SCLERODERMA (ACROSCLEROSIS)

II. TRYPTOPHAN METABOLISM BEFORE AND DURING TREATMENT BY CHELATION (EDTA) *

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Many factors affect the metabolism of tryptophan along the pathway which leads to the formation of nicotinic acid. The initial step in this pathway involves cleavage of the pyrrole ring of the indole nucleus (1) to yield formylkynurenine (2). This enzyme system, which was named tryptophan pyrrolase by Kotake (1), has also been called the tryptophan peroxidase-oxidase system (2). This enzyme system is adaptive in that administration of large doses of the substrate (L-tryptophan) to rats has been shown to increase the activity of the enzyme system in the liver as much as 10 fold (3, 4). The activity of this enzyme system may also be increased by substances such as cortisone, histamine, kynurenine and a variety of other substances (4). Evidence has been presented to show that the effect of these substances other than tryptophan is mediated via the adrenal-pituitary system (4). Another factor that appears to affect this reaction, according to Dalgliesh (5), is thiamin.

Formylkynurenine has been found to be rapidly converted to kynurenine by formylase (2, 6) or kynurenine formamidase (7). The high activity of this enzyme system in liver probably accounts for the fact that formylkynurenine could not be detected in urine (6, 8).

The normal metabolism of kynurenine has been shown to depend upon the presence of nicotinamide in the form of triphosphopyridine nucleotide (9), and vitamin B$_6$ in the form of pyridoxal phosphate (10–12). Evidence from several laboratories indicates that riboflavin may also be involved in tryptophan metabolism (13–16).

Evidence has been presented that pyridoxal phosphate probably functions in the form of a complex with a metal cation (17). In this regard it should be pointed out that Jakoby and Bonner (18) found that purified Neurospora kynureninase was activated by magnesium ions. A highly purified kynureninase obtained by Wiss and Weber (19) contained vitamin B$_6$ but was not activated by the addition of other cofactors.

Thus one might expect some alteration of tryptophan metabolism produced by changes in tissue levels of tryptophan, by alterations in the amounts of the active forms of niacin, pyridoxine, riboflavin or thiamin in the tissues, by factors affecting the activity of the pituitary-adrenal system, and alterations in tissue

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levels of metal cations which may participate in the action of pyridoxine. The large number of factors involved make possible several combinations of changes which might be expected to be reflected in the relative amounts of the various metabolites of tryptophan excreted in the urine.

Previous studies from these laboratories have shown that antagonists of pyridoxine like isoniazid and deoxypyridoxine profoundly alter tryptophan metabolism in patients with tuberculosis (20). The patterns of tryptophan metabolites excreted in the urine of patients treated with these drugs were very different even though the administration of pyridoxine was shown to restore tryptophan metabolism to normal when either drug was ingested. No conclusive evidence was obtained to indicate that riboflavin, thiamin or niacin had any influence on the effects of these drugs on tryptophan metabolism (20).

Many patients with bladder cancer (21—23) and certain other types of cancer (24) have also been found to have abnormal tryptophan metabolism. The pattern of urinary metabolites was very different in the cancer patients from the patterns observed in patients treated with isoniazid or deoxypyridoxine. The patterns of urinary metabolites have been found to be so characteristic that one may readily determine from examination of the analytical data whether the patient received isoniazid, deoxypyridoxine, or was a patient with cancer. Studies in progress on patients with other clinical conditions indicate that there may be other specific patterns of urinary tryptophan metabolites. However, patients with a variety of other diseases appear to have completely normal tryptophan metabolism.

Attention was directed toward scleroderma because of the known disturbance of inorganic metabolism which eventually leads to deposition of calcium and perhaps other metal ions in the tissues (25). It was anticipated that this abnormal inorganic metabolism might be reflected in the action of pyridoxine in tryptophan metabolism. It was found that these subjects did have abnormal tryptophan metabolism of a type unlike that seen in any other clinical condition thus far studied. This report concerns the nature of tryptophan metabolism in scleroderma (acrosclerosis), and the effect of pyridoxine, niacinamide and disodium ethylenediaminetetra-acetic acid on the urinary excretion of products of this metabolic pathway.

EXPERIMENTAL

Subjects. The subjects used in these studies have been described in a previous publication (26). They were kept in a special metabolic research ward adjacent to the laboratories.

Analytical Methods. The 24 hour urine collections were made as previously described (27). Routine determinations of urinary N-methyl-2-pyridone-5-carboxamide, kynurenic acid xanthurenic acid, aromatic amine fraction A, anthranilic acid glucuronide, o-aminohippuric acid, anthranilic acid, kynurenine, N-acetylkynurenine, and 3-hydroxykynurenine were made as previously described (27—29). Paper chromatography was done on all of the aromatic amine fractions to provide a qualitative check on the quantitative determinations. (9, 28).

Supplements. The disodium salt of ethylenediaminetetra-acetic acid (Endrate)1 was given intravenously (26). Before and during treatment 24 hour urine collections were made and

1 "Endrate" was kindly furnished by Rodney P. Gwinn, M.D., Abbott Research Laboratories, North Chicago, Illinois.
2.0 gm. (9.8 mmoles) of L-tryptophan were administered in the form of four 0.5 gm. tablets. The vitamin supplements were standard pharmaceutical preparations.

Study Schedule. Case I (P.W., hospital number 309815) and Case II (M.M., hospital number 311414) were studied on the first admission prior to the start of Na₂EDTA treatment. These patients were given 3 weekly courses of 5 days each of intravenous Endrate as described (26). During the last 4 days of the third course of Endrate the studies were repeated. A 24 hour urine sample was collected and 2.0 gm. of L-tryptophan was administered. At the end of the second 24 hour period the subject was started on 50 mgm. of pyridoxine hydrochloride twice daily intramuscularly. After 24 hours of pyridoxine treatment a second 2.0 gm. of L-tryptophan was given and the 24 hour urine collection following the administration of tryptophan was saved. Pyridoxine was given during this last 24 hour collection. Thus 24 hour urine collections were obtained before and after tryptophan without and with pyridoxine administration and Endrate was given daily during this period.

Case III (G.M., hospital number 312597) was admitted at the same time that Cases I and II returned for further studies. Before therapy was started the biochemical studies were repeated on the 3 subjects. Two 24 hour urine collections were made and 2.0 gm. of L-tryptophan were administered. Two more 24 hour urine collections were made after the tryptophan and the subjects were started on 50 mgm. of pyridoxine hydrochloride twice daily intramuscularly. After another 24 hour urine collection was made the subjects were again given 2.0 gm. of tryptophan. Two more 24 hour urine collections were made and the pyridoxine administration was discontinued. The patients were then started on 3 weekly 5-day courses of Endrate intravenously. During the last 2 days of each course of Endrate a 24 hour urine was collected before and after administration of 2.0 gm. of L-tryptophan. At the end of the third course of Endrate the subjects were all started on 400 mgm. of nicotinamide daily, given in four equal doses between 8 a.m. and 8 p.m. After 48 hours on niacin each subject collected a 24 hour urine before and after a 2.0 gm. dose of tryptophan. Intramuscular pyridoxine was then given in addition to the niacin supplement and the patients collected another 24 hour urine sample before and after a 2.0 gm. tryptophan supplement.

The urine samples were analyzed promptly after they were collected, usually within 2 or 3 days after collection. The samples were stored at 0-3°C. from the time of collection until they were analyzed.

Since the colorimetric method used for xanthurenic acid determination (30) in the first study on Case I and Case II was unsuitable in the presence of the large amount of Endrate in the urine a new fluorometric determination was developed for xanthurenic acid measurement. This procedure gave excellent recoveries of added xanthurenic acid and will be described in detail elsewhere.

RESULTS

The pathway of tryptophan metabolism under consideration is shown in Fig. 1. In these studies the other pathways open to this amino acid have not been considered.

Since these subjects excreted the metabolites of tryptophan in normal quantities prior to the ingestion of tryptophan the basal or pre-tryptophan values were not shown. The usual basal values found with these methods may be obtained from previous publications (28, 31, 32). All of the data have been recorded in terms of the increased excretion of the metabolites after ingestion of the 2.0 gm. supplement of the amino acid. The average normal values obtained for the in-

The tryptophan was compressed into 0.5 gm. tablets for these studies through the courtesy of Rodney P. Gwinn, M.D., Abbott Research Laboratories, North Chicago, Illinois.

Fig. 1. An abbreviated diagram of the metabolic pathway from tryptophan to nicotinic acid and N-methyl-2-pyridone-5-carboxamide, showing the relationship of the various metabolites to one another.

Fig. 2. The metabolic response of subjects P.W. and M.M. to treatment with Na₂EDTA (EDTA) and Na₂EDTA plus pyridoxine (EDTA + B₆). The values before any treatment (0) were abnormally high for both subjects.

Increased urinary excretion of these metabolites are shown in Fig. 4. These values represent the average of 20 subjects with no known disease. The greatest increase in excretion of these metabolites (in micromoles) by normal subjects after ingestion of 2 gm. of L-tryptophan were as follows: kynurenine, 60; acetylkynurenine, 14; o-aminohippuric acid, 58; kynurenic acid, 75; 3-hydroxykynurenine, 45; xanthurenic acid, 37.

Fig. 2 shows the data obtained during the first admission of P.W. and M.M. It was evident that both of these subjects had profoundly abnormal tryptophan
metabolism under the conditions used in these studies, and subject P.W. was the more abnormal of the two. Both subjects excreted abnormal amounts of kynurenine, kynurenic acid, acetylkynurenine, and hydroxykynurenine following tryptophan “loading” prior to treatment. The metabolism of both subjects changed toward normal after 2 weeks of treatment on Na₂EDTA, and subject M.M. had essentially a normal response to tryptophan. When the pyridoxine was added to the treatment there was further improvement from a metabolic standpoint.

Fig. 3 shows that the response to tryptophan supplementation was abnormal in all 3 subjects prior to treatment. Subjects M.M. and G.M. had a normal response to tryptophan during the parenteral administration of pyridoxine, but subject P.W. only partially responded to this treatment. During administration of Na₂EDTA subjects M.M. and G.M. remained normal except that G.M. tended to become abnormal again on the 15th day of treatment. The administration of nicotinamide had little effect on the metabolic patterns, but on nicotinamide plus pyridoxine subjects M.M. and G.M. responded in a normal manner.

Subject P.W. was almost normal after 10 days treatment with Na₂EDTA in the second study (Fig. 3). Otherwise she had an abnormal response to tryptophan “loading” in every instance.

Since the response to a tryptophan load by these subjects resembled the pattern
FIG. 4. A comparison of the pre-treatment metabolic response of subject P.W. to tryptophan loading (Fig. 3), as compared with the average response of 20 subjects with no known disease and a subject who ingested isoniazid and later deoxypyridoxine. As in Figs. 2 and 3, the values recorded represent the micromoles of increase in the excretion of the various metabolites after the ingestion of 9.8 millimoles of L-tryptophan (post-tryptophan values minus the average pre-tryptophan values). The metabolic patterns seen in subject P.W., in isoniazid treatment, and in deoxypyridoxine treatment were different from one another and all three conditions were abnormal. The subject receiving isoniazid or deoxypyridoxine had normal metabolism prior to administration of the drugs, or if he was also given pyridoxine (75 mgm. daily) (20). The “patterns” of metabolites for subjects P.W., M.M., and G.M. were similar to each other but the quantities of the various metabolites excreted by subjects M.M. and G.M. were less than those excreted by subject P.W.

of metabolites seen in pyridoxine deficiency, the metabolic “pattern” shown by P.W. was compared with that seen in a patient who was ingesting either isoniazid or deoxypyridoxine (Fig. 4). These values were taken from data collected in a previous study (20). It is evident that the pattern of metabolites in the scleroderma patient was different from that seen in the subject ingesting isoniazid or deoxypyridoxine. Furthermore, these effects of isoniazid and deoxypyridoxine on tryptophan metabolism could be completely overcome by the administration of 75 mg. of pyridoxine hydrochloride daily, while subject P.W. responded only partially to 100 mg. of this vitamin daily.

It should also be pointed out that the subjects with scleroderma (acrosclerosis) excreted less hydroxykynurenine than kynurenine. When isoniazid or deoxypyridoxine were given to human subjects 3-hydroxykynurenine was the chief urinary metabolite of tryptophan (20). It would appear that in the present studies some defect in kynurenine hydroxylation was evident (Fig. 3 and 4).

DISCUSSION

There appears to be little doubt that these 3 subjects with scleroderma (acrosclerosis) had abnormal tryptophan metabolism. The pattern of the metabolites in the urine after ingestion of tryptophan resembled, but was not identical with,
results obtained in studies on the antimetabolites of pyridoxine (20). Normal metabolism of kynurenine and hydroxykynurenine requires vitamin B₆ as a cofactor for the kynureninase reaction (10) and for kynurenine transaminase (11, 12). Metzler, Ikawa, and Snell (17) have presented evidence that pyridoxal phosphate functions with a metal cation to form a chelate which may be the active form of the coenzyme. The metal ion which functions in these reactions involving the metabolism of kynurenine and hydroxykynurenine in vivo are unknown. However, Jakoby and Bonner (18) found that the activation of purified kynureninase by calcium ions was quite variable, but magnesium ions always activated their preparations.

One would expect, therefore, that enzymes dependent upon pyridoxal phosphate would show decreased activity if there was a deficiency of pyridoxine or in the presence of a deficiency or an imbalance of metal cations. A defect involving only pyridoxine would be expected to respond to pyridoxine administration, while a defect involving a metal ion imbalance or deficiency might respond to large amounts of pyridoxine or to a correction of the metal ion imbalance. Since these subjects responded biochemically to either pyridoxine or to Na₂EDTA, it is suggested that the abnormal tryptophan metabolism in scleroderma (acrodermatitis) may be due to a metal ion imbalance in the tissues. In view of the fact that kynureninase has been activated by magnesium ions, one might suspect that the abnormal tryptophan metabolism observed depends upon a functional deficiency of magnesium ions. Na₂EDTA removed large amounts of zinc in these (26) and other (33) studies, and probably removed large amounts of calcium (34). Since patients with scleroderma often eventually develop calcium deposits in the soft tissues (25), it is possible that the abnormal metabolism observed in these patients was the result of a functional deficiency in tissue magnesium. A patient with a severe metal ion imbalance would not be expected to respond well to pyridoxine, and such was the case with P.W. This patient was most nearly normal in her response to tryptophan when she received pyridoxine followed by Na₂EDTA.

When human subjects were given the pyridoxine antagonists, isoniazid or deoxypyridoxine, severe disturbances in tryptophan metabolism resulted, which were quantitatively more severe than the results obtained with the most abnormal subject with scleroderma (20). These antagonists were completely overcome by the administration of pyridoxine, probably because there was a normal balance of tissue metal ions to effectively utilize the pyridoxine supplements.

The metabolism of tryptophan supplements during ingestion of isoniazid or deoxypyridoxine led to the urinary excretion of quantities of 3-hydroxykynurenine far in excess of the quantities of kynurenine (20). In untreated scleroderma (acrosclerosis) the kynurenine excretion always exceeded the excretion of 3-hydroxykynurenine. In this disease, therefore, there must be some partial inhibition of kynurenine hydroxylase. Further evidence for this was the fact that kynurenic acid and o-aminohippuric acid excretion remained high or increased when the kynurenine excretion decreased following supplementation with vitamin B₆. Since kynurenine hydroxylase requires niacin in the form of triphosphopyridine nucleotide (9), large amounts of niacin were administered to the 3 subjects.
However, niacin or niacin plus vitamin B₆ failed to correct the metabolism of subject P.W.

The administration of CaNa₂EDTA or Na₂EDTA to subjects with other conditions often resulted in symptoms suggesting pyridoxine deficiency (33, 34). These symptoms disappeared when pyridoxine supplements were given. One might expect, therefore, that overtreatment of patients with scleroderma might result in abnormal tryptophan metabolism. This may explain the fact that subject P.W. had a nearly normal pattern of urinary tryptophan metabolites after 10 days of chelation (Fig. 3) but a very abnormal pattern on the 15th day.

Since the patients improved clinically and biochemically by treatment with Na₂EDTA it would appear that tryptophan metabolic studies may be of use in diagnosis and in following experimental treatment of the subjects. These biochemical studies appear to be a definite indication of a metabolic defect in scleroderma. Whether this metabolic defect is of causal significance in scleroderma is not known. It is more likely a reflection of a disturbance in inorganic metabolism, possibly involving calcium, magnesium or other metal ions, which is reflected through pyridoxal phosphate to an abnormal metabolic disposition of tryptophan.

These studies illustrate, as have previous studies from these laboratories (20, 21, 24), that the determination of urinary xanthurenic acid as an indication of abnormal tryptophan metabolism cannot be expected to reveal all cases of disturbed metabolism of this amino acid. Xanthurenic acid has been detected in all normal human urine samples tested and must therefore be regarded as a normal metabolite of tryptophan (35). Xanthurenic acid has often been called an “abnormal” metabolite of tryptophan because the colorimetric methods for its determination will not produce clear-cut evidence for its presence in normal urine (30), while in certain disease states (36), in pregnancy (37) and other conditions xanthurenic acid has been detected in the urine. Although subjects with scleroderma did not excrete abnormal amounts of xanthurenic acid either before or after supplementation with tryptophan, it was readily detected in the urine by methods previously described (35).

From a metabolic standpoint it would appear that subjects M.M. and G.M. may be maintained within the normal limits of these studies by the administration of pyridoxine. Subject P.W., however, responded poorly to pyridoxine. The significance of these observations remains unknown.

**SUMMARY**

Three female patients with scleroderma (acrosclerosis) were found to have abnormal tryptophan metabolism, characterized by an abnormally large urinary excretion of kynurenine, hydroxykynurenine, kynurenic acid, and N-acetyl-kynurenine. The subjects excreted normal amounts of xanthurenic acid, before and after the ingestion of loading doses of L-tryptophan.

During therapy with disodium ethylenediaminetetraacetic acid (Na₂EDTA) the tryptophan metabolism became nearly normal in one patient and normal in a second. The simultaneous administration of Na₂EDTA and pyridoxine consider-
ably improved the tryptophan metabolism in the subject who was the less responsive to Na₂EDTA alone.

During a second course of Na₂EDTA both subjects responded in a manner similar to the first clinical trial. A third patient was found to have normal tryptophan metabolism after treatment with Na₂EDTA or pyridoxine.

The simplest explanation of the biochemical data on tryptophan metabolism in these three patients would be as follows: In scleroderma (acrosclerosis) there was an abnormal urinary excretion of kynurenine and its metabolites after oral administration of tryptophan. The administration of pyridoxine or pyridoxine plus nicotinamide partially corrected the metabolic abnormality. The efficacy of pyridoxine plus Na₂EDTA could be explained on the basis of a decrease in tissue calcium, zinc (and possibly other cations) making it possible for the metal ions normally functioning with pyridoxal phosphate to be utilized more advantageously. The data in the literature suggest that this metal which is unblocked for normal function may be magnesium.

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