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STUDIES OF PYRIDOXAL–PENICILLAMINE ANTAGONISM IN THE HUMAN*

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Abstract—The pyridoxal-L-penicillamine antagonism previously studied by others in lower animals has now been studied in man by means of urinary xanthurenic acid (XA) excretion levels. Subjects of the present study, all Chinese, included 21 normal individuals and 2 patients with Wilson's disease. The studies involved urinary XA determinations, with and without tryptophan-loading tests, and with varying dosage combinations of penicillamines and pyridoxine hydrochloride. It was found that urinary XA excretions remained normal after DL-penicillamine or DL-tryptophan alone but increased when the two were taken together; pyridoxine hydrochloride prevented the increase. No increases in urinary XA excretion occurred when D-penicillamine or N-acetyl-DL-penicillamine were used in place of the DL-penicillamine during tryptophan loading. These results support the thesis that a pyridoxal-L-penicillamine antagonism can operate in the human, both in normal individuals and in those with Wilson's disease.

THE possibility of a biological antagonism between L-penicillamine and pyridoxal was recognized first by du Vigneaud and co-workers[1–5] and Kuchinskas[6] in their studies of rat nutrition. The existence of such an antagonism was confirmed by studies in which rats fed the penicillamines with and without pyridoxal dietary supplementation exhibited changes in urinary excretion of compounds with vitamin B_6 activity[4] as well as changes in blood[7] and tissue transaminase activities.[4] Transaminases are known to be included among those enzymes that require pyridoxal phosphate as a co-factor.[8–11] Results of *in-vitro* studies of rat liver transaminase[5] with penicillamines and their derivatives provided further support for the existence of the biological antagonism and indicated that it involved specifically L-penicillamine with both the thiol and amino groups unsubstituted. Inhibition by L-penicillamine of other enzyme systems requiring the pyridoxal phosphate cofactor also has been reported.[12,13] The existence of an L-penicillamine–pyridoxal antagonism, therefore, is well documented, but its occurrence in the human has not previously received attention.

Remission, by means of pyridoxine therapy, of optic axial neuritis symptoms which developed in a Wilson's-disease patient during a period of intensive DL-penicillamine therapy[14] has indicated the likelihood that the same antagonism does operate in the human. In preliminary work[14] to investigate the relationship further, brief studies of urinary xanthurenic acid (XA) excretion were made on the same patient after restoration of his normal vision; results showed that DL-penicillamine could induce

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an increased urinary XA excretion after a tryptophan-loading test, in the manner characteristic of vitamin B_6 deficiency.[3,15–21]

With the assumptions that under the conditions of our work, urinary XA excretion levels can be taken as reliable indices of tryptophan metabolism and alterations in urinary XA levels primarily are due to changes in the biological availability of pyridoxal and its derivatives, we have extended the study of the pyridoxal–penicillamine antagonism in the human. The present report includes results of further studies of the original male Wilson's patient,[14] as well as those of a younger female Wilson's-disease patient and a group of normal Chinese adults. For sake of completeness the XA excretion data from the previous clinical report[14] are included here. In both patients and the normal Chinese adults the effects of DL-penicillamine, D-penicillamine, and N-acetyl-DL-penicillamine upon the biological availability of pyridoxal have been investigated.

In the present study the principal objective was to determine the likelihood that a pyridoxal-L-penicillamine antagonism could operate in the human. At the same time, is such an antagonism were found, it was of interest to obtain data concerning the quantitative relationship between pyridoxal and penicillamine in that antagonism and to search for possible differences in the expression of the antagonism in Wilson's-disease patients. As shown below, it has been possible to demonstrate the occurrence of the biological antagonism and to obtain approximate estimations of the molecular ratio between the two compounds, but no clearly defined difference was found between normal and Wilson's-disease individuals in the operation of the antagonism.

EXPERIMENTAL PROCEDURE

Twenty-one presumably healthy and normal Chinese adults, including 15 males and 6 females ranging in age from 20 to 40 years, were used for control studies. Their normal levels of urinary XA excretion and normal responses to our modified tryptophan-loading test were determined. Seven of the 21 normal adults were given additional tryptophan tests in conjunction with ingestion of DL-penicillamine, with and without supplemental oral pyridoxine hydrochloride. Three other normal Chinese male adults were used for tryptophan-loading studies with D-penicillamine and N-acetyl-DL-penicillamine. Two Wilson's-disease patients were studied: a male patient aged 18, described in the previous report,[14] and a female patient, aged 6. During their periods of testing, all subjects were placed on a similar nutritious diet containing a variety of typical Chinese foods. Except for consecutive daily XA excretion studies, tests on individuals were spaced several days or weeks apart to avoid possible interference.

Urinary XA excretion was the sole criterion used in assessing results in the present study. In all cases the total 24-hr urine specimens were pooled and XA determinations[19] made on an aliquot. Single determinations were made on samples during control periods, and duplicate determinations were made during periods of tryptophan-load testing; results are reported in milligrams of XA excreted per 24 hours. Total amounts of XA below 0·5 mg/24 hr could not be determined with precision; therefore, in the two instances where an amount in this range was found it was treated as "below 0·5 mg." For reasons covered in the discussion (*vide infra*) all tryptophan tests were made with loading doses of 0·1 g DL-tryptophan/kg body weight.

Except for some periods in which the Wilson's-disease patients received 2 g/day, the standard daily dose of DL-penicillamine was 1 g given orally. Both patients took

DL-penicillamine daily throughout the 15-month period of the present report except for short periods at the beginning and other brief intervals during the study when it was discontinued to allow specific test periods under other conditions. By contrast, the normal control subjects ingested the penicillamines only on days when their specific tests required it. Most of the present work was done with DL-penicillamine; however, studies also were made with 1-g daily doses of D-penicillamine and N-acetyl-DL-penicillamine. Because of its high cost, L-penicillamine has not been used thus far in our studies but will be studied shortly. On two occasions in the preliminary study pyridoxine hydrochloride was administered i.m. at levels of 100 and 200 mg; all other pyridoxine hydrochloride supplementation was provided orally in amounts varying from 10 to 300 mg/day.

RESULTS

Urinary XA excretion results are summarized in Table 1 and Fig. 1 and 2. As indicated in the table, the mean control value for the single 24-hr determinations

TABLE 1. SUMMARY OF URINARY XANTHURENIC ACID EXCRETION LEVELS (mg/24 hr)

Testing procedure	Normal Chinese adults*	Male Wilson's-disease patient	Female Wilson's-disease patient
Control, w/o tryptophan-loading tests	1·0 ± 0·3 (0·5 – 1·7) 21*	0·9 (0·8 – 1·1) 4†	1·0 ± 0·4 (0·5 – 1·9) 14†
DL-Penicillamine, 1 g daily, 4th-6th month, w/o tryptophan loading tests		1·6 ± 0·6 (0·6 – 3·0) 69†	1·4 ± 0·5 (0·7 – 3·3) 69†
Tryptophan-loading test	1·6 ± 0·6 (0·7 – 3·2) 21*	1·1	1·1
Tryptophan-loading tests w/ DL-penicillamine	6·8 (5·0 – 8·1) 7*	20·2	10·2
Tryptophan-loading tests w/ DL-penicillamine and pyridoxine HCl	1·0 (<0·5 – 1·4) 5*	2·2	<0·5
Tryptophan-loading test w/ D-penicillamine	1·5 (1·4 – 1·6) 3*	0·8	1·0
Tryptophan-loading test w/ N-acetyl-DL-penicillamine	1·3 (1·2 – 1·3) 3*	1·5	0·9

* The number of normal Chinese adults included in each testing procedure is starred. The values for their urinary XA excretion are given in terms of mean ± standard deviation, with ranges shown in parentheses.

† The numbers shown for both Wilson's-disease patients indicate the total number of daily determinations included in each patient's summarized values.

made on each of the 21 normal Chinese adults was 1·0 ± 0·3 mg. No difference was found between male and females in the XA excretion. In 14 consecutive daily determinations of urinary XA excretion made on one normal male adult the mean was 1·0 ± 0·4 with a range of 0·6 to 1·5 mg/24 hr. From these results a provisional normal range of 0·5 to 1·7 mg/24 hr can be used for the urinary XA excretion in adequately nourished normal Chinese adults consuming typical Chinese diets.

The control period used for determination of the urinary XA excretion levels shown in Table 1 for both Wilson's -disease patients was the same 14-day interval used for the study of the normal male adult. During that time all three subjects consumed identical diets. These data indicate that the control XA excretion of the two Wilson's-disease patients was in the normal range.

In a later series of 69 determinations, made in the 4th to 6th months of a period during which they received 1 g DL-penicillamine daily without pyridoxine supplementation, both patients showed slight increases in their urinary XA excretion.

When tryptophan-loading tests were made without DL-penicillamine in the 21 normal Chinese adults, slight increases in urinary XA excretions were noted; under the same conditions the Wilson's-disease patients excreted urinary XA within the normal range.

Obvious increases in urinary XA excretion occurred after tryptophan-loading tests in the presence of DL-penicillamine. The tests on the seven normal Chinese adults were made with single 1-g oral doses of DL-penicillamine taken on the day of the tryptophan-loading test. For the Wilson's-disease patients several loading tests were made at intervals during and after the variety of conditions indicated in Fig. 1 and 2. The data shown in the table for XA excretion after tryptophan loading in the presence of DL-penicillamine, 20·2 mg for the male and 10·2 mg for the female patient, were the initial results obtained. The responses of the Wilson's-disease patients to tryptophan taken in the presence of DL-penicillamine were greater than those shown by the seven normal Chinese subjects.

These results indicate that in the absence of DL-penicillamine therapy the Wilson's-disease patients excreted normal amounts of XA; furthermore, their XA excretion after tryptophan loads taken without DL-penicillamine was in the same range as those found during similar periods in the normal Chinese adults. In one series of XA determinations, not shown in Table 1, the male Wilson's disease patient was administered daily 2-g doses of DL-penicillamine without pyridoxine supplementation for an 8-day period preceding 18 Sept. 1962, when the initial tryptophan-loading test with penicillamine was made. XA excretions on six of those days were, in order, 1·0, 1·4, 1·2, 1·7, 1·1, and 3·3 mg. The last value, 3·3 mg, indicates a trend toward higher XA excretion in the presence of 2 g DL-penicillamine/day. After several months, during which both patients took daily 1-g doses of DL-penicillamine without dietary pyridoxine supplementation, their mean urinary XA excretion increased noticeably but remained at reasonably low levels. By contrast, both patients showed large increases above the normal range of urinary XA excretion when tryptophan-loading tests were run in the presence of DL-penicillamine and without supplemental dietary pyridoxine.

Tryptophan taken in the presence of DL-penicillamine and with pyridoxine supplementation caused no increase in urinary XA excretion. All amounts of oral pyridoxine tested, from 10 to 200 mg/day, were effective in preventing an elevation in XA excretion. In two instances 100- and 200-mg quantities of pyridoxine administered i.m. to the male patient also were effective; in the latter case the 24-hr XA excretion was reduced below 0·5 mg/day, which was the limit of precise measurement.

Both Wilson's-disease patients were given tryptophan loads in conjunction with 1-g oral doses of D-penicillamine and of N-acetyl-DL-penicillamine. As indicated in Fig. 1 and 2, the drugs were tested after medication-free periods when DL-penicillamine

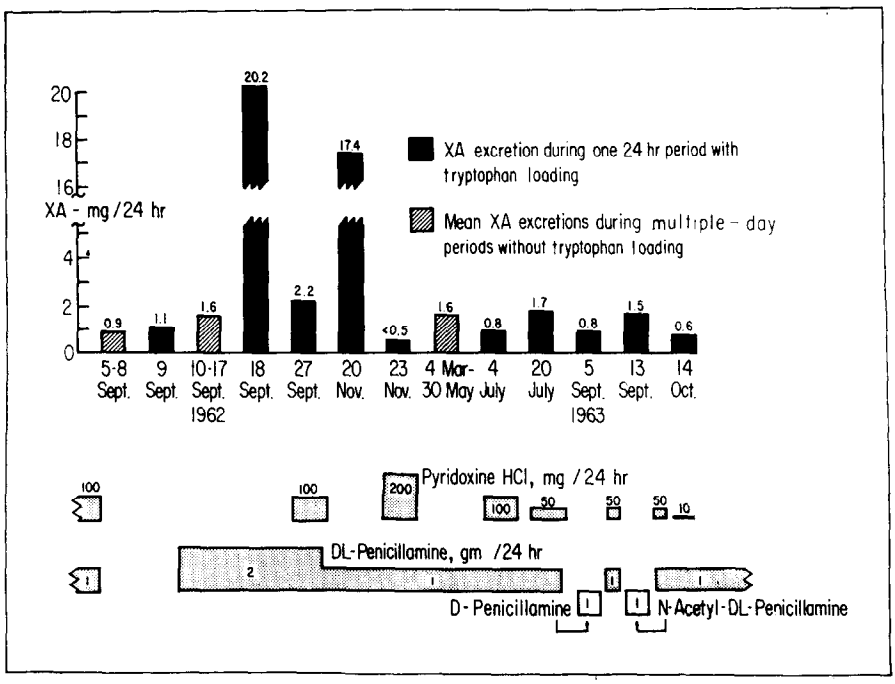

FIG. 1. Xanthurenic acid excretion studies in Wilson's disease. Patient L. C. W., male, aged 18 years.

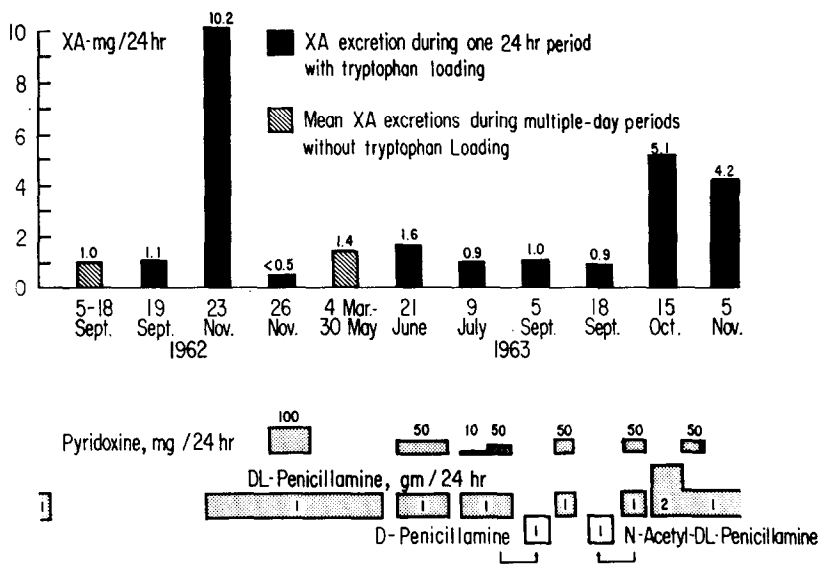

FIG. 2. Xanthurenic acid excretion studies in Wilson's disease. Patient C.L.C., female, aged 6 years.

and pyridoxine hydrochloride had been discontinued for several days to reduce possible interference. No increases of XA excretion were noted with either drug. In both patients, therefore, the only substantial increases in urinary XA excretion occurred when tryptophan-loading tests were made in conjunction with the ingestion of oral DL-penicillamine and in the absence of supplements of pyridoxine hydrochloride.

Three normal subjects not included in the other studies were given D-penicillamine and N-acetyl-DL-penicillamine during separate tryptophan-loading tests; no elevation in urinary XA above the normal range was observed. The 24-hr XA excretions in the three subjects after ingestion of 1 g D-penicillamine were 1·4, 1·6, and 1·4 mg; the corresponding results after 1 g N-acetyl-DL-penicillamine were 1·2, 1·3, qnd 1·3 mg. Therefore, in the normal Chinese subjects, as in the two Wilson's-disease patients, neither the D-penicillamine nor the N-acetyl-DL-penicillamine elicited changes in urinary XA excretion.

DISCUSSION

Results of the present study indicate that either DL-tryptophan or DL-penicillamine ingested alone has little effect upon the daily urinary XA excretion. By contrast, when the two are taken together, the urinary XA excretion invariably increases. Pyridoxine hydrochloride, by oral or intramuscular route, prevents the increase. These results are consistent with the existence of a penicillamine-pyridoxal antagonism; furthermore, the antagonism operates in normal individuals as well as in Wilson's-disease patients.

Although the definitive studies in the human with L-penicillamine have not been made, the absence of any elevation of urinary XA levels after ingestion of D-penicillamine and of N-acetyl-DL-penicillamine provides presumptive evidence that the biological antagonism in the human, as in the other species studied previously, involves specifically L-penicillamine and pyridoxal.

In the absence of pyridoxine supplementation, DL-penicillamine appeared to induce in the two Wilson's-disease patients studied a greater response of urinary XA excretion to the ingestion of DL-tryptophan than that shown by normal Chinese adults. However, no conclusions can be drawn on this point until further cases are studied. The previous exposure of the individuals to DL-penicillamine, rather than any inherent differences between normal persons and Wilson's-disease patients, may have been responsible for the differences noted. The normal individuals studied had no prolonged periods of exposure to DL-penicillamine to match those of the patients. For example, the male patient had received DL-penicillamine during more than 3 years of intermittent therapy prior to the beginning of the present study period. During that time he had ingested approximately 1 kg of DL-penicillamine and had experienced the visual disturbance previously reported.[14] The normal subjects received only a few grams of DL-penicillamine.

Investigators frequently[22] have used a standardized 10-g dose of DL-tryptophan, irrespective of body weight, in their tryptophan-loading tests on adult Caucasian subjects. For our Chinese adults, whose body weights ranged from 45 to 65 kg instead of the heavier weights characteristic of the Caucasian subjects, a somewhat smaller dose seemed desirable. Accordingly, the standardized loading dose of DL-tryptophan used for the present study was 0·1 g/kg body weight. For the 6-year-old female Wilson's-disease patient, whose weight remained nearly constant during the 15 months of the study, the amounts used were all approximately 2·7 g; the male patient, whose weight gain was 5 kg during the 15-month study period, ingested amounts ranging from 4·5 to 5·0 g in his ten tryptophan-loading tests.

In our earlier unpublished observations it was found that administration of 10 g of DL-tryptophan to the average normal Chinese adult frequently produced unpleasant

symptoms of sufficient intensity to make the subject unwilling to cooperate in additional loading studies. In the present study, in spite of the lower amount of DL-tryptophan used, most of the subjects complanied of dizziness, drowsiness, malaise, loss of appetite, and a feeling of fullness. Some subjects in the present series felt that the symptoms were more severe when the tryptophan was taken along with DL-penicillamine. The symptoms appeared to be most marked in the male Wilson's-disease patient; however, he took part in more loading tests than any of the other subjects and may have developed a more pronounced aversion to the procedure. Both patients became less cooperative after repeated loading tests. Similar unpleasant reactions to L-tryptophan were reported recently by Smith and Prockop.[23]

Our results indicate that the present loading dosage of DL-tryptophan is adequate to provide a sensitive indication of changes in XA production. The 24-hr levels of urinary XA excretion reported here are lower than those generally reported [22]in other tryptophan-loading studies, including those concerned with pyridoxal–isoniazid and pyridoxal–deoxypyridoxine antagonisms.[16,20,24] Although our lower DL-tryptophan dosage is one logical reason for the difference, it appears possible that dietary factors, such as relative intake of the B-complex vitamins, and ethnic differences also may influence the relative distribution of excess tryptophan among the several alternative pathways of tryptophan metabolism.

Tryptophan is one of the limiting amino acids in most diets; however, in the Chinese diet, which has a lower total protein content and a higher relative proportion of vegetable protein compared to typical Western diets, the tryptophan intake may be comparatively more marginal. Under these conditions it would appear likely that appreciable amounts of the extra L-tryptophan provided in the loading doses of DL-tryptophan would serve to supply the tissues with more favorable ratios of essential amino acids for protein synthesis and consequently leave relatively less of the extra L-tryptophan for catabolic pathways, including those leading to XA synthesis. The almost universal appearance in our subjects of the unpleasant symptoms attending the ingestion of tryptophan, however, strongly suggests that some significant portion of the tryptophan dose is not utilized for anabolic purposes but, instead, is converted to physiologically active metabolites such as tryptamine.

Its antagonism with pyridoxal is only one of the biological activities of L-penicillamine.[25] Although a variety of differing activities has been established for the L- and D-forms, and for derivatives such as the N-acetyl compounds, much remains to be learned. The close structural similarities between the amino acid penicillamine and the more common branched-chain amino acids such as valine, isoleucine and leucine, and the sulfhydryl amino acid cysteine account for some of its biological activity.[25] Penicillamine reacts *in vitro* at alkaline pH with carbonyl compounds[3] to form thiazolidine derivatives in the same way as that known for cysteine[26, 27]; the reaction with pyridoxal proceeds with ease,[28] and the thiazolidine compound has been characterized.[28] It has been suggested[3] that such a reaction might occur *in vivo* and be the basis by which the L-penicillamine reduces the biological availability of pyridoxal; however, except for the report[4] of increased urinary vitamin B_6 activity in rats fed L- penicillamine, no specific urinary excretion studies to investigate this possibility appear to have been made. Such information awaits further studies of detoxication mechanisms for penicillamine in the human.[25] Although D-penicillamine is known to be excreted by cystinurics as a disulfide-compound with cysteine,[29]

its excretory forms, under daily loads of a gram or more either in normal individuals or in Wilson's-disease patients, have not been studied adequately.

It is of interest to consider the effective molecular ratios of pyridoxal to L-penicillamine in the present work and to compare them with those of other workers. In rat-feeding experiments Heddle *et al.*,[7] judging from the effects of DL-penicillamine upon changes in body weight, food intake, and whole blood transaminase activity, concluded that incomplete but nearly maximal reversal of the penicillamine effects were obtained with vitamin B_6 to DL-penicillamine molecular ratios of 1 to 42. The corresponding ratio in terms of the L-penicillamine in the racemic mixture was 1 to 21. In the studies of du Vigneaud and co-workers[4, 5] the ratio, based on liver transaminase activities determined in rats after intravenous injections of combinations of pyridoxine hydrochloride and L-penicillamine, was about 1 to 5. In the present study it was noted that for a 1-day determination, as little as 10 mg of supplemental pyridoxine hydrochloride prevented any rise in urinary XA excretion after a standard tryptophan load taken along with 1 g of DL-penicillamine. Lower amounts also might have been effective but were not tried. From this effect, and without considering the dietary vitamin B_6, the molecular ratios of pyridoxal to DL- and L-penicillamine respectively would be 1 to 140 and 1 to 70. Our routine pyridoxine supplementation to accompany DL-penicillamine therapy is a more conversative ratio of 50 to 100 mg pyridoxine hydrochloride/g DL-penicillamine. It is of interest that Heddle at *al.*[7] predicted from their rat studies that in the human nearly maximal protection against 1·5-g doses of DL-penicillamine should be provided by 50 mg of vitamin B_6.

The present results implicating L-penicillamine are in agreement with previous reports[2, 12, 25, 30–32] which emphasize the lower toxicity of D-penicillamine; from all available information the latter is the logical choice of the isomers for treatment of Wilson's disease. Because of its lower cost, however, DL-penicillamine remains the practical choice to treat the disease when the higher cost of the D-isomer precludes its use.

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