THE NICOTINIC ACID CONTENT OF SOUTH AFRICAN MAIZE

AND

THE POSSIBILITY OF BREEDING VARIETIES WITH A HIGH NICOTINIC ACID CONTENT

Thesis

Presented for the Degree of Doctor of Philosophy

by

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CONTENTS

INTRODUCTION

Maize is a crop of primary importance in South Africa as it serves as the principal food of seven million Africans. Pellagra endemic areas are almost always found where maize constitutes a large proportion of the diet of the population. The countries of South Eastern Europe, Turkey, Egypt, and the Southern States of the United States of America constitute well known examples of such areas. Dietary deficiencies seldom prove to be simple single factor deficiencies. Lack of nicotinic acid was considered as the main factor in pellagra but recent investigations (65) have revealed that adequate tryptophane in the diet reduces the minimal level of nicotinic acid intake that is necessary to prevent pellagra symptoms and that the presence of the plant auxin indole-3-acetic acid demands an enhanced intake of nicotinic acid (82). Evidence has also been presented that tryptophane may be the precursor of nicotinic acid in the body (68)(69) and very recent work (76)(77) has indicated that pyridoxine plays a vital role in this process. In the light of the foregoing the association of maize with pellagra is no longer surprising since it is known that maize is low in nicotinic acid and tryptophane and high in indole-3-acetic acid.

When maize forms a large part of the diet of any group of people, pellagra can be prevented by increasing the amount of nicotinic acid or by increasing the amount of tryptophane in the diet. The high cost and inadequate supplies of most foods rich in tryptophane such as milk, meat, peas and beans, may preclude the latter alternative. On the other hand the possibility of increasing the nicotinic acid content of maize by genetical means presents a promising field.

The great diversity of soil and climatic conditions which characterise the maize growing region of South Africa necessitate the cultivation of a number of varieties. Current breeding work aims primarily at developing high yielding varieties, resistant to drought and disease, which will be capable of growing well on soils of low fertility. There is in the country today a demand for the large, flat, white type of seed and this preference is particularly unfortunate in view of the higher nutritional standard of yellow maize. It is clear that in order to improve the nutritive value of a crop a balance must be struck between breeding solely for yield and the so called market qualities and breeding to improve the nutritional quality.

This investigation covers the first stages in a programme of work to determine the possibilities of selecting and breeding strains of maize with adequate amounts of nicotinic acid. The first step involves the choice of a suitable method for the determination of nicotinic acid in maize; the survey of the nicotinic acid content of commercial varieties, of pure lines and of crosses between pure lines in order to determine whether the nicotinic acid content is governed by genetic factors.

In the presentation of the first paper it has been considered advisable to review the literature over a much wider field than the limited aspects covered by this investigation. A wide survey serves the purpose of setting the limited aims of the present paper in its correct perspective relative to the larger issues; it may also assist other investigators who follow in this field of work.

The production of a maize with a high level of nicotinic acid would be a step of considerable value and immediate consequence in reducing the incidence of pellagra. It remains, however, essential to determine whether the process of improving the one factor does not simultaneously produce a serious deficiency of another. This might mean that concurrently with reduction of the incidence of one disease there might occur the progressive development of another. This research confines itself to nicotinic acid but it is hoped that an extensive study of the other B complex vitamins and essential amino acids will be initiated along similar lines. In view of the interesting relationship between nicotinic acid, tryptophane and indole-3-acetic acid in the production of pellagra an investigation of these three compounds in maize might also prove of considerable interest and importance.

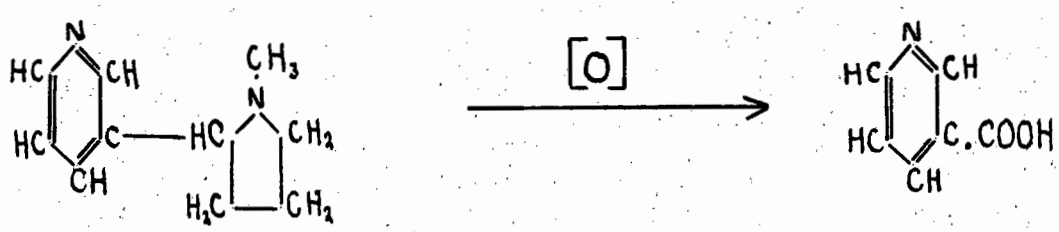
It is necessary to indicate here certain limitations that were imposed on the present investigation. The material for the chemical analyses was supplied by the Potchefstroom College of Agriculture where the cultivation and breeding of the maize was carried out. In view of the rigid seasonal factor involved in their programme of growing pure lines in order to produce material for crosses, analytical data on the pure lines had to be available by certain dates. Whilst the method used for determining the nicotinic acid in the routine analysis of these samples was later shown to give low figures the reproducibility was nevertheless of a sufficiently high order to continue the method for all the subsequent crosses so that the data for the analyses of varieties, pure lines and crosses are strictly comparable.

Unavailability of chemicals also placed a very strict limitation to the final choice of method to be used for analysis. In the continuation of this work improvements in the chemical estimation of nicotinic acid or the adoption of microbiological methods can be and probably will be made but the value of the data presented here will remain and can be related to later results through correction factors as between different methods of estimation.

REVIEW OF LITERATURE

Basic Role of Nicotinic Acid in Plants and Animals

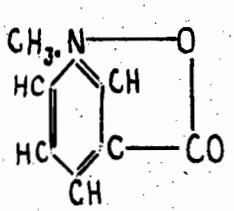
Nicotinic acid was first produced by Huber (1) in 1867 by oxidising nicotine with potassium chromate and sulphuric acid.



I — Nicotine

II — Nicotinic acid

Nicotinic acid is widely distributed in nature in the form of its ubiquitous methyl betaine, trigonelline, isolated from the plant Trigonella foenum graecum by Jahns (2) in 1885.



III

Trigonelline

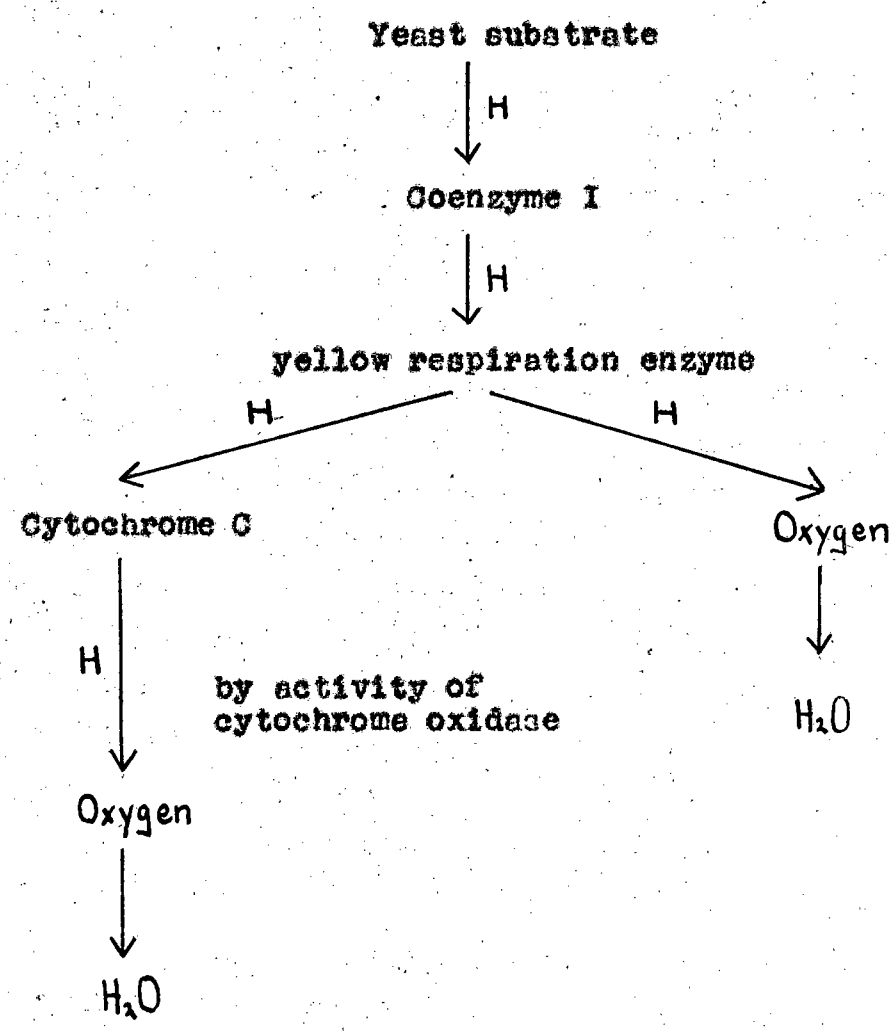
Recent work has shown that whereas raw coffee beans are a particularly rich source of trigonelline (430 mg. per cent) they are a relatively poor source of nicotinic acid (1.6 - 4.4 mg. per cent). Roasting the coffee produced a

five to six fold increase in the amount of nicotinic acid and it was shown that this increase was due to the decomposition of trigonelline to nicotinic acid during the roasting process (3). In 1912 nicotinic acid was isolated from rice (4) and fourteen years later Szymanska and Funk (5) studied the nutritional value of nicotinic acid and nicotinamide and attributed to them a food sparing and weight preserving action. Even with this finding nicotinic acid still remained of purely theoretical interest. In 1935 it was the work of Warburg and Christian (6)(7) and von Euler, Albers and Schlenk (8) in identifying nicotinamide as a constituent of coenzymes I and II that focussed considerable attention on the possible role of nicotinic acid derivatives in the body.

Nicotinic acid is distributed in small quantities in plant and animal tissues. Krehl and his associates (9) showed that animal tissues contained more nicotinic acid than plant tissues and that in the former it was present mostly as bound nicotinamide. Plant tissues on the other hand contained nicotinic acid in both the free state and as bound nicotinamide.

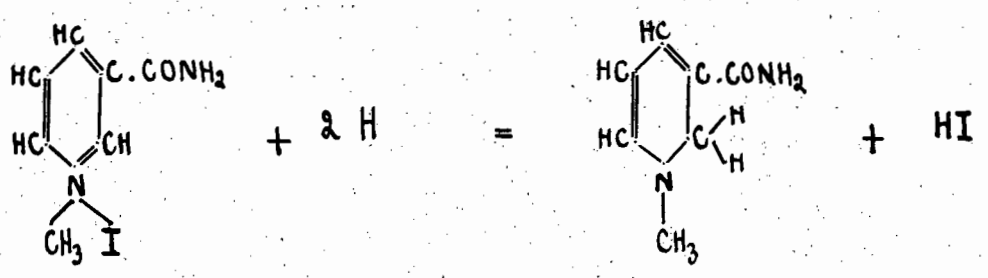
In both animal and plant tissues nicotinamide is a constituent of coenzymes I and II. These coenzymes in the animal body are concentrated in the red blood cells. Coenzyme I, diphosphopyridine-nucleotide or DPN and coenzyme II, triphosphopyridine-nucleotide or TPN play an important role in cell oxidation-reduction processes. For example in yeast coenzyme I receives hydrogen from the substrate and is transformed to dihydrocoenzyme I. This is stable in air but is dehydrogenated by a yellow respiration enzyme which functions as the hydrogen acceptor. The yellow enzyme is

altered to the leuco-form which can give up its hydrogen to the oxygen of the air with the formation of water or can yield its hydrogen to another acceptor namely cytochrome C, and is thus in turn reoxidised. The process then repeats itself. The following scheme attempts to set out in its most simple form the chain of reactions whereby hydrogen given off by the yeast substrate is eventually oxidised to water by atmospheric oxygen.



The work of Warburg and Christian (10) demonstrated that it was the nicotinamide unit of the coenzyme II which could accept one hydrogen atom and undergo hydrogenation to the ortho-dihydrocompound. In the presence of suitable hydrogen acceptors this ortho dihydrocompound could undergo dehydrogenation to its original form. Free nicotinamide could not

be hydrogenated to the reversible dihydrocompound and therefore could not serve as a hydrogen carrier. The process of hydrogenation and dehydrogenation of pyridine compounds can be traced in the following way (10). When pyridine compounds are converted to the reversible dihydrocompounds these give characteristic absorption bands known as dihydrobands with ultra violet light. The dihydropyridine compounds fluoresce when subjected to ultra violet light whereas the non hydrogenated forms do not. A simple pyridine compound that undergoes reversible hydrogenation is N'-methyliodonicotinamide (IV). It will be observed that the nitrogen atom of the pyridine ring is pentavalent and Karrer et al. (11) represented the reaction in which the N'-methyl-o-dihydronicotinamide (V) is produced as follows :-



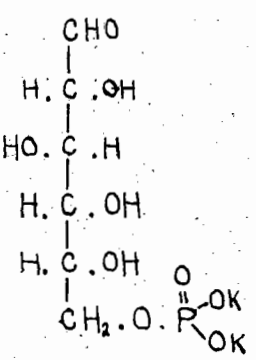
IV V

N'-methyliodonicotinamide N'-methyl-o-dihydronicotinamide

N'-methyliodonicotinamide (IV) when dissolved in sodium bicarbonate solution does not show a white fluorescence. On addition of sodium hyposulphite the white fluorescence appears due to the presence of the newly formed dihydrocompound. When oxygen is passed through the solution to remove excess

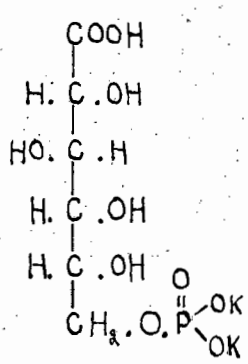
sodium hyposulphite the fluorescence persists due to the stability of the dihydrocompound in presence of free oxygen. On the other hand if the yellow respiration enzyme is added the fluorescence disappears because the enzyme dehydrogenates the dihydrocompound (V). Karrer et al. (11) deduced from these findings that nicotinamide was bound in coenzymes I and II in the form of its quarternary pyridinium salt and that the reversible hydrogenation property of nicotinamide was related to this type of linkage. This concept was supported by the finding that the reduction of quarternary pyridinium salts proceeded easily.

Carbohydrates or their phosphoric acid esters cannot reduce pyridine nucleotides in aqueous solution. However in presence of a specific carrier protein this reduction takes place (10). Addition of the potassium salt of hexosemonophosphoric acid (VI) to a solution of triphosphopyridine nucleotide produces no fluorescence when subjected to ultra violet light. On the other hand if the specific carrier protein is added to the T.P.N solution, addition of the potassium salt of hexosemonophosphoric acid produces with ultra violet light a fluorescence which is characteristic of the dihydropyridine nucleotides. It was found that phosphohexonic acid (VII) was produced in the reaction.



VI

Potassium salt of hexosemonophosphoric acid

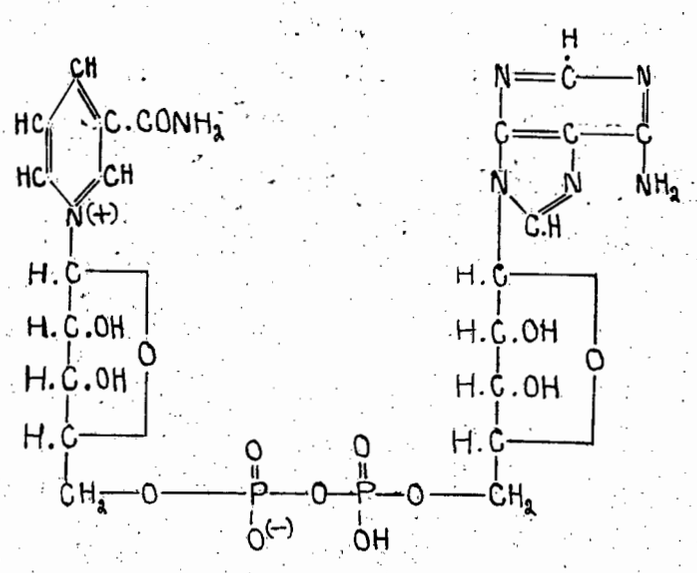


VII

Potassium salt of phosphohexonic acid

In the presence of oxygen and the yellow respiration enzyme the pyridine complex is dehydrogenated and restored to its original form. Small quantities of this pyridine catalytic complex can be used to oxidise large quantities of hexosemonophosphoric acid to phosphohexonic acid.

Warburg and his associates established that triphosphopyridine nucleotide which they had isolated from the red blood cells of the horse was constituted by one molecule of nicotinamide, two molecules of a pentose sugar, three molecules of phosphoric acid and one molecule of adenine. This immediately brought into question the work of von Euler and Myrbäck (12) on the composition of cozymase isolated from yeast by Harden and Young (13) in 1904. von Euler and Myrbäck described cozymase as an adenine mononucleotide with a molecular weight of 350. In the light of Warburg's findings with triphosphopyridine nucleotide the work on cozymase was repeated and it was found to have a molecular weight of 700 and to distinguish itself from TPN by having one molecule less of phosphoric acid. Cozymase is therefore a diphosphopyridine nucleotide (VIII).



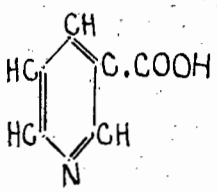
VIII

Diphosphopyridine nucleotide (Coenzyme I)

Nicotinic acid is required in the body for the synthesis of coenzymes I and II. It has been shown (14) that administration of nicotinic acid dramatically elevates the coenzyme I and II content of the human erythrocytes. Nicotinamide is at best only a third as potent as nicotinic acid in stimulating coenzyme synthesis. The exact path of coenzyme synthesis remains still to be discovered. A nicotinic acid deficiency was held to cause failure of the tissue respiratory mechanism which involves the pyridine nucleotides. In their work to ascertain the manner in which nicotinic acid deficiency caused the development of pellagra symptoms and finally death Handler and Dann (15) conducted in vitro studies with normal dogs and dogs with blacktongue and showed no difference in the ability of the tissues to oxidise glucose or lactic acid. The authors concluded that death was not due to a simple deficiency of coenzyme and the consequent failure of tissue respiration but must be the result of some other combination of circumstances. The parenteral administration of physiological saline solution to dogs with blacktongue resulted in alleviation of the deficiency syndrome and their lives were prolonged as much as 180 days. Although all the animals finally succumbed to the nicotinic acid deficiency their state in extremis seldom resembled the classical picture of blacktongue. Handler and Dann regarded dehydration and electrolyte imbalance as being possibly of primary importance in causing death in the case of nicotinic acid deficiency but it is obvious that the complete set of reactions involved in producing the characteristic pellagra symptoms is not yet understood.

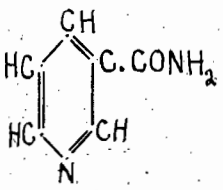
The results of Woolley and his associates (16) strongly indicated that a highly specific structure was required for curing blacktongue. Of the following seven

pyridine derivatives found effective all have the α-carbon atom unsubstituted :



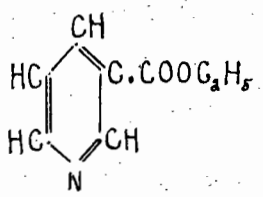
IX

Nicotinic Acid



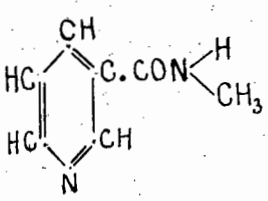
X

Nicotinamide



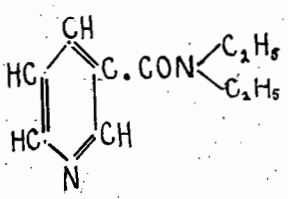
XI

Ethylnicotinate



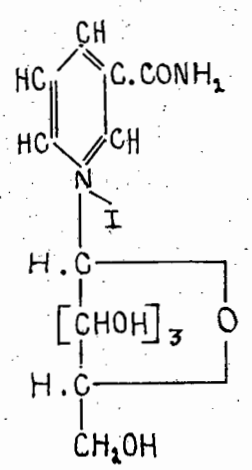
XII

Methylnicotinamide



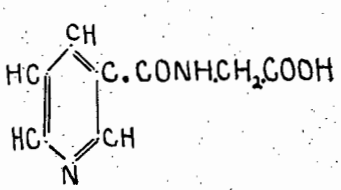
XIII

Diethylnicotinamide



XIV

Nicotinamide-glucosidoiodide



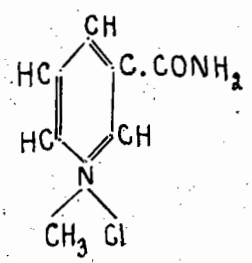
XV

Nicotinuric acid

It was suggested (16) that only those substances which can be converted to nicotinic acid or its amide by either hydrolysis or oxidation in the body possess anti black tongue activity.

Metabolism of Nicotinic acid derivatives

An excellent review of this subject is given by Ellinger (17). Ingestion of nicotinamide (X) is followed by the excretion of N'-methylchloronicotinamide (XVI) and small quantities of nicotinic acid (IX) and nicotinamide (X) in the human urine. The above derivatives together with Trigonelline (III) and nicotinuric acid (XV) are found in urine in amounts that vary with the diet. Pellagrins excrete hardly any N'-methylchloronicotinamide which is the chief end product of nicotinamide metabolism in the human being.

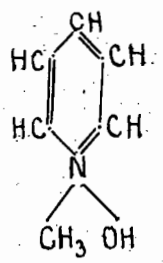


XVI

N'-methylchloronicotinamide

It is pointed out that only 10-15% of a single dose of nicotinamide is eliminated as N'-methylchloronicotinamide. In our present state of knowledge about four-fifths of the ingested nicotinamide cannot be accounted for in a balance study.

The methylation of pyridine in the body was observed by His (18) who found that pyridine was converted to N'-methylpyridiniumhydroxide (XVII).



XVII

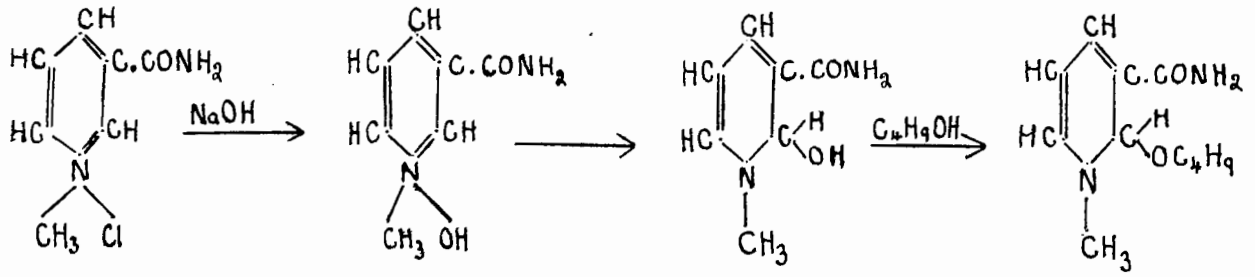
N'-methylpyridiniumhydroxide

Huff and Perlzweig (19) later observed that nicotinic acid derivatives were also methylated in the body and in another paper Perlzweig and his associates (20) reported that nicotinamide and not nicotinic acid underwent methylation. These authors also showed, using the rat as their test animal, that the seat of methylation was in the liver and that this process did not take place in kidney or muscle tissue. The reaction required the presence of oxygen and intact living cells and usually but not always methylation was increased by the addition of methionine. Najjar and Deal (21) gave evidence that N'-methylchloronicotinamide (XVI) was demethylated with the release of nicotinamide. The livers of rats which were given N'-methylchloronicotinamide contained half the quantity of fat which was present in the controls. In the light of their finding Najjar and Deal believed that the methyl group made available in the demethylation process might be biologically active. The work of Najjar and Deal (21) was called into question by Ellinger who showed that liver slices in vitro did not destroy N'-methylchloronico-

tinamide. Recent work by Ellinger and Hardwick (22) claimed that a portion of this metabolite (produced in the liver) was eliminated with the bile into the small intestine where it was destroyed by the intestinal flora. This finding, if correct, would account for at least part of the nicotinamide "lost" in the metabolic process. Najjar and co-workers (23) (24) attributed considerable biological activity to N'-methylchloronicotinamide which they regarded as a growth factor for Hemophilus influenzae. The identification of this substance is of practical importance because pellagra patients do not excrete as much N'-methylchloronicotinamide in urine as do normal individuals. (Physically fit persons excrete 6.0 mg., pellagrins 0.45 mg. per day (22).) This led to the establishment of a N'-methylchloronicotinamide urinary excretion test for the early detection of nicotinic acid deficiency.

It was originally thought that orally administered nicotinic acid was partly eliminated as trigonelline in urine (25)(26). In 1940 Najjar and Wood (27) reported that a whitish blue fluorescent substance was present in the urine of normal subjects and was increased in amount after the ingestion of nicotinamide and related substances. This fluorescent substance was termed F_2. Of a number of pyridine derivatives tested for their power to increase F_2 only nicotinamide and diethylnicotinamide were effective and both of these substances were active in the cure of pellagra. The composition of F_2 aroused great interest. Najjar et al. (23) showed that F_2 was a pyridine compound by producing a positive cyanogen bromide test after alkaline hydrolysis. These workers realised there were similarities between F_2 and N'-methylnicotinamide derivatives but did not succeed in identifying F_2 with any of them. Simultaneously with the publication of the findings of Najjar and his associates (23)

Huff and Perlzweig (19) published their report claiming to have identified F_2 with N'-methylchloronicotinamide (XVI). They showed that most of the trigonelline found by early workers (25)(26) in urine after nicotinamide ingestion was in reality N'methylchloronicotinamide. The work of Ellinger and Coulson (28) proved that F_2 could not possibly be N'-methylchloronicotinamide because this substance did not fluoresce when examined in the light of the 366 and 311 mercury lines. They demonstrated however that when this compound was treated with alkali it became slightly fluorescent and that this fluorescence was greatly intensified on extraction with isobutanol. The non fluorescent precursor of F_2 was identified as N'-methylchloronicotinamide (28) (29). Facts lead to the conclusion (28) that alkinization of N'-methylchloronicotinamide caused a change in the nicotinamide molecule with the production of a quarternary base (XVIII). This was unstable and underwent an intramolecular change in which the migration of the hydroxyl group from the ring nitrogen atom to the pyridine ring α-carbon atom took place (XIX). This was accompanied by the simultaneous loss of one double bond in the pyridine nucleus. Najjar and White (30) accepted this argument of Ellinger and Coulson (28) and postulated that the carbinol (XIX) then reacted with the isobutanol to produce a carbinol ether (XX) which they claimed was the strongly fluorescent compound F_2.



XVI	XVIII	XIX	XX
Quarternary salt	Quarternary base	Carbinol	Carbinol-ether
(non fluorescent)	(slightly fluorescent)	(slightly fluorescent)	(strongly fluorescent)

Ellinger (17) did not accept this view of Najjar and White (3) on the ground that F_2 could also be produced by reacting the carbinol (XIX) with ethyl acetate. He also observed that the increase in fluorescence was only brought about by solvents of F_2 e.g. isobutanol, methanol, ethanol, amyl alcohol and ethyl acetate. The definite composition of F_2 has as yet not been established.

Biosynthesis of Nicotinic acid derivatives

Many factors are involved in determining the nicotinic acid requirement of man and in 1944, with the finding that nicotinic acid derivatives were synthesised by the intestinal flora, another factor was added to the list. In their work on the metabolism of nicotinic acid Ellinger and Coulson (31) examined the effect of the ingestion of nicotinic acid and its derivatives on the daily elimination of N'-methylchloronicotinamide. From the relation of the excreted metabolite to the dietary nicotinamide intake they suspected the presence of an extra source of nicotinamide.

This was proved to be correct by Ellinger and his associates (32)(33) who showed that a considerable proportion of the human requirements of nicotinamide was met by the synthesis of this substance by the intestinal flora, a fact which throws new light on the etiology of pellagra. Using the "sterilising" drugs sulphaguanidine and succinylsulphathiazole these workers (32) reduced the output of urinary N'-methylchloronicotinamide by 30%. This action was attributed to the bacteriostatic effect of the sulpha-drugs on the intestinal bacteria normally synthesising nicotinamide. In 1937 Knight (34) showed that bacteria consumed nicotinamide and later the work of Koser and Baird (35) and Benesch (36) proved that bacteria destroyed nicotinamide. In the same study (36) Benesch showed that a suspension of bacteria from the caecum destroyed nicotinamide under anaerobic conditions and produced it under aerobic conditions. From these facts it can be deduced that a disturbance in the intestinal tract against the anaerobic bacteria ought to produce an increase in the synthesis of nicotinamide. This was confirmed by Ellinger and Emmanuelova's use (37) of a sulphonamide drug, Ambamide (p-aminomethylbenzene-sulphonamide) which, being selective against the anaerobic bacteria, brought about an increase in the output of N'-methylchloronicotinamide. These authors explained their results as follows. Nicotinamide was produced in vitro by the aerobic coliform bacteria. During the Ambamide dosing period an increase was observed in the number of coliforms and in the amount of N'-methylnicotinamide excreted. The increase of the metabolite might be due solely to the increase of nicotinamide-producing coliforms or to the simultaneous decrease of nicotinamide-consuming or destroying anaerobes. Coulson and Stewart (38) carried out experiments with infants less than two days old and showed that they excreted at least

3.2 mg. N'-methylchloronicotinamide per 24 hours. This was far more than could have been derived from the amount of nicotinic acid derivatives in the small quantity of breast milk ingested. Najjar and co-workers (39) placed human subjects on a purified diet containing 1.5 - 2.0 mg. nicotinamide per day. No signs of deficiency developed and the authors attributed the maintenance of good health and normal excretion of N'-methylchloronicotinamide to biosynthesis of nicotinamide in the intestine. It may be generally stated that if conditions in the intestinal tract are not optimal for the growth of nicotinamide-producing bacteria then this source of supply of the vitamin will be severely restricted.

Pellagra and the identification of the Pellagra Preventive factor

Pellagra is a disease endemic in those parts of the world where maize constitutes the main food. Casal in 1735 was the first to observe that the incidence of pellagra was associated with the consumption of maize. This relationship though very common is not invariably found. Classical pellagra affects three main systems -- skin, alimentary tract and nervous system. The skin shows characteristic pigmentation and thickening of the epithelium on those parts of the body exposed to sunlight. The deleterious effect of sunlight on the skin was observed by Frapolli as early as 1771. Diarrhoea, nausea, vomiting, loss of appetite with concurrent loss of weight together with sore red tongue manifest themselves. Mental disturbances and changes in the spinal cord are also observed. Some cases have shown such severe mental disorder that they have been sent to mental asylums. In 1928 Goldberger and Wheeler (40) demonstrated that canine black tongue, a disease characterised by loss of appetite,

loss of weight, vomiting, bloody diarrhoea and lesions of mouth and tongue was analogous to human pellagra. This was a very important finding as dogs could now be used as test subjects in place of human beings. Every substance of value in the cure or treatment of pellagra was of value in black tongue. Every agent that was worthless in the treatment of the one disease was worthless in the other.

There is still much controversy as to the cause of pellagra. Most people still hold the view that it is due to a vitamin deficiency but there are two other schools of thought -- one that it is due to lack of suitable protein, the other that it is due to the presence of a toxic substance in maize.

In 1914 Voegtlin (41) postulated that a deficiency of certain amino acids in the diet might be a factor in the incidence of pellagra. Goldberger and Wheeler (42) definitely showed that pellagra was a food deficiency disease. They observed that pellagrins were encountered mainly among a section of people whose diet chiefly consisted of maize meal, maize grits and syrup. Goldberger decided to test out such a diet on eleven volunteers in a Mississippi prison and of these six contracted the disease. Goldberger experimented with various pellagra inducing diets of which the following is a good example :

	%
Ground white maize	76.4
Pea meal	10.8
Crude casein	6.4
Cod liver oil	4.4
Salt mixture	2.5

In 1920 Chick and Hume (43) reported the production in monkeys of symptoms closely resembling those of pellagra by prolonged feeding of a low protein diet. Administration of

tryptophane to one monkey staved off death for many weeks. Two years later, Goldberger and Tanner (44) observed some beneficial results when they treated pellagrins with tryptophane and cystine. These workers in 1924 were able to prevent pellagra by incorporating high protein foods such as milk, lean meat and legumes in the diet (45). Despite this however Goldberger came to the conclusion that the disease was due to deficiency of a vitamin which he termed the pellagra preventive (P.P.) factor. The search to identify this factor now started.

In view of the newly discovered water soluble vitamins in liver Goldberger and Sebrell (46) tested liver extract on dogs with black tongue and found it had a beneficial result. In 1933 Ramsdell and Magness (47) cured 22 cases of pellagra with liver extract. Chick (48) in the same year stated however that pellagra was not due to a B vitamin nor was it in her opinion due to an amino acid because neither view explained the association of endemic pellagra with maize. She proposed that pellagra was caused by a toxic substance in maize and that this could be corrected by sufficient first class protein and perhaps by vitamins of the B complex. In the search through the B complex for the P.P. factor thiamine was considered and rejected on the grounds that whereas the P.P. factor was stable to heat thiamine was a heat labile compound. Rhoads and Miller (49) tried to produce black tongue with a diet deficient in riboflavin without success and Elvehjem and Koehn (50) and Birch et al. (51) failed to cure it by means of this vitamin. Vitamin B6, the anti pellagra-like dermatitis factor in rats (52) could not be the P.P. factor because it was present in black tongue producing diets (51) and because pellagra could be cured by a diet not containing this factor (53).

In their work on the effect of liver extract on the pellagra-like condition in chicks Elvehjem and Koehn (50) prepared a flavin-free fraction which was effective in curing the chicks. The concentration of the pellagra preventive factor was carried out along the following lines (54). An aqueous solution of liver extract was treated with a large excess of ethyl alcohol and ether. A precipitate was produced and this was filtered and washed with a mixture of alcohol, ether and water. The filtrate was concentrated in vacuo, acidified with hydrochloric acid and shaken up with Fuller's earth to remove the flavin fraction. This was allowed to settle out and the supernatant liquid siphoned off and neutralised with sodium hydroxide. The sodium chloride was precipitated by means of excess ethyl alcohol and filtered off. The filtrate was highly active in curing black tongue in dogs. These authors continued in their efforts to concentrate and purify the P.P. vitamin and in 1937 developed another procedure (55) which is briefly described as follows. An amyl alcohol solution equivalent to 400 g. of liver extract was evaporated to dryness in vacuo. Making use of 95% ethyl alcohol to dissolve the residue and successive acetone treatments to precipitate out impurities, the residue freed from acetone by concentration to dryness was extracted with water. This solution was decolourised with a small quantity of norit and the filtrate and washings were found to contain 2.56 g. of solid matter. Attempts to obtain crystals from the filtrate by means of different solvents met with no success. When the solid concentrate was fed at the rate of 64 mg. per day to dogs suffering from black tongue it effected a complete cure.

While work on the concentration and purification of the P.P. factor in liver was in progress, a deep interest was being displayed in the possible role of nicotinic acid in the body following the report of Warburg and Christian (7) that nicotinamide was the active constituent of coenzyme II (Triphosphopyridine nucleotide). In 1935 Kuhn and Vetter isolated nicotinic acid from heart muscle (56) and during 1937 Funk and Funk (57) published their results on the nutritional value of nicotinic acid and nicotinamide. Using pigeons and rats as test animals these authors reported that nicotinamide and to a lesser extent nicotinic acid caused a great improvement in appetite and increase in weight. About the same time Frost and Elvehjem (58) showed a definite growth response in rats on a purified ration when they were fed adenine nucleotides and nicotinic acid. Adenine nucleotides alone produced an immediate but not continuous reponse. The reaction to nicotinamide was poor at first but afterwards gave a very definite and prolonged increase in the rate of growth. Combination of the two supplements gave the best results. The spotlight was now definitely on nicotinic acid and its derivatives and on comparing the properties of nicotinic acid with those observed for the P.P. vitamin, e.g. the high stability towards prolonged heat treatment, Elvehjem, Madden, Strong and Woolley (59) immediately recognised similarities and decided to test out pure nicotinic acid on dogs with black tongue. A dog with all the typical symptoms of the disease was dosed with 30 mg. of nicotinic acid and a phenomenal response was obtained. The appetite improved, the mouth lesions disappeared and the dog started putting on weight rapidly. In this way was nicotinic acid identified as the anti black tongue factor.

Elvehjem et al. (59) continued their efforts to isolate the vitamin from liver and this was eventually accomplished as follows. A concentrate of the anti pellagra factor was obtained from liver by an alkaline acetone treatment. Further purification was carried out in a molecular still, the distillate dissolved in alcohol and treated with excess alcoholic mercuric chloride. The white precipitate which formed was filtered, washed, dissolved in dilute hydrochloric acid and treated with hydrogen sulphide to remove the mercuric ions. When the resulting filtrate was concentrated to dryness long white crystals were obtained which proved to be highly effective in curing black tongue. The crystals melted at 227-228°C (uncorrected) and by means of the mixed melting point test were identified as nicotinamide hydrochloride. The free base was prepared by removing the hydrochloric acid by means of silver oxide and recrystallising from ethyl acetate. The base was identified as nicotinamide.

In view of the analogy between canine black tongue and human pellagra these authors suggested that nicotinic acid might be effective in the cure of that disease. Shortly afterwards Spies et al. (60) achieved successful results when they treated eleven cases of severe pellagra with nicotinic acid. During this study Spies observed that when the skin lesions were severely ulcerated and thickened they did not appear to respond to nicotinic acid therapy, and he recommended that pellagra patients be given a well balanced diet together with the nicotinic acid treatment. Time has completely justified this early view for it is known today that nicotinic acid gives superior and more consistent results when given together with a good daily diet containing first class protein.

Even with the identification of the P.P. factor as nicotinic acid there were still many doubts as to whether deficiency of this factor was the sole cause of pellagra. It was observed that diets rich in rye and oats which contain considerably less nicotinic acid than maize did not induce pellagra and this strengthened the belief that maize contained a pellagra inducing toxin. Other people continued to believe in the important role of protein in the etiology of pellagra.

Relationship between Nicotinic acid and Tryptophane

It was found that an increase of protein in the basal diet without corresponding increase of B complex vitamins resulted in the poor growth of rats (61). Further data indicated that the excretion of total nicotinic acid and riboflavin was highest during periods of low protein intake. An increase in the protein content of the diet produced a decrease in the urinary excretion of nicotinic acid by dogs. This decrease of nicotinic acid in the urine was attributed in part to a retention under conditions of high protein intake. Wintrobe et al. (62) working with pigs demonstrated that when these animals were fed a high protein diet (26.1% casein) supplemented with all the B vitamins except nicotinic acid they developed no deficiency symptoms. On the other hand when the protein content of the diet was low (10% casein) the omission of nicotinic acid was associated with marked deficiency symptoms such as impaired growth, diarrhoea, poor appetite and anaemia. This pointed to a close relationship between protein, and nicotinic acid and Wintrobe felt that when pigs were fed a diet with a high protein level the dietary requirement of nicotinic acid was somehow altered. It is interesting at this stage to point out that the usual diet of

the pellagrin is poor in protein and minerals as well as in vitamins and that the Goldberger experimental diet supplies protein which is poor qualitatively as well as quantitatively.

Despite the finding by Dann (63) that the rat was able to synthesise its own nicotinic acid and was therefore independent of a dietary source Krehl, Tepley and Elvehjem (64) succeeded in producing nicotinic acid deficiency symptoms in this animal by incorporating maize in its basal ration. These authors fed the rat a basal diet free of nicotinic acid and on replacing 40% of the diet with maize effected a pronounced retardation in growth. They further established that the growth depressing action of maize could be counteracted by supplying nicotinic acid at the rate of 1 mg. per 100 g. of diet. It was also shown that the type of carbohydrate and the level of protein supplied as casein modified the effect of the harmful action due to maize. With glucose as the carbohydrate or when the level of casein was raised to 20% the growth depression due to the maize was diminished. Of striking interest is the fact that when polished rice or rolled oats which contain less nicotinic acid than maize were fed under identical conditions they produced no such growth depression. Furthermore milk, although very low in nicotinic acid was active in combating the harmful action of maize. It is well worth remembering that Goldberger had advocated the use of milk in the treatment of pellagra patients as early as 1924. Since the protective action of casein cannot be explained on the basis of its nicotinic acid content which is very low Krehl and coworkers (65) carried out experiments to find what factor in casein was responsible. Maize is deficient in the essential amino acids lysine and tryptophane and it was argued that

casein might be contributing these amino acids in sufficient quantity to overcome the deficiency in the maize. It was found that lysine supplied at 0.5% of the ration did not alleviate the effect of maize. However when l-tryptophane was added at a level of 0.4% to the low casein diet plus maize grits a dramatic response in the growth of rats occurred. If in the basal ration 40% rice polishings were substituted for the 40% maize the growth retardation stopped and weight was put on again. Rice contains less nicotinic acid but more tryptophane than maize. The work of Krehl et al. (65) confirmed that the protein level had a profound effect on the nicotinic acid requirement. A high protein diet, i.e. a diet containing adequate supplies of tryptophane, reduced the amount of nicotinic acid necessary to prevent the onset of pellagra symptoms. In view of the fact that the deleterious action of a basal ration containing 40% maize grits could be prevented by the addition of either 1 - 1.5 mg. nicotinic acid or 50 mg. l-tryptophane it was evident that a close interrelationship existed between the vitamin and the amino acid tryptophane. When the maize constituent (40%) of the basal ration was replaced by the equivalent quantity of its principal protein zein this relationship between nicotinic acid and tryptophane still held. Thus rations which were deficient in nicotinic acid and marginal in their tryptophane content or vice versa were inadequate for good rat growth. It is pointed out that maize is deficient in tryptophane and low in nicotinic acid. Martin (66) had discovered that 2% succinyl-sulphathiazole in diets containing a mixture of ten essential amino acids as the sole source of nitrogen resulted in a great loss of weight and death of the test animal. Martin had concluded that intestinal bacteria must synthesise amino acids essential to the host. In the

light of these findings Krehl and his associates (65) explained the action of maize on the grounds that due to its low nicotinic acid content it set up an unfavourable set of conditions for the synthesis of tryptophane in the intestinal tract and that the decreased output of tryptophane by the intestinal flora would be reflected in a greater dietary requirement for this compound. In 1942 it was shown (67) that when either casein, glycine, amino-valeric acid or choline were fed to rats on a protein free basal diet the excretion of nicotinic acid derivatives in the urine was promptly increased. Rosen et al. (68) showed a significant decrease in the excretion of nicotinic acid derivatives in the rat when the casein of the diet was replaced by gelatin, a tryptophane deficient protein. On addition of 50 mg. l-tryptophane to the gelatin diet an increased excretion of nicotinic acid occurred within 24 hours and this increase was much greater than that caused by other amino acids. The addition of tryptophane to the casein diet likewise produced a large rise in nicotinic acid excretion. These authors concluded that tryptophane might be the important precursor of nicotinic acid in the rat. Sarett and Goldsmith (69) later showed that the work of Rosen et al. (68) also held for human beings in that administration of l-tryptophane increased the excretion of N'-methylchloronicotinamide. The horse does not require a dietary source of nicotinic acid (70) and when this animal was fed 6 g. of l-tryptophane in addition to that present in the basal diet Schweigert and his associates (71) demonstrated that a threefold increase in the amount of nicotinic acid excreted took place. In the horse nicotinic acid and not N'-methylchloronicotinamide is the chief end product in the urine. The conversion to nicotinic acid was very rapid as an increase in the amount excreted was noted within 24 hours

of tryptophane administration and this level was maintained for only 24 hours after tryptophane feeding had been discontinued. Experiments with the horse offered no indication that nicotinic acid could be converted to tryptophane.

In an effort to elucidate the relationship between nicotinic acid and tryptophane it was decided (72) to determine the effect of nicotinic acid on the utilisation of tryptophane by a balance study between ingested tryptophane and the tryptophane accounted for by gain in body weight plus that excreted in the faeces and urine. Good growth and concomitant increase in total nicotinic acid obtained on a low nicotinic acid ration could only be attributed to a mechanism of nicotinic acid synthesis. Krehl et al. (72) suggested that this mechanism was impaired by the presence of 40% maize in the basal ration. Addition of nicotinic acid to this ration (1 mg./100 g.) led to an immediate resumption in growth. They concluded that the tryptophane content of the maize containing ration became adequate when nicotinic acid was added. These results showed that addition of nicotinic acid stepped up the utilisation of tryptophane from 30 - 70%. These authors also concluded that the good growth effected in rats by addition of 50 mg. tryptophane to the maize containing ration might result from the action of tryptophane in improving nicotinic acid synthesis. They stated that it yet remained to be determined whether tryptophane served as the precursor of nicotinic acid in the rat or whether it simply stimulated nicotinic acid synthesis.

The normal metabolism of tryptophane gives rise to kynurenin, kynurenic acid and, as recently discovered by Rosen et al. (68), nicotinic acid. In 1937 Xanthurenic acid (4, 8 - dihydroxyquinoline - 2 - carboxylic acid) was isolated

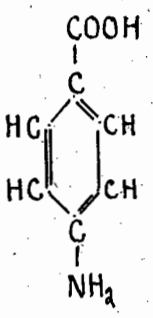
from the urine of animals fed diets high in tryptophane (73). Six years later Lepkovsky, Roboz and Haagen-Smit (74) isolated Xanthurenic acid from the urine of rats kept on a diet deficient in pyridoxine and they established that when l-tryptophane was fed to these rats the excretion of Xanthurenic acid was increased. The excretion of this abnormal metabolite stopped when pyridoxine was administered. Lepkovsky et al. concluded that tryptophane was a precursor of Xanthurenic acid and that pyridoxine was vitally concerned with tryptophane metabolism. Miller and Baumann (75) confirmed that Xanthurenic acid in the urine of mice was derived from tryptophane and that when this amino acid was fed to mice on a pyridoxine deficient diet it greatly shortened their lives.

The influence of pyridoxine on the conversion of tryptophane to nicotinic acid was investigated (76)(77) and it was shown that rats on a pyridoxine deficient diet were unable to convert appreciable amounts of tryptophane to nicotinic acid. The exact route by which this process proceeds still remains to be determined.

A Pellagra producing toxin in Maize

The old theory of a pellagra producing toxin in maize was sharply brought to light by the researches of Woolley (78)(79)(80). His work was based upon the competitive theory of analogous substances, e.g. a structural analogue of nicotinic acid would compete for the place of the nicotinic acid in a biologically active enzyme system but would be quite unable to fulfil the functions of the vitamin. This would then result in the development of the characteristic nicotinic acid deficiency symptoms.

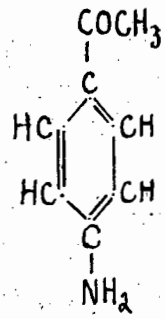
In 1942 Auhagen (81) reported that p-aminoacetophenone (XXII) was bacteriostatic and that this action was reversed by its analogue p-aminobenzoic acid (XXI).



XXI

p-aminobenzoic acid

Growth stimulant

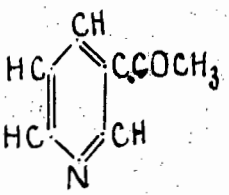


XXII

p-aminoacetophenone

Growth inhibitor

Woolley (78) decided to test out whether the replacement of the carboxyl group by the acetyl group was a general method for the formation of inhibiting analogues and accordingly he tested out 3-acetylpyridine (XXIII) on mice.



XXIII

3-acetylpyridine

Growth inhibitor

Woolley reported that 3-acetylpyridine caused a disease in mice (like rats, normally independent of a dietary source of nicotinic acid) with the characteristic deficiency symptoms of pellagra. Administration of nicotinic acid to

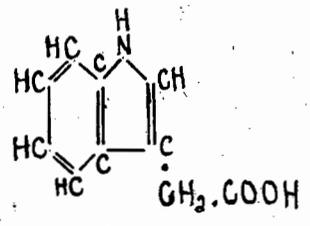
the 3-acetylpyridine containing diet prevented manifestation of the symptoms. 3-Acetylpyridine resembles in its action glucoascorbic acid, analogue of vitamin C, which allows production of scurvy in animals which do not require ascorbic acid in the diet.

Woolley observed that whereas 3-acetylpyridine was effective against mice it was completely ineffective against bacteria. In view of the similar reactions displayed by microorganisms and the higher animals to vitamins this point was rather puzzling.

In view of his findings with 3-acetylpyridine Woolley (79) offered the attractive hypothesis that the pellagragenic action of maize might be due to the presence in it of a structural analogue of nicotinic acid which competes with that vitamin just as 3-acetylpyridine does. Krehl's work had shown that tryptophane as well as nicotinic acid was able to counteract the harmful effect of maize (65). In turn Woolley (79) demonstrated that both tryptophane and nicotinic acid corrected the deficiency symptoms induced by 3-acetylpyridine. In a later paper (80) he examined maize for the presence of a structural analogue of nicotinic acid which would specifically induce pellagra. Mice differ from rats in that addition of maize to the nicotinic acid free basal ration brings about no retardation of growth. However if a sodium hydroxide - chloroform extract of maize was added to the basal ration the growth rate of the mice was markedly reduced and many of the animals developed mild diarrhoea and reddening of the skin and tongue. These symptoms could be prevented and cured by nicotinic acid. In contrast to the sodium hyrdoxide-chloroform extract commercial crude maize oil was not

deleterious. The factor was not isolated and identified but Woolley succeeded in preparing a highly active concentrate which when fed at a level of 1 mg. per 100 g. of the basic ration produced maximal inhibition of growth and severe pellagra-like symptoms. The responsible substance appeared to be a weakly basic water soluble compound since it was extracted from the chloroform by dilute sulphuric acid solution and remained in aqueous solution when the sulphate was removed by barium hydroxide. Woolley's work therefore presented a striking advance in the quest for a pellagra inducing toxin in maize.

The Cambridge group of workers headed by Kodicek (82) have recently taken this research one step nearer its goal. Maize is an unusually rich source of the plant hormone, heteroauxin (XXIV) (indole-3-acetic acid).



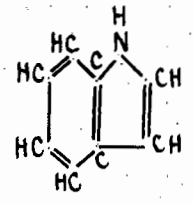
XXIV

Indole-3-acetic acid
(Heteroauxin)

According to different authorities (83)(84) yellow maize meal contains between 2-10 mg. per 100 g. of heteroauxin. It is present largely in the form of a precursor which can be converted into indole-3-acetic acid by mild alkali or enzymic digestion. A diet containing 40% maize will have an approximate content of 0.8 - 4.0 mg. of

heteroauxin per 100 g. diet. Young rats all gained weight steadily on a purified diet containing 10.5% casein as sole source of protein and administration of nicotinic acid and tryptophane did not increase the growth rate significantly. When rats were fed a similar diet supplemented by 1.5 mg. of indole-3-acetic acid per 100 g. diet about 60% showed severe depression of growth. When affected rats were dosed with 1 mg. nicotinic acid or 20 mg. tryptophane per day they recovered. It is notable that the effect of indole-3-acetic acid was similar to that produced by the incorporation of 40% maize in the basal diet. On a high protein diet (20 casein) neither maize meal nor indole-3-acetic acid was effective in stopping growth.

In 1943 Tatum and Bonner (85) showed that the mould Neurospora could synthesise tryptophane (XXVII) from indole (XXV) and serine (XXVI). Very recently Umbreit and associates (86) prepared a cell-free enzyme system from Neurospora which also converted indole and serine into tryptophane. The coenzyme of this system was found to be pyridoxal phosphate. The relationship between indole, tryptophane, indole-3-acetic acid and nicotinic acid can be represented schematically as follows :-



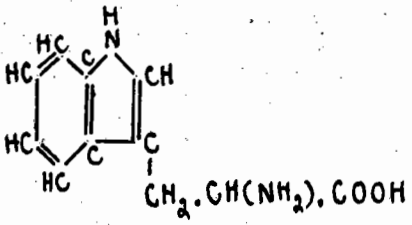
XXV

Indole

$CH_2OH.CH(NH_2).COOH$

XXVI

Serine

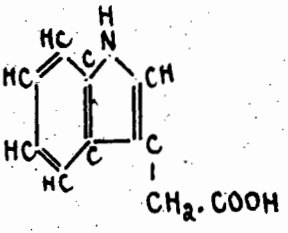


XXVII

Tryptophane

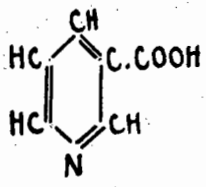
Tryptophane is a precursor of indole-3-acetic acid

Tryptophane is a possible precursor of nicotinic acid



XXIV

Indole-3-acetic acid



II

Nicotinic acid

Maize is poor in tryptophane and nicotinic acid and rich in indole-3-acetic acid and it appears that pellagra develops where a condition like this exists in a natural diet.

Influence of folic acid on utilisation of nicotinic acid

In 1945 it was demonstrated that dogs with black tongue responded considerably better to standard doses of nicotinic acid provided that folic acid was included in the synthetic basal ration (87). The cycle of producing black tongue by means of a synthetic ration and correcting it with nicotinic acid could be repeated a few times before the animal died. If however folic acid was included in the ration the cycle could go on many more times (88).

This account of the latest advances brings the picture up to date. The subject is very much alive at present and this makes any problem dealing with the nicotinic acid content of maize particularly interesting, especially in view of the fact that in every instance of artificially induced pellagra enumerated in the foregoing pages nicotinic acid was always successful as a corrective agent.

Inheritance in Maize

Maize belongs to the genera Zea of the family Gramineae and according to Mangelsdorf and Reeves maize had its origin in Paraguay, Bolivia and Brazil. The place of origin is regarded as the region in which the greatest diversity of types occur.

Classification of Maize (From Hayes and Immer - Methods of Plant Breeding)

1. Pod Maize - Each kernel is enclosed in a pod or between husks.
2. Flints - Starchy endosperm surrounded by a corneous starch.
3. Popcorn - Endosperm contains only a small proportion of soft starch and consists of mainly corneous starch.
4. Dents - Corneous starch located at the side of the seed and the soft starch extends to the summit. The soft starch dries more rapidly than the corneous and this causes the characteristic indentation.
5. Flour Maize - Comprised of almost all soft starch.
6. Sugary Maize - Translucent horny appearance of kernel and a wrinkled condition when dry.
7. Waxy Maize - Endosperm is waxy due to a carbohydrate present which is different to starch.

From the viewpoint of the geneticist maize is almost an ideal plant. Maize grows under a wide range of environmental conditions and exhibits many different characters. Pollination is relatively easy to control and a large number

of seeds can be procured on a single ear with a single pollination. A very important factor in favour of using maize as a weapon to solve genetical problems is the Xenia effect which is the immediate influence that pollination produces in the endosperm of the F1. In maize the endosperm has a sexual origin and is triploid in character. The pollen tube on entering the embryo sac discharges two sperms. One unites with the egg to produce the future embryo while the other unites with the two polar nuclei which undergo division and result in the eventual production of the endosperm. This explains the mechanism of Xenia and the transmission of heredity characters to the endosperm is one of the strongest evidences that exist of the sexual nature of the nuclear fusion in which the endosperm has its origin. From the morphological standpoint the endosperm appears to be a distinct individual with a high degree of differentiation which no other plant possesses. Many of these characteristics give clear cut responses in genetic experiments. In the course of experiments in the hybridization of other plants two generations must be grown before perceptible results are obtained and three generations are necessary for the production of a characteristic Mendelian ratio. The great advantage of maize rests in the fact that due to Xenia immediate results may be procured in the endosperm of the F1 generation.

Maize is characterised by possessing 10 chromosomes and these are numbered mainly in order of decreasing length from 1 - 10, number 1 being the longest and number 10 the shortest. The genes determining the characters in maize fall into 10 linkage groups corresponding to the 10 different chromosomes. The large chromosome number 1 carries linkage group no. 1 and the shortest no. 10 carries linkage group

No. 10. In all cases the orientation of the linkage group within the chromosome is known.

Burkholder, McVeigh and Moyer (89) in the United States of America studied the nicotinic acid content of a large number of maize varieties. They considered that locality appeared to exhibit no influence on the nicotinic acid content, and the fact that different strains on the same soil showed widely different amounts of nicotinic acid gave them grounds for thinking that nicotinic acid was a genetic factor. More direct support of this belief lay in the observation that hybrids between high and low nicotinic acid lines usually had an intermediate content and that hybrids of high nicotinic acid content usually came from parental lines high in this vitamin.

This was the state of knowledge when this work was started in 1945.

Ellis, Randolph and Matrone (90) demonstrated that in certain stocks of maize doubling the number of chromosomes was accompanied by an increase in the amount of nitrogen in the grain (increase of 15%) and in the stover (20 - 34%). The changes in chemical composition that accompanied the transformation from diploidy to tetraploidy they assumed to be due to the cumulative action of certain genes concerned with protein metabolism and the non-cumulative action of other genes. During 1946 in their comparative studies of the vitamin content of the leaves of disomic and trisomic maize, Giles et al. (91) determined that in all but a few instances the average vitamin content of the trisomes was higher than in the comparable diploids. The increases were relatively slight for extra chromosomal types but pronounced for others. As pointed out the normal number

of chromosomes in maize is 10 and the most striking increases were found in the following instances :-

Nicotinic acid	- chromosomes	3		8	9	
Riboflavin	- "	3	5	8	9	
Thiamine	- "		5	8		

Their results indicated that the addition of an extra dose of almost any chromosome had some effect on increasing the content in the leaf cells of the B vitamins studied. From this they deduced that several genes might be involved in the synthesis of each vitamin and that these genes were widely distributed in the chromosomal complement. Certain chromosomes were clearly more important than others in causing an increased content of any particular vitamin. In conclusion these authors stated that as trisome 8 showed a considerable increase for all three vitamins it might carry genes controlling certain basic reactions common to the synthesis of all three substances.

The next step lay in finding with what other factor nicotinic acid was associated. The results of Burkholder et al. (89) and Barton-Wright (92) showed that the nicotinic acid content of sweetcorn was much higher than that of starchy maize. In a later report Mather and Barton-Wright (93) stated that the difference between sugary and starchy grain could not be attributed to the greater shrivelling of sugary seeds in drying, for the ratio of mean weights of neighbouring sugary and starchy seed from two mixed ears (due to open pollination between sugary and starchy varieties) was 1:11. Their argument ran as follows. Sweetcorn characteristically carries the "sugary" allelomorph (su) which is represented in starchy maize by the dominant "starchy" alternative (Su). The endosperm is triploid, that in

sugary seeds being sususu and that in true breeding starchy seed being SuSuSu. In hybrids the endosperm is always phenotypically starchy whether the mother is sugary or starchy. The starchy grain from sugary mothers is Sususu. Besides appearing starchy these had a nicotinic acid content within the range given by seed coming from starchy mothers and being therefore SuSuSu or SuSusu in endosperm constitution. It will be noted that whereas the nicotinic acid content of sugary maize, endospermic constitution sususu, is high that of grains with endospermic constitution Sususu is low. This is most significant as it signifies that the nicotinic acid content follows the action of the Su-su gene. Mather and Barton-Wright emphasised that there must be an element of doubt until grain has been obtained which differs only in this gene, the genetic background being the same in all the samples. They also held the opinion that there were indications of the influence of other genes.

AIM OF THIS WORK

1. The examination of the nicotinic acid content of a wide range of maize varieties grown in South Africa for commercial and breeding purposes.

2. To determine whether the nicotinic acid content is a genetic factor in maize with the ultimate object of making possible the breeding of a maize with a relatively high level of this vitamin..

The following example may serve to demonstrate the importance of the work :-

The most widely grown maize in South Africa, the white dent varieties Hickory King and Potchefstroom Pearl, have a nicotinic acid content of the order 15μg./g.

Daily nicotinic acid requirement of adult	= 18 mg./day
Average daily consumption of maize by a native	= 600 g.
Nicotinic acid supplied by 600 g. maize	= 9 mg.

i.e. the amount of maize consumed supplies only half of the daily requirement. On the basis of a high maize diet it would be necessary to consume 1200 g. to meet the demand and this for the ordinary man is impossible. On the other hand if the maize consumed contained 30μg./g nicotinic acid the present diet with all its disadvantages would at least be satisfactory with respect to this vitamin.

EXPERIMENTAL

Climatic and soil conditions at Potchefstroom

Maize is a summer growing crop which is extensively cultivated in the high veld of the Transvaal and in the North and North Eastern parts of the Orange Free State. These regions lie in the summer rainfall area of South Africa.

All the maize dealt with in this research was grown at the College of Agriculture, Potchefstroom, Transvaal, under the direction of Mr. F.X. Laubscher. The following information on Potchefstroom was obtained from "Field Experiments at Potchefstroom" by Dr A.R. Saunders.

Potchefstroom is situated at 26° 44' South latitude and 27° 05' longitude at an altitude of 4,430 feet. The surrounding countryside is generally flat with scattered low hills and the vegetation is typically grassveld.

Climate

About 85% of the average annual rainfall falls in the six summer months October to March. The rainfall is very variable. Over the 38 year period 1903-1941 the mean annual rainfall was 23.6 inches. The highest level (34.6 inches) was reached in the 1924-1925 season and the lowest level (13.9 inches) was registered in the 1932-1933 season. The efficiency of the rainfall is not only reduced by its variability but also by the high evaporation rate. The mean total evaporation per month or per year is in all cases far in excess of the mean rainfall. The distribution of the rainfall during the period of this experiment (October 1945 - June 1947) is given in Table I.

TABLE I

Monthly Rainfall at Potchefstroom College of Agriculture, 1945-1947

Inches

	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Total
1945-46	0.08	0.00	0.00	0.12	1.72	2.72	4.55	2.74	4.02	0.85	0.76	0.00	17.56
1946-47	0.00	0.00	0.00	2.37	1.82	3.27	4.36	2.98	3.83	2.14	0.22	0.00	21.19

Frost

The first frosts usually occur by the middle of April and freezing temperatures may continue until the middle of September. The degrees of frost registered during this experiment are set out in Table II.

TABLE II

Degrees of Frost registered at Potchefstroom College of Agriculture, 1945-1947

°F

	May	June	July	August	September
1945	18	24	25	25	12
1946	4	20	25	14	8
1947	3	18			

Wind

Potchefstroom does not lie in a windy area for nearly 90% of the wind has a velocity of less than 14 m.p.h.

Soil

The soil at the experiment station is in the main shallow. It is a sedimentary reddish brown loam which is about 12 inches deep over the greater part of the area. The subsoil is largely composed of lateritic iron stone or "ou-klip" concretions which are also dispersed to some extent throughout the surface soil. Below 3 feet the amount of clay increases and the colour changes to a reddish yellow. The reaction of the soil fluctuates closely round pH 6.8.

Favourable features of the soil from an experimental point of view are its good drainage, remarkable uniformity of texture, fertility and evenness of slope which is only about 0.75%. Erosion presents no serious problem and moisture retention is good.

Cultural practice

The ground is ploughed as soon as the summer crop is removed. Ploughing operations commence as early as March or April and are usually complete by May. All fertilisers and manures are generally worked into the soil during this period. After the first rains in spring or early summer the soil is disced and harrowed before planting which is all done by hand with the greatest accuracy. The crop grows under dry-land conditions and cultivation after planting is confined to the control of weeds.

Layout

The experimental area is divided into ranges 22 yards wide with 12 ft. pathways between them and the plots lie across the ranges. The randomised block design is in general use.

Procedure of making the crosses

The crossings are carried out during the months of January and February. Briefly the procedure is as follows. A paper bag of appropriate size is inverted over the young ear before the silks emerge and is clipped securely to the stem. As soon as the pollen begins to dehisce the tassels are covered by other bags which are tied tightly round the peduncles to prevent the escape of pollen. After the silks have emerged inside the ear bag and a quantity of pollen has collected in the tassel bag of the selected parent plants the pollen is carefully removed and poured on

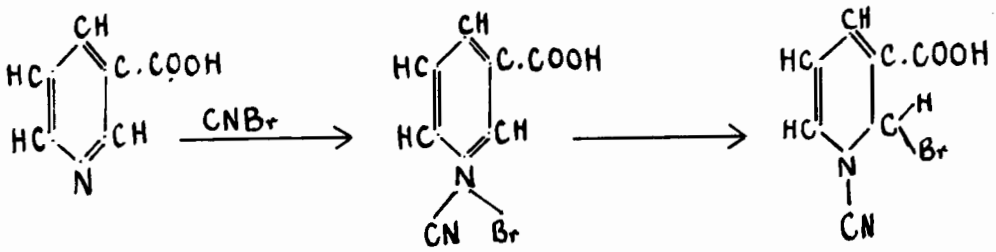
to the silks of the appropriate plant. The paper bags are immediately replaced after each operation.

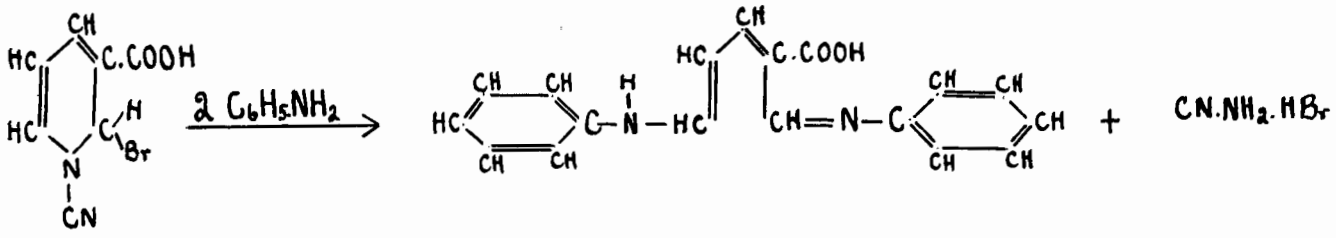
After the maize for these experiments was harvested the grain was picked from the ear and then despatched in paper bags to the author.

All the maize for this experiment was grown under climatic and soil conditions fairly characteristic of the South African maize growing region. The experiment was continued over a period of two years in order to compare results obtained under varying climatic conditions. Of a total of 44 crosses, 33 were carried out during the first season and the remainder during the second season. It is fully realised that two seasons are insufficient to draw any definite conclusions about the influence of the climate.

Chemical Estimation of Nicotinic Acid

Modern chemical methods for the estimation of nicotinic acid are based upon the reaction discovered by Konig (94) in 1904 that pyridine reacts with cyanogen bromide and an aromatic amine to produce a yellow coloured complex. This reaction was developed by Kulikow and Krestovosdwigenskaja (95) and later by Parry-Jones and Strafford (96) for the detection and colourimetric estimation of small quantities of pyridine. The reaction depended on the fact that the pyridine ring was opened by cyanogen bromide and that the reaction product combined with aromatic amines to build a yellow coloured complex. The nitrogen atom of the ring was liberated in the test solution as cyanogen amine. The reaction is still not completely understood but the following scheme represents what is generally thought to take place :-





XXVIII

Yellow coloured complex

Specificity of reaction

The great disadvantage of all chemical methods based on the Konig reaction is that the reaction is not specific for nicotinic acid but is given by a number of pyridine derivatives. Pyridine, nicotine, nicotinic acid, nicotinamide, nipecotic acid, nicotinuric acid and α - aminopyridine give the reaction while trigonelline, quinolinic acid and pyridoxine do not. When penta-valent nitrogen is present such as in the pyridinium compounds no colour reaction is given and another important requirement for the reaction is an unsubstituted α-carbon atom in the pyridine ring. The only known exception to this last rule is α-aminopyridine. Another disadvantage is that the colour complex is extremely sensitive. It is influenced by a large number of variable factors all of which cannot be absolutely controlled.

Decolourisation of extract

Perlzweig,Levy and Sarrett (97) were the first to realise the importance of preparing a colourless solution for application of the Konig reaction. If any pigment remained in the extract when the colour reaction was carried out it might develop increased absorption on the addition of the reagents. The methods of handling the blank compensated for initial absorption due to the pigment itself but there was no way of compensating for new absorption produced by the interaction of the reagents and the pigment. Such new absorption would be evaluated as nicotinic acid and would contribute towards a high result. Therefore to obviate this possibility an efficient decolourisation of the extract was necessary.

Swaminathan (98) decolourised the extracts with charcoal. A little later Pearson (99) reported that in decolourising the extract with charcoal the nicotinic acid was adsorbed and could not be completely eluted. Melnick and Field (100) stated that while losses of nicotinic acid due to charcoal adsorption were high in alcoholic or acid solutions, coupling the mediums was followed by a synergistic action which prevented the adsorption of nicotinic acid especially if a carefully controlled amount of vegetable charcoal (Darco G 60) was used.

One of the major disadvantages of the method used in this work which is based upon that of Melnick et al. (101) is that the extract is not completely decolourised.

Preparation of Blank

There exists a great deal of controversy about the correct way of preparing the blank.

(a) Elimination of cyanogen bromide.

Harris & Raymond (102) reported the presence of interfering substances of aldehydic nature in urine which gave colour with *p*-aminoacetophenone even in the absence of cyanogen bromide. Harris & Raymond accordingly substituted water for cyanogen bromide in the blank.

(b) Elimination of the amine.

Other workers (100) claimed that the presence of cyanogen bromide exercised an inhibitory action on the side reactions due to the aniline reacting with interfering complexes and they replaced the amine with water.

(c) Incorporation of cyanogen bromide and aniline.

Later Melnick and his associates (101) showed that both cyanogen bromide and aniline were necessary and their method of preparing the blank, which was adopted throughout this work, is fully described under "Chemical Procedure".

Some factors influencing colour development

1. Salts

(a) Sodium chloride in high concentration increases the colour intensity. This is a severe handicap as variations in the amount of this salt in the test solution will lead to variations in the amount of colour produced.

(b) Acetate ions interfere by causing fading and this excludes the use of acetate buffers in the estimation.

2. pH

The pH of the solution to which the cyanogen bromide is added is critical. The optimum pH for the development of maximum colour intensity is 6.8 and the colour intensity diminishes for values on either side of this point.

3. Addition of reagents

The interval between the addition of cyanogen bromide and aniline is also critical. The greater the interval the deeper the colour. In order to obtain comparable results test solutions must be given exactly the same treatment and strict timing is most important. In a collaborative method Melnick (103) put this increase of photometric density to use by adding the cyanogen bromide and allowing exactly ten minutes to elapse before adding the aniline. Lamb (104) showed that the interval could be made less critical if the order of adding the reagents was reversed. He first added the aniline which was followed immediately by the cyanogen bromide. Lamb's modification is used throughout this work.

4. Cyanogen bromide

For a given nicotinic acid concentration the maximum intensity varies slightly with the different cyanogen bromide preparations brought into use from time to time.

Choice of amine

In attempts to improve reproducibility, by making the reaction more specific towards nicotinic acid and increasing the stability of the colour, various aromatic amines have been tested. These have been reviewed by Teeri and Shimer (105) who also suggested the use of *m*-phenylenediamine together with 20 per cent hydrochloric acid. The following workers used aniline (106)(98)(107)(99)(100)(101)(104). *p*-Methylaminophenol was employed by (108)(109) and Harris and Raymond (102) after investigating eleven aromatic amines selected *p*-aminoacetophenone which Kodicek (110) later also used. The cyanogen bromide aniline method, despite various improvements, still lacks much that is desired from the standpoint of precise quantitative analysis. In very recent

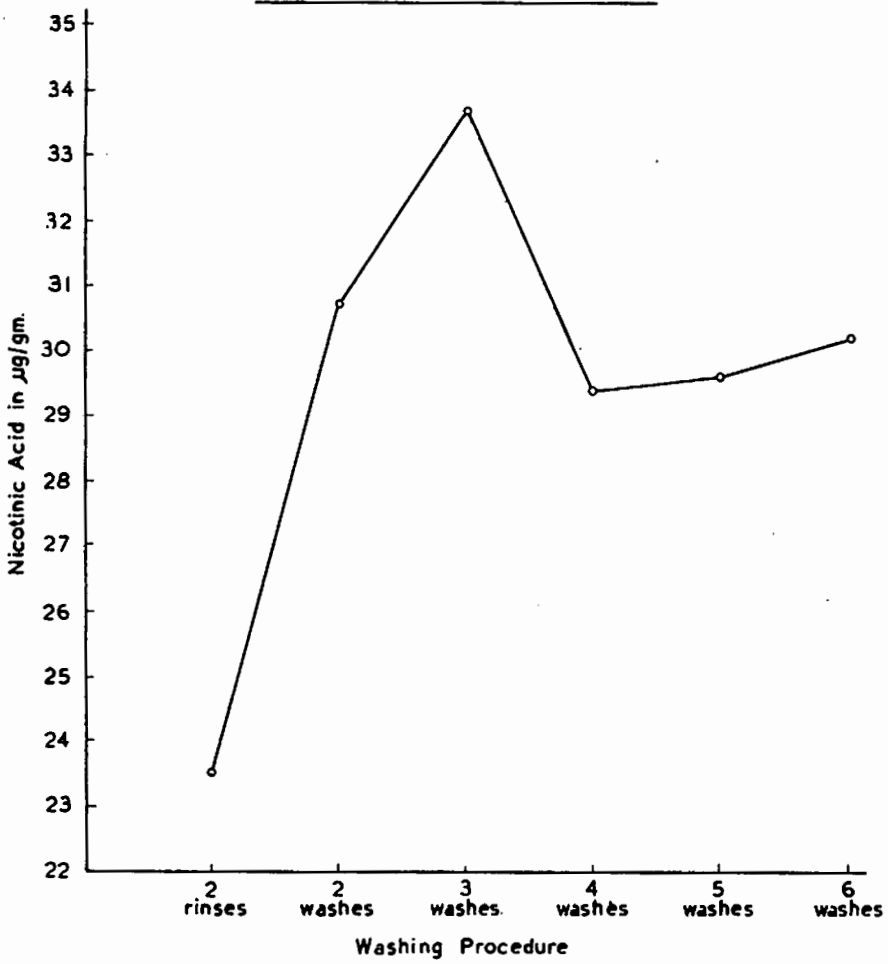
fering compounds in the extract. A good chemical method involving the use of Lloyd's reagent (109)(115) could not be adopted because a supply of this reagent was not available in South Africa and had to be ordered from overseas. Exactly a year after the commencement of the research the first consignment was delivered. The method of microbiological assay could not be undertaken due to lack of certain essential constituents of the basal medium such as biotin. In October 1945 this research was initiated and an immediate start had to be made because the analytical results for 102 lines of maize had to be available by January 1946. A chemical method involving the use of charcoal for decolourisation purposes therefore had to be chosen. This method while it contains a number of modifications remains basically that of Melnick, Oser and Siegel (101). It will be realised that once over a hundred samples of maize which included parental material for future crosses were analysed by this method, all the subsequent hybrid material had to be analysed by exactly the same procedure in order to obtain a basis for the purpose of comparing the results.

Brief digest of method

The maize sample is extracted with water and the solution is separated from the residue by centrifuging. The residue is rinsed with boiling water and the solution combined with the extract. The extract is concentrated to 3 - 5 ml. and then hydrolysed with hydrochloric acid. Absolute ethyl alcohol is added until the solution contains about one-third alcohol and the decolourisation by means of charcoal is carried out on this medium. The clear filtrate is neutralised, made up to volume, and aliquots taken for colour development.

FIG. II

EFFECT OF NUMBER OF WASHES



Extraction of nicotinic acid from maize

Various extraction times ranging from 15 minutes to 2 hours were tried out (Table III). The results are presented in graphical form in Fig. I.

TABLE III

Effect of time of extraction on nicotinic acid content of maize*

Time minutes	Nicotinic acid µg./g.
15	30.6
30	33.4
45	33.6
60	33.6
90	34.9
120	35.3

* 3 gram sample

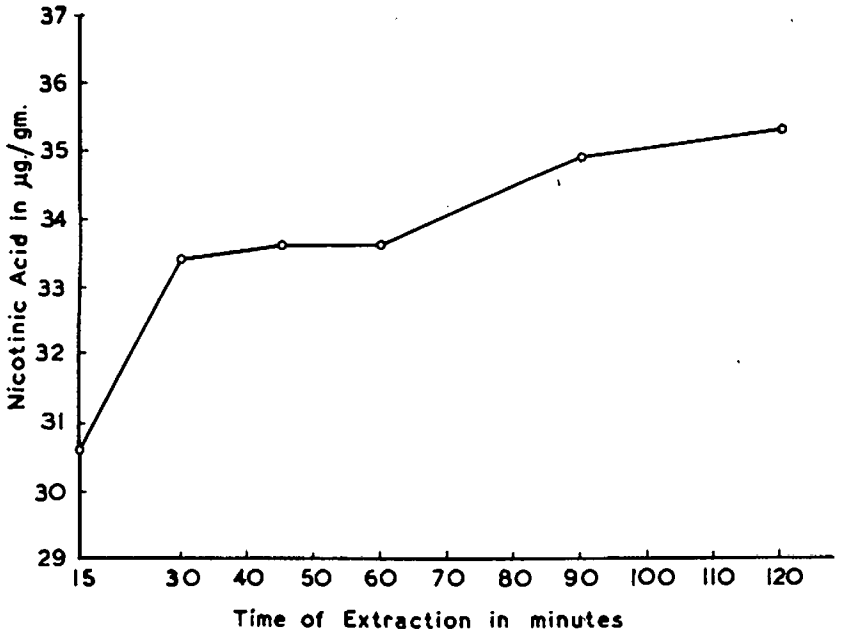
It appears that two fractions are being extracted. In 30 minutes about 95 per cent of the water soluble nicotinic acid is recovered and after 60 minutes the yield is still the same. When the time however is increased to 90 minutes an increase from 33.6 to 34.9 µg./g. is observed and then the curve appears to flatten out again. In view of the fact that the readily soluble fraction is completely extracted in 30 minutes and that this result is in any case only 5.3 per cent lower than the 2 hour extraction value, an extraction time of 30 minutes was adopted for this work. It is suggested that the longer time of extraction brings into solution some interfering pyridine complexes which are subsequently evaluated as nicotinic acid.

Washing the maize residue

After the extraction the suspension is quantitatively transferred to two large centrifuge tubes and centrifuged. The supernatant liquid is decanted into an

FIG. I

INFLUENCE OF TIME OF EXTRACTION



Erlenmeyer flask and the residue is treated with boiling water to remove any nicotinic acid. The effect of simply rinsing out the centrifuge tubes containing the residue and of actually washing the residue up to six times was determined. (Table IV)

A rinse consisted of adding 25 ml. boiling water to each of the tubes, centrifuging and decanting the liquid into the receiving flask.

A wash consisted of adding 25 ml. boiling water to each tube, stirring for half a minute, centrifuging and decanting as before.

TABLE IV

Effect of washing the maize residue*

Washing Procedure	Nicotinic acid µg./g.
2 Rinses	23.5
2 Washes	30.7
3 "	33.7
4 "	29.4
5 "	29.6
6 "	30.2

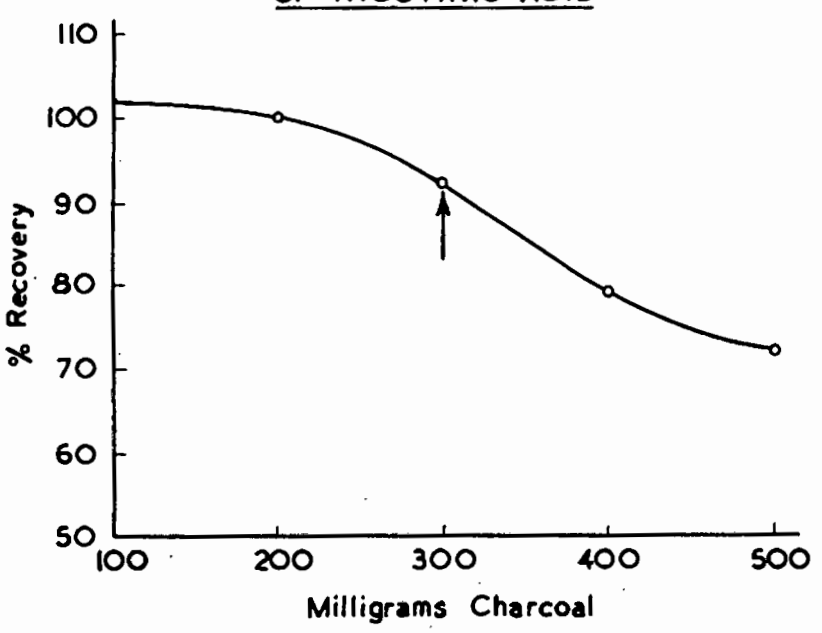
* 5 gram sample

It is readily deduced from Fig. II that by merely rinsing each tube twice a fairly large amount of nicotinic acid is left behind in the residue. At the same time it is seen that two washes give a result of strictly the same order as the values obtained for four, five and six washes and therefore two washes are considered sufficient. The value for three manipulations gives a suspicious peak.

The first large set of samples were rinsed and for the purpose of comparing the results the rinsing technique

FIG. III

INFLUENCE OF CHARCOAL ON RECOVERY OF NICOTINIC ACID



had to be followed throughout the remainder of the work. The method using this procedure gave an average reproducibility of 3.9 per cent.

Decolourisation of Extract

The influence of charcoal (Darco G 60) on the recovery of nicotinic acid in a pure system is shown in Fig. III. Up to 800 mg. of charcoal cause no loss of nicotinic acid but larger amounts of the decolourising agent do cause loss by adsorption.

TABLE V

Influence of charcoal on the recovery of nicotinic acid

Charcoal mg.	Recovery of nicotinic acid %
100	102
200	100
300	92
400	79
500	72

It was decided to use 300 mg. charcoal for this work because it gave a recovery of over 90% (92%). Even this quantity did not yield completely clear solutions and the blanks on the average contributed 18 per cent of the colour.

Colour development

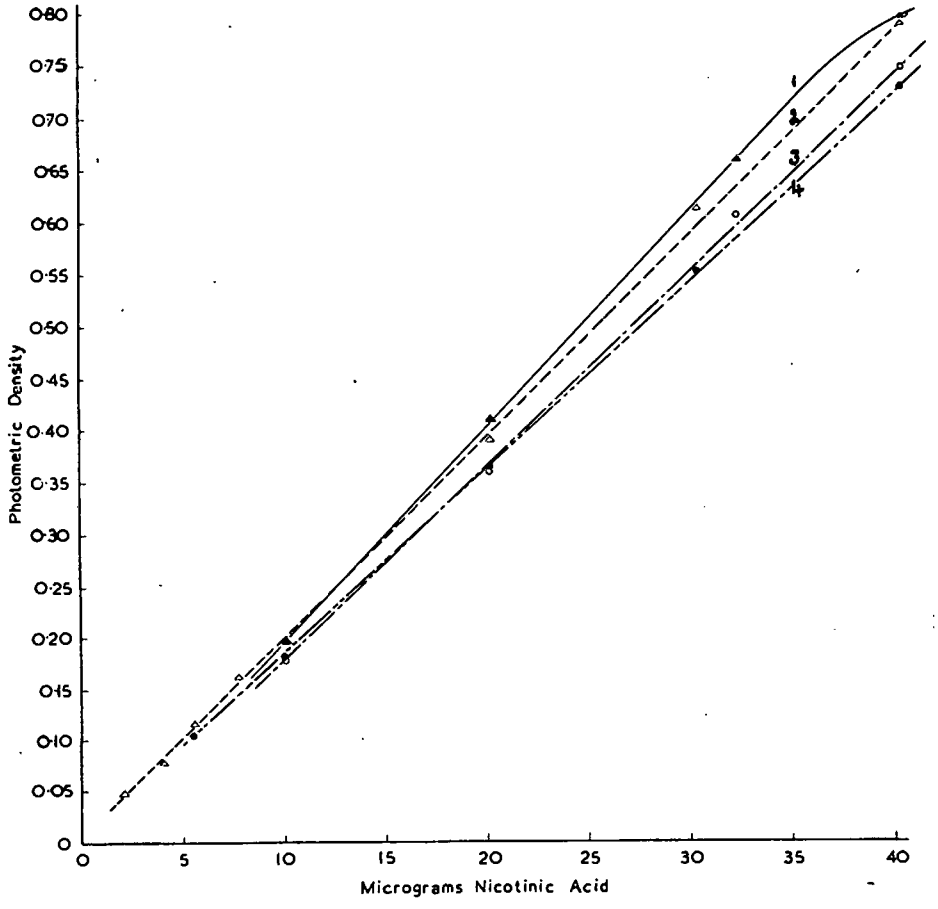
1. Effect of light

Light has a definite influence on colour development. For a given quantity of nicotinic acid the intensity of the colour developed in the dark is greater than that developed in room light (Table VI -- compare procedures 1 and 3). It is clearly shown in Fig. IV by virtue of the diverging lines (1 and 3) that between the limits of 10 and 34 μg. nicotinic acid this effect increases with increasing

FIG IV

INFLUENCE OF LIGHT AND TEMPERATURE ON DEVELOPMENT OF COLOUR

1. Cyanogen bromide at 5°C and colour developed in the dark.
2. Cyanogen bromide at 5°C and colour developed in colourimeter
3. Cyanogen bromide at 5°C and colour developed in roomlight
4. Cyanogen bromide at 20°C and colour developed in colourimeter



concentration. The difference in photometric density at the 20 μg. level is just over twice the difference at the 10 μg. level. This influence of light immediately suggests the use of a standard light condition for actual colour development and it is recommended that following the addition of the reagents the tube be immediately inserted in the colourimeter. An advantage of permitting the colour to develop in this way is that with cyanogen bromide used at 5° C the colour intensities for the normal working range of 5 - 20 μg. nicotinic acid correspond on the whole to the maximum intensities which develop in the dark. This is an important consideration when it comes to estimating quantities of nicotinic acid between 2 - 12 μg..

TABLE VI

Variation in Photometric Density* under different conditions of colour development

Procedure	Nicotinic acid, μg./ml.	0.02	0.04	0.06	0.08	1	2	3	4	5
1	Cyanogen bromide at 5° C. Colour developed in the dark					0.196	0.409	0.622	0.796	
2	Cyanogen bromide at 5° C. Colour developed in the colourimeter	0.046	0.078	0.115	0.161	0.196	0.390	0.611	0.789	0.959
3	Cyanogen bromide at 5° C. Colour developed in room light					0.174	0.357	0.556	0.748	
4	Cyanogen bromide at 20° C. Colour developed in the colourimeter			0.102		0.179	0.359	0.549	0.727	0.939

* The colour is read in an Evelyn Photoelectric colourimeter and the photometric density = 2 - log. G
where G = galvanometer reading.

2. Effect of temperature

An increase in temperature causes a lowering of the maximum colour intensity. Variations due to differences in temperature can be fairly well controlled by adding the cyanogen bromide at 5°C to the test solution. This procedure appears to ensure a more complete reaction as it yields a greater intensity of colour. It is interesting to note from Table VI that the use of cyanogen bromide at 20°C (compare procedures 4 and 2) and the effect of room light (compare procedures 3 and 2) reduce the intensity by about the same amount and the curves 4 and 3 of Fig. IV practically correspond with one another between the 7.5 and 25 μg. limits.

Of the four curves in Fig. IV number 2 represents practically a direct proportion between photometric density and the concentration of nicotinic acid. Such a relationship is of the highest importance especially where an increment method of evaluation is used and the calculation depends upon the ratio of the value of the test solution to test solution plus known amount of nicotinic acid.

3. Readings

For the purpose of obtaining comparable results all readings must be taken at the maximum intensity. There is definite evidence against the system of reading the colour after a given set time.

(a) The time (after addition of cyanogen bromide) for the development of maximum intensity differs with varying amounts of nicotinic acid. (Fig. V) A maize extract test solution containing 1.17 μg. nicotinic acid per ml. reaches maximum intensity in 3.5 minutes whereas the same solution plus 10 μg. nicotinic acid attains maximum intensity in 4.5 minutes.

FIG. V

TIME FOR DEVELOPMENT OF MAXIMUM COLOUR INTENSITY IN A MAIZE EXTRACT SYSTEM

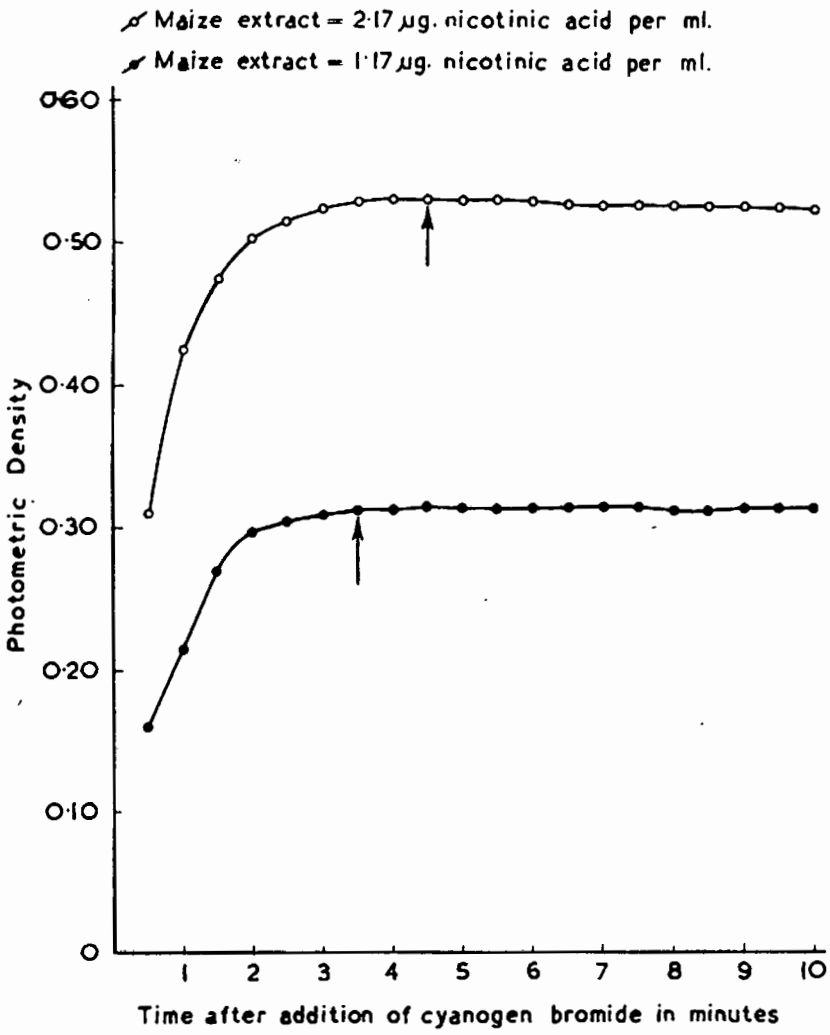


TABLE VII

The time for development and stability of maximum colour for different amounts of nicotinic acid*

Time after addition of cyanogen bromide minutes	Photometric Density	
	1.17 μg. nicotinic acid per ml.	2.17 μg. nicotinic acid per ml.
0.5	0.161	0.310
1.0	0.215	0.426
1.5	0.272	0.475
2.0	0.297	0.502
2.5	0.305	0.516
3.0	0.312	0.523
3.5	0.314	0.527
4.0	0.314	0.528
4.5	0.314	0.530
5.0	0.314	0.530
5.5	0.314	0.530
6.0	0.314	0.530
6.5	0.314	0.527
7.0	0.314	0.527
7.5	0.314	0.527
8.0	0.312	0.527
8.5	0.312	0.527
9.0	0.312	0.525
9.5	0.312	0.525
10.0	0.312	0.523

* The aniline is added first and the time is noted on the addition of the cyanogen bromide.

(b) It will be observed from Fig. V that the stability of the colour also varies with the amount of nicotinic acid in the test solution. A maize extract containing 1.17 μg. nicotinic acid per ml. gives a maximum colour which remains stable for 4 minutes but the same solution containing 2.17 μg. nicotinic acid per ml. gives a colour which only remains stable for 1$\frac{1}{2}$ minutes.

These two points in addition to the influence of light already considered stress the importance of having the tube in the colourimeter immediately after adding the reagents in order to ensure reading the maximum value.

TABLE VIII

NICOTINIC ACID CONTENT OF MAIZE

Sample	Variety	Nicotinic Acid on Absolute Dry Weight Basis µg./g.	Sample	Variety	Nicotinic Acid on Absolute Dry Weight Basis µg./g.	Sample	Variety	Nicotinic Acid on Absolute Dry Weight Basis µg./g.	Sample	Variety	Nicotinic Acid on Absolute Dry Weight Basis µg./g.
Hickory King	white dent	14.9	118	(2 x Hotnot)F2	19.0	427	yellow dent	20.8	264	yellow flint	24.4
Potchefstroom Pearl	white dent	15.3	46	yellow flint	19.0	399	yellow dent	20.8	379	yellow dent	24.5
360	yellow flint	15.7	U.S.13	yellow dent	19.0	Tecmaize	yellow flint	20.9	252	yellow flint	24.9
22	white dent	15.8	6	white dent	19.0	290	yellow flint	21.0	267	yellow flint	24.9
138	(white dent x Hotnot)F2	15.9	588	yellow dent	19.1	151	(50 x Hotnot)F2	21.0	408	yellow dent	25.2
15	white dent	16.9	155	(50 x Hotnot)F2	19.1	242	yellow flint	21.1	389	yellow dent	25.4
404	yellow dent	16.9	356	yellow flint	19.3	Anveld	white dent	21.2	9	white dent	25.5
2	white dent	17.0	50	yellow flint	19.3	51	yellow flint	21.2	347	yellow flint	25.6
Synthetic Anveld	white dent	17.1	13	white dent	19.5	Sahara	yellow dent	21.2	293	yellow flint	26.7
146	(49 x Hotnot)F2	17.1	405	yellow dent	19.5	272	yellow flint	21.3	301	yellow flint	26.8
136	hybrid of Hotnot F2	17.2	315	yellow flint	19.7	Synthetic Tecmaize	yellow flint	21.3	383	yellow dent	27.5
123	hybrid of Hotnot F2	17.2	Golden Standard	yellow dent	19.7	Robyn	yellow dent	21.8	418	yellow dent	28.7
Hotnot	yellow flint	17.2	49	yellow flint	19.7	406	yellow dent	21.8	422	yellow dent	28.8
7	white dent	17.4	375	yellow flint	19.8	21	white dent	21.9	351	yellow flint	28.9
327	yellow flint	17.5	48	yellow flint	20.0	122	hybrid of Hotnot F2	21.9	271	yellow flint	31.3
19	white dent	17.5	346	yellow flint	20.1	247	yellow flint	22.0			
Synthetic Potchefstroom Pearl	white dent	17.5	359	yellow flint	20.3	300	yellow flint	22.1	86	Sweetcorn	36
			149	(49 x Hotnot)F2	20.3	116	(2 x Hotnot)F2	22.1	93	Sweetcorn	46
1	white dent	17.6	20	white dent	20.3	430	yellow dent	22.2	Golden Cross	yellow Sweetcorn	48
4	white dent	17.9	368	yellow flint	20.4	378	yellow dent	22.2	95	Sweetcorn	49
129	(22 x Hotnot)	18.1	16	white dent	20.6	5	white dent	22.5	90	Sweetcorn	51
329	yellow flint	18.2	17	white dent	20.7	8	white dent	22.9	Potchefstroom synthetic	white Sweetcorn	52
390	yellow dent	18.3	12	white dent	20.7	10	white dent	22.9	94	Sweetcorn	55
119	(2 x Hotnot)F2	18.5	14	white dent	20.8	3	white dent	23.0	89	Sweetcorn	56
392	yellow dent	18.5				407	yellow dent	23.4	96	Sweetcorn	63
18	white dent	18.8				47	yellow flint	23.5	85	Sweetcorn	72
126	hybrid of										

The K value which is obtained by dividing the photometric density by the nicotinic acid concentration of the test solution is constant for amounts of nicotinic acid varying between 4 - 50 μg. However the actual value of K varies not only with the different batches of cyanogen bromide but also with the temperature at which the reagent is used and the light conditions prevailing during colour development. Due to this variation of K no previously determined standard curve can be used and instead a K value has to be obtained for each series of test solutions.

Preparation of Sample

The normal size of a sample ranged from 20 - 200 g. The grains were spread out on brown paper and separated from any extraneous matter such as straw by hand picking. By means of a coffee grinder the whole sample was ground up until it all passed a 60 mesh sieve. This entailed several operations as after each sieving the rejected portion was put through again. Different types of electrically driven mills were tried out but these were too large in capacity to handle the small samples. The fine sample was spread out on brown paper and thoroughly mixed. In order to secure a good uniform mixture the sample was divided into six parts and spread by means of a spatula in the centre of the paper. The spatula treatment of the six small heaps was conducted at random. The resulting mixture was then rolled about by manipulating the ends of the paper and the sample was stored in a glass bottle with a bakelite screw-type lid.

Moisture estimation

In order to have a sound basis of comparison all results were expressed on the moisture-free basis. 5 grams of

the finely divided sample were heated overnight in an air oven at 105° C and the loss of weight was expressed as moisture.

Chemical Procedure

Principle - The lemon yellow colour produced when nicotinic acid reacts with cyanogen bromide and aniline is measured in an Evelyn Photoelectric colourimeter using a 420 mμ filter.

Reagents

Cyanogen bromide - Water saturated with bromine at 5 - 10° C is decolourised in the cold by the addition of a cold solution of 10% sodium cyanide (A.R.). In order to avoid a large excess of sodium cyanide the last stage of the titration is carefully accomplished with a 1% solution. When the reagent is stored in a brown bottle in the refrigerator at about 5° C, it keeps for at least 6 weeks.

Aniline solution - Redistilled aniline is dissolved in absolute ethyl alcohol to make a 4% solution. Stored in a brown bottle at room temperature this reagent will keep for months.

Standard nicotinic acid solution - Contains 100 μg. nicotinic acid per ml. absolute ethyl alcohol. If stored in the refrigerator keeps many months.

Buffer solution - water 1960 ml.
85% phosphoric acid 10 ml.
15% sodium hydroxide solution 30 "
Absolute ethyl alcohol 333 "

This alcoholic phosphate buffer used in the evaluation of the blank, while possessing the same titratable acidity and pH as that produced in the test solution by cyanogen bromide and aniline gives the same value as distilled water in the photoelectric colourimeter.

Charcoal - Darco G 60

Hydrochloric acid - Concentrated hydrochloric acid S.G. 1.18

1 Normal Solution

Sodium hydroxide - 18 Normal Solution

1 Normal Solution

Absolute ethyl alcohol

Indicators - Phenolphthalein (1% in alcohol)

Bromothymol blue (0.04% in alcohol)

Procedure

Five grams of the finely divided uniform sample are weighed into a 250 ml. wide-neck Erlenmeyer flask. 100 ml. distilled water are added and after stirring for one minute the suspension is autoclaved for 30 minutes at 15 pounds pressure. In order to prevent loss due to frothing the pressure is gradually reduced. The mixture is well stirred and quantitatively transferred while still hot to two 75 ml. centrifuge tubes using small quantities of hot water to wash out the flask. The tubes are centrifuged and the supernatant liquid decanted into a 250 ml. wide neck Erlenmeyer flask. The residue in each tube is rinsed twice with 25 ml. boiling water and each time after centrifuging the solution is combined with the extract. The solution is concentrated overnight to a volume of 2 - 5 ml. The solution should not be taken to dryness. If this occurs, the concentrated hydrochloric acid (added in the next step) reacts with the extract producing a very dark

brown colour which later at the colourimeter stage yields a high blank value and this affects the validity of the result.

To the concentrated aqueous extract 5 ml. of concentrated hydrochloric acid are added and the material substantially dissolved by immersing the Erlenmeyer flask in a boiling water bath for at least two minutes. During this process the flask is removed and rotated to ensure that the liquid is brought into contact with the sides. The suspension is transferred by means of a policeman to a 15 ml. calibrated test tube. The flask is washed twice with very small amounts of hot water and the washings are added to the test tube. The tube is cooled and the contents made up to the mark with distilled water. The contents are mixed by shaking and the test tube is then immersed in a boiling water bath and hydrolysis allowed to proceed for 45 minutes with occasional shaking of the solution. The sample is cooled to room temperature and the volume restored to the 15 ml. mark with distilled water.

10 ml. of absolute ethyl alcohol are added and the solution is shaken up and transferred to a 125 ml. Erlenmeyer flask. 300 mg. charcoal (Darco G 60) are added, the flask shaken for 3 minutes and using Whatman no. 42 (or 542) paper the solution is filtered at room temperature.

A one half aliquot of the filtrate i.e. 12.5 ml. is pipetted into a clean 15 ml. calibrated test tube, one drop phenolphthalein is added, and the solution is neutralised in the cold to pH 6.8. The final pH adjustment is made using bromthymolblue as external indicator. The volume is then made up to the mark and the contents mixed by shaking. For the neutralisation 1N Normal sodium hydroxide is used at first but the last stage is effected using normal solutions

of sodium hydroxide and hydrochloric acid. During the neutralisation the solution becomes distinctly more yellow in colour and a white gelatinous precipitate forms which after making the solution up to volume is removed by centrifuging. The clear supernatant liquid is decanted and from this the aliquots are taken for the development of colour. For this stage 3 aliquots of 3 ml. are taken and introduced into specially matched tubes. A 3 ml. aliquot represents one-tenth of the original sample.

Treatment of Aliquots

Aliquot (a) - 7 ml. alcoholic buffer solution are added and mixed by rotating tube rapidly.

Aliquot (b) - 1 ml. aniline solution is added, the tube rotated rapidly, and this is immediately followed by the addition of 6 ml. cyanogen bromide solution. The contents are again mixed.

Aliquot (c) - 0.1 ml (= 10μg.) standard nicotinic acid solution is added followed by the reagents as in (b).

In the case of (b) and (c) immediately after adding the cyanogen bromide solution the tube is inserted in the colourimeter and the maximum intensity recorded. In the case of maize extracts this usually develops in 3 - 6 minutes. The colour is read in an Evelyn Photoelectric colourimeter with the 420 mμ filter.

By subtracting the photometric density of solution (a) (due to residual colour) from that of (b) the photometric density of the reacted nicotinic acid is obtained. This is converted into absolute units of nicotinic acid by correlating the increment of photometric density of solution (c) minus solution (b) with the amount of nicotinic acid added.

Centre settings

In the colourimetric measurement it is necessary to have two centre settings I and II, centre setting I for evaluating the residual colour of the test solution and centre setting II for the colour developed by the chemical reaction.

Solution A for centre setting I

water	2 ml.
Absolute ethyl alcohol	1 "
Alcoholic buffer solution	7 "

Solution B for centre setting II

water	2 ml.
Absolute ethyl alcohol	1 "
Aniline solution	1 "
Cyanogen bromide solution	6 "

The colourimeter is set to give a galvanometer reading of 100.0 with solution A and the blanks are read in turn to determine the residual colour of the test solution. The colourimeter is then set to give a reading of 100.0 with solution B and all the subsequent solutions containing reacted nicotinic acid are read using this centre setting.

Galvanometer readings are converted into photometric density by the formula

$$L = a - \text{Log. } G$$

where L = photometric density
G = galvanometer reading.

(Most instruments are supplied with a conversion table.)

General remarks

The method is long and tedious taking one operator 4 - 5 days to work 6 samples through in duplicate.

The average reproducibility is 3.9% and reproducibility ranges from 0.4 - 6.9%. This is good in comparison with the chemical and microbiological methods available today.

Statistical Treatment of Results

(a) Determination of standard deviation of a large number of samples.

$$\sigma' = C\sqrt{\frac{\Sigma f(d')^2}{N} - \left(\frac{\Sigma f d'}{N}\right)^2} \qquad 1$$

where σ' = standard deviation uncorrected for grouping of data

d' = deviation of midpoint of class interval from arbitrary origin in terms of class intervals

f = frequency of items in class intervals

C = size of class interval

N = number of items

Sheppard's correction for grouping is then applied as follows -

$$\sigma^2 = \sigma'^2 - \frac{C^2}{12} \qquad 2$$

where σ = corrected standard deviation

σ' = uncorrected standard deviation

C = class interval

(b) Determination of standard deviation of a small number of samples.

In cases where the number of items is of the order 30 and below the following formula is used

$$\sigma' = \sqrt{\frac{\Sigma f d^2}{N-1}} \qquad 3$$

where d = deviation of midpoint of class interval from the mean

Then σ' is corrected for grouping of data as in (2).

(c) Significance of difference between two means.

In common practice with other workers, if the difference between two means is greater than twice the standard error of difference between the means it is regarded as significant. The standard error of difference between the means M_1 and M_2 is given by the formula

$$\sigma_D = \sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}} \qquad 4$$

where σ_D = standard error of difference between two means M_1 and M_2

σ_1 = standard deviation of first sample

σ_2 = standard deviation of second sample

N_1 = number of items in first sample

N_2 = number of items in second sample.

The difference between the means M_1 and M_2 is significant if:

$$M_1 - M_2 > 2\sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}} \qquad 5$$

Nicotinic acid content of South African Maize

In Table VIII are set out in ascending order of nicotinic acid content the data for 102 lines of maize. The most striking feature of the table is the very high nicotinic acid content of sweetcorn which in the ten varieties studied ranges from 56 - 72 μg./g. and gives an average of 65 μg./g. which is of the same order as wheat. This information concerning sweetcorn was put to use in an endeavour to establish the factorial basis of inheritance of nicotinic acid by making crosses between sugary and starchy varieties. The mean nicotinic acid content of 92 samples of starchy maize is 20.8 μg./g. ($\sigma = \pm$ 5.8). A very significant point is the wide range which extends from 14.9 - 51.5 μg./g. In view of the fact that the samples were grown on the same soil in the same locality the range is encouraging of the possibility of positive results in an inheritance study. Burkholder, McVeigh and Meyer (39) studied the nicotinic acid content of 253 strains and hybrids of yellow and white maize in the U.S.A. In Table IX the work of Burkholder, McVeigh and Meyer is lined up for purposes of comparison with the corresponding data gathered for South African grown maize in this research programme. It will be observed that the nicotinic acid content of South African maize conforms to that obtained in the U.S.A.

Of immediate importance is the fact that the data set out in Table VIII supplies a basis for choosing a variety with a relatively high level of nicotinic acid. It is stressed that the white dent varieties Hickory King and Potchefstroom Pearl, the most widely grown maize in South Africa, pathetically occupy the lowest positions in the table. Some of the popular varieties of maize grown in South Africa are listed below.

TABLE VIII

NICOTINIC ACID CONTENT OF MAIZE

Sample	Variety	Nicotinic Acid on Absolute Dry Weight Basis µg./g.	Sample	Variety	Nicotinic Acid on Absolute Dry Weight Basis µg./g.	Sample	Variety	Nicotinic Acid on Absolute Dry Weight Basis µg./g.	Sample	Variety	Nicotinic Acid on Absolute Dry Weight Basis µg./g.
Hickory King	white dent	14.9	118	(2 x Hotnot)F2	19.0	427	yellow dent	20.8	264	yellow flint	24.4
Potchefstroom Pearl	white dent	15.3	46	yellow flint	19.0	399	yellow dent	20.8	379	yellow dent	24.5
360	yellow flint	15.7	U.S.13	yellow dent	19.0	Teomaize	yellow flint	20.9	252	yellow flint	24.9
22	white dent	15.8	6	white dent	19.0	290	yellow flint	21.0	267	yellow flint	24.9
138	(white dent x Hotnot)F2	15.9	388	yellow dent	19.1	151	(50 x Hotnot)F2	21.0	408	yellow dent	25.2
15	white dent	16.9	155	(50 x Hotnot)F2	19.1	242	yellow flint	21.1	389	yellow dent	25.4
404	yellow dent	16.9	356	yellow flint	19.3	Anveld	white dent	21.2	9	white dent	25.5
2	white dent	17.0	50	yellow flint	19.3	51	yellow flint	21.2	347	yellow flint	25.6
Synthetic Anveld	white dent	17.1	13	white dent	19.5	Sahara	yellow dent	21.2	293	yellow flint	26.7
146	(49 x Hotnot)F2	17.1	405	yellow dent	19.5	272	yellow flint	21.3	301	yellow flint	26.8
136	hybrid of Hotnot F2	17.2	315	yellow flint	19.7	Synthetic Teomaize	yellow flint	21.3	383	yellow dent	27.5
123	hybrid of Hotnot F2	17.2	Golden Standard	yellow dent	19.7	Robyn	yellow dent	21.8	418	yellow dent	28.7
Hotnot	yellow flint	17.2	49	yellow flint	19.7	406	yellow dent	21.8	422	yellow dent	28.8
7	white dent	17.4	375	yellow flint	19.8	21	white dent	21.9	351	yellow flint	28.9
327	yellow flint	17.5	48	yellow flint	20.0	122	hybrid of Hotnot F2	21.9	271	yellow flint	31.3
19	white dent	17.5	346	yellow flint	20.1	247	yellow flint	22.0			
Synthetic Potchefstroom Pearl	white dent	17.5	359	yellow flint	20.3	300	yellow flint	22.1	86	Sweetcorn	36
			149	(49 x Hotnot)F2	20.3	116	(2 x Hotnot)F2	22.1	93	Sweetcorn	46
1	white dent	17.6	20	white dent	20.3	430	yellow dent	22.2	Golden Cross	yellow Sweetcorn	48
4	white dent	17.9	368	yellow flint	20.4	378	yellow dent	22.2	95	Sweetcorn	49
129	(22 x Hotnot)	18.1	16	white dent	20.6	5	white dent	22.5	90	Sweetcorn	51
329	yellow flint	18.2	17	white dent	20.7	8	white dent	22.9	Potchefstroom synthetic	white Sweetcorn	52
390	yellow dent	18.3	12	white dent	20.7	10	white dent	22.9	94	Sweetcorn	55
119	(2 x Hotnot)F2	18.5	14	white dent	20.8	3	white dent	23.0	89	Sweetcorn	56
392	yellow dent	18.5				407	yellow dent	23.4	96	Sweetcorn	63
18	white dent	18.8				47	yellow flint	23.5	85	Sweetcorn	72
126	hybrid of										

TABLE IX

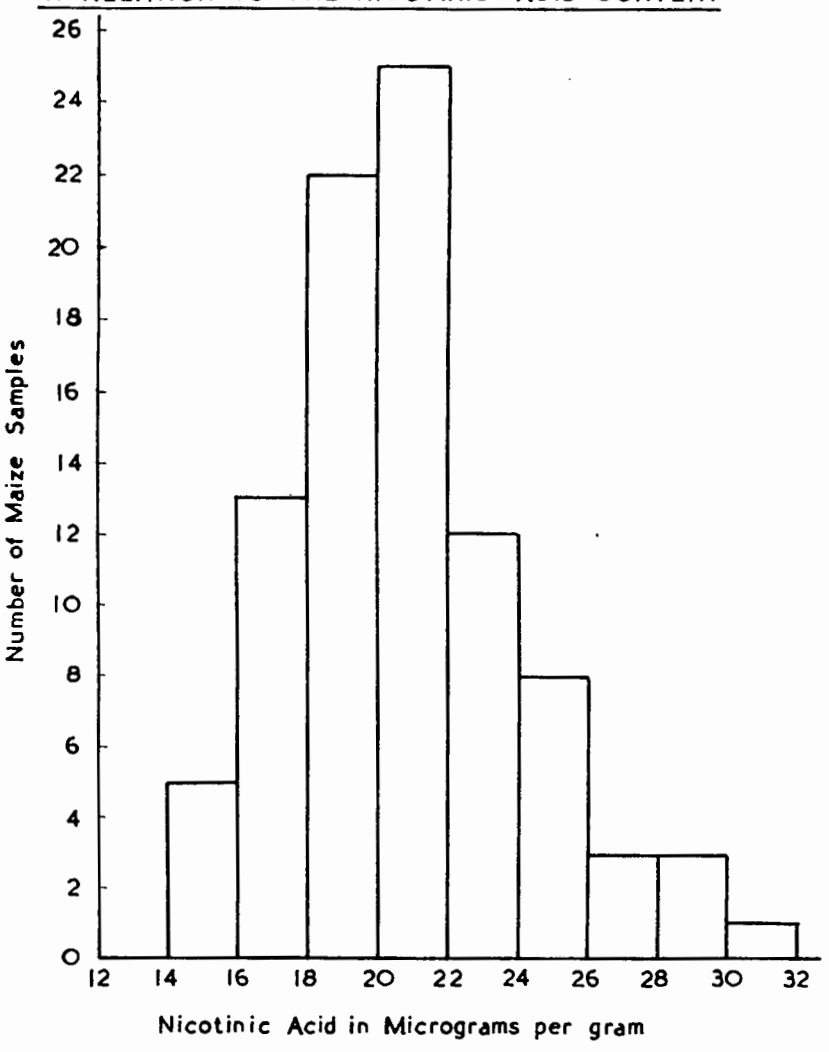
Comparison of the Nicotinic Acid content of maize grown in the United States of America and in South Africa

Data of Burkholder et al. on U.S.A. maize			Data of author on S.A. maize		
Results expressed on air-dry basis			Results expressed on moisture-free basis		
Maize	Range µg./g.	Average µg./g.	Maize	Range µg./g.	Average µg./g.
46 Strains of Sweetcorn	18.2-62.1	34.6	10 Strains of Sweetcorn	36 - 72	53
94 Varieties of yellow field maize	11.3-36.3	21.4	53 Varieties of yellow field maize	15.7-31.3	21.8
86 Varieties of white field maize	12.7-29.4	20.1	25 Varieties of white field maize	14.9-25.5	19.6
7 Strains of popcorn	7.9-21.6	17.4			

FIG. VI

DISTRIBUTION OF STARCHY MAIZE

IN RELATION TO THE NICOTINIC ACID CONTENT



		nicotinic acid µg./g.
white dent		
	Hickory King	14.9
	Potchefstroom Pearl	15.3
	Anveld	21.2
yellow dent		
	Sahara	21.2
	Robyn	21.8
yellow flint		
	Hotnot (Improved Boesman)	17.2

It will be observed that none of these values lie in the upper region of table VIII. There is a general movement afoot to try and supplant the cultivation of white maize with the nutritionally superior yellow maize (higher provitamin A content) and this transitional step presents the ideal opportunity for selecting lines with a high nicotinic acid content.

From the histogram (Fig. VI) showing the distribution of starchy maize with respect to nicotinic acid content it is seen that the dispersion about the mean corresponds fairly well with a normal distribution.

The results of some hybrids nos. 129, 119 118 116, 155 151, 146 149, are shown in Table X.

TABLE X

Nicotinic acid content of some hybrids

Cross No.	Parents	Nicotinic acid content of parents µg./g.	Nicotinic Acid content of hybrids µg./g.	Average Nicotinic acid content of hybrids µg./g.
1	22 x Hotnot	15.8 x 17.2	18.1	18.1
2	2 x Hotnot	17.0 x 17.2	18.5, 19.0, 22.1	19.9
3	50 x Hotnot	19.3 x 17.2	19.1, 21.0	20.1
4	49 x Hotnot	19.7 x 17.2	17.1, 20.3	18.7

From these results it appears that where two parents have a low nicotinic content of the same order the nicotinic acid is additively determined. In the two cases where this is not found the nicotinic acid content of the hybrid is only a shade lower than that of the lower parent. Crosses 1 to 4 are arranged in ascending order from 15.8 x 17.2 to 19.7 x 17.2 and it is seen that with the exception of cross 4 the average of the hybrids from cross 5 is greater than that of cross 2 which in turn is greater than that of cross 1. More generally, the higher the nicotinic acid content of the one parent the higher the nicotinic acid value of the hybrid and this strongly suggests that a genetic influence may be at work. These results although extremely interesting cannot be regarded as conclusive in view of the fact that the parents are not sufficiently inbred.

Nicotinic acid content of Yellow and White Maize

A comparison is made of the nicotinic acid content of starchy varieties of white and yellow maize in Table XI. It is of interest to observe with respect to range that in each of the groups white dent, yellow dent and yellow flint, the nicotinic acid can very nearly or just about double itself. Therefore the characteristic found for the total number of samples examined is also a feature of the component groups. It is apparent that the mean of white maize (19.6 μg./g.) is lower than the mean of yellow maize (21.8 μg./g.) and using formula 5 the difference of 2.2 μg./g. is found to be significant.

$$\sigma_D = \sqrt{\frac{(3.36)^2}{53} + \frac{(2.76)^2}{25}}$$

$$= 0.72$$

$$\text{ie } 2\sigma_D = 1.44$$

TABLE XI

Nicotinic Acid Content of Yellow and White Maize

White dent		Yellow Dent		Yellow Flint	
Sample	Nicotinic Acid µg./g.	Sample	Nicotinic Acid µg./g.	Sample	Nicotinic Acid µg./g.
Hickory King	14.9	404	16.9	360	15.7
Potshefstroom Pearl	15.3	390	18.3	Hotnot	17.2
15	16.9	392	18.3	327	17.3
2	17.0	U.S.13	19.0	329	18.2
Synthetic Anveld	17.1	388	19.1	46	19.0
7	17.4	405	19.3	356	19.3
19	17.3	Golden Standard	19.7	50	19.3
Synthetic Potchefstroom Pearl	17.3	427	20.8	316	19.7
1	17.6	399	20.8	49	19.7
4	17.9	Sahara	21.2	375	19.8
18	18.8	Robyn	21.8	48	20.0
6	19.0	406	21.8	346	20.1
13	19.3	430	22.2	339	20.3
20	20.3	378	22.2	368	20.4
16	20.6	407	23.4	Tecmaize	20.9
17	20.7	387	23.8	290	21.0
12	20.7	379	24.3	242	21.1
14	20.8	408	25.2	51	21.2
Anveld	21.2	389	25.4	272	21.3
21	21.9	383	27.3	Synthetic Tecmaize	21.3
5	22.3	418	28.7	247	22.0
8	22.9	422	28.8	300	22.1
10	22.9			47	23.3
3	23.0			264	24.4
9	23.3			252	24.9
				267	24.9
				347	25.6
				293	26.7
				301	26.8
				351	28.9
				271	31.3
Number of samples 25		Number of samples 22		Number of samples 31	
Range 14.9-25.3 µg./g.		Range 16.9-28.8 µg./g.		Range 15.7-31.3 µg./g.	
Mean 19.6 (σ=±2.76)		Mean 22.3 µg./g. (σ=±3.31)		Mean 21.8 µg./g. (σ=±3.55)	

Total number of yellow maize samples = 53

Range = 15.7 - 31.3 µg./g.

Mean = 21.8 µg./g. (σ = ± 3.36)

The observed difference of 2.2 is much greater than $2\sigma_D$ and the odds against such a difference being due to chance is of the order 369:1. The difference in favour of yellow maize is an indication that the search for a line of high nicotinic acid content is best made among the yellow varieties and in addition lends support to the move to replace white maize with yellow maize for human consumption. The means of yellow dent and yellow flint are close together and the difference of 0.5 µg./g. is not significant.

Comparison of early, medium and late varieties of maize

Growing period classes are not clear cut but out of interest to see whether there is any variation in the nicotinic acid content the maize samples in Table XII are sorted into the arbitrary divisions early, medium and late. The mean of the medium group (19.8 µg./g.) is lower than the mean of the early group (21.4 µg./g.) and the mean of the late group (21.3 µg./g.). It is highly interesting to point out on the basis of the significance test that though the means of early and late lines are practically the same only the difference between early and medium maturing maize is significant.

Selection of material for crossing purposes

102 lines of maize have been investigated for their nicotinic acid content (Table VIII) but of these relatively only a few are sufficiently inbred to ensure the high degree of homozygosity necessary for breeding purposes. The following nine lines were selected as the parental stock:-

white dent	nos. 15, 2, 3, 9.
yellow dent	" 428, 430.
yellow flint	" 301, 271.
sweetcorn	no. 96.

TABLE XII.

Nicotinic Acid Content of Early, Medium and Late Varieties of Starchy Maize.

Sample	EARLY Variety	Nicotinic Acid on Absolute Dry weight basis µg./g.	Sample	MEDIUM Variety	Nicotinic Acid on Absolute Dry weight basis µg./g.	Sample	LATE Variety	Nicotinic Acid on Absolute Dry weight basis µg./g.
360	yellow flint	15.7	22	white dent	15.8	Hickory King	white dent	14.9
2	white dent	17.0	138 hybrid	(white dent x Hotnot)F2	15.9	Petchefstroom Pearl	white dent	15.3
Synthetic Amveld	white dent	17.1	15	white dent	16.9	404	yellow dent	16.9
Hotnot	yellow flint	17.2	146 hybrid	(49xHotnot)F2	17.1	392	yellow dent	18.5
7	white dent	17.4	136 hybrid	(of Hotnot)F2	17.2	U.S.13	yellow dent	19.0
327	yellow flint	17.5	123 hybrid	(of Hotnot)F2	17.2	50	yellow flint	19.3
1	white dent	17.6	19	white dent	17.5	405	yellow dent	19.5
4	white dent	17.9	Synthetic Petch.Pearl	white dent	17.5	375	yellow flint	19.8
129 hybrid	(22xHotnot)F2	18.1				368	yellow flint	20.4
329	yellow flint	18.2	390	yellow dent	18.3	427	yellow dent	20.8
6	white dent	19.0	119 hybrid	(2xHotnot)F2	18.5	399	yellow dent	20.8
118 hybrid	(2xHotnot)F2	19.0	18	white dent	18.8	Sahara	yellow dent	21.2
386	yellow flint	19.3	126 hybrid	(of Hotnot)F2	18.9	Synthetic Tecomaize	yellow flint	21.3
315	yellow flint	19.7	46	yellow flint	19.0			
Golden Standard	yellow dent	19.7	388	yellow dent	19.1	Robyn	yellow dent	21.8
			155 hybrid	(50xHotnot)F2	19.1	406	yellow dent	21.8
48	yellow flint	20.0	13	white dent	19.5	430	yellow dent	22.2
346	yellow flint	20.1	49	yellow flint	19.7	407	yellow dent	23.4
339	yellow flint	20.3	149 hybrid	(49xHotnot)F2	20.3	387	yellow dent	23.8
242	yellow flint	21.1	20	white dent	20.3	379	yellow dent	24.5
Amveld	white dent	21.2	16	white dent	20.6	408	yellow dent	25.2
272	yellow flint	21.3	17	white dent	20.7	418	yellow dent	28.7
122 hybrid	of Hotnot F2	21.9	12	white dent	20.7	422	yellow dent	28.8
247	yellow flint	22.0	14	white dent	20.8			
116 hybrid	(2xHotnot)F2	22.1	Tecomaize	yellow flint	20.9			
5	white dent	22.5	290	yellow flint	21.0			
8	white dent	22.9	151 hybrid	(50xHotnot)F2	21.0			
10	white dent	22.9	51	yellow flint	21.2			
3	white dent	23.0	21	white dent	21.9			
264	yellow flint	24.4	300	yellow flint	22.1			
252	yellow flint	24.9	378	yellow dent	22.2			
267	yellow flint	24.9	389	yellow dent	25.4			
9	white dent	25.5	383	yellow dent	27.5			
347	yellow flint	25.6						
293	yellow flint	26.7						
301	yellow flint	26.8						
351	yellow flint	28.9						
271	yellow flint	31.3						

Number of samples 37	Number of samples 42	Number of samples 22
Range 15.7-31.3 µg./g.	Range 15.8-27.5 µg./g.	Range 14.9-28.8 µg./g.
Mean 21.4 µg./g. ($\sigma_{\pm}$3.8)	Mean 19.8 µg./g. ($\sigma_{\pm}$2.47)	Mean 21.3 µg./g. ($\sigma_{\pm}$3.6)

In order to determine whether nicotinic acid is a genetic factor and at the same time in an effort to throw as much light as possible on the mechanism of its inheritance the following types of crosses were made during the 1945 - 1946 season.

1. High x high

White dents 3 x 9	and the reciprocal	9 x 3
yellow flints 301 x 271	" " "	271 x 301

2. Low x low

White dents 15 x 2	" " "	2 x 15

3. Medium x low

yellow dent x white dent 428 x 2

4. Medium x medium

yellow dent x yellow dent 428 x 430 and the reciprocal 430 x 428

5. High x low

yellow flint x yellow dent 271 x 428	" " "	428 x 271
white dents 9 x 15	" " "	15 x 9
sweetcorn x white dent 96 x 2	" " "	2 x 96
yellow dent x yellow flint 430 x 271	" "	

Results of crosses

The results of the crosses are set out in Table XIII. Although the number of crosses is fairly limited a definite tendency is apparent. It will be observed that in cases where the parents have a content of about 22.2 μg./g. or less the nicotinic acid level of the hybrid appears to be additively determined and exceeds that of both parents. This is true in 12 out of 13 hybrids. Where the content of the parents, or of one of the parents, is higher than 22.2, the cross is intermediate and this is true in the case of 19 out of 20 hybrids.

TABLE XIII

Results of Crosses

Nicotinic acid in μg./g.

Parents	15 wd 16.9	2 wd 17.0	428 yd 20.0	430 yd 22.2	3 wd 23.0	9 wd 25.5	301 yf 26.8	271 yf 31.3	96 sc 63
15 wd 16.9		22.0 22.1				21.9			
2 wd 17.0	24.4								26.3
428 yd 20.0		26.0) 26.9)26.5		24.0) 22.5) 24.4)22.8 20.4)				20.8)21.7 22.6)	
430 yd 22.2			24.4) 23.7)26.1 26.0) 30.2)					25.2) 26.5)25.9 26.0)	
3 wd 23.0						24.3			
9 wd 25.5	24.7				23.3				
301 yf 26.8								30.8	
271 yf 31.3			26.9				24.4)26.5 28.5)		
96 sc 63		26.2) 25.3) 25.1)25.8 25.3) 26.6) 26.2)							

wd = white dent yd = yellow dent yf = yellow flint sc = sweetcorn

Example of reading the table :-

The result of a cross 3 x 9 is determined by selecting the first enumerated parent (in this case 3) in the left-hand vertical column and running the finger along the horizontal column until opposite column 9. The answer is 24.3 μg./g. In the same way the reciprocal cross 9 x 3 gives a value of 23.3 μg./g.

Some very interesting crosses are grouped together in Table XIV and these crosses bear out the existence of a common ancestry effect.

TABLE XIV

Common ancestry effect

Cross No.	Inbreds crossed to make hybrids	Nicotinic acid content of inbreds µg./g.	Nicotinic acid content of hybrids µg./g.
1	428 x 271	20.0 x 31.3	20.8, 22.6 } 21.7
2	430 x 271	22.2 x 31.3	25.2, 26.5, 26.0 } 25.9
3	301 x 271	26.8 x 31.3	30.8
4	271 x 428	31.3 x 20.0	26.9
5	271 x 301	31.3 x 26.8	28.5

The results are not only intermediate between the parents but Table XIV very forcibly indicates that hybrids from lines high in nicotinic acid come from parents high in nicotinic acid acid for it is seen that the hybrid of cross no. 3 is richer in nicotinic acid than no. 2 which in turn is richer than no. 1. The same is true of hybrids derived from crosses 4 and 5.

On the basis of these findings nicotinic acid is definitely a genetically inherited character in maize.

The nicotinic acid content of sweetcorn is much higher than that of starchy maize and the results of the cross 96 x 2 and its reciprocal 2 x 96 bear out the view

held by Mather and Barton-Wright (93) that nicotinic acid follows the action of the Su-su gene even to its dominant starchy (Su) relation. The hybrid of the cross 96 x 2, between sugary and starchy maize, which must naturally contain the dominant starchy (Su) gene is not only starchy in character but its nicotinic acid content of 25.8 µg./g. lies well within the range of nicotinic acid given by seed coming from starchy mothers. The data shows that the very high nicotinic acid content of sweetcorn cannot be used for enhancing the nicotinic acid content of starchy maize through breeding.

In an effort to throw light on the dosage principle compound crosses were made between the following :-

A. [2 x 15]

B. [271 x 301]

C. [96 x 2]

TABLE XV

Nicotinic acid content of compound crosses

Cross	Parents	Nicotinic acid content of parents µg./g.	Nicotinic acid content of hybrid µg./g.
A x B	[2 x 15] x [271 x 301]	24.4 x 26.5	26.0
	[271 x 301] x [2 x 15]	26.5 x 24.4	26.7
B x C	[271 x 301] x [96 x 2]	26.5 x 25.8	28.7
	[96 x 2] x [271 x 501]	25.8 x 26.5	29.4
A x C	[2 x 15] x [96 x 2]	24.4 x 25.8	26.4
	[96 x 2] x [2 x 15]	25.8 x 24.4	26.9

A x B gives (low x low) x (high x high) and as the result of the hybrid is strictly of the same order as that of the parents no combining ability is demonstrated.

B x C and A x C were carried out to see what effect the sugary gene had in various doses against different genetic backgrounds but here again the results which are only slightly higher than the parents do not exhibit any striking variations from which the dosage principle can be deduced.

Eleven crosses were carried out during the 1946-1947 season and the results are recorded in Table XVI.

TABLE XVI

Nicotinic acid content of the 1946-1947 Crosses

No.	Parents	Nicotinic acid content of parents µg./g.	Nicotinic acid content of hybrids µg./g.
1	390 yd x 271 yf	18.3 x 31.3	24.3
2	96 sc. x 2 yd	63 x 17.0	23.4
3	2 yd x 96 sc	17.0 x 63	25.8
4	15 wd x 96 sc	16.9 x 63	21.0
5	96 sc x 15 wd	63 x 16.9	20.3
6	383 yd x 271 yf	27.5 x 31.3	25.2
7	9 wd x 271 yf	25.5 x 31.3	24.8
8	15 wd x 329 yf	16.9 x 18.2	22.2
9	9 wd x 329 yf	25.5 x 18.2	23.1
10	2 yd x 15 wd	17.0 x 16.9	26.0
11	15 wd x 2 yd	16.9 x 17.0	21.9

WHERE yd = yellow dent wd = white dent
yf = yellow flint sc = sweetcorn

These results generally corroborate the findings deduced from the first set of crosses. The crosses between sugary and starchy maize, namely 96 x 2 and 96 x 15, confirm the previous finding that nicotinic acid follows the action of the Su-su gene even to its dominant relation. The crosses 15 x 329 and 15x2 substantiate the result that hybrids from parents low in nicotinic acid have a higher nicotinic acid content than either parent.

Four crosses were repeated and the results obtained for both seasons are grouped in Table XVII.

TABLE XVII

Comparison of Crosses made during the 1945-1946 season and the 1946-1947 season

No.	Parents	Nicotinic acid content of parents µg./g.	Nicotinic acid content of hybrid 1945-1946 µg./g.	1946-1947 µg./g.
1	2 x 96	17.0 x 63	26.3	25.8
2	96 x 2	63 x 17.0	25.8	23.4
3	15 x 2	16.9 x 17.0	22.1	21.9
4	2 x 15	17.0 x 16.9	24.4	26.0

These results are very important in that not only are they in close agreement but that they also follow the same trend. It will further be observed that with the exception of the cross 2 x 15 the results for 1946-1947 are lower than the corresponding values for 1945-1946. The cross 2 x 15 gives a high value of 26.0 µg./g. but this result follows the same trend as that observed in the 1945-1946 season where 2 x 15 gave a higher result than that of the reciprocal 15 x 2.

The results of all the crosses have been combined and are presented in Table XVIII.

TABLE XVIII

Complete set of Crosses

Nicotinic acid in µg./g.

Par-ents	15 wd 16.9	2 wd 17.0	329 yf 18.2	390 yd 18.3	428 yd 20.0	430 yd 22.2	3 wd 23.0	9 wd 25.5	301 yf 26.8	383 yd 27.5	271 yf 31.3	96 sc 63
15 wd 16.9		21.9 22.0 22.1	22.2					21.9				21.0
2 wd 17.0	24.4 26.0											25.8 26.3
329 yf 18.2												
390 yd 18.3											24.3	
428 yd 20.0		26.0 26.9				24.0 22.5 24.4 20.4					20.8 22.6	
430 yd 22.2					24.4 23.7 26.0 30.2						25.2 26.5 26.0	
3 wd 23.0								24.3				
9 wd 25.5	24.7		23.1				23.3				24.8	
301 yf 26.8											30.8	
383 yd 27.5											23.2	
271 yf 31.3					26.9				24.4 28.5			
96 sc 63	20.3	23.4 25.1 25.3 25.3 26.2 26.2 26.6										

wd = white dent yf = yellow flint yd = yellow dent sc = sweetcorn

This table is read in the same way as Table XIII

CONCLUSIONS

Starchy maize exhibits a wide range of nicotinic acid content which for the 92 lines investigated extends from 14.9 - 31.3 μg./g. In view of the fact that all these plants grew on the same soil type, in the same locality during the same year it would not be an extremely wide guess to infer that nicotinic acid might be a genetic factor. That this is so is supported by the following facts :-

1. In cases where the nicotinic acid content of one or both parents exceeds 22.2 μg./g. the crosses have an intermediate content. Where the parents do not exceed this value of 22.2 μg./g. the nicotinic acid of the hybrids appears to be additively determined.

2. Hybrids high in nicotinic acid come from parents high in nicotinic acid.

3. The fact that much greater variations in nicotinic acid content exist in the case of inbred lines than in the hybrids indicates very strongly that the variations are due to genetic influences. Whereas the range of parents extends from 16.9 - 31.3 μg./g. that of the crosses is much smaller extending from 20.8 - 30.8 μg./g. From this significant fact it is presumed that if an open pollinated starchy variety has a wide enough genetic basis the various genotypes will show a blending effect so that the content of the middle twenties is achieved.

Results of the crosses between starchy and sugary maize indicate that the high nicotinic acid content of sugary maize cannot be used to increase the nicotinic acid content of starchy varieties by means of crossing. Unfortunately no crosses were made between varieties of sugary maize and it remains to be discovered whether the high nicotinic acid content of sugary maize is also a genetic factor.

While this work supports the belief that nicotinic acid is a genetically inherited factor it fails to throw light on the mechanism of its inheritance and the dosage principles involved. This aspect definitely merits immediate attention as its successful elucidation will have far reaching and valuable application in maize breeding.

Discussion

It is felt that this work supplies a basis for selecting and breeding a maize with a relatively high nicotinic acid content. Especially in view of the present propaganda to switch over from white to yellow maize production an ideal opportunity is offered to the responsible authorities to select maize varieties with a suitable level of nicotinic acid. The most widely grown maize in South Africa Hickory King and Potchefstroom Pearl have an extremely poor nicotinic acid content of about 15 μg./g. In the interests of the large native population this can and must be improved upon by growing maize with a content of the order 25 - 30 μg./g. Such an increase, representing an increment of 65 - 100%, is well within the bounds of possibility and would be a profound step in the right direction towards improving the existing inadequate diet of the native. It is realised that the Department of Agriculture would have to contend with many factors apart from those normally involved in selecting a new variety such as uniformity of stand, high yield and drought resistivity. These other factors would include prejudices on the part of the farmer (unfortunately no mean factor to overcome) and the requirements of the trade which has an established predilection for the "flat white type" of maize.

Removal of the bran and germ by the milling process which involves concomitant loss of nicotinic acid and other

B vitamins such as thiamin and riboflavin will greatly reduce the advantage that could accrue by cultivating a maize suitable in respect of its nicotinic acid content. Propaganda should be made among the natives to consume whole maize meal and the sale of refined mealie meal made from white mealies should be prohibited. A wartime measure to the effect that all mealie meal must contain at least 50% of yellow maize meal was lifted in May of this year. This retrogressive step is to be deeply deprecated. Le Riche and Le Riche (116) have indicated that the white highly refined meal is extremely low in fat, iron, calcium, phosphorus and in addition to being deficient in vitamin A contains only small quantities of nicotinic acid (6 μg./g.), thiamin and riboflavin.

Various suggestions have been made on the subject of improving the nutritive value of mealie meal. Addition of Soya bean meal, Food Yeast and fish meal have been recommended. It is pointed out that the South African fishing industry is expanding rapidly and the employment of fish meal in maize would doubtlessly help to encourage the production of this useful by-product. Food Yeast (Torula utilis) is high in B vitamins and protein and due to the fact that it is harmless to man and can be produced in bulk from cheap raw materials (waste sugar molasses) makes Food Yeast eminently suitable for incorporation in this cereal. Le Riche and Le Riche (116) in their work on the fortification of maize have also demonstrated that lucerne meal and guava meal can be used to good advantage in a maize enrichment programme.

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