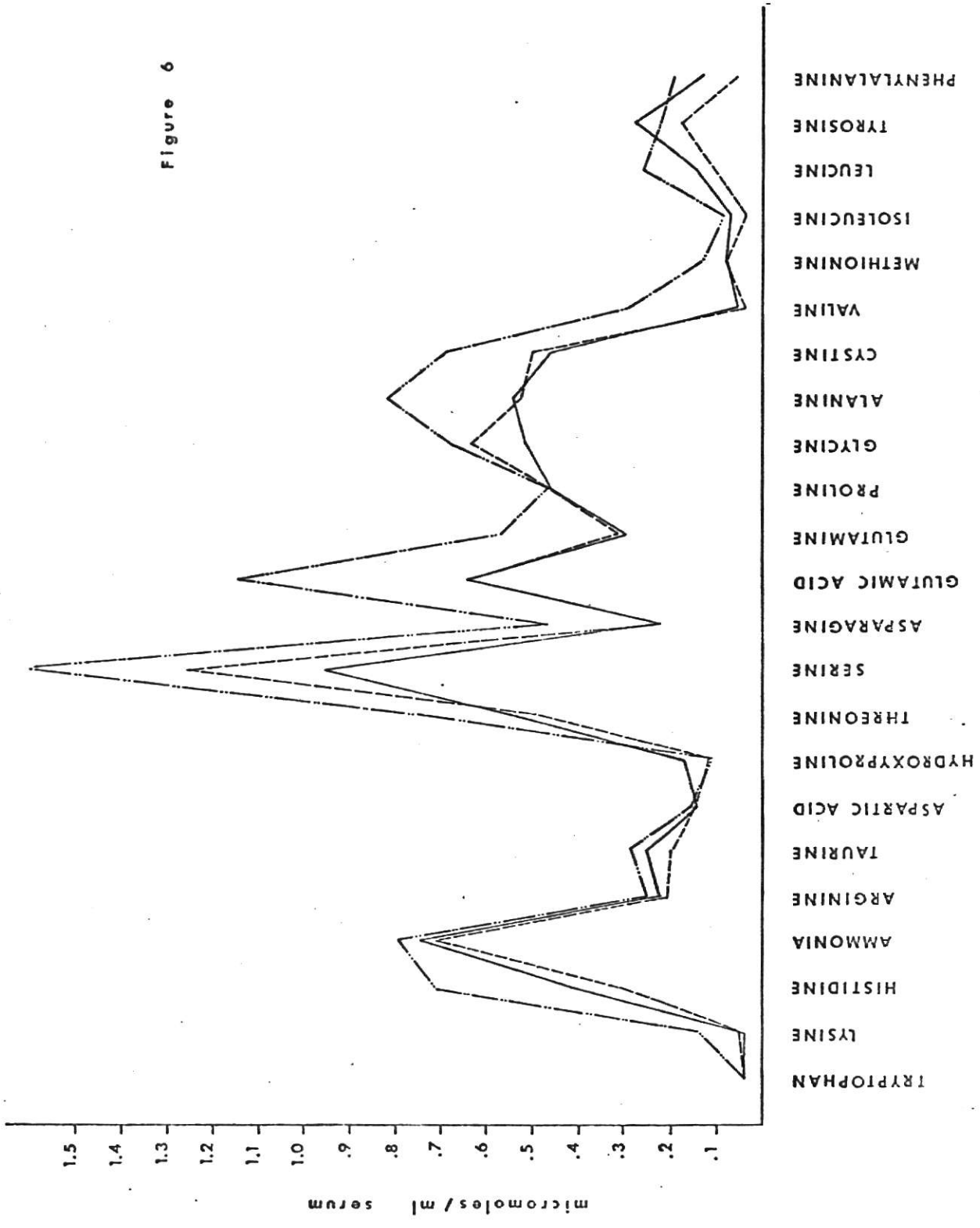
Figure 5

Fig. 6 Serum free amino acid patterns of chicks fed a 10% protein diet, Experiment III.

– – – – – – – – Control group fed basal diet with normal amount of vitamin A (3000 IU/kg) for 22 days.

———————— Deficient group fed vitamin A-free diet for 22 days.

– •• – •• – •• – Depleted birds were supplemented with high vitamin A (10,000 IU/kg) for 6 days.


micromoles / ml serum
1.5
1.4
1.3
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1.1
1.0
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TRYPTOPHAN
LYSINE
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SERINE
ASPARAGINE
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CYSTINE
VALINE
METHIONINE
ISOLEUCINE
LEUCINE
TYROSINE
PHENYLALANINE

Figure 6

and minor increases in leucine and isoleucine.

2) Deficient birds supplemented with high vitamin A for 6 days had increased concentrations of practically all serum amino acids, especially histidine, glutamic acid, serine, alanine and leucine, as compared to serum of controls.

Data from figure 7 illustrate that chicks fed a high vitamin A diet for 48 days had decreased concentrations of basic amino acids, methionine and proline in the serum, as compared to those of control-fed birds. Serine, glycine, and alanine were increased, with lesser or no changes in the other amino acids.

Chickens fed a 30%-protein diet had free serum amino acid patterns as illustrated in figures 8 and 9. Numerical values for figures 8 and 9 are shown in Appendix, table 7.

Data from figure 8 can be summarized as follows:

1) As compared to controls, concentrations of all 23 amino acids decreased in serum of vitamin A-deficient birds.

2) Deficient chicks supplemented with high vitamin A had higher concentrations of all serum amino acids, except lysine, aspartic acid and glutamine, which were considerably lower. Concentrations of all amino acids were greater in serum of supplemented birds than in that of deficient birds.

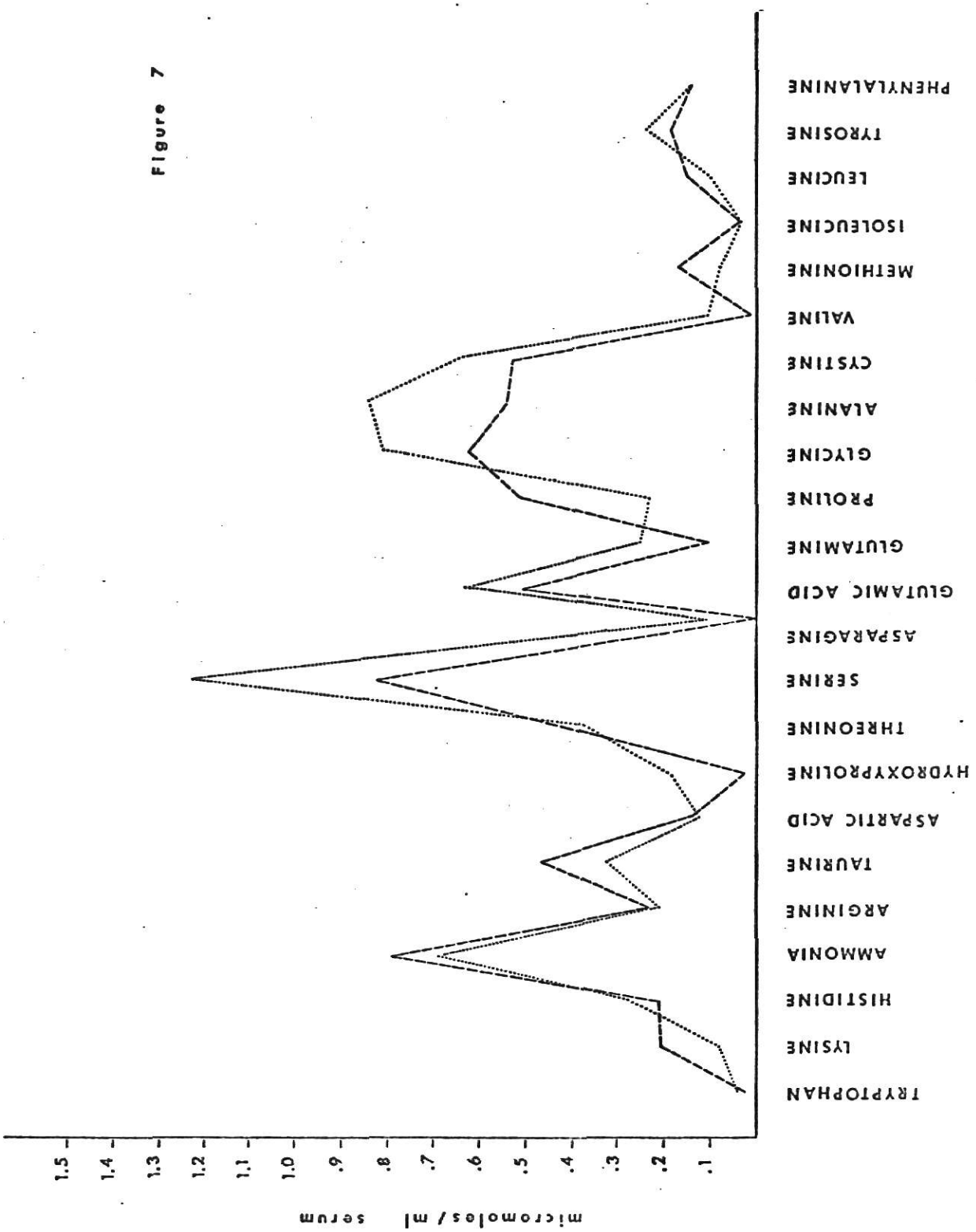
Data from figure 9 illustrate that, as compared to controls, feeding high vitamin A produced marked increases in serum concentrations of lysine, arginine, proline and leucine and decreases in taurine, threonine, serine, asparagine and alanine.

Comparisons of the results of serum amino acid concentrations of chicks

Fig. 7 Serum free amino acid patterns in chicks fed a 10% protein diet, Experiment III.

– – – – – – – – Control group fed basal diet with normal amount of vitamin A (3000 IU/kg) for 48 days.

..... High vitamin A diet (10,000 IU/kg) fed for 48 days.


micromoles / ml serum
1.5
1.4
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TRYPTOPHAN
LYSINE
HISTIDINE
AMMONIA
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TAURINE
ASPARTIC ACID
HYDROXYPROLINE
THREONINE
SERINE
ASPARAGINE
GLUTAMIC ACID
GLUTAMINE
PROLINE
GLYCINE
ALANINE
CYSTINE
VALINE
METHIONINE
ISOLEUCINE
LEUCINE
TYROSINE
PHENYLALANINE

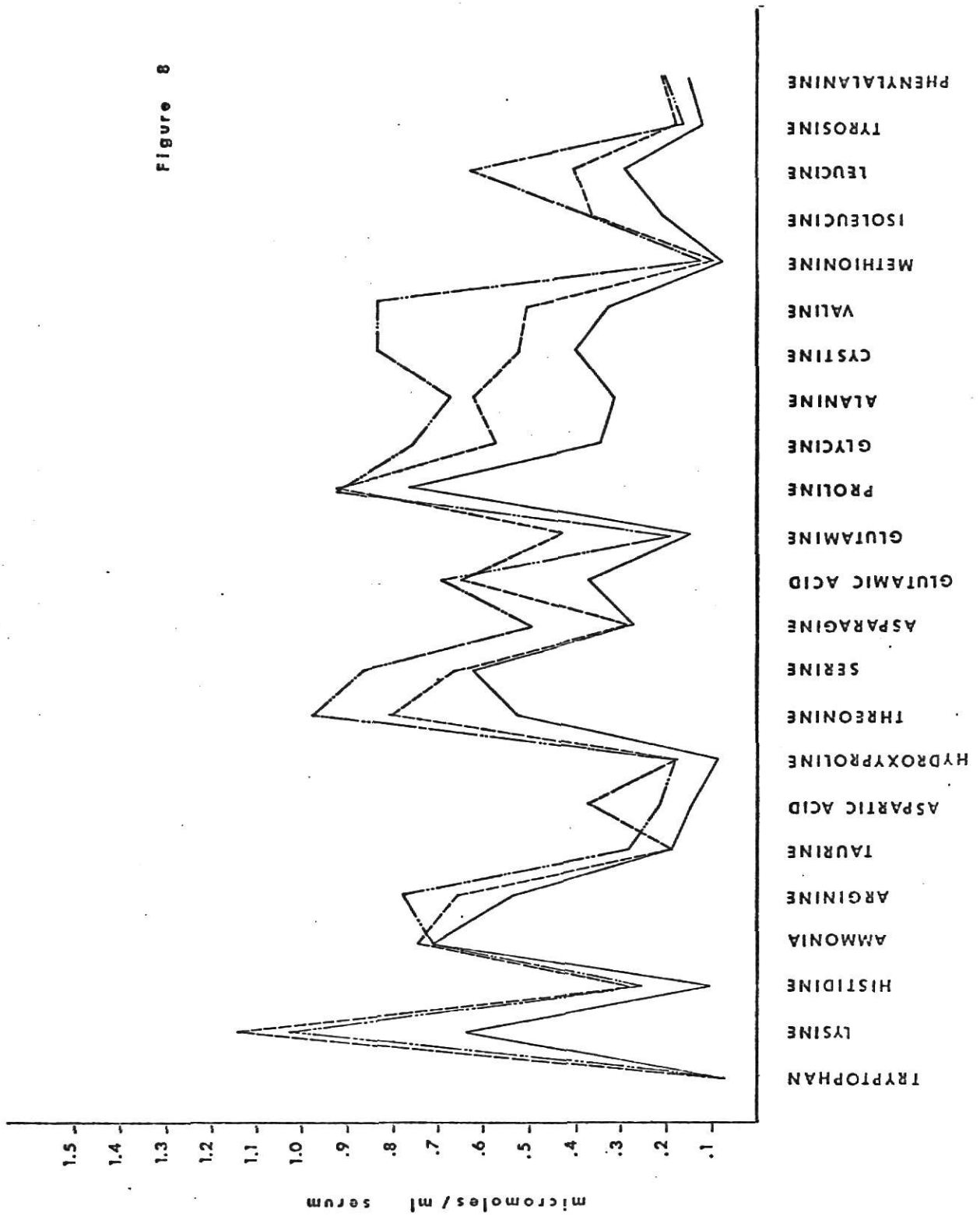
Figure 7

Fig. 8 Serum free amino acid patterns in chicks fed a 30% protein diet, Experiment III.

– – – – – – – – Control group, fed basal diet with normal amount of vitamin A (3000 IU/kg) for 22 days.

———————— Deficient group fed vitamin A-free diet for 22 days.

– •• – •• – •• – Depleted birds were supplemented with high vitamin A (10,000 IU/kg) for 6 days.


micromoles / ml serum
1.5
1.4
1.3
1.2
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TRYPTOPHAN
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HYDROXYPROLINE
THREONINE
SERINE
ASPARAGINE
GLUTAMIC ACID
GLUTAMINE
PROLINE
GLYCINE
ALANINE
CYSTINE
VALINE
METHIONINE
ISOLEUCINE
LEUCINE
TYROSINE
PHENYLALANINE

Figure 8

Fig. 9 Serum free amino acid patterns in chicks fed a 30% protein diet, Experiment III.

– – – – – – – – Control group, fed basal diet with normal amount of vitamin A (3000 IU/kg) for 48 days.

•••••••••••••• High vitamin A diet (10,000 IU/kg) for 48 days.

Figure 9
micromoles / ml serum
1.5
1.4
1.3
1.2
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
TRYPTOPHAN
LYSINE
HISTIDINE
AMMONIA
ARGININE
TAURINE
ASPARTIC ACID
HYDROXYPROLINE
THREONINE
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GLUTAMIC ACID
GLUTAMINE
PROLINE
GLYCINE
ALANINE
CYSTINE
VALINE
METHIONINE
ISOLEUCINE
LEUCINE
TYROSINE
PHENYLALANINE

fed the three different protein levels indicate that chicks fed a control 10%-protein diet had a high serum concentration of serine, somewhat less of valine, isoleucine and leucine, and much less of lysine (90%), as compared to chicks fed a 20%-protein diet. Other amino acid concentrations were approximately the same in serums of the two groups. High serine and low lysine concentrations were found in serum of all experimental groups fed the 10%-protein diet.

The control 30%-protein diet resulted in a similar serum amino acid profile to that of the control 20%-protein diet.

Comparisons of the ratios of essential to nonessential serum amino acids were made among all groups of experiment III (table 5). In general, increasing dietary protein from 10% to 20% to 30% increased the ratios of essential to nonessential amino acids accordingly.

Vitamin A-deficiency elevated the E/NE ratio of serum amino acids in chicks fed diets of all three levels of protein as compared to their controls. Chicks fed vitamin A-free diets at all three protein levels grew less than the controls.

The E/NE serum amino acid ratio of depleted birds supplemented with high vitamin A for 6 days was higher than that of control-fed birds. The increase in the E/NE ratio for 6-day supplemented birds fed 10%- and 20%-protein levels was even greater than for vitamin A-deficient birds. At the 30%-protein level, the ratio of essential to nonessential amino acids for the deficient plus 6-day-supplemented chicks was essentially the same as for deficient chicks.

Feeding chickens high vitamin A and 10%- and 20%-protein diets resulted in a decrease in the E/NE ratios as compared to those in serum of 45-day-old

control chicks. However, 30%-protein and a high vitamin A diet increased the E/NE ratio considerably.

TABLE 5

Ratio of essential to nonessential amino acids, Experiment III.

| Experiment group | E/NE | | |
|---|---|---|---|
| | 10% protein | 20% protein | 30% protein |
| Control (22 day-old chicks) | $\frac{1.4416}{3.6824} = 0.39$ | $\frac{4.2960}{4.2720} = 1.00$ | $\frac{4.5464}{3.8160} = 1.20$ |
| Deficient (22 day-old chicks) | $\frac{1.7560}{3.0488} = 0.57$ | $\frac{3.6168}{3.5024} = 1.03$ | $\frac{2.9488}{2.2552} = 1.30$ |
| Deficient, plus 6-day high vitamin A supplementation (28 day-old chicks) | $\frac{2.8152}{4.7936} = 0.59$ | $\frac{3.7552}{3.3704} = 1.11$ | $\frac{5.2880}{4.0984} = 1.29$ |
| Control (48 day-old chicks) | $\frac{1.6304}{3.1568} = 0.52$ | $\frac{3.5048}{3.6360} = 0.97$ | $\frac{4.4968}{3.7856} = 1.19$ |
| High vitamin A (48 day-old chicks) | $\frac{1.4792}{3.8760} = 0.38$ | $\frac{1.9680}{2.6880} = 0.73$ | $\frac{5.1280}{3.9304} = 1.30$ |

# IV. DISCUSSION

Malathi et al. (57) stated that tyrosine and phenylalanine were markedly increased and threonine decreased, while other amino acids were insignificantly affected, in serum of vitamin A-deficient rats as compared to their pair-fed controls. Other investigators (55, 56, 58, 59) were in agreement with these observations.

This investigation partially verified also the results of Malathi et al. but differences were observed. In this study, higher concentrations of tyrosine and phenylalanine were found in serum of vitamin A-deficient chicks fed a 10%-protein diet, which was in agreement with the investigators cited. In the present study, low lysine and serine concentrations were found in serum of chicks fed the vitamin A-free diet, as compared to their 20%-protein controls.

At a 20%-protein level, a marked decrease in threonine and increase in tyrosine concentration in serum of vitamin A-deficient chicks was found, as did the above investigators, but increased levels of valine, isoleucine and leucine also were present as compared to the control.

At a 30%-protein level, decreases were found in all 23 serum amino acids of vitamin A-deficient chicks. A lower tyrosine concentration was found in serum of deficient chicks fed the 30%-protein diet as compared to that in serum of chicks fed the corresponding vitamin A-normal, control diet.

Attempts have been made to explain the increased concentrations of tyrosine and phenylalanine in vitamin A-deficiency. Malathi et al. (57) speculated that accumulation of phenylalanine might be due to damaged adrenal glands which impaired hydroxylation reactions that are required for the production of adrenaline from phenylalanine.

It is tempting to suggest that in vitamin A deficiency, the retinol-binding protein in serum might be broken down. Retinol-binding protein was found to be extremely high in tyrosine and tryptophan (68). If vitamin A deficiency caused breakdown of retinol-binding protein, increased quantities of free tryptophan and tyrosine might be freed to the serum. However, the essential amino acid tryptophan might be metabolized immediately by the animal and thus not be elevated in the serum. In this study, tryptophan was not found to be elevated in serums of vitamin A-deficient chicks fed 10%- and 20%-protein diets but tyrosine was elevated.

Results of this study differing from those in the literature might have been caused by two facts: 1) Chickens were used as experimental animals, rather than rats which were used by other investigators (55, 56, 58, 59). 2) Feeding was ad libitum rather than pair-feeding. These two differences might account at least in part for changes in concentrations of individual amino acids in this study as compared to results reported in the literature.

Short-time high vitamin A supplementation of deficient birds fed all protein levels resulted in an amino acid pattern which can be considered intermediate between that of chicks fed the vitamin A-deficient diet and that of chicks fed the high-vitamin A diet.

Studies of the ratio of essential to nonessential amino acids indicated that increasing dietary protein (10% to 20% to 30%) increased E/NE ratios in chick serum. Increases especially in concentrations of the essential amino acids, which are not synthesized and must be furnished by dietary protein, were mainly responsible for this change. In the E/NE ratios, the numerator changed more than the denominators.

Vitamin A deficiency increased the serum E/NE ratio at all three protein

levels as compared to the corresponding control, suggesting that the essential and, to a lesser degree, the nonessential amino acids were utilized less efficiently. Deficient animals also grew less than their controls.

The serum E/NE ratios of depleted birds supplemented with high vitamin A for 3 or 6 days also was raised as compared to the controls at all three protein levels. However, when deficient birds were vitamin A-supplemented for 10 days, the E/NE ratio was more nearly normal, indicating that shorter time supplementation was insufficient to bring about the changes in amino acid utilization.

Forty-eight-day-old chicks fed high vitamin A in the 10%- and 20%-protein diets had a lower serum E/NE ratio than 48-day-old chicks fed a diet normal in vitamin A and the same protein content, suggesting a more efficient utilization of essential amino acids. High vitamin A fed with a 30%-protein diet, however, resulted in higher concentrations of essential amino acids as compared to the control, which suggests less efficient utilization but may merely reflect an excess available when the highest protein diet was fed.

Comparison of essential to nonessential amino acid (E/NE) ratios in chick serum suggested that, in general:

a) E/NE ratio in serum varied directly with levels of dietary protein.

b) Vitamin A deficiency impaired protein utilization, as it also depressed growth rate.

c) High vitamin A supplementation of deficient chicks at all three protein levels apparently had beneficial effects on the E/NE ratios only when supplementation was given for at least 10 days.

d) As measured by E/NE ratios, high vitamin A fed with 10%- and 20%-protein diets resulted in more efficient utilization of dietary protein than when normal levels of vitamin A were fed. This effect was not apparent with a 30%-protein diet.

# V. SUMMARY

The serum free amino acid patterns of chicks fed diets containing various protein levels varied with different vitamin A treatments. The pattern variations were not consistent according to vitamin A treatment within any one protein level but there were several similarities among protein levels.

Vitamin A deficiency with 10%-protein diets caused an increase in serum concentrations of tyrosine and phenylalanine and a decrease in concentrations of lysine and serine, as compared to 20%-protein diets with normal vitamin A levels. Vitamin A deficiency with 20%-protein diets decreased the concentration of threonine, and increased concentrations of tyrosine, valine, isoleucine and leucine as compared to the same diet with normal vitamin A levels. Vitamin A deficiency with 30%-protein diets caused a decrease in all 23 serum amino acid concentrations as compared to control birds. It was speculated that the high serum tyrosine content in the vitamin A deficiency state might be caused in part by the breaking down of retinol-binding protein, which has a high tyrosine content. At all three dietary protein levels the amino acid patterns in serum of vitamin A-deficient chicks supplemented with high vitamin A for 3 or 6 days were approximately intermediate between those of chicks fed vitamin A-deficient diets and those of chicks fed high-vitamin A diets. In general, it was found that:

a) The E/NE ratio in serum varied directly with levels of dietary protein.

b) Vitamin A deficiency impaired protein utilization, as well as depressing growth rate.

c) High vitamin A supplementation of vitamin A-deficient chicks

fed all three protein levels seemed to have a beneficial effect on the E/NE ratios only when supplementation was given for at least 10 days.

d) As judged by E/NE ratios, high vitamin A fed with 10%- and 20%-protein diets resulted in more efficient utilization of dietary protein than when normal vitamin A levels were used. This effect was not apparent at the 30%-protein level.

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# APPENDIX

APPENDIX, TABLE 1

Composition of experimental diets[1]

| | Ingredients | Protein content, % 20 | 10 | 30 |
|---|---|---|---|---|
| | | lb | lb | lb |
| 1. | Corn, white, ground | 30.0 | 49.0 | 23.5 |
| 2. | Sorghum grain, ground | 31.5 | 41.5 | 20.0 |
| 3. | Soybean oil meal, 44% solvent-extracted | 29.0 | - | 39.0 |
| 4. | α-Protein | - | - | 8.0 |
| 5. | Brewer's dried yeast | 2.0 | 1.0 | 2.0 |
| 6. | Non-fat milk solids | 2.0 | 2.0 | 2.0 |
| 7. | Steamed bone meal | 2.0 | 3.0 | 2.0 |
| 8. | Calcium carbonate | 1.0 | 1.0 | 1.0 |
| 9. | Sodium chloride | 0.5 | 0.5 | 0.5 |
| | | g | g | g |
| 10. | Cottonseed oil | 300.0 | 300.0 | 300.0 |
| 11. | Manganese sulfate premix | 138.5 | 138.5 | 138.5 |
| 12. | Vitamin premix | 469.5 | 469.5 | 469.5 |
| Total | | 100 lb | 100 lb | 100 lb |

Vitamin premix

| | g |
|---|---|
| Vitamin $B_{12}$, 12 μg/lb | 30 |
| Vitamin $D_3$ (15,000 ICU/gm) | 4 |
| DL-methionine | 46 |
| B-vitamin supplement | 23 |
| Choline chloride | 6 |
| Wheat middlings | 360.5 |
| | mg |
| Vitamin K (menadione) | 23 |
| Total | 469.5 g |

Manganese sulfate premix

| | g |
|---|---|
| $MnSO_4H_2O$ | 25 |
| Wheat middlings | 113.5 |
| Total | 138.5 g |

[1]Vitamin A levels not shown, see text.

APPENDIX, TABLE 2

Concentration of free amino acids in chick serum[1], Experiment I

| Amino Acids | micromoles/ml serum | | | |
|---|---|---|---|---|
| | control (4 weeks) | deficient +3 day Vit. A (4 weeks) | control (4 month) | deficient[2] (4 month) |
| Tryptophan | - | - | - | - |
| Lysine | 1.1044 | 1.0268 | .4958 | .6080 |
| Histidine | - | - | .0880 | .1480 |
| Ammonia | .2272 | .3096 | .3763 | .5560 |
| Arginine | .1572 | .2172 | .4316 | .3792 |
| Taurine | .2412 | .4136 | .2230 | .3648 |
| Aspartic acid | .0736 | .0880 | .0988 | .0976 |
| Hydroxyproline | - | - | - | - |
| Threonine | - | - | - | - |
| Serine | - | - | - | - |
| Asparagine | - | - | - | - |
| Glutamic acid | .1364 | .1688 | .1565 | .2104 |
| Glutamine | - | - | - | - |
| Proline | .1376 | .1504 | .2767 | .2816 |
| Glycine | .3916 | .4728 | .5142 | .5504 |
| Alanine | .3648 | .4760 | .4416 | .3032 |
| Valine | .1640 | .2764 | .2225 | .2856 |
| Half cystine | .1880 | .1660 | .0487 | .0768 |
| Methionine | .0336 | .0792 | .0920 | .1008 |
| Isoleucine | .1092 | .1456 | .1125 | .1128 |
| Leucine | .1720 | .2168 | .1336 | .1992 |
| Tyrosine | .1032 | .0896 | .1114 | .1000 |
| Phenylalanine | .0712 | .0684 | .0536 | .0576 |

[1]Values for pooled serum from 3 birds.

[2]Chickens were fed commercial feed for 9 weeks and then were fed vitamin A-free diet for 7 weeks.

APPENDIX TABLE 3

Vitamin A determinations in serum and liver

| | control | deficient |
|---|---|---|
| Experiment I | | |
| serum[1] | 97.86 µg/100 ml | 1.77 µg/100 ml |
| liver[2] | 881.82 units/g | 5.35 units/g |
| Experiment II | | |
| serum[3] | 105.20 µg/100 ml | 1.65 µg/100 ml |
| liver[4] | 832.00 units/g | 7.60 units/g |
| Experiment III[6] | | |
| liver[5] (20% prot.) | 612.00 units/g | 0.718 units/g |
| liver[5] (10% prot.) | 71.24 units/g | 0.146 units/g |
| liver[5] (30% prot.) | 639.27 units/g | 0.69 units/g |

[1]Pooled serum from 3 birds.

[2]Average of 3 birds.

[3]Pooled serum from 4 birds.

[4]Average of 4 birds.

[5]Individual values.

[6]Control birds receiving basal diet supplemented with vitamin A.

APPENDIX TABLE 4

Concentration of free amino acids in chick serum[1], Experiment II

| Amino Acids | micromoles/ml serum | | | | |
|---|---|---|---|---|---|
| | control (35 days) | deficient (35 days) | def.+3 days high Vit. A supplement (38 days) | control (45 days) | def.+10 days high Vit. A supplement (45 days) |
| Tryptophan | .0924 | .0616 | .1056 | .0792 | .1552 |
| Lysine | 1.0164 | .7544 | .7760 | .9856 | .5168 |
| Histidine | .5464 | .6000 | .2888 | .3552 | .1440 |
| Ammonia | - | - | - | - | - |
| Arginine | .9048 | .6800 | .8504 | .9352 | .5952 |
| Taurine | .3916 | .4632 | .4768 | .3632 | .3200 |
| Aspartic acid | .2404 | .2968 | .1944 | .2744 | .2720 |
| Hydroxyproline | .1656 | .1472 | - | .1536 | .1688 |
| Threonine | .5820 | .6248 | .5536 | .6184 | .7680 |
| Serine | .7524 | .5856 | .5600 | .6744 | .8816 |
| Asparagine | - | - | .3680 | - | - |
| Glutamic acid | .5648 | .5504 | .4968 | .7808 | .4680 |
| Glutamine | .6492 | .5392 | .5192 | .8400 | .5304 |
| Proline | .6908 | .4272 | .4696 | .8322 | .6568 |
| Glycine | .7944 | .6904 | .6720 | .9256 | .7248 |
| Alanine | .7164 | .7640 | .5936 | .6704 | .7604 |
| Valine | .3872 | .4792 | .2104 | .4024 | .3744 |
| Half cystine | .6708 | .5064 | .5288 | .6000 | .5600 |
| Methionine | .1524 | .2648 | .0832 | .1192 | .1304 |
| Isoleucine | .1676 | .3272 | .1088 | .1664 | .2000 |
| Leucine | .3294 | .5344 | .2552 | .3512 | .3720 |
| Tyrosine | .2304 | .2240 | .1544 | .2488 | .2672 |
| Phenylalanine | .1476 | .1312 | .1296 | .1416 | .1416 |

[1]Values for pooled serum from 4 birds.

APPENDIX, TABLE 5

Concentration of free amino acids in chick serum[1], Experiment III

| Amino Acids | micromoles/ml serum | | | | |
|---|---|---|---|---|---|
| | control[2] (22 days) | deficient[2] (22 days) | def.+6 days[2] high Vit. A supplement (28 days) | control[2] (48 days) | high Vit. A[2] (48 days) |
| Tryptophan | .0704 | .0560 | .0496 | .0704 | .0424 |
| Lysine | 1.0064 | .7640 | .6952 | .7784 | .4072 |
| Histidine | .4208 | .3544 | .2984 | .2192 | .1128 |
| Ammonia | .7782 | .7576 | .7136 | .6840 | .6608 |
| Arginine | .6136 | .5896 | .6576 | .5544 | .4528 |
| Taurine | .2488 | .2440 | .2648 | .5016 | .1416 |
| Aspartic acid | .2544 | .1728 | .1616 | .2304 | .1272 |
| Hydroxyproline | .2224 | .1144 | .3672 | .1536 | .1400 |
| Threonine | 1.0400 | .7056 | 1.1216 | .6272 | .4416 |
| Serine | .9992 | .8008 | .6072 | .8648 | .7608 |
| Asparagine | .6000 | .4000 | .3800 | .2688 | .0752 |
| Glutamic acid | .8016 | .6264 | .6888 | .6816 | .6016 |
| Glutamine | .4664 | .3536 | .3184 | .2096 | .1944 |
| Proline | .7680 | .7168 | .6648 | .6248 | .1808 |
| Glycine | .6792 | .6296 | .5408 | .6912 | .4680 |
| Alanine | .7728 | .5560 | .7072 | .5432 | .5496 |
| Valine | .3886 | .2808 | .2344 | .4168 | .1592 |
| Half cystine | .6128 | .5464 | .5416 | .5864 | .4800 |
| Methionine | .0976 | .1248 | .1648 | .0984 | .0576 |
| Isoleucine | .1704 | .2424 | .1264 | .1880 | .0784 |
| Leucine | .3440 | .3568 | .2704 | .3472 | .1040 |
| Tyrosine | .0776 | .1248 | .1304 | .2536 | .1064 |
| Phenylalanine | .1432 | .1424 | .1368 | .2048 | .1120 |

[1]Values for pooled serum from 4 birds.

[2]20% protein level.

APPENDIX, TABLE 6

Concentration of free amino acids in chick serum[1], Experiment III

| Amino Acids | micromoles/ml serum | | | | |
|---|---|---|---|---|---|
| | control[2] (22 days) | deficient[2] (22 days) | def.+6 days[2] high Vit. A supplement (28 days) | control[2] (48 days) | high Vit. A[2] (48 days) |
| Tryptophan | .0352 | .0352 | .0280 | .0208 | .0424 |
| Lysine | .0472 | .0376 | .1344 | .2000 | .0760 |
| Histidine | .3096 | .4144 | .7000 | .2120 | .2896 |
| Ammonia | .7160 | .7472 | .7928 | .7928 | .7136 |
| Arginine | .2064 | .2216 | .2400 | .2304 | .2120 |
| Taurine | .2088 | .2520 | .2880 | .4680 | .3280 |
| Aspartic acid | .1520 | .920 | .1480 | .1480 | .1272 |
| Hydroxyproline | .1286 | .1776 | .1064 | .0328 | .1944 |
| Threonine | .4848 | .5456 | .7432 | .4352 | .3776 |
| Serine | 1.2512 | .9544 | 1.5912 | .8152 | 1.2240 |
| Asparagine | .2240 | .1280 | .4592 | - | .1128 |
| Glutamic acid | .6440 | .4720 | 1.1344 | .5080 | .6328 |
| Glutamine | .3184 | .1144 | .5664 | .1184 | .2568 |
| Proline | .4608 | .4608 | .4432 | .5136 | .2416 |
| Glycine | .6376 | .5288 | .6624 | .6288 | .8088 |
| Alanine | .5368 | .5408 | .8144 | .5432 | .8416 |
| Valine | .0464 | .0536 | .2944 | .0104 | .1056 |
| Half cystine | .5016 | .4616 | .6928 | .5328 | .6400 |
| Methionine | .0872 | .0880 | .1336 | .1720 | .0840 |
| Isoleucine | .0472 | .0744 | .0912 | .0464 | .0376 |
| Leucine | .1128 | .1552 | .2592 | .1544 | .1056 |
| Tyrosine | .1872 | .2808 | .2208 | .1912 | .2480 |
| Phenylalanine | .0648 | .1304 | .1912 | .1488 | .1488 |

[1]Values for pooled serum from 4 birds.

[2]10% protein level.

APPENDIX, TABLE 7

Concentration of free amino acids in chick serum[1], Experiment III

| Amino Acids | micromoles/ml serum | | | | |
|---|---|---|---|---|---|
| | control[2] (22 days) | deficient[2] (22 days) | def.+6 days[2] high Vit. A supplement (28 days) | control[2] (48 days) | high Vit. A[2] (48 days) |
| Tryptophan | .0704 | .0704 | .0744 | .0704 | .0704 |
| Lysine | 1.1320 | .6288 | 1.0440 | .8400 | 1.0696 |
| Histidine | .2920 | .1104 | .2608 | .2792 | .3008 |
| Ammonia | .7432 | .7136 | .7136 | .8320 | .7728 |
| Arginine | .6504 | .5320 | .7776 | .6376 | .9200 |
| Taurine | .1864 | .1896 | .2824 | .4952 | .3576 |
| Aspartic acid | .3752 | .1480 | .2136 | .2656 | .3568 |
| Hydroxyproline | .1776 | .0888 | .1848 | .0832 | .1672 |
| Threonine | .8064 | .5288 | .9872 | .6928 | .5616 |
| Serine | .6672 | .6324 | .8512 | .6224 | .4976 |
| Asparagine | .2820 | .2720 | .4952 | .4096 | .2376 |
| Glutamic acid | .6512 | .3760 | .6816 | .7104 | .7264 |
| Glutamine | .4264 | .1408 | .1824 | .2336 | .2176 |
| Proline | .9216 | .7680 | .9168 | .7256 | .8872 |
| Glycine | .5784 | .3498 | .7504 | .7232 | .8312 |
| Alanine | .6224 | .3120 | .6848 | .7384 | .6312 |
| Valine | .5000 | .3256 | .8336 | .7360 | .8096 |
| Half cystine | .5288 | .4040 | .8304 | .6576 | .6352 |
| Methionine | .1088 | .0880 | .1336 | .1968 | .1864 |
| Isoleucine | .3664 | .2144 | .3442 | .3240 | .3384 |
| Leucine | .4088 | .2912 | .6288 | .5120 | .6664 |
| Tyrosine | .1872 | .1200 | .1664 | .2168 | .2848 |
| Phenylalanine | .2112 | .1592 | .2048 | .2080 | .2048 |

[1]Values for pooled serum from 4 birds.

[2]30% protein level.

# EFFECT OF VITAMIN A ON SERUM AMINO ACID PATTERNS

by

CHRISTA MARIA STANTON

B. S., Justus-Liebig-University, Giessen, Germany, 1966

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AN ABSTRACT OF A MASTER'S THESIS

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The purpose of this study was to investigate the effect of different levels of dietary vitamin A on serum free amino acid patterns in chickens fed three different levels of protein (10%, 20% and 30%). Serum amino acid analysis was done according to the procedure of Spackman et al., 1958. Total vitamin A in liver and serum also was determined to check on vitamin A reserve status.

The serum free amino acid patterns varied with different vitamin A treatments.

Vitamin A deficiency with 10%-protein diets caused an increase in serum concentrations of tyrosine and phenylalanine and a decrease in concentrations of lysine and serine as compared to 20%-protein diets with normal vitamin A levels. Vitamin A deficiency with 20%-protein diets decreased the concentration of threonine and increased concentrations of tyrosine as well as valine, isoleucine and leucine as compared to the same diet with normal vitamin A levels. Vitamin A deficiency with 30%-protein diets caused a decrease in all 23 serum amino acid concentrations as compared to corresponding controls.

The high tyrosine concentration found in vitamin A deficiency state when 10%- and 20%-protein diets were fed might have resulted in part from break down in retinol-binding protein, a protein reported to be of high tyrosine content.

High vitamin A supplementation to previously vitamin A-deficient birds fed all three dietary protein levels resulted in serum amino acid patterns intermediate between those of chickens fed vitamin A-deficient diets and chickens fed high-vitamin A diets.

Studies of E/NE amino acid ratios indicated changes in the ratio were caused primarily by changes in the concentrations of the essential amino

acids. Increasing dietary protein from 10% to 20% to 30% increased the E/NE ratios in chick serum. Vitamin A deficiency caused an increase in the E/NE ratio at all three protein levels as compared to the corresponding controls, suggesting that vitamin A deficiency impaired utilization of essential amino acids. At all three dietary protein levels high-vitamin A supplementation to previously vitamin A-deficient chicks seemed to have a beneficial effect on lowering the E/NE ratios only when supplementation was given for at least 10 days. As measured by E/NE ratios, high vitamin A fed with 10%- and 20%-protein diets resulted in more efficient utilization of dietary protein than when normal vitamin A levels were fed. This effect was not apparent on 30%-protein diets.