THE TRYPTOPHAN LOAD AS A TEST FOR PYRIDOXINE DEFICIENCY IN HOSPITALIZED PATIENTS*

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Measurement of urinary excretion of xanthurenic acid after administration of an oral dose of tryptophan has been the technique most widely used for detection of subclinical pyridoxine deficiency. Although alterations in urinary excretion of this and other metabolites of tryptophan have been described in a wide variety of conditions and diseases,[1] comparison of results obtained by different investigators has been difficult because of variability in dose and kind of tryptophan, urine collections, method of analysis and definitions of "abnormality." Recently, as a result of the development of more accurate methods by Price and associates[2] and the plea for standardization made by Coursin,[3] greater agreement has been reached concerning proper methods of study.

For the past ten years our laboratory has been concerned with the investigation of the prevalence of biochemical evidence of deficiency of several of the water-soluble vitamins in ill patients in our hospital. Several years ago we began to assess the relative "sensitivity" of several methods proposed for the detection of pyridoxine deficiency in normal adults placed upon a pyridoxine-deficient diet supplemented with vitamin-free casein. Studies included serial determinations of absolute lymphocyte count, serum and erythrocyte glutamic-oxalacetic transaminase and glutamic-pyruvic transaminase, taurine excretion after a cysteine load, estimation of phosphorylated and nonphosphorylated derivatives of pyridoxine in plasma, urinary excretion of 4-pyridoxic acid and 24-hour urinary excretion of xanthurenic acid and hydroxykynurenine after a 5 g dose of L-tryptophan. After the study of three patients, we arrived at the same conclusions as those in the then just published papers of Yess, Swan and colleagues[4-6] that the earliest and most consistent abnormalities observed were increases in excretion of hydroxykynurenine and xanthurenic acid after a tryptophan load.

At this time, Coursin made his plea for standardization of methods and for utilization of a 2 g dose of L-tryptophan.[3] For this reason and also because we had observed considerable interindividual variation in excretion of tryptophan metabolites in our normal subjects tested during control periods before and after the period of deficiency, a comparison of the 2 g and 5 g dose of L-tryptophan was made in sixteen healthy adults both prior to and following administration of pyridoxine.[7] Urinary hydroxyanthranilic acid was also determined, since the ratio of excretion of hydroxyknurenine to that of hydroxyanthranilic acid has been proposed as a helpful adjunct in the assessment of pyridoxine deficiency.[8]

In order to clarify the rationale for this approach, FIGURE 1 presents a diagram of the metabolic pathway of tryptophan metabolism of importance in the tryptophan load test. Tryptophan pyrrolase, which catalyzes the first reaction in this pathway, is inducible by substrate (tryptophan) and by adrenocortical steroids. The vitamin B_6-containing enzymes are not subject to adaptive change (enzyme induction),[9] and therefore a relative or absolute decrease in activity of these enzymes could bring about alterations in tryptophan metabolism. Since the

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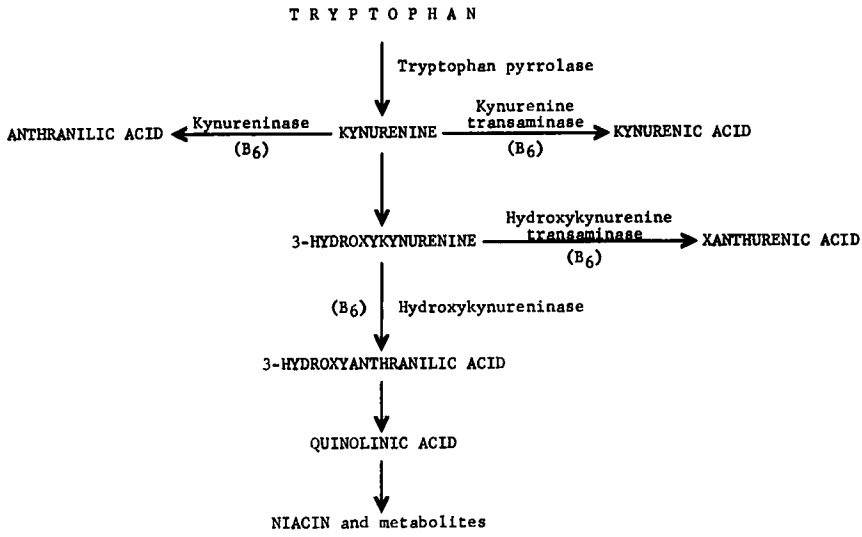

FIGURE 1. Abbreviated outline of pathway of tryptophan metabolism of importance in the tryptophan load test for pyridoxine deficiency.

increase in excretion of kynurenic acid in pyridoxine-deficient animals and humans is of a lesser magnitude than the increase in xanthurenic acid (XA), kynurenine transaminase may be decreased to a greater extent by vitamin B_6 deficiency than hydroxykynurenine transaminase.[10] Several alternative explanations have been offered for the somewhat paradoxical increase in xanthurenic acid in pyridoxine-deficient animals and humans. Although the activity of hydroxykynurenine transaminase in the soluble supernatant fraction of tissue is decreased markedly, a major fraction of hydroxykynurenine transaminase activity is mitochondrial in origin and is only slightly decreased by pyridoxine deficiency.[11] On the other hand, Korbitz and colleagues[10] have proposed that an alternate, non-B_6-dependent pathway may be responsible for increases in xanthurenic acid excretion. If a significant "block" in metabolism of hydroxykynurenine (HK) in pyridoxine deficiency were brought about by a decrease in activity of hydroxykynureninase, then one might also expect a decrease in formation of hydroxyanthranilic acid (HAA) and an increase in the HK/HAA ratio.

Our findings after administration of 2 g and 5 g doses of L-tryptophan to normal subjects provided data in support of Coursin's recommendation. After a 5 g dose of tryptophan, individual variability in excretion of these metabolites was so great that "tolerance limits" for range of excretion of them in a population of normal subjects were too broad to be meaningful. In addition, when effects of administration of the 2 g and 5 g doses of tryptophan were compared in the same subjects, the larger dose brought about a several-fold rise in percent of load excreted as XA (0.62%) and HK (0.51%), but percent of dose excreted as HAA (0.44%) had decreased. After the 2 g load, the values were XA 0.35%, HK (male) 0.14% HK (female) 0.28%, and HAA 0.71%. These differences have been interpreted as probably resulting from induction of higher tryptophan pyrrolase activity by the larger dose of substrate; the percent of dose

of tryptophan excreted as HAA was thought to be decreased with the larger dose because hydroxykynureninase, which does not increase adaptively, does not, under normal conditions, have sufficient activity to catalyze the conversion of the greatly increased amounts of HK to HAA.

In these same subjects, when the 2 g of tryptophan load was repeated after administration of 62.5 mg of pyridoxine hydrochloride in divided doses, percent of dose excreted as HAA was doubled (1.47%). These findings demonstrate that hydroxykynureninase can increase in activity, even in normal individuals, if more vitamin is available for formation of coenzyme. Significant decreases in percent of the 5 g dose excreted as XA (0.27%) and HK (0.16%) after vitamin B_6 supplementation can be explained by the same mechanism.

On the basis of these findings, we have concluded that in those prior studies utilizing doses of L-tryptophan much greater than 2 g, no statistical criteria for normality or abnormality can be established. Since many previous studies in which abnormalities have been reported in patients with a wide variety of diseases were conducted with larger doses of tryptophan, racemic mixtures and less reliable methods of analysis, we have attempted to reassess the validity of the tryptophan load test, with the standards recommended by Price and coworkers[2] and Coursin,[3] in the screening of selected hospitalized patients for possible subclinical pyridoxine deficiency.

Methods

The following groups of subjects were selected for study:

1. Patients with nutritional problems of gastroenteric origin.
2. Patients with advanced atherosclerotic cardiovascular disease.
3. Patients with malignant neoplasms:
   a. Subgroup with nonmetastatic cancer,
   b. Subgroup with metastases.

Included in addition for comparison were a group of patients with normal nutrition hospitalized for management of diseases of lesser severity (chronic venous insufficiency, obesity, pilonidal sinus, etc.), also patients with cancer who had been receiving supplemental vitamins and several subjects being treated with steroid hormones.

All patients were assessed with respect to height, weight, history of recent weight loss and recent dietary and drug history. None had porphyria, scleroderma, rheumatoid arthritis, schizophrenia, tropical or nontropical sprue, carcinoma of the urinary bladder, Hodgkin's disease or leukemia. None were pregnant or receiving oral contraceptives. Any patients receiving drugs thought to have an influence on tryptophan metabolism or to interfere with the analytic procedure (soluble sulfonamides, procaine, para-amino-salicylic acid, acetophenetidin, diphenylhydantoin, para-aminobenzoic acid, or isoniazid)[2] were excluded.

Two grams of L-tryptophan were given preprandially in gelatin capsules. Twenty-four hour acidified urine specimens were collected in dark bottles kept under refrigeration. Analyses for xanthurenic acid, hydroxykynurenine and 4-pyridoxic acid were performed by methods recently summarized by Price, Brown and Yess.[2] Similar to the experience of these authors, in several samples no HK or added standard was recovered. Determination of hydroxyanthranilic acid was by the method of Tompsett.[12] The Aminco-Bowman spectrophotofluorometer was utilized for spectrofluormetric analyses, and the Beckman-DU spectrophotometer for spectrophotometric readings.

Determinations of excretion of 4-pyridoxic acid (4-PA) were used as a measure of recent dietary intake of vitamin and to detect possible vitamin supplementation of which the patient himself was unaware. Any patient with 24-hour excretion of 6.5 micromoles or more of 4-PA was arbitrarily excluded.

From the data obtained from our prior studies of normal adults, tolerance limits[13] were calculated for the range of values to be expected in 99% of a similar population with 99% confidence (P = 0.99; gamma = 0.99). Utilizing these statistical criteria, the upper limits for "normality" in 24-hour excretion of metabolites after a 2 g load of tryptophan were: XA, 70 micromoles; HK (males), 58 micromoles; HK (females), 83 micromoles, and HAA, 137 micromoles. Tolerance limits were too broad to define lower limits for excretion of these metabolites.

Results

TABLE 1 presents a summary of the abnormalities detected in the several groups of subjects who have been studied. All patients with increased excretion of XA (Column 1) had increases in excretion of HK as well (with the exception of one in whom no recovery of HK was obtained). Column 2 lists the numbers of additional patients who, according to our calculated tolerance limits, had normal levels of excretion of xanthurenic acid but increased values for HK. In our normal subjects the mean excretion of HK was significantly lower in males than in females, and therefore separate limits for each sex were estimated. However, since Price and collaborators[2] have reported a higher mean value for males and have not found a significant difference between values for excretion of HK

TABLE 1

ABNORMALITIES IN THE EXCRETION OF XANTHURENIC (XA) AND HYDROXKYNURENINE (HK) IN VARIOUS CATEGORIES OF SUBJECTS

Categories of Subjects	Number of Subjects Studied	Number of Patients with Abnormalities		
		Column 1 — XA > 70 (micromoles/ 24 hr)	Column 2 XA ≤ 70 HK Male: > 58 Female: > 83 (micromoles/ 24 hr)	Column 3* XA ≤ 70 HK > 83 (micromoles/ 24 hr)
Nutritionally normal patients	11	0	0	0
Patients with gastroenteric disease	14	0	1	1
Patients with atherosclerotic cardiovascular disease	21	1	2	0
Patients with localized cancer	15	0	3	2
Patients with metastatic cancer	16	4	4	3
Patients with cancer receiving vitamin B_6	9	0	4	3
Patients receiving steroid hormones	8	5	3	3

* Column 3 is a retabulation of the number of patients with abnormalities in HK excretion listed in Column 2 with exclusion of those males with levels of HK excretion between 59 and 83 micromoles per 24 hours. The number of subjects in each group with abnormalities is a sum of Columns 1 and 2 or 1 and 3 (depending on the criteria selected).

in males and females, the tolerance limit for males, established on the basis of our data, may be too low. For this reason, Column 3 is a retabulation of abnormalities in HK excretion based upon the higher tolerance limit for females (83 micromoles/24 hours). This limit would appear to be more compatible with the range of values for normals reported by Price and associates.[2]

A small group of "nutritionally normal" patients hospitalized for treatment of diseases of recent onset and of lesser severity were included as a check of the validity of the tolerance limits calculated for "normals." No abnormality in excretion of tryptophan metabolites was detected in these subjects.

Patients with gastroenterologic problems known to entail possible nutritional abnormalities were also screened. These subjects had cirrhosis of the liver or were patients with longstanding weight loss following operative removal of portions of the stomach or intestinal tract. Although the highest frequency of relatively low values for 4-pyridoxic acid excretion (1.5 micromoles or less) was found in this group (six of 13 subjects), none displayed an elevated excretion of XA, and only one had an increase in urinary HK.

The demonstration by Rinehart and Greenberg[14] that monkeys on a pyridoxine-deficient diet develop arterial lesions resembling atherosclerosis in man prompted the inclusion of a group of patients with severe atherosclerotic cardiovascular disease. Abnormalities were found after the tryptophan load test in three of 21 subjects and, of these, two male patients had only a slight elevation in excretion of HK. The third subject with definite abnormalities in excretion of both XA and HK was acutely ill with ischemia of one leg secondary to a femoral arterial embolus, presumably arising from the heart following a recent myocardial infarct.

The group of patients with malignant neoplasms was subdivided into those individuals without metastatic disease and those with clinical or roentgenologic evidence of dissemination of tumor. This was an attempt at differentiation according to severity of disease and by selection, within the latter category, of those subjects most likely to be in negative nitrogen balance. As a reflection of these differences, mean weight loss in the group of patients with metastatic cancer was 22 pounds as opposed to 3½ pounds in the subjects without evidence of metastasis. Since many patients with extensive neoplasms were receiving vitamins at the time they were first encountered, a small group of these patients were also tested and are reported separately.

A special effort was made to test as a separate group patients receiving steroid therapy, since changing levels of steroid hormones have been shown to influence tryptophan metabolism and are thought to act as an important variable affecting results of the tryptophan load test in ill subjects.[15]

TABLE 2 is a tabulation of the values obtained in patients with elevated excretion of XA (steroid-treated group not included). The four subjects with metastatic cancer had relatively low values for excretion of 4-PA. Excretion of HAA was within normal limits in all. The mean value for excretion of HAA in normal subjects with 70 ± 13 micromoles/24 hours.

TABLE 3 lists the results obtained in those subjects who had increased levels of HK in the urine but normal values for XA. Excretion of 4-PA and HAA was highly variable. Of interest is that four of nine patients with cancer receiving supplemental pyriodoxine for at least three days in doses varying from 2 mg to 50 mg per day excreted HK in amounts above 99% tolerance limits for normal. The four patients who did display abnormalities in HK excretion, however, were receiving 5 mg or less of pyridoxine per day.

TABLE 2

PATIENTS WITH URINARY XANTHURENIC ACID (XA) GREATER THAN 70 MICROMOLES/24 HOURS

Sex	Age	Diagnosis	XA	Hydroxy-kynurenine	Hydroxy-anthranilic Acid	4-Pyridoxic Acid	Recent Weight Loss (lb)
M	42	Cancer of stomach (M)*	99	†	43	1.5	30
M	73	Cancer–mesothelioma (M)	256	488	118	1.2	35
M	52	Cancer of colon (M)	102	171	93	1.4	25
M	72	Cancer of stomach (M)	160	98	51	0.7	30
F	52	Femoral arterial embolus; ischemia of leg	226	275	99	3.1	O – obese

* (M) indicates the presence of metastatic disease in patients with cancer.

† No recovery.

TABLE 3

PATIENTS WITH INCREASE IN URINARY HYDROXYKYNURENINE
(XANTHURENIC ACID WITHIN NORMAL LIMITS)
(Micromoles/24 hr)

Sex	Age	Diagnosis	Xanthurenic Acid	Hydroxy-kynurenine	Hydroxy-anthranilic Acid	4-Pyridoxic Acid	Recent Weight Loss (lb)
M	56	*Cirrhosis of liver*	16	121	94	0.7	10
M	56	Atherosclerotic vascular disease	41	77	102	2.6	0
M	60	Atherosclerotic vascular disease	32	59	103	2.6	0
M	72	Cancer of tongue	18	81	44	2.5	0
M	55	Cancer of colon	35	101	145	4.4	0
M	50	Cancer of colon	27	335	86	1.0	0
M	60	Cancer of colon (M)*	47	96	51	4.8	12
F	79	Cancer of colon (M)	19	90	17	1.5	26
M	71	Cancer of colon (M)	15	175	97	2.2	30
M	65	Cancer of stomach (M)	33	66	36	2.7	5
		Patients Receiving Supplemental Vitamins:					
M	70	Cancer of stomach (M)	64	93	56	6.6	10
M	74	Cancer of stomach (M)	46	385	59	6.8	11
M	73	Cancer of stomach (M)	56	396	230	13.8	15
M	55	Cancer of colon (M)	19	60	17	7.0	20

* (M) indicates the presence of metastatic disease in patients with cancer.

TABLE 4

INCREASE IN URINARY XANTHURENIC ACID OR HYDROXYKYNURENINE IN PATIENTS RECEIVING STEROIDS
(Micromoles/24 hr)

Sex	Age	Diagnosis	Xanthurenic Acid	Hydroxy-kynurenine	Hydroxy-anthranilic Acid	4-Pyridoxic Acid	Dosage of Steroid (daily)
F	43	Cancer of breast (M)*	152	128	80	2.2	200 mg hydrocortisone
F	48	Cancer of breast (M)	90	142	65	3.8	140 mg hydrocortisone
F	47	Cancer of breast (M)	44	244	69	2.1	2 mg dexamethasone
M	50	Diabetic neuropathy	23	121	277	2.3	4 mg methylprednisolone
M	67	Interstitial fibrosis of lung	26	119	65	0.2	25 mg prednisolone
F	74	Cancer of breast (M)	549	106	93	0.6	15 mg diethylstilbestrol
F	72	Cancer of breast (M)	147	93	45	1.6	15 mg diethylstilbestrol
M	59	Cancer of Colon (M)	144	295	28	2.3	20 mg norethandrolone

* (M) indicates the presence of metastatic disease in patients with cancer.

TABLE 4 records the results obtained after administration of the tryptophan load to patients being treated with either adrenocortical steroids, stilbestrol, or an androgen. The three patients receiving adrenal corticoids who had normal values for XA had all been on steroid therapy for months, while the three with elevated values for this metabolite had received the steroid for much shorter periods. This possible relationship between duration of administration and level of XA excretion did not hold, however, for the subjects receiving estrogen or androgen, since all had been receiving these medications for a prolonged period.

In each group of subjects (with the exception of vitamin- and steroid-treated groups), statistical analysis of mean values in individuals not displaying one of the above abnormalities has been performed. Although, as expected, the variance was greater than that reported for normal controls, no significant difference between group means was found. Fourteen subjects, scattered throughout the various groups, had levels of excretion of 4-PA at or below 1.5 micromoles/24 hours, while values for XA and HK were normal. Calculations of ratio of excretion of HK to that of HAA were not of interpretive value, since excretion of HAA was highly variable and increases in HK were not accompanied by any predictable or characteristic change in excretion of HAA. Mean level of excretion of HAA was not significantly decreased in patients with increased excretion of XA or HK.

Discussion

Several factors complicate the interpretation of the significance of deviations from "normality" in excretion of the several metabolites of tryptophan after a tryptophan load is administered to hospitalized patients. The "stress" of the primary illness brings about an increase in secretion of glucocorticoids by the adrenals. Increased amounts of adrenal corticosteroids stimulate adaptive increases in activity of tryptophan pyrrolase in animals and man.[9,15] Altman and Greengard[15] have reported that hydrocortisone causes a two- to fourfold increase in level of activity of tryptophan pyrrolase in human liver and that the level of activity of this enzyme is linearly related to level of urinary excretion of kynurenine after a 2 g dose of L-tryptophan. An increase in activity of tryptophan pyrrolase brings about shunting of a larger fraction of free tryptophan via the kynurenine pathway, resulting in increased excretion of kynurenine and hydroxykynurenine and other related metabolites. Altman and Greengard propose that adaptive increases in activity of tryptophan pyrrolase induced by adrenocortical hormones may be the common factor leading to increased excretion of these metabolites in humans with various diseases.

In a nutritionally normal subject with elevated activity of tryptophan pyrrolase and increased urinary excretion of kynurenine (after a tryptophan load) brought about by administration of hydrocortisone, administration of pyridoxine was followed by a return of excretion of urinary kynurenine to normal values, while tryptophan pyrrolase remained elevated.[15] Since vitamin B_6-containing coenzymes are not normally present at saturation levels, enzyme activity (kynureninase, hydroxykynureninase, etc.) is increased when this vitamin is provided in greater amounts. Since this phenomenon is demonstrable in nutritionally normal subjects,[7,16] one cannot assume that correction of an abnormality in urinary content of these metabolites after administration of pyridoxine is *de facto* evidence of a pre-existing deficiency unless one accepts the premise that anything less than maximal activity of these pyridoxal-containing enzymes represents a clinically significant deficiency.

The mechanism by which sex hormones influence tryptophan metabolism has not yet been defined. Elevated excretion of xanthurenic acid, kynurenine, hydroxykynurenine and other metabolites has been observed in pregnant women[17] and in women receiving oral contraceptives.[18] To our knowledge, the similar pattern observed in our patient receiving norethandrolone has not been previously reported in man. In an isolated experiment, Schor and Frieden[19] have reported that administration of testosterone to rats is followed by a twofold increase in activity of tryptophan pyrrolase. In support of the possible role of androgens in enzyme induction is the recent demonstration that several drug-metabolizing enzymes increase in activity after administration of 19-nortestosterone derivatives to mice.[20] Norethynodrel, a component in an oral contraceptive, is also a 19-norsteroid and has been shown to have an effect similar to that of norethandrolone in another system.[21] However, estrogenic substances, as well as androgens and progestational agents, may also have an influence, since stilbestrol, which has been shown to induce the formation of a new protein in male chickens, might also act to stimulate synthesis of apoenzyme.[22] In addition, the influence of one steroid upon the metabolism of another steroid has not yet been fully clarified. Since several androgenic steroids have been shown to inhibit the degradation of cortisol, increasing its plasma half-life and decreasing its turnover rate,[23,24] it is possible that androgens may influence tryptophan metabolism through their effect upon metabolism of cortisol.

Another major variable affecting results obtained with the tryptophan load test is the subject's level of protein intake and general state of protein metabolism. Both influence the amount of free tryptophan available for metabolism via the kynurenine pathway. In addition, increases in amount of free tryptophan bring about adaptive increases (induction by substrate) in tryptophan pyrrolase activity and further shunting of tryptophan into this pathway. Tryptophan pyrrolase activity is higher in rats on a high-protein diet.[9] The higher the protein intake, the greater the excretion of tryptophan metabolites in experimental subjects receiving a pyridoxine-deficient diet.[6] On the other hand, increased catabolism of endogenous protein and negative nitrogen balance are also associated with increased levels of free tryptophan and increased activity of tryptophan pyrrolase. Wood, Rivlin and Knox[25] have shown that tryptophan pyrrolase activity increases in mice in the terminal phases of cancer as protein catabolism increases. As Knox[9] has recently pointed out, in some instances at least, it may be the discrepancy between a high induced level of tryptophan pyrrolase and the level of the nonadapting kynureninase and hydroxykynureninase that permits the accumulation of metabolites of kynurenine in the urine. In other words, the observed "block" in metabolism, thought to be caused by pyridoxine deficiency, is, in a sense, caused by the dose of tryptophan used in the test and the additional free tryptophan arising from dietary and endogenous sources. In support of the role played by tryptophan in substrate induction of tryptophan pyrrolase in man is the recent demonstration by Hankes, Brown and associates,[26] utilizing ^{14}C-labeled tryptophan in man, that simultaneous administration of a 2 g load of L-tryptophan produces a two- to fourfold increase in urinary radioactivity in kynurenine and hydroxykynurenine and xanthurenic acid; they explain their findings on this basis.

Another variable, about which we are still not fully knowledgeable, is the influence of certain drugs upon the performance of the test. Price and colleagues[2] have shown that some agents interfere with the analytic technique itself. The mechanism of action of other drugs such as diphenylhydantoin has not yet been

explained; we, too, have noted abnormalities in hydroxykynurenine excretion in several patients (not included in this study) who were receiving this compound. Canal and Maffei-Faccioli[27] have reported that reserpine causes an increase in tryptophan pyrrolase activity which is not adrenal-dependent. Perhaps other drugs have a similar effect.

The current techniques that we have utilized for measurement of tryptophan metabolites and 4-pyridoxic acid, although accurate and apparently specific (provided certain drugs are excluded), are laborious and would not be easily adaptable to performance in a routine laboratory for clinical biochemistry. Since these procedures are very time-consuming, no attempt has been made to study a segment of the hospital population as a whole. The groups of patients subjected to this preliminary screening were selected for specific reasons. The "nutritionally normal" patients were studied to provide one "internal control." In addition, after this investigation was in progress, it became apparent that solitary abnormalities in excretion of hydroxykynurenine would be particularly difficult to interpret. For this reason, patients with neoplasms who were receiving vitamins were selected as another group that might be useful for comparison. The frequent finding of elevated HK excretion in this group of vitamin-supplemented patients with cancer could result from incomplete correction of a pre-existing deficiency of pyridoxine or could be interpreted in support of the premise that other variables that affect tryptophan metabolism may be primarily responsible for this abnormality.

The influence of steroids, particularly adrenocorticoids, has been investigated to demonstrate that a major variable that may influence results and their interpretation may be the effect of the "stress" of the primary illness or of measures used in its treatment upon adrenocortical hormonal secretion.

The very low incidence, almost total absence, of abnormalities detectable in patients with "nutritional diseases" may be related to errors in selection of patients or, perhaps more likely, to the combination of a relatively low protein intake in a group of patients with longstanding illnesses in which the stress of acute disease and its accompanying adrenocortical hypersecretion are absent.

The rarity of detectible abnormalities in excretion of hydroxykynurenine or XA in patients with atherosclerotic cardiovascular disease by no means excludes a possible relationship between subclinical pyridoxine deficiency and the development of atherosclerotic disease in man but does demonstrate that this particular methodologic approach is unsatisfactory for demonstrating any such relation.

The absence of significant increases in xanthurenic acid in patients with nonmetastatic neoplastic disease, the infrequent occurrence of increased excretion of HK and the appearance of increased amounts of HK in the urine of four of nine vitamin-supplemented patients with cancer complicate interpretation of the significance of elevated levels of HK alone. One patient with nonmetastatic cancer had increases in HK that might be termed "borderline," while in another, increased excretion of HK was accompanied by simultaneous increases in HAA. The higher prevalence of abnormalities in patients with metastatic cancer could be secondary to a higher freqeuncy of deficiency of vitamin B_6 in more debilitated patients. However, it could also be related to the effect of greater "stress" of the primary disease, resulting in more protein catabolism, increased secretion of adrenal corticoids, and induction of tryptophan pyrrolase, with "funneling" of considerably greater amounts of tryptophan through the "kynureninase pathway." Actually all three factors (decreased amounts of

vitamin, increased protein catabolism, and adaptive increases in tryptophan pyrrolase) could be acting in concert.

The interpretation of the tryptophan load test in hospitalized patients is complicated by our inability to weigh accurately the influence of these multiple and sometimes conflicting variables on excretion of metabolites of tryptophan. Either a high protein intake or, conversely, a profound negative nitrogen balance could bring about "abnormal" increases in excretion of several metabolites of tryptophan that might not have occurred had neither of these variables been present. In their absence, the activity of kynureninase and related enzymes might have been adequate to handle a lesser "load" of free tryptophan. On the other hand, it is quite possible that more abnormalities might have been detected in some of our more ill patients had they not been ingesting a diet relatively low in protein. In addition, although an attempt has been made to exclude those patients receiving drugs that Price, Brown and Yess[2] have shown may interfere in the analytical procedures for several of these metabolites, other drugs, about which we are still unaware, might also interfere.

The absence of a significant decrease in level of excretion of HAA poses the question raised by Knox of whether the tryptophan load itself is primarily responsible for the production of observed abnormalities, or whether a decrease in the functional adequacy of the kynureninase pathway is of practical significance. Hernandez[28] has shown that after administration of a tryptophan load to pregnant women, the increase in excretion of xanthurenic acid is accompanied by simultaneous increases in HAA and N^1-methylnicotinamide. Excretion of the latter product did not differ significantly in those women with high or with low XA excretion. Increased excretion of niacin metabolites in pregnant women has also been reported by Brown and coworkers.[17] Brown and others[29] have recently presented further data concerning elevated quinolinic acid excretion in experimental pyridoxine deficiency and have discussed the still unresolved problem of the role of vitamin B_6 in niacin synthesis.

In normal subjects studied under carefully controlled conditions, statistical criteria can be used to define "abnormality." As has been pointed out, this is much more difficult when the tryptophan load test is used for screening for these "abnormalities" in hospitalized patients in whom, for practical reasons, some other influential variables cannot be controlled. This does not mean that the abnormalities in excretion of tryptophan metabolites observed in some of these patients might not have been secondary to decreases in activity of vitamin B_6-containing enzymes. Unfortunately, however, the demonstration that these abnormalities are correctable by pyridoxine cannot be used as presumptive evidence of a significant pre-existing pyridoxine deficiency. At our present state of knowledge, there is no clinical condition or histopathologic lesion that can be used to correlate with and confirm the significance of these biochemical abnormalities. Rare clinical entities such as pyridoxine-responsive anemia[30] and the pyridoxine-dependency syndrome[31] appear to be secondary to inborn errors in normal utilization of pyridoxine, since extremely large quantities of vitamin are usually needed for correction of the clinical manifestations. In the absence of clinical-anatomic correlates serving to define "deficiency" with respect to some of the other vitamins, a "post hoc" type of reasoning has been utilized in some instances to define "subclinical deficiency" of pyridoxine when certain abnormalities in urinary excretion of tryptophan metabolites appear after a tryptophan load. The whole concept of "subclinical deficiency" of a vitamin is still in limbo and will remain so until one can demonstrate that that subclinical de-

ficiency is interfering with the "optimal" health of the individual. Determinations of erythrocyte transketolase activity may be used to follow the whole spectrum of thiamine deficiency of varying severity, culminating in the demonstration of most extreme abnormalities in Wernicke's disease,[32] In this instance, there is more presumptive evidence that this abnormality is specific for thiamine deficiency and that lesser decreases in activity of thiamine-containing enzymes might have a deleterious effect upon carbohydrate metabolism. On the other hand, for example, there is as yet no definitive evidence that the many people with blood and buffy coat ascorbic acid values far below levels compatible with tissue saturation are in less satisfactory physical condition or are less able to recover from an illness than persons having very high blood and tissue levels in this vitamin. Such considerations are particularly pertinent with respect to pyridoxine, a vitamin quite ubiquitous in dietary distribution. Assessment of the adequacy of vitamin B_6 nutriture may require the development of other techniques more specific than those currently available. Direct measurement of vitamin B_6 in blood would be desirable, but many difficulties have been encountered with currently available enzymatic, microbiological and chemical methods. The presence of the vitamin in phosphorylated and protein-bound complexes requires the development of more satisfactory procedures for preliminary extraction of the vitamin before this approach can be successfully utilized.[33]

Summary

The tryptophan load test has been administered to selected groups of hospitalized patients. Twenty-four hour urinary levels of xanthurenic acid or hydroxykynurenine above 99% tolerance limits for "normals" were most frequently observed in patients with extensive cancer. Several alternative explanations for the appearance of these abnormalities, other than the presence of a subclinical pyridoxine deficiency, are discussed. In view of the possible lack of specificity of the "tryptophan load test," when utilized for the detection of pyridoxine deficiency in ill patients, considerable caution is required in interpretation of results obtained. Further investigation, with the development of other approaches to diagnosis, will be necessary before the prevalence and practical significance of deficiency of vitamin B_6 in ill adults can be assessed.

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