cells shrink considerably. Erythrocytes show a similar but smaller shift both of electrolytes and water.

Indoleacetic acid also causes shrinkage of the tumor cells, and loss of potassium chloride. With erythrocytes the effect was quite small, although definite shrinking was seen(3). In correspondence the indoleacetate ion showed a smaller effect than pyridoxal in the present experiments (Fig. 1).

Evidence obtained with tumor cells from pyridoxine-deficient mice implicates pyridoxal in the normal process of amino acid accumulation by these cells(3,4). A close relationship to the transfer of potassium into cells is also indicated(3).

Summary. Pretreatment of erythrocytes with 10 mM pyridoxal makes the cells subsequently more resistant to lysis by hypotonic solutions. Indoleacetic acid shows the same behavior to a smaller degree. These effects are attributed to a net loss of cell solutes in the form of potassium and chloride which the agents produce.

1. Wilbrandt, W., *Arch. ges. Physiol.*, 1941, v245, 22.

2. Raker, J. W., Taylor, I. M., Weller, J. M., and Hastings, A. B., *J. Gen. Physiol.*, 1950, v33, 691.

3. Christensen, H. N., Riggs, T. R., and Coyne, B., mss. in preparation.

4. Riggs, T. R., Coyne, B., and Christensen, H. N., *Biochim. Biophys. Acta*, 1953, v11, 303.

Received December 23, 1953. P.S.E.B.M., 1954, v85.

Effect of Isoniazid on Vitamin B_6 Metabolism; Its Possible Significance in Producing Isoniazid Neuritis.* (20891)

J. PARK BIEHL† AND RICHARD W. VILTER. (Introduced by L. H. Schmidt.)
(With the technical assistance of Florence C. Beall and Cora Ellen Kennedy.)
From the Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati.

Peripheral neuritis occurs as a side-effect of isoniazid (isonicotinic acid hydrazide, INH) therapy in tuberculosis, particularly when large doses are employed(1-6). This usually consists of paresthesia and numbness of the fingers and toes, advancing centripetally, with muscle soreness and weakness in some cases if medication is not discontinued. Vibratory sense may be impaired, and patchy hypesthesia has been seen. Reflexes may be diminished or exaggerated. The symptoms usually disappear within a few weeks if INH is promptly discontinued at the onset; however, late residuals such as burning feet, atrophy, fasiculation, and paresthesia may persist for months. In our experience, using a dose of 20 mg/kg/day of INH, neuritis has appeared in a predictable percentage of patients within definable limits of time. The similarity of this side effect to the neuritis induced by the vit. B_6 antagonist, desoxypyridoxine(7), has led us to investigate vit. B_6 metabolism in patients who were receiving INH for tuberculosis. The data to be reported indicate that INH does interfere with B_6 metabolism, and that the neuritis may be prevented with large doses of B_6.

Materials and methods. Subjects. All subjects studied were adult patients with active tuberculosis either on the wards of the Cincinnati General Hospital, or at Dunham Hospital, Cincinnati. During the study, all were receiving streptomycin (SM), one g daily or twice weekly. All patients were given the regular hospital diet. The various aspects of the study were carried out concurrently. Leading questions concerning symptoms of neuritis were avoided in evaluating the course of the patients, in order to eliminate the factor of suggestion. Any remarks by the patient, on the other hand, possibly connected with the onset of neuritis were pursued carefully. Neurologic testing was performed, but served no useful purpose in detecting the onset of neuritis. *Incidence of*

* This work supported by grants from the Trustees of Dunham Hospital, Cincinnati, the National Tuberculosis Association, and the National Vitamin Foundation.

† Teaching Fellow, American Trudeau Society.

neuritis. Thirty-six patients received 20 mg/kg/day of INH in order to establish the incidence of neuritis at this dose level. This is an extension of the previously reported series(5), in which neuritis appeared in 5 of 12 (42%) cases. *Effect of pyridoxine in preventing and reversing INH neuritis.* Twenty patients were given 150-450 mg of pyridoxine daily, beginning when INH therapy (20 mg/kg/day) was started, and were observed for at least 10 weeks on this regimen. Other patients were treated with 300-450 mg of pyridoxine daily, in addition to INH, at the onset of INH neuritis. The effects of pyridoxine 50 mg and of a combination of thiamin 80 mg and niacin 200 mg in 2 groups of matched cases of late residual INH neuritis were observed. *Effect of INH on B_6 metabolism.* Twenty-one patients were studied for the effects of INH on B_6 metabolism. Following admission to the hospital, these patients were observed for a few days, and control samples of urine were obtained. Twelve then received 20 mg/kg/day of INH, 6 received 3-5 mg/kg/day, and one received no INH. The latter case received instead p-aminosalicylic acid, 12 g daily. *Laboratory determinations.* Serial 24-hour urine samples were analyzed for excretion of vit. B_6(8,9), pyridoxic acid(10), and N_1methylnicotinamide(11). Xanthurenic acid (XA) excretion was measured in 24-hour urine samples before and after an oral test dose of 10 g of dl-tryptophane(12). A rise in XA excretion to at least 30 mg/24 hours after tryptophane was accepted as indicating a deficiency or metabolic block of B_6.

Results. Neuritis occurred in 14 of 36 patients (40%) receiving 20 mg/kg/day of INH. Its rate of occurrence in the later cases agreed with that of the earlier series previously reported(5). The length of time for neuritis to appear in the 14 cases after the start of therapy is indicated in Table I. The

TABLE I. Time of Onset of Neuritis after Starting of INH (20 mg/kg/Day).

	Wk of INH therapy										
	1	2	3	4	5	6	7	8	9	10	17
No. of cases with onset of neuritis	0	0	0	4	3	4	1	1	0	0	1

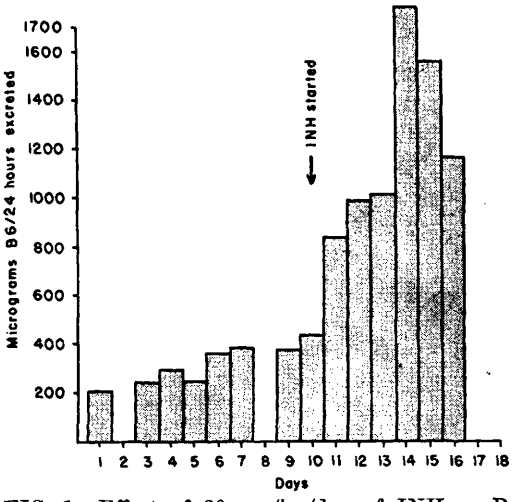

FIG. 1. Effect of 20 mg/kg/day of INH on B_6 excretion. INH was started on 10th day in this patient.

20 patients who received pyridoxine from the beginning of INH therapy did not develop neuritis, after at least 10 weeks of treatment.

On 2 occasions, at the time of the onset of neuritis, pyridoxine failed to reverse the symptoms, after 3 and 16 days. Another case responded to pyridoxine, and it and INH (20 mg/kg/day) were continued for 9 weeks. Neuritis reappeared when pyridoxine was inadvertently discontinued. Late residual neuritis in 22 patients did not respond either to pyridoxine or to thiamine and niacin.

An example of the effect of INH on the daily excretion of B_6 can be seen in Fig. 1. A control period of 9 days, during which the patient received SM only, preceded the administration of INH. On the day after INH was added, there was a striking increase in B_6 excretion. Fig. 2 shows the average B_6 excretion in all cases. Each patient receiving INH exhibited significantly increased excretion of B_6, the most striking elevations appearing in the 20 mg/kg/day group. No increase was seen when PAS was used in place of INH. B_6 excretion would promptly fall to normal if INH were stopped.

Excretion of pyridoxic acid and N_1-methylnicotinamide were normal.

XA excretion following tryptophane was greatly increased, but in an irregular fashion, in patients receiving 20 mg/kg/day, as is

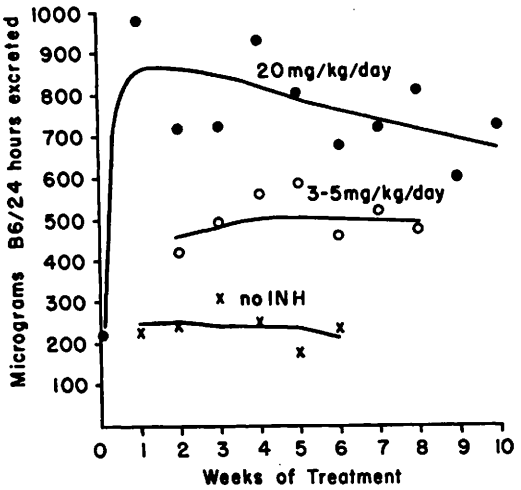

FIG. 2. Average values of B_6 excretion during treatment with 20 mg/kg/day of INH, 3-5 mg/kg/day of INH and no INH.

seen in Table II. If B_6 were given when the XA was elevated, a prompt fall to normal would occur. Lower XA levels were observed with the lower dose of INH, but even some of these tended to be abnormally high while INH was being given. The absence of pretreatment values in the latter group is unfortunate, since abnormal values for XA excretion after tryptophane may be present at the time the patient is admitted to the hospital, presumably due to relative malnutrition during the toxic stage of tuberculosis. Such elevations, however, fall rapidly to normal when B_6 is given and somewhat more slowly on the hospital diet. These patients had been observed in the hospital for from 3 to 6 weeks before the tests were performed. By this time XA levels are ordinarily normal.

Discussion. The incidence of neuritis, under the conditions described, was 40%. It usually appeared during the fifth to seventh week. In only one patient did it appear as late as the 17th week. In sharp contrast, none of the 20 patients on pyridoxine and INH developed neuritis while on treatment for over 10 weeks. On this basis, it would appear that pyridoxine in large doses has prevented the appearance of neuritis. It cannot be concluded, however, that factors unrelated to B_6 may have played some part in producing neuritis in our patients. The incidence of neuritis in tuberculosis cases in general is considerable, and B_6 abnormality may serve only as a precipitating factor in the neuritis associated with INH. Sufficient studies were not permitted to determine whether large doses of pyridoxine would reverse the neuritis at its onset, even though INH was continued. In one out of 3 cases this appears to have happened.

Metabolic antagonism with nicotinic acid has been suggested as a mechanism for the production of this neuritis(1-3). If this were the case, pellagra should be the clinical result, and there is no clear cut evidence that INH has induced this syndrome. Neuritis is not a part of nicotinamide deficiency in human beings(13), and in our cases normal N_1methylnicotinamide values were obtained in the urines. On the other hand, neuritis has been produced by pyridoxine deficiency in suckling pigs(14) and by B_6 antagonism in human beings(7).

B_6 deficiency has been demonstrated in our series by the elevation that occurred in XA excretion after tryptophane. At the same time a sharp increase in B_6 excretion has been observed. The data do not support the thesis of metabolic antagonism. They suggest either a disorder in renal conservation of B_6, which seems a remote possibility, or a chemical alteration of this vitamin by INH and its excretion. Mutual interference between pyridoxal and hydrazines or hydrazides has been shown. Minute concentrations of hydrazine (0.001 molar) completely inactivate amino acid decarboxylases that are known to require pyridoxal phosphate as coenzyme, whereas hydrazine is virtually ineffective against those enzymes which do not require pyridoxal(15). Pyridoxine has been claimed to be an antidote to certain other toxic effects of INH and thiosemicarbazide(16,17). "Metabolic antagonism" between INH and pyridoxal has been thought to exist in the case of colon bacillus tryptophanase and arginine decarboxylase(18,19), both of which require pyridoxal phosphate as coenzyme. The presence of the hydrazide moiety in each of these B_6 "antagonists", suggests that pyridoxal is inactivated by the formation of a hydrazone. That pyridoxal isonicotinyl hydrazone may have been formed and excreted

TABLE II. Excretion of Xanthurenic Acid (mg/24 Hr) following 10 g of DL-Tryptophane before and during INH Therapy (20 mg/kg/Day), in 12 Patients.

Wk of INH therapy												
0	1	2	3	4	5	6	7	8	9	10	11	12
45		55	156		116	250		192		31*		
3		16	150		95		81		21	22	52	66
18		24	68		11	65	34					
29		21		11	11			17.5	11			
87	57	72	24	40	94	13.5*	13*	13*				
	23	180						43				
75	41	33	105									
40		63		52								
16		28	20	30		19	13*	13*				
	43	50	72	81	80		22		9	5	37	
5.2	11	18		16	0							
126	61	188	199	119	121	112	55	107				
35		5	25									

* Patients receiving pyridoxine at these times.

in our subjects, with re-hydrolysis into pyridoxal during the preparation of the filtrate for B_6 assay, appears to be the most likely mechanism for explaining the phenomena we have observed.

The influence of pyridoxine on the course of the tuberculosis of the patients studied has not been evaluated. So far, *in vitro* studies have failed to demonstrate any effect of pyridoxine in altering the effectiveness of INH against the H37Rv strain of *M. tuberculosis.*

Summary and conclusions. The excretion of an excess of vit. B_6 has been demonstrated in patients receiving isoniazid, with the irregular appearance of abnormal amounts of xanthurenic acid following a test dose of tryptophane. The effect is more marked in those getting a higher dose of INH. Such laboratory evidence of B_6 abnormality provides a cause of peripheral neuritis. Preliminary results suggest that the addition of pyridoxine to the high dose of INH prevents neuritis, which otherwise occurs in 40% of the cases.

The additional technical assistance of Helen Glazer is appreciated.

Generous supplies of pyridoxine were furnished by Dr. Elmer Alpert, Merck and Co., Rahway, N. J., Portions of the Isoniazid were furnished by Dr. Lawrence B. Hobson, E. R. Squibb and Sons, N. Y. City, and by Dr. W. G. Morson, Wm. S. Merrill Co., Cincinnati.

1. Pegum, J. S., *Lancet,* 1952, v2, 536.

2. Jones, W. A., and Jones, G. P., *ibid.,* 1953, v1, 1073.

3. Buchegger, G., and Weinreick, J., *Klin. Wochenschr.,* 1953, v31, 788.

4. Gammon, G. D., Burge, F. W., and King, G., *A. M. A. Arch. Neurol. and Psychiat.,* 1953, v70, 64.

5. Biehl, J. P., and Skavlem, J. H., *Am. Rev. Tuberc.,* 1953, v68, 297.

6. Lubing, H. N., *ibid.,* 1953, v68, 458.

7. Vilter, R. W., Mueller, J. F., Glazer, H. S., Jarrold, T., Abraham, J. P., Thompson, C., and Hawkins, V. R., *J. Lab. and Clin. Med.,* 1953, v42, 335.

8. Atkin, L., Schultz, A. S., Williams, W. L., and Frey, C. N., *Indust. and Engr. Chem. Anal. Ed.,* 1943, v15, 141.

9. Rabinowitz, J. C., and Snell, E. E., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 235.

10. Sarett, H. P., *J. Biol. Chem.,* 1951, v189, 769.

11. Huff, J. W., and Perlzweig, W. A., *ibid.,* 1943, v150, 395.

12. Glazer, H. S., Mueller, J. F., Thompson, C., Hawkins, V. R., and Vilter, R. W., *Arch. Biochem. and Biophysics,* 1951, v33, 243.

13. Goldsmith, G. A., Sarett, H. P., Register, V. D., and Gibbens, J., *J. Clin. Invest.,* 1952, v31, 533.

14. Follis, R. H., and Wintrobe, M. M., *J. Exp. Med.,* 1945, v81, 539.

15. Taylor, E. S., and Gale, E. F., *Biochem. J.,* 1945, v39, 52.

16. Reilley, R. H., Killam, K. F., Jenney, E. H., Marshall, W. H., Tausig, I., Apter, N. S., and Pfeiffer, C. C., *J.A.M.A.,* 1953, v152, 1317.

17. Parks, R. E., Kidder, G. W., and Dewey, V. C., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 287.

18. Yoneda, M., Kato, N., and Okajuna, M., *Nature,* 1952, v170, 803.

19. Yoneda, M., and Asano, N., *Science,* 1953, v117, 277.

Received December 23, 1953. P.S.E.B.M., 1954, v85.