The Effect of Isonicotinic Acid Hydrazide and Vitamin B_6 on Glutamic-Oxalacetic Transaminase Levels in Whole Blood

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GROUP A served as a control and was selected at random from a large group of medical and surgical patients. Selection of patients was limited by the exclusion of cases with myocardial infarction, cirrhosis of the liver or hepatitis. Group B consisted of patients from the Chest Service of this hospital, all of whom had been receiving INH (300 mg/day) for at least 30 days. Group C consisted of nine men and four women volunteers selected from laboratory and ward personnel. All of these subjects were in good health, of average income, with presumed average dietary habits. In contrast to Group A, Group C was not fed from the hospital kitchen. Two additional groups of patients (D and E) who had never before received INH were selected at random from the Chest Service. Group D was placed on INH therapy (500 mg/day) and Group E received INH (500 mg/day) plus pyridoxine (25 mg/day).

Whole blood transaminase activity was measured at the start and at suitable intervals throughout the 6 to 12 week experimental period. Complete hematologic studies were done on these patients at the start, middle and end of the study. At the end of this experimental period, three patients in each group, D and E, received a tryptophan load test.[10]

The last group (Group F) consisted of selected patients with the lowest blood transaminase levels achieved after the administration of INH (500 mg/day) for 6 to 12 weeks. These patients continued to receive the same dose of INH plus supplementary multivitamin tablets without pyridoxine. Blood transaminase activity was measured at suitable intervals.

Laboratory Methods: Blood was collected in dry oxalate (Heller and Paul) and a 1:20 dilution was prepared in distilled water. This dilution of hemolyzed blood was centrifuged at 2500 rpm for 15 minutes to remove cell stroma. The clear supernatant was analyzed on the same day. In several cases, when necessary, the hemolysate was stored for 2 to 4 days at −20° C prior to analysis. The stability of the transaminase enzyme under these conditions is well established.

A modification[6] of the spectrophotometric procedure of Karmen and associates[7] was used for the estimation of glutamic-oxalacetic transaminase. This procedure is much more sensitive than that used by other investigators and does not present any of the reported difficulties[3,4] encountered with the use of the older procedure of Tonhazy, White and Umbreit.[8]

For this assay 0.2 to 0.3 ml of a 1:20 dilution of whole blood was analyzed. Readings were taken in a Beckman DU spectrophotometer, one unit representing a change in optical density of 0.001 O.D. units per minute, in a total volume of 3.0 ml under the conditions specified. All operations were carried out at room temperature. The temperature coefficients developed by Steinberg and Ostrow[9] were used to correct the values to a temperature of 25° C.

Lactic dehydrogenase (L.D.H.) levels were determined by an enzymatic technic based on the oxidation of reduced coenzyme I in the interconversion of pyruvic to lactic acid.[10]

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The concentration of hemoglobin in the dilution of blood analyzed was determined by spectrophotometric estimation of oxyhemoglobin at 540 mμ using a Beckman DU spectrophotometer. This instrument was standardized for hemoglobin determination by the manometric procedure of Van Slyke.[11] All other hematologic data were obtained by standard technics.

Studies in the pyridoxine-deficient state have been less revealing. In monkeys, vitamin B_6 deficiency produced through dietary restriction resulted in a reduction of blood transaminase levels below those of control animals.[3] No such differences have been reported in humans in whom pyridoxine deficiency is not so easily established. In one study of pregnant women whose response to tryptophan loading was characteristic of pyridoxine deficiency, blood transaminase values did not differ from those of non-pregnant controls.[4] To our knowledge, no other studies have been reported on this problem.

Recent reports indicate that the administration of isonicotinic acid hydrazide (INH) in high doses can produce a syndrome in humans suggestive of pyridoxine depletion.[5] In animals, INH has been used to produce a presumably more severe pyridoxine deficiency than can be produced by dietary means or by the administration of desoxypyridoxine.[2] These observations suggested the study of blood glutamic-oxalacetic transaminase (G.O.T.) levels in patients receiving INH in routine tuberculosis therapy.

EXPERIMENT

Subjects: Six groups of patients were studied in all. Three of these (Groups A, B and C) were studied to determine whether differences in whole blood G.O.T. levels existed in individuals receiving INH as compared with individuals not receiving this drug.

Pyridoxine or its metabolites are known to act as a prosthetic group for transaminase enzymes in animals.[1,2] While this relationship is presumably true in humans, evidence for this is indirect. Recent investigations in normal subjects[3] and in pregnant women[4] have indicated that supplementation with pyridoxine results in a significant elevation of blood transaminase activity. When supplementation is discontinued there is a slow return of blood enzyme activity to pre-administration levels.

RESULTS

The data in Table I indicate that the mean whole blood G.O.T. level of patients receiving 300 mg INH per day (Group B) is lower than that of the control series of hospital patients (Group A). Surprisingly enough, the mean blood transaminase level in non-hospitalized volunteers (Group C) is almost identical with that of Group B rather than with that of Group A, as might be expected.

TABLE I

Glutamic-Oxalacetic Transaminase and Lactic Dehydrogenase Activity in the Blood of Patients with and without Prolonged INH Therapy* (Mean ± S. E.)

Group	G.O.T. Units/ml	G.O.T. Units/100 mg Hb	L.D.H. Units/mg Hb
A (Control)	723 ± 25 (23)	526 ± 20	154 ± 3.1 (12)
B (INH)*	550 ± 38 (19)	390 ± 26	152 ± 1.0 (16)
C (Volunteers)	590 ± 29 (13)	420 ± 6	157 ± 1.7 (13)
Significance (P)			
A vs. B	<0.01	<0.01	0.62
A vs. C	<0.01	<0.01	0.69
B vs. C	0.57	0.62	0.48

* INH therapy consists of 300 mg/day for a period of at least 30 days (numbers in parentheses refer to number of patients).

"P" values (Table I) indicate that the differences between Groups A and B and Groups A and C are highly significant. These differences did not change appreciably when the transaminase units were calculated on the basis of hemoglobin content indicating that variations in cell count, as reflected by hemoglobin content, did not account for the differences observed. All subsequent G.O.T. values are reported in units per 100 mg hemoglobin.

Blood lactic dehydrogenase levels (L.D.H.) of the three groups were also studied to

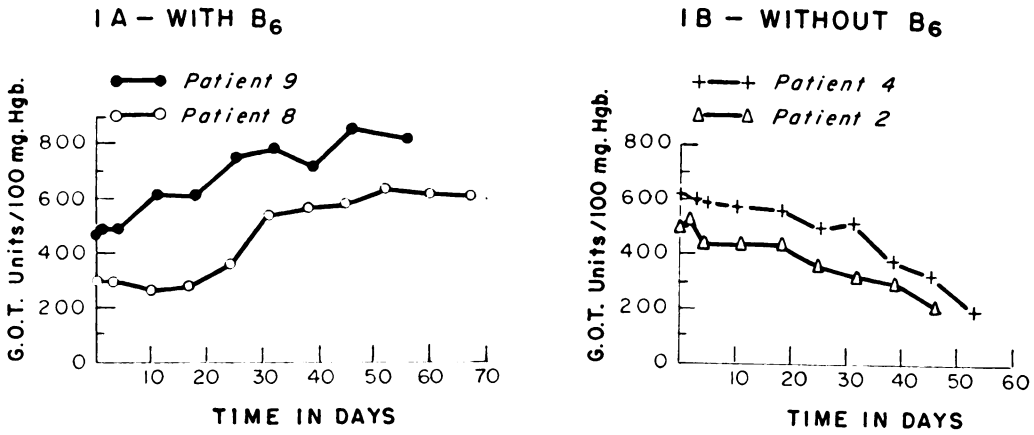

Fig. 1. Blood G.O.T. levels during INH therapy.

determine the specificity of the presumed effect of INH administration (Table I). The high "P" values indicate that there are no significant differences among the three groups.

Serial Studies: The blood G.O.T. levels during the experimental period of INH and vitamin B_6 administration are presented in Figure 1 for two representative patients in each of groups D and E. The increase in G.O.T. level during INH plus vitamin B_6 therapy (Fig. 1A) is marked beginning within 10–15 days after the start of pyridoxine administration. The decrease in G.O.T. activity observed during INH therapy alone, on the other hand, is limited and does not occur until 20–25 days of INH therapy at a level of 500 mg/day.

The transaminase values for each patient in groups D and E at the beginning and end of the experiment are presented in Table II. The differences between the mean pre- and post-therapy values for each group have been found to be statistically significant in contrast to the difference between the mean G.O.T. values for the two groups at the start of therapy. Plasma G.O.T. levels in all patients were within the normal range and could not influence whole blood transaminase levels significantly.

At the end of this experimental period three patients in each group were selected for a tryptophan load test. Twenty-four-hour urine specimens were collected on each patient for control levels. At the end of this period,

TABLE II

Change in Transaminase Activity in Whole Blood of Patients on INH Therapy with and without Vitamin B_6* (units/100 mg Hemoglobin)

	Patient	Pre-therapy	Post-therapy	Per cent change
Group D INH therapy	1	625	435	−31
	2	510	210	−59
	3	375	280	−25
	4	605	345	−43
	5	360	315	−12
	6	385	380	—
		Mean 475	330	−31
Group E INH and vitamin B_6 therapy	7	510	750	+47
	8	305	705	+130
	9	475	830	+75
	10	495	655	+32
	11	375	610	+62
		Mean 432	710	+69

* Therapy: 25 mg vitamin B_6/day and/or 500 mg INH/day over a period of from 5 to 11 weeks.

10 g DL-tryptophan* were administered by mouth and another 24-hour-urine collection was obtained. All urines were analyzed for xanthurenic acid[12] and the results are presented in Table III. They indicate that none of the patients in either group exhibited the marked increase in xanthurenic acid excretion indicative of pyridoxine deficiency.[13]

The mean hematologic values for each of the two groups at the beginning and end of the

* Generously supplied by Merck, Sharpe & Dohme, Inc.

TABLE III

Xanthurenic Acid Excretion after Tryptophan Load Test

Patient	Therapy	Xanthurenic acid mg/24 hours	
		Control*	Post-tryptophane
2	INH	29.8	48.0
3	INH	20.4	55.0
4	INH	28.8	34.4
8	INH and B_6	–	30.4
10	INH and B_6	16.4	1.9
11	INH and B_6	25.0	27.0

* 24-hour urine collection prior to administration of 10 g DL-tryptophan.

experiment are presented in Table IV. Since the hemoglobin content of the blood was used as a reference in calculating the G.O.T. level, any changes in the hemoglobin concentration per red cell (MCHC) could exaggerate changes in G.O.T. These changes did not occur. The significance of the apparent macrocytosis indicated by the elevated mean corpuscular volumes (MCV) is not apparent at the present time.

In order to evaluate the effect of vitamins other than pyridoxine, four additional patients (Group F) were studied. This group of patients had been receiving INH (500 mg/day)

TABLE IV

Hematologic Indices before and after Experimental Period

	MCV μ^3	MCH $\mu\mu g$	MCHC %	RBC *mill/mm³*	Hb *g/100 ml*	Hct %
Group D Pre-therapy	102	31.6	31.3	4.79	15.1	49
Group D Post-therapy	102	30.9	30.8	5.07	15.3	50
Group E Pre-therapy	101	29.1	28.9	4.66	13.5	47
Group E Post-therapy	100	30.2	30.4	4.70	14.2	47

MCV = mean corpuscular volume
MCH = mean corpuscular hemoglobin
MCHC = mean corpuscular hemoglobin concentration
RBC = red blood cells
Hb = hemoglobin
Hct = hemocrit

TABLE V

Transaminase Activity in the Blood of Patients on INH before and after Multivitamin Therapy* (Units/100 mg Hemoglobin)

Patient	Pre-therapy†	Post-therapy	% Change
2	210	210	0
4	280	200	−29
5	280	240	−16
6a	200	190	− 5
			Mean −12.5

* Two multivitamin tablets/day plus 500 mg INH/day for $6\frac{1}{2}$ weeks.

† Mean of two determinations one week apart.

for 6 to 12 weeks and were selected on the basis of low whole blood G.O.T. levels. They were continued on the same INH therapy and were given, in addition, a daily dose of two multivitamin* tablets without pyridoxine. G. O. T. levels were measured at intervals. The results (Table V) indicate that the administration of vitamins other than B_6 did not increase blood G.O.T. levels in the dosages employed.

DISCUSSION

In animals, INH has been demonstrated to produce a pyridoxine deficiency that is more severe than can be evoked by dietary restriction or by the administration of desoxypyridoxine.[2] In humans, peripheral neuropathy has been noted after doses of INH ranging from 24 mg per kg per day to as low as 3 to 5 mg per kg per day.[14] These toxic manifestations, amenable to pyridoxine therapy, are presumably due to the formation of an INH-pyridoxine complex,[5] effectively removing pyridoxine from the available pool for tissue utilization. The relative infrequency of overt clinical signs of toxicity at lower doses of INH, however, is no assurance that more subtle biochemical changes do not occur.

Our studies indicate that the administration

* Each tablet contains the following vitamins:

Vitamin A	— 5,000 U
Vitamin D	— 450 U
Thiamine	— 2 mg
Riboflavin	— 3 mg
Nicotinamide	— 20 mg
Ascorbic acid	— 75 mg

of INH results in a drop in blood transaminase activity despite the absence of clinical signs of toxicity. The mean G.O.T. activity in the blood of patients receiving 300 mg INH per day for a period averaging approximately four months (Table I, Group B) was found to be significantly lower than that of a control group on the same diet (Table I, Group A). This dosage level constitutes the usual regimen in the therapy of tuberculosis. More recently, some investigators have concluded that this dose is inadequate therapy and have suggested the use of a minimum of 8 mg per kg per day.[15-18] At approximately this level (500 mg per day) INH has been shown to depress blood transaminase activity within a period of from 6 to 11 weeks (Fig. 1B; Table II, Group D). This effect could be reversed by the administration of 25 mg pyridoxine per day (Fig. 1A; Table II Group E). Vitamins A and D, thiamine, riboflavin, nicotinamide and ascorbic acid were ineffective in the doses employed (Table V).

The significance of measurements of blood transaminase activity is certainly unclear at present. High doses of INH have already been shown to produce metabolic alterations indicative of pyridoxine deficiency.[5] With the dose used in this study (500 mg per day), tryptophan load tests at the end of the period of therapy were normal (Table III) in agreement with a previous report.[5] It is difficult, therefore, to avoid the suggestion that blood transaminase levels may serve as a more sensitive indicator of the adequacy or inadequacy of pyridoxine intake. The surprising finding that the mean G.O.T. activity of hospitalized control patients (Table I, Group A) exceeded that of non-hospitalized volunteers (Table I, Group C) could be a reflection of this relationship. It is certainly conceivable that the vitamin B_6 content of the routine, well-controlled hospital diet received by Group A was greater than that of the uncontrolled, non-institutional diet chosen by Group C. Unfortunately, no such measurements could be made.

Until further information becomes available on the meaning of the observed changes in transaminase activity during INH therapy, it would seem prudent to prevent their occurrence by the administration of small doses of pyridoxine whenever long-term INH therapy is necessary. The minimal dose of pyridoxine adequate to accomplish this has not as yet been determined. The amount used in this study has already been found to be effective in preventing peripheral neuritis in patients receiving 8 mg INH per kg per day.[17,18] This is probably in excess of that required to prevent changes in blood G.O.T. activity. It is expected that further study with smaller doses will indicate more precisely the amount of vitamin B_6 required to "neutralize" the effects of known amounts of INH.

SUMMARY AND CONCLUSIONS

The effect of INH and pyridoxine on whole blood glutamic-oxalacetic transaminase (G.O.T.) levels has been investigated. The mean blood G.O.T. activity of patients receiving 300 mg INH per day has been shown to be lower than that of control hospital patients but not significantly different from the mean of a group of normal, non-hospitalized subjects.

Serial studies at higher doses (500 mg INH per day) indicated that a significant drop in blood transaminase activity occurred after 6 to 11 weeks of therapy. At this time, tryptophan load tests were normal. These changes in blood transaminase activity were reversed by the administration of 25 mg pyridoxine per day and not by a group of other vitamins.

In view of these findings, it is suggested that blood G.O.T. activity may reflect the level of pyridoxine intake even before tryptophan metabolism becomes markedly abnormal. Until the actual significance of these changes becomes more clearly defined, it would seem advisable to combine the administration of pyridoxine with INH to prevent a depression in blood transaminase activity.

REFERENCES

1. SNELL, E. S.: Evidence that B_6 derivatives serve as coenzymes of transaminase. *Physiol. Rev.* 33: 509, 1953.
2. MEISTER, A., and DOWNEY, P. F.: Evidence for participation of vitamin B_6 in glutamine-alpha-

keto acid transamination-deamidation reaction. *Proc. Soc. Exper. Biol. & Med.* 91:49, 1956.

3. MARSH, M. E., GREENBERG, L., D., and RHINEHART, J. F.: The relationship between B_6 investigation and transaminase activity. *J. Nutrition* 56:115, 1955.
4. GLENDENING, M. B., COHEN, A. M., and PAGE, E. W.: Influence of B_6 on transaminase activity of human placenta, maternal and fetal blood. *Proc. Soc. Exper. Biol. & Med.* 90:25, 1955.
5. BIEHL, J. P., and VILTER, R. W.: Effect of isoniazid on vitamin B_6 metabolism: Its possible significance in producing isoniazid neuritis. *Proc. Soc. Exper. Biol. & Med.* 85:389, 1954.
6. STEINBERG, D., and OSTROW, B. H.: Serum transaminase as a measure of myocardial necrosis. *Proc. Soc. Exper. Biol. & Med.* 89:31, 1955.
7. KARMEN, A., WROBLEWSKI, F., and LADUE, J. S.: Transaminase activity in human blood. *J. Clin. Investigation* 34:126, 1955.
8. TONHAZY, N. F., WHITE, N. G., and UMBREIT, W. W.: A rapid method for the estimation of glutamic-aspartic transaminase in tissues and its application to radiation sickness. *Arch. Biochem.* 28:36, 1950.
9. STEINBERG, D., and OSTROW, B. H.: A clinical method for the assay of serum G.O.T. *J. Lab. & Clin. Med.* 48:144, 1956.
10. HILL, R. R., and LEVI, C.: Elevation of a serum component in neoplastic disease. *Cancer Res.* 14:513, 1954.
11. VAN SLYKE, D. D., and NEILL, J. M.: The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. I. *J. Biol. Chem.* 61:523, 1924.
12. ROSEN, F., LOWY, R. S., and SPRINCE, H.: A rapid assay for xanthurenic acid in urine. *Proc. Soc. Exper. Biol. & Med.* 77:399, 1951.
13. GLAZER, H. S.: A study of urinary excretion of xanthurenic acid and other tryptophane metabolites in human beings with pyridoxine deficiency induced by desoxypyridoxine. *Arch. Biochem.* 33:243, 1951.
14. BIEHL, J. B., and VIMITZ, H. J.: Studies on the use of a high dose of isoniazid. I. *Am. Rev. Tuberc.* 70:430, 1954.
15. MANDEL, W., COHEN, M. L., RUSSELL, W. F., JR., and MIDDLEBROOK, G.: Serum isoniazid levels and catalase activities of tubercle bacilli from isoniazid treated patients. *Am. J. M. Sc.* 233:66, 1957.
16. MIDDLEBROOK, G., COHEN, M. L., and SCHAEFER, W. B.: Studies on isoniazid and tubercle bacilli. III. *Am. Rev. Tuberc.* 70:852, 1954.
17. KUKRAL, A. J., CROW, J. B., MIDDLEBROOK, G., and DRESSLER, S. H.: The handling of the poor risk patient with pulmonary tuberculosis: antimicrobial therapy, cardio-pulmonary function and surgery. *Dis. Chest* 29:266, 1956.
18. CARLSON, H. B., ANTHONY, E. M., RUSSELL, W. F., JR., and MIDDLEBROOK, G.: Prophylaxis of isoniazid neuropathy with pyridoxine. *New England J. Med.* 255:118, 1956.