TYROSINE METABOLISM IN THE CHICKEN

by

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B.S., The Catholic University, China, 1943

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1951

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INTRODUCTION

Although considerable work has been done on the metabolism of tyrosine, at the present time our knowledge of this field is still far from clear. Many investigations concerning tyrosine metabolism in the scorbutic guinea pig have been made and it has been shown that ascorbic acid plays a very important role in tyrosine metabolism. Considerable knowledge concerning the possible intermediary metabolism of this aromatic amino acid has been provided by studies of the metabolites found in the urine of many species under various experimental conditions. In this regard, the ingestion of extra tyrosine by the ascorbic acid deficient guinea pig resulted in the urinary excretion of the partial metabolites of tyrosine. The metabolism of tyrosine in the chicken has not been investigated. It is generally accepted that the chicken does not need ascorbic acid in the diet, but studies with purified rations have revealed that the ingestion of ascorbic acid proved beneficial. The effect of ascorbic acid on egg production or growth of birds on a normal diet has not been definitely determined.

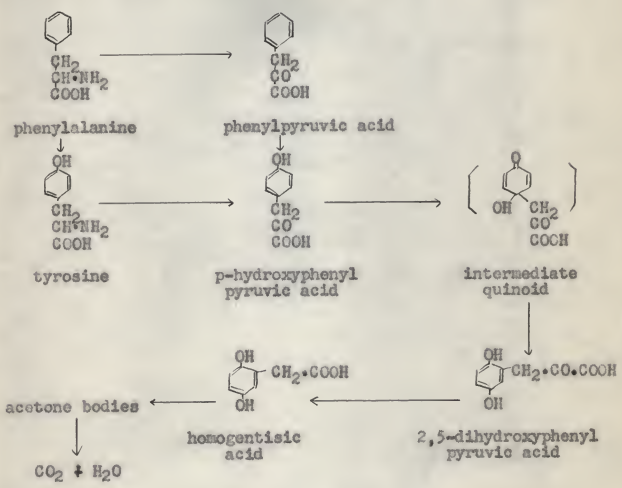
The following investigation was undertaken to study the relationship between tyrosine metabolism and ascorbic acid in the chicken with the ultimate purpose of attempting to determine the site, method and amount of synthesis of ascorbic acid in the chicken. The original goal proved too ambitious but the results did show that ascorbic acid and tyrosine were definitely related

in the chicken and the investigation did initiate the first step in the overall investigation mentioned above.

HISTORICAL

Tyrosine, an aromatic amino acid, was first discovered by Liebig (1) in 1846. Since that time, tyrosine has been used for experimental and theoretical work on the metabolic fate of this amino acid. Alcaptonuria, an inborn error of metabolism, was first reported by Boedeker (2) in 1861. He reported that the urine of his patients contained a substance which was easily oxidized and turned brown to black in alkaline solution. He called this substance alcapton. Thirty years after the original announcement on alcaptonuria, Wolkow and Baumann (3) isolated this substance "alcapton" from urine in the pure state and identified it as homogentisic acid, 2,5-dihydroxyphenylacetic acid. Quantitative studies on the homogentisic acid in the urine indicated that when extra tyrosine was fed, an increased amount of homogentisic acid was found in the urine. This indicated that tyrosine was the substance from which homogentisic acid was formed in the organism. In 1909, Neubauer (4) reported the oxidation of tyrosine in an alcaptonuric patient. He found that not only tyrosine and phenylalanine, but phenylpyruvic acid and p-hydroxyphenylpyruvic acid as well, were converted into homogentisic acid. This evidence led him to postulate a scheme for the intermediary metabolism of the aromatic amino acids. He

assumed that homogentisic acid is a normal intermediate in the catabolic reaction, and that the peculiarity of alcaptonuria consists in the lack of ability to carry the oxidation to completion beyond homogentisic acid. The mechanism postulated by Neubauer involves oxidation of p-hydroxyphenylpyruvic acid, and subsequent intramolecular rearrangement of the intermediate and unstable quinoid structure thus formed.



In 1938 Papageorge and Lewis (5) reported that after the daily oral administration of l-phenylalanine to experimental rats in amounts in excess of 0.3 gram per 100 grams body weight over a considerable period of time, homogentisic acid was excreted in the animal's urine. Excretion of homogentisic acid was demonstrated not only by qualitative and quantitative tests, but also by the preparation of dibenzoylhomogentisamide. The amide was characterized by its melting point and its elementary analysis. In the same year, Butts, Dunn and Hallman (6) studied the metabolism of dl-phenylalanine and dl-tyrosine in the normal rat. They found that dl-phenylalanine, fed at a level of 28.6 grams per sq. m. evoked an excretion of homogentisic acid. When dl-tyrosine was fed in an isomolecular amount, no trace of homogentisic acid appeared in the urine.

It has been shown that ascorbic acid was intimately connected with the metabolism of tyrosine. In 1939 Sealock and Silberstein (7) reported that when tyrosine was fed to the scorbutic guinea pig, homogentisic acid was excreted, and could be prevented by the administration of l-ascorbic acid. They also demonstrated that tyrosine fed to guinea pigs on a diet deficient in ascorbic acid resulted in the excretion of homogentisic, p-hydroxyphenyl-pyruvic and p-hydroxyphenylactic acids, and that the administration of l-ascorbic acid prevented the abnormal excretion of these metabolites. A new role for ascorbic acid, that of participating either directly or indirectly in tyrosine metabolism, was therefore uncovered. In 1939 Levine, Marples and Gordon (8) noted also

that premature infants fed diets of ascorbic acid free cow's milk at the rate of five grams or more of protein per kilogram of body weight excreted p-hydroxyphenylpyruvic acid and p-hydroxyphenyllactic acid. The excretion of these metabolites was prevented by the administration of ascorbic acid. The evidence suggested that the metabolic aberration in premature infants was an interrelated function of the level of intake of the aromatic amino acids, phenylalanine and tyrosine, and the degree of saturation of the tissues with ascorbic acid. In 1942, Sealock (9) found that the administration of a relatively small single dose of glutamic acid to the guinea pig receiving an extra dose of tyrosine with an ascorbic acid deficient diet will cause the removal of tyrosine metabolites from the urine. The effect observed is not permanent, however, for repeated doses of glutamic acid fail to produce the same effect. Later investigations of Tien Ho Lan and Sealock (10) contributed the further studies in vitro of the metabolism of tyrosine by liver and kidney tissues of normal and ascorbic acid deficient guinea pigs.

Chicks were used as experimental animal in 1945 by Hill, Slinger and Marcellus (11). The feeding of l-tyrosine in quantitative amounts varying from 0.5 to 5 percent of the ration fed to day old chicks showed no ill effects after a three weeks' feeding period. However, the feeding of a 9 percent level resulted in retarded growth. They emphasized that no other adverse effects were observed. They did not attempt to correct the condition by adding ascorbic acid to the diet. When the quantities of

l-tyrosine fed were increased, there was a decrease in the ascorbic acid content of the liver. This evidence pointed to an interrelationship between ascorbic acid and tyrosine metabolism in the chick.

In 1946, Basinski and Sealock (12) investigated the structural specificity of tyrosine in relation to the metabolic action of ascorbic acid. In this study a series of phenylalanine and tyrosine derivatives were fed to scorbutic guinea pigs with and without ascorbic acid supplementation. The compounds used were l-tyrosine, N-acetyl-l-tyrosine, diacetyl-l-tyrosine, d-phenylalanine, l-p-methoxyphenylalanine, dl-phenylaminobutyric acid and l-S-benzylcysteine. Each represents a specific modification of the original amino acid structure. The value reported for daily urinary excretion of keto acid and tyrosyl value for each compound exhibited no correlation with the state of ascorbic acid nutrition. From these observations it may be concluded that each one was metabolized independently of ascorbic acid by the guinea pig.

In 1946, Luckey et al. (13) worked on the activity of synthetic folic acid in purified rations for the chicks. The addition of 25 gamma percent of synthetic folic acid to the basal ration prevented the reduced growth, poor feathering, and low hemoglobin and hematocrit values ordinarily obtained when the ration was fed to chicks. Almost similar results were obtained when 25 gamma percent of crystalline vitamin B_c was added to the basal ration. Growth was improved with ascorbic

acid or whole liver powder in addition to the folic acid. The addition to the basal diet of 50 gamma percent of alpha-pyracin alone or with 10 to 50 gamma percent of synthetic folic acid produced slightly better growth.

Painter and Zilva (14) had observed that guinea pigs receiving large doses of l-tyrosine, orally or parenterally, while being deprived of l-ascorbic acid, excreted hydroxyphenyl compounds including p-hydroxyphenylpyruvic acid. The failure to rupture the benzene ring on a scorbutic diet requires the ingestion of at least 0.1 gram of tyrosine. This failure becomes effective very soon after the ascorbic acid is omitted from the diet and reaches a maximum in four or five days. The total amount of excreted hydroxyphenyl compounds increased as the dose of tyrosine increased. Homogentisic acid could not be found in urine even after continued administration of 0.5 to 0.8 gram tyrosine to guinea pigs on a scorbutic diet. In 1947 Neuberger et al. (15), reporting studies on an alcaptonuric patient, showed that homogentisic acid is the only aromatic substance in the urine in abnormal and detectable amount. About 80 to 85 percent of the phenylalanine or tyrosine is converted into homogentisic acid and other catabolic pathways can not account for more than 5 to 20 percent of the aromatic amino acids catabolized. Neither ascorbic acid, cysteine, nor methionine affect significantly the excretion of homogentisic acid. It was suggested that tyrosine is oxidized normally to dl-2,5dihydroxyphenylalanine. which is converted to homogentisic acid. Since the homogentisic acid level in plasma

is very low, and the renal clearance is very high, Neuberger assumed that either the structure was actually formed in the kidney or was actively and rapidly excreted by the tubules.

In 1947, Lepp, Moore, Elvehjem and Hart (16) reported the effect of ascorbic acid on the hemoglobin production in chicks. Chicks maintained on a purified diet containing dextrin as the carbohydrate and various levels of folic acid up to 200 micrograms percent showed increased hemoglobin values upon the addition of ascorbic acid to the diet. No effect of ascorbic acid was observed on dextrin diets supplemented with 500 micrograms percent folic acid. Ascorbic acid gave positive hemoglobin responses on all levels of folic acid up to 500 micrograms percent when sucrose was used as the carbohydrate. An indirect action of ascorbic acid on hemoglobin formation in the chick was suggested.

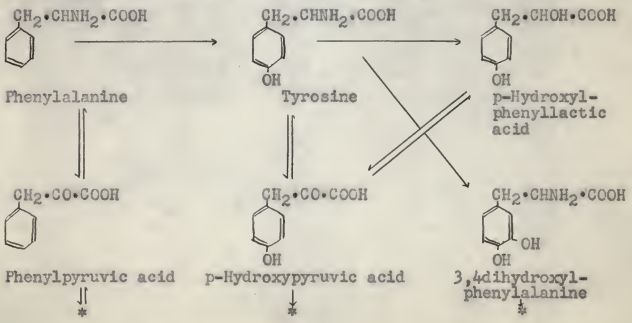
Rodrey, Swendseid and Swanson (17) studied tyrosine oxidation by livers from rats with a sulfasuxidine induced pteroylglutamic acid deficiency. This suggested an investigation to determine whether or not pteroylglutamic acid influenced the oxidation of tyrosine. Pteroylglutamic acid deficiency was produced in rats by placing them on a purified diet containing sulfasuxidine until leucopenia developed. Liver from the pteroylglutamic acid deficient rats were compared to livers from normal animals as to their effect on tyrosine oxidation in the Warburg technique. Addition of sulfasuxidine to normal liver suspension caused only a negligible inhibition of oxygen uptake compared with that of pteroylglutamic acid deficient liver. In other experiments, when crystalline

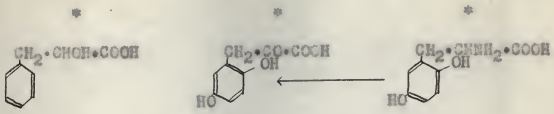
pteroylglutamic acid was added to flasks containing liver suspension from pteroylglutamic acid deficient rats, the effect on the oxidation of tyrosine was observed. The results indicated that pteroylglutamic acid influenced tyrosine metabolism. In 1948, Woodruff and Darby (18) observed in vivo the effect of pteroylglutamic acid upon tyrosine metabolism in the scorbutic guinea pigs. Phenolic compounds were increased in the urine of the patients with untreated pernicious anemia. Liver suspensions from pteroylglutamic acid deficient rats were able to better oxidize tyrosine after the addition of pteroylglutamic acid. This observation led the author to study the effect of this vitamin upon the metabolism of tyrosine in the scorbutic guinea pig. This defect in tyrosine metabolism was influenced by either pteroylglutamic acid or ascorbic acid.

Woodruff, Cherrington, Stockell and Darby (19) in 1949 observed the effect of pteroylglutamic acid and related compounds upon tyrosine metabolism in the scorbutic guinea pig. The hydroxyphenyl urea produced in guinea pigs, which were fed scorbutigentic diets containing 5 percent l-tyrosine, was elminated by both pteroylglutamic acid and its triglutamic homologue as well as by ascorbic acid. These pteroylglutamic acid derivatives did not protect the guinea pig against scurvy. The metabolism of tyrosine was further studied in four patients with scurvy and in three normal individuals by Rogeres and Gardner (20). In six of these individuals, 20 grams of tyrosine per day was given by mouth for periods varying from 5 to 21 days. The patients with scurvy, while on an ascorbic acid free diet and upon oral administration of tyrosine, excreted large amount of "tyrosyl" derivatives, and

the ability of the urine to reduce phosphomolybdic acid increased markedly. This was presumably due, in part, to the presence of p-hydroxyphenylpyruvic acid. The addition of ascorbic acid to the diet in the scorbutic patients resulted in a rapid decrease in the excreation of "tyrosyl" derivatives and the disappearance of the abnormal reducing material in the urine. It was concluded that individuals with scurvy had a marked defect in the metabolism of tyrosine and hydroxyphenyl compounds and that previous experiments with scorbutic animals and premature infants were, in general, in agreement with the findings reported here. It seemed unlikely that this defective metabolism of tyrosine played a significant role in the chemical picture of scurvy since the excretion of "tyrosyl" derivatioves were not present unless added tyrosine was given.

The mechanism of the metabolism of phenylalanine and tyrosine was postulated by Folling in Norway mentioned in Human Biochemistry (21). He observed that feebleminded children frequently excreted a considerable amount of phenylpyruvic acid.

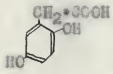




Phenyllactic acid

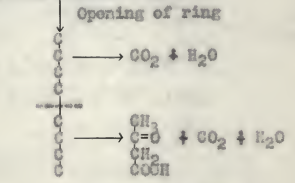
2,5-Dihydroxy-phenylpyruvic acid

2,5-Dihydroxyl-phenyl-alanine



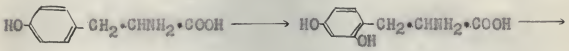
2,5-Dihydroxyphenyl acetic acid (Homogentisic acid)

Opening of ring



Acetoacetic acid

Another scheme for the intermediary metabolism of the aromatic amino acid was postulated by Neuberger (22) in 1948.



Tyrosine

2,4-Dihydroxylphenylalanine

2,4-Dihydroxylphenyl pyruvic acid

3,4-Dioxyphenyl acetic acid

6-Hydroxyl-3,4-dioxy-phenyl acetic acid

3,4,6-Trioxyphenyl acetic acid

3,6-Dihydroxyl-4,5-dihydroxyl-phenyl acetic acid

Homogentisic acid

Although, as indicated by the review of the literature, tyrosine metabolism has received considerable study, there has been up to the present time no investigation correlating the tolerance of animals on a tyrosine diet and the synthesis of ascorbic acid. In most cases scorbutic guinea pigs were used as experimental animals and in a few cases rats were employed. However, the results were different in different experimental animals. As previously mentioned the ingestion of ascorbic acid by chicks fed a purified ration has been beneficial, especially when the amount of folic acid was low. Therefore, it was considered desirable to undertake a study of tyrosine metabolism in chickens and to observe the amount of ascorbic acid needed for tyrosine metabolism in this experimental animal. Based on this information an attempt could be then made in the future to correct the purified rations and to ultimately determine how and where the ascorbic acid was biosynthesized.

EXPERIMENTAL

Two methods which could be adapted to study the relationship of tyrosine to ascorbic acid were used in this investigation. The first investigated the effect of long term feeding on the growth and well being of the chicks, and the other, the effect of certain tyrosine metabolites in the urine of the chicken. Although the growth experiments and the metabolism experiments were run concurrently, they are discussed separately.

Methods

Growth Experiments. In order to demonstrate the effect of various levels of tyrosine on growth and survival, a typical chick starter ration was supplemented with 0, 2.5, 5.0, 7.5 and 10 percent tyrosine. The composition of the chick starter ration (basal ration) is recorded in Table 1. In each case, the tyrosine supplementation was accomplished by adding the percentage of tyrosine in grams to 100 minus the percentage of tyrosine in grams of the basal ration. No attempt was made to determine the amount of tyrosine in the basal ration. Groups of 10 day old chicks were fed the chick starter ration and the various tyrosine supplemented ration. Ascorbic acid was administered both orally and by mixing with thediet in the amounts indicated. No attempt was made to administer the folic acid by mouth. The chicks were observed daily and weighed weekly.

Metabolism Experiments. The method of feeding and administering the tyrosine, ascorbic acid and folic acid varied according to the experiment and will be described subsequently in the appropriate experiment.

The method of collecting the urine for analysis was essentially that of Coulson and Hughes (23) and was performed as follows: The hen was restrained on a specially constructed holder (see Coulson and Hughes). Then the holder was placed at an angle of about 45 degree so that the urine would drain form the cloaca by means of a suitable catheter made by flaring a glass tube at one

Table 1. The composition of chick starter ration (Basal ration).

Ingredients	lbs.
Ground yellow corn	65
Wheat bran	4
Dehydrated alfalfa meal	1
Meat and bone scraps	5
Fish meal	5
Soybean oil meal	18
Steamed bone meal	1
$CaCO_3$	1
$MnSO_4$	25 g
Delsterol	40 g
Riboflavin	5 g
Prot-A	100 g
Ca pantothenate	100 mg

end so that the tube would be held in position by the constriction of the cloaca. Before inserting the catheter it was necessary to plug the intestine with cotton in order to prevent contamination of the urine with feces. The period of time need to collect a urine samples varied from bird to bird and with the conditions of the experiment. Since the amount of urine secreted depended upon the water intake, in most cases 50 ml of water was forced into the bird ten to thirty minutes before starting the urine collection. The water probably diluted the urine but should not have changed the relative amounts of tyrosine metabolites present. This method was limited in its application since it was not possible to obtain the total daily urine excretion, and it was impractiable to leave the hen trussed up on the board for a 24 hour period.

Analytical Procedures

Determination of Keto Acid Values. The method of Penrose and Quastal (24) was used for the determination of p-hydroxylphenylpyruvic acid in the urine. The keto acid reacts with 2,4-dinitrophenylhydrazine to form the corresponding 2,4dinitrophenylhydrazone which dissolves in alkaline solution with the development of a red color.

The chicken urine was centrifuged, and 1 ml of the clear urine added to 4 ml of water was treated with 5 ml of the filtered dinitrophenylhydrazine reagent. The mixture was well shaken. In

all cases of phenylketourea there is formed, almost immediately, a yellow opalescence, or a yellow precipitate. After 30 minutes at room temperature, the mixture was again well shaken and 2 ml were transferred to a test tube, to which 4 ml of 1N sodium hydroxide were added, the mixture was gently shaken, allowed to stand for a few minutes and then transferred to a flask containing 24 ml of water. The samples were compared in a Klett-Summerson Photoelectric Colorimeter using filter No. 540.

Lithium pyruvate was not available for the preparation of a standard curve. Since the analysis of normal urine was always negative, the keto acid value is recorded directly in Klett-Summerson Photoelectric Colorimeter readings.

Determination of Homogentisic Acid Values. The homogentisic acid content was determined by the method of Briggs(25). One ml of the chicken urine was diluted to about 15 ml in a 25 ml graduate test tube. Two ml of the molybdate solution and 2 ml of the phosphate solution were added, and the mixture diluted with water to the 25 ml mark. The flask was inverted for a few times so that the contents were well mixed. The samples were compared in a Klett-Summerson Photoelectric Colorimeter after 5 minutes using filter No. 420.

Hydroquinone was used as the standard in the quantitative determination of homogentisic acid. For this purpose 200, 400, 600, 800, 1000, 1200 and 1400 micrograms of hydroquinone were used as color was developed by the addition of 2 ml of 5 percent ammonium molybdate in 5 N sulfuric acid solution. The intensity of color was compared in a Klett-Summerson Photoelectric Colorimeter after 5 minutes by using filter No. 420.

Determination of Tyrosyl Values. The tyrosyl value was determined as described by Folin and Ciocalteau (26) 0.5 ml of urine was diluted with water to 250 ml. To 5 ml of the diluted urine 10 ml of 0.5 N sodium hydroxide and 3 ml of the phenol reagent were added. The solution was whirled during the addition of the phenol reagent. The color produced was read in the Klett-Summerson Photoelectric Colorimeter after 5 minutes.

The standard curve was determined by using 25, 50, 75, 100, 125 and 150 micrograms of tyrosine in solution. The intensity of the color produced by the addition of phenol reagent in alkaline solution by Folin's method was recorded by means of Klett-Summerson Photoelectric Colorimeter using filter No. 520.

Results

Growth Experiments. In the preliminary growth experiments it was necessary to determine the effect of ingested tyrosine on the growth and well being of the chick. This information was needed so that the amount of tyrosine necessary to produce an adverse effect could be determined. The basal ration was supplemented with 0, 2.5, 5.0 and 10 percent tyrosine and fed to groups of ten day old chicks for a period of four weeks. The results are recorded in Table 2.

In Table 2 the chicks fed 2.5 percent tyrosine (group II) grew as well as groups I (basal). Groups I and II averaged 211 and 212 grams respectively at four weeks of age, whereas group III averaged only 156 grams. Group IV, which received the 10

percent tyrosine weighed only 105 grams at the fourth week. It should be noted, however, that only one bird survived in the 10 percent tyrosine supplementation for four weeks. A further investigation of the data in Table 2 demonstrates that after the first week the number of birds surviving in group IV declined rapidly until one bird remained at the fourth week. This effect was not noticed in Group III which received 5 percent tyrosine. Two effects were therefore evident: first, the slower growth of the chicks receiving the ration containing 5 and 10 percent tyrosine and second, the inability of the chicks to survive on the ration containing 10 percent tyrosine.

The second growth series was undertaken to determine the effect of ascorbic acid on chicks fed a ration high in tyrosine. As previously mentioned, various investigators have shown that ascorbic acid was necessary for the metabolism of tyrosine. Most results to date have indicated that the chicks did not need added ascorbic acid when fed what could be considered a normal ration. In this case, however, the addition of tyrosine to the ration created a stress on the ascorbic acid synthesis by the bird. Therefore, the addition of ascorbic acid to the ration, should, to some degree, correct this condition. In this experiment one series of chicks were fed 0, 5, and 10 percent tyrosine in the ration and another group on similar diets fed 100 mg of ascorbic acid daily. For this purpose 100 mg of ascorbic acid were dissolved in 1 ml of water and fed to the chicks by pipette. The results of this experimental series are summarized in Table 3.

Table 2. The effects of tyrosine on the growth and survival of chicks.

Group	Diet	Weight in grams				
		Initial wt.	1 week	2 weeks	3 weeks	4 weeks
I	Basal	33 (10)[a]	45 (8)	95 (8)	159 (8)	211 (8)
II	Basal + 2.5% tyrosine	29 (10)	43 (9)	72 (9)	157 (9)	212 (9)
III	Basal + 5% tyrosine	31 (10)	52 (10)	73 (10)	106 (10)	156 (9)
IV	Basal + 10% tyrosine	33 (10)	37 (10)	46 (5)	79 (2)	105 (1)

[a]The numbers in brackets are the chicks in each group included in the average.

Table 3. The effect of ascorbic acid* on the growth and survival of chicks fed tyrosine.

Group	Diet	Weight in grams			
		Initial wt.	1 week	2 weeks	3 weeks
V	Basal	38 (10)[a]	68 (10)	110 (10)	170 (10)
VI	Basal + 100 mg ascorbic acid daily	38 (10)	69 (10)	96 (10)	116 (10)**
VII	Basal + 5% tyrosine	40 (16)	54 (14)	74 (14)	123 (14)
VIII	Basal + 5% tyrosine + 100 mg ascorbic acid daily	38 (16)	52 (16)	80 (14)	116 (14)
IX	Basal + 10% tyrosine	39 (16)	44 (14)	64 (4)	90 (4)
X	Basal + 10% tyrosine + 100 mg ascorbic acid daily	38 (10)	56 (10)	62 (10)	73 (8)**

*Ascorbic acid fed by pipette daily.
**Due to ascorbic acid feeding - soft heads, etc.
[a]The number in brackets are the chicks in each group included in the average.

The chicks fed with 0, 5 and 10 percent tyrosine, Group V, VII and IX, respectively, confirmed the results demonstrated in the first series. At the third week the basal chicks averaged 170 grams, the chicks fed with 5 percent tyrosine averaged 123 grams, the chicks fed with 10 percent tyrosine averaged 90 grams. In the same manner the survival of bird fed the 5 percent tyrosine (Group VII) was excellent, whereas only four chicks remained in Group IX which received 10 percent tyrosine.

A further examination of the data in Table 3 shows that the survival of the birds on fed 10 percent tyrosine, Group IX, was much better when the 100 mg of ascorbic acid was fed daily than Group X. The experiment was complicated, however, by the fact that in all cases the birds fed with ascorbic acid did not grow as well as those not receiving the ascorbic acid supplement. In all of the groups receiving ascorbic acid the beaks of the chicks became soft and in many cases bloody, and it was thought that probably the tender beaks of these chicks prevented the birds from eating normally.

The third series of experiments were run in which the ascorbic acid was mixed directly in the ration. For this series 500 mg of ascorbic acid were mixed with 100 grams of the ration. The percentage of tyrosine fed in the diet was 0, 5 and 7.5. In this case the 7.5 percent was used in order to conserve tyrosine. As in the previous series, three groups of chicks received 0, 5 and 7.5 percent tyrosine and three groups received similar ration containing 500 mg of ascorbic acid per 100 grams of diet. The results of

this phase of the investigation are recorded in Table 4.

As noted in the previous experiment, the chicks fed the tyrosine plus ascorbic acid did not die as fast as those receiving tyrosine supplementation. In this case, however, the chicks fed ascorbic acid in the ration grew at a faster rate than those that did not receive ascorbic acid. When ascorbic acid was added to the basal ration no effect was noted.

Various investigators have indicated that folic acid was related to tyrosine metabolism in scorbutic animals. Therefore the effect of added folic acid on the growth and well being of chicks on a high tyrosine diet was investigated. In the begining an attempt was made to employ a "Folic Acid Free" test diet which was supplied by a commercial firm. However this basal ration failed to support growth even when folic acid was added at the rate of 150 mg per 100 grams of diet. It was necessary therefore, to employ the same chick starter ration used in the previous growth experiments. Forty day old chicks were divided equally into four groups, and given a typical chick starter basal ration. This was supplemented with 7.5 percent tyrosine and 100 mg of folic acid per 100 grams of diet. In this series of experiments, the following rations were used, Group XVII, basal, Group XVIII, basal plus folic acid, Group XIX, basal plus 7.5 percent tyrosine and Group XX, basal plus 7.5 percent tyrosine, plus folic acid. The results are recorded in Table 5. The data in this table show that during the three weeks of the experimental period the growth of the chicks receiving 100 mg of folic acid per 100 grams of the

Table 4. The effect of ascorbic acid* on the growth and survival of chicks fed tyrosine.

Group	Diet	Weight in grams				
		Initial wt.	1 week	2 weeks	3 weeks	4 weeks
XI	Basal	38 (10)[a]	64 (10)	115 (10)	214 (10)	239 (10)
XII	Basal + ascorbic acid	39 (10)	62 (10)	113 (10)	191 (10)	323 (10)
XIII	Basal + 5% tyrosine	38 (10)	57 (10)	77 (10)	133 (9)	198 (9)
XIV	Basal + 5% tyrosine + ascorbic acid	38 (10)	58 (10)	94 (10)	151 (10)	231 (10)
XV	Basal + 7.5% tyrosine	38 (10)	52 (9)	70 (5)	103 (4)	134 (4)
XVI	Basal + 7.5% tyrosine + ascorbic acid	39 (10)	55 (9)	78 (8)	112 (8)	150 (8)

* Ascorbic acid mixed with the ration daily.
[a] The numbers in brackets are the chicks in each group included in the average.

Table 5. The effect of folic acid* on the growth and survival of chicks fed tyrosine.

Group	Diet	Weight in grams			
		Initial wt.	1 week	2 weeks	3 weeks
XVII	Basal	36 (10)[a]	75 (10)	132 (10)	227 (10)
XVIII	Basal + folic acid	41 (10)	75 (10)	137 (10)	206 (10)
XIX	Basal + 7.5% tyrosine	41 (10)	55 (9)	67 (8)	86 (7)
XX	Basal + 7.5% tyrosine + folic acid	40 (10)	53 (9)	65 (5)	108 (4)

* Folic acid: 100 mg of folic acid / 100 g of feed.

[a] The numbers in brackets are the chicks in each group included in the average.

basal ration was just the same as the chicks receiving the basal ration. On the other hand the growth of the birds receiving 7.5 percent tyrosine and folic acid was comparatively poor and the number of surviving birds declined, until only four chicks remained at the third week. This effect was duplicates in the 7.5 percent tyrosine feeding group and the addition of folic acid did not lower the death rate in this group. This experiment was repeated using five chicks per group and the results were essentially the same. In this case it was necessary to reduce the number of chicks to five because of the lack of sufficient supplement.

It has been demonstrated that the addition of high percentage of tyrosine to a basal ration will decrease the growth and chance of survival of chicks. This effect of tyrosine could be partially reversed by adding ascorbic acid to the ration. The addition of folic acid to the diet did not alleviate the conditions caused by tyrosine feeding.

Exhibits

Some idea of the physical condition of the birds fed these ration can be obtained from Figs. 1, 2, 3 and 4. Figure 1 is a composite picture of birds characteristic of the groups. There were no difference observed in Group V (basal) and VI (basal plus 100 mg ascorbic acid daily). Group IX which was fed 10 percent tyrosine shown in the Fig. 1 exhibited a peculiar feather condition

and in addition the feathers were chalk white. Group X, which was fed 10 percent tyrosine plus 100 mg of ascorbic acid daily, were more vigorous, and exhibited slightly is peculiar feather condition. The feathers however were chalk white.

The difference between the birds in Group IX and X are more noticeable in Fig. 2. The peculiar dropping of the wing was characteristic of the chicks fed the tyrosine plus ascorbic acid. The ascorbic acid led to better growth, better feathering and more active chicks.

Figure 3 is a comparison of Group V, fed basal and Group IX fed basal plus 10 percent tyrosine. The peculiar feathering is noticeable in Group IX.

Figure 4 is a comparison of Group VI, fed basal ration plus ascorbic acid, and Group X, fed basal ration plus 10 percent tyrosine plus ascorbic acid, the chalky appearance of the feathers in Group X is clearly evident in this picture.

Metabolism Experiments. Although the feeding and metabolism investigations were run concurrently, it soon became evident from the feeding experiments that the extra tyrosine in the ration was adversely effecting the chicks and that this effect could be corrected by incorporating ascorbic acid in the ration. From this it was surmised that the tyrosine feeding was depleting the ascorbic acid stores of the chick. It has been shown previously (7) that guinea pigs on an ascorbic acid deficient diet excreted tyrosine metabolites in the urine. Therefore, if the chicken could

IX X VI V

Fig. 1. Comparison of Chicks in Groups V, VI, X and IX.

V: Basal.

VI: Basal + ascorbic acid.

X: Basal + 10% tyrosine + ascorbic acid.

IX: Basal + 10% tyrosine.

IX X

Fig. 2. Comparison of Chicks in Groups IX and X.

IX: Basal + 10% tyrosine.

X: Basal + 10% tyrosine + ascorbic acid.

IX V

Fig. 3. Comparison of Chicks in Groups V and IX.

V: Basal.

IX: Basal + 10% tyrosine.

X VI

Fig. 4. Comparison of Chicks in Groups VI and X.

VI: Basal + ascorbic acid.

X: Basal + 10% tyrosine + ascorbic acid.

be shown to excrete these metabolites and that ingested ascorbic acid would correct this condition, it would be reasonable to assume that the high tyrosine diet was depleting the ascorbic acid stores of the bird.

Adult, non laying hens were used in the following metabolism investigations. The cost of the tyrosine and the other supplements led to the use of a few chickens in each experiment. In addition it should be emphasized that the metabolism phase was undertaken in an attempt to discover some metabolic relationship which would support the growth experiments and which could be developed later in purely metabolic investigation.

In a preliminary experiment a hen that had been on a normal diet was used, after taking an initial sample of urine she was fed 7 grams of tyrosine and another sample of urine collected 140 minutes after the feeding. It was shown that the tyrosyl value, homogentisic acid and keto acid values were practically zero throught the experiment. Since the ingestion of 7 grams of tyrosine did not upset the metabolism of the bird fed a normal diet and it was necessary in the following experiments to precondition the birds by feeding them tyrosine daily until the tyrosine metabolites appeared in the urine.

In the first metabolism series an attempt was made to demonstrate that adult chickens would excrete tyrosine metabolites when fed a high tyrosine supplement. Three pullets were fed a basal ration (Table 1) for the duration of the experiment. First, the urine of the birds were analyzed for tyrosyl, homogentisic acid

and keto acid values. The birds were then fed 1.5 grams of ascorbic acid for two days and the urine collected and analyzed on the fifth day. After the fifth day, 7 grams of tyrosine were administered daily for the remainder of the experiment. Urine samples were collected at certain intervals and analyzed for the metabolites mentioned above. The results of this series are reported in Table 6 and illustrated in Plate I.

As shown in Plate I, there was no regular trend in the tyrosyl value. Bird A excreted urine of a high tyrosyl value until the twelfth day of the experiment and then leveled off at a fairly constant value. Bird B, showed approximately the same results except that there was a general tendency for the tyrosyl value to increase after the eleventh day. Bird C exhibited a very high tyrosyl value from eleventh day through the twenty-third day.

In the case of homogentisic acid content, the analysis indicated a fairly constant value until the end of the experiment at which time they rose to a high value of about 150 mg per 100 ml of urine. In the same manner, the keto acid values were low in the early stages of the experiment and then rose to about 200 mg per 100 ml of urine. In every case the keto acid value increased before an increase occured in homogentisic acid content.

After the hens had been conditioned by feeding tyrosine for twenty three days, extra tyrosine was fed after the initial urine samples were obtained and urine samples collected at different intervals of time after the tyrosine was ingested. The results

EXPLANATION OF PLATE I

The Effect of Long Term Tyrosine Feeding on the Appearance of Tyrosine Metabolites in the Urine

Tyrosyl Value: mg per 100 ml of urine

Homogentisic Acid Value: mg per 100 ml of urine

Keto Acid Value: Klett-Summerson Photoelectric Colorimeter reading Filter 540

PLATE I

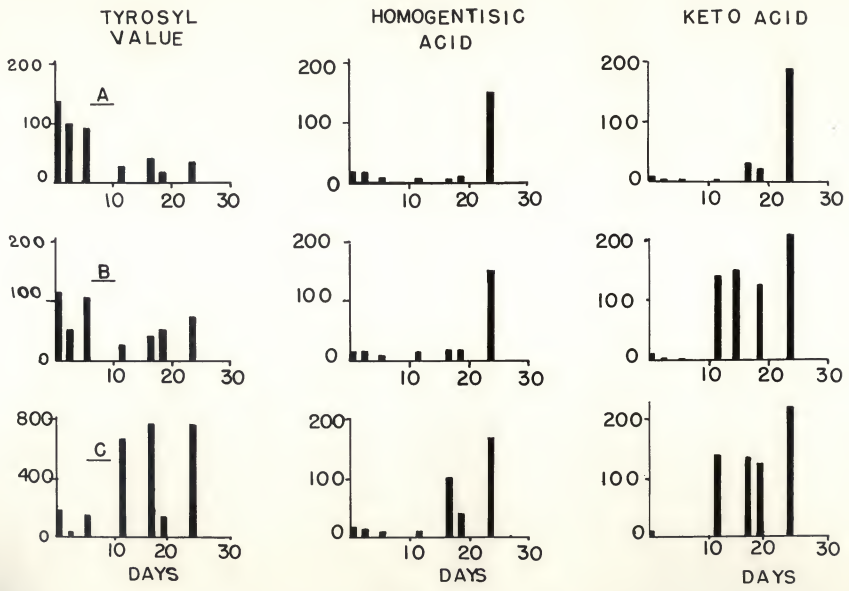

TYROSYL VALUE
HOMOGENTISIC ACID
KETO ACID
A
B
C
200
100
0
800
400
10
20
30
DAYS

Table 6. The effect of long term tyrosine feeding on the appearance of tyrosine metabolites in the urine.

Time	Tyrosyl Value[a]			Homogentisic Acid Value[b]			Keto Acid Value[c]		
	Chicken number								
	A	B	C	A	B	C	A	B	C
Zero Time	137	112	175	17	12	17	5	10	10
2 Days (d)	100	50	37	16	13	12	0	-	-
5 Days (d)	93	106	143	8	8	10	0	-	-
11 Days (e)	25	25	675	6	12	10	0	10	140
16 Days (e)	40	40	775	4	18	104	35	95	135
18 Days (e)	17	50	125	8	17	40	20	60	125
23 Days (e)	35	72	730	150	170	170	180	210	210

(a) mg/100 ml of urine
(b) mg/100 ml of urine
(c) Klett-Summerson Photoelectric Colorimeter reading Filter 540
(d) Fed 1.5 grams of ascorbic acid
(e) Fed 7 grams of tyrosine daily from the 5th day

of this experiment are shown in Plate II and Table 7. Different levels of tyrosine were fed: birds in experiment A received 5 grams of tyrosine, birds in experiments B, C and E received 10 grams of tyrosine and birds in experiment D received 7 grams of tyrosine. Tyrosine was fed in capsules shortly after the initial urine samples were obtained, and tyrosyl, homogentisic and keto acid values were determined at different intervals of time after the tyrosine was ingested.

In the case of tyrosyl value considerable variation occured in the results and, as shown in the previous experiment, no general trend was indicated. When 5 and 10 grams of tyrosine were fed, in Plate II, experiment A and C, the tyrosyl values increased whereas in experiment D and E in which 7 and 10 grams of tyrosine were fed, there was no great difference in tyrosyl value at different intervals. Experiment B was intermediate in that a slight increase of tyrosyl vlaue occured at about 210 minutes after the tyrosine was fed.

In these experiments, as in the proceeding experiment, much better agreement was obtained in the homogentisic acid and keto acid values after the tyrosine was ingested by the birds. Both homogentisic acid and keto acid values were increased from zero time up to 450 minutes. In one case, however, experiment D, which was fed 7 grams of tyrosine, the homogentisic acid values and keto acid values were increased from zero time to 140 minutes and then decreased at 200 minutes. This indicated that the birds fed tyrosine for a period of time before the metabolism test was run could not handle the extra tyrosine, as well as those birds which had not received the pre-condition tyrosine feeding.

EXPLANATION OF PLATE II

The Effect of Extra Ingested Tyrosine on the Appearance of Tyrosine Metabolites in the Urine of Chicken Pre-conditioned by Tyrosine Feeding

Tyrosyl Value: mg per 100 ml of urine

Homogentisic Acid Value: mg per 100 ml of urine

Keto Acid Value: Klett-Summerson Photoelectric Colorimeter reading, Filter 540

PLATE II

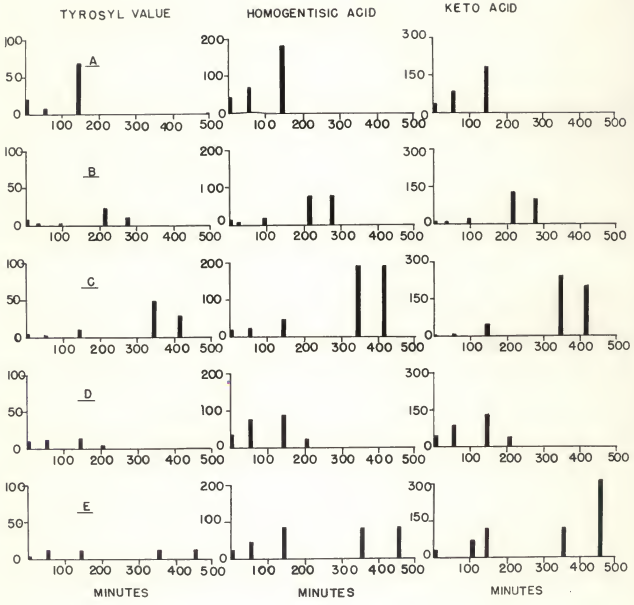

TYROSYL VALUE
HOMOGENTISIC ACID
KETO ACID
A
B
C
D
E
MINUTES
MINUTES
MINUTES

Table 7. The effect of extra ingested tyrosine on the appearance of tyrosine metabolites in the urine of chicken pre-conditioned by tyrosine feeding.

Feeding condition*	Time in minutes	Tyrosyl value		Homogentisic acid value		Keto acid value	
		Chicken number					
		A	B	A	B	A	B
A	0	16		41		35	
	50	7		69		80	
	140	70		180		185	
B	0	7		12		10	
	30	3		8		10	
	90	3		18		20	
	210	22		73		130	
	270	10		73		100	
C	0	3		19		0	
	50	3		21		5	
	140	10		43		50	
	340	48		190		240	
	410	27		190		200	
D	0		10		34		45
	50		12		77		90
	140		14		88		130
	200		3		23		35
E	0		3		21		30
	50		12		43		70
	140		12		84		115
	350		12		84		120
	450		12		84		300

* A - 5 grams of tyrosine
B -10 grams of tyrosine
C -10 grams of tyrosine
D - 7 grams of tyrosine
E -10 grams of tyrosine

Other investigators have shown that tyrosine metabolites characteristic of scurvy could be corrected by the addition of ascorbic acid. In the same manner it has been shown, previously in this investigation, that ascorbic acid would partially correct the effect of added tyrosine in the diet of the chicken. Therefore, the effect of ascorbic acid on the tyrosine metabolism of chickens, pre-conditioned by tyrosine feeding and then subjected to the metabolism test, was investigated.

In order to investigate the effect of added ascorbic acid on the appearance of tyrosine metabolites in the urine, the experimental birds which were pre-conditioned by tyrosine feeding, were fed ascorbic acid and tyrosine after the zero time urine samples had been obtained. The results of this experiment are summarized in Table 8 and Plate III.

In all cases in this experiment, a very high homogentisic acid value was obtained shortly after the tyrosine and ascorbic acid were fed. In the fourth case this rise was delayed until after 300 minutes and then a very high value was obtained. The ketoacid values, however, did not rise from the initial value as far as the keto acid values of the birds fed with tyrosine alone. An examination of Plate II (experiment B) and Plate III (experiment C) shows that in the case of feeding tyrosine the rise in the keto acid value was very great, whereas, with but one exception, the keto acid values shown in Plate III did not rise very far from the zero time sample. From this it was possible to speculate that the action of ascorbic acid in the metabolism of tyrosine

EXPLANATION OF PLATE III

The Effect of Ascorbic Acid and Extra Ingested Tyrosine on the Appearance of Tyrosine Metabolites in the Urine of Chickens Pre-conditioned by Tyrosine Feeding

Tyrosyl Value: mg per 100 ml of urine

Homogentisic Acid Value: mg per 100 ml of urine

Keto Acid Value: Klett-Summerson Photoelectric Colorimeter reading, Filter 540

PLATE III

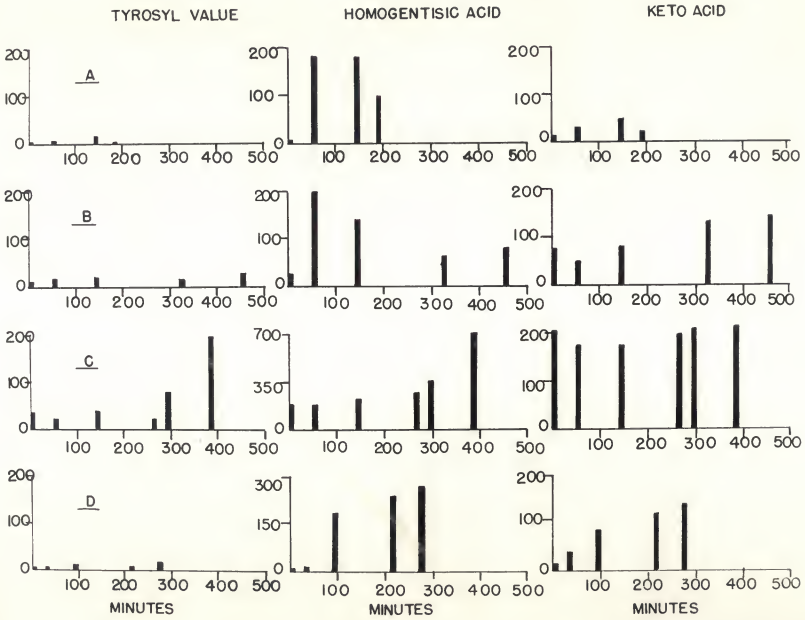
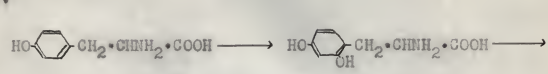

TYROSYL VALUE
HOMOGENTISIC ACID
KETO ACID
A
B
C
D
MINUTES
MINUTES
MINUTES

Table 8. The effect of ascorbic acid and extra ingested tyrosine on the appearance of tyrosine metabolites in the urine of chickens pre-conditioned by tyrosine feeding.

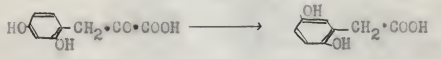
Feeding condition*	Time in minutes	Tyrosyl value		Homogentisic acid value		Keto acid value	
		Chicken number					
		A	B	A	B	A	B
A	0	2		6		10	
	50	7		180		25	
	140	12		180		45	
	185	3		97		20	
B	0		10		25		75
	50		18		200		50
	140		18		140		80
	320		12		56		130
	450		25		78		140
C	0	33		174		200	
	50	21		174		175	
	140	33		240		175	
	200	21		260		190	
	290	79		358		200	
	380	195		716		200	
D	0	3		8		15	
	30	3		12		40	
	90	10		186		85	
	210	7		240		120	
	270	17		270		140	

A - 1.0 gram of ascorbic acid and 7 grams of tyrosine
B - 1.6 grams of ascorbic acid and 10 grams of tyrosine
C - 1.3 grams of ascorbic acid and 10 grams of tyrosine
D - 1.3 grams of ascorbic acid and 10 grams of tyrosine

might be at the keto acid stage. For example, in the scheme for the intermediary metabolism of the aromatic acid by Neuberger (22).



tyrosine — 2,4-Dihydroxyphenylalanine



2,4-Dihydroxyphenyl pyruvic acid — Homogentisic acid

According to this scheme, therefore, the rise in homogentisic acid values and the leveled off keto acid value might be due to the action of ascorbic acid at the keto acid stage of tyrosine metabolism and the reaction might be blocked at the homogentisic acid and keto acid stage. In the presence of ascorbic acid, however, the metabolism of tyrosine would not be blocked at the keto acid stage. From the above we could conclude that the addition of ascorbic acid does, to a certain extent, alleviate the condition arising from the tyrosine ingestion and that we have an agreement between the feeding and metabolism phases of the experiment. The ascorbic acid did not entirely relieve the condition, however, and this led to a search for other means of lowering the amount of tyrosine metabolites in the urine.

Some evidence in the literature has indicated that tyrosine metabolism was altered in pernicious anemia (17) and that a

pteroylglutamic acid deficiency existed in this disease. It has been shown also that sulfasuxidine would interfer in the metabolic function of pteroylglutamic acid. In the following metabolism series, the influence of sulfasuxidine and pteroylglutamic acid on tyrosine metabolism was investigated.

Three birds were deprived of pteroylglutamic acid by placing them on a basal ration supplemented with 0.5 gram of sulfasuxidine daily. At the sixth day, 10 grams of tyrosine were fed, after the first urine sample was collected and then, the urine samples were collected at definite period of time and the metabolites determined. The results of this experiment are reported in Table 9 and Plate IV.

The experiments A, B, C and D (Plate IV) in which the birds were fed sulfasuxidine and then fed a dose of tyrosine were remarkably similar to those obtained when birds were pre-conditioned by tyrosine feeding (Plate II). From this it was assumed that folic acid was involved in tyrosine metabolism in the chicken. This possibility was investigated with a bird which had been preconditioned by tyrosine feeding and then fed 7 grams of tyrosine and 250 mg of folic acid shortly after the zero time urine sample was obtained. The results of this experiment were shown in Plate VI and Table 11. It should be noted that bird A showed the lower tyrosyl, homogentisic and keto acid values. Plate VI also contains a summary of birds B and C which had been preconditioned with sulfasuxidine feeding and then fed tyrosine and folic acid after the zero time urine sample was obtained. The

EXPLANATION OF PLATE IV

The Influence of Sulfasuxidine Feeding on the Effect of Extra Ingested Tyrosine on the Appearance of Tyrosine Metabolites in the Urine of Chickens Pre-conditioned by Sulfasuxidine Feeding

Tyrosyl Value: mg per 100 ml of urine

Homogentisic Acid Value: mg per 100 ml of urine

Keto Acid Value: Klett-Summerson Photoelectric Colorimeter reading, Filter 540

PLATE IV

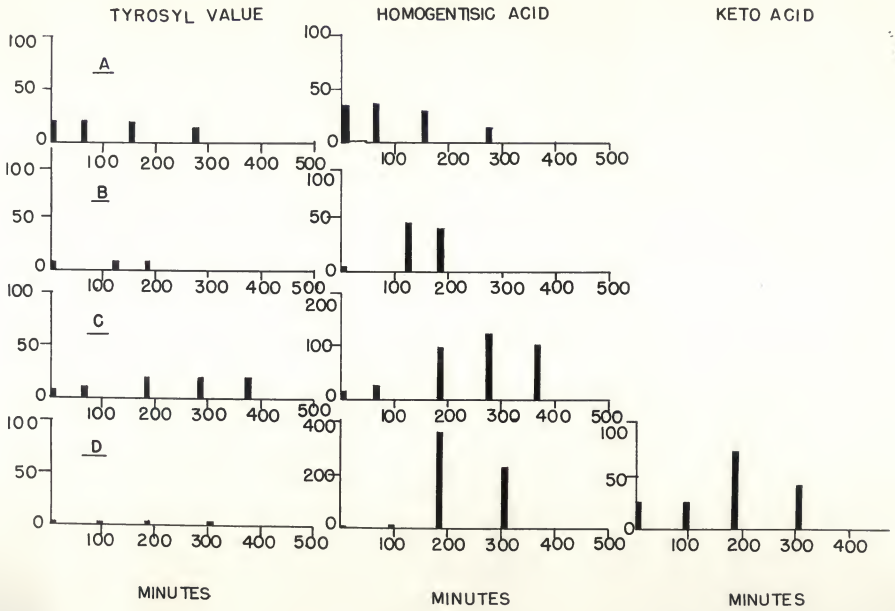

TYROSYL VALUE
HOMOGENTISIC ACID
KETO ACID
A
B
C
D
100
50
0
200
300
400
500
MINUTES

Table 9. The influence of sulfasuxidine feeding on the effect of extra ingested tyrosine on the appearance of tyrosine metabolites in the urine of chickens pre-conditioned by sulfasuxidine feeding.

Feeding condition*	Time in minutes	Tyrosyl value		Homogentisic acid value		Keto acid value	
		Chicken number					
		A	B	A	B	A	B
A	0	19		35		0	
	60	19		35		0	
	150	19		27		0	
	270	12		13		0	
B	0	7		4		-	
	120	7		45		-	
	180	7		40		-	
C	0	7		15		-	
	60	10		25		-	
	180	18		96		-	
	270	19		123		-	
	360	19		101		-	
D	0		2		1		25
	90		2		10		25
	180		3		340		73
	300		2		230		40

*
A - 0.5 gram of sulfasuxidine and 10 grams of tyrosine
B - 0.5 gram of sulfasuxidine and 10 grams of tyrosine
C - 0.5 gram of sulfasuxidine and 10 grams of tyrosine
D - 3.0 grams of sulfasuxidine and 10 grams of tyrosine

EXPLANATION OF PLATE V

The Influence on Ascorbic Acid upon the Extra Ingested Tyrosine on the Appearance of Tyrosine Metabolites in the Urine of Chickens Pre-conditioned by Sulfasuxidine Feeding

Tyrosyl Value: mg per 100 ml of urine

Homogentisic Acid Value: mg per 100 ml of urine

Keto Acid Value: Klett-Summerson Photoelectric Colorimeter reading, Filter 540

PLATE V

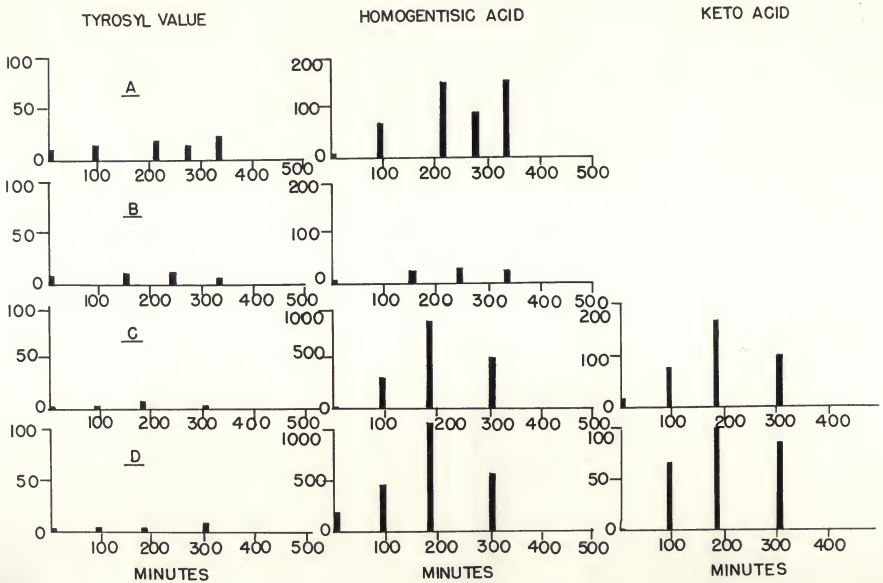

TYROSYL VALUE
HOMOGENTISIC ACID
KETO ACID
A
B
C
D
100
50
0
200
100
1000
500
100 200 300 400 500
MINUTES

Table 10. The influence of ascorbic acid upon the extra ingested tyrosine on the appearance of tyrosine metabolites in the urine of chickens pre-conditioned by sulfasuxidine feeding.

Feeding condition*	Time in Minutes	Tyrosyl value		Homogentisic acid value		Keto acid value	
		Chicken number					
		A	B	A	B	A	B
A	0	10		9		-	
	90	12		67		-	
	210	18		146		-	
	270	12		86		-	
	330	21		146		-	
B	0		75		7		-
	150		10		24		-
	240		12		25		-
	330		7		33		-
C	0		2		3		15
	90		2		290		75
	180		7		840		165
	300		2		480		95
D	0		2		190		50
	90		3		450		68
	180		3		1060		100
	300		7		560		65

*

A - 0.5 gram of sulfasuxidine, 0.4 gram of ascorbic acid and 10 grams of tyrosine

B - 0.5 gram of sulfasuxidine, 0.4 gram of ascorbic acid and 10 grams of tyrosine

C - 3.0 grams of sulfasuxidine, 0.4 gram of ascorbic acid and 10 grams of tyrosine

D - 3.0 grams of sulfasuxidine, 0.4 gram of ascorbic acid and 10 grams of tyrosine

EXPLANATION OF PLATE VI

The Effect of Pteroylglutamic Acid and Extra Ingested Tyrosine on the Appearance of Tyrosine Metabolites in the Urine

Tyrosyl Value: mg per 100 ml of urine

Homogentisic Acid Value: mg per 100 ml of urin

Keto Acid Value: Klett-Summerson Photoelectric Colorimeter reading, Filter 540

PLATE VI

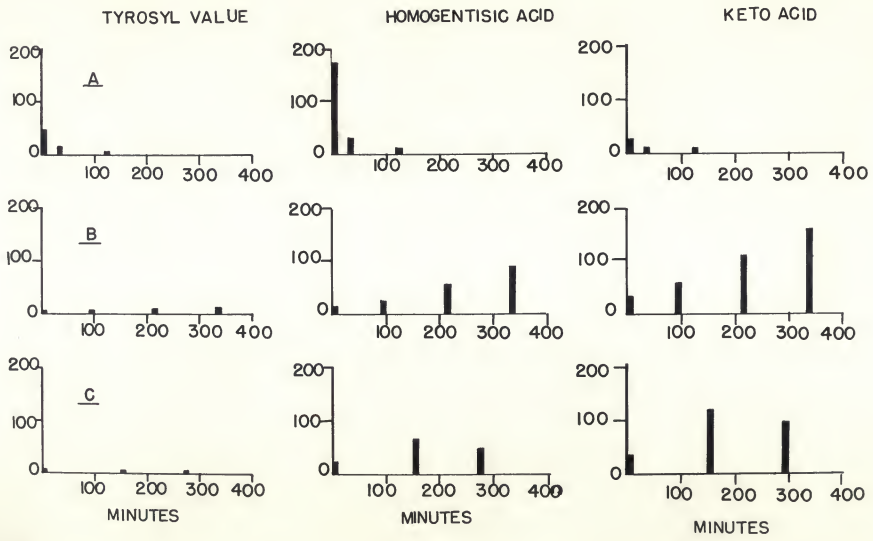

TYROSYL VALUE
HOMOGENTISIC ACID
KETO ACID
A
B
C
200
100
0
100
200
300
400
MINUTES

Table 11. The effect of pteroylglutamic acid and extra ingested tyrosine on the appearance of tyrosine metabolites in the urine.

Feeding condition*	Time in minutes	Tyrosyl value			Homogentisic acid value			Keto acid value		
		Chicken number								
		A	B	C	A	B	C	A	B	C
A	0	47			170			25		
	30	15			29			10		
	120	3			10			10		
	210	-			-			-		
B	0		3			13			31	
	90		7			22			56	
	210		10			56			109	
	330		12			86			165	
C	0			3			22			35
	150			3			58			120
	270			3			48			100

*

A - 100 mg of folic acid and 7 grams of tyrosine
B - 0.5 gram of sulfasuxidine, 250 mg of folic acid and 10 grams of tyrosine
C - 250 mg of folic acid and 10 grams of tyrosine, another 250 mg of folic acid as extra dose

Bird A pre-conditioned by tyrosine feeding
Birds B and C pre-conditioned by sulfasuxidine feeding

results of this experiment were similar to those in Plate IV when no folic acid was fed. Evidently the pre-feeding with sulfasuxidine had an adversed effect upon the ability of bird to metabolize tyrosine even when folic acid was ingested, possibly a high dose of folic acid would overcome the effect of sulfasuxidine. The expense involved in throughly investigating all the metabolic pathway was beyond the scope of this study. However, an attempt was made to determine whether or not ascorbic acid would reverse the effect of sulfasuxidine feeding. The results of this experiment are summarized in Table 10 and Plate V. Bird A was pre- conditioned by sulfasuxidine feeding, and then fed 10 grams of tyrosine and 0.4 gram of ascorbic acid shortly after the zero time samples were obtained. Ascorbic acid did prevent the large increase in keto acid and homogentisic acid values.

It was concluded that (a) chickens pre-conditioned by tyrosine feeding would excrete homogentisic acid and keto acid in the urine after being on this feed for 12 to 20 days, (b) when the birds were pre-conditioned by tyrosine feeding or by feeding sulfasuxidine, large increase in homogentisic acid and keto acid values would be found shortly after a large dose of tyrosine was fed, (c) when ascorbic acid was added along with tyrosine there was no large increase of keto acid although the homogentisic acid would rise to a high value, (d) in the experiment, in which folic acid was fed to birds previous tyrosine feeding, the folic acid appeared to reverse the action of sulfasuxidine. Ascorbic acid was unable

to prevent the rise of tyrosine metabolism in the birds fed sulfasuxidine.

DISCUSSION

Both the growth experiments and the metabolism experiments demonstrated that ascorbic acid plays a significant role in tyrosine metabolism in the chicken. Various levels of tyrosine in the diet influenced the growth and well being of the chicks. Symptoms, such as slower growth and chalk white wings, were shown by the chicks fed a diet containing 5 percent tyrosine, and chicks fed 10 percent tyrosine did not survive. The magnitude of these effects was corrected to a certain extent by the addition of ascorbic acid. The effect of added pteroylglutamic acid on the growth and well being of the chicks on a high tyrosine diet has been investigated, and folic acid did not alleviate the conditions caused by tyrosine feeding.

In the metabolism investigation, the administration of extra dose of tyrosine caused an increase in the amount of tyrosine metabolites in the urine and ingested ascorbic acid partially corrects this condition. On this point, therefore, there was agreement between the feeding and metabolism phases of this investigation. On the other hand, although ingested sulfasuxidine also interfered with the metabolism of tyrosine, neither ascorbic acid nor folic acid corrected the abnormal condition caused by the sulfasuxidine. In one experiment, ingested folic acid corrected the condition caused by the high tyrosine diet. It may be a matter of metabolic balance between the compounds used but further investigation along this line was beyond the scope of this investigation.

Hill and his co-workers (11) reported that the feeding of tyrosine at a 9 percent level to day old chicks caused retarded growth, but they did not attempt to correct this condition by adding ascorbic acid to the diet. The fact that the birds did not survive the 10 percent tyrosine supplementation was not observed by these investigators.

Sealock and Silberstein (7) in 1939, reported that when tyrosine was fed to the scorbutic guinea pigs, homogentisic acid was excreted, but could be prevented by the administeration of ascorbic acid. They also demonstrated that tyrosine fed to guinea pigs on a diet deficient in ascorbic acid resulted in the excretion of homogentisic acid and that the administration of ascorbic acid prevented the abnormal excretion of these metabolites. It is possible that the ascorbic acid stores of the chickens were depleted by high tyrosine diet and therefore, they excreted tyrosine metabolites.

The chickens fed 7 grams of tyrosine daily for 23 days did eventually excrete tyrosine metabolites in the urine. On the basis of 1 mg of ascorbic acid for 100 mg of tyrosine, which has been shown by Sealock (27) to be the ratio of ascorbic acid to tyrosine needed for normal metabolism, the chicken probably did not synthesize the 70 mg of ascorbic acid needed to metabolize the 7 grams of tyrosine. However, there was individual variation for all the chickens on the tyrosine supplement did not excrete tyrosine metabolites at the same time. Much more work is needed on this phase of work.

It was definitely shown that the chicken needs ascorbic acid and that the birds in all probability, synthesized this vitamin.

At the present time, the site and mechanism of this synthesis is not known and in future investigations, the more modern methods of studying enzyme action may be the best means of discovering the site and the method of synthesis of this vitamin.

SUMMARY

1. The effect of ingested tyrosine on the growth and well being of the chicks was shown by the slower growth of the chicks receiving the 5 and 10 percent tyrosine, and by the inability of the chicks on 10 percent tyrosine to survive.

2. Chicks, which had been fed upon a high tyrosine ration to which ascorbic acid was added, showed better growth, better feathering and a greater general activity than upon such rations which contained no ascorbic acid.

3. The addition of folic acid to the diet did not alleviate the conditions caused by tyrosine feeding.

4. The chicken pre-conditioned by high tyrosine feeding excreted tyrosine metabolites, and this conditon was partially corrected by the addition of ascorbic acid to the diet.

5. The chicken, pre-conditioned by high sulfasuxidine feeding, showed a large increase in homogentisic acid and keto acid values after an extra dose of tyrosine was fed. Ascorbic acid was unable to eliminate the tyrosine metabolites in the urine of the chickens fed sulfasuxidine.

6. The ingested folic acid did not correct the tyrosine metabolites in the urine of the chicken fed sulfasuxidine.

ACKNOWLEDGMENT

The author wishes to express her sincere appreciation to Dr. R.E. Clegg, Associate Professor in the Department of Chemistry, for the aid and guidance tendered during the course of this investigation. The author wishes to thank Dr. Paul E. Sanford, Associate Professor in the Department of Poultry Husbandry for his constant encouragement and his provision of the facilities for the animal experimentation required for this study.

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TYROSINE METABOLISM IN THE CHICKEN

by

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B.S., The Catholic University, China, 1943

AN ABSTRACT OF
A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1951

This investigation was undertaken to study the relationship between tyrosine metabolism and ascorbic acid in the chicken. The experimental results did show that tyrosine and ascorbic acid were definitely related in the chicken.

In this investigation two effects were studied, first the effect of long term tyrosine feeding on the growth and well being of the chicks and second the effect of certain dosages of tyrosine and ascorbic acid on the amount of excretion of certain tyrosine metabolites in the urine of the chicken.

The preliminary experiments had demonstrated the effect of various levels of tyrosine on growth and survival of the chicks. This information was needed so that the amount of tyrosine necessary to produce an adverse effect could be determined. The basal ration was supplemented with different levels of tyrosine and fed to groups of ten, day old chicks for a period of four weeks. Ascorbic acid was administered both orally and by mixing with diet. No attempt was made to administer the folic acid orally. The chicks were observed daily and weighed weekly.

The effect of ingested tyrosine on the growth and well being of the chicks was shown by the slower growth of the chicks receiving the 5 and 10 percent tyrosine. The influence of ascorbic acid on chicks fed a ration high in tyrosine was shown by better growth, better feathering and better survival of the chicks. The addition of folic acid to the diet did not alleviate the conditions caused by tyrosine feeding.

In the metabolism investigations, adult, non-laying hens were used as experimental animals. The extra ingested tyrosine did not upset the metabolism of the birds fed a normal diet, therefore, it was necessary to pre-condition the birds by feeding them tyrosine daily until the tyrosine metabolites appeared in the urine. The urine of the birds were analyzed for tyrosyl, homogentisic and keto acid values. After the hens hadbeen pre-conditioned by feeding tyrosine, the extra dose of tyrosine, ascorbic acid with tyrosine and folic acid were fed after the initial urine samples were obtained and urine samples collected at different intervals. Some evidence in the literature had indicated that tyrosine metabolism was altered in prenicious anemia and that a pteroylglutamic acid deficiency existed in this disease. It has also been shown that sulfasuxidine would interfer the metabolic function of pteroylglutamic acid. Therefore, three hens were deprived of pteroylglutamic acid by placing them on a basal ration supplemented with sulfasuxidine. After the birds were pre-conditioned by sulfasuxidine feeding, extra dose of tyrosine, ascorbic acid, together with tyrosine and folic acid were fed after the first urine samples were collected. The urine samples were collected at different period of time and the metabolites determined.

The metabolism investigations showed that the chicken, pre-conditioned by high tyrosine feeding, excreted tyrosine metabolites and that this condition was partially corrected by the addition of ascorbic acid to the diet. The chickens pre-conditioned by high sulfasuxidine feeding gave a large increase in homogentisic acid

and keto acid after an extra dose of tyrosine was fed. Ascorbic acid was unable to eliminate these metabolites in the urine of the chickens fed sulfasuxidine. In addition, ingested folic acid did not eliminate the tyrosine metabolites in the urine of the chickens fed sulfasuxidine.