TABLE II. Response of Rabbit #1 to Heat-Killed *E. coli* 086 and of Rabbit #2 to Viable *E. coli* 086. Rabbits were injected intravenously on day 1, 5, 9 and bled on day 1 and on day 21.

Sera—rabbit #1	Anti-B hemagg. titer	*E. coli* 086 agg. titer with heat-killed organisms	Bactericidal antibody titer *E. coli* 086	*E. coli* 02
1st day—pre-immunization	2	64	2.5	2.5
21st day—post-immunization	1024	1024	1960	2.5
Idem, after absorption with group B cells	2	1024	1560	2.5
Sera—rabbit #2				
1st day—pre-immunization	16	16	2.5	2.5
21st day—post-immunization	256	1024	666	2.5
Idem, after absorption with group B cells	2	1024	600	2.5

to determine the protective effect, if any, of anti-B against *E. coli* 086 or, indeed, of either isoantibody against any microbial agent which may possess blood group antigens. Nevertheless, cross-reactivity between blood group substance B and *E. coli* 086 affords a model for the possible influence of blood groups in resistance to infection against those microbial agents which may possess blood group antigens.

1. Rosenfield, R. E., *Blood,* 1955, v10, 17.

2. Race, R. R., Sanger, R., *Blood Groups in Man,* 2nd ed. (Charles C Thomas, Springfield, Ill., 1954), p.358.

3. McConnell, R. B.,*Ann. N. Y. Acad. Sci.,* 1956, v65, 12.

4. Jungeblut, C. W., Karowe, H. E., Braham, S. B., *Ann. Int. Med.,* 1947, v26, 67.

5. Manuila, A., *J.A.M.A.,* 1958, v167, 2047.

6. Oliver-Gonzalez, J., *Ann. Rev. Microbiol.,* 1954, v8, 353.

7. Springer, G. F., *J. Immunol.,* 1956, v76, 399; Springer, G. F., *Naturwissenschaften,* 1956, v43, 94.

8. Felix, A., Pitt, R. M., *J. Hyg.,* 1951, v49, 92.

9. Webster, M. E., Landy, M., Freeman, M. E., *J. Immunol.,* 1952, v69, 135.

10. Landy, M., Lamb, E., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 953.

11. Wiener, A. S., *Blood Groups and Transfusion,* 3rd ed., (Charles C Thomas, Springfield, Ill., 1943), p333.

12. Muschel, L. H., Chamberlin, R. H., Osawa, E., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 376.

13. Muschel, L. H., Treffers, H. P., *J. Immunol.,* 1956, v76, 1.

14. Mollison, P. L., *Blood Transfusion in Clinical Practice,* 2nd ed. (Charles C Thomas, Springfield, Ill., 1956), p504.

15. Boyd, W. C., Warshaver, E. R., *J. Immunol.,* 1945, v50, 101.

Received June 4, 1959. P.S.E.B.M., 1959, v101.

Pyridoxine Deficiency in Congestive Heart Failure. (25037)

HOWARD A. LEVY, MICHAEL G. WOHL AND ANTON SZUTKA
(Introduced by M. J. Boyd)
Nutrition Project, Philadelphia General Hospital and Divisions of Medicine and Biological Chemistry, Hahnemann Medical College, Philadelphia, Pa.

Our previous studies have shown the occurrence of thiamine deficiency in patients with advanced congestive heart failure. The deficiency was demonstrated by the thiamine test dose(1), pyruvic acid levels in the blood (2), and determination of thiamine and cocarboxylase content in myocardial tissues of patients dying of congestive heart failure(3). In view of the fact that Vit. B_6 is a member of the Vit. B complex and is required for a great variety of enzymatic reactions, it appeared desirable to investigate Vit. B_6 metabolism in congestive heart failure.

Methods and material. The presence of Vit. B_6 complex is required for metabolic breakdown of tryptophan. In induced Vit.

B_6 deficiency, derangement of tryptophan metabolism is manifested by increased excretion of xanthurenic acid after a tryptophan load(4,5). Administration of Vit. B_6 corrects this aberration and xanthurenic acid excretion is greatly decreased or becomes normal(6). We have used the increased excretion of xanthurenic acid (XA) after a load test of tryptophan and its correction by administration of pyridoxine as an index of a relative pyridoxine deficiency. Twenty patients (14 males and 6 females) were selected from the medical services of the Hahnemann Hospital. The patients were hospitalized for treatment of congestive heart failure. The causes leading to heart failure were hypertension, arteriosclerotic coronary artery disease and rheumatic heart disease. No patient with heart failure was studied unless organic heart disease was clinically demonstrable and objective evidence of peripheral or pulmonary edema, tachycardia, tachypnea were present. Elevation in venous pressure and prolonged arm-to-tongue circulation time (Decholin) were demonstrated in each patient by the standard technics. *Controls.* Seventeen patients free from congestive failure served as controls. They were patients of similar age and sex distribution as the cardiac group (13 males and 4 females), hospitalized for treatment for chronic osteomyelitis, anxiety neurosis, cerebral thrombosis, fracture of the femur, phlebothrombosis and malignancy of gall bladder. The daily diet of both groups of patients was the standard Hahnemann Hospital 1.0 g sodium diet containing on the average 2900 calories with protein, carbohydrate and fat and vitamin content in excess of that recommended by NRC (Food and Nutrition Board). Every effort was made to encourage patients under study to consume the daily allowed food. However, it is possible that in a few instances, a portion of the food was not eaten. We are aware that this factor may contribute to variation in amounts of xanthurenic acid excretion. However, the validity of the results of the overall study are not impaired since our aim is to evaluate the status of Vit. B_6 nutrition in patients with congestive heart failure as they are observed in a general hospital under ordinary hospital care. The food intake of our patients represents probably the usual dietary experiences of hospitalized cardiac patients. None of the patients in our series, to our knowledge, were taking supplementary vitamins prior to the study. Xanthurenic acid excretion was determined by a modified method of Rosen(7) on a 24 hour urine specimen. A control XA excretion before the tryptophan load test was done. On the morning of the second day, a 10 g dose of d, l-tryptophan (Dow Chemical Co.) was given mixed in fruit juice and a second 24-hour urine collection obtained. On the morning of the third day, another 10 g dose of tryptophan was given as on the previous day and the third 24-hour urine was collected. On the morning of the fourth day, 50 mg of pyridoxine-HCl (U.S.P.) was administered intramuscularly 30 minutes before a third dose of 10 g of tryptophan was given, and this was followed by the fourth and final 24-hour urine collection. Urine specimens were collected on the ward under toluene, the reaction was adjusted to pH 5.5 with 5 normal hydrochloric acid, aliquots were stored in amber glass bottles at 4°C. Initially, all urine was refrigerated as soon as it was voided, but this was not continued since it was found that no significant loss of XA occurred when urine was maintained at room temperature. Accuracy of collections was checked by carrying out urinary creatinine determinations by the method of Folin(8). More accurate collections were obtained if collection bottles were kept at the bedside or carried with the patients to X-ray or other hospital locations during the study. When a variation in creatinine of more than 25% was found, the patient was dropped from the study. Complete collections were deemed essential, since preliminary studies had revealed that the largest amounts of xanthurenic acid excretion in both controls and patients with heart failure appeared in the first 8 hours after oral ingestion of tryptophan. During the period of study, all patients were given supplements of 10 mg of thiamine and 5 mg of riboflavin by mouth daily, since studies by Dalgliesh(9) had pointed out the role of these vitamins in metabolism of tryptophan. None of the patients taking tryptophan experienced any severe side

TABLE I. Xanthurenic Acid Excretion in Control Patients.

		Before trypt.			After trypt. 1			After trypt. 2			After trypt. + B_6		
Sex	Age	Vol	Creat.	XA	Vol	Creat.	XA	Vol	Creat.	XA	Vol	Creat.	XA
♂	49	3100	1.2	3.9	2000	1.0	15.8				2600	1.1	13.6
♀	69	1280	1.0	3.1	1680	1.1	5.8	1200	1.0	7.2	1720	1.1	7.5
♂	67	750	.9	5.9	1490	1.0	14.9				1110	1.2	7.0
♂	74	530	.9	5.6	1100	1.8	15.7				575	.8	5.2
♂	19	900	.6	3.2	1740	1.7	9.9	1240	.9	11.9	1300	1.2	8.4
♂	62	1220	1.1	7.8	1440	.9	7.1	840	.6	7.1	1880	1.0	10.4
♂	78	2660	1.4	7.8	1160	1.2	22.2				2610	2.5	18.0
♂	42	2240	.7	1.0	880	.5	9.2	1470	1.2	5.3	420	.5	1.0
♂	46	550	1.1	2.1	1670	1.0	10.2	2370	1.3	17.0	2200	1.6	7.0
♀	30	700	.9	8.8	1560	.9	14.2				1310	1.2	12.3
♀	80	1340	.6	2.8	1140	1.0	11.9				840	.8	10.5
♂	25	1185	1.8	2.6	1745	1.4	6.1				680	1.1	1.2
♂	72	1000	1.2	8.0	800	1.2	17.8	980	1.5	18.8	1820	1.4	16.7
♂	44	1090	1.6	7.7	2550	1.5	20.1				1800	1.5	11.5

TABLE II. Xanthurenic Acid Excretion in Heart Failure Patients.

		Before trypt.			After trypt. 1			After trypt. 2			After trypt. + B_6		
Sex	Age	Vol	Creat.	XA	Vol	Creat.	XA	Vol	Creat.	XA	Vol	Creat.	XA
♀	58	530	.6	3.8	840	.8	52.4	760	.7	74.7	700	.5	4.6
♀	75	750	.8	5.0	1000	.7	55.8	770	.5	101.0	900	.8	14.6
♂	75	435	.5	1.1	800	.6	12.0	1125	.5	40.9	1860	1.0	13.0
♂	42	1970	1.0	1.0	2670	.9	14.9	920	.6	22.2	860	.7	6.4
♀	50	430	.5	2.2	500	.5	43.6				800	.8	14.9
♂	40	1180	1.3	4.0	1100	1.4	77.9				920	1.1	8.8
♂	72	1940	1.1	9.6	2375	1.4	8.3	500	.9	31.8	510	1.2	4.2
♂	48	2370	2.1	3.3	655	1.4	24.9	450	.9	26.5	505	.8	2.9
♂	62	1220	.7	3.4	1030	1.1	8.2				780	.7	7.0
♂	68	1600	.8	4.5	1780	.9	69.0	1380	.7	42.3	2530	1.4	20.9
♂	63	2850	1.9	3.1	2590	1.5	38.6				1920	1.2	12.2
♀	72	395	.9	3.0	430	.5	37.8	795	.3	38.7	700	.5	2.6
♂	70	1838	1.1	3.2	1420	1.0	14.1	1320	.7	32.7	1370	.7	18.0
♂	59	540	1.0	7.3	510	.6	40.8				1585	.7	14.1

reactions, although 2 patients were omitted from the study because of persistent nausea and vomiting on each occasion they were given the material, no matter how it was disguised. No change in blood urea nitrogen, or liver function, was noted in any of the patients during the study. Serum glutamic-oxaloacetic transaminase determinations were carried out by the method of Cabaud *et al.*(10) on each patient, since this enzyme is known to be involved in pyridoxine metabolism. The correlation between the S.G.O. transaminase levels and xanthurenic acid excretion will be published separately.

Results. Tables I and II present results of xanthurenic acid excretion, creatinine, and volume of 24 hr urine collections on all subjects. Fourteen heart failure patients and 14 control patients were selected for analysis who fulfilled the following criteria: 1. There was a pre-tryptophan, a post-tryptophan, and a post-tryptophan and pyridoxine value. 2. The level of creatinine in all urine collections was 0.4 g per day or higher. Collections with levels of 0.3 g or less were considered as fair evidence of poor collection of urine and were discarded. Means and standard deviations for these subjects are shown in Fig. 1.

The heart failure group and control group

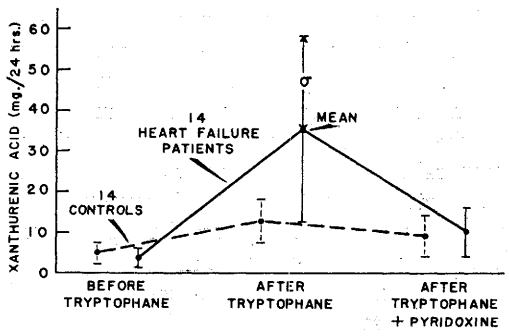

FIG. 1. Xanthurenic acid excretion in heart failure and control patients.

were quite comparable with respect to both mean and standard deviation in pre-tryptophan level of xanthurenic acid excretion with means of 3.9 and 5.0 mg/24 hr respectively. Following tryptophan load, each showed a highly significant mean increase over the pre-tryptophan level (probability of "t" less than 0.001 in each case). The mean increase for the heart failure group was, however, significantly greater than that for the control group (probability of "t" less than 0.01) so that the former reached a significantly higher mean level of excretion (35.6 *vs.* only 12.9 mg/24 hr). In addition, the variance for the heart failure group was considerably and significantly greater than that of the control group (standard deviation of 22.8 and 5.2 mg/24 hr respectively). This greater variance in the heart failure group was to be expected because these patients differed greatly in severity of condition while the control group was more homogeneous in the sense that none suffered from heart failure. The highest level attained by a control patient was 22.2 mg/24 hr while two-thirds of the heart failure patients exceeded this value.

When a tryptophan load was given after pyridoxine, the level was, for each group, significantly lower than with tryptophan load alone but significantly higher than in the pre-tryptophan period. In 20 of the 28 subjects, this pattern (lowest in the pre-tryptophan, highest in the post-tryptophan and intermediate in the post-tryptophan plus pyridoxine period) was followed exactly. The heart failure group, which had attained a higher level than the control group in the post-tryptophan period, showed a greater decline from post-tryptophan to post-tryptophan plus pyridoxine so that the 2 groups were at approximately the same level after pyridoxine (10.3 mg/24 hr for the heart failure group and 9.3 mg/24 hr for the control group). They also showed comparable variances after pyridoxine (standard deviation of 5.9 and 5.0 respectively). While the level after tryptophan plus pyridoxine was higher than that in the pre-tryptophan period, the difference was significant only in a statistical sense; however, it is apparent that pyridoxine decreased xanthurenic acid excretion levels comparable to those of the control pyridoxine treated group.

There were 16 subjects (8 heart failure and 8 control) to whom a second tryptophan load had been given. In these patients, the difference between results of the first tryptophan and second tryptophan loads were analyzed. The heart failure group showed an additional mean rise of 11.8 mg/24 hr with a standard deviation of 21.0, the control group showed an additional mean rise of 1.7 mg/24 hr with a standard deviation of 3.1. This indicates that the tryptophan load caused a true biochemical abnormality and not an artefact, and that in fact, abnormality became more severe after a second load test. The ability of pyridoxine to restore the body's response to tryptophan to normal in both controls and heart failure must be considered a specific effect.

Discussion. The statistically significant high levels of xanthurenic acid excretion in patients with congestive heart failure, tend to indicate that the nutritional status of the patients with respect to Vit. B_6 was subnormal. The biochemical response to administration of pyridoxine with improvement in derangement of tryptophan metabolism would further strengthen this conclusion. The only reference in the literature in regard to tryptophan metabolism in cardiac patients is that by Wachstein and Lobel(11); they found in 4 patients with cardiac decompensation, 24 hour urinary excretion of xanthurenic acid after a tryptophan load varying between 3 and 19 mg (3 mg, 10 mg, 14 mg and 19 mg respectively). These figures parallel some of our non-cardiac controls. It is to be regretted that no data are given as to the clinical status of the patients or as to whether compensation was restored by the conventional cardiac therapy. Furthermore, the patient in whom xanthurenic acid excretion after the tryptophan load was 19 mg responded to 100 mg of pyridoxine with zero xanthurenic acid excretion. We studied tryptophan metabolism in 9 patients after compensation was restored with bed rest, salt free diet, diuretics and digitalis. After restoration of compensation, xanthurenic acid excretion was within the limits of our controls (Table III). The question arises as to the factors accounting for the ab-

TABLE III. Xanthurenic Acid Excretion in Compensated Heart Failure Patients.

		Before trypt.			After trypt.			After trypt. + B_6		
Sex	Age	Vol	Creat.	XA	Vol	Creat.	XA	Vol	Creat.	XA
♂	46	550	1.1	2.1	1670	1.0	10.2	2200	1.6	7.0
♀	70	838	1.1	3.2	800	.75	17.4	600	.7	10.5
♂	74	503	.9	5.6	1100	1.8	15.7	575	.7	5.2
♀	70			8.9	520	.8	8.3	1620	1.1	1.8
♂	60	1200	.5	1.2	1260	.9	15.2	1240	1.0	14.9
♀	60	2480	1.4	3.9	970	1.0	18.2	900	1.0	10.3
♀	65	2380	1.4	9.7	2045	1.1	19.0	1800	1.1	13.5
♀	58	1985	1.5	7.9	2080	1.6	18.1	2100	1.6	18.5
♀	68	2930	1.2	4.3	3070	2.1	11.3	2425	1.2	7.7

normal tryptophan metabolism in congestive heart failure. It is quite likely that the basic difficulties in absorption and utilization of food factors present in heart failure may play an important role. The anorexia with inadequate food intake, anoxia of the intestinal tract due to passive congestion with resultant interference of absorption of pyridoxine supplied by the inadequate diet, may be a further reason for the abnormal tryptophan metabolism. That a similar situation exists with regard to thiamine in heart failure was pointed out(12). On the other hand, the abnormal tryptophan metabolism may indicate only a relative deficiency of pyridoxine: rate of synthesis of pyridoxine-containing coenzymes in the body may be less than rate of utilization.

The patients considered in these studies did not show gross clinical manifestations of pyridoxine deficiency. It is possible that pyridoxine deficit may occur in cardiac muscle not unlike that of thiamine, whereas peripheral organs may be free of that deficiency.

The limited studies available on pyridoxine metabolism in heart failure are indicative of the need for much more extensive investigation particularly at the blood and tissue level both from a clinical and laboratory point of view. Likewise, an evaluation of the effect of daily pyridoxine supplementation of the conventional therapy for congestive failure is desirable.

Summary. 1. Fourteen patients with congestive heart failure and 14 control patients without heart failure were studied with the tryptophan load test. 2. Urinary xanthurenic acid excretion following tryptophan load was significantly greater in the cardiac group than in controls. Mean XA excretion of the group with congestive failure was 35.6 mg/24 hours with standard deviation of 22.8. Mean excretion of the control group was 12.9 mg/24 hours with standard deviation of 5.2 mg/24 hours. 3. The heart failure group, which attained a higher level of XA excretion than the control group in the post tryptophan period, showed a greater decline in XA excretion after pyridoxine than the controls. 4. Since pyridoxine is required for orderly catabolism of tryptophan and pyridoxine corrects this metabolic aberration of tryptophan, it is not unreasonable to state that in congestive heart failure availability of Vit. B_6 is limited.

We acknowledge our appreciation to Dr. H. Menduke for statistical analysis of the data and to Elsie M. Faust for technical assistance.

1. Wohl, M. G., Shuman, C. R., Turner, R., Fittipoldi, J. J., Jr., *Circulation,* 1953, v7, 744.

2. Alper, C., Wohl, M. G., Shuman, C. R., Fittipoldi, J. J., Jr., *Fed. Proc.,* 1955, v14, 426.

3. Wohl, M. G., Brody, M., Shuman, C. R., Turner, R., Brody, J., *J. Clin. Invest.,* 1954, v33, 1580.

4. Lepkowsky, S., Roboz, E., Haagen-Smith, A. J., *J Biol. Chem.,* 1943, v149, 195.

5. Lepkowsky, S., Nielsen, E., *ibid.,* 1942, v144, 135.

6. Wachstein, M., Lobel, S., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 624.

7. Rosen, F., Huff, J. W., Perlzweig, W. A., *J. Nutr.,* 1947, v33, 561.

8. Folin's photometric method modified from Hawk, Oser and Somerson: *Practical physiological chemistry,* 12th edition, p840, 1949.

9. Dalgliesh, C. E., *Biochim. Biophys. Acta,* 1954, v15, 295.

10. Cabaud, P., Leper, R., Wroblewski, F., *Am. J. Clin. Path.,* 1956, v26, 1101.

11. Wachstein, M., Lobel, S., *ibid.,* 1956, v26, 910.

12. Wohl, M. G., Shuman, C. R., Alper, C., *A.M.A. Arch. Intern. Med.,* 1955, v96, 11.

Received June 9, 1959. P.S.E.B.M., 1959, v101.