RELATION OF CORN AND TRYPTOPHANE-LOW PROTEINS TO THE DIETARY REQUIREMENT FOR NICOTINIC ACID IN CHICKENS

By

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INTRODUCTION

The year 1937 climaxed the classical work of Goldberger and associates in their search for the human pellagra-preventive factor by the announcement of Elvehjem and co-workers from the Wisconsin laboratory that nicotinic acid would cure canine blacktongue. Soon thereafter investigators in the south showed that nicotinic acid was active in curing human pellagra, thus firmly establishing its vitamin nature. Since that time an ever increasing volume of research work has indicated the growing complexity of nicotinic acid metabolism and that the requirement of numerous animals for nicotinic acid is related to certain dietary contingencies. Among the dietary factors found to increase an animal's requirement for nicotinic acid are corn (rat, chick, dog, swine, man) and tryptophane-low proteins such as gelatin, zein, acid hydrolyzed casein (rat, chick, dog). Ample supplementation of tryptophane to diets deficient in nicotinic acid has been shown to negate the need for dietary nicotinic acid (rat, dog, chick, swine). In this connection proteins containing large amounts of tryptophane such as casein and fibrin behaved similarly to the action of tryptophane. More recently a "pellagragenic factor" has been isolated from corn. This factor acts as an anti-vitamin of nicotinic acid thereby causing a deficiency state in mice.

Because such a variety of dietary factors have been shown to influence the nicotinic acid requirement of several species of animals it seemed desirable to investigate some of these apparent dietary misbehaviors as they affect the requirement for nicotinic acid in chickens. Obviously, corn commanded immediate attention for investigation because it is the most widely used single grain in poultry feeding - estimated to be one-third of the total feed consumed by poultry (Heuser 1946). Information obtained regarding the mechanism by which corn, under certain dietary conditions, increases the nicotinic acid requirement of growing chicks would be of extreme practical value as well as fundamental interest. Knowledge of the same nature concerning the action of certain tryptophane-low proteins, such as gelatin and zein, would also be valuable in order to appreciate more thoroughly the metabolism of nicotinic acid in chickens. Accordingly, this investigation was undertaken to determine: (1) Why the feeding of corn increases the nicotinic acid requirement of chicks. (2) Why the feeding of gelatin and zein increases the nicotinic acid requirement of chicks and whether the actions of corn, gelatin, and zein are related. (3) Whether hens require a dietary source of nicotinic acid.

It should be pointed out, however, that early in the course of experiments on the feeding of corn to chicks it was found that corn contained an unidentified factor which was required by the chick, in addition to all the known nutritional essentials - including nicotinic acid, for maximum growth. Another study was undertaken to identify this growth factor. Therefore, to facilitate description and preserve clarity this thesis is presented in two parts. Part I contains the results of nicotinic acid experiments with chicks and laying hens. Part II contains the results of studies with chicks on the identification of the unknown growth factor present in corn.

LITERATURE REVIEW

Elvehjem, Madden, Strong, and Woolley (1938) isolated nicotinic acid amide from a liver concentrate and reported that it was active in curing canine blacktongue, a deficiency disease analogous to pellagra in humans. Nicotinic acid was equally effective in this respect. Numerous clinical reports followed showing that both nicotinic acid and the amide cured human pellagra (Spies et al. 1939).

Previous to the finding of Elvehjem and coworkers (1938), Warburg and Christian (1934) isolated nicotinamide from coenzyme II and demonstrated its' function as part of a hydrogen-transporting coenzyme (Warburg and Christian (1935)). Shortly thereafter, Evler, Albers, and Schlenk (1936) obtained nicotinamide from coenzyme I, and it was shown that both coenzymes were nicotinamide - adenine - dinucleotides, but that coenzyme II contained three molecules of phosphoric acid while cozymase or coenzyme I contained two. Thus it was established that a vitamin was a component part of an important respiratory enzyme system.

That young chickens require a dietary source of nicotinic acid was first demonstrated by Briggs, Mills, Elvehjem, and Hart (1942). These workers fed White Leghorn chicks a purified diet containing dextrin 61, casein 18, gelatin 10, soybean oil 5, minerals and vitamins except nicotinic acid. This diet caused a marked depression in growth rate and the appearance of a deficiency condition similar to canine blacktongue. The chick blacktongue was characterized by an extremely inflamed condition of the entire mouth cavity and base of the tongue. Normal growth and health was obtained by supplementing the diet with 1.8 milligrams of nicotinic acid per 100 grams.

Previous to this finding Dann and Handler (1941) and Snell and Quarles (1941) had presented evidence that the chick embryo synthesized considerable amounts of nicotinic acid during the incubation period. They inferred from their results that the hatched chick did not require a dietary source of nicotinic acid.

Briggs, Luckey, Teply, Elvehjem, and Hart (1943) extended the findings of Briggs et al. (1942). They observed additional deficiency symptoms in chicks; namely, poor feathering, perosis, diarrhea, and low feed consumption. Moreover, they showed that the breast muscle of deficient chicks contained less nicotinic acid and coenzyme I than the breast muscle of the controls.

Briggs (1946) studied a nicotinic acid deficiency in turkey poults employing a highly purified diet similar to that used by Briggs et al. (1942). He reported that the young turkey requires from 3 to 5 milligrams of nicotinic acid per 100 grams for the prevention of deficiency symptoms such as inflammation of the mouth, diarrhea, low feed consumption, poor efficiency of feed utilization, poor feathering and perosis. However, his data indicated that higher levels of nicotinic acid were needed for maximum growth. Jukes, Stokstad, and Belt (1947) confirmed the findings of Briggs (1946). These workers found that 5 milligrams of nicotinic acid per 100 grams would allow maximum growth and prevent the occurrence of perosis in turkey poults. Their diet was also highly purified and consisted of starch 55.5, casein 20, gelatin 8, corn oil, mineral mixture and vitamins except nicotinic acid.

Hegsted (1946) reported that young ducklings rapidly develop a nicotinic acid deficiency on a highly purified diet (similar to diet of Briggs et al. 1942). In contrast to the severity of the deficiency in the chick, the only symptoms observed in the duckling were poor growth, diarrhea, and general weakness. Approximately 2.5 milligrams of nicotinic acid per 100 grams were required for normal health and rapid growth.

Chick, Macrae, Martin, and Martin (1938) were the first to demonstrate the need for nicotinic acid in the nutrition of the pig. The diet used by these workers contained a large amount of corn (77.5 percent) and was poor in protein quality. The deficiency symptoms in pigs receiving this diet were poor growth, rough coat, slight inflammation of the gums, and necrotic enteritis. Recoveries were obtained by giving large doses of nicotinic acid. These findings were later confirmed by Braude, Kon, and White (1946) using the very same diet. They reported that on such a diet the pig requires about 5 to 10 milligrams of nicotinic acid per day for normal growth and health.

Wintrobe, Stein, Folis, and Humphreys (1945) fed young pigs a high protein (26.1 percent casein) purified diet containing all of the known vitamins in crystalline form and observed normal growth. The omission of nicotinic acid from this diet still allowed normal growth, much to their surprise. However, by lowering the protein content of this diet (10 percent casein) and omitting nicotinic acid, typical deficiency symptoms appeared (greatly impaired growth, rough coats, diarrhea, and poor appetite). It was concluded that a close relationship existed between protein and nicotinic acid in the nutrition of the pig.

For many years dogs have been used as an experimental animal in the study of pellagra. Goldberger and Wheeler (1928) described a condition in dogs which closely resembled pellagra in humans. The disease was characterised by a loss of weight, reddening of the gums, and swelling of the tongue (blacktongue), salivation, diarrhea, poor coat, and dermatitis of the scrotum.

The symptoms were produced by feeding a diet high in cornmeal (74 percent) and low in protein (casein 9, pea meal 8). Goldberger, Wheeler, Lillie, and Rogers (1928) later reported that the addition of 1.8 percent of yeast to the blacktongue producing diet completely protected the dog from the deficiency disease. Since the early work of Goldberger, numerous investigators have used various modifications of the Goldberger diet in studies of blacktongue in the dog. Elvehjem et al. (1938) showed that the deficiency in dogs caused by feeding a modified Goldberger diet was a nicotinic acid deficiency.

Schaefer, McKibbin, and Elvehjem (1942) were the first to produce a nicotinic acid deficiency in the dog by feeding an entirely synthetic diet almost completely lacking in nicotinic acid. It was composed of sucrose 66, casein 19, cottonseed oil 8, cod liver oil 3, salt mixture 4, and thiamine, riboflavin, pyridoxine, Ca pantothenate, and choline. They reported weight losses and typical blacktongue in dogs receiving this diet. Handler (1943) employed the same diet and reported that although some weight loss and blacktongue occurred in his dogs the severity of the deficiency was not nearly so great as that produced by feeding a modified Goldberger diet. Because of this difference Handler suggested that the presence of cornmeal per se may be an etiological factor in pellagra. It is interesting to note in this connection that Chick (1933) cognizant of the apparent close relationship between pellagra in humans and the ingestion of large amounts of corn, proposed the theory that: "Pellagra is caused by a toxic substance derived from the maize diet, which can be corrected by sufficient 'good' protein, or perhaps by sufficient vitamin B_2."

Birch (1939) fed rats a purified diet composed of casein 20, lard 20, sucrose 55, salts 4, and Cod liver oil 1. This regime was low in several

B vitamins, including nicotinic acid, but the rats grew very well. No increase in growth rate was observed upon the addition of nicotinic acid. Birch concluded that nicotinic acid is not an essential dietary substance for the rat. Dann (1941) fed rats a highly purified diet low in nicotinic acid but adequate in all the other known nutritional essentials. It was calculated that the animals ingested 5 micrograms of nicotinic acid daily whereas an analysis of their tissues indicated that the vitamin increased daily by an average amount of the order of 200 micrograms. This evidence supported the finding of Birch (1939) that the rat did not need a dietary source of nicotinic acid. Krehl, Teply, and Elvehjem (1945a) fed rats a highly purified diet essentially free of nicotinic acid but adequate in the other known vitamins (with the exception of folic acid). A gain in weight of 30 grams in 4 weeks was made on this diet. However, when 40 percent of the entire diet was replaced by yellow corn or corn grits a marked growth depression was observed (13 grams gain in 4 weeks). The addition of 0.5 to 1.0 milligrams of nicotinic acid completely counteracted the growth depressing action of corn. Addition of folic acid in place of nicotinic acid had no effect. When the casein level was raised from 15 to 20 percent some of the growth inhibitory effect of corn was overcome.

In a similar study with dogs Krehl, Teply, and Elvehjem (1945b) reported that the inclusion of 60 percent corn grits, at the expense of sucrose, in a synthetic diet caused marked loss of weight and death of the animals. Without corn the diet allowed normal growth and health in dogs. The addition of 5 milligrams of nicotinic acid per 100 grams of diet counteracted the deleterious action of corn. Whereas corn increased the nicotinic acid requirement of dogs, milk, which contains much less nicotinic acid, tended to decrease the requirement. The authors explained the action of milk as being probably due to the establishment of an intestinal flora which favored the synthesis of nicotinic acid. On the other hand, the action of corn was explained as being due to the suppression of a favorable intestinal flora, or it was suggested that corn might contain substances which combine with nicotinic acid thereby making the vitamin unavailable to the animal.

Sarma and Elvehjem (1946) working with chicks showed that the inclusion of corn in a highly purified diet low in nicotinic acid depressed growth. This effect of corn was completely counteracted by the addition of nicotinic acid.

In a very dramatic study Krehl, Teply, Sarma, and Elvehjem (1945) reported that the growth retarding action of corn on rats receiving nicotinic acid-low diets was counteracted by tryptophane as well as by nicotinic acid. This discovery immediately explained the beneficial action of good quality proteins, such as casein, milk, and meat in the diet of pellagrins (Goldberger, and Wheeler (1915), Goldberger, Waring, and Willets (1915)). It also showed that a very close relationship existed between an amino acid and a vitamin in an animal's metabolism.

The importance of intestinal synthesis of nicotinic acid in the rat was further emphasized by Krehl, Sarma, Teply, and Elvehjem (1946). They reported that the kind of carbohydrate in the diet and the level of tryptophane influenced the extent of the undesirable effect of corn. Glucose, dextrin and lactose were beneficial in their action. Non-corn diets which were low in tryptophane and nicotinic acid gave poor growth when sucrose was used as the carbohydrate. In the latter diets normal growth was obtained by adding tryptophane or nicotinic acid, or by using a carbohydrate which produced a favorable intestinal flora. These workers pointed out further that though polished rice, rolled oats, and rye all contain significantly less nicotinic acid than yellow corn, yet they did not produce growth depression.

Woolley (1945) fed oral doses of 3-acetylpyridine daily (analogue of nicotinic acid) to mice and produced a marked depression in growth and some reddening of the tongue. The effect of this substance was counteracted upon the addition of nicotinic acid, or tryptophane (Woolley (1946a)) to the diet. It was explained that 3-acetylpyridine produced its effect by competing with nicotinic acid in certain enzyme systems in the body. Woolley suggested that corn may contain a similar toxic material which is responsible for the growth depressing action of this grain. This point was investigated and a concentrate was obtained from corn which when fed to mice acted in a similar manner as 3-acetylpyridine (Woolley (1946b)). Its action was counteracted with nicotinic acid. The potent "pellagragenic" agent was soluble in a mixture of chloroform and sodium hydroxide. The active material has not yet been chemically characterized.

Krehl, Sarma, and Elvehjem (1946) extended their previous findings on the action of corn on the growth of rats. They showed that by adding certain tryptophane-low proteins such as zein, gelatin, and acid hydrolyzed casein to diets low in nicotinic acid a growth depression resulted which was similar to that caused by the feeding of corn. Moreover, they found that the growth inhibiting of corn was related to the nature of its protein. In addition it was shown that when the casein of the basal diet was replaced by fibrin, egg albumin, or soybean globulin the deleterious action of corn in the diet was prevented. This was explained as being due to the higher tryptophane content of these proteins.

Working with chicks Briggs (1945) showed that the presence of gelatin in a highly purified diet low in nicotinic acid caused growth depression and the occurrence of blacktongue. The addition of 0.2 percent dl-tryptophane or 5 milligrams of nicotinic acid per 100 grams of diet counteracted the inhibitory action of gelatin.

In studies with swine Luecke, McMillen, Thorp and Tull (1947) reported that the feeding of a high protein diet (19 percent) composed of corn, casein, soybean oil meal and a mineral mixture produced only mild symptoms of a nicotinic acid deficiency and these were confined entirely to inflammation of the colon. No symptoms were observed when corn was replaced with oats. On the other hand, the lowering of the protein content (14 percent) of the corn diet caused severe inflammation of the colon, loss of weight, and diarrhea. Supplementing this diet with 200 milligrams of dl-tryptophane per pig per day gave excellent growth and normal health.

Krehl, de la Huerga, and Elvehjem (1946) reported that nicotinic acid improved the utilization of tryptophane in rats receiving purified diets containing corn grits. In balance studies it was found that rats which did not receive nicotinic acid only used 30 percent of the total tryptophane ingested for the synthesis of body protein while rats which received nicotinic acid utilized 70 percent.

The recent work of Rosen, Huff, and Perlzweig (1946), Singal, Briggs, Sydenstricker, and Littlejohn (1946) with rats, and Sarett and Goldsmith (1947) with humans has shown that the feeding of tryptophane leads to the excretion of very high amounts of N^1-methylnicotinamide in the urine. These workers have indicated that tryptophane may be an important precursor of nicotinic acid (in the rat and man) and may explain the antipellagragenic activity of certain foods such as milk which are low in nicotinic acid, but rich in good protein.

Krehl, Henderson, de la Huerga, and Elvehjem (1946) investigated the possibility that the growth depressing action of tryptophane-low proteins such as zein may be caused by an amino acid imbalance. Only a few amino acids were fed in their experiments, but the results they obtained led them to conclude that the total effect produced by feeding tryptophane-low proteins is due to an amino acid imbalance. Of the single amino acids tested, glycine appeared to have the greatest growth depressing action. They further stated that the inhibitory action of corn seemed to be totally related to its predominant protein, zein.

In the light of our newer concepts of nutrition and the analytical data at hand on the nicotinic acid content of various foodstuffs, Frasier and Friedemann (1946) have re-evaluated the various diets used by Goldberger and associates in their studies of human pellagra. They calculated that the minimum daily intake of nicotinic acid in a marginal diet containing corn products was 7.5 milligrams per day. On a diet containing corn, but with small quantities of milk or milk products, or on a diet without corn, the minimum requirement was calculated to be about 4 milligrams per day. It was emphasized, however, that a liberal diet with generous amounts of green vegetables and good proteins (milk, meat and eggs) appeared to be the best safeguard against pellagra in humans.

PROCEDURE

Over three thousand chicks were used in these investigations, but only the most important experiments were selected for presentation in this thesis. In all of the chick experiments, day-old New Hampshires of mixed sexes were used. They were progeny of the University of Maryland farm flock. Their dams had received a good practical breeder mash. At the beginning of each experiment the chicks were divided into uniform groups, composed usually of six chicks each, 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 and the experiments were conducted for a period of 4 weeks. When each experiment was terminated, total feed consumption was determined for calculating feed efficiency thus: $\frac{\text{Total weight gained}}{\text{Total feed consumed}} =$ Efficiency of feed utilization.

The basal diets employed in the experiments with chicks were composed of highly purified ingredients and are given in Table I. Diets 108GN, 113GN, and 114GN were used interchangeably in nicotinic acid studies. These diets were all low in nicotinic acid and were deficient in the amino acids glycine and arginine. Diets 108GN and 114GN differed only in their sulfur amino acid supplement; 0.3 percent l-cystine was used in the former whereas 0.3 percent dl-methionine was used in the latter. Diet 113GN was similar to diet 114GN except that liver fraction "L" (Wilson), used to supply the chicks' requirement for folic acid, was replaced by synthetic folic acid (Lederle). When the requirements for glycine, arginine, and nicotinic acid were met, all three diets produced normal growth and health in chicks.

TABLE I

BASAL DIETS EMPLOYED IN EXPERIMENTS WITH CHICKS

DIETARY INGREDIENT	CHICK BASAL DIETS			
	108GN	113GN	114GN	113
Cerelose	68.4	71.4	68.4	61.4
Casein (crude)	18.0	18.0	18.0	18.0
Gelatin	-	-	-	10.0
Salts IM *	6.0	6.0	6.0	6.0
Soybean oil	4.0	4.0	4.0	4.0
Liver fraction "L" (Wilson)	3.0	-	3.0	-
dl-Methionine	-	0.3	0.3	0.3
l-Cystine	0.3	-	-	-
Choline chloride **	0.2	0.2	0.2	0.2
i-Inositol **	0.1	0.1	0.1	0.1
	100.0	100.0	100.0	100.0
(Vitamins - MG/100 gms diet)				
Nicotinic acid	-	-	-	10.0
Thiamine HCl	0.4	0.4	0.4	0.4
Riboflavin	0.8	0.8	0.8	0.8
Ca pantothenate	2.0	2.0	2.0	2.0
Pyridoxine HCl	0.6	0.6	0.6	0.6
Biotin	0.02	0.02	0.02	0.02
P-Amino benzoic acid	0.2	0.2	0.2	0.2
Vitamin K	0.1	0.1	0.1	0.1
Vitamin E	0.5	0.5	0.5	0.5
Folic acid	-	0.2	-	0.2
Vitamins A and D_3	1200 U.S.P. and 170 A.O.A.C. units respectively by dropper weekly			

*Salts IM are composed of the following ingredients by weight:

$CaCO_3$	150.0	$MgSO_4 7H_2O$	50.0	$CuSO_4 5H_2O$	0.2
K_2HPO_4	90.0	$Fe(C_6H_5O_7)_2\ 6H_2O$	14.0	H_3BO_3	0.09
Na_2HPO_4	73.0	$MnSO_4 4H_2O$	4.1	$CoSO_4 7H_2O$	0.01
$Ca_3(PO_4)_2$	130.0	KI	0.4		500.00
NaCl	88.0	$ZnCl_2$	0.2		

When fed at a level of 6% the mixture supplies 1.11 gms of calcium, 0.58 gm of phosphorus, and 0.01 gm of manganese per 100 gms of diet.

**These compounds are essential vitamins, but since they are required in amounts higher than the usual vitamin levels they are included as a bulk component of the diet.

On the basis of nicotinic acid values reported for crude casein by Cannon, Boutwell, and Elvehjem (1945) and for liver fraction "L" by Hegsted (1946), the nicotinic acid content of diets 108GN, 114GN, and 113GN was calculated to be 0.64, 0.54, and 0.06 milligrams per 100 grams respectively. According to the values given by Block (1945) the 18 percent of casein contained in these diets would supply 0.25 grams of tryptophane, which is the chick's requirement per 100 grams of feed as established by Grau and Almquist (1944).

Diet 113 (Table I) is a highly purified diet and contains all the known essential nutrients, including nicotinic acid, required by the chick for normal growth. It was used as the basal diet for the identification of an unknown growth factor contained in corn.

Substitutions in all the above mentioned diets were made at the expense of the carbohydrate. Details of the various supplements which were made to the diets will be presented later under "Results".

In a majority of the experiments with chicks observations were made on the condition of feathering at 4 weeks of age. Table II gives the system employed in determining the feather score.

TABLE II

SYSTEM USED IN FEATHER SCORING

ADJECTIVE SCORE	NUMERICAL SCORE
Very poor	0
Very poor plus	25
Poor	50
Poor plus	60
Fair	70
Good	80
Very good	90
Excellent	100

The studies with laying hens (New Hampshire pullets) were conducted in conventional-type steel laying batteries. Artificial inseminations were performed twice weekly with the pooled semen of several New Hampshire roosters. Eggs were collected daily and stored in a refrigerator at 50°F. until they were set. Weekly settings of eggs were made and hatchability recorded. On the twenty-first day, all of the eggs that did not hatch were broken out and time of embryo mortality was determined.

Two experiments were conducted with laying hens. The first experiment was composed of two groups of hens, four hens per group. It was of thirteen weeks duration. The second experiment was composed of six groups of hens, four hens per group. Four groups of these hens were removed from experiment after twelve weeks, while the remaining two groups were continued until nineteen weeks.

In Table III the diets which were used in the nicotinic acid experiments with hens are given. Diet 201N, highly purified, was employed in the first experiment. It was designed to be low in nicotinic acid and high in a source of protein low in tryptophane. In the chick experiments it was found that bone ossein was as efficacious as gelatin in producing a nicotinic acid deficiency and had the advantage of not sticking to the beaks of birds when used at high levels. Therefore, a high level of bone ossein was utilized in diet 201N instead of gelatin.

Diets 202N, 203N, and 204N were used in the second experiment with hens. Diet 202N was designed to determine the effects of high levels of corn and corn gluten meal as component parts of a nicotinic acid low purified diet on the production of a nicotinic acid deficiency in hens. This diet was further varied by omitting the casein, or by adding high levels of glycine.

TABLE III

DIETS EMPLOYED IN EXPERIMENTS WITH HENS

DIETARY INGREDIENT	HEN DIETS			
	201N	202N	203N	204N
Corn starch (Globe Pearl 144)	52.55	-	62.55	53.55
Corn gluten meal (44% protein)	-	50.00	-	-
Ground yellow corn	-	28.55	-	-
Casein (crude)	15.00	4.00	15.00	9.00
Gelatin	-	5.00	10.00	25.00*
Bone casein	20.00	-	-	-
Salts- IM	6.00	6.00	6.00	6.00
Soybean oil	3.00	3.00	3.00	3.00
Liver fraction "L" (Wilson)	3.00	3.00	3.00	3.00
dl-Methionine	0.15	0.15	0.15	0.15
Choline HCl	0.20	0.20	0.20	0.20
i-Inositol	0.10	0.10	0.10	0.10
	100.00	100.00	100.00	100.00
(Vitamins - mgs/100 gms diet)				
Thiamine HCl	0.40	0.40	0.40	0.40
Riboflavin	0.80	0.80	0.80	0.80
Ca pantothenate	2.00	2.00	2.00	2.00
Pyridoxine HCl	0.60	0.60	0.60	0.60
Biotin	0.02	0.02	0.02	0.02
p-Amino benzoic acid	0.20	0.20	0.20	0.20
Vitamin K	0.10	0.10	0.10	0.10
Vitamin E	0.50	0.50	0.50	0.50
Vitamins A and D_3	8400 U.S.P. and 1190 A.O.A.C. units weekly by dropper			

* Flaked gelatin.

Diet 203N was similar to diet 201N except that all of the bone casein was replaced with 10 percent of gelatin. This diet was used to determine the effect of high levels of glycine in a purified diet on the production of a nicotinic acid deficiency in hens.

Diet 204N was similar to diet 203N. However, it differed by having more gelatin and less casein. The gelatin used in this instance was flaked and when fed at a level of 25 percent of the diet did not cause excessive "gumming" of the beak. Diet 204N was designed to determine the effect of a low level of casein and a high level of gelatin on the production of a nicotinic acid deficiency in hens.

On the basis of nicotinic acid values reported for casein and liver fraction "L" (mentioned previously) and for corn and corn gluten meal (Hale et al. 1940) the nicotinic acid content of diets 201N, 202N, 203N, and 204N were calculated to be 0.53, 2.65, 0.53, and 0.51 milligrams per 100 grams respectively.

In diet 202N the substitutions were made at the expense of yellow corn. In the other three diets substitutions were made at the expense of corn starch. In all instances, feed, water, and ground oyster shell were offered ad libitum.

RESULTS, PART I

A. Nicotinic Acid Experiments with Chicks

In Table IV a summary is given of an initial study involving a number of experiments dealing with the effect of the addition of various levels of gelatin in diet 1080N with and without nicotinic acid. Results obtained with the presence of corn are also given.

As the level of gelatin (which supplies ample amounts of arginine and glycine for the chick when fed at a level of 10 percent) was increased in the diet above the 5 percent level an actual depression in growth rate occurred (Compare groups 1, 3, 5, and 9). Growth depression was accompanied by increased mortality, perosis, and blacktongue. Other symptoms associated with nicotinic acid deficiency, such as poor feathering, accumulation of feed under the tongue ("food canker"), diarrhea, and dehydration were noted in a majority of the chicks (See figure 1). The growth depression was most marked with the highest levels of gelatin fed, but was overcome by the addition of nicotinic acid or tryptophane to the diet. The latter point shows that dietary nicotinic acid is not necessary when ample tryptophane is present in the diet.

It is evident, as would be expected, that sufficient arginine and glycine (supplied by gelatin) must be present before nicotinic acid can give maximum growth (Compare groups 2, 4, and 6). Higher levels of gelatin, above 20 percent, caused the diet to be too gummy when eaten by the chicks.

Bone ossein, from which gelatin is derived, caused a similar depression of growth in the absence of nicotinic acid.

TABLE IV

EFFECT OF GELATIN AND CORN ON GROWTH, BLACK TONGUE, PEROSIS, FEED EFFICIENCY, AND NICOTINIC ACID REQUIREMENT OF CHICKS

GROUP NO.	SUPPLEMENT TO BASAL DIET - 108GN (With no gelatin)	NO. CHICKS	NO. DIED	AVE. WT. 4 WKS. GMS	% WITH BLACK TONGUE	% WITH PEROSIS	FEED EFFICIENCY
1	None	12	1	88	8	25	.350
2	5 mg % Nicotinic acid	12	2	101	0	0	.275
3	5% Gelatin	35	1	247	43	20	.505
4	As 3 + 5 mg % Nicotinic acid	18	0	298	0	0	.521
5	10% Gelatin	72	12	138	69	64	.358
6	As 5 + 5 mg % Nicotinic acid	65	1	328	0	15	.565
7	As 5 + 10 mg % Nicotinic acid	12	1	333	0	8	.512
8	As 5 + 0.2% dl-Tryptophane	12	0	343	0	25	.588
9	20% Gelatin	6	5	75	83	0	.148
10	As 9 + 5 mg % Nicotinic acid	12	1	333	33	50	.561
11	Bone ossein - 10%	6	2	116	83	17	.296
12	As 11 + 5 mg % Nicotinic acid	12	0	311	0	9	.600
	Following groups contain corn in place of cerelose.						
13	10% Gelatin	12	2	130	92	75	.370
14	As 13 + 5 mg % Nicotinic acid	6	0	373	0	33	.647
15	As 13 + 0.2% dl-Tryptophane	12	0	306	0	66	.599

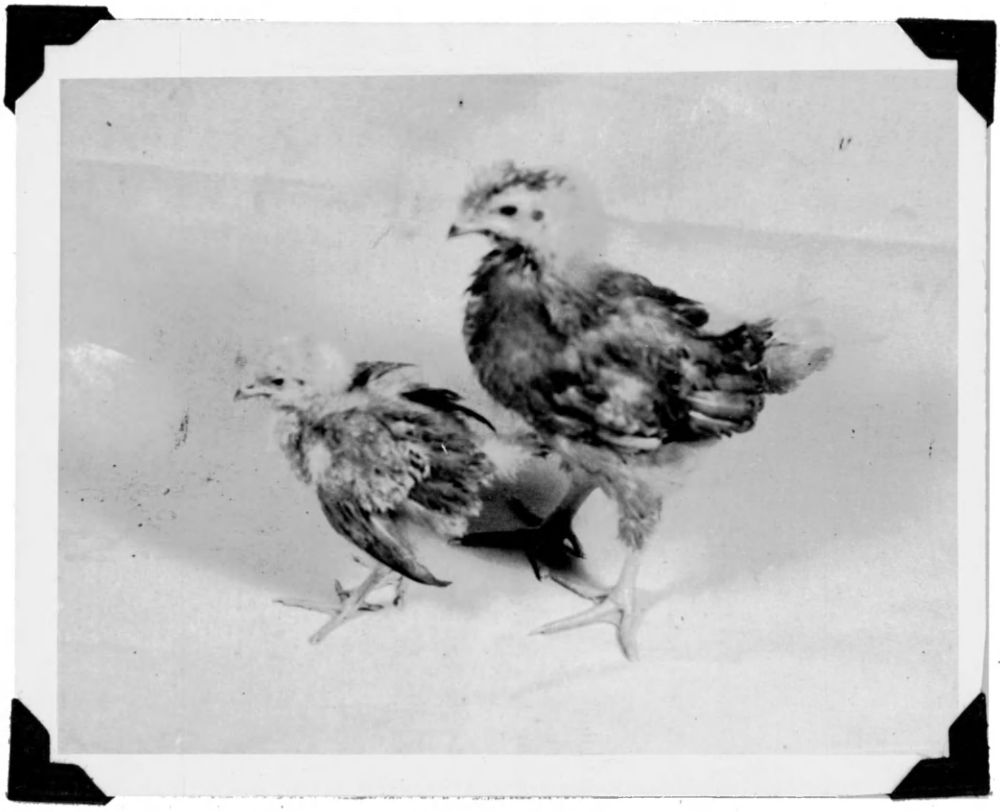

Figure 1. NICOTINIC ACID DEFICIENCY IN THE CHICK

Both chicks are the same age (4 weeks) but the chick on the left did not receive nicotinic acid.

The feeding of large amounts of corn with gelatin gave results similar to those with gelatin alone. Apparently the action of corn and gelatin was not additive with 10 percent of gelatin in the diet. The addition of nicotinic acid to the corn diets resulted in an unusually fast rate of growth for the chicken (appreciably greater growth than when nicotinic acid was added to the cerelose diets), (Compare groups 6 and 14). This suggested that corn contained an unidentified growth-promoting factor or factors not present in the basal diet. Similar results were obtained with corn when added to the basal diet 113GH in the presence of 10 percent gelatin and nicotinic acid. (A later portion of this thesis is devoted to the identification of the growth factor in corn.) Tryptophane appeared to be somewhat less effective when added to the corn diet than when added to the cerelose diet (Compare groups 8 and 15).

Because fairly good growth was obtained by the inclusion of only 5 percent gelatin in the diet, whereas higher levels caused a marked depression of growth, it seemed desirable to determine the critical level of gelatin supplementation where maximum growth could be obtained in the absence of nicotinic acid. This point was investigated employing diet 114GH which is essentially the same as diet 108GH. The results are given in Table V.

It is evident that of the various levels of gelatin used 3 percent gave the greatest growth response in the absence of nicotinic acid. Above this level feed efficiency and feathering became poorer while the incidence of blacktongue, perosis, and mortality increased. However, the maximum growth, efficiency of feed utilization, and feathering that was obtainable with gelatin alone was greatly surpassed by 5 or 10 percent of gelatin in combination with nicotinic acid (Compare group 2 with groups 8 and 9).

TABLE V

DETERMINATION OF CRITICAL LEVEL OF GELATIN SUPPLEMENTATION IN DIET 114GN

GROUP NO.	SUPPLEMENT TO BASAL DIET 114GN (with no gelatin)	NO. CHICKS	NO. DIED	AVE. WT. 4 WKS. GMS	NO. BLACK TONGUE	NO. PEROSIS	FEED EFFICIENCY	FEATHER SCORE
1	1% Gelatin	6	0	155	0	1	.413	28
2	3% Gelatin	6	0	260	0	1	.478	77
3	5% Gelatin	6	0	230	3	2	.459	59
4	6% Gelatin	6	0	214	5	3	.486	48
5	8% Gelatin	6	1	122	6	4	.408	10
6	10% Gelatin	6	1	117	6	2	.340	10
7	As 1 + 5 mg % Nicotinic acid	6	0	162	0	0	.408	27
8	As 3 + 5 mg % Nicotinic acid	6	0	343	0	0	.555	93
9	As 6 + 5 mg % Nicotinic acid	6	0	334	0	0	.570	87

As previously found, no growth response was obtained with nicotinic acid when the diet was deficient in arginine and glycine (Compare groups 1 and 7).

Another experiment of the same nature was performed using diet 113GN. Table VI summarizes the results. In this instance, best performance of gelatin in the absence of nicotinic acid was obtained with 4 percent of gelatin. This level was not fed in the previous trial, but it appears that gelatin exerts a maximum growth response in the vicinity of 3 or 4 percent in the absence of nicotinic acid.

It will be noted that growth was generally poor in this last experiment. This was due to different battery conditions. We have observed better growth in our steel type battery than in our wooden frame type in which this experiment was conducted. The poor growth displayed by group 8 was due to an illness of several days duration believed to have been brought about by a growth of mold occurring in the water fount.

The foregoing experiments clearly indicated that as the level of gelatin was increased, in the absence of nicotinic acid, growth was increased until a level of 3 to 4 percent was reached and beyond this level additional gelatin depressed growth. At this point it was realized that we had a very good method for assaying the growth depressing action of various supplements. The assay method was based upon the depressing effect of the supplements when added to a basal diet containing 5 percent of gelatin.

The results of such an assay are given in Table VII. The addition of high levels of gelatin (groups 2 and 3) and ossein (group 4) to the basal assay diet had severe depressing effects. Casein, soybean oil meal, oats, and wheat (groups 5 to 8) had no depressing effect.

TABLE VI

DETERMINATION OF CRITICAL LEVEL OF GELATIN SUPPLEMENTATION IN DIET 113GN

GROUP NO.	SUPPLEMENT TO BASAL DIET 113GN (with no gelatin)	NO. CHICKS	NO. DIED	AVE. WT. 4 WKS. GMS	NO. BLACK TONGUE	NO. PEROSIS	FEED EFFIC- IENCY	FEATHER SCORE
1	2% Gelatin	6	0	129	0	2	.366	43
2	3% Gelatin	6	0	181	0	0	.442	65
3	4% Gelatin	6	0	198	1	1	.477	69
4	5% Gelatin	6	0	175	2	0	.480	52
5	10% Gelatin	6	2	133	5	2	.290	18
6	As 1 + 10 mg % Nicotinic acid	6	0	112	0	1	.313	27
7	As 2 + 10 mg % Nicotinic acid	6	1	180	0	1	.443	45
8	As 3 + 10 mg % Nicotinic acid	6	1	141	0	1	.348	27
9	As 4 + 10 mg % Nicotinic acid	6	0	235	0	0	.510	78
10	As 5 + 10 mg % Nicotinic acid	6	0	244	0	0	.545	76

TABLE VII

ASSAY FOR GROWTH DEPRESSING ACTION OF VARIOUS FEEDSTUFFS

GROUP NO.	SUPPLEMENT TO DIET 1086N CONTAINING 5% of GELATIN	NO. CHICKS	NO. DIED	AVE. WT. 4 WKS. GMS.	% BLACK TONGUE	% PEROSIS	FEED EFFICIENCY
1	None	35	1	247	43	20	.505
2	5% Gelatin	72	12	138	89	64	.358
3	15% Gelatin	6	5	75	83	0	.148
4	15% Bone ossein	6	5	57	100	0	-
5	15% Casein	6	0	284	0	33	.603
6	15% Soybean oil meal	6	0	335	33	17	.568
7	25% Oats	6	0	299	83	67	.566
8	25% Wheat	6	0	272	50	33	.492
9	25% Corn	12	0	203	75	33	.524
10	25% Corn gluten meal (44% protein)	18	1	176	94	61	.473
11	15% Zein	6	0	155	83	67	.452
12	As 9 + 5 mg % Nicotinic acid	6	0	295	0	0	.559
13	As 10 + 5 mg % Nicotinic acid	24	0	385	0	0	.608

By comparing the activities of corn, corn gluten meal, and zein (groups 9 to 13), it is evident that the depressing action of corn is due to its protein content, as Krehl, Sarma, and Elvehjem (1946) have likewise reported in an independent investigation. The depressing action of corn and corn gluten meal was again prevented by the presence of nicotinic acid. It is interesting to note that the nicotinic acid which was supplied by the supplementation of oats, corn, and corn gluten meal was 0.50, 0.575, and 0.750 milligrams per 100 grams respectively as calculated from values reported for these products by Hale et al. (1942). Although the corn and corn gluten meal supplied more nicotinic acid to the diet than did oats, the latter grain stimulated growth whereas corn and corn gluten meal depressed growth. These results showed that factors other than the nicotinic acid content of corn and corn gluten meal are responsible for their growth depressing action.

Briggs et al. (1942) determined the requirement of nicotinic acid for White Leghorn chicks which were fed a purified diet containing 10 percent of gelatin. They reported that that species required 1.8 milligrams of nicotinic acid per 100 grams of diet for maximum growth. Since our studies were conducted with New Hampshires, a heavy breed, it seemed desirable to investigate their requirement for nicotinic acid. Accordingly, this point was investigated quantitatively, employing the highly purified diet 113GN. Moreover, the requirement was determined at two levels of gelatin, 5 percent and 10 percent.

Table VIII shows that New Hampshire chicks require approximately 1.6 milligrams of nicotinic acid per 100 grams of diet for maximum growth when the diet contains 5 percent of gelatin. The nicotinic acid contributed by

TABLE VIII

DETERMINATION OF THE NICOTINIC ACID REQUIREMENT FOR GROWTH OF NEW HAMPSHIRE CHICKS EMPLOYING DIET 113GN + 5 PERCENT OF GELATIN

GROUP NO.	LEVEL OF NICOTINIC ACID ADDED TO BASAL DIET 113GN (with 5% gelatin)	NO. CHICKS	NO. DIED	AVE. WT. 4 WKS. GMS	NO. BLACK TONGUE	NO. PEROSIS	FEED EFFICIENCY	FEATHER SCORE
1	None	6	0	202	2	0	.495	47
2	0.5 mg %	6	0	178	1	2	.488	46
3	1.0 mg %	6	0	204	1	1	.514	52
4	1.5 mg %	6	1	268	0	0	.538	74
5	2.0 mg %	6	0	273	2	0	.559	75
6	2.5 mg %	6	0	276	0	2	.557	77
7	5.0 mg %	6	0	280	0	0	.536	74
8	10.0 mg%	6	0	284	0	0	.541	78

the diet was calculated to be 0.06 milligrams per 100 grams. Thus, for the sake of round figures, the requirement for maximum growth may be set at 1.6 milligrams per 100 grams of diet. However, the requirement for the prevention of blacktongue and perosis is probably slightly in excess of 2.5 milligrams, or approximately 1 milligram above the requirement for growth. That nicotinic acid is required for normal feathering is apparent (Compare groups 1, 2, 3, and 4). The requirements for maximum growth and feathering appear to be the same.

The data presented in Table IX show that when 10 percent of gelatin is present in the diet the nicotinic acid requirement for maximum growth is increased, being about 2.1 milligrams per 100 grams (taking into account, again, the nicotinic acid contributed by the diet). The requirement for the prevention of blacktongue and perosis is probably slightly greater than 3.0 milligrams per 100 grams of diet with this level of gelatin. Again, there is evidence that nicotinic acid is required for normal feathering and that the requirement for maximum growth satisfies the requirement for feathering.

Having been placed on a quantitative basis, the foregoing data clearly show that an increase of gelatin in the diet from 5 percent to 10 percent increased the nicotinic acid requirement of the chick.

In an effort to determine the cause of the growth inhibition in chicks brought on by the feeding of gelatin, in the absence of nicotinic acid, an investigation involving amino acid studies seemed to be the most logical approach. Accordingly, pure amino acids were fed, alone and in various combinations, simulating in most instances their relative occurrences in gelatin. The composition of the amino acid mixture used and the results of the feeding trial are given in Table X. It should be pointed out, however, that proline occurs in gelatin in fairly high amounts. At the

TABLE IX

DETERMINATION OF THE NICOTINIC ACID REQUIREMENT FOR GROWTH OF NEW HAMPSHIRE CHICKS EMPLOYING DIET 113GN + 10 PERCENT OF GELATIN

GROUP NO.	LEVEL OF NICOTINIC ACID ADDED TO BASAL DIET 113GN (with 10% gelatin)	NO. CHICKS	NO. DIED	AVE. WT. 4 WKS. GMS	NO. BLACK TONGUE	NO. PEROSIS	FEED EFFIC-IENCY	FEATH-ER SCORE
1	None	6	0	151	6	3	.475	32
2	1.0 mg %	6	0	222	6	5	.547	56
3	1.5 mg %	6	1	252	3	3	.568	63
4	2.0 mg %	6	0	297	3	3	.613	73
5	2.5 mg %	6	0	305	0	3	.608	71
6	3.0 mg %	6	0	296	1	1	.640	66
7	5.0 mg %	6	0	290	0	0	.614	81
8	10.0 mg %	6	0	328	0	0	.594	80

TABLE X

DATA SHOWING THAT THE GROWTH DEPRESSING ACTION OF GELATIN IS DUE TO ITS AMINO ACID CONSTITUENTS

GROUP NO.	SUPPLEMENT TO BASAL DIET 114GN (with no gelatin)	NO. CHICKS	% DIED	AVE. WT. 4 WKS. GMS	% BLACK TONGUE	% PEROSIS	FEED EFFICIENCY
1	5% Gelatin	18	6	218	45	16	.494
2	10% Gelatin	18	28	118	100	27	.370
3	As 2 + 5 mg % Nicotinic acid	18	0	312	0	0	.602
4	As 1 + 2% Glycine	6	17	188	67	17	.460
5	As 1 + 4% Glycine	12	25	145	100	9	.437
6	As 4 + 5 mg % Nicotinic acid	6	0	314	0	0	.575
7	As 5 + 5 mg % Nicotinic acid	6	0	300	0	0	.570
8	As 1 + 4% Glycine + 25 mg % Nicotinic acid	6	0	313	0	0	.574
9	As 1 + 6% Glycine + 25 mg % Nicotinic acid	6	0	295	0	0	.586
10	As 1 + 5% Amino acid mixture*	12	42	131	100	0	.457
11	As 10 + 5 mg % Nicotinic acid	5	0	308	0	0	.577
12	As 1 + 2% Glycine, 0.5% Arginine	6	0	131	100	33	.461
13	As 12 + 0.5% Alanine	6	17	118	100	17	.397
14	As 10 minus Glycine and Arginine	6	17	251	100	17	.513
15	As 1 + 3% Urea	6	17	208	83	17	.492
16	As 15 + 5 mg % Nicotinic acid	6	0	300	0	0	.527

* Glycine 2.0, dl Aspartic acid 0.6, dl Alanine 0.5, l(+) Arginine HCl 0.5, l(+) Glutamic acid 0.5, l(-) Leucine 0.2, l(+) Lysine HCl 0.2, dl Phenylalanine 0.1, dl Serine 0.2, dl Valine 0.1, l(-) Tyrosine 0.1

time this experiment was performed proline was not available so glycine was increased in the amino acid mixture proportionally to the amount of proline which was lacking. A later experiment showed proline to behave in a similar manner to glycine so this procedure was justified.

That the feeding of glycine had growth-inhibiting properties under the conditions employed is evident (groups 4 and 5). Furthermore, certain other symptoms associated with nicotinic acid deficiency, namely chick blacktongue, diarrhea, perosis, and poor feed utilization were also aggravated by the feeding of glycine. But, most important was the complete prevention of these cachectic conditions by the feeding of nicotinic acid (groups 6 and 7). Even 6 percent of glycine was tolerated when the diet contained sufficient nicotinic acid (group 9).

The feeding of the amino acid mixture (group 10) and combinations of some of the components of this mixture demonstrated that the growth inhibiting effect of gelatin was not entirely due to glycine. Arginine and glycine together (group 12) and especially arginine, glycine, and alanine in combination (group 13) showed marked inhibitory action. The other amino acids in the mixture, even in the presence of alanine, apparently were not involved in the growth inhibition at the levels fed, although the incidence of blacktongue was increased.

Urea nitrogen in contrast to amino acid nitrogen had no growth depressing action (groups 15 and 16). These results indicate that the growth depression caused by the feeding of gelatin is due mainly to its amino acid constituents - principally glycine, arginine, and alanine. They also indicate that nicotinic acid is concerned, in some manner, with the metabolism of these amino acids.

The next step involved a similar investigation of the corn protein, zein. The composition of the amino acid mixture, simulating 15 percent zein is given in Table XI together with the results obtained. Zein, as shown previously (Table VIII) decreased growth, feed efficiency, and caused poor feathering while increasing the incidence of mortality, blacktongue, and perosis (Compare groups 1 and 5); but not quite as much as did gelatin (Compare groups 2 and 5). However, the feeding of the amino acid mixture produced much more severe results than did zein (Compare groups 5 and 6). Less severe results were obtained with the modified amino acid mixture (Compare groups 6 and 7), but the severity of its action was still greater than zein (Compare groups 5 and 7). Thus, it appears that the amino acids which are primarily involved in the growth depression effect of zein are glutamic acid, leucine, alanine, proline, and phenylalanine. These are the amino acids which occur in the greatest quantities in this protein. The residual amino acids were additive in producing a further growth depressing action. The reason that zein was less depressing in action than the amino acid mixture was probably due to the presence of small amounts of naturally occurring tryptophane and nicotinic acid in zein. The most significant point to observe, however, is the counteraction of the effects of the amino acid mixture with nicotinic acid (Compare groups 3, 4, 8, and 9). These results show that the depressing action of zein is due to its amino acid constituents. Furthermore, as in the case of gelatin, it has been demonstrated that nicotinic acid is involved in the metabolism of amino acids.

In relating the actions of gelatin and zein it was evident that certain dissimilarities were apparent, relative to their amino acid constituents. Since zein contains no glycine and very little arginine,

TABLE XI

DATA SHOWING THAT THE GROWTH DEPRESSING ACTION OF ZEIN IS DUE TO ITS AMINO ACID CONSTITUENTS

GROUP NO.	SUPPLEMENT TO BASAL DIET 115GN (with no gelatin)	NO. CHICKS	NO. DIED	AVE. WT. 4 WKS. GMS	NO. BLACK TONGUE	NO. PEROSIS	FEED EFFIC-IENCY	FEATHER SCORE
1	5% Gelatin	6	0	175	2	0	.480	52
2	10% Gelatin	6	2	133	5	2	.290	18
3	As 1 + 10 mg % Nicotinic acid	6	0	235	0	0	.510	78
4	As 2 + 10 mg % Nicotinic acid	6	0	244	0	0	.545	76
5	As 1 + 15% Zein	6	1	155	5	1	.382	32
6	As 1 + 15% Amino acid mixture*	6	5	61	6	0	.067	0
7	As 1 + 11.5% modified A.A.Mix**	6	1	110	5	0	.412	24
8	As 5 + 10 mg % Nicotinic acid	6	0	221	0	1	.460	78
9	As 6 + 10 mg % Nicotinic acid	6	0	226	0	0	.540	78

* l(+) Glutamic acid 3.90, l(-) Leucine 3.60, dl Alanine 1.55, l(-) Proline 1.45, dl Phenylalanine 1.00, l(-) Tyrosine .85, dl Isoleucine .65, dl Aspartic acid .50, dl Methionine .35, dl Threonine .35, dl Valine .35, dl Serine .15, l(+) Histidine .15, l(+) Arginine .15

**This mixture contained Glutamic acid, Leucine, Alanine, Proline and Phenylalanine at the same levels as used in the above Amino acid mixture.

these amino acids obviously were not primarily involved in the results obtained with this protein whereas they were with gelatin. This suggested that perhaps all the amino acids had growth inhibiting potentialities in the absence of nicotinic acid if fed at high enough levels. Accordingly, 17 amino acids were individually tested at a level of 4 percent of the diet. The results are given in Table XII. It may be seen that a growth depression occurred in each instance, but that they varied in intensity in the order listed. Since glycine was known to depress growth when fed at a level of 4 percent (from previous experiments), and that its action could be counteracted with nicotinic acid, this amino acid was used as a standard in measuring severity of inhibitory action. Thus, it may be seen that tyrosine, histidine, and arginine, when fed at a level of 4 percent, were about equal to glycine in producing growth inhibition, while proline, and especially cystine, lysine, and methionine were more severe. Methionine was extremely toxic and all of the chicks died by the end of the third week.

The chicks which received 4 percent of lysine exhibited tremors throughout the entire period as did the chicks receiving 5 percent of glycine. However, this condition was less severe in the latter case. The chicks receiving 4 percent of cystine had very ragged wing feathering. Other than these observations, only growth depression and the typical deficiency symptoms were noted in the other chicks.

Table XIII summarizes the effect of nicotinic acid in counteracting the inhibitory action of the amino acids found to be most severe in this respect. The data show that the detrimental action of arginine, glycine, glutamic acid, and proline was overcome with nicotinic acid. In fact,

TABLE XII

ASSAY FOR GROWTH DEPRESSING ACTION OF INDIVIDUAL AMINO ACIDS

GROUP	SUPPLEMENT TO BASAL DIET 113GH (with 5% gelatin)	NO. CHIX	NO. DIED	AVE. WT. 4 WKS. GMS	NO. BLACK TONGUE	NO. PEROSIS	FEED EFFICIENCY	FEATHER SCORE	% OF BASAL WT.
1	None*	6	0	234	1	0	.550	68	-
2	2% Glycine	6	0	164	5	2	.421	40	70
3	3% Glycine	6	0	137	5	1	.458	22	59
4	4% Glycine	6	0	116	5	1	.392	8	50
5	5% Glycine	6	2	87	6	2	.354	6	37
6	4% dl Valine	3	0	186	0	1	.466	47	80
7	4% l(-) Leucine	6	0	185	3	0	.513	53	79
8	4% dl Serine	4	1	184	2	1	.483	50	79
9	4% dl Threonine	3	1	167	1	1	.475	38	71
10	4% dl Aspartic acid	6	1	167	1	0	.520	41	71
11	4% dl Phenylalanine	4	0	151	1	0	.453	50	65
12	4% dl Isoleucine	6	1	151	2	0	.470	20	65
13	4% l(+) Glutamic acid	6	0	142	3	0	.442	17	61
14	4% dl Alanine	6	0	137	4	0	.440	20	59
15	4% l(-) Tyrosine	6	0	115	5	2	.348	14	49
16	4% l(+) Histidine	6	0	114	5	0	.398	20	49
17	4% l(+) Arginine	4	2	107	4	2	.365	0	46
18	4% l(-) Proline	4	0	100	4	1	.394	13	43
19	4% l(+) Cystine	6	1	79	1	1	.313	0	34
20	4% l(+) Lysine	4	0	77	2	0	.277	0	33
21	4% dl Methionine	6	6	-	-	-	-	-	-

* Basal group

TABLE XIII

COUNTERACTION OF THE GROWTH DEPRESSING ACTION OF INDIVIDUAL AMINO ACIDS WITH NICOTINIC ACID

GROUP	SUPPLEMENT TO BASAL DIET 113GN (with 5% gelatin and 10 mg % nicotinic acid)	NO. CHIX	NO. DIED	AVE. WT. 4 WKS. GMS	NO. BLACK TONGUE	NO. PEROSIS	FEED EFFICIENCY	FEATHER SCORE	% OF BASAL WT.
1	None*	12	1	232	0	0	.507	82	-
2	4% l(+) Arginine	3	0	306	0	0	.602	97	132
3	4% Glycine	6	0	266	0	2	.583	87	115
4	4% l(+) Glutamic acid	6	0	256	0	0	.522	93	110
5	4% l(-) Proline	3	0	226	0	0	.592	73	97
6	4% dl Alanine	6	0	207	0	0	.525	56	89
7	4% l(-) Cystine	6	0	148	0	0	.454	53	64
8	4% l(-) Tyrosine	6	0	145	0	0	.390	43	63
9	4% dl Isoleucine	4	1	144	0	0	.412	53	62
10	4% l(+) Histidine	4	1	136	0	0	.408	57	59
11	4% l(+) Lysine	3	2	103	0	0	.242	0	44
12	4% dl Methionine	6	1	59	0	0	.176	0	25

* Basal group

arginine and glycine gave a growth response considerably above that of the control group. In the case of alanine the growth was not quite so good as the control. Moreover, a peculiar physical condition was observed in these chicks. They displayed a kind of hypnotic state and tended to be unconcerned of their surroundings. They were inclined to stand for long periods of time with their heads down and necks extended.

Some improvement was noted with cystine, tyrosine, isoleucine, and histidine, while no improvement was observed with lysine or methionine. The ragged wing-feathering observed previously in the chicks receiving cystine was not improved by nicotinic acid feeding nor were the tremors in the chicks which were fed lysine ameliorated. The fact that nicotinic acid reversed the deficiency states produced by the feeding of arginine, glycine, glutamic acid, proline, and alanine is positive evidence that the vitamin is concerned with the metabolism of these amino acids.

Since glycine, in the presence of nicotinic acid, appeared to be tolerated by chicks at a level of 6 percent (Table X, group 9), whereas methionine and lysine were extremely toxic at 4 percent, it seemed of interest to investigate glycine further to determine its minimum toxic level. Furthermore, we were especially concerned about this point since Almquist et al. (1940) reported that only 2 percent of glycine was toxic to chicks.

Table XIV summarizes the results of feeding 8 percent of glycine to chicks in combination with various levels of nicotinic acid. It is apparent that glycine at this level inhibits growth and that the growth depression is not overcome by feeding nicotinic acid in amounts as high as 100 milligrams per 100 grams. Mortality was observed in each group, perosis was aggravated, and feathering was inferior. However, no blacktongue was seen. The majority

TABLE XIV

EFFECT OF VARIOUS LEVELS OF NICOTINIC ACID ON CHICKS RECEIVING HIGH LEVELS (8 PERCENT) OF GLYCINE

GROUP NO.	SUPPLEMENT TO BASAL DIET - 113GN CONTAINING 8% GELATIN	NO. CHICKS	NO. DIED	AVE. WT. 4 WEEKS	NO. PEROSIS	FEED EFFICIENCY	FEATHER SCORE
1	10 mg % Nicotinic acid	6	0	235	0	.510	78
2	10 mg % Nicotinic acid + 8% glycine	6	1	157	2	.501	62
3	50 mg % Nicotinic acid + 8% glycine	6	1	152	1	.551	60
4	100 mg % Nicotinic acid + 8% glycine	6	1	158	1	.504	57

of birds had very ragged wing and tail feathers. From the start of the experiment the chicks exhibited periods of extreme prostration accompanied by tremors which seemed to be greatest after eating. Sometimes the chicks would be extremely comatos and would appear to be at the point of death when they would gradually rally. Constant weight gains were made. After the first week the chicks appeared to out-grow the tendency toward prostration, but slight tremors existed in the chicks throughout the whole experiment. They seemed to be constantly tired and would stretch their wings and legs at frequent intervals. They were extremely sensitive to sound, the least noise being capable of producing a bodily twitch of muscles. At about the third week a peculiar puffiness about the eyes was noticed. By the fourth week it was evident that either the eyeballs were becoming enlarged or that an edemic condition existed in the vesicle which was causing the eye to be forced out as occurs in exopthalmic goiter. The eye condition at this point was severe enough to interfere with the movements of the nictitating membrane. After four weeks had terminated, three chicks from each group receiving glycine were selected and the total of 9 chicks were given the diet containing 8 percent of glycine and 10 milligrams of nicotinic acid per 100 grams of diet. They were continued to seven weeks of age with the 6 control chicks. From the fourth week to the seventh week the "popped" eye condition became worse. From about the fifth week on, the eyes were so bulged that normal vision was impaired to the point where the eyes could not be focused on objects close at hand. Consequently, the feed hoppers had to be kept quite full so that the chicks could partake of food. Otherwise, vain attempts were made to obtain feed from the bottom of the feed hopper.

At seven weeks of age all the chicks were killed by severing the jugular vein. All had been starved for a period of 6 hours previous to killing. The heart, liver with bile sac, spleen, thyroids, and one eye from each chick were excised and weighed immediately. In the case of the eye, all adhering muscular tissue was removed before being weighed. In Table XV the values obtained for the various organs are given - expressed in terms of percent of body weight. The data show that the percent of total body weight of the heart, liver plus bile sac, and spleen were slightly higher on the average for the chicks fed glycine than for the controls. Little difference was noted between thyroids. However, a marked difference existed in the weight of the eyeballs. The eyes from the chicks which received 8 percent of glycine were almost twice as great on a percent of body weight basis than were the eyes from the controls.

This unusual eye condition compared with normal eyes may be seen in figures 2, 3, and 4. Figure 5 shows the difference in size of two excised eyeballs, one from a control chick and the other from a chick which received 8 percent of glycine.

The production of an apparent overgrowth of the eyeballs seems to be related to the form in which the amino acid is fed. The same basal diet supplemented with 10 percent of gelatin and 25 percent of bone ossein did not cause an enlargement of the eyeball, although the diet contained approximately 8 percent of glycine but in the peptide form. Furthermore, the dramatic effect of free glycine is confined to the growing chick because in experiments with laying hens, to be discussed later, no such condition was observed by feeding free glycine at a level as high as 12 percent.

TABLE XV

EFFECT OF 8 PERCENT GLYCINE IN THE DIET OF CHICKS ON THE SIZE OF VARIOUS ORGANS OF THE BODY

DIET FED - 113GM + 5% gelatin, 10 mg % nicotinic acid (control group)						
CHICK NO.	BODY WEIGHT 7 WEEKS GMS	PERCENT OF BODY WEIGHT				
		EYE*	THYROID**	HEART	LIVER AND BILE SAC	SPLEEN
1	392	.375	.008	.587	2.684	.191
2	400	.362	.008	.537	2.762	.262
3	500	.396	.009	.400	3.302	.188
4	550	.334	.006	.500	2.625	.262
5	574	.301	.006	.458	2.761	.249
6	775	.289	.012	.536	2.523	.109
Average -	532	.343	.008	.503	2.776	.210
DIET FED - 113GM + 5% gelatin, 10 mg % nicotinic acid, 8% glycine						
7	231	.762	.006	.593	2.983	.182
8	347	.692	.006	.568	2.499	.164
9	368	.660	.009	.516	3.141	.242
10	394	.591	.008	.520	3.051	.215
11	395	.686	.008	.489	3.380	.342
12	402	.619	.005	.522	2.684	.214
13	423	.595	.007	.501	2.991	.288
14	437	.565	.008	.545	2.858	.183
15	590	.575	.009	.581	2.451	.254
Average -	399	.638	.007	.537	2.893	.232

* Values given are for one eye.

** Values given are for both thyroids.

Figure 2. DORSAL HEAD VIEW OF A 7 WEEK OLD NORMAL CHICK

Figure 3. DORSAL HEAD VIEW OF A 7 WEEK OLD CHICK WHICH RECEIVED 8 PERCENT OF GLYCINE IN ITS DIET. Note the bulging eyes.

Figure 4. FRONTAL VIEW OF A 7 WEEK OLD CHICK WHICH RECEIVED 8 PERCENT OF GLYCINE IN ITS DIET.

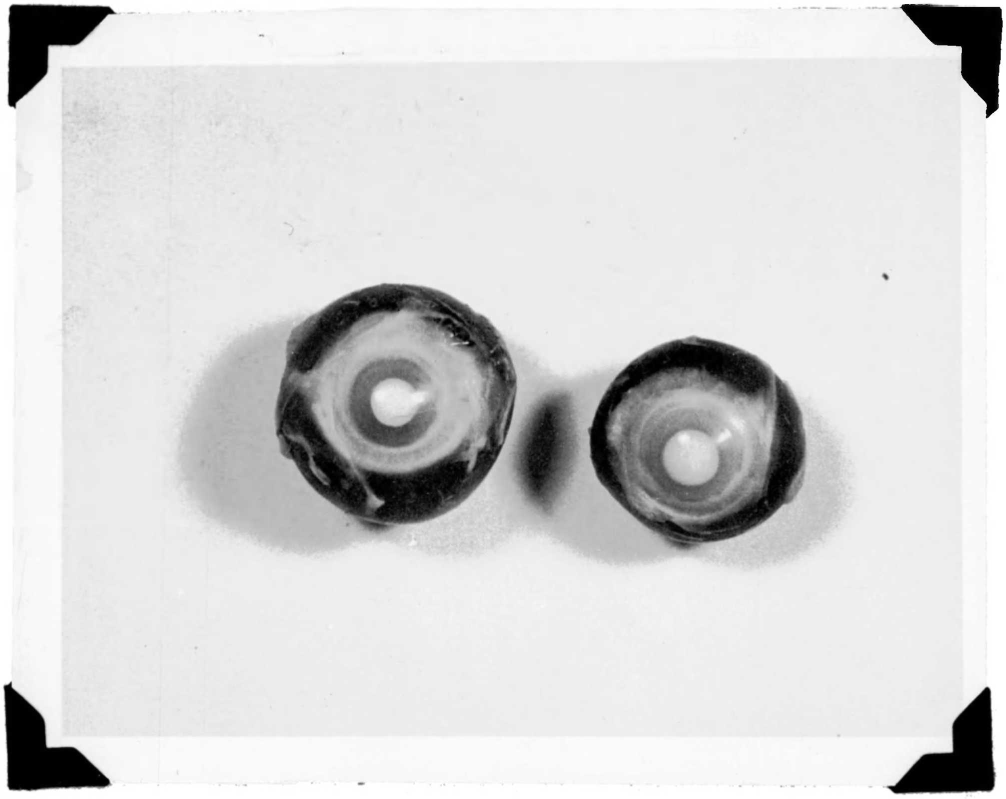

Figure 5. CONTRAST IN SIZE OF EYEBALLS

The eyeball on the left is from a 7 week old chick which received 8 percent of glycine; its' body weight was 395 grams. The eyeball on the right is from a normal 7 week old chick; its' body weight was 574 grams.

B. Nicotinic Acid Experiments with Hens

The work with chicks showed that as additions of gelatin above the 5 percent level were made to the basal diets low in nicotinic acid the severity of nicotinic acid deficiency became greater. This point was used to advantage in producing a nicotinic acid deficiency in hens.

In the first experiment two groups of New Hampshire pullets, four pullets per group, were fed diet 201N (Table III) with and without nicotinic acid. The four hens which received the basal diet without nicotinic acid (group A) began to lose weight rapidly and showed a decline in egg production as well as a decrease in hatchability (Chart 1 and Table XVI). The hens in group B which received the basal diet plus 50 milligrams of nicotinic acid per 100 grams of diet maintained their weight and exhibited normal egg production and hatchability. After a period of six weeks when the hens in group A had lost considerable weight and one had become quite ill, the diets were reversed. Within a few days the hens in group A, which now received nicotinic acid, began to gain weight and continued to improve, except for the sick hen which died. Concurrently, with the gain in weight, group A showed a recovery in egg production and hatchability. On the other hand, group B, which received no nicotinic acid after the reversal of diets, began to show a loss of weight and a decrease in egg production and hatchability. No blacktongue was observed in any of the hens. Fertility averaged approximately 88 percent. The experiment was terminated after 13 weeks because the supply of bone casein became exhausted.

Although only a few hens were involved in this experiment the results demonstrate quite conclusively, and for the first time, that under

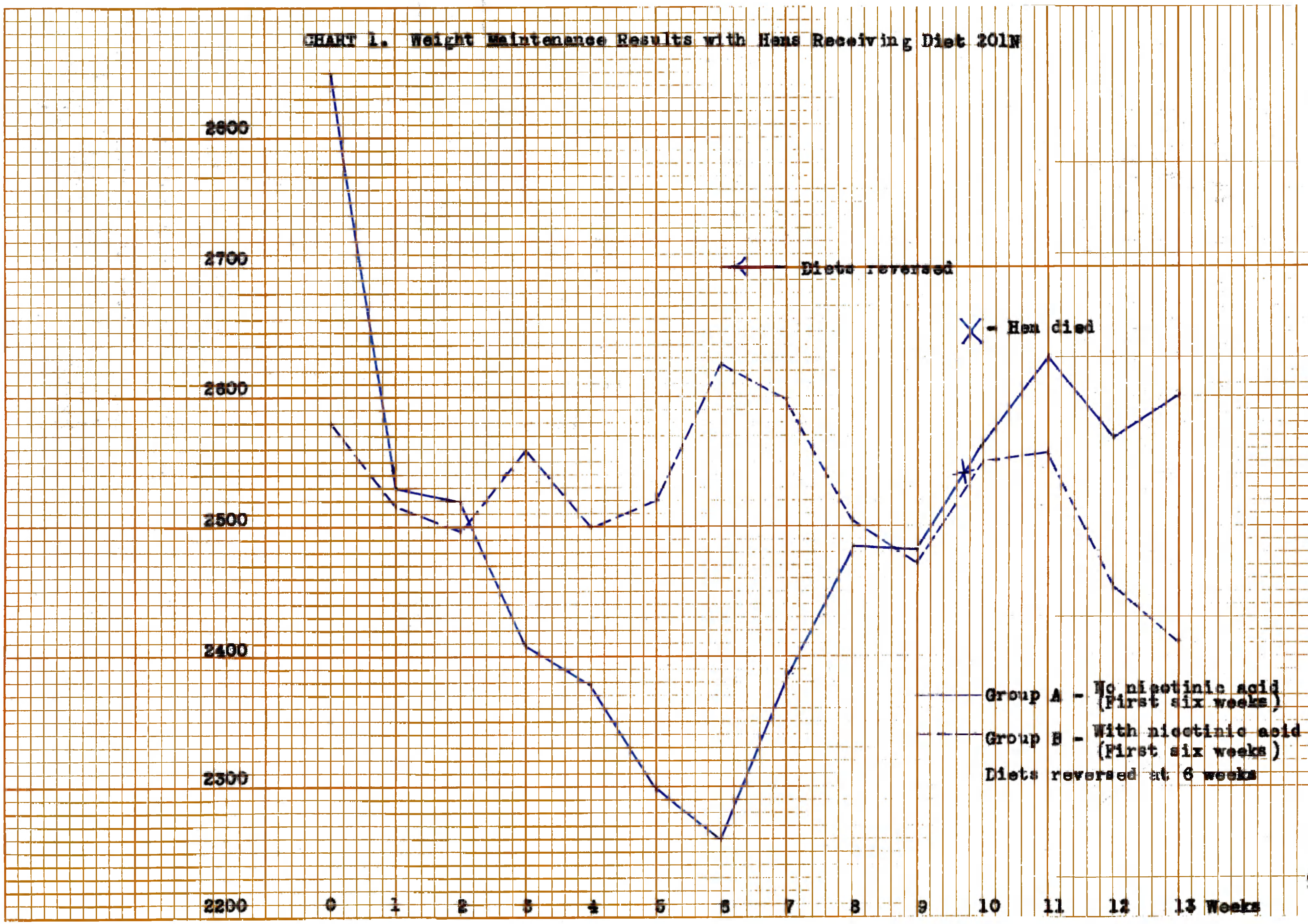

CHART I. Weight Maintenance Results with Hens Receiving Diet 201N

TABLE XVI

DATA SHOWING THE NEED FOR NICOTINIC ACID IN THE DIET OF HENS FOR NORMAL EGG PRODUCTION AND HATCHABILITY

GROUP A FED DIET 201N			GROUP B FED DIET 201N + 50 MG % NICOTINIC ACID		
WEEKS ON DIET	EGGS/HEN/DAY	% HATCH.	WEEKS ON DIET	EGGS/HEN/DAY	% HATCH.
1	.392	100	1	.678	72.7
2	.285	50	2	.500	55.6
3	.250	0	3	.642	90.0
4	.211	0	4	.714	87.5
5	.285	0	5	.678	72.7
6	.107	0	6	.642	81.8
DIETS REVERSED					
7	.035	0	7	.642	62.5
8	.211	80	8	.535	67.0
9	.250	67	9	.500	0.0
10	.240	100	10	.392	28.6
11	.190	100	11	.250	33.3
12	.238	-	12	.250	-
13	.333	-	13	.285	-

extreme dietary conditions the laying hen requires nicotinic acid for normal health, egg production, and hatchability.

A second experiment was performed to determine the effects of high amounts of corn, corn gluten meal, and glycine on the production of a nicotinic acid deficiency in hens since these materials proved quite effective with chicks. The experiment was composed of six groups of New Hampshire pullets, 4 pullets per group. The diets fed were 202N, 203N, and 204N (Table III). A plan of the experiment together with the results is given in Table XVII.

No marked differences in egg production or hatchability were observed during the first 5 weeks (period 1) by the feeding of diet 202N alone, by adding 4 percent of glycine, or by omitting casein from this diet (groups 1, 3, and 5). The addition of nicotinic acid to these dietary regimes was without further value (groups 2, 4, and 6). All of the groups, except group 3, gained weight and showed normal egg production and hatchability. Since this feeding trial extended over a period of 5 weeks and good results were obtained, it is evident that the basal diet 202N was nutritionally adequate even without added nicotinic acid.

Supplementation of 10 percent of gelatin in diet 202N in period 2 appeared to cause a slight lowering in body weight initially, but with no further decrease thereafter. Egg production declined slightly, but no effect was noted on hatchability. Results were normal in the group receiving nicotinic acid (Compare groups 1 and 2 with 5 and 6).

The effects of 8 percent and 12 percent of glycine in diet 202N caused a gradual decline in egg production (group 3, periods 2 and 3). The decline in egg production was not affected by adding nicotinic acid (group 4, periods 2 and 3). Maintenance of body weight and hatchability

TABLE XVII

RESULTS OF FURTHER EXPERIMENTS ATTEMPTING TO PRODUCE A NICOTINIC ACID DEFICIENCY IN HENS, EMPLOYING DIETS HIGH IN CORN PRODUCTS AND GLYCINE

PERIOD	WEEKS	GROUP 1			GROUP 2		
		DIET FED					
		202N			202N + 50 MG % NICOTINIC ACID		
		AVE. BODY WT. GMS	PERCENT EGG PROD.	PERCENT HATCH.	AVE. BODY WT. GMS	PERCENT EGG PROD.	PERCENT HATCH.
	1	2835	42.9	80.0	2835	53.6	100.0
	2	2838	42.9	75.0	2836	53.6	78.6
1	3	2938	45.3	87.5	2846	57.1	77.8
	4	2956	57.1	62.5	2824	87.1	94.4
	5	3009	52.4	92.3	2816	71.4	100.0
		202N + 10% GELATIN			202N + 10% GELATIN + 50 MG % NICOTINIC ACID		
	6	2858	57.1	100.0	2830	87.1	69.2
2	7	2821	52.4	70.0	2825	67.8	82.4
	8	2874	42.9	85.7	2869	64.3	94.1
		203N + 8% GLYCINE			203N + 8% GLYCINE + 50 MG % NICOTINIC ACID		
	9	2837	42.9	100.0	2806	71.4	94.4
3	10	2808	61.9	75.0	2773	60.7	88.2
	11	2740	66.7	76.9	2814	60.7	100.0
	12	2745	57.3	75.0	2720	60.7	94.1
		204N			204N + 50 MG % NICOTINIC ACID		
	13	2585	52.4	40.0	2616	50.0	81.8
	14	2535	19.1	16.7	2615	21.4	83.3
	15	2524	0.0	-	2522	28.5	71.4
4	16	2510	4.7	No fertile eggs	2525	21.4	100.0
	17	2454	9.5	"	2549	17.8	100.0
	18	2359	14.3	*	2519	25.0	*
	19	2235	4.7	*	2569	21.4	*

* Hatchability data still forthcoming at the time this thesis was compiled.

TABLE XVII CONTINUED

RESULTS OF FURTHER EXPERIMENTS ATTEMPTING TO PRODUCE A NICOTINIC ACID DEFICIENCY IN HENS, EMPLOYING DIETS HIGH IN CORN PRODUCTS AND GLYCINE

PERIOD	WEEKS	GROUP 3			GROUP 4		
		DIET FED					
		202N + 4% GLYCINE			202N + 4% GLYCINE + 50 MG % NICOTINIC ACID		
		AVE. BODY WT. GMS	PERCENT EGG PROD.	PERCENT HATCH.	AVE. BODY WT. GMS	PERCENT EGG PROD.	PERCENT HATCH.
1	1	2926	39.3	67.0	2893	60.7	71.4
	2	2931	50.0	60.0	3003	42.8	66.7
	3	2950	67.8	77.8	3036	57.1	81.8
	4	2984	60.7	92.8	3034	57.1	71.4
	5	2988	53.6	88.9	3046	60.7	77.8
		202N + 8% GLYCINE			202N + 8% GLYCINE + 50 MG % NICOTINIC ACID		
2	6	2985	57.1	75.0	2996	57.1	92.8
	7	2994	42.8	42.9	2996	57.1	76.9
	8	2938	39.3	66.7	2960	46.4	72.7
		202N + 12% GLYCINE			202N + 12% GLYCINE + 50 MG % NICOTINIC ACID		
3	9	2970	32.1	100.0	2943	46.4	63.7
	10	2933	35.7	85.7	2884	25.0	85.7
	11	3046	25.0	80.0	2954	10.7	100.0
	12	2993	28.5	77.1	2911	17.8	75.0

TABLE XVII CONCLUDED

RESULTS OF FURTHER EXPERIMENTS ATTEMPTING TO PRODUCE A NICOTINIC ACID DEFICIENCY IN HENS, EMPLOYING DIETS HIGH IN CORN PRODUCTS AND GLYCINE

PERIOD	WEEKS	GROUP 5			GROUP 6		
		DIET FED					
		202N MINUS CASEIN			202N MINUS CASEIN + 50 MG % NICOTINIC ACID		
		AVE. BODY WT. GMS	PERCENT EGG PROD.	PERCENT HATCH.	AVE. BODY WT. GMS	PERCENT EGG PROD.	PERCENT HATCH.
	1	3035	39.3	100.0	2895	46.4	100.0
	2	3079	50.0	80.0	2956	46.4	83.3
1	3	3080	46.4	100.0	3035	46.4	66.7
	4	3138	57.1	81.8	3065	42.8	91.7
	5	3101	39.3	100.0	3054	60.7	81.8
		202N			202N + 50 MG % NICOTINIC ACID		
	6	3139	57.1	83.3	3066	42.8	60.0
2	7	3213	46.4	81.8	3144	32.1	75.0
	8	3209	57.1	78.5	3138	50.0	75.0
		202N			202N + 50 MG % NICOTINIC ACID		
	9	3264	64.3	78.5	3188	33.3	100.0
	10	3231	50.0	93.3	3180	42.8	83.3
3	11	3328	50.0	90.0	3014	52.4	87.5
	12	3268	48.6	88.9	2930	42.8	87.5

appeared not to be disturbed by the high levels of glycine (Compare groups 3 and 4 with 5 and 6 in periods 2 and 3). In contrast to the results obtained with chicks by feeding high amounts of glycine it is interesting to note that no toxic symptoms or evidence of bulging eyes were observed in any of the hens.

Glycine, at a level of 8 percent, did not appear to affect egg production or hatchability when added to the highly purified diet 203H with or without nicotinic acid. Weight losses occurred, however, in the presence or absence of nicotinic acid. (Compare groups 1 and 2, period 3).

The feeding of diet 204H, which is low in casein (9 percent) and high in gelatin (25 percent) gave the most conclusive evidence of producing a nicotinic acid deficiency state in the second experiment with hens. The hens which received diet 204H alone exhibited a rapid loss in body weight, egg production and hatchability (group 1, period 4), while the hens which received the supplement of nicotinic acid showed an initial loss of weight which was soon halted. Body weight, thereafter, was maintained on the lower plateau. Egg production declined and was maintained on a lower level while hatchability remained normal (group 2, period 4). Again, as in the first experiment with hens, no blacktongue was observed. It is clear, however, that the results of second experiment with hens indicate that the adult chicken is more resistant to a nicotinic acid deficiency than is the chick.

DISCUSSION

The data presented in this thesis show conclusively that the deleterious effect of feeding gelatin or zein to chicks receiving diets low in nicotinic acid is caused by an amino acid imbalance of these proteins. More specifically, it was found that the amino acids which were principally involved in the growth depression caused by the feeding of gelatin were glycine, arginine, alanine, and probably proline. In zein the amino acids which were found to be principally involved in this respect were glutamic acid, leucine, alanine, proline, and phenylalanine. It is significant to note that the amino acids named are the ones which occur in the greatest amounts in each protein, respectively.

The fact that nicotinic acid reversed the deleterious actions of gelatin and zein, and amino acid mixtures simulating these proteins, definitely associates nicotinic acid with the metabolism of amino acids. Evidence in support of this finding has recently been reported by Dann and Huff (1947) for arginine. These workers showed that the excrement of chickens contained ornithine, a cleavage compound of arginine, conjugated with two moles of nicotinamide forming dinicotinylornithine. Moreover, Sarett, Klein, and Perlzweig (1942) in balance studies with dogs and rats inferred that nicotinic acid was concerned in protein anabolism. They found that less nicotinic acid was excreted in these animals on a high protein than on a low protein diet.

Since zein was shown to be growth depressing when fed to chicks receiving nicotinic acid-low diets, it is logical to assume that at least part of the growth depressing action of corn is due to its protein, zein. Whether an additional depressing action is caused by the presence of a "pellagragenic" agent reported to exist in corn by Woolley (1946) is unknown.

The experiments on the feeding of individual amino acids at a level of 4 percent each revealed a varying tolerance for each of these substances by chicks when nicotinic acid was present or absent in the diet. The fact that nicotinic acid failed to bring about recoveries and produce normal growth in chicks fed some of the amino acids, particularly methionine and lysine, should not be interpreted that the vitamin is not concerned in the metabolism of these amino acids. Different thresholds of tolerance probably exist in the chick for each amino acid. Thus, when this threshold is surpassed a general toxicosis is established which negates any beneficial action which may be contributed by nicotinic acid. A situation like this may explain the inability of nicotinic acid to counteract the deleterious effects of methionine and lysine. Similarly, cases which borderline the tolerance threshold may only give partial responses to nicotinic acid supplementation. A situation like this may explain the slight recoveries obtained in the chicks receiving cystine, tyrosine, isoleucine, and histidine when nicotinic acid was supplied in the diet.

It is suggested that the toxic condition may be due not only to the amino acid per se but also to the production of a toxic metabolic end product of the amino acid. It is possible that the latter alternative may have caused the peculiar physical condition observed in the chicks fed alanine and nicotinic acid. Alanine, upon deamination, forms pyruvic acid which in thiamine deficiency is not metabolized and causes a paralytic state. The formation of pyruvic acid by the feeding of high amounts of alanine might conceivably be too great for the normal metabolic channels to handle. Thus, the pyruvic acid in excess would be free to affect nervous disorders.

That the feeding of extremely high levels of amino acids in spite of nicotinic acid supplementation may cause growth depression and certain physical peculiarities, which in all probability are characteristic for each amino acid, was further demonstrated with glycine. The phenomenal enlargement of the eyeballs caused by the feeding of 8 percent of the free amino acid is indeed interesting, but the mechanism involved in producing the condition remains obscure. Whether the enlargement is caused by an actual stimulation in growth of the eye tissues or is brought about by a disturbance in osmotic relationships must await further experimentation. It seems certain, however, that the condition is specific to glycine in the free form since the same amount of glycine as the peptide allowed normal growth in the chick. Furthermore, the effect of free glycine is confined to the growing chick; no enlargement of the eyeballs or other apparent physical abnormalities were produced in hens receiving as high as 12 percent of free glycine.

The manner in which nicotinic acid is involved in the metabolism of amino acids is not known at this time. A likely theory, however, is the system outlined by Harrow (1945). It is believed that the first step in the deamination of amino acids involves the catalytic action of a dehydrogenase, together with a hydrogen acceptor. The hydrogen acceptor is coenzyme I which contains a mole of nicotinamide. The dehydrogenase removes two hydrogen atoms from the amino group. The hydrogens are taken up by coenzyme I. The imino acid which is formed as a result of dehydrogenation then hydrolyzes to form the corresponding alpha keto acid and ammonia. Thus, nicotinic acid (coenzyme I) in addition to its role as a hydrogen carrier in carbohydrate metabolism may play an important function in the deamination of amino acids.

By progressing in this same line of thought it is possible to relate the functions of tryptophane and nicotinic acid in amino acid metabolism. In our work the feeding of tryptophane was able to replace nicotinic acid, although much higher levels had to be used. The recent work of Rosen, Huff, and Perlzweig (1946) and Singal, Briggs, Sydenstricker, and Littlejohn (1946) with rats and Sarett and Goldsmith (1947) with humans indicates that tryptophane may be an important precursor of nicotinic acid synthesis. The site of the synthesis is unknown. Assuming that nicotinic acid is synthesized from tryptophane, then the action of tryptophane is readily understood and the growth depressing action of tryptophane-low proteins, such as gelatin and zein, may be explained in the following manner: In the absence of dietary nicotinic acid the chick is forced to use dietary tryptophane for the synthesis of nicotinic acid in order that coenzyme I may be maintained in the body to carry out vital life processes. By adding gelatin or zein to this diet a very great strain is placed on the system because more nicotinic acid (as the coenzyme) is needed for the deamination of the excess amino acids. Consequently, the chick must use still more tryptophane for the synthesis of nicotinic acid. At length the level of gelatin or zein in the diet may be so great that the chick is unable to synthesize enough nicotinic acid from available dietary tryptophane to metabolize these proteins and a nicotinic acid deficiency is called forth. By supplementing nicotinic acid to the diet the vitamin has a sparing action on tryptophane, thus allowing the amino acid to be utilized in the synthesis of protein, and simultaneously carries out its role in the deamination of amino acids.

Krehl et al. (1946) in studies with rats have emphasized the importance of intestinal synthesis of nicotinic acid. They state that corn alters the intestinal flora in such a manner that less nicotinic acid is synthesized in the tract. Thus, the animal loses a valuable source of the vitamin. They attribute the beneficial action of tryptophane and other proteins high in tryptophane as being due to the re-establishment of a desirable intestinal flora resulting in an increased synthesis of nicotinic acid in the tract. We feel, however, that in the case of the chick intestinal synthesis of nicotinic acid is of little consequence and that most of the synthesis occurs within the body tissues. Briggs et al. (1948) showed that only a small portion of the chick's nicotinic acid requirement, on diets similar to those used in our studies, is synthesized within the intestinal tract.

Since the tryptophane requirement of chickens is dependent upon the amount of nicotinic acid in the diet (and vice versa), it is difficult to attempt to set the dietary requirements for nicotinic acid or tryptophane unless the composition of the diet is considered. It is clear from our results that the amount of tryptophane supplied by 18 percent of casein (approximately 0.25 grams per 100 grams according to Block, 1945) is sufficient when ample nicotinic acid is present. The requirement of tryptophane for chicks, as determined by Grau and Almquist (1944), was 0.25 percent of the diet.

Our studies show that under extreme dietary conditions the laying hen requires nicotinic acid for maintenance of body weight, egg production, and hatchability. It is unlikely, however, that a nicotinic acid deficiency in hens ever occurs in the field for we have found hens to be extremely resistant toward this type of deficiency. On the other hand, the growing chicken is readily susceptible to a nicotinic acid deficiency. It is interesting to note in this connection that Berry, Carrick, Roberts, and Hauge (1943) and Gericke (1944) have presented evidence that certain practical chick mashes may be deficient in nicotinic acid. Richardson, Hogan, and Kempster (1945) noted an actual increase in the incidence of perosis in growing chickens when nicotinic acid was omitted from a practical diet "diluted" with a synthetic diet. Briggs et al. (1943) had previously observed perosis in nicotinic acid-deficient chicks.

Prange, Hauge, and Carrick (1927) reported that gelatin caused a depression in growth rate of chickens when added to a practical diet containing large amounts of corn and meat meal. Massengale (1929) reported that high levels of meat scraps (which contain considerable gelatin) in diets for poultry resulted in poor growth and in a condition similar to perosis. The addition of yeast or milk corrected the condition. It is quite possible in the latter instances that a nicotinic acid deficiency was produced although the knowledge of nicotinic acid in nutrition was not known at the time.

In the light of the present study and the recent work by Sarma and Elvehjem (1946) and Scott, Singsen, and Matterson (1946) on the influence of corn on the nicotinic acid requirement of growing chickens it is extremely important that nicotinic acid as well as protein quality be considered in formulating practical diets for chicks.

RESULTS, PART II

Experiments with Chicks on the Identification of a Growth Factor in Corn

When it became apparent that diet 113 (Table I) which was considered complete in all the known essential nutrients required by the chick, including nicotinic acid, could be further improved by the addition of 25 percent of ground yellow corn it was obvious that corn contained a factor which was lacking in diet 113 and which was needed by the chick for maximum growth. It should be emphasized, however, that diet 113, although highly purified, has consistently produced growth in chicks considerably greater than that obtained by feeding a good practical mash. The fact that diet 113 could be further improved then was somewhat surprising.

In reviewing the literature it was found that the studies reported by Stokstad, Manning and Rogers (1940) on a chick growth factor in polished rice indicated that several cereal products including corn promoted growth equally as well as polished rice when added to their simplified basal diet.

Later, Almquist, Stokstad, Mecchi, and Manning (1940a) presented evidence to show that the growth stimulating effect of polished rice could be duplicated by two substances, glycine and chondroitin, fed jointly. Further evidence by Almquist and co-workers (1940b) showed that the active constituent of chondroitin was glucuronic acid. Stokstad et al. (1941) demonstrated that several substances were capable of serving as the carbohydrate component (glucuronic acid) of the "rice factor" for chicks. These included gum arabic, arabinose, and xylose.

Our diet 113 differed from those employed by the aforementioned workers in that it was more highly purified. Furthermore, it was not deficient in glycine. It lacked, however, sources of the carbohydrate moiety which have been shown to be part of the "rice factor". With this information at hand several experiments were undertaken to determine whether our "corn factor" was related to some of the components of the "rice factor".

Experiment 1. In this experiment polished rice, chondroitin, pectin, and gum arabic were tested for potency, as was corn protein (zein). On hydrolysis, chondroitin yields a fairly high amount of glucuronic acid, pectin yields arabinose and galacturonic acid, and gum arabic yields arabinose and glucuronic acid. Thus, all these materials contain substances which have been shown to promote growth in chicks. In this experiment, pectin was fed at a lower level than gum arabic or chondroitin because of its tendency to form a gummy mass when moistened.

It was considered a possibility that the growth stimulus obtained with corn was produced indirectly through the action of intestinal bacteria which utilized something in corn for the synthesis of a factor which the chick used for growth. Therefore, sulfasuxidine was fed with corn, chondroitin, and gum arabic in an effort to suppress bacterial activity and thereby enforce a change in growth promoting ability. The results are summarized in Table XVIII.

It is apparent from these data that polished rice, like corn, gave a growth response when added to diet 113. Chondroitin and gum arabic behaved similarly in this respect whereas pectin was inactive. Apparently the chick is incapable of hydrolyzing pectin. Corn gluten meal, which contains more protein than corn, was active. However, when the purified corn protein, zein, was fed (in amount approximately 6 times greater than it occurs in 25 percent yellow corn) no significant increase in growth was obtained. This is evidence which precludes the growth factor in corn being related to protein. Sulfasuxidine was ineffective in reducing the

TABLE XVIII

EFFECT OF CORN PROTEIN, MATERIALS HIGH IN URONIC ACIDS AND OF SULFASUXIDINE ON CHICK GROWTH

GROUP NO.	SUPPLEMENT TO DIET 113	NO. CHICKS	NO. DIED	AVE. WT. 4 WEEKS GMS	FEED EFFIC-IENCY	FEATHER SCORE
1	None	6	0	277	.555	79
2	25% Ground yellow corn	6	0	372	.644	86
3	25% Corn gluten meal (44% protein)	6	0	369	.626	86
4	15% Zein	6	0	297	.610	80
5	25% Ground polished rice	6	0	338	.563	82
6	5% Chondroitin	6	0	346	.574	79
7	5% Gum arabic	6	1	367	.596	78
8	3% Pectin	6	0	283	.611	78
9	As 2 + 0.5% Sulfasuxidine	6	0	355	.628	80
10	As 6 + 0.5% Sulfasuxidine	6	2	356	.577	81
11	As 7 + 0.5% Sulfasuxidine	6	1	360	.572	83

growth promoting activity of corn, chondroitin, and gum arabic. Therefore, if bacteria are involved in this problem they are refractive to sulfasuxidine. The chicks which received corn and corn gluten meal utilized their feed most efficiently. No significant differences in feathering were observed.

Experiment 2. Diet 113 is devoid of fiber. Obviously fiber was supplied to this diet by the supplementation of corn. Therefore, it seemed desirable to see whether the effect of corn was due to its fiber content. Several sources of fiber were used. They were; ground corn cobs (prepared by coarse grinding of the cobs in a screw type grinder and then pulverizing in a Wiley mill), Cellu flour (a commercial product prepared from plant cellulosic residues and rated as having no feeding value), beet pulp (ground fine in a coffee grinder), oat hulls, and wheat bran (fed as coarse matter). Furfural was also tested because it is derived very easily from such fibrous materials as corn cobs and oat hulls by treatment with hot HCl. Table XIX gives the results obtained with these materials.

At a level of 3 percent wheat bran and ground corn cobs were equally as good as 25 percent of corn in promoting growth. The same levels of cellu flour, beet pulp, and oat hulls were somewhat less effective, but they did give a growth response. Most interesting, however, was the activity of furfural which, at a level of 0.5 percent, promoted growth equally as well as 25 percent corn. The groups receiving ground corn cobs, beet pulp, furfural, and corn had a slight advantage in feathering over the basal group.

Experiment 3. Gum arabic and chondroitin were shown to be active in experiment 1. In the present experiment the pentose arabinose (a hydrolytic product of gum arabic) and glucuronic acid (a hydrolytic product of both chondroitin and gum arabic) were tested. Ground corn cobs and furfural

TABLE XIX

EFFECT OF VARIOUS HIGH FIBER MATERIALS AND FURFURAL ON CHICK GROWTH

GROUP NO.	SUPPLEMENT TO DIET 113	NO. CHICKS	NO. DIED	AVE. WT. 4 WEEKS GMS	FEED EFFIC-IENCY	FEATHER SCORE
1	None	6	1	309	.634	81
2	25% Ground yellow corn	6	0	342	.609	85
3	3% Ground corn cobs	6	0	353	.617	88
4	3% Cellu flour	6	0	337	.609	79
5	3% Oat hulls (coarse)	6	0	325	.648	74
6	3% Ground beet pulp	6	1	354	.631	88
7	3% Wheat bran	6	0	354	.658	83
8	0.5% Furfural	6	0	342	.591	93

were retested. Xylose was assayed for growth promoting activity because it is a hydrolytic product of corn cobs which is rich in xylans. Although the chicken does not require a dietary source of ascorbic acid, this vitamin was tried because Luckey et al. (1946a) using a diet similar to our 115 reported it to be growth promoting. Hemoglobin values were determined for chicks on the basal group and the corn supplemented group since Luckey et al. (1946b) recently reported higher hemoglobin values as well as increased growth of chicks receiving a highly purified diet supplemented with corn. The results of this experiment are summarized in Table XX.

The growth responses which were obtained with ground corn cobs and furfural in experiment 2 were again obtained. One percent of arabinose gave a fair response while 0.3 percent of glucuronic acid and 0.3 percent of xylose showed slight activity. A good response was obtained, however, with 0.5 percent of ascorbic acid. Higher hemoglobin values were obtained when the diet contained 25 percent of corn. This confirms the observations of Luckey et al. (1946b). Little differences in feed efficiency were noted among the experimental groups. Corn, corn cobs, arabinose, furfural, and xylose produced better feathering.

Experiment 4. This experiment was designed to determine the growth promoting activity of wheat and oats as compared with corn; also, whether the energy portion of corn (corn starch) had activity. Ascorbic acid was retested at two levels. Sorbitol, chemically related to ascorbic acid, was also assayed.

The results of this experiment given in Table XXI show that 25 percent of wheat was as active as 25 percent of corn in promoting growth while 25 percent of oats was not so good as corn. Corn starch was inactive at

TABLE XX

EFFECT OF CORN ON HEMOGLOBIN VALUES AND THE EFFECT OF VARIOUS SUGARS, GLUCURONIC ACID, AND ASCORBIC ACID ON CHICK GROWTH

GROUP NO.	SUPPLEMENT TO DIET 113	NO. CHICKS	NO. DIED	AVE. WT. 4 WEEKS GMS	FEED EFFICIENCY	FEATHER SCORE	AVE. HEMOGLOBIN GMS %
1	None	6	0	325	.611	85	8.24
2	25% Ground yellow corn	6	0	406	.638	98	8.60
3	3% Ground corn cobs	6	0	383	.650	95	
4	1% Arabinose	6	0	363	.571	94	
5	0.5% Furfural	6	0	359	.640	93	
6	0.5% Ascorbic acid	6	0	370	.635	87	
7	0.3% Xylose	6	0	342	.600	93	
8	0.3% Glucuronic acid	6	0	350	.625	87	

TABLE XXI

EFFECT OF CORN STARCH, OATS, WHEAT, SORBITOL, AND ASCORBIC ACID ON CHICK GROWTH

GROUP NO.	SUPPLEMENT TO DIET 113	NO. CHICKS	NO. DIED	AVE. WT. 4 WEEKS GMS	FEED EFFIC-IENCY	FEATHER SCORE
1	None	6	2	270	.528	83
2	25% Ground yellow corn	6	0	358	.615	95
3	25% Corn starch	6	1	292	.527	74
4	25% Ground oats	6	0	335	.600	91
5	25% Ground wheat	6	0	365	.598	93
6	3% Sorbitol	6	0	280	.578	78
7	0.1% Ascorbic acid	6	1	288	.580	81
8	0.5% Ascorbic acid	6	0	373	.608	85

25 percent. Ascorbic acid at 0.5 percent was again active. However, 0.1 percent of ascorbic acid was inactive. Three percent of sorbitol was inactive. Better feathering was obtained in the groups receiving corn, wheat, and oats.

Experiment 5. Various levels of corn were tested to determine which level of supplementation produced maximum activity. Wheat and oats were retested as was polished rice. Xylose was retested at higher levels. Ground corn cobs and Cellu flour were also retested at higher levels. Stokstad et al. (1940) showed that small increases in growth occurred when either 5 percent soybean oil, wheat germ oil, or corn oil was added to their basal diet. Accordingly, corn oil was tried in this experiment alone and in combination with xylose. The results of the addition of all of these supplements are given in Table XXII.

It may be seen that maximum growth-promoting activity of corn is obtained at a level of 15 percent. Very good growth was obtained with 25 percent of wheat and slightly less growth was obtained with polished rice. In this trial, however, oats showed no activity. A marked growth response was obtained with 0.4 percent of xylose but no improvement was realized when the level was increased to 0.8 percent. Extremely good growth was observed with 5 percent of corn cobs whereas Cellu flour was much less active at the same level of supplementation. Corn oil at a level of 3 percent gave a good growth response. However, the activity of 3 percent of corn oil was lessened when in combination with 0.4 percent of xylose. This suggests that these two materials are incompatible in the diet.

TABLE XXII

EFFECT OF VARIOUS LEVELS OF CORN AND OF WHEAT, OATS, XYLOSE AND CORN OIL ON CHICK GROWTH

GROUP NO.	SUPPLEMENT TO DIET 113	NO. CHICKS	NO. DIED	AVE. WT. 4 WEEKS GMS	FEED EFFIC-IENCY	FEATHER SCORE
1	None	6	0	323	.654	79
2	5% Ground yellow corn	6	0	350	.636	93
3	15% Ground yellow corn	6	0	367	.677	91
4	25% Ground yellow corn	6	0	377	.651	92
5	Corn replacing cerelose(61.4%)	6	0	396	.664	96
6	25% Ground wheat	6	0	405	.657	94
7	25% Ground oats (coarse)	6	0	306	.621	81
8	25% Polished rice	6	0	368	.662	91
9	0.4% Xylose	6	1	385	.660	85
10	0.8% Xylose	6	0	365	.617	93
11	5% Ground corn cobs	6	0	411	.645	92
12	5% Cellu flour	6	0	351	.606	86
13	3% Corn oil (Mazola)	6	0	381	.643	87
14	As 13 + 0.4% Xylose	6	0	341	.573	84

DISCUSSION

The foregoing experiments demonstrate the existence of a growth promoting factor present in yellow corn. The factor is not related to corn protein (zein) nor to the energy yielding portion of corn (corn starch). Some activity was shown in corn oil. The greatest activity of a corn product was found in ground corn cobs. Moreover, this material possessed all the activity of yellow corn, 3 to 5 percent being as active as 15 to 25 percent yellow corn. Wheat bran was as active as ground corn cobs. Wheat and polished rice were equally as active as corn while oats gave inconclusive results. It is believed that the coarseness of grind of the oats which were used may have caused the inconclusive results; a pulverized grind would probably have been better tolerated by the chicks.

That fiber per se is not principally involved in this problem is evidenced by the fact that Cellu flour, although growth stimulating to some extent, was consistently inferior to ground corn cobs. Oat hulls and ground beet pulp also were less active than ground corn cobs. It is significant to note in this connection, however, that Woolley and Sprince (1945) found that a mixture of cellulose (Cellu flour), arginine, cystine, and glycine replaced an unidentified growth factor termed GPF-2 in guinea pigs.

A variety of pure substances were also shown to be active. They were chondroitin, gum arabic, glucuronic acid, arabinose, xylose, furfural, and ascorbic acid.

The data obtained in this investigation confirm the findings of Stokstad et al. (1940) that cereal products contain an essential factor required for maximum growth of chicks. They also confirm the reports of

(1) Almquist et al. (1940a), (2) Almquist et al. (1940b) and (3) Stokstad et al. (1941) that (1) chondroitin (2) glucuronic acid and (3) gum arabic, arabinose, and xylose have growth promoting activity in chicks. In addition, we have found independently that furfural and ground corn cobs are also growth promoting.

At first glance it may appear very confusing that such a variety of materials could have similar growth promoting abilities. But on closer examination it is apparent that certain relationships exist which reduce themselves to a more or less common basis for each material. These known relationships may be outlined as follows: One of the hydrolytic products of chondroitin is glucuronic acid. Gum arabic on hydrolysis yields arabinose and glucuronic acid. Glucuronic acid on decarboxylation gives xylose. Corn cobs on hydrolysis yield xylose. Thus it is apparent that active materials such as chondroitin, gum arabic, glucuronic acid and corn cobs can yield the pentose sugars xylose or arabinose which are themselves active. Furthermore, fibers such as Cellu flour contain small amounts of hemicelluloses which on hydrolysis yield some pentose sugars. It is therefore inferred that growth promoting activity of corn is related to its pentose sugar content or to substances which have the activity of pentoses.

Although arabinose and xylose have been shown to be growth promoting in chicks, very little is known regarding the physiology of these substances. It is known however, that the pentose sugar ribose is an important constituent of nucleic acids and pyridine and flavin nucleotides. It would appear then that the requirement of the growing chick for ribose would be very great because the process of growth involves an appreciable synthesis of nucleic acids. The fact that diet 113, which is devoid of pentose sugars produces very good growth in chicks suggests that the chick has the ability to synthesize appreciable quantities of ribose, but not enough for maximum growth. The source of material for this synthesis in the chick is unknown. However, it is conceivable that by supplying arabinose or xylose to the chick these pentoses may readily be converted to ribose, thus supplementing a limited ribose synthesis, thereby increasing growth. Since sufficient ribose was unavailable for a feeding experiment to determine the validity of a limited synthesis of ribose in the chick this viewpoint must still be proven.

Thus far, the growth promoting activities of furfural, ascorbic acid, and corn oil are still unexplained. Since furfural is a 5-carbon compound it is logical to assume that the chick can utilize the 5-carbon stem nucleus for the synthesis of its pentose sugar requirement. However, an explanation of the activities of corn oil and ascorbic acid are not so easily explained. If it is assumed that the chick has a limited capacity for ribose synthesis then one might explain the action of corn oil as being due to a sparing action on the ribose precursor, especially if the precursor were derived from carbohydrate. Or, in the case of corn oil and ascorbic acid the products of their metabolism in the tissues may lead to configurations from which pentoses can be readily synthesized.

With a view toward a practical application of these findings it would seem unlikely that chickens receiving a practical poultry mash would suffer from insufficient pentoses in their diet. Normally, such diets contain large quantities of corn, wheat, and wheat bran which are potent sources of pentose sugars. It would be interesting, however, and perhaps profitable, to investigate the extent to which ground corn cobs or corn and cob meal can be used in a practical mash as a substitute for wheat bran or wheat middlings.

In contrast to practical diets it is apparent that certain highly purified diets are deficient in sources of pentose sugars. It is important that where such diets are used in nutrition experiments a source of pentose sugars be provided as a part of the dietary.

CONCLUSIONS

1. The growth depression in chicks caused by the feeding of corn, in the absence of dietary nicotinic acid, is due in part at least to the nature of its' protein (zein).

2. The growth depression in chicks caused by the feeding of gelatin or zein, in the absence of dietary nicotinic acid, is due to the cumulative action of their amino acid constituents. Glycine, arginine, alanine, and probably proline are principally involved in the case of gelatin. Glutamic acid, leucine, alanine, proline, and phenylalanine are principally involved in the case of zein.

3. The growing chicken does not require dietary nicotinic acid when adequate amounts of tryptophane are present in the diet.

4. Nicotinic acid is concerned in some manner with the metabolism of amino acids.

5. The nicotinic acid requirement for maximum growth and the prevention of blacktongue and perosis in the New Hampshire chick is slightly in excess of 3.0 milligrams per 100 grams on a highly purified diet containing 10 percent of gelatin. With only 5 percent of gelatin in the diet the requirement is slightly in excess of 2.5 milligrams per 100 grams.

6. Feeding a high level of free glycine (8 percent) to chicks causes a peculiar enlargement of the eyeballs. The condition is not produced by feeding the same amount of glycine in the peptide form.

7. Under extreme dietary conditions the laying hen requires dietary nicotinic acid for normal egg production, hatchability, and maintenance of body weight. The adult chicken is more resistant to a nicotinic acid deficiency than the growing chicken.

8. Corn is an excellent feedstuff when properly supplemented with nicotinic acid and contains an essential dietary factor which is required by the chicken for maximum growth when fed highly purified diets. The factor is carbohydrate in nature since certain pentoses, especially xylose, or materials containing pentoses gave growth responses similar to that produced by corn.

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