

**THE INFLUENCE OF AMINO ACID IMBALANCES ON THE GROWTH
RATE AND METABOLISM OF THE CHICK**

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INTRODUCTION

As guides in formulating rations for growing chicks, nutritionists have endeavored to, first, find what substances are necessary for growth and, second, determine the amount of each substance needed to allow maximum growth rate. A ration has usually been considered satisfactory if the minimum requirement for each essential nutrient was met. With the exception of minerals, there has been little concern about excesses of any one substance as long as the excess did not limit the amounts of other essential nutrients. The protein requirement of the chick has been further broken down to a requirement for ten essential amino acids. Three other amino acids are essential under certain conditions. Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are considered essential. Cystine can replace part of the methionine requirement and tyrosine can replace part of the phenylalanine requirement. Glycine is needed for the most rapid rate of gain. Under certain conditions glutamic acid and proline may be needed for rapid growth.

Following the findings that certain amino acids are essential for the chick, studies have been carried on to determine what level of each of the various amino acids is essential for maximum growth rate of the chick. Much of the work to date has been done at the University of California.

Almquist ('47) has summarized most of this work. The requirements for the various amino acids were determined using a diet with 20 per cent protein. Since then several reports have been made that indicate that the requirement for several amino acids varies with the level of protein in the diet. Grau ('48) reported that as the protein level of the diet was increased, the chick's requirement for lysine was increased. Almquist ('49) found that the chick's requirement for methionine was approximately 0.75 per cent, if the diet contained 30 per cent protein. This is proportional to the earlier established requirement of 0.50 per cent of a 20 per cent protein diet. Almquist and Merritt ('50) investigated the arginine requirement of the chick as affected by the protein level of the diet. The requirement was found to be approximately six per cent of the total protein content of the diet. Thus it is apparent that the requirements for several, if not all of the essential amino acids, varies with the protein content of the diet, and that somewhat of a balance must be maintained among the various amino acids.

In the studies reported in this thesis, amino acid imbalances were produced by the addition of a single amino acid or an incomplete amino acid mixture to the diet of chicks. This work has developed from earlier studies on the growth depression produced by the addition of gelatin or zein to niacin-low diets fed chicks. Groschke and Briggs ('46) and Briggs, Groschke and Lillie ('46) found that the glycine, alanine, and arginine were at least partly responsible for the

growth depressing action of gelatin in the niacin-low diet. The fact that zein contains only limited quantities of these amino acids suggested that other amino acids may be involved. Accordingly, 17 different amino acids were added individually, at a level of four per cent, to niacin-low chick diets. All of these amino acids depressed the growth rate of the chicks. In another experiment, eleven of the amino acids were added separately, at a level of four per cent, to chick diets containing niacin. The data from both experiments are given later in this thesis. The growth depression which was noted when arginine, alanine, glycine, proline, or glutamic acid was added to the niacin-low diet, was not observed when the diet contained adequate niacin. Isolencine, tyrosine, lysine, methionine, cystine, and histidine depressed growth even if the diet contained adequate niacin.

The fact that the five amino acids, which did not depress growth when added to the diet containing adequate niacin, are all glucogenic suggested that niacin may have some function in converting excesses of these amino acids into intermediates normally produced in carbohydrate metabolism. It was believed that one of the most important reasons why the feeding of the excess amino acids depressed growth was that there was an imbalance of amino acids in the protein synthesizing centers of the chick. It seemed probable that even with sufficient niacin tyrosine, methionine, lysine, histidine, isolencine, and cystine, would themselves depress growth, or that some of their metabolic products would

depress growth of the chicks.

If the growth depression, resulting from the omission of niacin from the diet, was the result of the inability of the chick to correct the amino acid imbalance, it would be expected that a deficiency of any vitamin needed to correct the imbalance would have a similar effect. Pyridoxin is believed to be needed for transamination which is one reaction which would aid in the correction of the imbalance caused by feeding excess amounts of most of the amino acids. If the pyridoxin-deficient chicks were not able to correct the imbalance it was expected that the blood nonprotein nitrogen and amino nitrogen levels would be increased. Therefore, 16 amino acids were fed chicks individually, at a level of four per cent, in diets with low and adequate pyridoxin to determine whether their rate of gain, blood nonprotein nitrogen level, and amino nitrogen level would support this reasoning. Early in the work it was found that feeding the excess amino acids altered the blood sugar level. Several studies have been made to determine what relation the altered blood sugar level may have to the depression of growth of the chicks.

LITERATURE REVIEW

Prior to 1938 many studies were made to determine the cause of diseases associated with high corn diets, namely, pellagra in humans and blacktongue in dogs. In that year Elvehjem, Madden, Strong, and Woolley ('38) reported the isolation of a factor which would cure the canine blacktongue. This factor was identified as nicotinamide. Since then it has also been shown that a deficiency of niacin is at least one factor in pellagra. Corn is the most widely used grain in feeding poultry. Corn grits were found by Sarma and Elvehjem ('46), to depress the growth of chicks fed a purified diet low in niacin. The growth depression could be overcome by feeding niacin. Scott, Singsen and Matterson ('46) reported that niacin would prevent the perosis noted on high corn diets.

A large part of the protein of corn is zein. The growth depressing action of zein and gelatin in niacin-low diets, has already been mentioned in the early part of this thesis.

This action is noted even though there is no excess of any amino acid present in the diet as great as that produced by four per cent of one amino acid. The main difference between these proteins and most others is that they are almost completely devoid of the amino acid tryptophan. In 1945 it was reported by Krehl, Teply, Sarma and Elvehjem, that tryptophan, as well as niacin, would overcome the growth-depressing effects caused by feeding rats niacin-low diets high in corn.

Briggs ('45) working with chicks, showed that the addition of gelatin to a purified diet low in niacin caused a growth depression which could be overcome by the addition of either 0.2 per cent DL-tryptophan or five milligrams per cent niacin to the diet. Since then many studies have been done to determine the way in which tryptophan exerts this action. It is apparent that most animals are able to convert tryptophan into niacin or niacinamide. The work of Meidelberger, Abraham and Lepkovsky ('48) indicates that kynurenine, and 3-hydroxyanthranilic acid are intermediates; Henderson's ('49) work suggests that 3-hydroxyanthranilic acid is converted into quinolinic acid, which is known to be able to replace niacin both for the rat and chick.

There have been several reports on the effects of adding various amino acids to rat diets low in niacin. Krehl, Henderson, de la Hueraga and Elvehjem ('46) fed several mixtures of glycine, glutamic acid, tyrosine, phenylalanine, valine, and leucine. Of these amino acids, glycine seemed to be the one most responsible for the growth depression. Singal, Sydenstricker, and Littlejohn ('47) reported that a mixture of histidine, lysine, valine, and threonine caused a marked growth depression when added to niacin-low diets of rats. This mixture was added at a level of 1.47 per cent.

Henderson, Deodhar, Krehl and Elvehjem ('47) found that the growth of rats was depressed by the addition, to a niacin-low diet, of two per cent glycine, two per cent acid-hydrolyzed casein, or the equivalent amino acids. Two per cent

sodium pyruvate, two per cent sodium glycolate, or two per cent sodium lactate were fed but did not depress the rate of growth. Later Hankes, Henderson, Brickson and Elvehjem ('48) fed rats the amino acids found in two per cent casein individually; only DL-threonine and DL-phenylalanine depressed the growth of the rats. The entire growth depression resulting from feeding the acid-hydrolyzed casein seemed to be caused by the threonine and phenylalanine present. Hankes, Henderson and Elvehjem ('49) reported that the L-threonine and the D-phenylalanine are the isomers responsible for the growth depressing effect. In their nine per cent casein diet, several amino acids are below the level required for maximum growth. When calculated on the basis of Rose's suggested minimal levels for normal rat growth, methionine, histidine, threonine, lysine, phenylalanine, and tryptophan are limiting. A level of 0.2 per cent cystine is added to the diet in which the addition of threonine and phenylalanine depressed growth. When cystine was omitted from the diet, the growth depression caused by the addition of these amino acids was not as great. To account for their results they suggested that the addition of both threonine and cystine made the tryptophan deficiency more acute than when only one amino acid was added. The addition of tryptophan to the diet corrected the growth depression caused by feeding the extra amino acids. They point out that reports have been made in which multiple vitamin deficiencies were not as damaging as a single vitamin deficiency.

This seems to be a reasonable explanation for the depression caused by feeding threonine. These authors did not extend the same explanation to the depression caused by feeding phenylalanine. However, it would be expected that the addition of cystine, threonine, and phenylalanine to the same diet would make the tryptophane deficiency even more acute than the addition of only threonine and cystine. Their data showed that the addition of the three amino acids to the niacin-low diet did not depress growth as much as the threonine and cystine combination. The fact that the D-phenylalanine was the isomer which depressed growth, makes it obvious that making a more complete protein is not the cause of the depression noted when DL-phenylalanine was fed since both isomers are available to the rat.

In each of the five previous reports the growth depressions were overcome by the addition of either niacin or tryptophan to the diet.

The growth depression which occurs when amino acids other than tryptophan are added to a niacin-low diet, might be explained by assuming that the excess amino acids decrease the efficiency of the conversion of tryptophan to niacin. There have been some studies done on the effects of feeding proteins low in tryptophan, or amino acids, on the excretion of N-methylnicotinamide. Schweigert and Pearson ('48) found that the addition of gelatin to the diet of rats did not decrease the excretion of N-methylnicotinamide. The dietary change did inhibit the growth of the rats. Sarett ('50)

found that the daily addition of 20 to 30 grams of gelatin, or 20 grams of glycine, to niacin and protein-low diets of humans, did not decrease the excretion of N-methylnicotinamide. The addition of casein to the diet did not increase N-methylnicotinamide excretion, but did increase the excretion of tryptophan. In experiments done by Rosen and Perlzweig (149), rats fed a ten per cent casein, six per cent gelatin diet, gained only six grams during a four week period, rats fed the same diet without the gelatin gained 33 grams during the same period. The excretion of N-methylnicotinamide was determined during the last three days of the period. The rats fed the gelatin excreted slightly more N-methylnicotinamide during this period. At the end of the four week period a test dose of L-tryptophan was given the rats. During the next three days the rats fed casein increased their N-methylnicotinamide excretion by 465 micrograms; those fed casein and gelatin increased the excretion by only 72 micrograms. The authors believed that their data indicated clearly that the presence of an excess of gelatin in the diet resulted in an impairment of the conversion of tryptophan to niacin. This defect in the conversion, if once permitted to develop for several weeks, was not remedied by the addition of small amounts of tryptophan to the diet. This same amount of tryptophan was sufficient to allow maximum growth if feeding was started before the defect developed.

While it seems reasonable that feeding gelatin would impair the conversion of tryptophan to niacin, it is questioned

that the data of Rosen and Perlzweig indicate that such is true. Another group of rats, to which they fed the ten per cent casein, six per cent gelatin diet with five milligrams per cent niacin added, showed the greatest increase in N-methylnicotinamide excretion after the intraperitoneal injection of the L-tryptophan. Two other groups of rats were also fed the diet with gelatin and no niacin for the first three weeks. During the fourth week one group of rats was given 50 milligrams per cent of DL-tryptophan in the diet, but failed to respond by increased weight gain; the other group was given five milligrams per cent of niacin in the diet and during the next week gained weight as fast as the positive controls. At the end of the fourth week, when L-tryptophan was given, the former excreted N-methylnicotinamide in amounts comparable to the rats fed casein and gelatin; the latter's increased excretion was about six times the former. These facts seem to indicate that it was not the presence of the gelatin, per se, which impaired the conversion of tryptophan to niacin, but that the impairment was associated with the poor rate of growth of the rats.

Sarett and Perlzweig ('43) found that the liver concentration of riboflavin and niacin varied directly with the level of protein in the diet. The rest of the carcass had approximately the same amount regardless of protein level. Wright and Skeggs ('46) found that both the hepatic storage of niacin, and the fecal elimination of niacin and its metabolites, were increased as the protein level of the diet

was increased. Hundley ('47) found that the niacin content of both the liver and muscle increased, as the level of casein in the diet increased, when either diets with or without added niacin were fed. The last three references were all reports of work done with rats. Whether or not this extra niacin is needed in the liver when the diet contains greater amounts of protein, is not known. The increased niacin level of the liver suggests that the niacin requirement may be increased as the protein level of the diet is increased. Hartman, Dryden, and Cary ('49) have shown that the vitamin B₁₂ requirement apparently increases as protein level of the diet is increased.

Glycine toxicity on low pyridoxin diets has been noted by Page and Gingras ('46). The toxicity could be overcome by the addition of one milligram per cent of pyridoxin to the diet. The addition of 10 per cent glycine, to a rat diet without folic acid, resulted in a growth suppression, mild leucopenia, and an elevated creatinurea, all of which responded to folic acid, (Martel, Page and Gingras, '47). Liver extract and vitamin C overcame the depression to a lesser extent, (Dinning, Keith, Day and Tetter, '49).

Martin ('46a) found that cystine, tryptophan, tyrosine, histidine, glycine, and glutamic acid were all more toxic if fed to rats in a riboflavin-deficient diet than they were when the same diets were supplemented with riboflavin. The addition of tyrosine resulted in the death of rats within six months if the diet contained pyridoxin; if pyridoxin was

omitted from the diet all the rats lived for the six months' experimental period, (Martin '46).

The four papers cited prior to the last one indicate that certain amino acids may cause a growth depression when added to diets deficient in vitamins other than niacin, and that the depression can be overcome, at least to some extent, by the addition of the deficient vitamin to the diet.

Excesses of any amino acid become toxic to the rat if the level in the diet is great enough. Wretling ('49) found that the amounts needed to become toxic varied from three to twenty-four per cent. Methionine was the most toxic of all. These depressions are noted even though the diet contains adequate amounts of the known vitamins. The depressions have been noted to be influenced by additions of other amino acids to the diet. Sullivan, Hess and Sebrell ('32) found that when five per cent cystine was added to diets containing either five or ten per cent tyrosine, the toxicity of the tyrosine was reduced. Martin ('47) reported that the unnatural form of tyrosine is relatively non-toxic. The toxicity of L-tyrosine was found to be decreased by feeding cystine or glycine; tryptophan or glutamic acid had no effect.

Brown and Allison ('48) fed rats a diet containing 12 per cent casein and 4.8 per cent DL-methionine and found that the rats lost weight faster than rats pair fed the same diet without the methionine. The addition of arginine, to the diet containing methionine, resulted in less weight loss. The decrease in weight when methionine was fed was associated

with a loss in body nitrogen and fat. Roth and Allison ('49) found that the addition of excess glycine or glycine and arginine to the high methionine diet would also counteract the weight loss. In this work it was noted that feeding excess methionine, alone or in combination with the other two amino acids, increased slightly the excretion of creatinine and creatine. The loss of weight was noted even though the rats were in positive nitrogen balance, which they interpreted to mean that there was extensive catabolism of fat. They proposed that if the observed effects of feeding methionine were due to the increased demands of the body to metabolize methyl groups, either by oxidation or by elimination as the methyl group of creatinine, it would be expected that feeding excessive methyl groups as choline would also have the same effect. However, when this was tried it was found that choline, at a level of 1.35 per cent of the diet, was well tolerated by the rat; the addition of choline to the high methionine diet did not significantly change the results (Roth and Allison, '50). Therefore, it was thought that the effects noted are due to the homocysteine portion of the molecule, and state that du Vigneaud has obtained evidence for this conclusion. Since it was demonstrated that glycine could, in part, counteract the effects of excess methionine it was proposed that glycine is utilized to convert to serine which with homocysteine forms cysteine.

The growth depression caused by feeding excess DL-methionine seems to be mostly due to the L-isomer as indicated

by the work of Van Pilsum and Berg ('50).

Since the feeding of high levels of amino acids appear to alter carbohydrate metabolism, and since the regulation of carbohydrate metabolism in birds appears to be quite different from that of mammals, a brief summary of what is known will be given. The summary is taken from a paper by Golden and Long ('42). The blood sugar level of birds is about twice that of most common mammals. The fed level was found by these authors to be about 192 milligrams per cent, and the fasted level about 182 milligrams per cent for 300 gram birds. This value decreases slightly with age. The total removal of the pancreas of birds does not produce the permanent diabetes found in dogs and other mammals. The pancreas of the birds does contain insulin, however. Some have suggested that the avian pancreas, when present, functions in the regulation of carbohydrate metabolism in a manner similar to that of the mammalian pancreas, but its presence is not essential. It has been shown that birds may be given large doses of insulin without any manifestations of convulsions or insulin shock. When insulin is given in amounts of one unit per kilogram of body weight there is a marked lowering of blood sugar. Liver glycogen is quite variable but averages about three per cent in fed birds and declines to about 0.4 per cent after 24 hours fasting. The pectoral muscle contains about one per cent glycogen in fed birds and declines to 0.75 to 0.80 per cent during a 24-hour fast. The pectoral muscle contains three to four times more glycogen than the gastrocnemius muscle. Adrenal cortical

extract administered to fasted chicks produces a significant deposition of liver glycogen and raises the blood sugar to supernormal levels. Protein seems to be the source of the new carbohydrate. The hyperglycemic and glycogenolytic properties of epinephrine have also been shown in the chick. Birds are extremely sensitive to hypophysectomy and adrenalectomy. Hypophysectomy, however, does not seem to cause a very marked reduction in blood sugar level.

There have been reports of amino acids or their breakdown products having an insulin-like action. Frank, Nothmann and Wagner ('26) reported that guanidine produced a progressive hypoglycemia in rabbits. The effect was also noted with several substituted guanidines, one of which was agmatine, produced by decarboxylation of arginine. This suggests that arginine itself may have this effect; if not, at least a possible metabolic product does. Landauer ('45) noted that when insulin was injected into eggs prior to 72 hours incubation, a large increase in the per cent of rumpless chicks resulted. When insulin was reversibly inactivated, this action was lost but reactivation of the insulin restored the action (Landauer and Lang, '46). Significant increases in rumplessness was noted when cysteine hydrochloride, cystine, glutamic acid, or malic acid was injected into the egg, thus suggesting that these amino acids have an insulin-like activity. Johlin ('49) found that repeated injections of three milligrams of glycine per kilogram of body weight into rabbits, appeared to increase the action of subsequent injections of insulin for a prolonged

period of time. Martin ('47) found that rats fed diets high in tyrosine did not show as marked hypoglycemia when insulin was injected.

Niacin has been shown to influence the action of insulin. Burke and McIntyre ('39) reported that niacin decreased duration of hypoglycemia following the injection of a standard dose of insulin. Landauer ('48) found that nicotinamide and alpha-ketoglutaric acid prevented some of the micromelia and beak defects which resulted when insulin was injected into the yolk sac of the hen's egg between 96 and 120 hours of incubation. Zwilling ('49) found that nicotinamide and alpha-ketoglutaric acid reduced the hypoglycemia following the injection of insulin into the egg incubated for 120 hours.

These papers suggested that arginine, glycine, glutamic acid, and cystine may have some insulin-like action and that niacin can reverse, to a certain extent, the action of insulin. Our early work had indicated that the same amino acids depressed growth of chicks on a niacin-low diet and this effect was reversed when niacin was added to the diet. This naturally raised the question as to whether the glucogenic amino acids have an insulin-like action.

The work of Lotspeich ('49) has shown that insulin will cause a decrease in the blood amino nitrogen of fasting dogs. He found that there was a correlation between the proportions of each amino acid removed from the blood and the proportions of each amino acid in a representative body protein, skeletal muscle. This observation suggested that insulin promotes the

synthesis of protein from circulating amino acids.

It was first noted that the additions of amino acids, at the four per cent level to chick diets, would affect the growth rate of the chicks. The work reported in this thesis has been done to determine how these amino acids cause the effects.

EXPERIMENTAL PROCEDURE

In the experiments reported here, unless otherwise noted, day-old New Hampshire chicks of both sexes were used. They were progeny of the University of Maryland flock being fed a practical breeding mash. At the beginning of each experiment the chicks were divided into uniform groups and raised in electrically-heated batteries with wire floors. Feed and water were given ad libitum. Weighings and other observations, except feed consumption, were made weekly. The experiments were conducted for four weeks. At the termination of each experiment the total feed consumption was determined and the efficiency of feed utilization was calculated by dividing the total gain by the total feed consumed.

The basal diet 113, and the rations used in experiments 38 and 40, are given in table 1. In much of the work reported here diet 113 with five per cent of the gelatin replaced by Cerelese, was used. In this thesis this ration is designated 113-5 per cent gelatin. When all of the niacin was omitted, the diets are designated as 113N or 113N-5 per cent gelatin. Substitutions in these diets were made at the expense of Cerelese.

In some of the experiments the University of Maryland poultry department's starting mash was fed. This is a commercial type mash having approximately 21 per cent protein.

In these experiments the level of several blood constituents has been determined. Unless otherwise indicated, the birds were fasted for approximately 12 hours before the blood samples were

TABLE I
Composition of purified basal diets employed
in experiments with chicks

INGREDIENTS	RATION 113	RATION USED IN EXP. 38	RATION USED IN EXP. 40		
Grams per 100 grams of diet					
Glucose (Cerelease)	61.4	58.0	60.4		
Casein (crude)	18.0	24.0	24.0		
Gelatin	10.0	4.5	5.0		
Salts 1M*	6.0	6.0	6.0		
Soybean oil	4.0	4.0	4.0		
DL-Methionine	0.3	0.1	0.3		
Liver paste	-	0.1	-		
Celuniflour	-	3.0	-		
Vitamins					
milligrams per 100 grams of diet					
Choline chloride	200.0	200.0	200.0		
4-Inositol	100.0	100.0	100.0		
Niacin	10.0	10.0	10.0		
Ca pantothenate	2.0	2.0	2.0		
Riboflavin	0.8	0.8	0.8		
Thiamin HCl	0.4	0.4	0.4		
Pyridoxin HCl	0.6	0.6	0.6		
Para-aminio benzoic acid	0.2	0.2	0.2		
Biotin	0.02	0.02	0.02		
Folic acid	0.2	0.2	0.2		
alpha-Tocopherol acetate	0.5	0.5	0.5		
Menadione	0.1	0.1	0.1		
Vitamin A and D	1200 U.S.P. and 170 A.C.A.C. unit respectively by dropper weekly.				
*Salts 1M are composed of the following ingredients by weight:					
CaCO ₃	150.0	MgSO ₄ ·7H ₂ O	50.0	CuSO ₄ ·5H ₂ O	0.2
K ₂ HPO ₄	90.0	Fe(C ₆ H ₅ O ₇)·3H ₂ O	14.0	H ₂ BO ₃	0.09
Na ₂ HPO ₄	73.0	MnSO ₄ ·H ₂ O	4.1	CoSO ₄ ·7H ₂ O	0.01
Ca ₃ (PO ₄) ₂	130.0	KI	0.4		
NaCl	88.0	ZnCl ₂	0.2		

taken. The blood used in all the determinations was withdrawn by heart puncture. Approximately equal amounts of blood were withdrawn from each bird, and the blood from all chicks in a group was pooled and a group sample was used in making the determinations. In all experiments, in which amino nitrogen or nonprotein nitrogen was determined, the protein-free blood filtrate was prepared by the method of Folin and Wu ('19). In some experiments only blood sugar was determined and the blood proteins were precipitated by 0.15 N barium hydroxide and 2.5 per cent zinc sulfate. Determination of nonprotein nitrogen was by the method of Folin and Wu, loc. cit. Amino nitrogen was determined by the method of Danielson as modified and given by Hawk, Gser and Summerson ('47), with the following exceptions: In the determinations of amino nitrogen in the blood of the chicks of the pyridoxin experiments (data given in tables 3 and 4), the final colored solution was diluted to 35 milliliters instead of 15 milliliters as the instructions give. All of these amino nitrogen values are higher than would be expected. The amino nitrogen in a few of the samples were later determined by the method given below and lower amino nitrogen levels were found. These values, although lower, were correlated with the higher values determined earlier. Since it is felt that the differences between groups are the most significant part of the results, no attempt has been made to correct the level. All other amino nitrogen levels were determined as given in the instructions, except that two milliliters of the blood filtrate and three milliliters of distilled water were used instead of

the five milliliters of the blood filtrate.

The sugar content of the filtrates was determined by the method of Benedict ('31). In all determinations, after developing the color, the color density was determined by a Leitz Rouy-photrometer.

In some experiments the birds were subjected to a glucose tolerance test. Six representative birds of a group were used for each test. Blood samples were taken just before giving glucose, one hour later, and two and one-half or three hours later, as indicated. Two blood samples were taken from each bird; four of the six birds were used each time blood was drawn, two of which were used to obtain each of the other two samples. The glucose (Cerelese) was given at the rate of three grams per kilogram of body weight as a 30 per cent solution in water.

Procedures other than have been given previously, were used in several experiments. Since they usually apply to only one experiment the procedure is given with the results.

RESULTS

Effect of additions of individual amino acids to chick diets with and without added niacin

The first experiments with high levels of individual amino acids were done in 1947 by Dr. A. C. Groschke with whom the author was working at that time. The results of these experiments have not been published in detail. Since they form the background of the work which follows in this thesis the data are presented in table 2. In the absence of niacin, a growth depression occurred with the feeding of high levels of several different amino acids, but this depression varied in degree. Methionine was extremely toxic, all chicks being dead by the end of the third week. As the level of glycine was increased from two to five per cent, the growth depression became greater. Leucine depressed the growth rate of the chicks least of all.

When the same amino acids were included in a diet which contained niacin, improved growth was noted with all amino acids except isoleucine. The growth depressing action of arginine, glycine, glutamic acid, and proline, all of which are glucogenic amino acids, was overcome. It will be apparent in a later summary of all work that the glucogenic amino acids do not depress growth as much as most of the ketogenic amino acids. This difference between the action of the ketogenic and glucogenic amino acids suggested that niacin functioned in the metabolism of the excesses of the amino acids.

The chicks fed 4 per cent lysine in diet 113-5 per cent

TABLE 2

Growth depressing action of individual amino acids
in the presence and absence of niacin

GROUP NO.	AMINO ACID SUPPLEMENT TO DIET 113-5 PER CENT GELATIN	WITHOUT NIACIN		WITH NIACIN	
		NO. CHICKS	AVE. WT. 4 WKS.	NO. CHICKS	AVE. WT. 4 WKS.
			grams		grams
1	None	12	220	6	234
2	4% DL-alanine	6	137	6	207
3	4% L-arginine HCl	2	107	3	306
4	4% DL-aspartic acid	5	167		
5	4% L-cystine	5	79	6	148
6	4% L-glutamic acid	6	142	6	256
7	2% glycine	6	164		
8	3% glycine	6	137		
9	4% glycine	6	116	6	266
10	5% glycine	4	87		
11	6% glycine			6	201
12	4% L-histidine HCl	6	114	3	136
13	4% DL-isoleucine	5	151	3	144
14	4% L-leucine	6	185		
15	4% L-lysine HCl	4	77	1	103
16	4% DL-methionine			5	59
17	4% DL-phenylalanine	4	151		
18	4% L-proline	4	100	3	226
19	4% L-serine	3	184		
20	4% DL-threonine	2	167		

TABLE 2
continued

Growth depressing action of individual amino acids
in the presence and absence of niacin

GROUP NO.	AMINO ACID SUPPLEMENT TO DIET 113-5 PER CENT GELATIN	WITHOUT NIACIN		WITH NIACIN	
		NO. CHICKS	AVE. WT. 4 WKS.	NO. CHICKS	AVE. WT. 4 WKS.
21	4% L-tyrosine	6	grams 115	6	grams 145
22	4% DL-valine	3	186		

gelatin, in this experiment and in subsequent experiments, have exhibited tremors throughout the period. This has also been noted when glycine was fed at levels above four per cent, but the condition was less severe.

A peculiar condition has been noted in the chicks fed alanine. These chicks tend to stand in a fixed position and do not move about the pen as much as normal chicks. The movements which are made are very slow and deliberate. This condition seems to be somewhat overcome by the end of a four week experimental period.

Effects of adding individual amino acids to chick diets containing low levels of pyridoxin

In later studies the effects of feeding glycine in diets low in pyridoxin were studied more extensively than any other amino acid. In one experiment, diet 113-5 per cent gelatin was fed; the levels of pyridoxin fed were 0, 50, 75, 100, 150,

300, and 600 micrograms per cent. Another experiment was done with diet 113 and the same pyridoxin levels. The results of both experiments are presented in Figure 1. The addition of four per cent glycine to diet 113-5 per cent gelatin did not depress the growth rate of the chicks with any of the pyridoxin levels fed. In diets containing from 100 to 600 micrograms per cent pyridoxin, the addition of glycine to the diet increased the growth rate. Only when glycine was added to the diet without pyridoxin was there any deleterious effect; here the chicks fed glycine died at an earlier age.

The growth of chicks fed diet 113 with four per cent glycine, was depressed with all pyridoxin levels below 300 micrograms per cent. Slightly greater growth was noted when glycine was added to diet 113 with 600 micrograms per cent of pyridoxin. Thus the addition of glycine depressed growth of chicks if the pyridoxin level was inadequate, but the growth depression was overcome if an adequate level of pyridoxin was added to the diet. This action is similar to that noted with glycine in the niacin-low diet, although ten per cent gelatin was required in the diet before the similarity could be noted.

Blood nonprotein nitrogen and amino nitrogen levels were determined for all groups of chicks in the experiment in which diet 113-5 per cent gelatin was fed. Both were increased by the addition of glycine to the diet at all pyridoxin levels. There was no correlation between pyridoxin level in the diet and either the nonprotein nitrogen or amino nitrogen levels in the chick blood.

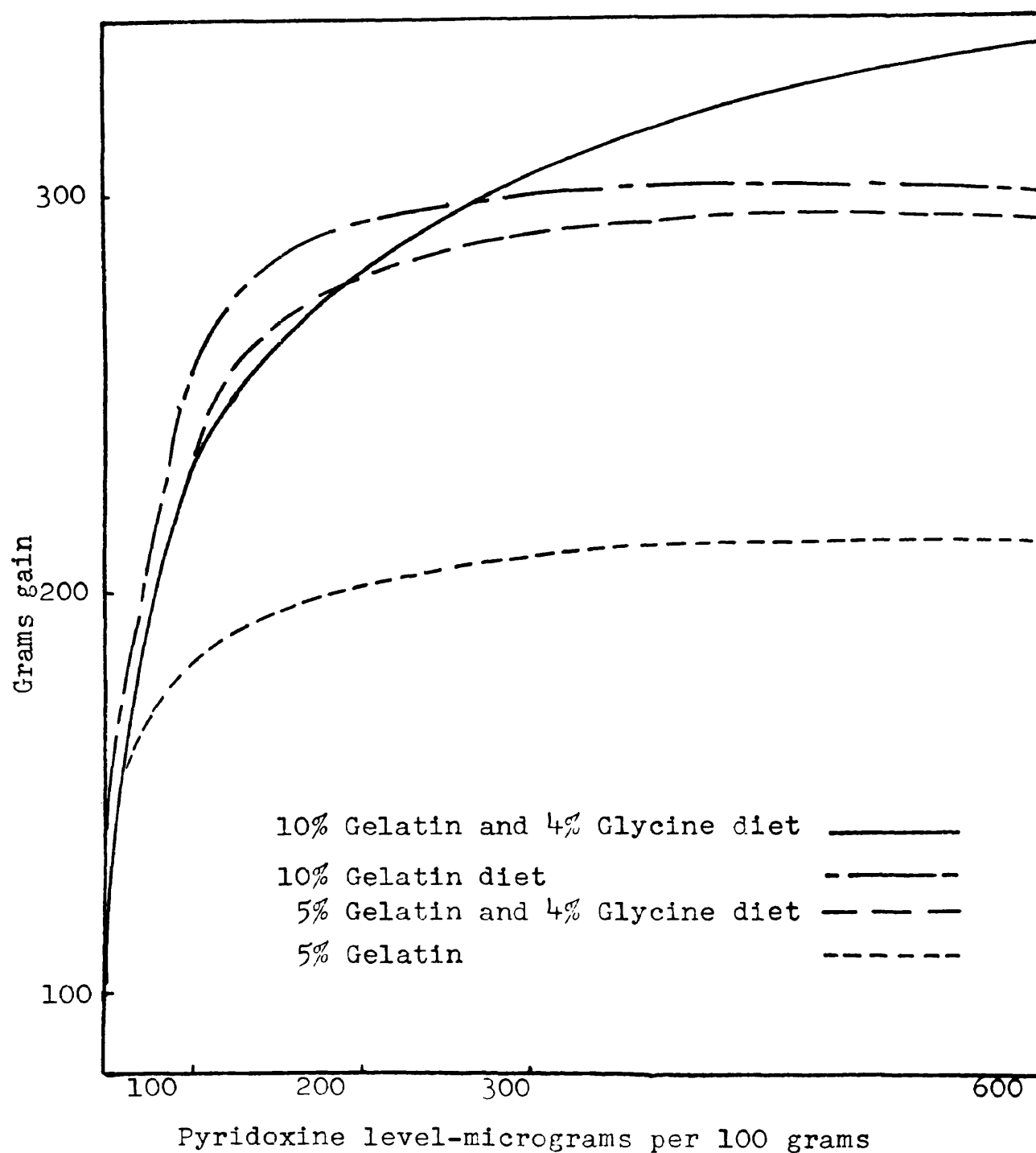


Fig. 1 Effects of adding four per cent of glycine to chick diets containing various levels of pyridoxin. The data from which this figure is drawn are given in the appendix.

Since reducing the pyridoxin level to 75 micrograms per cent of the diet depressed the growth of chicks to about the same extent as omitting niacin from the diet, this pyridoxin level was selected to study the effects of additions of other amino acids. In two experiments 10 amino acids were added, individually, at a level of four per cent, to diet 113-5 per cent gelatin. Pyridoxin levels of 75 and 600 micrograms per cent were fed. The data are given in tables 3 and 3a. Six amino acids were also compared using diet 113, the results of which are given in table 4.

In the pyridoxin-low diet 113-5 per cent gelatin the addition of the allothreonine-threonine mixture caused a growth depression which was overcome if adequate pyridoxin was added to the diet. Additions of arginine or glutamic acid, to the pyridoxin-low diet, increased the growth rate; these same amino acids had produced marked growth reductions in the niacin-low diet. These experiments also confirmed the fact that feeding any one of the ketogenic amino acids studied, except leucine, depresses growth of chicks even if ample niacin and pyridoxin are present in the diet.

When the ten per cent gelatin basal was fed, the addition of leucine depressed growth of chicks fed the pyridoxin-low diet, and the depression was not observed when adequate pyridoxin was added to the diet. The other five amino acids either decreased or increased growth of chicks when added to either of the diets.

Blood amino nitrogen and nonprotein nitrogen were determined in both of the experiments and are given with the results. Most

TABLE 3

A comparison of the effects of feeding chicks various amino acids in diets containing low or adequate levels of pyridoxin (experiment 35)

GROUP NO.	ADDITION TO DIET 113-5% GELATIN	PYRIDOXINE LEVEL	BLOOD LEVEL OF		AVG. WT. 4 WKS. CHICKS	NO.
			NPN	AMINO N.		
			milligrams per cent		grams	
1	None	0.075	47.5	26.7	139	10
2	None	0.600	51.0	28.8	183	8
3	4% DL-methionine	0.075	42.5	-	34	1
4	4% DL-methionine	0.600	44.5	27.6	51	4
5	4% L-cystine	0.075	51.1	31.1	120	9
6	4% L-cystine	0.600	47.7	29.7	129	9
7	4% DL-phenylalanine	0.075	52.0	30.0	81	5
8	4% DL-phenylalanine	0.600	66.0	30.0	96	6
9	4% L-tyrosine	0.075	44.5	30.0	100	9
10	4% L-tyrosine	0.600	52.5	34.2	115	10
11	4% L-leucine	0.075	-	-	138	9
12	4% L-leucine	0.600	52.5	30.0	252	8
13	4% DL-valine	0.075	-	-	93	5
14	4% DL-valine	0.600	49.8	33.0	137	5
15	4% DL-isoleucine	0.075	51.0	32.4	89	6
16	4% DL-isoleucine	0.600	57.7	34.2	123	6
17	4% L-glutamic acid	0.075	53.0	30.0	166	10
18	4% L-glutamic acid	0.600	50.0	30.0	194	9
19	4% DL-alanine	0.075	56.0	32.0	135	8
20	4% DL-alanine	0.600	62.5	34.2	180	7

TABLE 3A

A comparison of the effects of feeding chicks various amino acids in diets containing low or adequate levels of pyridoxin (experiment 37)

GROUP NO.	ADDITION TO DIET	PYRIDOXIN LEVEL	BLOOD LEVEL OF		AVE. WT. 4 WKS.	NO. CHICKS
	113-5% GELATIN		NPW	AMINO N.		
		milligrams per cent			grams	
1	None	0.075	57.0	30.0	201	10
2	None	0.600	55.5	31.8	232	8
3	4% DL-lysine HCl	0.075	51.0	30.6	83	8
4	4% DL-lysine HCl	0.600	46.0	28.8	77	7
5	4% L-arginine HCl	0.075	-	22.4	247	4
6	4% L-arginine HCl	0.600	-	29.1	348	3
7	4% DL-threonine DL-allothreonine	0.075	58.0	37.2	158	5
8	4% DL-threonine DL-allothreonine	0.600	57.0	35.7	247	6
9	4% L-histidine HCl	0.075	71.0	30.9	97	5
10	4% L-histidine HCl	0.600	65.0	31.8	137	5
11	4% DL-tryptophan	0.075	53.5	20.4	85	4
12	4% DL-tryptophan	0.600	63.5	32.7	109	4

TABLE 4

A comparison of the effects of feeding chicks various amino acids in diet 113 containing low and adequate levels of pyridoxin (experiment 37)

GROUP NO.	ADDITION TO DIET 113-5% GELATIN	PYRIDOXIN LEVEL	BLOOD LEVEL OF NPN	AMINO N.	AVE. WT. 4 WKS.	NO. CHICKS
		milligrams per cent			grams	
1	None	0.075	53.0	29.4	184	9
2	None	0.600	38.5	23.1	287	10
3	4% DL-aspartic acid	0.075	42.5	22.8	119	3
4	4% DL-aspartic acid	0.600	52.0	26.4	240	5
5	4% L-arginine HCl	0.075	-	24.9	214	5
6	4% L-arginine HCl	0.600	-	27.3	290	6
7	4% L-glutamic acid	0.075	48.5	25.8	185	10
8	4% L-glutamic acid	0.600	47.0	24.0	312	9
9	4% L-leucine	0.075	55.0	30.6	149	9
10	4% L-leucine	0.600	54.0	30.6	289	9
11	4% DL-alanine	0.075	54.0	30.6	188	10
12	4% DL-alanine	0.600	57.5	28.8	275	8
13	4% L-tyrosine	0.075	45.0	26.1	125	10
14	4% L-tyrosine	0.600	46.5	24.6	270	9

of the values for nonprotein nitrogen are higher than the values reported by Scott, Serfontein and Sieling ('33), of 38.8 and 42.4 milligrams per cent in adult White Leghorn pullets and cockerels, respectively. Nonprotein nitrogen values were usually found to be higher when an amino acid was added to the diet, most of the amino acids, which did not increase nonprotein nitrogen levels, were those which depressed growth to the greatest extent. The blood amino nitrogen level was increased by the addition of each amino acid except methionine, lysine, and arginine to the diet containing five per cent gelatin. The blood amino nitrogen level was decreased also by increasing the gelatin level of the diet from five to ten per cent.

The blood nonprotein nitrogen and amino nitrogen levels did not appear to be altered consistently with the change in pyridoxin level from 75 to 600 micrograms per cent.

An attempt was made to determine the relative levels of tryptophan and arginine, by chemical color tests, in the blood filtrates from the chicks fed the basal diet and those fed tryptophan or arginine in experiment 37. These amino acids could be detected only in the filtrates from the chicks which had been fed the amino acids. As judged by the intensity of the color produced, the chicks fed the diet with 600 micrograms per cent pyridoxin had a higher blood level of the amino acid than those fed the low level of pyridoxin. Thus, there was no indication that the blood amino acid balance was restored to normal by the addition of adequate pyridoxin and niacin to

the diet.

Glycine requirement of the chick

The increase in growth noted with the addition of glycine to the five per cent gelatin diet, and to a lesser extent to the ten per cent gelatin diet, raised a question as to the glycine requirements of the chick. According to presently accepted standards the former diet should have ample glycine and the latter diet should have at least twice that needed for optimum growth. There was a possibility that extra protein was needed for optimum growth and that glycine was increasing growth rate of the chicks by raising the protein level of the diet. Therefore the diet listed in table 1, under experiment 38, was prepared to contain a higher level of protein. It was calculated to contain 1.0 per cent glycine and 1.2 per cent arginine, which are the presently accepted requirements for optimum growth of the chick. The addition of glycine to this diet, at levels from 0.25 to 2.0 per cent, resulted in a slightly decreased rate of gain (table 5). Addition of five per cent gelatin, however, increased the average four-week gain about 100 grams. It is believed that the arginine in casein is not completely available to the chick; gelatin is a good source of arginine.

TABLE 5

Glycine requirement of chicks fed a 26 per cent protein diet
(experiment 38)

GROUP NO.	SUPPLEMENT TO BASAL DIET	NO. CHICKS	NO. DEAD	AVE. WT. 4 WKS. grams	FEED EFF.
1	None	21	0	252	.536
2	0.25% glycine	18	1	228	.498
3	0.50% glycine	18	0	239	.496
4	1.0% glycine	18	1	235	.499
5	2.0% glycine	18	1	244	.470
6	5.0% gelatin	18	0	355	.614

Effect of arginine, glutamic acid and gelatin on the growth depression caused by feeding chicks a high level of methionine

As has been stated earlier, the addition of DL-methionine to the diet of chicks at a level of four per cent results in almost a complete cessation of growth. This has also been noted in rats by Brown and Allison ('48). These workers had also reported that arginine and glycine would partially overcome some of the effects of feeding excess methionine.

This raised the question as to whether or not arginine would overcome any of the growth depression that feeding excess methionine had caused in chicks. A diet very similar to that used in the last study on glycine was made. Its composition is given in table 1, experiment 40. This diet was calculated to contain slightly over the presently accepted

requirement of the chick for arginine, if it is completely available. Graded levels of arginine were added to the diet with and without three per cent added DL-methionine. The arginine is also compared with gelatin and glutamic acid. The results of the experiment are given in table 6. The rate of gain of the chicks fed the extra arginine was twice that of the chicks fed the basal diet. The addition of three per cent DL-methionine to the basal diet depressed the growth rate of the chicks to less than one-half of the rate of the chicks fed the basal diet. This depression of growth rate was somewhat overcome by arginine, gelatin, or glutamic acid but none of these chicks grew at a rate equal to those fed the basal diet. Results of determinations of blood nonprotein nitrogen, amino nitrogen, nonfasted glucose levels of all the groups, and blood glucose levels in a glucose tolerance test on seven groups are in table 6. As the level of arginine added to the basal diet was increased the nonprotein nitrogen, amino nitrogen, and nonfasted blood sugar levels decreased. These trends were not as apparent after addition of methionine to the diet.

The glucose tolerance tests, on the chicks of experiment 40, were done 11 days following the termination of the experiment. During the ten days following the experiment all chicks were fed the University of Maryland starting mash without the addition of any amino acid. The results of the test indicate that the blood sugar level was still being influenced by the diet fed during the experimental period. The fasting blood sugar level of the chicks which had been fed arginine

TABLE 6

Effect of arginine, glutamic acid and gelatin on the growth depression caused by feeding chicks methionine (experiment 40)

GROUP NO.	SUPPLEMENT TO BASAL DIET	NO. CHICKS	AVE. WT. 4 WKS. grams	AMINO N	NPN	BLOOD SUGAR LEVEL TOLERANCE TEST*			
						FAST	0'	60'	180'
1	None	10	160	21.3	53.5	207	173	204	180
2	0.5% L-arginine HCl	10	319	19.0	52.5	199	-	-	-
3	1.0% L-arginine HCl	9	280	19.8	49.5	178	-	-	-
4	2.0% L-arginine HCl	10	330	15.8	39.5	163	148	256	181
5	3.0% DL-methionine	6	89	19.8	50.0	193	188	366	218
6	As 5 / 0.5% L-arginine HCl	5	92	19.8	49.0	205	-	-	-
7	As 5 / 1.0% L-arginine HCl	9	133	18.1	45.5	191	-	-	-
8	As 5 / 2.0% L-arginine HCl	8	111	18.6	51.0	195	162	280	210
9	5% gelatin	10	309	21.3	49.5	200	174	238	212
10	As 5 / 5% gelatin	10	123	18.9	54.5	206	187	252	197
11	As 5 / 2.0% L-glutamic acid	7	114	21.0	59.0	219	178	238	195

*The glucose tolerance test was done 11 days after the feeding part of the experiment was finished; the chickens were fed a commercial type mash during this period.

was lower, and those which had been fed methionine was higher than the chicks not fed either of the amino acids.

Effect of feeding a high level of one amino acid on the performance of chicks in a glucose tolerance test

Later three tests were conducted to determine the effects of feeding a high level of a single amino acid, usually methionine, on the performance of chickens in a glucose tolerance test. The first was done on chicks which had been used in another experiment not reported here. A group of niacin-deficient chickens and a group of chickens fed a diet containing adequate niacin were available. The niacin deficient chickens all weighed less than 125 grams at four weeks of age; the mean weight of the chickens fed the diet with adequate niacin was over 300 grams. Each of these groups was divided into two equal groups on the basis of body weights and fed the diets listed in table 7 for two days. The chickens were then fasted for approximately 12 hours and a glucose tolerance test performed. The fasting blood sugar levels of the niacin-deficient chickens of both groups were lower than that of the chickens fed the diets containing added niacin. The feeding of methionine increased the fasting blood sugar level of the chickens fed either diet 113 or 113M. This difference was even more apparent 2.5 hours after glucose was given.

Glycine, leucine, lysine, and methionine were later fed, individually, at a level of five per cent, to six week-old chickens; the remainder of the diet was the department's starting mash. These chickens were fed the diet for six days after

TABLE 7

Influence of eight per cent DL-methionine in the diet on blood sugar levels found in glucose tolerance test on niacin-deficient chicks and chicks which had received adequate niacin

DIETARY TREATMENT	BLOOD SUGAR LEVEL FOUND IN TEST		
	0'	60'	150'
	milligrams per cent		
113N	136	230	188
113N / 8% DL-methionine	142	241	218
113	158	194	177
113 / 8% DL-methionine	166	232	204

which they were fasted for 12 hours and a glucose tolerance test performed. The results of the experiment are given in table 8.

TABLE 8

Effect of high dietary levels of single amino acids on blood sugar levels found in a glucose tolerance test

GROUP NO.	AMINO ACID SUBSTITUTED FOR 5 PER CENT OF DIET	SIX-DAY GAIN	BLOOD SUGAR LEVEL FOUND IN TEST		
			0'	60'	150'
		grams	milligrams per cent		
1	None	86	172	228	192
2	Glycine	36	177	264	215
3	L-leucine	50	190	246	207
4	DL-lysine HCl	28	174	214	215
5	DL-methionine	-42	186	226	242

All of the chickens fed amino acids gained less weight than birds fed the starting mash alone; those fed 5 per cent methionine lost weight during the experiment. The fasting blood sugar level of all chickens fed amino acids was higher than those not fed amino acids. In the glucose tolerance test the chickens fed methionine or lysine had higher blood sugar levels 150 minutes after the glucose was given than 90 minutes earlier. Neither level was above 215 milligrams per cent in the case of the lysine-fed chickens. The birds fed glycine or leucine both had higher blood sugar levels 150 minutes after the glucose was given than the birds fed the basal diet. However, in both cases this level was below the blood sugar level found 60 minutes after giving glucose. This appeared to be significant, since glycine and leucine had increased chick growth rates when added to diet 113-5 per cent gelatin, and lysine and methionine both decreased growth rates when added to the same diet.

Another experiment similar to the last was done in which methionine was the only amino acid fed. Two groups of chickens, average weight about 500 grams, were fed the poultry department's starting mash. Two more similar groups were fed the same diet with five per cent DL-methionine added. At the end of a ten-day period a glucose tolerance test was done. In this test, one group fed the basal diet and one group fed extra methionine, received 150 milligrams of methionine per kilogram of body weight in addition to the glucose. The results are given in table 9. Three hours after giving the glucose, the

TABLE 9

Effect of feeding methionine and giving methionine with the sugar in a glucose tolerance test

GROUP NO.	DIETARY AND TEST TREATMENT	TEN DAY GAIN	BLOOD SUGAR LEVEL FOUND IN TEST		
			0'	60'	180'
		grams	milligrams per cent		
1	Starting mash	160	171	199	171
2	As 1 / DL-methionine in test	144	168	187	177
3	As 1 / 5% DL-methionine	-38	171	216	202
4	As 3 / DL-methionine in test	-84	167	254	202

blood sugar level of both groups of chicks fed the basal diet during the ten-day feeding period had returned almost to the original level. Both groups which had been fed excess methionine had blood sugar levels considerably above their original fasting levels. The addition of methionine to the glucose solution given in the tolerance test, had little effect on the final blood glucose level.

Effect of methionine on the rate at which chickens lose weight during a fasting period

In these experiments the addition of four per cent methionine to the diet of chickens, resulted in almost complete cessation of growth; if five per cent was added, the chickens lost weight. This raised the question as to whether the methionine had this action by increasing the rate at which body proteins were broken down.

Experiment 46 was done to see if methionine would increase the rate that chickens would lose weight during fasting. In this experiment 72 birds were equally distributed into 18 groups. The average weight of each bird at the beginning of the experiment was 430 grams. One day prior to beginning fasting each bird in six of the groups received a gelatin capsule with approximately 350 milligrams of methionine; six other groups were given an equal amount of glutamic acid. These birds received the same amounts of the amino acids each day as long as they were on experiment. One group of birds from each treatment was taken off experiment at the different times indicated in table 10, and the weight loss and blood sugar level was determined. It is apparent from the results that giving the methionine did not increase the weight loss. There is a suggestion in the data that giving glutamic acid decreased the weight loss. The blood sugar levels of birds fed either of the amino acids are, in almost all cases, higher than the blood sugar levels of the fasted birds which did not receive the amino acids. This was especially true of the birds given methionine before fasting was started. This difference was not as great after 12 hours fasting.

A similar experiment, number 48, was done in which birds were starved except for glucose, or glucose and methionine, or glucose and glutamic acid. Thirty-six birds, with an average weight of 557 grams, were divided into three equal groups, and offered free choice of the mixtures listed in table 10. In this experiment the birds offered the glucose

TABLE 10

Influence of methionine and glutamic acid on the weight loss of chickens during starvation, or feeding only glucose

GROUP NO.	TREATMENT	HOURS ON EXPERIMENT						
		0	12	24	36	48	60	72

Experiment 46 - No glucose given

1	No feed							
	Weight loss*	0	21	48	66	73	-	105
	Blood sugar**	166	163	163	167	152	-	161
2	350 mg. DL-methionine per day							
	Weight loss	0	26	52	64	81	-	96
	Blood sugar	203	165	159	173	157	-	164
3	350 mg. L-glutamic acid per day							
	Weight loss	0	14	51	59	71	-	80
	Blood sugar	179	158	165	174	160	-	174

Experiment 48 - Birds offered free choice of feed listed

1	Glucose (Cerelese)							
	Weight loss	0	-	34	-	53	-	-
	Blood sugar	205	-	-	-	188	-	-
2	95% Glucose / 5% DL-methionine							
	Weight loss	0	-	45	-	66	-	-
	Blood sugar	-	-	-	-	205	-	-
3	95% Glucose / 5% L-glutamic acid							
	Weight loss	0	-	34	-	52	-	-
	Blood sugar	-	-	-	-	216	-	-

Experiment 49 - All birds given 20 gm. Cerelese per day

1	Glucose#							
	Weight loss	0	60	-	95	-	130	135
2	Glucose and 350 mg. DL-methionine per day							
	Weight loss	0	58	-	85	-	112	115
	Blood sugar	-	-	-	-	-	-	198
3	Glucose and 350 mg. L-glutamic acid per day							
	Weight loss	0	67	-	99	-	128	130
	Blood sugar	-	-	-	-	-	-	190

*All weight losses are given in grams.

**All blood sugars are given in milligram per cent.

#Blood sample lost.

and methionine lost more weight than birds offered only glucose. The difference is statistically highly significant. However, the methionine fed chicks consumed only 140 grams of the mixture during the two-day experiment; those given glucose consumed 582 grams of the glucose. When the weight losses in this experiment are compared with those in experiment 46, it is apparent that the birds given methionine utilized the smaller amount of feed consumed more efficiently. Therefore, experiment 49 was done in which the amount of glucose given remained constant for all groups. The average weight of the 24 birds used in this experiment was 825 grams. The amount of glucose given, 20 grams, was intermediate between the amounts which had been consumed by the glucose and glucose and methionine fed birds of experiment 48. The glucose was force fed as a 40 per cent solution in two equal feedings per day. Methionine or glutamic acid was added to the solutions as indicated. In this experiment the birds given methionine lost significantly less weight than birds given only glucose. Thus it seems apparent that the increase loss in weight of birds offered the glucose with methionine in experiment 48 was the result of the self restricted glucose intake.

In experiment 48 both groups of birds offered the amino acids had higher blood sugar levels, after being on the experiment for 48 hours, than birds offered glucose. The blood sample, from the birds given glucose only, in experiment 49, was lost when the test tube containing it was broken. Blood sugar levels of the two groups with the added amino acids

were higher than would be expected after 12 hours fasting.

Further studies on the effects of adding single amino acids
to niacin-low chick diets

Following the finding that feeding amino acids influenced the blood sugar level, further studies were carried on to determine what happens when niacin was omitted from the diets with added amino acids.

In the first experiment seven amino acids and two keto-acid analogues were fed as additions to diet 113-5 per cent gelatin with and without added niacin. The results are given in table 11. In this experiment the action of sodium ethyl-oxalacetate was compared with aspartic acid. The sodium ethyl-oxalacetate depressed growth both with and without added niacin in the diet. It is believed that this depression was not entirely a result of the presence of oxalacetic acid, but that the extra sodium added may have been toxic. This amount of the material added over one per cent sodium to the diet, for which there was no adjustment made in the diet. This salt, no doubt, raises the pH of the diet. Therefore no comparison can be made with the action of aspartic acid.

The phenylpyruvic acid depressed growth both in the presence and absence of added niacin. This growth depression, however, in neither case was as great as with the groups of chicks fed phenylalanine. Arginine, glycine, glutamic acid, alanine, and aspartic acid all depressed the growth of chicks fed the niacin-low diet. When niacin was added to the diet the

TABLE 11

Influence of individual amino acids on blood sugar levels and growth of chicks fed diets with and without added niacin

GROUP NO.	ADDITION TO DIET	NO. CHICKS	NO. DEAD	AVE. FEED WT.	FEED EFF.	BLOOD SUGAR LEVEL			
						NOT FASTED	0'	60'	180'
	113N-5% GELATIN			grams		milligrams	per cent		
1	None	12	2	191	.483	188	165	191	190
2	Niacin	12	0	279	.600	170	168	189	186
3	6.3% Na ethylhexal-acetate	10	0	161	.369	215	158		
4	As 3 \neq niacin	10	2	183	.366	194	168		
5	4% DL-aspartic acid	10	7	157	.301	211	144		
6	As 5 \neq niacin	6	0	270	.517	199	181		
7	4% phenylpyruvic acid	10	3	160	.432	195	165		
8	As 7 \neq niacin	10	1	178	.469	193	148		
9	4% DL-phenylalanine	10	1	126	.312	191		214	229
10	As 9 \neq niacin	6	0	153	.402	200	196		296
11	4% L-arginine HCl	10	5	119	.319	171	146	249	208
12	As 11 \neq niacin	6	0	333	.592	196	187	251	
13	4% glycine	12	3	166	.428	220	166	232	172
14	As 13 \neq niacin	12	1	302	.6	194		234	208
15	4% DL-alanine	12	3	160	.348	189	146		
16	As 15 \neq niacin	10	0	234	.547	191	155		
17	4% DL-lysine HCl	12	3	99	.251	255	188		
18	As 17 \neq niacin	12	1	148	.422	188	176	231	304
19	4% L-glutamic acid	12	1	197	.393	196	160		
20	As 19 \neq niacin	10	0	295	.555	217	181		
21	5% gelatin					199	159	239	187

growth depression was entirely overcome with each amino acid except alanine; lysine depressed growth when added to either diet. These growth results are all essentially the same as found in the earlier experiments. The nonfasted and fasted blood sugar levels were determined on chickens of all groups; glucose tolerance tests were done on nine groups and the results are also given in the table. The blood filtrates were stored for several days before the glucose was determined. During this time four of the filtrates became cloudy due to bacterial action; no glucose values are given for these four samples. The glucose levels found in this experiment will be discussed later along with values found in other experiments.

A group of chicks fed diet 113N was raised to compare with the other chicks of this experiment in a glucose tolerance test. Only chicks which showed a marked growth reduction as a result of being fed the niacin-deficient diet were selected for use. These chicks are listed as group 21 in table 11.

In another experiment the growth depressing actions of alanine and aspartic acid were compared with their keto-acid analogues using diet 113-5 per cent gelatin with and without added niacin. The sodium salt of the diethylester of oxalacetic acid was used in this experiment as in the last experiment. In this experiment all the sodium chloride and sodium acid phosphate were omitted from the diet of the chicks to be fed the sodium ethylmalacetate; the chloride and phosphate were restored by adding calcium chloride and extra calcium phosphate and omitting part of the calcium carbonate.

Even with this adjustment the sodium level of the diet was in excess of that used in the basal diet. This diet became very rancid within two days after preparation. The results are given in table 12, but it is believed that the chicks fed the sodium ethyloxalacetate should not be compared with the chicks fed aspartic acid.

In this experiment pyruvic acid depressed growth of chicks even more than alanine when added to the niacin-low diet; this was not in agreement with the results of Henderson et al. (1947), who fed the sodium salt of pyruvic acid to rats, receiving a niacin-low diet, without any growth depression. The depression noted here was almost completely overcome by the addition of niacin to the diet.

Since omitting niacin from the diet in previous experiments had resulted in a decreased blood sugar level, the fasting blood sugar level of each chick of this experiment was determined to find out if there was any correlation between the growth rate and the blood sugar level of the chick. The samples of blood were stored in a refrigerator for 3 days before the filtrates could be prepared. During this period the red cells settled and could not be resuspended uniformly in the plasma. Therefore, only the plasma was used in making the sugar determinations. The values found are higher than for whole blood, which is to be expected since the plasma is known to contain more of the sugar than the red cells.

The plasma sugar levels of the chicks fed niacin were, with three exceptions, between 180 and 210 milligrams per cent.

TABLE 12

Comparative effect on growth and blood sugar level of alanine and aspartic acid and their keto-acid analogues

GROUP NO.	ADDITION TO DIET 113N-5% GELATIN	NO. CHICKS	AVE. WT. 4 WKS. grams	FEED EFF.	PLASMA SUGAR LEVEL mg per cent
1	None	10	221	.465	178
2	Niacin	10	277	.507	163
3	4% pyruvic acid	9	177	.397	187
4	As 3 and niacin	10	255	.481	189
5	4% DL-alanine	8	193	.538	184
6	As 5 and niacin	6	209	.422	200
7	6.3% Na ethyloxal- acetate	8	104	.361	166
8	As 7 and niacin	9	144	.403	-
9	4% DL-aspartic acid	8	195	.546	164
10	As 9 and niacin	3	332	.558	205

There was a greater variation in the plasma sugar levels of the chicks fed the niacin-low diet, 14 of 25 chicks had plasma sugar levels below 180 milligrams per cent. There did not appear to be any definite relation between plasma sugar level and growth rate. Eleven of the 14 chicks with the low plasma sugar levels were chicks whose weights were between 78 and 113 per cent of their group mean weight; in other words, they were the "average" chicks.

Effect of adding proteins and amino acid mixtures to chick diets low in niacin

It had been noted that the addition of gelatin to diet 113N-5 per cent gelatin depressed growth of chicks more than the addition of an equal amount of zein. This was observed by Briggs et al. (1946). It appeared that the growth depression might be influenced by the amino acid content of the protein other than the content of tryptophan. Gelatin, zein, and casein are quite different in their amino acid composition. In the next experiment acid-hydrolyzed gelatin, zein, and casein were to be compared in their growth-depressing action when added to chick diets low in niacin. The proteins were hydrolyzed with hydrochloric acid on a steam bath for approximately 12 hours. None of the proteins were completely hydrolyzed but all gave a negative test for tryptophan. During the hydrolysis, the gelatin and casein turned black. After heating for 12 hours, the hydrolysates were concentrated to a thick syrup on a steam bath. Sodium bicarbonate was added to neutralize one half the acid added.

In preparing the rations, all the sodium chloride was omitted.

The sodium added as bicarbonate was equivalent to that omitted as the chloride when the hydrolysate was added at a level equivalent to five per cent of the protein. There is sufficient calcium carbonate in the mineral mixture to more than neutralize the remaining acid. Sufficient carbohydrate was added to the protein hydrolysates to make them granular and all were passed through a sieve before being incorporated in the diet.

The results of feeding the hydrolyzed proteins to chicks, as compared with the intact proteins, are given in table 13. There were two amino acid mixtures also fed in this experiment. The first was a mixture of arginine, threonine (mixed isomers), glutamic acid, aspartic acid, glycine, and alanine in the same proportions they are found in gelatin; the second was a mixture of lysine, tyrosine, phenylalanine, methionine, isoleucine, and valine in the same proportions that they are found in casein.

The chicks fed the acid hydrolyzed gelatin and casein did not grow as well as the chicks fed the basal diet even with added niacin. Evidently the hydrolysis resulted in changes in the casein and gelatin, other than splitting part of the peptide linkages, which caused growth of the chicks to be depressed. The addition of the intact zein to the niacin-low diet resulted in a growth depression much greater than had been noted before, and more than was produced by an equal amount of gelatin. It is believed that other factors, other

TABLE 13

Effects of adding acid hydrolyzed proteins and amino acid mixtures to diets with and without added niacin

GROUP NO.	SUBSTITUTION IN DIET 113-5% GELATIN	NO. CHICKS	AVE. WT. 4 WKS.	FEED AMINO EFF. NIT.		BLOOD SUGAR LEVEL
				grams	mg per cent	
1	None	8	182	.494	20.7	143
2	Niacin	10	250	.536	22.5	160
3	5% acid-hyd. gelatin	8	149	.406	21.8	158
4	As 3 and niacin	8	183	.461	19.2	183
5	5% gelatin	9	180	.458	20.7	149
6	As 5 and niacin	9	288	.630	18.4	165
7	5% acid-hyd. zein	9	175	.443	19.4	160
8	As 7 and niacin	8	271	.623	19.2	165
9	5% zein	8	94	.264	18.6	172
10	As 9 and niacin	9	258	.537	20.7	164
11	5% acid hyd. casein	8	129	.386	21.4	171
12	As 11 and niacin	8	173	.449	20.6	173
13	5% casein	10	193	.515	20.7	168
14	As 13 and niacin	9	276	.519	20.6	170

TABLE 13
(continued)

Effects of adding acid hydrolyzed proteins and amino acid mixtures to diets with and without added niacin

GROUP NO.	SUBSTITUTION IN DIET 113-5% CELRITIN	NO. CHICKS	AVE. WT. 4 WKS.	FEED AMINO APP.	MIT.	BLOOD SUGAR LEVEL	Glucose tolerance		
			grams		mg per cent		0'	60'	180'
15	5% glucogenic amino acids*	7	137	.402	21.3	172	215	192	
16	As 15 and niacin	7	299	.623	16.8	174	225	192	
17	5% ketogenic amino acids**	8	118	.336	19.6	163	-	205	
18	As 17 and niacin	10	104	.301	19.4	186	223	211	

*Amino acid used were L-arginine HCl 0.77, threonine (mixture) 0.12, L-glutamic acid 0.84, DL-aspartic acid 0.51, glycine 1.94, and DL-alanine 0.82.

**Amino acid used were DL-lysine HCl 0.97, L-tyrosine 0.90, DL-phenylalanine 0.73, DL-methionine 0.49, DL-isoleucine 0.92, and DL-valine 0.99.

than the diet fed, caused the poor growth noted with the chicks fed zein.

The addition of the glucogenic amino acids to the niacin-low diet resulted in a growth depression which was not noted in the chicks fed the same diet containing niacin. The ketogenic amino acid mixture depressed the growth rate of the chicks when added to diets with or without niacin. In this experiment the chicks fed the ketogenic amino acids gained more when niacin was omitted from the diet; the difference is not considered

significant, however.

The intact gelatin and the acid-hydrolyzed zein caused very little growth depression when added to the niacin-low basal. The growth depressions found when most of the glucogenic amino acids or gelatin was added to the niacin-low diet, in this or the two previous experiments, was not as great as had been observed in earlier experiments. Also in these experiments the niacin-deficiency symptoms, blacktongue, and the presence of food cankers in the mouth, were rarely seen. This suggested that the casein used in these experiments contained more niacin than that used in earlier experiments.

Amino nitrogen and fasting blood sugar level were also determined on all groups of chicks; a glucose tolerance test was done on the last four groups. As was observed in a previous experiment, the amino nitrogen level of the chicks was decreased by the addition of either five per cent gelatin, or the amino acid mixture containing arginine to the basal diet containing niacin. The amino nitrogen level was higher when niacin was omitted from each diet except the basal diet and the diet with added zein. The blood glucose levels are discussed later.

In these experiments the fasting blood sugar level was lower than normal when niacin was omitted from the basal diet or the diets with added glucogenic amino acids. The chicks fed extra ketogenic amino acids usually had blood sugar levels higher than normal. This suggested the possibility that the depressing action of the glucogenic amino acid on the blood

sugar level and growth rate of niacin-deficient chicks, might be partly overcome by the addition of the ketogenic amino acids. However, when two per cent of a mixture of equal parts, of valine, lysine, methionine, and tyrosine was added to diet 113N with two per cent glycine, no increased growth was obtained. It seemed probable, however, that a mixture of ketogenic and glucogenic amino acids would not depress growth of chicks, when added to the niacin-low diet, as much as an equal level of either glucogenic or ketogenic amino acids alone. This was tested in the next experiment.

Since in the last experiments there seemed to be an indication that the casein was not as free of niacin as is needed to produce a marked deficiency, the casein to be used in following experiment was washed with water. After washing, the casein was dried in an incubator at 38°C and passed through a screen before being incorporated into the diet. Since it was possible that washing the casein removed the vitamin B₁₂ present, it was decided to eliminate any possible deficiency of vitamin B₁₂ by giving the vitamin to the chicks fed the washed casein. During the experiment each chick received two injections of 0.3 micrograms of crystalline vitamin B₁₂. The chicks were hatched from eggs from hens which were being fed animal protein. The various amino acid mixtures used and the results obtained are given in table 14. The mean four-week weights of the chicks fed diet 113N-5 per cent gelatin was only 121 grams. During the early part of the experiment, the electrical heating element used to provide heat for this group became defective and the chicks

TABLE 14

Effects on growth rate of mixtures of glucogenic and ketogenic amino acids in a diet without added niacin

GROUP NO.	ADDITION TO DIET 113N- 5% GELATIN	CHICKS STARTED	PER CENT MORTALITY	AVE. WT. 4 WKS. EFF.	FEED EFF.
				grams	
1	None	12	8.3	(200)	.421
2	Niacin	10	0	237	.518
3	1% L-arginine HCl / 1% DL-aspartic acid / 1% glycine	15	13.3	134	.441
4	1% DL-valine / 1% DL-isoleucine / 1% L-tyrosine	15	20.0	157	.440
5	As 3 and amino acids of 4	15	66.7	127	-
6	As 3 / 1% L-glutamic acid / 1% DL-alanine / 1% L-histidine HCl·H ₂ O	12	66.7	74	.128
7	As 4 / 1% DL-lysine HCl / 1% DL-phenylalanine / 1% DL-methionine	15	20.0	77	.251
8	As 7 and niacin	15	26.6	91	.280
9	2% ammonium chloride	10	10.0	129	.407
10	As 9 and niacin	8	0	169	.443

were without heat for a maximum of two days; by the end of the experiment some of the chicks were gasping. Therefore in table 14, the actual mean weight of the chicks is given as 200, which is approximately what has been obtained in several previous experiments.

The addition of amino acids to the niacin-low diet resulted

in marked growth depression. Many of the chicks showed niacin deficiency symptoms. Thus it appears that washing the casein removed some of the niacin present.

The addition of three glucogenic or three ketogenic amino acids to diet 113N-5 per cent gelatin reduced the average four-week weight of the chicks to about 145 grams. When six glucogenic or six ketogenic amino acids were added to the same diet the average weight at four weeks, was only about 75 grams. If instead a mixture of three glucogenic amino acids and three ketogenic amino acids are added, growth was depressed only slightly more than with three glucogenic amino acids.

The per cent mortality of these chicks was very high, however, the high mortality rate was also noted with chicks fed the six glucogenic amino acids. The growth depression caused by the six ketogenic amino acids was not overcome by the addition of niacin to the diet.

In this experiment two per cent ammonium chloride was fed to chicks in diets with and without added niacin. This amount of ammonium chloride added about the same amount of nitrogen as four per cent serine. The ammonium chloride depressed growth of chicks when added to either diet.

Effect of insulin on growth rate and blood sugar level of chicks

As was stated in the literature review, there was a question as to whether the growth depression noted when four per cent of one of the glucogenic amino acids was added to chick diets, was in part due to an "insulin-like" action of the amino acids.

In the next experiment chicks fed diet 113-5 per cent gelatin with and without added niacin, were compared with chicks fed the same diet and receiving injections of protamine-zinc insulin. In another experiment it was found that one unit of regular insulin, injected subcutaneously into 900 gram chickens, depressed the blood sugar level to about one-half of the normal level. In the chick experiment all chicks were injected subcutaneously with one-quarter unit of protamine-zinc insulin per bird per day for the first experimental week; during the second experimental week this amount was doubled. All injections were given about 9:00 A.M. The chicks used in the experiment were all fed diet 113N-5 per cent gelatin for the first five days. At the end of this period, 15 of the largest and smallest chicks were discarded, and the remaining 44 chicks were equally distributed into four groups and fed the diets indicated in table 15. The injections of insulin and the experimental period were started two days later.

The results indicate that injection of insulin did not depress growth any more in chicks fed a niacin-low diet than it does in chicks fed a diet with adequate niacin. After the experiment was terminated, the chicks were fasted for six hours and a blood sample was taken for sugar determination. This was 30 hours after the last insulin injection. Again the chicks fed the niacin-low diet had a blood sugar level below those given niacin in the diet. However, the chicks fed the niacin-low diet and injected daily with insulin had a normal blood sugar level after six hours of starvation. No further

TABLE 15

Influence of injecting insulin on the growth and fasting blood sugar level of chicks fed diets with and without niacin

TREATMENT	TWO WEEK GAIN	BLOOD SUGAR LEVEL
	grams	milligrams per cent
113N-5% gelatin diet	52	157
113N-5% gelatin diet injected with insulin	43	189
113-5% gelatin diet	63	182
113-5% gelatin diet injected with insulin	56	193

determinations were made to determine how long this level was maintained.

Chickens fed the starting mash with five per cent of glycine, glutamic acid, arginine, tyrosine, leucine, or methionine were compared with chickens fed only the starting mash, in order to determine the influence that injecting regular insulin would have on the blood sugar level. The birds' average weight was about 900 grams. They were fed the various diets for five days, after which they were fasted for 16 hours. A blood sample was then taken and regular insulin was injected subcutaneously. The amount of insulin injected varied slightly with each experiment, but was about one unit of insulin per bird. Additional blood samples were taken 1, 2.5, and 5 hours after giving insulin. Thus four samples were taken from each bird, and the glucose

level in each sample was determined.

In the first experiment the blood glucose level of chickens, which had previously been fed arginine, did not decrease as much as it did in chickens previously fed the starting mash, or the mash with methionine. In a second experiment chickens, which had been previously fed glycine, increased their blood sugar level after regular insulin was injected. Chickens previously fed glutamic acid or tyrosine showed moderately decreased blood sugar levels after injection of insulin; leucine-fed chickens showed a greater decrease than any of the others. In a third experiment about 25 per cent more insulin was injected than was injected in the second experiment. In this experiment chickens fed the starting mash, or the same mash with added leucine or glycine, all showed about equal decrease in blood sugar level. The minimum levels reached were between 40 and 70 milligrams per cent.

The results of the three experiments were not consistent, but there was some indication in the first two experiments that the blood glucose level, of the chickens previously fed arginine or glycine, was not decreased as much by insulin as that of chicks previously fed leucine or methionine.

**Effect of certain proteins and amino acid mixtures on
the growth depression caused by feeding chicks high
levels of lysine or tyrosine**

Throughout this work a growth depression has been noted when certain amino acids were added at a level of four or five per cent, to the diets with adequate niacin and pyridoxin. In

work in which lysine was added to the starting mash, growth did not appear to be depressed as much as adding it to the synthetic diet 113-5 per cent gelatin. The starting mash contains something which decreased the growth depressing action of lysine. The idea that a balance between the various amino acids is required suggested that if the other amino acids were built up around this high lysine level, the depressing action of lysine would be decreased. The data given in table 16 support this idea. Some of the data were gleaned from experiments previously mentioned and some are from experiments done to study this effect. If lysine was added at a level of four per cent to diet 113-5 per cent gelatin, the rate of gain of the chicks was only 20 to 45 per cent of the rate of gain of chicks fed the same diet without lysine. In experiments 50 and 51, three per cent lysine was added to the diet, and the diet contained three per cent liver meal; the rate of gain of the lysine-fed chicks was 64 per cent of those not fed lysine. The addition of extra gelatin, or gelatin and corn, or gelatin and zein further increased the rate of growth and the ratio of the growth of the lysine-fed chicks to the growth of chicks fed the same diet without lysine. Gelatin contains lysine but also contains a high level of arginine, which is submarginal in the five per cent gelatin diet. Zein contains no lysine and corn only a relatively small amount of it.

In experiment 56, the action of seven glucogenic amino acids, at levels equal to that found in five per cent gelatin

TABLE 16

Amino acid balance and growth depressing action of DL-lysine

GROUP	DIET	NO. CHICKS	AVE. WT. 4 WKS.	RATIO OF GAIN	FEED EFF.	BLOOD SUGAR		
						0'	60'	180'
			grams			mg per cent		
Experiment 37								
1	113-5% gelatin	8	232		.494	-	-	-
2	As 1 / 4% DL-lysine	7	77	.213	.207	-	-	-
Experiment 43								
1	113-5% gelatin	12	279		.600	168	189	186
2	As 1 / 4% DL-lysine	11	148	.452	.422	176	231	304
Experiment 50								
1	113	9	332		.547	179	200	198
2	As 1 / 3% DL-lysine	9	248	.717	.524	197	209	199
3	113 / 3% liver meal / 25% corn	10	404		.579	192	206	200
4	As 3 / 3% DL-lysine	10	327	.791	.525	195	202	222
Experiment 51								
1	113-5% gelatin / 3% liver	10	272		.488			
2	As 1 / 3% DL-lysine	8	189	.640	.311			
3	113 / 3% liver meal	9	350		.544			
4	As 3 / 3% DL-lysine	10	251	.679	.432			
5	As 3 / 5% zein	10	320		.511			
6	As 5 / 3% DL-lysine	8	272	.828	.496			
Experiment 56								
1	As 5 in exp. 51	10	3 wks. 202		.571			

TABLE 16
(continued)

Amino acid balance and growth depressing action of DL-lysine

GROUP	DIET	NO. CHICKS	AVS. WT. 4 WKS.	RATIO OF GAIN	FEED EFF.	BLOOD SUGAR		
						0'	60'	180'
			grams			mg per cent		
			3 wks.					
Experiment 56 (continued)								
2	As 1 / 3% DL-lysine	10	150	.675	.549			
3	113-5% gelatin / 3% liver meal / amino acid mix*	8	241		.601			
4	As 3 / 3% DL-lysine	8	174	.663	.460			

*L-Arginine HCl 5, L-histidine HCl·H₂O 1, mixture of threonines 2, L-glutamic acid 21, DL-aspartic acid 4, glycine 11, and DL-alanine 9 grams per kilogram of feed.

and five per cent zein, were compared with the intact proteins as to their ability to overcome the growth depressing action of lysine. During the last week the chicks of group 1 gained only about one-half as much weight as expected. This decreased gain during the fourth week apparently was not due to the diet fed. Therefore, the average weights at the end of the third week are presented. The ratios of gain of the groups fed the diets with and without lysine are lower than was noted in experiment 51. Experiment 56 would indicate that these seven glucogenic amino acids are equal to the whole proteins in overcoming the growth depression produced when three per cent lysine is added to the diet. With only one experiment this equality may be questioned

since no chicks were fed the diet without the addition of extra gelatin and zein, and the ratio of gain between chicks fed lysine and those fed the same diet without lysine is lower than previously noted.

There are also indications in the previous data that the depression of growth, caused by the addition of tyrosine to the diet, is less with the ten per cent gelatin diet than with the five per cent gelatin diet.

In experiment 35, table 3, addition of four per cent tyrosine to the five per cent gelatin diet, depressed the growth of the chicks to 54 per cent of the growth of the chicks fed the same diet without tyrosine. In experiment 37, table 4, the same amount of tyrosine was added to the 10 per cent gelatin diet and the growth of the chicks was 93 per cent of the growth of the chicks fed the same diet without tyrosine.

A summary of the effects of feeding amino acids
on glucose metabolism in the chick

In all the experiments in which the blood glucose levels were determined, there have been consistent changes noted when the amino acids were fed or niacin was omitted from the diet. In the course of the studies the fasting blood glucose levels, of chickens fed the poultry department's starting mash, have been determined in five experiments. The values found are 172, 171, 171, 167, and 174 milligrams per cent. The blood glucose values for chickens, reported in the older literature, are mostly in excess of 200 milligrams per cent. Most of the

later reports give values between 170 and 190 milligrams per cent. The range of fasting blood glucose levels of the chicks fed diet 113-5 per cent gelatin as determined in these experiments, was from 160 to 168 milligrams per cent after 12 hours fasting. For the chicks fed diet 113 fasting levels from 158 to 179 milligrams per cent have been observed. Generally, there seems to be a slight increase in the fasting blood glucose level as the per cent protein in the diet is increased.

The chicks fed the niacin-low basal diets were observed to have lower blood glucose levels than those fed the same diet containing niacin. Only one exception has been noted, table 12, here the value found for those chicks fed the niacin-low basal diet is higher than usual. The mean decrease in glucose level resulting from the omission of niacin from the diet, in six experiments in which both diets were fed, is 10 milligrams per cent. In each diet, in which added glucogenic amino acids have been fed, the blood glucose level has been lower if niacin was omitted than it was if niacin was included in the diet. In one experiment (see table 11) the chicks fed the niacin-low diet with added, arginine, alanine, or aspartic acid had blood sugar levels of 146, 146, and 144 milligrams per cent, respectively. However, the glucogenic amino acids, with one exception, alanine, table 11, increased the blood glucose level when fed in the diet with added niacin. This includes an increased plasma sugar level when alanine was fed in another experiment, table 12.

Feeding the ketogenic amino acids, methionine, lysine, phenylalanine, or the mixture of ketogenic amino acids shown in table 13, were observed to increase the blood sugar level when added to diet 113-5 per cent gelatin, either with or without niacin. The chicks fed the diet with phenylpyruvic acid and niacin (table 11) had a low blood glucose level. The blood glucos levels determined before fasting do not show the trends which are noted after fasting.

The glucose tolerance tests have shown differences in the glucose metabolism of the chicks fed the amino acids which decrease growth rate and those which increase the rate of growth when added to diet 113-5 per cent gelatin. Five glucose tolerance tests were done on chickens previously fed a high level of methionine. Each time these chickens did not return their blood glucose level toward normal as well as chickens fed the diet without methionine. In one experiment the blood glucose level was higher 2.5 hours after glucose was given, than it was after one hour.

Chickens previously fed four or five per cent lysine also did not return their blood glucose level toward normal as well as the chickens not fed lysine. In experiment 50, table 16, one group of chickens fed three per cent lysine returned their blood sugar level as well as the chickens which were not fed lysine; another group fed a different diet and three per cent lysine did not. The maximum glucose level noted with most of the chickens fed a high level of lysine was lower than noted with chicks fed other amino acids.

Two groups of chicks previously fed four per cent phenylalanine failed to return their blood glucose level as well as the chicks fed the basal diets (table 11).

In experiment 52 (table 13) the two groups of chicks fed a mixture of ketogenic amino acids had higher blood glucose levels three hours after being given glucose than the two groups of chicks fed the glucogenic amino acid mixture.

Chickens fed a high level of arginine, glycine or leucine were also subjected to glucose tolerance tests. The leucine and glycine fed chicks (table 8) did not return their blood glucose level as well as the chicks which were not fed any extra amino acids, but the level was lower 150 minutes after glucose was given than it was 60 minutes after giving glucose. This was not found in the chickens fed methionine or lysine.

The chicks fed glycine in diet 113N-5 per cent gelatin (table 11), did not return their blood glucose level as well as did the chicks fed the basal diet, but the level decreased during the last two hours. The same was true of the chicks fed arginine in the diet without niacin. The chicks fed glycine in the diet without niacin returned their blood sugar as well or better than the chicks fed the basal diet. This, in comparison with the results with the phenylalanine or lysine fed chicks in the same experiment, clearly indicates that the impairment in ability to reduce the blood glucose level, follows the feeding of phenylalanine or lysine and is not a characteristic of chicks whose growth had been retarded.

In these glucose tolerance tests, the blood glucose level of the chicks fed the amino acids was consistently higher, 60 minutes after the glucose is given, than that of the chicks fed the basal diets without the addition of excess amino acids. This was true in the presence and absence of added niacin and with either ketogenic or glucogenic amino acids.

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DISCUSSION

In the first experiment in which four per cent of various amino acids were fed to chicks in diet 113-5 per cent gelatin, the growth of the chicks during the four-week experimental period varied from 266 grams to almost nothing. Since then these amino acids and others have been fed to chicks, using the same basal diet. In all the work there has been good agreement as to the influence that the addition of a given amino acid to the chick diet will have on the growth rate of the chick. In these experiments it was noted that arginine, glycine, glutamic acid, and proline were the only amino acids, which if added to the chick's diets containing ample niacin, resulted in no growth depression or an increased growth rate of the chicks. However, if niacin was omitted from the chick diet, each of these amino acids depressed the rate of growth. Feeding a level of four per cent of a single amino acid obviously created an amino acid imbalance in the chick diet. Since it is known that glucose could be formed from each of these four amino acids it suggested that niacin might be concerned in converting excesses of these amino acids into glucose. It was reasoned that if the chick could not convert these excess amino acids into glucose, there would be a large excess of the one amino acid in the tissues of the chick, which was thought to be the cause of the growth depression. Omitting any other vitamin, which has a role in the conversion of these excess amino acids to glucose, should result in approximately the same chick growth depression that was noted when niacin was omitted

from the diet. A preliminary study, omitting several vitamins individually from diet 113 with four per cent glycine added, indicated that decreasing the level of pyridoxin of the diet of the chick resulted in a decreased growth rate. Pyridoxal is considered to be part of a coenzyme needed in transamination, and transamination is probably one of the reactions which would be needed to dispose of the amino acid excesses. Therefore, 15 other amino acids were added individually, at a four per cent level, in order to determine if their addition to the pyridoxin-low chick diets would depress the growth of chicks in a manner similar to that noted with the niacin-low diet.

Figure 2 is a summary of seven experiments in which four per cent of 17 amino acids were added, individually, to chick diets containing adequate levels of niacin and pyridoxin; also most of these have been added to the niacin-low and the pyridoxin-low diet. The amino acids are listed in declining order of growth of chicks resulting when each amino acid is added, at the four per cent level, to the complete chick diet. The length of each bar is proportional to the mean chick growth; a bar which reaches the vertical line indicates that the growth of chicks fed the diet with the amino acid equaled the growth of the chicks fed the same diet without the amino acid.

Of the amino acids fed, only threonine caused a growth depression by its addition to the low-pyridoxin diet which was not observed if there was adequate pyridoxin in the diet. This is reminiscent of the work of Hanks et al. ('48) in which in the case of rats threonine was found to be the amino

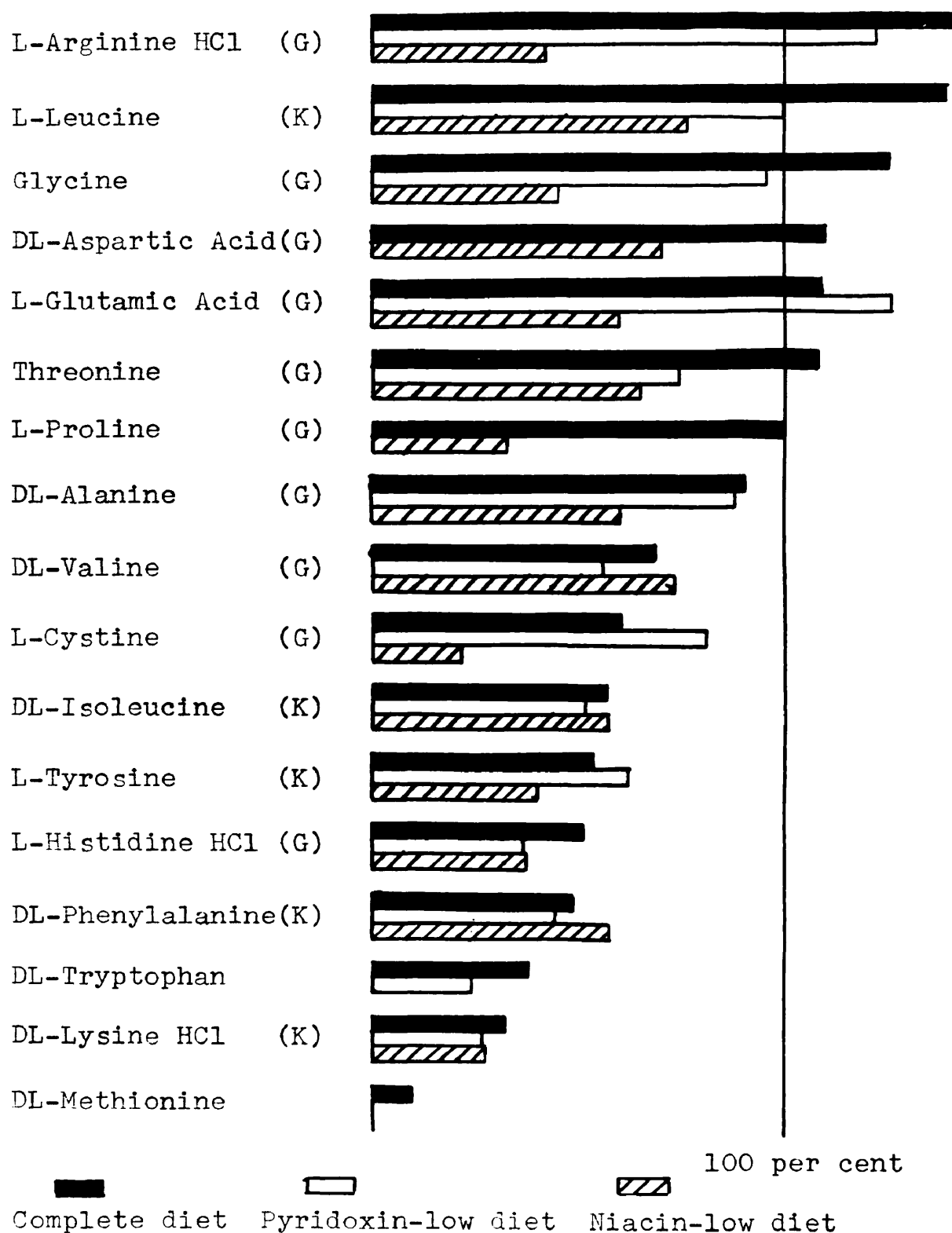


Fig. 2 Summary of the effect on growth when individual amino acids are added to the diet of chicks. The length of each bar is proportional to the gain of the chicks fed four per cent of the amino acid as a per cent of the gain of chicks fed the same diet without the amino acid.

acid mainly responsible for the growth depression noted upon the addition of acid hydrolyzed casein to the diet. A growth depression was noted upon the addition of four per cent glycine or leucine to the pyridoxin-low diet 113, and this depression was overcome when the pyridoxin level was increased to 600 micrograms per cent. This depression was not noted when these amino acids were added to diet 113-5 per cent gelatin. In this work, decreasing the pyridoxin level of the basal diet to 75 micrograms per cent decreased the chick growth rate to about the same extent as omitting the niacin. However, when the glucogenic amino acids were added to the pyridoxin-low diet some did not depress growth, while others depressed growth but not to as great an extent as in the niacin-low diets. Especially note arginine, glycine, glutamic acid, alanine, and cystine. Thus, it must be said that either the chicks fed the low pyridoxin diet are able to dispose of the excesses of the amino acids, or that the overcoming of the growth depression, when niacin was added to the niacin-low diet, is not due to the ability of the chick to retain a normal amino acid balance in the tissues while an excess of one amino acid is in the diet. Two other facts tend to indicate that the latter is true. First, the level of arginine and tryptophan in the blood of chicks fed these amino acids, was higher than in chicks which were not fed the amino acids, even if adequate niacin and pyridoxin were in the diet. Second, pyruvic acid depressed growth of chicks fed the niacin-low diet to as great an extent as alanine. This growth depression was also overcome by the addition of

niacin to the diet. The results given in table 5 indicate that the increase in growth noted with the addition of glycine to the five per cent gelatin diet is not due to a specific need of the chick for more glycine. The increased growth which was noted in these experiments when glycine was added to the five per cent gelatin diet probably results from a need for extra protein. If the amounts of the various amino acids in diet 113-5 per cent gelatin are calculated, only arginine is below the presently accepted minimum requirement.

In figure 2 each glucogenic amino acid is indicated by the letter (G) following the name, and each ketogenic amino acid has the letter (K) following the name. The listing used here is taken from Mitchell's "Textbook of Biochemistry" (p. 447). Tryptophan and methionine are not listed. Tryptophan is usually considered to be ketogenic. Several list valine as a ketogenic amino acid. The classification of methionine is doubtful.

It is apparent from figure 2 that ketogenic amino acids depress growth to a greater extent, under the conditions of these experiments, than the glucogenic amino acids. Leucine and histidine are the only two amino acids which are exceptions. The chick has a very low requirement for histidine. The work of Almquist and Grau (1947) indicates that a level of 0.15 per cent of the diet is almost adequate, and 0.30 per cent is definitely adequate. In feeding four per cent histidine between 10 and 20 times as much histidine is added as is

required by the chick. This is obviously a greater imbalance than is caused by feeding four per cent arginine, glycine, or leucine which is only three to four times the normal requirement. It is believed that if histidine was added with a comparable imbalance the growth depression would be much less.

Omitting niacin from a diet containing four per cent of a ketogenic amino acid causes little difference in the rate of gain of the chicks, and the blood sugar level of the chicks is not decreased. If niacin is omitted from a diet with a high level of a glucogenic amino acid, there is a decrease in blood sugar level and a marked decrease in growth rate.

Since the ketogenic amino acids have similar effects on growth and blood sugar level, and mixtures of ketogenic amino acids have about the same effects on the chick as a high level of a single ketogenic amino acid, it is believed that the ketogenic amino acids have some property in common which influences metabolism in the chick. This property does not appear to be related to their ability to form ketone bodies. Leucine is usually considered to be ketogenic, but does not depress the growth rate of the chicks as the other ketogenic amino acids.

The amino acids which appear to have this property are valine, isoleucine, tyrosine, phenylalanine, tryptophan, lysine, and methionine. Valine is included in this group because it behaves like the other ketogenic amino acids when added to chick diets, and because of its structural similarity to isoleucine and leucine.

It is apparent also that the glucogenic amino acids have

some property in common which influences metabolism in the chick. Furthermore, this does not appear to be related to their relative ability to form glucose. Included in this group are arginine, glycine, aspartic acid, glutamic acid, threonine, proline, alanine, cystine, and histidine. It is difficult to place leucine in either category on the basis of this work.

There was a consistent increase in blood amino nitrogen levels of the chicks fed the diets with additional amino acids. This was noted with the addition of each amino acid except methionine, lysine, and arginine. The chick fed the diets with methionine or lysine gained very slowly and consumed very little feed. In this case, it is believed that the low amino nitrogen level only reflected the condition of the birds. The chicks fed arginine, however, grew and ate well. It was also noted that the addition of five per cent gelatin to the diet decreased the blood amino nitrogen level. In diet 113-5 per cent gelatin, arginine is the amino acid which limits the rate of gain. Gelatin is a good source of arginine, which probably explains the reason that the addition of gelatin has the same action on blood amino nitrogen level as arginine. The addition of either gelatin or arginine to diet 113-5 per cent gelatin, results in a more rapid gain of the chicks. It seems reasonable that the addition of arginine, allowing more rapid growth would result in a greater utilization of all the amino acids and thus reduce the quantity of amino nitrogen in the blood. The data also indicate that the addition of niacin to most

of the niacin-low diets allowed a greater growth rate and produced lower blood amino nitrogen levels. Charkey, Wilgus, Patton and Cassner ('50) found that the addition of vitamin B₁₂ to an all-vegetable-protein diet fed chicks, resulted in increased growth rates and a decreased blood level of the seven amino acids which were determined. All this would indicate that any alteration of a diet, which would allow better growth of chicks, should also decrease blood amino nitrogen level. The fact that there was no consistent difference in the blood amino nitrogen level of the chicks fed the pyridoxin-low diets and those fed the diets with adequate pyridoxin, detracts from this relationship.

The results of the seven experiments which are summarized in figure 2, have raised several questions: 1. Why does the omission of niacin, from the chick diets containing a high level of a glucogenic amino acid, result in such a great growth depression? 2. Why is there such a great variation in the growth of chicks fed the complete diet with the addition of various amino acids? 3. Why do the amino acids, which are usually classed as ketogenic, depress growth of chicks more than the glucogenic amino acids? 4. What can be done to overcome the growth depressions? If these questions could be answered completely they, no doubt, would answer other questions about protein metabolism in the chicken. It is felt that the studies reported in this thesis and related studies of others may partly answer these questions, or at least suggest reasons as to why the effects were noted.

The proposal of Hanks et al. ('49), that the tryptophan deficiency is accentuated by the addition of amino acids, which are present in suboptimal levels in the basal diet, could not account for the depression of growth of chicks noted in these experiments when each of the amino acids was added to the niacin-low diet. It may, however, be a factor in the growth depression caused by feeding arginine in the niacin-low diet. It must be pointed out that in these experiments the amino acids were added at a level several times greater than was fed by Hanks et al., and the results would be expected to be different.

In this work the blood sugar studies suggest why the addition of the glucogenic amino acids depress growth of chicks fed the niacin-low diet. These studies have indicated that the omission of niacin from either five or ten per cent gelatin basal diets, resulted in a decrease of the blood sugar level. This work is somewhat supported by the work of Landauer ('48) who found that niacin would prevent some of the teratogenic effects of injecting insulin into the incubating egg, and also by the work of Zwilling ('49), who found that nicotinamide reduced the duration of the insulin-induced hypoglycemia of the incubating egg. These studies reported in this thesis indicate that the hypoglycemia was most apparent when niacin was omitted from chick diets with added glucogenic amino acids. These same amino acids, if added to the diets with niacin resulted in an increased blood sugar level. On the basis of previous work with rats, this might have been expected. Mirski, Rosenbaum, Stein and Wertheimer ('38) found that rats

fed a diet high in carbohydrates had more liver glycogen than rats fed a high protein diet if the glycogen was determined before fasting. However, if the rats were fasted before the liver glycogen was determined, the protein-fed rats had a greater quantity of liver glycogen. Guest ('41) confirmed this action of protein and also reported that there was no demonstrable relationship between blood sugar level and liver glycogen in fed rats. If the rats were fasted, the blood sugar level varied directly with the logarithm of the percentage of liver glycogen. Todd, Barnes and Cunningham ('47) reported that after 24 hours of fasting, rats which had previously been fed a diet containing ten per cent glycine for 48 hours had a higher level of liver glycogen than rats which had been fed the same diet without glycine. Feeding 18 per cent alanine had the same effect but to a lesser extent. Rats fed glutamic acid or leucine did not show the effect. Cunningham, Barnes and Todd ('48) reported that after 13 hours fasting and 5 hours under the influence of insulin, rats prefed a basal diet with 10 per cent glycine had 2.5 times as much muscle glycogen, 10 times as much liver glycogen, and a blood sugar level 40 milligrams per cent higher than rats which had been prefed the basal diet. Todd and Talman ('49) have since reported that rats similarly treated have six times as much extra carbohydrate in their carcasses after eight hours of fasting as can be accounted for by direct conversion of the excess glycine found in the rat before fasting. These workers believe that feeding the excess glycine stimulates the general

processes of glyconeogenesis, probably by increasing adrenal cortical activity. This "protein effect" was not noted in adrenalectomized rats.

In the studies reported here, the same effects in fasting blood sugar level have been noted with chicks fed glycine, arginine, aspartic acid, alanine, or glutamic acid in the diet containing niacin. There was also some indication that chickens fed extra arginine or glycine were not affected as much by insulin as chicks not fed these amino acids. Therefore, it seems that this "protein effect" is apparent in the chick as well as the rat. This immediately raises the question as to why this "protein effect" is not noted in chicks fed the niacin-deficient diet. It is generally believed that hormones secreted by the adrenal cortex stimulate gluconeogenesis. This is a property of the C_{21} steroids, which possess an oxygen atom in the 11 position; of these, 17-hydroxy-11-dehydrocorticosterone (Kendall's compound E) is the most active.

In the fasting experiments the blood sugar level was maintained at a higher level, even after 72 hours fasting, than was noted after 12 hours fasting of the chicks fed arginine, alanine, or aspartic acid in the niacin-low diet. This suggests that the endocrine systems, which control blood sugar level in the chicks fed the glucogenic amino acids in the niacin-low diet, do not function properly, possibly because of a deficiency in the secretion of the hormones of the adrenal cortex which stimulate gluconeogenesis. Handler and Bann ('42) found that the parenteral administration of physiological saline solution

to dogs with blacktongue resulted in alleviation of the deficiency syndrome and prolonged life as much as 180 days. All animals finally succumbed. This suggests that the salt metabolism, which is also under the influence of hormones of the adrenal cortex, is altered in a niacin deficiency. Thus, there is an indication that the normal functioning of the adrenal cortex is impaired by the niacin deficiency, and that feeding high levels of the glucogenic amino acids tends to aggravate the condition and makes it more apparent. The ketogenic amino acids apparently do not influence the blood sugar level by exactly the same mechanism as do the glucogenic amino acids.

As far as can be determined, there seems to be no single property of the amino acids that could account for all the differences in growth depressions noted when the individual amino acids are added to diet 113-5 per cent gelatin.

It is apparent that the degree of imbalance has its effect. Within the glucogenic amino acids, four per cent arginine, or glycine, for which the normal requirements are 1.2 and 1.0 per cent of the diet, respectively, depress growth less than the same level of histidine or cystine, for which the chick has a requirement of less than 0.4 per cent. In these experiments it was found that four per cent lysine depressed growth more than three per cent, and five per cent methionine depressed growth more than four per cent methionine.

Heir (1947) found that the plasma level of arginine, histidine, isoleucine, leucine, methionine, phenylalanine,

threonine, tyrosine, and valine rose when the corresponding amino acid was given by gavage to dogs. The methionine level of the plasma tended to remain high longer than the other amino acids. It seems probable that the same would happen in the experiments in which chicks were fed four per cent of the various amino acids. This was found to be true when arginine or tryptophan was fed. If the chick has the same difficulty in removing the excess methionine from the blood, as the dog was found to have, the great extent to which methionine depresses growth may be explained. It seems reasonable that an excess of one amino acid in the protein synthesizing centers of the body would decrease the rate of protein synthesis.

Since it was found that feeding ammonium chloride depressed the rate of growth of chicks, it might be expected that the degree by which an amino acid depressed growth would be related directly to its nitrogen content. No trend such as this was noted, however, in these experiments.

The glucose tolerance tests have revealed a rather significant difference between the chicks fed arginine, glycine, or leucine and those fed methionine, lysine, or phenylalanine. In each experiment in which a glucose tolerance test was done on chicks fed arginine, glycine, or leucine, the chicks showed a lower blood sugar level three hours after, than they did one hour after giving glucose. In some cases the three-hour values were such as would be considered a normal fasting sugar level. The chicks fed methionine, lysine, or phenylalanine at a level of four per cent, sometimes showed an increase in blood sugar

level between the first and third hour. In no test was the blood sugar level three hours after giving glucose as low as that of chicks which were not fed these amino acids. This delay in the return to the normal blood sugar level is also found in mammals under certain pathological conditions, namely, hyperthyroidism, diabetes mellitus, and nephritis. What causes the blood sugar to remain high in the test is not entirely clear. In these experiments the blood sugar level of the chickens fed the ketogenic amino acids were found to be higher than normal even though these chickens consume less feed. This suggests that feeding excesses of the ketogenic amino acids causes an excessive conversion of protein to glucose, and may be a factor in the growth depression noted when high levels of these amino acids are fed.

The experiments in which the influence of methionine on weight loss of chickens during fasting or feeding only glucose was studied, throw some light on why the chicks fed methionine grow slowly. Experiment 46 (table 10) indicates that the methionine did not cause starved birds to lose weight any faster. In experiment 48, the birds offered methionine and glucose lost more weight, even though their blood sugar level was higher, than the chicks which were not fed amino acids. This suggests that the breakdown of body protein in the chickens fed methionine and glucose more nearly approaches the rate of breakdown noted during a complete fast. It is apparent from experiment 49 that the greater weight loss of the chickens fed methionine in experiment 48, was due to the low feed consumption. Whether or not this is due to the methionine being

unpalatable or to some other factor is not known. The smaller weight loss of the birds given methionine in experiment 49, indicates that the metabolic rate of these birds is decreased. Brown (1949) found that rats fed a diet with 4.8 per cent methionine had slightly enlarged thyroids.

In most of the studies the effects noted were the results of the addition of a single amino acid at a level of three, four, or five per cent. In these studies the glucogenic amino acids have exhibited somewhat similar actions, and the same can be said of the ketogenic amino acids. These actions, however, are not dependent on the high level of a single amino acid. The results given in tables 13 and 14 indicate that the amino acid mixtures used have essentially the same effects. In the last experiment six glucogenic amino acids, at a level of one per cent each, caused a greater growth depression than three glucogenic amino acids or than had been noted earlier when four per cent of any one amino acid was added to the same diet. The ketogenic amino acid mixtures also depressed the growth rates of the chicks upon addition to diets with or without added niacin. This action is similar to that which was noted when four per cent of a single ketogenic amino acid is added to the diet. The fact that the ketogenic and glucogenic amino acid mixtures have the same actions as the individual amino acids is the best evidence that the amino acids of each group have some common additive property which influences metabolism in the chick. It is believed that there is a variation in the amount of these properties which each glucogenic

and each ketogenic amino acid possess. It was suggested that one common action of the glucogenic amino acids was an insulin-like action. Since injection of insulin did not have the same effect on chicks fed the niacin-low diet as feeding the glucogenic amino acids, it is doubtful that the growth depression observed when these amino acids are fed, is due to an insulin-like action.

The fact that addition of ketogenic amino acids to the diet increased blood sugar level suggested that their addition, to the niacin-low diets with excesses of glucogenic amino acids, may have a beneficial effect. However, when this was tried, no beneficial effect on growth rate was noted. Evidently these opposing effects on the blood sugar level which were observed are not a result of opposing effects which these two groups of amino acids have on a specific reaction.

While the addition of ketogenic amino acids does not overcome the growth-depressing action of glucogenic amino acids in niacin-low diets, it is apparent from the results given in table 14, that a mixture of glucogenic and ketogenic amino acids do not depress growth as much as an equal level of either group alone. There is additional support for this fact in table 13. The mixture of glucogenic amino acids, in the same ratio that they are found in gelatin and fed at a level of five per cent, depressed growth more than five per cent gelatin. Thus, it is apparent that the degree by which growth of chicks is depressed when certain proteins are added to niacin-low diets, depends not only on their relative lack of tryptophan, but also upon the balance between the glucogenic

and ketogenic amino acids. Briggs et al. ('46) found that gelatin was about three times as active as zein in depressing the growth rate of young chicks when added to a niacin-low diet. By using the amino acid composition of gelatin and zein, as given by Block and Bolling ('45), it is found that the glucogenic amino acids constitute 85.1 per cent of gelatin, and 60.3 per cent of zein. In calculating these values, the leucine content was included in the ketogenic total. Thus, it seems probable that one reason that gelatin depresses growth of chicks more than zein is because of its higher content of glucogenic amino acids.

In both experiments, in which mixtures of ketogenic amino acids were fed, growth of the chicks was depressed even if sufficient niacin was added to the diet. The most significant results are given in table 13, group 18. The mixture contained six ketogenic amino acids in approximately the same ratio that they are found in casein. Fifteen per cent casein would have added the same amount of these amino acids to the diet. In an earlier experiment, the addition of either 12 or 22 per cent casein to a similar diet increased the growth rate. Therefore it is apparent that for maximum growth, chickens not only require the proper level of each essential amino acid, but also a balance between the glucogenic and ketogenic amino acids. It is clear from this work that an oversupply of ketogenic amino acids depresses the growth rate of chicks. An oversupply of the glucogenic amino acids does not appear to affect growth as much unless the diet is deficient in niacin.

Of the ten amino acids which are considered absolutely essential for chicks, seven are ketogenic. The tentative requirements for these seven ketogenic amino acids total 6.35 per cent; the requirements for the three glucogenic amino acids total 1.95 per cent. If glycine, cystine, and tyrosine are added to this list, the totals are 5.95 per cent for the ketogenic amino acids, and 3.35 for the glucogenic amino acids. Most of the non-essential amino acids are glucogenic. It would be expected that if a diet was made which contained only the essential amino acids in this ratio, there would be an excess of the ketogenic amino acids. Luckey, Moore, Elvehjem and Hart (1947) have fed a diet in which the protein was supplied by the ten essential amino acids and glycine. Twenty per cent of the diet was ketogenic amino acids and nine per cent of the diet was glucogenic amino acids. Two other amino acid mixtures were fed which contained some of the non-essential amino acids. The ketogenic amino acids constituted less than one-half of the protein of these two diets. None of the chicks fed the amino acid mixtures gained weight as fast as chicks fed a diet in which casein, arginine, glycine, and cystine provided the protein. The chicks fed the diet with only the essential amino acids did not gain as much as the chicks fed diets which contained the non-essential amino acids also. It seems probable that one reason why the chicks fed non-essential amino acids gained more, was because there was a better balance between the glucogenic and ketogenic amino acids.

There are indications that this balance between the glucogenic and ketogenic amino acids is important in rats and mice. The essential amino acids required by rats and mice are also mostly ketogenic. Maddy and Elvehjem ('49) have fed 50 different combinations of amino acids to mice. Some of these were rations which contained only essential amino acids. They found that a ration with 5.8 per cent essential amino acids produced a greater rate of gain than a diet with 8.7 per cent essential amino acids. Glycine and glutamic acid increased growth rates when added to a diet containing 5.8 per cent of the essential amino acids. The growth rate of the mice increased as the level of glutamic acid in the diet was increased up to 11.6 per cent.

Ramasarma, Henderson, and Elvehjem ('49) fed rats diets containing only essential amino acids and glutamic acid. A decrease in growth rate of the rats was noted when the levels of essential amino acids were increased from 5.8 per cent to 7.7 or 11.6 per cent with corresponding decreases in glutamic acid content of the diet.

The importance of amino acid balance in chick diets is shown in the lysine experiments, the results of which are given in table 16. The growth depression noted when lysine was added to the diet was somewhat overcome by the addition of gelatin, corn, or zein to the diet. Gelatin contains lysine but also contains a comparatively high level of arginine, which is low in the five per cent gelatin diet. Zein has no lysine, and the protein of corn is low in lysine. Thus it appears that the increased growth rate, noted when these proteins were

included in the diet, is due to the fact that the level of other amino acids are built-up around the high lysine level which provides a better amino acid balance. This work is related to the report by Grau (1948) who found that increasing the dietary protein level increased the lysine requirement. The growth depression produced by the addition of tyrosine to the diet was also increased by the additions of other amino acids to the diet.

Addition of arginine increased slightly the rate of gain of the chicks fed three per cent methionine. Evidently it is more difficult to overcome the growth depressions caused by feeding high levels of methionine. In experiment 40 arginine or gelatin had very little effect on the growth depression noted when three per cent methionine was added to the diet.

SUMMARY

Chicks were fed 17 different amino acids, individually, at a level of four per cent in a diet which contained 18 per cent casein, 5 per cent gelatin, and 0.3 per cent DL-methionine. Arginine, leucine, glycine, aspartic acid, glutamic acid, and threonine increased the rate of chick growth when added to this diet. Proline and alanine caused a slight decrease in growth rate. The following amino acids decreased the growth rate: valine, cystine, isoleucine, tyrosine, histidine, phenylalanine, tryptophan, lysine, and methionine. The amino acids are listed in descending order of the growth which resulted when each was added to a chick diet at a level of four per cent. It is apparent that the ketogenic amino acids, except leucine, depress growth to a greater extent than most of the glucogenic amino acids. When niacin was omitted from the diet, the addition of any amino acid depressed growth. When pyridoxin was omitted from the diet only a mixture of allothreonine and threonine caused a growth depression which could be overcome by the addition of pyridoxin to the diet. Thus it seems that niacin must have an important role in metabolism in the chick when the diet contains high levels of glucogenic amino acids. It was first thought that this role was in the conversion of the excess glucogenic amino acids to compounds which are normally produced in carbohydrate metabolism. However, if pyridoxin has a role in transamination, it should also be needed to aid in the disposal of the excess amino acids. However, when the chick diets contained a high level of glucogenic amino acids,

omitting niacin caused a greater growth depression than decreasing the pyridoxin level. Two other observations indicate that the beneficial effect of niacin is not entirely a result of an increase in the ability of the chick to maintain a normal amino acid balance in the tissues. First, chicks fed tryptophan or arginine had more of the amino acid in the blood than chicks which were not fed the amino acid, even though, there was adequate niacin and pyridoxin in the diet. Second, pyruvic acid was found to have a similar action to that which was noted when alanine was added to the diet.

Blood amino nitrogen, nonprotein nitrogen, and glucose levels were determined in the chicks of several experiments. The nonprotein nitrogen level was increased by the addition of most amino acids to the diet. The amino nitrogen level also was increased by the addition of each amino acid except arginine, lysine, and methionine. The decrease noted when methionine and lysine were fed is believed to be due to low feed consumption by the chicks. Arginine is contained in the basal diet in a level which is below that needed for maximum growth. It is believed that feeding arginine decreased the blood amino nitrogen level by causing a more efficient utilization of the protein. Lower blood amino nitrogen levels were found in chicks fed diets containing niacin than were found in chicks fed the same diet without niacin.

The fasting blood sugar level was increased by the addition of any amino acid studied to a diet containing niacin. The increased fasting blood sugar level has been noted before

in rats after feeding high levels of casein or glycine, and has been termed "protein effect". If niacin was omitted from the basal diet, or a diet to which a high level of a glucogenic amino acid had been added, the fasting blood sugar level was decreased. It is suggested that feeding one of the glucogenic amino acids stimulates gluconeogenesis by causing the adrenal cortex to secrete more of the hormones which stimulate this process. Since the fasting blood sugar level is decreased when niacin is omitted from the chick diets containing a high level of a glucogenic amino acid, it is believed that the secretion of hormones from the adrenal cortex which stimulate gluconeogenesis is hindered by the niacin deficiency. Secretions of other hormones by the adrenal cortex may also be hindered by the deficiency.

The addition of the ketogenic amino acids to the diet depressed growth rate even when the diet contained adequate niacin. When chickens which had been fed a high level of methionine, lysine, phenylalanine, or a mixture of six ketogenic amino acids were subjected to a glucose tolerance test, they were unable to return their blood sugar level toward normal as fast as chicks which had been fed the same diet without added amino acids, or with glycine, leucine, or arginine added. It is believed that feeding high levels of most ketogenic amino acids results in excessive conversion of glucogenic amino acids to glucose. The feed consumption of the chicks fed the ketogenic amino acids is low.

Effects similar to those noted when a single ketogenic amino acid was added to the chick diet, were observed when a mixture of ketogenic amino acids was added to the diet. Glucogenic amino acid mixtures also had an action similar to single glucogenic amino acids. Thus it is apparent that the ketogenic amino acids as a group, and the glucogenic amino acids as a group, each have a common additive influence on the chicken.

It was observed that the growth depression produced, when amino acid mixtures were added to niacin-low chick diets, is not as great if the mixture contains both glucogenic and ketogenic amino acids as it is if the mixture contains only glucogenic or ketogenic amino acids. The ketogenic amino acid mixtures depress growth even when the diet contains adequate niacin. The chick seems to be very sensitive to dietary excesses of ketogenic amino acids. Apparently for maximum growth rate they require not only an adequate level of each essential amino acid, but also a balance between the glucogenic and ketogenic amino acids.

It was found that the growth depressing action of lysine and tyrosine could be somewhat overcome by the addition of proteins which would build up the level of other amino acids around the high lysine or tyrosine level. This is further evidence that the amount of any of the essential amino acids needed for maximum growth rate of the chick, depends on the levels of other amino acids in the diet.

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APPENDIX

Effect of adding four per cent glycine to chick diets
containing various levels of pyridoxin

GROUP NO.	DIETARY PYRIDOXIN	DIET 113		DIET 113 $\frac{1}{4}$ PER CENT GLYCINE	
		NO. CHICKS	AVE. WT. 4 WKS.	NO. CHICKS	AVE. WT. 4 WKS.
	micrograms %		grams		grams
1	0	0	-	1	47
2	50	9	142	6	103
3	75	7	209	5	180
4	100	11	228	10	228
5	150	11	306	10	266
6	300	11	310	11	305
7	600	11	292	11	338
		Diet 113-5% gelatin		Diet 113-5% gelatin $\frac{1}{4}$ 4% glycine	
8	0	0	-	0	-
9	50	9	108	5	109
10	75	9	175	8	164
11	100	9	202	9	229
12	150	8	192	10	270
13	300	10	209	9	290
14	600	10	213	9	293

*Number of chicks at the termination of the experiment is given. At the start of the experiments each of the first seven groups contained 11 chicks; each of the last seven groups contained 10 chicks.